Band 45,
Supplement 1,
September 2018

Transfusion Medicine and Hemotherapy

Founded in 1973 as «Die Infusionstherapie» by H. Reissigl, H. Grobecker, U. Henneberg, K.H. Bäßler
Continued by: H.-D. Viering (1973–1977), A. Grünert (1982–1992), G. Kleinberger (1982–1988),
K. Widhalm (1988–1992), G. Wolfram (1990–1992), J. Eckart (1992–1997), H. Forst (1992–1997),
K. Meßmer (1992–1997), K. Peter (1992–1997), K.H. Usadel (1992–1997), B. Zwißler (1995–1997),
K. Gutensohn (1998–2001), V. Kretschmer (1990–2002), W. Stangel (1992–2005),
W. Dzik (1998–2005), H.G. Klein (1998–2005), W. Sibrowski (2003–2014)

Official Organ of
German Society for Transfusion Medicine and Immunohematology

dgti
Die Gesellschaft für Blut, Zellen & Gewebe

Deutsche Gesellschaft für Transfusionsmedizin und Immunhämatologie

KARGER
Basel · Freiburg · Paris · London · New York · Chennai · New Delhi · Bangkok · Beijing · Shanghai · Tokyo · Kuala Lumpur · Singapore · Sydney

Disclaimer: The statements and data contained in this publication are solely those of the individual authors and contributors and not of the publisher and the editors. The appearance of advertisements in the journal is not a warranty, endorsement, or approval of the products or services advertised or of their effectiveness, quality or safety. The publisher and the editors disclaim responsibility for any injury to persons or property resulting form any ideas, methods, instructions or products referred to in the content or advertisements.

Die veranstaltenden Gesellschaften, das Programm- und Organisationskomitee sowie die Kongressorganisation und der S. Karger Verlag übernehmen keine Gewähr für die Richtigkeit von Angaben in den Abstracts.

Die Abstracts und das Autorenverzeichnis wurden ohne Lektorierung publiziert, für den Inhalt der Abstracts sind die Autoren verantwortlich.

Imprint

ISSN Print Edition: 1660–3796
ISSN Online Edition: 1660–3818

Journal Homepage: http://www.karger.com/tmh

Publication Data: Volume 45, 2018 of 'Transfusion Medicine and Therapy' appears with 6 issues.

Disclaimer: The statements, opinions and data contained in this publication are solely those of the individual authors and contributors and not of the publisher and the editor(s). The appearance of advertisements in the journal is not a warranty, endorsement, or approval of the products or services advertised or of their effectiveness, quality or safety. The publisher and the editor(s) disclaim responsibility for any injury to persons or property resulting form any ideas, methods, instructions or products referred to in the content or advertisements.

Subscription Rates: Subscriptions run for a full calendar year.
Prices are given per year.

Print:	EUR 192.00 + postage and handling
Online:	EUR 192.00
Combined (print+online):	EUR 242.00 + postage and handling

Postage and handling (added to print and print+online)**:**
EUR 19.00 (Germany), EUR 25.00 (Rest of World)

Discount subscription prices:
Please enquire about reduced rates for members of related societies.

Back Volumes and Single Issues: Information on availability and prices of single print issues and print or electronic back volumes can be obtained from Customer Service at aboservice@karger.com

For customers in Germany: Please contact your local bookstore or
S. Karger Verlag für Medizin und Naturwissenschaften GmbH
Wilhelmstr. 20A, 79098 Freiburg (Germany)
Tel. +49 761 45 20 70, Fax +49 761 45 20 714
aboservice@karger.com

For customers in all other countries: Please contact your bookshop or
S. Karger AG
Allschwilerstr. 10, 4009 Basel (Switzerland)
Tel. +41 61 3 06 11 11, Fax +41 61 3 06 12 34
karger@karger.com

Advertising: Correspondence should be addressed to the publisher.
S. Karger Verlag für Medizin und Naturwissenschaften GmbH
Attn. Ellen Zimmermann (Head of Marketing)
e.zimmermann@karger.com
Price list No. 32 of January 1, 2018 is effective.

Publisher: S. Karger Verlag für Medizin und Naturwissenschaften GmbH
Wilhelmstr. 20A, 79098 Freiburg (Germany)
www.karger.de, information@karger.com

V.i.S.d.P. (Person responsible according to the German Press Law):
Susanne Meister

Type setting and printing: Kraft Premium GmbH, 76275 Ettlingen, Germany.
Printed on acid-free and non-aging paper (ISO 9706).

Indexing/Abstracting
Listed in bibliographic services, including:
PubMed / PubMed Central / Web of Science / Science Citation Index / Science Citation Index Expanded / Current Contents – Clinical Medicine / BIOSIS Previews / BIOSIS Reviews Reports And Meetings / Google Scholar / Scopus / Embase / Pathway Studio / WorldCat

Supplement 1/2018
ISBN 978-3-318-06429-2
e-ISBN 978-3-318-06430-8

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

Accessible online at:
www.karger.com/tmh

Transfusion Medicine and Hemotherapy

Band 45,
Supplement 1,
September 2018

51. Jahrestagung der Deutschen Gesellschaft für Transfusionsmedizin und Immunhämatologie (DGTI)

19.–21. September 2018, Lübeck

dgti
Die Gesellschaft für
Blut, Zellen & Gewebe

ABSTRACTS

Gast-Herausgeber

Prof. Dr. Siegfried Görg, Lübeck

Prof. Dr. Holger Hennig, Lübeck

KARGER

Basel · Freiburg · Paris · London · New York · Chennai · New Delhi ·
Bangkok · Beijing · Shanghai · Tokyo · Kuala Lumpur · Singapore · Sydney

Transfusion Medicine and Hemotherapy

Contents

Transfus Med Hemother 2018;45(suppl 1):IV

Plenarsitzungen	PL-1	Aktuelle Herausforderungen der Transfusionsmedizin	1
	PL-2	Stammzelltransplantation und Zelltherapie II – Joint session DGHO	1
	PL-3	Perioperatives Anämie-Management	2
	PL-4	Transfusion Medicine in Denmark (Neighbour Day)	3
	PL-5	Infektionssicherheit III	3
Zentrale klinische Fortbildung	ZF-2	Zentrale klinische Fortbildung II	5
	ZF-3	Zentrale klinische Fortbildung III	5
Vortragssitzungen	VS-1	Präparative und therapeutische Hämapherese	6
	VS-2	Automation und Datenverarbeitung	8
	VS-3	Stammzelltransplantation und Zelltherapie I – Joint session GSCN	10
	VS-4	Immunhämatologie	12
	VS-5	Präparative und therapeutische Plasmapherese – Joint session ARGE Plasmapherese	15
	VS-6	Infektionssicherheit I	18
	VS-7	Gewebezubereitungen	20
	VS-8	Hämostasiologie I	21
	VS-9	Gentherapie – Joint session DG-GT	25
	VS-10	Blutspende und Weiterverarbeitung	25
	VS-11	Hämostaseologie II – Joint session GTH	30
	VS-12	Infektionssicherheit II	30
	VS-13	Transplantationsimmunologie und Immungenetik – Joint session DGI	32
	VS-14	Hämotherapie (Neighbour Day)	34
	VS-15	Pränatale Diagnostik und Therapie	36
	VS-16	Zell- und Gentherapie	38
	VS-17	Immunity	42
	VS-18	Hämostaseologie III	43
	VS-19	Neighbour Day – Molecular Typing (Neighbour Day)	43

DGHO = Deutsche Gesellschaft für Hämatologie und Onkologie e.V.; GSCN = German Stem Cell Network – Deutsches Stammzellnetzwerk e.V.; ARGE Plasmapherese = Arbeitsgemeinschaft Plasmapherese e.V.; DG-GT = Deutsche Gesellschaft für Gentherapie e.V.; GTH = Gesellschaft für Thrombose- und Hämostaseforschung e.V.; DGI = Deutsche Gesellschaft für Immungenetik e.V.

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

Accessible online at:
www.karger.com/tmh

Transfusion Medicine and Hemotherapy

Contents

Transfus Med Hemother 2018;45(suppl 1):VI

Posterbeiträge

PS-1A	Hämatopoetische Stammzellen	47
PS-1B	Zelltherapie	50
PS-2A	Blutspende	53
PS-2B	Herstellung und Qualitätskontrolle	56
PS-3A	Immunhämatologie	61
PS-3B	Hämotherapie und Patienten-Blut-Management	66
PS-4A	Infektionssicherheit von Blutprodukten	68
PS-4B	Hämostaseologie	73
PS-5A	Immunhämatologische Genetik	75
PS-5B	Immungenetik	79
PS-5C	Präparative und Therapeutische Apherese	81
PS-5D	Qualitätssicherung	82

Author Index 85

Imprint II

Die Zahlen in den Poster-IDs bezeichnen das ePoster Terminal des jeweiligen Themas.

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

Accessible online at:
www.karger.com/tmh

Transfusion Medicine and Hemotherapy

Abstracts

Transfus Med Hemother 2018;45(suppl 1):1–90
DOI: 10.1159/000492927

Plenarsitzungen

PL-1
Aktuelle Herausforderungen der Transfusionsmedizin

PL-1-1

Demographic change und blood

Katalinic A., Pritzkuleit R.

Universität zu Lübeck, Institut für Sozialmedizin und Epidemiologie, Lübeck, Deutschland

Background: The ratio of young to old people in Germany is shifting more and more towards the elderly. Because risk of most disease rises with age, an aging society will be confronted with a higher burden of diseases. This might have also an impact on the supply with blood.
Methods: Because of missing data on age, sex and disease-specific blood supply rates we used selected diseases or therapies as a proxy for blood supply. Based on sex- and age-specific rates and on official predictions of population figures, we did projections of absolute disease numbers and number of therapies. Additionally we examined further data sources to estimate the current demand for blood supply.
Results: The number of persons older than 65 years will rise from by about 30% until 2030 (from 2010). For diseases or therapies which occur in higher age, an increase in disease/therapy frequency was estimated for the next decades. E.g. number of new diagnosed cancer patients will rise from 2010 to 2030 by about 30%. Crude incidence rate will increase by 37%. Number of femoral neck fractures will rise by about 80%. Endoprosthetic surgery of hips and knees will increase by 22%, colon surgery by about 13%.
Conclusion: Blood supply is needed for therapy of many of the investigated diseases. Therefore, it can be assumed that future demand of blood will rise in a similar manner as the rising number of diseases. The projected developments describe an extraordinary challenge for the German health care system and in particular for transfusion medicine.

Literature:

1. Katalinic A, Peters E, Beske F, Pritzkuleit R.: Projection of Morbidity 2030 and 2050: Impact for the National Health System and Blood Supply. Transfus Med Hemother. 2010;37(3):155–159.
2. Beske F, Brix F, Katalinic A, Peters E, Pritzkuleit R.: Versorgungsprognose 2060. Kiel. 2012.
3. Pritzkuleit R, Katalinic A.: Demografische Entwicklung und Krebsentwicklung in Deutschland. In: Keck T, Hopt UT, editors. Onkologische Chirurgie bei alten Patienten. Berlin, Heidelberg: Springer-Verlag; 2016:3–11.

Conflict of interest: none

PL-2
Stammzelltransplantation und Zelltherapie II – Joint session DGHO

PL-2-1

CAR T cells – promise and challenges

Rössig C.

Universitätsklinikum Münster, Pädiatrische Hämatologie und Onkologie, Münster, Deutschland

Treatment with T cells expressing recombinant chimeric antigen receptors (CARs) has emerged as a powerful strategy to target B-lineage leukemias and lymphomas. CARs combine the ability of antibodies to interact with tumor-associated surface antigens with the capacity of stimulatory signaling domains to induce T cell effector responses. In independent clinical trials in patients with refractory CD19-positive cancers, T cells that were gene-modified to express CD19-specific CARs have induced complete, even molecular, remissions in the majority of patients.
A toxic side effect of CAR T cell therapy is cytokine release syndrome (CRS), with high temperatures and sometimes life-threatening arterial hypotension and respiratory failure. CRS has become manageable by administration of IL-6 receptor antagonists upon early clinical signs. CAR gene-engineered T cells have the ability to persist *in vivo* for prolonged periods up to many years. They can mediate protective tumor-antigen specific memory responses, with durable remissions. Another advantage is effective penetration of CAR T cells into the central nervous system where they efficiently eliminate leukemic cells and thus prevent relapses in this compartment. Relapses after CAR T cell therapy are a consequence of either limited persistence of CAR T cells, or emergence of CD19-negative resistant clones by either CD19 splice variants or outgrowth of CD19-negative myeloid subclones.
Today, broad implementation of CAR T cell therapy in B cell cancers is still challenged by the need for individualized manufacturing of the T cell products. And to extend the promise of the strategy towards other cancers, including solid tumors, adequate CAR target antigens in cancers outside the B cell lineage have to be identified. Moreover, methods and combination strategies to overcome immune-inhibitory barriers against the engineered T cells in the tumor microenvironment will have to be developed.

Conflict of interest: none

PL-2-2

A novel CAR T cell approach eliminates prostate cancer in a mouse tumor model

Cathomen T.

Universitätsklinikum Freiburg, Institut für Transfusionsmedizin und Gentherapie, Freiburg, Deutschland

Although chimeric antigen receptor (CAR) T cell immunotherapy targeting CD19 has shown remarkable success in patients with hematologic malignancies, the potency of CAR T cells in solid tumors, such as prostate

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

Accessible online at:
www.karger.com/tmh

cancer, has been low thus far. Several CARs targeting prostate cancer associated antigens, such as prostate-specific membrane antigen (PSMA), have been described. Although PSMA is abundantly expressed on prostate cancer epithelial cells, the potency of those CARs seems modest both *in vitro* and *in vivo*. One way to overcome moderate potency or unsatisfactory specificity is to develop novel CARs based on highly affine and highly specific scFv. Here, we report the development of novel 2nd generation CARs based on a scFv derived from the monoclonal antibody 3/F11, which was shown to bind extracellular PSMA with higher affinity and specificity than previously described monoclonals. Potency of these novel CARs was tested by retroviral transfer to T cells and assaying the resulting CAR T cells *in vitro* and *in vivo*. The generated CAR T cells could be expanded to large numbers while keeping a T stem cell memory phenotype. Upon antigen sensitization *in vitro,* these CAR T cells released pro-inflammatory cytokines and completely eliminated PSMA-positive tumor cells at a low effector-to-target ratio. Moreover, as determined histologically and by *in vivo* imaging, the generated CAR T cells were able to eradicate solid PSMA-positive tumors in a mouse xenotransplantation model in combination with chemotherapy. In conclusion, the preclinical evaluation of our novel PSMA targeting CARs demonstrates that the derived CAR T cells combine excellent specificity with high antigen-specific cytolytic activity *in vitro* and *in vivo,* hence supporting the translation of these CAR T cells to a clinical setting.

Conflict of interest: none

PL-3
Perioperatives Anämie-Management

PL-3-1

Use of Erythropoietin

Jelkmann W.

ehem. Universität zu Lübeck, Lübeck, Deutschland

Erythropoietin (Epo) is the primary factor for the maintenance of the mass of red blood cells (RBCs), as indicated by normal blood hemoglobin concentration ([Hb]) and hematocrit (Hct). Epo is a 30.4-kDa glycoprotein hormone composed of 165 amino acids, 3 tetra-antennary *N*-linked glycans and 1 small *O*-linked glycan. Epo acts *via* specific cell-membrane receptors (EpoR) of myeloid erythrocytic progenitors and precursors. In response to Epo, colony forming units-erythroid (CFU-Es) and their progeny divide several times, thus generating 8–64 erythroblasts within 7–8 days. Therefore, Epo increases RBC mass in a delayed way.

Recombinant human Epo (rhEpo, Epoetin) is an established therapeutic for anemic patients suffering from chronic kidney disease or cancer requiring chemotherapy. Two decades ago, coinciding with the growing awareness of the possibility of viral infections through blood transfusions, rhEpo was also licensed for the stimulation of RBC production in the perioperative setting in the European Union. There are two approved indications for rhEpo.

First, rhEpo (Epoetin alfa, beta or zeta) can be administered in pre-donation programs to increase the yield of autologous blood in mildly anemic (*e.g.* (Hb) 100–130 g/l; Hct 0.33 to 0.39), non-iron deficient, adult patients undergoing major elective surgery accompanied by considerable blood loss requiring pre-deposit of ≥4 units of blood and a high perceived risk for transfusion complications. The ability to donate blood depends predominantly on the patient's blood volume and baseline packed cell volume (PCV). RhEpo is administered at relatively high doses (*e.g.* 600 IU *per* kg body weight 1–2 times weekly for 3 to 4 weeks prior to surgery). Concomitant iron replacement is important for the effectiveness of rhEpo therapy.

Second, rhEpo (Epoetin alfa or zeta) can be administered to reduce the exposure to allogeneic blood in patients undergoing major elective orthopedic surgery. Here, the therapy is intended to augment an already stimulated erythropoiesis at the time of surgery and consecutively reduce the extent and duration of postoperative anemia. If [Hb] reaches ≥150 g/l (9.38 mmol/l) during the preoperative period rhEpo administration should be stopped. Of note, patients scheduled for major elective orthopedic surgery should receive adequate antithrombotic prophylaxis, as thrombotic and vascular events may occur, especially in patients with underlying cardiovascular disease. Special precaution should be taken in patients with predisposition for development of deep vein thrombosis (DVT). RhEpo should not be used in patients with baseline [Hb] of >130 g/l (>8.1 mmol/l).

In addition to its use in licensed indications, rhEpo was applied in unapproved clinical situations («off-label» use), such as in patients undergoing cardiac surgery, and in Jehovah´s witnesses who do not accept blood transfusions.

Conflict of interest: Der Autor hat Honorare von Firmen erhalten, die Epoetine vermarkten bzw. entwickeln.

PL-3-2

Towards evidence-based patient blood management: The International Consensus Conference in Frankfurt/Main, Germany in April 2018

Seifried E.[1], Van Remoortel H.[2], Meybohm P.[3], Vandekerckhove P.[2], Aranko K.[4], Müller M.[1]

[1]DRK-Blutspendedienst Baden-Württemberg-Hessen, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt am Main, Deutschland
[2]Belgian Red Cross, Centre for Evidence-Based Practice, Mechelen, Belgien
[3]Universitätsklinik Frankfurt, Abteilung für Anästhesiologie, Intensivmedizin und Schmerztherapie, Frankfurt am Main, Deutschland
[4]European Blood Alliance, Amsterdam, Niederlande

Introduction: Patient Blood Management (PBM) is a comprehensive initiative in quality management focussing on improving patients outcome by diagnosing and treating preoperative and perioperative anaemia, implementing blood saving measures throughout the courses of diagnosis and treatment, and transfusing patients following accepted and evidence-based transfusion triggers.

Aim: The international consensus conference (ICC) on PBM was initiated in order to evaluate current evidence regarding three different topics in PBM. Firstly, red blood cell concentrate (RBC) transfusion triggers are available for different perioperative and clinical settings, but the evidence quality in different indications is variable ranging from no or very low-quality evidence available to high-quality evidence. Secondly, pre- and perioperative anaemia is not uniformly accepted as a perioperative risk factor, while definition, diagnosis and treatment are still a matter of debate. Thirdly, PBM measures are not uniformly implemented in different countries and not even within one single country. The ICC PBM took place in Frankfurt/Main, Germany on April, 24 and 25, 2018.Together with partners from Australia, Canada and further international scientific groups, the European Blood Alliance (EBA), the AABB, formerly known as the Association of American Blood Banks, the International Society of Blood Transfusion (ISBT) and the French (SFTS), Italian (SIMTI) and German (DGTI) scientific Societies of Blood Transfusion co-sponsored these activities.

Methodology: PBM for the ICC was defined according to the World Health Organization (WHO) definition. PBM is a patient-focused, evidence-based and systematic approach to optimize the management of patients and transfusion of blood products for quality and effective patient care. To identify the available scientific evidence, the Scientific Committee (SC) phrased different PICO (Population, Intervention, Comparison, Outcome) questions according to the three chosen topics: 1) preoperative anaemia, 2) RBC transfusion triggers and 3) implementation of PBM. The Centre for Evidence-Based Practice (CEBaP) carried out a systematic literature review and developed search strategies in four different biomedical databases (Pubmed, Embase, Cochrane Library and Transfusion Evidence Library). CEBaP screened approx. 18,000 papers and included more than 140 relevant papers within the three PBM topics. The evidence-based con-

clusions as well as the quality of the evidence using the GRADE methodology was presented at the Frankfurt consensus conference by the SC. A transparent evidence-to-decision framework according to the GRADE approach was used by three multidisciplinary expert panels to formulate recommendations and compile consensus statements.
Results: Consensus statements with the supporting evidence will be presented during the lectures. A comprehensive summary of all consensus statements will be published later.

Conflict of interest: none

PL-4
Transfusion Medicine in Denmark (Neighbour Day)

PL-4-1
Transfusion Medicine in Denmark and Germany – Differences and similarities

Homburg K. M.

Region Zealand, Dept of Clinical Immunology, Transfusion and Tissue Services, Naestved, Dänemark

Even though Denmark and Germany are neighbouring countries, we have a lot of differences in the way we manage the procurement of donors, blood production, testing and issue of blood components. We have similar culture and share many values and work under legislation derived from the same EU Directives, but the implementation of these are different as well as the organisation of our services.
The major differences and similarities will be discussed in the talk.

Conflict of interest: none

PL-4-2
Transfusion databases

Titlestad K.

South Danish Transfusion Service, Department of Clinical Immunology, Odense C, Dänemark

Background: Denmark has a long tradition for clinical databases, e.g. the national Cancer Registry was established already in 1943. In the 1990ies, the transfusion practices in Denmark had only been superficially described, even though the transfusion rate was among the highest in the world. On this background, the first database for monitoring transfusion practices were established based exclusively upon data from existing computerised registers.
Methods: Reliable linking of data requires a unique and consistent identification of records in all included registers. In Denmark, this is possible as the central authorities have provided all inhabitants with a unique and permanent Personal Identification Number (PIN) since 1968. This number is used for all official registrations. Hence it is possible to link data from blood transfusion registers, diagnosis and procedure registers, clinical biochemistry registers, and several national registers.
Results: Late in the 1990ies, the first transfusion database with linked data was developed, leading to a PhD thesis in 2000: Computerised Monitoring of Transfusion Practices – A Survey of Two University Hospitals. This work was developed further, leading to the Danish Transfusion Database (DTDB). DTDB has collected data on transfusions, diagnoses, and biochemical tests since 2000. The first annual report was published in 2002. DTDB is now undergoing reprocessing; hence the last report was published in 2016. Collaboration between Denmark and Sweden conducted the next major development: the Scandinavian Donations and Transfusions (SCANDAT) database. SCANDAT has been in operation since 2006, and includes all registered blood donors and transfusion recipients in Sweden and Denmark since the start of computerised registration in 1966. By linking these records to nationwide population and health registers, follow-up for up to 36 years was possible. This not only for donation and transfusions, but also for reproduction, hospital morbidity, cancer, and death. SCANDAT contained 1.1 million blood donors who contributed 15 million records of donations and 1.3 million recipients who received almost 12 million transfusions. In 2016, SCANDAT was updated to attain complete follow-up for up to 47 years, containing 25 million donation records and 21 million transfusion records. SCANDAT now includes 3.7 million unique persons with valid identification, presently followed over 40 million person-years. This makes SCANDAT the probably most comprehensive transfusion database in the world. In the presentation, examples for the practical use of the described transfusion databases will be given.
Conclusion: Already existing computerized data, collected in routine health care, can make the basis for comprehensive transfusion databases. These databases can, in addition to describing transfusion practice, also monitor long-term outcome and disease concordance for transfusion recipients as well as blood donors.

Conflict of interest: none

PL-5
Infektionssicherheit III

PL-5-1
History and future of NAT blood donor screening

Roth W.K.

GFE Blut, Frankfurt am Main, Deutschland

The introduction of blood donor screening by virus NAT in the mid to late 1990-ies was driven by the so called AIDS and HCV catastrophe with thousands of infected haemophiliacs and transfusion recipients. Thus, plasma fractionators were the first to introduce NAT testing besides pathogen reduction procedures, to reduce the virus transmission risk through their products. To achieve a similar safety standard, NAT was then also introduced for labile blood components. German transfusion centres were the first to start in-house NAT testing of their donations in pools of up to 96 samples for HCV, HBV and HIV-1. Years later the diagnostics industry provided commercial HCV and HIV-1 and later HBV NAT tests on automated platforms. NAT tests for HIV-2, HAV and Parvovirus B19 followed, again driven by transfusion centres with their in-house tests. When SARS and WNV emerged it was the NAT that enabled the manufacturers and transfusion centres to introduce instantly sensitive and specific screening tests. The NAT has not only revolutionized the screening of blood donations with unprecedented viral safety standards but also the confirmation algorithms of test results which are now more precise and reliable. Subsequent automation including sample preparation has significantly reduced the costs and complexity of the procedure and made it affordable to low income countries as well. Currently more than 60 million donations per year are NAT tested worldwide and the remaining residual risk of virus transmission by blood components and products could be reduced to almost zero.
Automation rendered possible the reduction of pool size in conjunction with increased throughput and sensitivity. Thus, antibody and antigen testing may be dispensable in the long run, particularly in the combination of NAT testing with pathogen reduction. There are new technologies on the horizon like ddPCR, LAMP, NGS, lab-on-a-chip, micro arrays, digital antigen assays, which are comparably sensitive. However each of these has limitations either in through-put, costs, automation, time to result, specificity, or needs NAT as an integral part of the technology. Thus, NAT is still the shortest and most efficient way to the result. The are efforts to expand NAT to other pathogens than viruses. However, the particular

biology of those agents still limits the applicability of this excellent technology for blood donor screening.
Blood donor screening NAT contributed significantly to our knowledge on how fast viruses replicate, and on the respective diagnostic window. In conjunction with animal and patient studies we learned more about the minimal infectious dose and the epidemics in the donor population world wide.

Conflict of interest: none

PL-5-2

Seeding capacity of Amyloid-β – impact on transfusion safety

Offergeld R., Baier M., Beekes M.

Robert Koch-Institut, Berlin, Germany

Pathological prions remain a challenge for transfusion safety because no commercial tests for routine blood donor screening are available. In addition, these proteins cannot be inactivated by pathogen reduction strategies targeting nucleic acids. Recently, studies have revealed that apart from transmissible spongiform encephalopathies (TSE) other pathologies related to neurodegenerative disorders such as Alzheimer's disease (AD) or Parkinson's disease might also be transmissible. The possible transfer of pathologies or clinical features of neurodegenerative protein misfolding diseases by transfusion has therefore become a public health issue.
Amyloid-β (Aβ) is of special interest in this context. Aβ is a peptide deposited in the brain parenchyma in most cases of AD and in cerebral blood vessels, causing cerebral amyloid angiopathy (CAA). This condition is considered to be causative for 5–12% of all intracerebral hemorrhages in individuals with an age of 55 years or higher.
Experimentally, Aβ pathology was shown to be transmissible in animals, most effectively after intracerebral injection. However, even after i.v. application of Aβ seeds derived from human brain extracts from patients with AD, vascular Aβ amyloidosis was promoted in mice. In this setting, i.v.-injected Aβ seeds possibly passed the blood-brain barrier to trigger amyloid deposition in the brain.
In humans it was demonstrated that some patients who received intramuscular injections of human cadaveric pituitary-derived growth hormone developed not only iatrogenic Creutzfeldt-Jakob disease but also independent Aβ pathologies 28–39 years after exposure.
Apart from this very specific exposure, there is no epidemiological evidence from large retrospective cohort studies in blood recipients or hemophiliacs that blood transfusion is an important route of transmission of neurodegenerative diseases between humans. This was confirmed by case control studies in blood recipients. But it has to be kept in mind that observational studies have limitations especially with respect to the proposed long incubation period of neurodegenerative disorders caused by seeding of pathological proteins.
Blood safety measures to reduce the risk of TSE transmission have already been implemented. These include universal leucodepletion and implementation of specific donor eligibility criteria. Still, further research is required to identify factors contributing to the transmissibility of misfolded proteins and ultimately to prevent transmission of amyloid pathologies in particular by blood products in humans.

Conflict of interest: none

Zentrale klinische Fortbildung

ZF-2
Zentrale klinische Fortbildung II

ZF-2-1

Prevention of transfusion-transmitted infections

Juhl D.

Universitätsklinikum Schleswig-Holstein, Transfusionsmedizin, Lübeck, Deutschland

After the detection of the Hepatitis B virus, the implementation of the first HBsAg tests in blood donor screening thereafter was the first cornerstone in the prevention of transfusion-transmitted (TT) hepatitis, one of the most important TT infections at that time. Improvement of the HBsAg tests in the following decades and implementation of anti-HBc testing in blood donor screening further increased the safety of blood components.
Many other cases of Hepatitis after transfusion have been caused by Hepatitis C virus (HCV). First antibody tests became available at the beginning of the 1990´s, a further step toward an improved safety of blood components. However, due to the long-lasting window period of HCV, TT-HCV infections still occurred after implementation of anti-HCV antibody tests. This NAT-only positive, window period donations can now be detected efficiently by minipool NAT testing.
Also TT-HIV infections can be prevented by combining a serological screening assay with a NAT-based screening assay. Cases of TT infections with these three viruses meanwhile do very seldom occur according the data published in the haemovigilance report of the Paul-Ehrlich-Institute and thus, a high safety standard of blood components concerning these viruses has been reached in the past decades.
A challenge for safety of blood products are emerging viruses: many of these often vector borne viruses (West Nile, Chikungunya, Dengue or Zika virus) – at least to date – are not present in Germany. However, travellers, returning from countries in which these viruses are widespread, can transmit them potentially by their blood donation. Temporary rejection of these blood donors is a reasonable option to prevent TT infections beside NAT-testing of blood donations.
Hepatitis E virus (HEV) is also transmissible by transfusion, but this infection route seems to be of minor relevance as many infections are probably acquired via a food-borne, zoonotic route. While HEV infection poses a threat for the subgroup of severely immunosuppressed patients by inducing chronic infections, in many patients the infection probably go unnoticed with final recovery. The enactment of a mandatory screening of all blood donations for HEV-RNA is intended by the German authorities.
Bacterial contamination of blood products can result in a fatal clinical outcome by septicemia in patients. Especially in platelet concentrates, stored at room temperature, conditions necessary to growth for bacteria are favorable. Reduction of the storage life of platelet concentrates from 5 to 4 days was able to clearly reduce the rate of severe transfusion-associated septicemias, but also reduced the availability of these often scarce blood products. To reach a prolonged storage life and to reduce the risk of releasing a blood product which is contaminated by bacteria, either testing for bacterial contamination or pathogen reduction by photochemical treatment of blood products can be applied.

Conflict of interest: none

ZF-2-2

Instruction of physicians in the process of blood transfusion and documentation

Zeiler T.

DRK-Blutspendedienst West, Zentrum für Transfusionsmedizin Breitscheid, Ratingen, Deutschland

Chapter 6 of the German «Guideline Haemotherapy» revised in 2017 outlines the requirements of adequate instructions and trainings of physicians prior to performing their first blood transfusion. This paper describes simple and proved procedures for the instruction and training of physicians as well as the implementation and documentation of the process.

Conflict of interest: none

ZF-3
Zentrale klinische Fortbildung III

ZF-3-1

Current diagnostics of RhD

Wagner F.

DRK Blutspendedienst NSTOB, Springe, Deutschland

Introduction: The D antigen located on the RhD protein is considered the most important blood group antigen caused by a protein.
Methods: RhD frequency data were obtained from our donor registry (first time donors). An overview of current knowledge on RhD and the recommendations for transfusion is given.
Results: About 17.6% of Northern German blood donors lack the RhD protein («RhD negative»), almost 82.3% carry it in almost normal strength («RhD positive»). The remaining donors possess weakly expressed or variant proteins that may cause diagnostic and therapeutic problems. There are two phenomena: Partial D indicates qualitative changes of the D antigen with epitope loss. Individuals with partial D may (by definition) produce allo-anti-D, the risk of anti-D immunization heavily depends on the specific partial D. Weak D indicates weak expression of antigen D. There are more than 150 known weak D types, most of which are exceedingly rare. DEL indicates extremely weak D expression only detected by adsorption/elution; routinely such patients are typed as RhD negative. Partial D may be associated with weak D expression, and serologic distinction of rare weak and partial D types is difficult. For serologic RhD typing of patients, monoclonal anti-D that do not react with DVI are recommended. This strategy ensures D negative transfusion for patients with DVI, a partial D with high anti-D immunization potential. For patients with weak D expression, there is consensus that individuals with the frequent weak D types type 1, type 2 and type 3 cannot produce allo-anti-D; therefore, the current German transfusion guideline recommends molecular detection of these types to allow RhD positive transfusion and obviate the need of anti-D prophylaxis. Patients with weak D expression and other alleles and patients for whom the molecular cause is unknown are recommended to be given RhD negative blood. Patients with phenotype ccD.ee and weakened D expression are unlikely to carry weak D type 1 to 3. The borderline between normal D and weak D is not exactly defined; usually any even slightly reduced antigen D expression indicates a variant RhD protein, sometimes permissive of anti-D immunization. When defining a suitable borderline, it should be considered that even with the old strategy allowing D positive transfusion in patients weakly reactive with both anti-D anti-D immunization in D positives was much rarer than immunization to some other blood group antigens. The knowledge on *RHD* is continually growing, new techniques are emerging (molecular typing without serologic information) or entering widespread use (fetal typing from maternal blood), and a full definition of the allele – phenotype relation is getting more important every day
Conclusion: Problems in serologic RhD determination are rare; the «new» guideline introduced changes and clarifications.

Conflict of interest: Franz Wagner ist Miterfinder von Patenten über die Molekularbiologie der RH Blutgruppe

Vortragssitzungen

VS-1
Präparative und therapeutische Hämapherese

VS-1-1

Extracorporeal photopheresis in solid organ transplantation

Ahrens N.[1], *Hutchinson J. A.*[2]

[1]Universitätsklinikum Regensburg, Institut für Klinische Chemie und Laboratoriumsmedizin, Transfusionsmedizin, Regensburg, Deutschland
[2]Universitätsklinikum Regensburg, Klinik und Poliklinik für Chirurgie, Regensburg, Deutschland

Extracorporeal photopheresis (ECP) has been described as a bidirectional therapy because it ameliorates T cell-mediated pathologies while preserving protective immunity and possibly stimulating anti-tumour responses. The underlying immunological mechanism are not necessarily antagonistic, though. The immunological activities of ECP can be divided into (1) effects mediated by dead or dying cells, and (2) effects mediated by living cells rendered immunosuppressive by ECP. Apoptotic cells are rapidly cleared by recipient macrophages and dendritic cells (DC) which then up-regulate suppressor factors (eg. TGF-ß, IL-10, IDO and PGE2) and down-regulate costimulatory molecules. In turn, such «in situ-tolerised» macrophages and DC suppress T cell effector activity and support Treg function. In addition, the ECP process in itself has been characterized to impact the immune system by integrin- and platelet-dependent conversion of blood monocytes into DC-like cells. Theses are capable of phagocytosing, processing and presenting exogenous antigens to CD4+T cells, but also exhibit a remarkable capacity for cross-presentation of soluble, exogenous antigen to CD8+T cells. This leads to the proposition that ECP ex vivomodifies monocytes such as that they attain regulatory capacity like suppressor macrophages and tolerogenic DCs in vivoby further differentiation after re-administration to patients. In support of this hypothesis, ECP-modified monocytes share certain characteristics with «licensed monocytes," such as reduced CD14 and HLA-DR expression.
ECP may basically applied in three different setups.First, closed inline systems as provided by Therakos combine apheresis and UV irradiation in one device, and remain connected to the patient for the whole of the procedure. In Europe, closed inline systems are regulated as medical therapies. Sencond, open offline systems use separate devices for apheresis and UV irradiation in processes that require detachment of the leukapheresate from the patient. Open offline methods are mostly regarded as ATMP manufacturing process. Third, open inline systems combine separate devices operating at the bedside and thus allowing unbroken communication between the apheresate and patient. Because the apheresate is not detached from the patient, such processes may be regarded as medical treatments, which do not require a manufacturing permit and remain beyond the scope of pharmaceutical authority surveillance.
Either of these systems may be used for the treatment of cutaneous T cell-lymphoma and for steroid-refractory GvHD as well as in solid organ transplantation. It is recommended for prevention and treatment of cardiac transplant rejection, and is a valuable option for slowing functional deterioration of lung allografts in patients with treatment-resistant bronchiolitis obliterans syndrome. Hence, applying ECP as an induction, maintenance or rescue therapy in kidney, liver or other solid transplantation is a cogent suggestion.

Conflict of interest: none

VS-1-2

A novel simple and rapid quality control test for analysis of T cell proliferation inhibition after extracorporeal photopheresis

Schwab L.[1], *Michel G.*[1], *Hackstein H.*[2]

[1]Universitätsklinikum Gießen, Gießen, Germany
[2]Universitätsklinikum Erlangen, Transfusionsmedizin und Hämostaseologie, Erlangen, Germany

Introduction: Extracorporeal photopheresis (ECP) employing UVA irradiation in the presence of 8-Methoxypsoralen (8-MOP) is a widely used immunomodulatory cell-based therapy exhibiting efficacy in heterogenous T cell-mediated diseases such as cutaneous T cell lymphoma, graft versus host disease (GVHD) and organ allograft rejection. Despite its clinical relevance, only few centers regularly perform quality control assays analyzing ECP-induced T cell proliferation inhibition. We have developed a simple and rapid quality control assay determining CD71 expression as a T cell proliferation surrogate marker.
Methods: We systematically evaluated kinetics and magnitude of T cell proliferation surrogate markers including, Ki67, CD25, CD69 and CD71 by flow cytometry after different types of T cell stimulations in UVA+MOP-treated PBMC in comparison to untreated or partially treated controls. Results were compared with the gold-standard CFSE-T cell proliferation assay and standard apoptosis assay using Sytox/Annexin V staining.
Results: Detection of surface CD71 upregulation after CD3/CD28 T cell stimulation and suppression after UVA+8-MOP proved to be a reliable, simple and sensitive ECP quality control marker. Surface CD71 expression and ECP inhibition can be detected as early as 7–16h after ECP in contrast to the laborious CFSE-assay that needs a minimum culture time of 4–5 days.
Conclusion: Surface CD71 analysis represents a novel and simple quality control procedure for the detection of ECP-mediated T cell proliferation inhibition.

Conflict of interest: none

VS-1-3

Splitting of apheresis granulocyte concentrates manufactured with modified fluid gelatin does not affect granulocyte function and product quality

Offner R.[1], *Koo S.*[2], *Pfaehler S.*[2], *Lindner R.*[2], *Gruber M.*[2], *Ahrens N.*[1]

[1]Universitätsklinikumm Regensburg, Inst. für Klinische Chemie, Laboratoriumsmedizin, Transfusionsmedizin, Regensburg, Germany
[2]Universitätsklinikum Regensburg, Forschungslaboratorium der Klinik für Anästhesiologie, Regensburg, Germany

Background: In order to be able to supply several simultaneous requests from granulocyte concentrates (GC) from one single available donation, we have validated our procedure for inline-splitting. We investigated the influence of this mechanical act of splitting and subsequently storage of the granulocytes.
Study design and methods: We connected an additional platelet concentrate storage bag (Terumo BCT) to the apheresis collection bag and split the GC into two. The tested parameters covered both quality-related static and cellular function parameters immediately after splitting, at the end of the allowed shelf time (up to 24 h after apheresis) and after a further 24 h of storage. In order to examine the effect of splitting on modified fluid gelatin (MFG, Gelafundin 4%, B. Braun Melsungen AG) derived GC, we measured cell count, granulocyte yield, blood gas, annexin V-binding, 7-AAD incorporation, CD11b-, CD62L-, CD66b-expression, oxidative burst and granulocyte migration in IBIDI-chambers.

Results: Splitted GC (n = 6) met all product specification criteria. As expected, glucose and pO2 decreased rapidly, while lactose, pH, free hemoglobin and pCO2 increased to the end of shelf life. After storing GC for up to 48 h apoptotic cells decreased from 12,93% to 2,40% (average), and dead cells increased from 0,90% to 18,86%, while annexin V- and 7-AAD-negative cells remained unchanged. Expression of CD11b had a tendency to rise over the period of 48 h, albeit not significantly. Expression of CD62L and CD66b did not change. There was a slight decrease of reactive oxygen species (ROS) production upon PMA challenge after 48 h of storage. Time to unstimulated ROS production on slides, however, was unchanged by storage. NETosis response time was found not to be affected by prolonged storage. The ability to properly migrate was significantly reduced, as the absolute distance travelled decreased from 133,90 µm on average to 72,29 µm in a given time frame after 48 hours of storage. Similar parameters such as Euclidean distance, straightness and displacement did also reflect this change. None of the parameters changed due to the GC split.
Conclusion: Splitting of granulocyte concentrates by apheresis did not affect granulocyte function and product quality during the regular shelf life of up to 24 h. The investigated splitting procedure has clear benefits in terms of blood supply logistics and spares donor resources.

Conflict of interest: none

VS-2
Automation und Datenverarbeitung

VS-2-1

Preparation of a guideline for – «Practical validation of software systems and processes according to GAMP5» – «Validation of methods and qualification of equipment according to GMP Guide Annex 15»

Reinhardt F.[1], Steinke W.[2]

[1]DRK-Blutspendedienst Nord-Ost gemeinnützige GmbH, Qualitätsmanagement, Plauen, Deutschland
[2]DRK-Blutspendedienst West gemeinnützige GmbH, Qualitätsmanagement, Hagen, Deutschland

Background: On 03./04. March 2016 to the DGTI section meeting automation and data processing in Plauen the decision was made to working groups. In order to implement the requirements for the validation of methods, the qualification of devices according to GMP Guide Annex 15, as well as for risk-assessing decisions according to GAMP5, generalized templates should be made available.
In the Automation and Data Processing section, the preparation of a guideline for
– «Practical validation of software systems and processes according to GAMP5»
– «Validation of methods and qualification of equipment according to GMP Guide Annex 15»
is to be introduced
Material and methods: In order to implement the requirements for the validation of methods, the qualification of devices according to GMP Guide Annex 15, as well as for risk-assessing decisions according to GAMP5, generalized templates should be made available. At the same time, the exchange of experiences will take place within the framework of the section meetings, involving external speakers.
With the involvement of external speakers, institutions and authorities, examples of solutions for the implementation of GAMP5 and GMP Guide in the routine for hospitals, transfusion facilities, as well as medical facilities should be recorded in compliance with legally binding guidelines.
Results: At the meetings of the Automation and Data Processing (ADV) Section, four working groups discussed sample qualifications and validations, which will be presented in the ADV section's guide in the future.
Automation in the laboratory for infectious serology and future PCR techniques
Automation in the Laboratory Patient Serology and Immunohematology in close cooperation with the DGTI Section Immunohematology and Patient Serology (Dr. C. Geisen, Frankfurt)
Automation in plasmapheresis, in the manufacture and quality control of blood components
RFID logging when transporting blood products, delivery note floppy disk
Conclusion: For the session Automation and Data Processing in Lübeck the first solutions for illustration in the guideline are to be presented and discussed. Date: Wednesday, 19.09.2018; 09:30–11:00; Lübeck Music and Congress Halls GmbH, Willy-Brandt-Allee 10, 23554 Lübeck, Seminar room 3.
«Automation in the Laboratory of Infectious Serology and Future PCR Techniques»
«Quo vadis tube? Automation in Infectious Serology of the DRK Blood Transfusion Service West»
«Routine operation of the automated labeling and sorting system in ZTM Hagen of the DRK BSD West»
«Irradiation of blood products with the X-ray machine RS 3400»
«Qualification of Sysmex XN550 laboratory machines for haematological examinations taking into account the properties correctness, precision, linearity»

Conflict of interest: none

VS-2-2

Automation in the laboratory for serology and NAT methods

Schmidt M.[1], Hourfar K.[1], Schröder F.[1], Gubbe K.[2], Frank K.[2], Reinhardt F.[2], Karl A.[2], Tonn T.[3], Seifried E.[4]

[1]DRK BaWüHe Institut Frankfurt, Qualitätsmanagement, Frankfurt am Main, Germany
[2]DRK Nord-Ost Institut Plauen, Spenderscreening, Plauen, Germany
[3]DRK Nord-Ost Institut Dresden, Geschäftsführung, Dresden, Germany
[4]DRK BaWüHe Institut Frankfurt, Geschäftsführung, Frankfurt am Main, Germany

Für die Sektion Automation
Hintergrund: Der DRK Blutspendedienst ist sowohl zertifiziert nach DIN ISO 9001, als auch akkreditiert nach DIN ISO 15189 und 17025. In diesem Kontext stellt nicht nur ein Wechsel eines Screeningverfahrens eine hohe Anforderung an die Qualifizierung und Validierung dar, sondern auch ein regulierter Prozess in dem regelmäßig der Qualifizierungsstand der Geräte kontrolliert und dokumentiert wird.
Ziele: Qualifizierung und Validierung der Roche Cobas 8800/ 6800 PCR Automaten an den Standorten Frankfurt, Plauen und Ulm, Qualifizierung und Validierung der ABBOTT Alinity S Analyzer an den Standorten Frankfurt und Plauen und Qualifizierung und Validierung der Roche Cobas e801 Module am Standort Frankfurt
Methoden: Über ein geregeltes Change Control Verfahren bei dem sowohl der Projektplan und die Risikoanalyse die Grundelemente für die Qualifizierung und Validierung darstellen werden diese durch DQ, IQ, OQ und PQ ergänzt. Ferner wird der gesamte Einführungsprozess mit Hilfe eines Abweichungsdokuments begleitet, aufgetretene Abweichungen wurden dokumentiert und nachverfolgt.
Ergebnisse: In enger Zusammenarbeit zwischen den Fachabteilungen, der IT-Abteilung, der Haustechnik und den Herstellern konnten im Blutspendedienst Baden-Württemberg – Hessen und im Blutspendedienst Nord-Ost in den letzten 3 Jahren sowohl eine kommerzielle PCR Methode, und zwei neue infektionsserologische Screeningmethoden eingeführt werden. Die Leistungsvorgaben des Herstellers konnten dabei vor Ort überprüft werden. Eine besondere Aufmerksamkeit wurde bei der Einführung neuer Systeme auf eine nach GAMP 5 durchgeführte IT Validierung gelegt.
Zusammenfassung: Neue kommerzielle Screeningmethoden konnten erfolgreich qualifiziert und validiert werden. Dabei zeigte sich, dass die diagnostische Sensitivität und diagnostische Spezifität der neuen Verfah-

ren mit den Angaben der Hersteller übereinstammten. Eine besondere Herausforderung bestand bei der Validierung der IT Software in Übereinstammung mit GAMP 5. Insgesamt war eine Gesamtprozess-Risikoanalyse hilfreich, alle Prozessänderungen zu erfassen und in einem Validierungsplan zu berücksichtigen. Die Gesamtprozess-Risikoanalyse stellt auch die Grundlage für eine nachfolgende Re-Qualifizierung.

Conflict of interest: none

VS-2-3

Irradiation of blood products with the X-ray machine RS 3400

Reinhardt F.[1], Dreweck C.[2], Karl R.[2], Tilmann M.[2]

[1]DRK-Blutspendedienst Nord-Ost gemeinnützige GmbH, Qualitätsmanagement, Plauen, Deutschland
[2]DRK-Blutspendedienst Nord-Ost gemeinnützige GmbH, Institut Berlin, Berlin, Deutschland

Introduction: The irradiation of blood preparations is intended to prevent a graft-versus-host (GvH) reaction from immunocompetent residual leukocytes in blood preparations in immuno-incompetent specimen recipients. This can be done both by gamma rays and X-rays. A dose of at least 25gray / preparation must be guaranteed.
The radiation device with source of an X-ray tube «QuaStar X-ray Tube» is to be established in the institute Berlin, Hindenburgdamm in the future for the irradiation of blood products (Abb. 1).
The device has a significantly more efficiency than a standard x-ray tube.
– Longer tube life. Operates at lower temperature.
– Significantly more dose per unit of power than a standard x-ray tube
– 4 pi X-ray tube is repairable. A conventional x-ray tube is not.
Methods: In the DRK Blood Transfusion Service gGmbH North-East Institute Berlin Am Großen Wannsee, the irradiation of blood products with a gamma radiation source (IBL 437C) is performed. In the new institute on the Hindenburgdamm the irradiation of the blood products with X-rays with the device RS3400 was established.
Rad Source X-Ray Blood Irradiator der Firma RAD SOURCE Technologies, Inc.
480 Brogdon Rd. Suite 500
Suwanee, GA 30024
www.radsource.com
The care and maintenance in Germany is done by Fa. Römatec Service center and trading company for X-ray technology in industry and research Robert-Bosch-Strasse 10, D-59199 Bönen.
Result: The advantages of X-irradiation over gamma irradiation are:
The radiation source is active only when needed – safety for employees and preparations
The irradiation time is significantly shorter (5 minutes / cycle)
Irradiation of max.12 preparations (EK and TK at the same time) are possible in one cycle.
Irradiation of preparastions is more homogeneous
Lower requirements with regard to radiation protection
The risk of a terrorist attack is lower. Therefore, the process has long been established in the USA.
Conclusion: In the new institute Berlin at the Hindenburgdamm it could be proven that irradiation with X-ray tube as radiation source is equally good with regard to the equivalent dose, as with the formerly used irradiation by IBL 437 C: gamma radiation. In comparison, gamma radiation at dose: 27.5–40.1 in a period of 12–20 minutes show one with RS 3400 X-rays at the same dose
27.5–39.7 Gy of an irradiation time of 5 min distinct advantages (Abb. 2).

Conflict of interest: none

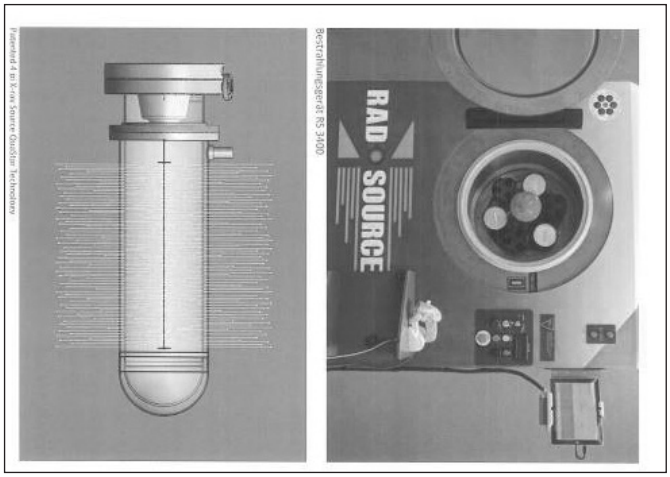

Fig. 1.

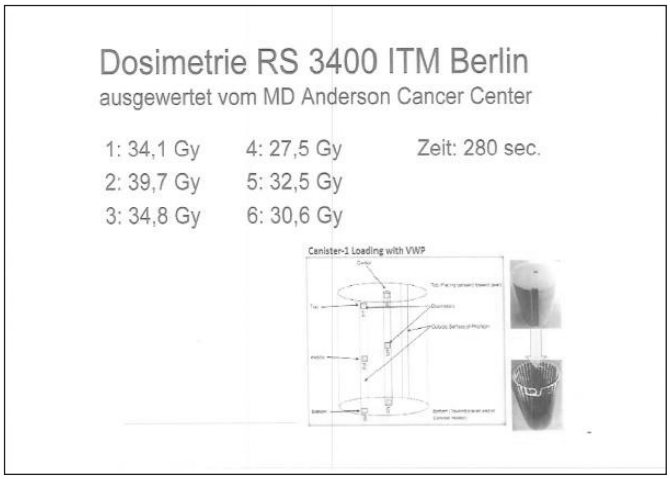

Fig. 2.

VS-2-4

Qualification of laboratory automats Sysmex XN550 for haematological investigations considering the characteristics correctness, precision, linearity

Reinhardt F.[1,2], Frank, K.[1]

[1]DRK-Blutspendedienst Nord-Ost gemeinnützige GmbH, Qualitätsmanagement, Plauen, Deutschland
[2]DRK-Blutspendedienst Nord-Ost gemeinnützige GmbH, Labor Qualitätskontrolle, Cottbus, Deutschland

Background: The verification of the functionality of new analysis systems, which are approved according to medical device law, as well as in vivo invitro diagnostics for device and reagents, require qualification measures for pharmaceutical companies. Since in an enterprise the introduction must take place in several locations, the effort for the qualification by comparative measurements against the system previously used should optimized.
Material and methodology: First, the device to be introduced will be evaluated on the basis of a risk analysis with regard to the parameters (leucocyte content, erythrocyte content, PLT platelet content, HGB hemoglobin, HKT hematocrit, MCV medium corpuscular volume) which are tested in various media with respect to linearity, accuracy and precision for RiLiBäk quantitative methods.Based on these results, the sample size to qualify the devices is defined for each site in the enterprise network.
Results: After successfully assessing the accuracy, precision, linearity, and reproducibility in the central laboratory of the group of companies The functionality for the devices at the other locations of the group of companies was checked for accuracy, for precision, and for the stated speci-

fication provided by the device manufacturer. The linearity was evaluated based on the quality controls.
Conclusions: The qualification algorithm is organized according to the standard set by the company's central quality management. By determining the necessary sample size based on a risk analysis, the qualification effort to prove the functionality of the devices can be optimally organized in the whole company.

Conflict of interest: none

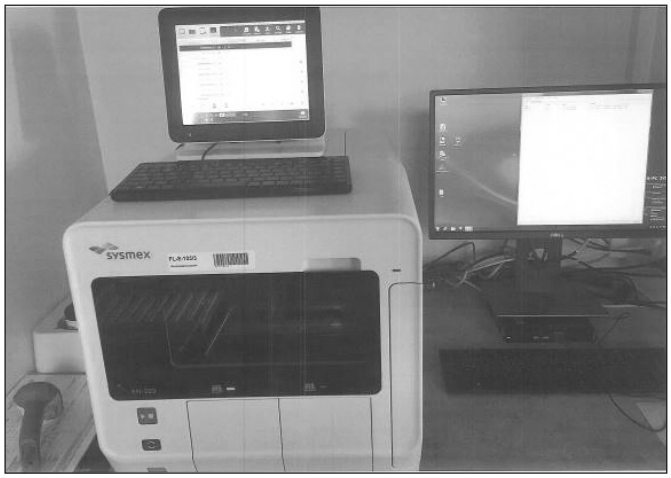

Fig. 1.

VS-3
Stammzelltransplantation und Zelltherapie I – Joint session GSCN

VS-3-1

En route to an artificial stem cell niche: biomaterials for directing hematopoietic stem cell behavior

Lee Thedieck C.

Karlsruhe Institute of Technology, Institute of Functional Interfaces, Eggenstein-Leopoldshafen, Germany

Blood is replenished with billions of fresh cells every day throughout the entire life span. The source of these cells are the hematopoietic stem cells (HSCs). Their ability to reconstitute the entire blood system makes them the key to the cure of many hematological diseases. However, this treatment is restricted by the limited availability of HSCs. To overcome that limitation, controlling HSC behavior in terms of proliferation or differentiation in vitro, is an important goal of nowadays HSC research.
In vivo HSCs are controlled by a highly specialized microenvironment – the niche – within the bone marrow. In this niche HSCs are supported by mutual cell-cell as well as cell-matrix interactions. While it is clear that biological and/or chemical parameters play an important role in this interplay, surprisingly little attention was paid to physical signals that are transmitted by the niche microenvironment. In the last years, we found that these physical signals include matrix stiffness, nanostructure as well as the three-dimensional architecture. In reductionist approaches, in which we studied only one parameter at a time, we could show that all of these parameters impact HSC behavior.
In order to achieve the goal of a synthetic stem cell niche to guide HSC behavior, the complexity of the natural HSC niche, which combines a variety of different signals, has to be taken into account. For this purpose, we increased the complexity of our systems to study the synergistic effects of different biological and/or physical signals. With these experiments we hope to get one step closer towards a synthetic stem cell niche that is as simple as possible but as complex as necessary to instruct HSCs.

Conflict of interest: none

VS-3-2

Engineering of inducible transcription factors (iTFs) to explore human ex vivo CD34+ cell expansion and differentiation

Soliman S.[1], Windisch R.[1], Pirschtat N.[1], Setzer S.[1], Shvartsman M.[1], Chen-Wichmann L.[1], Klump H.[2], Humpe A.[1], Wichmann C.[1]

[1]Klinikum LMU München, Abteilung für Transfusionsmedizin, Zelltherapeiutika und Hämostaseologie, München, Germany
[2]Universitätsklinikum Essen, Institut für Transfusionsmedizin, Essen, Germany

Background: Long term expansion of human CD34+ stem and progenitor cells has proven virtually impossible due to their rapid differentiation after detachment from the bone marrow stem cell niche. Despite the description of multiple local factors, which support self-renewal within the niche, it has not been possible to establish similar conditions in *ex vivo* CD34+ progenitor cultures, so far. Of note, a few leukemia-related transcription factors were recently shown to confer remarkable *ex vivo* expansion capacity in progenitor cells (Faridi et al.:, 2013).
Methods: The coding sequences (CDS) of chemically inducible-engineered transcription factors derived from *RUNX1* and *MLL* fusion genes were retrovirally transduced into and expressed in human CD34+ progenitors. *Ex vivo* liquid cultures were performed in cytokine supplemented medium without feeder cells. Expansion and differentiation of the transduced cells was monitored by gene marking and phenotypic analysis during *ex vivo* culture.
Results: Two independent inducible systems were applied to trigger CD34+ cell expansion and differentiation in *ex vivo* cultures. First, a chimeric RUNX1 transcription factor was constructed by dividing it into two non-functional parts, which were able to hetero-dimerize via a small molecule-dependent chemical dimerization system (CID) to fully reconstitute its activity. We were able to demonstrate RUNX1 activity exclusively in chemical dimerizer treated cultures, leading to a robust progenitor expansion. In a second approach, a MLL chimeric gene was fused to the CDS of a mutant FKBP12 protein destruction domain, rapidly leading to the degradation of the fusion protein. Upon Shield1 treatment, a chemical ligand that neutralizes the destruction domain, the inducible transcription factor (iTF) was stabilized and induced powerful expansion of vector transduced progenitor cells. After complete selection and outgrowth of Shield1 treated iTF expressing progenitor cells, removal of Shield1 rapidly led to lineage differentiation towards functional macrophages and granulocytes (Fig.1).
Conclusion: With the help of tunable protein switch elements, we explored the capacity of inducible and reversible transcription factor activities to expand and differentiate human myeloid progenitors in *ex vivo* cultures (Fig.1). Such iTFs, which are able to induce specific transcriptional programs, may be interesting tools for cell engineering projects aiming at gene correction and generation of patient-derived immune cells for experimental cell therapy.

Reference:

1. Faridi F, Ponusammy K, Quagliano I, Chen-Wichmann L, Grez M, Henschler R, and Wichmann C: Aberrant epigenetic regulators control expansion of human CD34+ hematopoietic stem/progenitor cells. Front. Genet. 2013;28:254

Conflict of interest: none

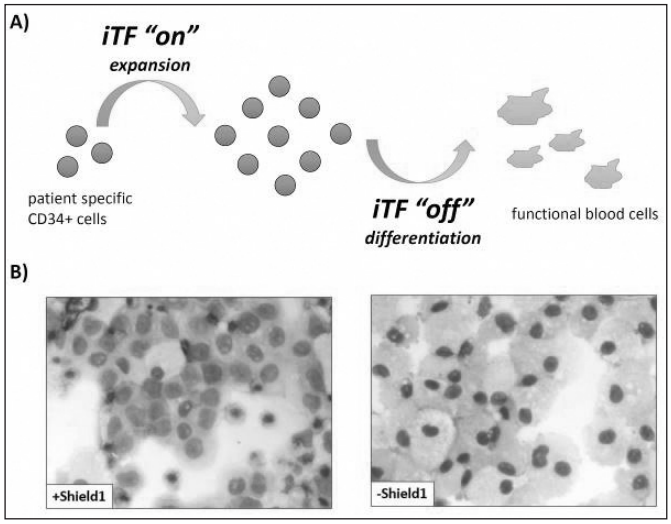

Fig. 1. (A) Schematic illustration of CD34+ cell expansion and differentiation by iTFs. (B) Shield1 controls iTF induced CD34+ progenitor expansion.

VS-3-3

Role of miR-30a-5p and miR-26a-5p in induction of terminal differentiation in human K562 erythroleukemia cells

Kronstein-Wiedemann R.[1], Milanov P.[2], Pasini E.[3], Tonn T.[1,4]

[1]Medizinische Fakultät der TU-Dresden, Dresden, Germany
[2]DRK-Blutspendedienst Badenwürttemberg/Hessen, Frankfurt am Main, Germany
[3]Biomedical Primate Research Centre, Rijswijk, Netherlands
[4]DRK Blutspendedienst Nord-Ost, Dresden, Germany

Background: *Plasmodium vivax* (*Pv*) infections affect ~300 million people every year. However, studies into this parasite species are hampered by the inability to set up a continuous, long-term *in vitro* blood stage culture for *Pv*. So far, only short-term cultures have been established requiring the repeated isolation of reticulocytes from blood donors. Establishing an *in vitro* «red blood cell matrix» that would allow continuous access to reticulocytes would facilitate the establishment of continuous, long-term *in vitro Pv* blood stage cultures and ultimately the study of parasite vulnerabilities that can be exploited in therapeutic approaches. Our study aims to establish culture conditions that allow for the differentiation of K562 cells into enucleated erythroid cells, serving as a constant source of reticulocytes for continuous, long-term *Pv* blood stage cultures.
Materials and Methods: Since the K562 cell line (K562) does not express the Duffy blood group antigen receptor (Fy), essential for *Pv* invasion, the Duffy FyB receptor was stably introduced into the K562 by lentiviral gene transfer. In addition, we combined different factors, which were known to enhance the terminal erythroid differentiation of K562 (knockdown of miR-30a-5p and -26a-5p, co-cultivation with macrophages, EPO, transferrin, insulin and mithramycin A). Effects were analysed by quantification of total cell numbers, CD45-CD71+CD235a+ cells and May-Grünwald-Giemsa staining. Hb expression was detected by Benzidin staining and PCR.
Results: K562 cells transduced with anti-miR-26a-5p and -30a-5p (C26a-30a) and stimulated with the induction cocktail comprising EPO, transferrin, insulin and mithramycin A (MIT) showed a significantly increase in the amount of enucleated reticulocytes as compared to cells transduced with the empty vector. This is reflected in a stronger down-regulation of CD45 and up-regulation of CD71 as well as in the number of enucleated reticulocytes and the increase in hemoglobin production. Stimulation of K562 cells transduced with the empty vector did not yield enucleation. CD235a expression was already higher in clone C26a-30a prior to stimulation with the induction cocktail as compared to unstimulated K562 empty cells. The Duffy receptor expression remained stable in stimulated cells of the clone C26a-30a, while it was downregulated in K562 empty vector cells over the stimulation period. Co-cultivation with macrophages did not further enhance the MIT-induced hemoglobin production and erythroid differentiation.
Conclusions: The data show that knockdown of miR-26a-5p and -30a-5p are necessary to induce terminal differentiation of K562 cells. Further optimisation of culture conditions to enhance the yield of RBC blasts from K562 is necessary, as is the confirmation of functionality and susceptibility to *Pv* infection of these cells.

Conflict of interest: none

VS-3-4

Enhanced erythroid differentiation from human iPSCs with minimal cytokine support

Bernecker C.[1], Lachmann N.[2], Ackermann M.[2], Zaehres H.[3], Schlenke P.[1], Dorn I.[1]

[1]Medizinische Universität Graz, Universitätsklinikum f Blutgruppenserologie & Transfusionsmedizin, Graz, Österreich
[2]MHH Hannover, Hannover, Deutschland3Biomedical Primate Research Centre, Rijswijk, Netherlands
[3]Ruhr Universität, Bochum, Deutschland

Objectives: Erythroid differentiation of human induced pluripotent stem cells (iPSCs) is ineffective, time consuming and expensive due to high amounts of cytokines. This excessive cytokine support might additionally influence cell differentiation in an un-physiological way and might be responsible for limited proliferation, low enucleation and failure in hemoglobin switching to adult hemoglobin.
Methods: Embryoid bodies (EB) of human iPSC lines derived from erythroblasts, cord blood CD34+ cells and fibroblasts were cultured in defined media with only SCF, EPO and IL3. EBs were allowed to attach to the plastic surface and form stromal layers. After 3 weeks of EB culture, hematopoietic single cells were released to the supernatant, harvested and brought into an established erythroid assay.
Results: Cells harvested from the EB supernatant were 95% CD43+ and >80% GPA+/CD36+, indicating hematopoietic and predominantly erythroid nature. CD43high cells showed a high colony forming potential with a majority of BFU-E / CFU-E formation. Cumulative expansion of the erythropoiesis culture was near 103-fold. The new protocol further led to a homogenous 98% GPA positive population with a remarkably high mean enucleation rate of 40% (up to 60% in single experiments). Formation of blistered EBs and stromal layers seemed crucial to gain these results.
Conclusion: This new simplified and cost saving model generates a homogenous population of hematopoietic- and finally erythroid cells. The formation of blistered EBs stromal layers might simulate in part the physiological niche and therefore enhance BFU-E potential, proliferation and enucleation. A possible co-localization of other cells within the adherent EB, e.g. macrophages is currently under investigation. Remarkably, the switch of the cytokine cocktail enables large scale production of monocytes and macrophages with this protocol, as demonstrated recently by our team members.

Conflict of interest: none

VS-4
Immunhämatologie

VS-4-1

F(ab´)$_2$ fragments as approach to circumvent problems of pretransfusion testing in daratumumab treated patients

Gebicka P. D., Thiele T., Wesche J., Selleng K.

Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Transfusionsmedizin, Greifswald, Germany

Introduction: Plasma of patients receiving the therapeutic antibody daratumumab (anti-CD38) induces panreactive agglutination of red blood cells in the indirect antihuman globulin test (IAT). Treatment of red blood cells by dithiothreitol can solve the interference of daratumumab in IAT but inhibits the binding of clinically relevant red cell alloantibodies of distinct specificities, e.g. anti-K or anti-Kp^a. Using daratumumab F(ab´)$_2$ fragments the binding of free daratumumab in the patient plasma on IAT test cells could be blocked. This may allow the detection of red blood cell alloantibodies with commercially available antibody screening tests.
Methods: F(ab´)$_2$ fragments were prepared from daratumumab by pepsin digestion and protein G purification. F(ab´)$_2$ fragment generation was confirmed by Western blot analysis. IAT was performed using the microcolumn gel card (ID-LISS/Coombs, BioRad). Plasmas with previously identified red blood cell antibodies of different specificities were titrated to a minimal reactivity of +1. These plasmas were tested with and without daratumumab spiking (500µg/ml). Red blood cells were preincubated (5 min, 37°C) with F(ab´)$_2$ fragments (50 µl/test, conc. 500µg/ml) in the reaction cavity of the gel card. Samples of daratumumab treated patients were tested to confirm the results in «real life» material.
Results: 8 plasma samples with different antibody specificities (anti-D, anti-Kell, anti-E, anti-Fy^a, anti-e, anti-M, anti-S, anti-C) were spiked with daratumumab and tested in the IAT. The addition of daratumumab F(ab´)$_2$ fragments completely blocked the binding of free daratumumab (up to 500µg/ml) in the patient plasma. All of the specific antibodies could be detected with the same agglutination strength compared to the IAT without addition of F(ab´)$_2$ fragments. Daratumumab induced agglutination in the antibody screening tests of daratumumab treated patients (n = 4) was successfully inhibited by addition of F(ab´)$_2$ fragments.
Conclusion: Daratumumab F(ab´)$_2$ fragments can be used for antibody screening and crossmatching of daratumumab treated patients to overcome the interference of free daratumumab in the patient plasma. The use of F(ab´)$_2$ fragments may generally overcome such interferences of therapeutic antibodies with pretransfusion antibody testing.

Conflict of interest: Keine Interessenkonflikte bei P.D. Gebicka und J. Wesche. K. Selleng erhielt Beraterhonorare und Reisekostenunterstützungen von Janssen, Forschungsunterstützung von Johnson & Johnson, Vortragshonorare von Aspen, Reisekostenunterstützung von NovoNordisk und Bayer. T. Thiele erhielt Vortragshonorare von Bristol Myerss Squibb, Bayer, Pfizer und Novartis, Reisekostenunterstützung von Bayer und Pfizer sowie Fortbildungsunterstützung von NovoNordisk.

VS-4-2

First experiences with DaraEx in cross-matching red blood cell concentrates under Daratumumab therapy

Rosner A.[1], Chocholi I.[2], Hölig K.[1]

[1]Universitätsklinikum Carl Gustav Carus Dresden an der Technischen Universität Dresden, Medizinische Klinik und Poliklinik I, Bereich Transfusionsmedizin, Dresden, Germany
[2]Universitätsklinikum Carl Gustav Carus Dresden an der TU Dresden, Institut für Klinische Chemie und Laboratoriumsmedizin, Dresden, Germany

Introduction: Daratumumab is the first anti-CD38 antibody that has been approved for the treatment of multiple myeloma. While more and more patients benefit from this new therapy, it has become an increasing challenge for blood bankers to supply these patients with compatible red blood cell (RBC) concentrates, because CD38 is expressed in most RBC rendering false positive results in screening for irregular antibodies and in RBC cross-matching. Protocols using dithiothreitol (DTT) or papain have been developed to overcome these problems but both methods tend to mask a couple of RBC antigens. In addition, the DTT method remains somewhat cumbersome and, particularly for crossmatching, time-consuming. DaraEx has recently been developed to specifically block the daratumumab interference in antibody screening and in RBC cross-matching.
Methods: EDTA plasma samples from 12 Daratumumab recipients were examined. Cross-matches were performed using the indirect antiglobulin test by gel column agglutination technique with anti-IgG present in the gel matrix using a 0.8% donor RBC suspension in low-ionic-strength solution (LISS). The donor RBC were (a) untreated, (b) after DTT treatment (modified after Chapuy et al.:), or (c) after the addition of 7,5 µl DaraEx solution, mixing and incubation at room temperature for 30 minutes within the cavity of the gel column (modified after Schneeweiß et al.:). Antibody screening tests were similarly performed using untreated, DTT-treated, or DaraEx-treated test erythrocytes. Quality controls with treated test erythrocytes were performed.
Results: Out of 12 samples, 11 had weakly positive cross-matches with untreated donor RBC (a), 2 were still positive using the DTT method (b), and all were negative in the DaraEx method (c). Our (c) method with a reduced DaraEx dosage as compared to manufacturer»s instructions worked also in 11 of the 12 antibody screening tests. Quality controls showed that the detection of patient»s Anti-K and Anti-Fy(a) antibodies was not impaired when using DaraEx-treated test erythrocytes.
Conclusion: Our first experiences with a modified DaraEx protocol were very encouraging for promptly and safely crossmatching RBC. Dose reduction of the substance gave acceptable results, was more cost-effective, and became our routine method for crossmatching under daratumumab therapy. For antibody screening, protocol (b) with ready-to-use DTT-pretreated test erythrocytes still remains the standard approach for our institution.

References:

1. Chapuy CI, Nicholson RT, Aguad MD, Chapuy B, Laubach JP, Richardson PG, Doshi P, Kaufman RM. Resolving the daratumumab interference with blood compatibility testing. Transfusion. 2015 Jun;55(6 Pt 2):1545–1554.
2. Schneeweiß C, Grüger D, Heuft H-G. DaraEx verhindert Daratumumab-Interferenz im indirekten Antihumanglobulintest. Transfusionsmedizin 2017;7(04): 229–232.

Conflict of interest: none

VS-4-3

Detection of masked irregular antibodies in Anti-CD38 containing plasma after neutralization with a novel recombinant CD38

Binda M.[1], Favaloro V.[2], Piel N.[2], Berry J. D.[2], Schwind P.[1]

[1]Medion Grifols Diagnostics, Duedingen, Switzerland
[2]Grifols Diagnostic Solutions, Emeryville, CA, United States

Background: Novel anti-CD38 drugs for treatment of multiple myeloma, such as daratumumab (DARA), interfere with diagnostic screening and identification of irregular antibodies causing pan-reactivity of Reagent Red Blood Cells (RRBC). Strategies to overcome this problem have been proposed, e.g.: 1) Pretreatment of RRBC with reducing agents; 2) Issuing phenotype/genotype matched RBC units; 3) Pre-incubation of patient plasma with soluble CD38 (sCD38) or anti-idiotype antibodies (1). The aim of this study was to evaluate the diagnostic utility of a newly developed sCD38.
Methods: A fusion protein containing the extracellular domain of CD38 was expressed in mammalian cells, purified and concentrated as soluble CD38. For evaluation of its diagnostic functionality, anti-CD38 (Darzalex, Janssen, Horsham, USA) spiked donor plasma (containing alloantibodies or not) were mixed and incubated for 15 minutes at 37°C with varying volumes/concentrations of i) sCD38; ii) a commercial recombinant hu-

man (rhCD38; R&D Systems, Minneapolis, USA) or iii) PBS as control. Antibody detection was then performed by Indirect Antiglobulin Test (IAT) in conventional tube technique or DG Gel technique (Medion Grifols Diagnostics, Duedingen, Switzerland; Diagnostic Grifols, Parets del Valles, Spain).

Results: A ratio of 2µl of recombinant sCD38 (~30mg/ml) per 25µl of plasma, allowed for complete inhibition of 0.5mg/ml anti-CD38. rhCD38 (~0.5mg/ml) used in the same experimental settings, could not inhibit the same anti-CD38 load (fig. 1).

Alloantibodies (anti-D, -E, -c, -Cw, -K, -Fya, -Jka, -S, -s, -M, -Lua, -Cob) spiked at barely detectable amounts into DARA-spiked donor plasma could be readily detected in 16/16 samples when 25µl of plasma are incubated with 2µl of sCD38 (fig. 2). In contrast, after incubation with 20µl and 200µl of more diluted preparations of sCD38, respectively 15/16 and 3/16 of the same antibodies spiked into DARA plasma could still be detected.

Conclusion: The presented results show the inhibition of therapeutic plasma concentrations (1,2) of daratumumab using a novel highly concentrated sCD38 at small volumes without interference in the detection of irregular antibodies. Moreover, it is demonstrated that a higher volume of more diluted CD38 protein may allow for the same inhibition effect on anti-CD38, however it will increase the risk of missing underlying antibodies of low potency.

The sCD38 presented in this work may provide, in combination with IAT, a rapid and accurate screening and identification method of even weakly reacting alloantibodies masked by anti-CD38, which is neutralized readily, with minimal plasma dilution during pre-treatment.

References:

1. Oostendorp M, et al.: Transfusion 2015;55:1555–1562.
2. De Vooght KMK, et al.: Current Opinion in Hematology 2016;23:557–562.

Conflict of interest: Matteo Binda and Peter Schwind are employees of Medion Grifols Diagnostics.

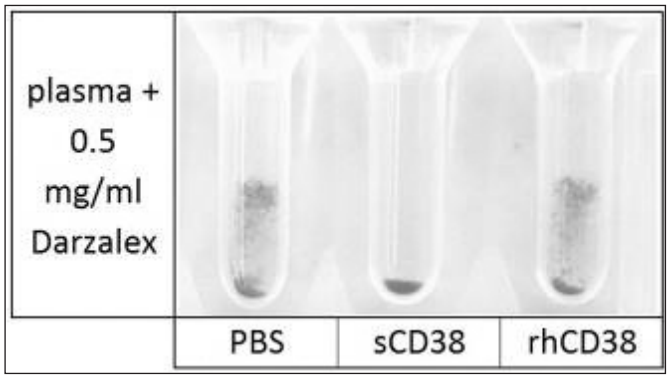

Fig. 1. sCD38 neutralizes 0.5mg/ml DARA spiked plasma.

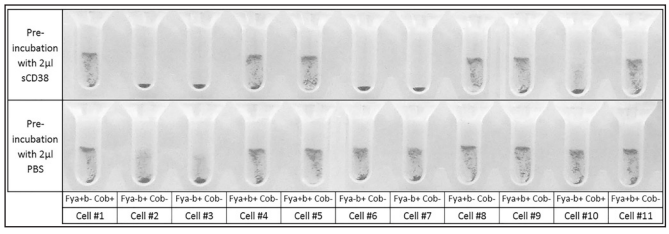

Fig. 2. sCD38 allows identification of a mixture of anti-Fya and anti-Cob antibodies in the presence of anti-CD38 in plasma.

VS-4-4

Daratumumab interference in indirect antiglobulin testing is eliminated by use of daratumumab Fab-fragments

Werle E., Ziebart J., Wasmund E., Eske-Pogodda K.

Institut für Laboratoriumsdiagnostik, Mikrobiologie und Transfusionsmedizin, Dietrich Bonhoeffer Klinikum, Neubrandenburg, Germany

Introduction: Monoclonal antibodies directed against CD38 (daratumumab) are used for therapy of haematological malignancies, e.g. refractive myeloma. Daratumumab binding to CD38 on erythrocytes, however, leads to panreactivity in indirect antiglobulin testing and possibly masks clinically relevant alloantibodies. Dithiothreitol (DTT) which destroys CD38 is widely used today but it also destroys several blood group antigens. In summer 2017, we started experiments with daratumumab Fab-fragments (dara-Fab) and have validated this method since autumn 2017 in our laboratory in parallel to the DTT treatment.

Methods: Dara-Fab were generated from daratumumab antibodies (Darzalex, Janssen, Beerse, Belgium) using the Pierce Fab Preparation Kit (Thermo Fisher). Antibodies were digested for 16 hrs at 37°C using immobilized papain in a spin column. Subsequently, the column was centrifuged, washed with 100 µl PBS and the flow-throughs pooled and stored at -20°C. Digestion was controlled by immunofixation electrophoresis (IFE; Sebia, Évry, France). For testing efficiency of dara-Fab to mask daratumumab binding sites, 15 µl of dara-Fab and 50 µl of 0.8% ID-DiaCell I-II-III screening cells or ID-DiaPanel cells for antibody identification (BioRad) were incubated with standard human plasma (Siemens, Erlangen Germany) spiked with daratumumab (final concentration of 100, 250, 500 mg/l) or plasma from patients under daratumumab therapy at 37°C for 15 min. Thereafter, cell suspensions were transferred to the microtubes of an ID-Card LISS/Coombs and centrifuged for 10 min as recommended by the manufacturer. To evaluate if dara-Fab incubation might impair detection of alloantibodies, plasma of patients with known alloantibodies was spiked with daratumumab (final concentration up to 500 mg/l). As controls, untreated screening cells were incubated with patients' plasma with or without daratumumab.

Results: IFE of native and papain-digested daratumumab antibodies showed complete fragmentation into Fc and Fab fragments, detected by anti-gamma heavy chain and anti-light chain kappa antiserum, respectively.

Dara-Fab (15 µl) efficiently prevented red blood cell agglutination by plasma spiked with daratumumab for all concentrations. In addition, 15 µl dara-Fab could override daratumumab-induced panreactivity in nearly all patients under daratumumab treatment. In a few cases, 30 µl dara-Fab were necessary. In addition, preincubation of test cells with dara-Fab fragments did not interfere with detection of alloantibodies as expected from the mode of action.

Conclusion: We present a quite easy, reproducible and cost effective method for daratumumab Fab fragment generation. Blocking the CD38 epitopes on antibody screening and identification cells with dara-Fab fragments is a novel and quick routine method to overcome daratumumab interference in patients' plasma.

Conflict of interest: none

VS-4-5

Daratumumab and Isatuximab – two different treatments for patients with multiple myeloma interfering with compatibility testing for blood transfusion

Enkel S., Nowak-Harnau S., Bakchoul T.

Center for Clinical Transfusion Medicine Tübingen, Tübingen, Germany

Background: Daratumumab targets CD38-expressing myeloma cells and is therefore used to treat patients with multiple myloma (MM). Isatuximab is another anti-CD38 monoclonal antibody undergoing clinical investigations. Positive reactions in indirect antiglobulin tests (IATs) for

antibody detection and crossmatches are observed for both therapeutic monoclonal antibodies. Several techniques have been described to overcome this problem. Addition of dithiothreitol (DTT) is a simple and practical method to allow alloantibody identification in presence of CD38 antibodies in patients» plasma.
Method: Clinical and serological data from patients with MM who were treated with daratumumab or isatuximab at our university hospital were retrospectively analyzed. Pre-transfusion testing included ABO and RH typing, antihuman globulin gel testing for antibody screen/crossmatch and direct antiglobulin test (DAT). Tests were performed with and without DTT-treatment.
Results: A total of 52 patients treated with daratumumab (n = 45, median age: 68 years, range 45–88) or isatuximab (n = 7, median age: 57 years, range 43–67) were included in the study. Serological investigations were performed for a median follow up of 28 days (range 1–483) and 176 days (range: 3–218) after administration of daratumumab and isatuximab, respectively. Samples from all patients showed positive reactions in routine antibody screening tests. All interferences could be negated using DTT-treated red blood cells (RBCs). In serum from one patient *antibodies* directed against the *Rh* antigen E could be identified. A follow up after therapy with daratumumab showed DTT-treatment for test cells as a sufficient method to recognize these known antibodies. 30/52 (58%) patients of the daratumumab-group received 240 compatible red blood products (median: 6 RBCs, range 1–39). 3/7 (43%) patients treated with isatuximab were transfused with 16 RBC-units (median: 3 RBCs, range 2–11) compatible red blood products. Most importantly, no clinically-relevant, transfusion-related hemolytic reaction was reported in both groups.
Conclusion: DTT treatment of test cells reagent and samples from RBC units seems to be a safe approach to overcome serological interference and false positive results in patients treated with anti-CD38 monoclonal antibodies. We observed a complete diminishing of the interference with indirect Coombs test for daratumumab- as well as for isatuximab-treated patients indicating that DTT can be implemented in pre-transfusion testing even for patients with irregular antibodies.

Conflict of interest: none

VS-4-6

Eryptosis in autoimmune haemolytic anaemia

Bartolmäs T., Herziger A., Balola A. H., Mayer B., Salama A.
Charité, Institut für Transfusionsmedizin, Berlin, Germany

Introduction: Autoimmune haemolytic anaemia of warm type (wAIHA) and of cold type (cAIHA) are rare diseases, which are caused by the production of autoantibodies (aabs) to red blood cells (RBCs). These aabs belong to IgG, IgM and less commonly IgA classes. Haemolysis is solely attributed to antibody and/or complement-mediated destruction and clearance of RBCs. Eryptosis is the suicidal death of red blood cells (RBCs) similar to the apoptosis of nucleated cells. Its hallmark is the breakdown of phosphatidylserine (PS) asymmetry. Until now, it has been unknown, whether eryptosis may play a role in AIHA.
Methods: RBCs from 24 patients with wAIHA, 7 patients with chronic cAIHA, and one patient with AIHA of mixed-type were analysed for phosphatidylserine (PS) exposed at the surface by treatment with phycoerythrin (PE)-labelled Annexin V. Cell-associated fluorescence was measured using a MACSQuant flow cytometer and results have been put in context to serological (monospecific DAT) and clinical data (parameter of haemolysis).
Results: Eryptosis has been detected in all patients with IgM aabs (all patients with chronic cAIHA, the patient with AIHA of mixed-type and in 6 of 13 patients with significant wAIHA) and one patient with significant wAIHA due to IgA aabs. In contrast, PS exposure was not increased in all patients with significant wAIHA only due to IgG aabs and in patients with wAIHA in complete remission. Patients with decompensated AIHA and eryptosis appear to respond to treatment with erythropoietin, which is a known inhibitor of eryptosis.
Conclusion: Eryptosis appears to occur frequently in AIHA related to IgM or IgA aabs. Inhibition of eryptosis may represent an additional therapeutic option in the treatment of decompensated AIHA.

Conflict of interest: none

VS-4-7

Establishment, optimization and validation of a highly sensitive flow cytometry assay for monitoring survival of transfused platelets: Tracking HLA mismatch between donor and recipient

Geisen C.[1], Fleck E.[2], Kjeldsen-Kragh J.[3,4], Vetlesen A.[5], Kjaer M.[4,6]
[1]DRK Blutspendedienst Baden-Württemberg - Hessen gemeinnützige GmbH, Abteilung für Immunhämatologie, Hämostaseologie und Molekulare Diagnostik, Frankfurt am Main, Germany
[2]DRK Blutspendedienst Baden-Württemberg - Hessen, Frankfurt am Main, Germany
[3]University and Regional Laboratories, Lund, Sweden
[4]Prophylix Pharma AS, Tromsö, Norway
[5]University Hospital, Oslo, Norway
[6]University Hospital North, Tromsö, Norway

Introduction: The large European collaborate study (PROFNAIT) is in the process of developing Hyperimmune anti-HPA-1a IgG (NAITgam) for the prevention of HPA-1a immunization and fetal and neonatal alloimmune thrombocytopenia (FNAIT) in HPA-1a positive children born by HPA-1a negative women. The ability of NAITgam to eliminate HPA-1a positive platelets transfused to HPA-1a negative healthy individuals will be tested in a phase 1/2 trial.
Traditionally, *in vivo* monitoring the survival of transfused platelets has been carried out by measuring radioisotope-labeled platelets. This labeling procedure requires significant manipulation of platelets and it is questionable if these platelets are comparable with un-manipulated platelets. Hence, establishment and validation of a high sensitive method for studying survival of un-manipulated transfused platelets would allow for measurement of a more true platelet survival kinetic for use in clinical settings. The aim was to establish, optimize and validate a high sensitive flow cytometric method that allowed the detection of minor amounts (<0.015%) of transfused platelets. The established method will be used in the PROFNAIT phase 0 and phase 1/2 clinical trials for measurement of *in vivo* platelet survival.
Methods: The established flow cytometric assay was based on the method published by Vetlesen et. al (Transfusion, 2012) using HLA-A2 or HLA-A9 mismatch between donor and recipient. Standards and quality control samples were prepared by adding small amounts of HLA A2 or HLA A9 positive platelets (0.015–1%) to different batches of platelet rich plasma (PRP) from HLA A2 or HLA A9 negative donors. A total of 10E6 platelets, at a flow rate of 10 µl/min, were collected and isolated in a FCS/CD41 (PERpCy5.5, Beckman Coulter) dot plot. Further, the targeted populations of HLA A2 or HLA A9 positive platelets were identified in a SSC/FL1 dot plot using FITC conjugated anti-HLA A2,28 IgG2a or anti-HLA A9 IgG2b monoclonal antibodies (One Lamda Inc). Parameters to be validated were; selectivity, carry-over, lower limit of detection (LLOQ), calibration curve, accuracy, precision, and robustness.
Results: HLA-A2 and HLA-A9 positive platelets exhibited strong signals (FL1) that allowed for discrimination of 0.015% positive platelets among 99,985% negative platelets. The validation confirmed the acceptance criteria of an accuracy and precision within 15% (respectively 20% for LLOQ), a linearity (R2) of >0.97 and a LLOQ of 0.015%.
Conclusion: We successfully established a high sensitive method for measurement of the natural clearance of transfused platelets. The method validation was according to EMAS guidelines and fulfilled the requirement of Good Laboratory Practice (GLP).

Conflict of interest: none

VS-4-8

Sialylation modification on human megakaryocytes by autoantibodies from patients with autoimmune thrombocytopenia

Marini I.[1], Zlamal J.[1], Rigoni F.[1], Althaus K.[2], Bakchoul T.[1,2]

[1]Medical Faculty Tübingen, University Hospital Tübingen, Tübingen, Germany
[2]Center for Clinical Transfusion Medicine Tübingen, Tübingen, Germany

Introduction: Megakaryocytes (MKs) are polypoid cells that release anucleated cells called platelets (PLTs). Immune thrombocytopenia (ITP) is a bleeding syndrome caused by autoantibodies (AAbs) directed against PLT glycoproteins (GPs). Recently, it has been shown that AAbs can induce glycan changes on the surface of PLTs leading to their destruction in the liver. In this study, we investigated the impact of AAbs from ITP patients on the glycan pattern of human MKs.
Methods: MKs were differentiated from human hematopoietic stem cells (HSCs) after 14 days of cultivation with thrombopoietin. The population of mature MK was defined as CD41 and CD42b double positive by flow cytometry (FC). The ploidy state of the cells was determined after propidium iodide (PI) staining. Lectin binding assay (LBA) was performed incubating MKs with ITP or healthy donor sera and the modifications in glycan pattern were assessed by FC using lectins; Ricinus communis agglutinin (RCA), Erythrina cristagalli lectin (ECL) and Peanut agglutinin (PNA) that bind respectively to beta-galactose, N-acetylglucosamine and N-acetylgalactosamine residues, respectively.
Results: In this work, the impact of 11 sera from ITP patients and 5 sera from healthy donors on MK glycan pattern were investigated. First of all, we performed a full characterization of the differentiated MKs. The analysis of the specific markers CD41 and CD42b showed a population of complete mature MKs after 14 days of cultivation (median% of CD41 and CD42b double positive cells: 25.12, range: 8.25–30.70). The DNA content of the differentiated cells was quantified by PI staining, showing a ploidy state of 2N, 4N, 8N and 16N in comparison to HSCs (2N), used as control. In the LBA we observed that the glycan expression of mature MKs can be modified by exogenous Neuraminidase. Interestingly, ITP-AAbs were able to change different glycan patterns on MKs surface. In particular, 7/11 sera induced a significant increase in RCA binding. 5/11 sera caused high ECL binding and 3/11 sera enhanced PNA binding. In contrast, no significant changes were induced by sera from healthy donors used as control.
Conclusion: Our study demonstrates that the mechanism of antibody-mediated modification of glycan pattern caused by AAbs from ITP patients can target human mature MKs, suggesting a relevant impact on PLT production.

Conflict of interest: none

VS-5
Präparative und therapeutische Plasmapherese – Joint session ARGE Plasmapherese

VS-5-1

Plasmapheresis-Part of Transfusion Medicine?

Storch,H.

Haemo Consult,Brotterode-Trusetal,Germany

Background: Plasmapheresis(PP) started in the transfusion medicine as a manual procedure and has made great progress in the automated version for the last 40 years. This reflect the number of plasma donations as preparative plasmapheresis(PPP)of all blood donations,the quality of the produced plasma and the technical development of automated plasma separators. The main reason for this is the increasing demand of patients for plasma derivatives and consequently the starting material the plasma for fractionation(PfF). The therapeutic version of the PP has been extended as treatment of severe diseases mainly in autoimmune disorders. An analysis of PPP as part of transfusion medicine may be helpful regarding research and decisions in the health policy. Furthermore,the concept of European self sufficiency (SS) declared 1989 in the Council Directive 89/381/EEC should be evaluated.
Methods: Survey of the scientific literature on PPP. Official,public information focused on Europe,USA and selected EU-member states(MS) have been analyzed.
Results: The most experience with PPP exist in the USA,the biggest global user of PPP and producer of PfF with more than 60% of the global available PfF. However, a strict separation can be observed between the classical transfusion medicine and the commercial private and industry owned plasmapheresis centers(PC). Austria(A) as the first MS of the EU with national SS has the same structure of PC as USA. The Czech Republic(CZ) and Hungary(H) are in a transition phase with the opportunity to achieve the cooperation with the transfusion medicine. Germany(G) is beside Sweden one of the MS in which the PPP was included as part of transfusion medicine. This reflect the structure of ARGE Plasmapherese,where Governmental Blood Establishments,Red Cross an PC work together in order to develop the PP and investigate the scientific questions. The results of the PEI-data in 2017 demonstrates that 38% of all blood donations in Germany were plasma donations. 66.9% of the collected PfF was Source Plasma(SP) from PPP,the main- and most important part of PfF in the current situation of decreasing procurement of plasma from whole blood donation(recovered plasma). Remarkable is the fact,that the 4 MS of the EU with national SS (A,G,CZ and H) of PfF have a compensated PP. The goal of national SS has never been achieved in MS with strong activities on PP like Netherlands,France and Italy using a non compensated plasma donation. The majority of MS demonstrate a low level of PP with a weak contribution of PfF for an European SS.Therefore,the EU is depending with PfF and the final plasma derivatives from the USA. The scientific output of papers dealing with the PPP showed clearly a lack of publications in all countries where no cooperation with the transfusion medicine exist. This is in opposite to the growth of PPP and the open questions of this procedure.
Conclusion: Europe has the challenge to make a substantial contribution to the increasing global demand on PfF in order to prevent an insufficient supply of plasma derivatives if epidemiological and political changes in the USA occur. The patient needs can be supported by national programs on PP in the MS of EU. The current need of IgG can be met by 30 L PfF/1.000 inhabitants. The scientific goals of PPP have to be directed on donor safety,plasma quality and IT-improvements of the PPP separators. This may be achieved by integration of the scientists in PC into the transfusion medicine.

Disclosure Statement: none

VS-5-2

IgG metabolism in plasma donors depending on individual conditions

Kiessig S.[1], Ulrich E.[2]

[1]Ruhr-Plasma-Zentrum Bochum, Bochum, Germany
[2]Haema AG, Leipzig, Germany

Background: German Plasmapheresis Guidelines determine a minimum IgG level of 6.0 g/L and require a permanent deferral after 3 times IgG below this limit. There is no scientific evidence for such a regulation.
Material & Methods: 6667 donors with IgG caused deferrals from an 8-year period (2001–2009) were selected from the donor data base. Three IgG-levels were extracted: IgG at the first donation (IgG1), IgG at the donation resulting in IgG deferral (IgG2), IgG at the first control after expired deferral period (IgG3). Additional data were extracted too: differences between IgG levels, donation frequencies, deferral periods, IgG synthesis rates per day were calculated and evaluated statistically. Only donors with deferral periods between 21–28 days were selected. 309 donors (4.63%) with initial IgG lower than acceptable also excluded. 1294 selected data sets could be evaluated by cross correlations.

Results: The Initial IgG levels varied from 1.94 to 16.3 g/L in all donors. In the selected population from the cross correlations no clear associations among the data was observed. The highest correlation coefficients were found in IgG3 vs. IgG1 (r = 0.52). The predefined deferral period of 21–28 d was long enough for regeneration of IgG in 4694 (70.4%) of the 6667 donors. The Synthesis rate varied from 0.004 to 0.319 g*d/L within the selected donor population. A weak correlation between synthesis rate and IgG1 was found (r = 0.48). There was also a weak correlation between IgG1 and total IgG synthesis rates.
Conclusion: The IgG metabolism is a highly heterogenous and individual process, depending most on the IgG ahead of any donation (IgG1). No general rules like the deferral rule at the German Guideline can be applied. Only one exception: IgG1 levels should determine the acceptable donation frequency:
Individualized donation frequency rules are required instead of generalized deferral rules:
6–8 g/L à 2/month
8–10 g/L à 1/week
> 10 g/L à 2/week
Individualized deferral rules and times are required according to IgG2 levels (at deferral) to avoid unnecessary donor deferral, to allow donations according to the donor capacities.

Disclosure Statement: none

VS-5-3

Long-term trending of IgG and TP values depending on the frequency and regularity of plasma donations.

Krull M., Ziyaie-Hochstrat O.

Plasma Service Europe GmbH, Dreieich, Germany

Introduction: The donation rate for plasma donations is highly dependent on the donor IgG & TP concentrations. The continued question from our DGTI-Poster 2017 (& the now strongly discussed guidelines regarding the exclusion of 3 x decreased IgG-readings) is to what extent a regular high-frequency donation activity is possible & how this 'aspired frequency' will affect the og. Concentrations and **can be preferred in the routine procedure**. The aim of this study was a retrospective comparison between 2 different donor rates for new donors and their long-term effects (with a special focus on the IgG value).
Method: The review of the IgG and TP development of new qualified donors for the year 2016 (N = 240) continued in 2017. The pool was formed from donors with at least 30 plasmapheres each in 2016 and 2017. Two groups were further formed: 1st group (N1 = 62) with a donation interval of 3 to 7 days and 2nd group (N2 = 67) of 8 to 14 days. IgG & TP measurement points were for every 5th donation. The MVs were compared from 15 measurement points spread over 2 years. (5th, 10th, 15th, etc. donation)
Results: The comparison of the IgG and TP concentration of the two groups revealed an analogous course. It turned out that with the start of the donation career, the IgG & TP concentrations decreased significantly in both groups. Between the 15th and the 30th donation, both groups remained at a relatively constant level and a long-term comparable course. The 'regeneration' with constant donation reaches in the first group a pronounced level of increase over the short-term development in the long term. In comparison to the 2nd group, which shows a low level increase over the long term. The TP showed analogous behavior in the short term, but in the long term a level decrease in both groups. The general regeneration effect is not significant in the long term.

Tab. 1.

IgG-Concentration	MV measuring point 0 in 2016		lowest mean in 2016		MV in 2017
N1	10,973 ± 1,8 g/l	→	8,395 ± 1,4 g/l	→	9,219 ± 1,9 g/l
N2	10,497 ± 1,9 g/l	→	8,668 ± 1,7 g/l	→	8,949 ± 1,9 g/l

Discussion / Conclusion: It is known that regular plasmapheresis leads directly to the decrease of the IgG value, which recover in the medium term with 'regularly repeated donations' in the context of a 'possible immunomodulation'. In our study, this phenomenon has occurred in both investigated groups. In the long term, this loss compensation will continue and remain at a comparable level. The assumption was that the humoral immune system quickly adjusted to 'regular IgG loss'. The results can be interpreted to suggest that high-frequency donation behavior favors the 'regeneration effect'. Larger groups of donors and longer-term studies are needed in our view.

Conflict of interest: Anstellungsverhältnis der Autoren bei der Plasma Service Europe GmbH; 100% Tochter der Biotest AG.

VS-5-4

Donor safety in haemapheresis – influence of frequent plasma donations on parameters of calcium-phosphate and bone metabolism

Hendig D.[1], Schmauder A.[1], Eberhardt M.[2], Heuser J.[1], Hinse D.[1], Knabbe C.[1]

[1]Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany
[2]TMD Gesellschaft für transfusionsmedizinische Dienste mbH, Kassel, Germany

Introduction: Periodic plasmapheresis is generally safe although loss of total protein and immunoglobulin G (IgG) might accompany plasma donation. The German guidelines «Hemotherapy» already include regular control measures of total protein and IgG concentration to ensure plasma donor»s safety. This study evaluates for the first time the impact of frequent plasma donations on parameters of calcium-phosphate and bone metabolism in a German blood donor cohort.
Methods: The study included 243 donors of whole blood, platelets and plasma (apheresis). We divided the study cohort into 4 subgroups. Group I consisted of whole blood donors (4 donations per year at most). Group II included low frequency plasma donors (maximal 10 plasma donations per anno). Group III and IV included donors with a higher frequency of plasma donations, while donors of group IV frequently donate plasma (more than 10 times per anno) for more than 5 years.
We measured selected biochemical parameters by standard laboratory methods. These parameters included concentrations of calcium, phosphate, magnesium, total protein (TP), albumin (ALB), alkaline phosphatase (AP), intact parathyroid hormone (PTH) and special markers of bone metabolism and an increased risk for vascular calcification: osteocalcin (OCN), collagen metabolites (ß-crosslaps, CTX and type 1 procollagen total N-terminal propeptide, TP1NP) and fetuin-A.
We performed statistical analysis using the SPSS23 software package. We tested all data for normal distribution using the Kruskal Wallis test. Multivariate variant analysis was used to test for overall differences between subgroups I to IV. Comparison between two subgroups was done using post-hoc Donn-Bonferroni testing. Statistical difference was assumed if p value was ≤ 0.05.
Results: No differences in blood concentrations were found for calcium, phosphate, magnesium, AP and ALB comparing the four subgroups. Further, no differences between subgroups were detected for the vascular calcification risk marker fetuin-A and the bone metabolism marker CTX. As expected, we observed significantly reduced TP concentration, with highest TP concentration in subgroup I and lowest TP levels in subgroup IV ($p < 0.05$). Moreover, we detected an increase of bone metabolism markers PTH, OCN and tP1NP with lowest levels in subgroup I and highest in subgroup IV ($p<0.05$). However, all measured values laid within the reference range of a healthy population.
Conclusion: Long-time plasma donors with more than 11 plasmapheresis sessions per year showed a decrease in TP and an increase in bone metabolism markers pointing towards activated bone turnover. Therefore, further studies on a larger blood donor cohort are of high interest, includ-

ing analysis of bone mineral density, to uncover the impact of potentially activated bone metabolism in plasma donors with frequent donations.

Conflict of interest: none

VS-5-5

Volumes exchange between the two major compartments (blood volume and interstitial volume) during preparative plasmapheresis

Kiessig S.T.

Ruhr-Plasma-Zentrum Bochum, Bochum, Germany

Aim: Some regulations require that a maximum extra-corporeal volume during automated apheresis should not be exceeded. Therefor we observed the exchange of volumes between the two main compartments, the blood volume and the interstitial volume in donors during and after plasmapheresis.
Methods: These two different main compartments were evaluated in 41 donors before and after plasmapheresis using impedance spectroscopy with a body composition monitor (BCM, Fresenius Medical Care AG, Hof/Saale, Germany). The donation volumes were adjusted according to German Guidelines 2010. Na-Citrate was added in a ratio of 1:16 to the whole blood collected with anticoagulant, 300 mL isotonic NaCl was given as volume substitute at the end of the procedure.
BCM measurements were carried out either at the same day before and immediately after (group 1, n = 21) or before and two days after plasmapheresis (group 2; n = 20). Procedure and donor data (donation time, hemogram, body weight, etc.) were collected. The different compartments were detected [intracellular water (ICW), extracellular water (ECW), total body water (TBW), urea distribution water] or calculated from the donor data (blood volume). Overhydration was calculated from ICW and TBW information by a validated body composition model. All data were cross correlated to identify depending parameters.
Results: An average of 798 mL plasma including citrate volume was collected from donors showing a mild but highly variable overhydration of 0.28 L (±0.96 L). An average of about 158 mL citrate was used for the plasma donation. About 133 mL citrate went into the product, 26 mL into the donor respectively. A total volume loss of 473 mL of TBW (about 1%) was found.
In group 1, ICW average was nearly uninfluenced by plasmapheresis (24.95 L before vs. 25.04 L after). ECW decreased by 0.23 L (19.53 L before vs. 19.30 L after). In group 2 we observed a decline of ICW by 0.34 L (25.21 L before vs. 24.88 after). ECW increased from 19.00 L to 19.09 L.
Conclusions: A loss of 130 mL of water volume immediately after plasmapheresis was found in group 1. The major changes happen in the ECW. This compartment is highly accessible during plasmapheresis. The available volume of ECW seems to be large enough and can easily be mobilized. The flow between the compartments has to be observed much more in detail to understand the regulation behind. Despite of the fact that there are no methods to measure the extracorporeal volume during the plasmapheresis procedure, there is no need until the donors have a sufficient body weight with the respective blood and interstitial volume. The exchange between the two compartments is finalized already with the end of the plasmapheresis procedure.

Conflict of interest: none

VS-6
Infektionssicherheit I

VS-6-1

Platelet bacteria screening: Where we are coming from and where we want to go

Störmer M., Gathof B.

Uniklinik Köln (AöR), Zentrale Dienstleistungseinrichtung für Transfusionsmedizin/Blutspendezentrale, Köln, Deutschland

Bacterial safety of blood products presents an ongoing challenge for physicians, manufacturers and regulators. Although there have been many new approaches to enhance the microbial safety during the last decade, established methods for microbiological control still need to be fully adapted to the special circumstances of blood components. The last years of evaluation demonstrate the variety of problems and risk factors for the development of new strategies for microbial safety.
Special attention has been taken on strategies focusing on the detection or reduction of bacterial contamination of platelet concentrates. But up to now none of the aimed strategies have become accepted worldwide. It became apparent that the development of new technologies and the implementation into the routine laboratory setting is a whole different problem. Moreover the expectation and requirement of new technologies, to achieve the same sensitivity as it is stated for the conventional culture method, limited the authority»s approval and implementation into the routine setting as well. As a result the classical microbiological control methods still represent the golden standard and the negative to date automated culture strategy seems to become the method of choice for platelet bacteria screening. Recently significant changes in regulatory requirements have been made by revising chapters of the European Pharmacopeia to address the need of next-generation therapies. These revisions may lead to authority»s acceptance of alternative methods for platelet bacteria screening as well.
In conclusion, the last decade findings in microbial safety of platelet concentrates enable us to understand that the detection or reduction of bacteria represents a more difficult challenge in comparison to viruses. Cellular products of a shelf-life of 4–7 days may present a bigger challenge than those of a 1 day shelf-life. But the change in regulation demonstrates that we are getting closer to the goal of a shift from the traditional view of sterility evaluation to a new thinking about applicable microbiological control.

Conflict of interest: none

VS-6-2

Comparison of ten rapid bacterial detection assays regarding the efficiency to improve blood safety

Schmidt M.[1], Hourfar K.[1], Seifried E.[2]

[1]DRK BaWüHe Institut Frankfurt, Qualitätsmanagement, Frankfurt am Main, Germany
[2]DRK BaWüHe Institut Frankfurt, Geschäftsführung, Frankfurt am Main, Germany

Background: Transfusion transmitted bacterial infections are still a risk in transfusion medicine. Platelets are on higher risk than packed red cell concentrates due to the storage conditions at room-temperature which represents good growth conditions for a broad range of bacteria. Blood safety can be improved by the diversion of the first 30–40 ml into a pre-donation satellite bag. In addition pathogen reduction methods like the Intercept, the Mirasol or the Theraflex methods are able to inactivate potential bacteria in platelet concentrates. An alternative to pathogen reduction methods could be the implementation of a bacterial screening method which can be classified into release methods and non-release methods.
Aims: Ten release methods (ATP measurement, BacTx, BacT/ALERT, Bactiflow, FACS, NAT, Haemonetic eBDS, Scansystem, Spetifast and Ve-

rax PGD) published in peer-reviewed manuscripts were compared with each other regarding the diagnostic sensitivity, the diagnostic specificity and the clinical performance to blood donor screening.
Methods: Medical publications (pubmed) were searched reading rapid bacterial detection methods between 2000 and 2018. The original publications were investigated and compared with each other.
Results: Bacterial screening tests can be classified into rapid detection tests (testing time <6h), combo tests (combination between culture methods and rapid tests (total test time app. 24h) and point of care tests (immediately before transfusion). The diagnostic sensitivity had a range between 101 to 105 CFU/ml. Currently in use are the Bactiflow method, Haemonetics eBDS and Verax PGD.
Summary / Conclusions: Bacterial release tests were able to improve blood safety to detect fast growing bacterial strains in platelets. The reduced diagnostic sensitivity will be covered by a late sample collection (at minimum 48h after blood donation). Slow growing bacteria strains like *Propionibcaterium acnes* are not in the focus of release tests. The implementation of a bacterial release test includes a release of untested platelet concentrates for the first 48h. Due to the fact that 1/3 of all platelet concentrates will be released within the first 48 hours only 66% of all platelets can be tested by this screening strategy. The adoption of culture system for a late sample collection (36h to 48h) reduced the difference between release and non-release screening systems.

Conflict of interest: none

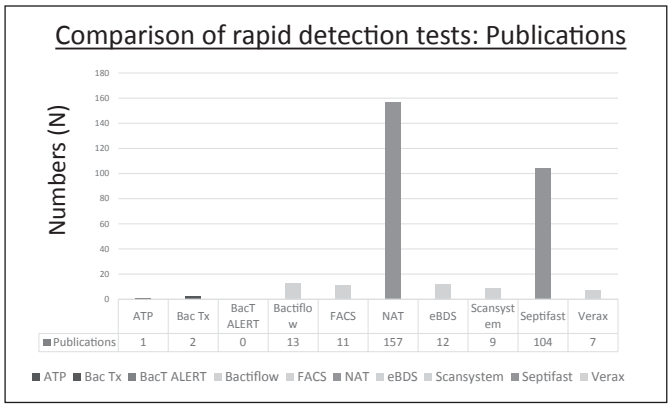

Fig. 1.

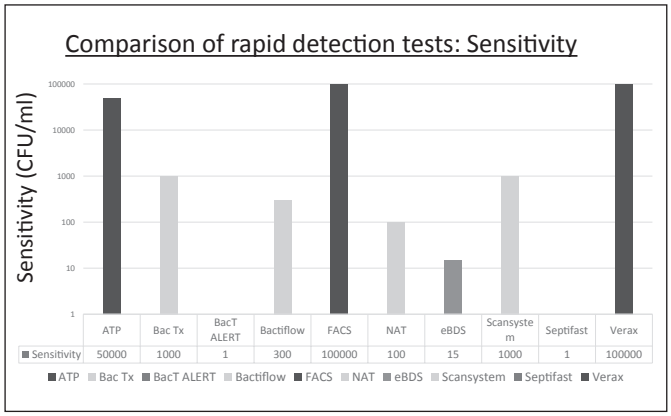

Fig. 1.

VS-6-3

Microbial safety of thrombocyte concentrates: from viewpoint of regulator

Prax M., Bekeredjian-Ding I., Krut O.
Paul-Ehrlich-Institut, Langen, Deutschland

Bacterial contamination of thrombocyte concentrates (TC) remains a persistent source of transfusion related sepsis. Based on international studies the estimated rate of contaminated TCs is up to 0.1%. Therefore, appropriate procedures need to be implemented in order to mitigate the risk of transmission of infection.
Worldwide, strategies to reduce the frequency of bacterial contamination are not harmonized and differ significantly among different countries. The measures include reduction of the shelf life of TCs, bacteriological screening of TCs by direct or growth-based methods or implementation of pathogen reduction systems. Each of these strategies has its benefits and limitations, which are still subject of current research and stakeholder discussions. However, from the microbial safety point of view the simple limitation of the shelf life cannot be considered sufficient to minimize the risk of transfusion-associated sepsis. Based on haemovigilance data and published reports, bacterial screening or pathogen inactivation of TCs should be generally considered as a preferred option.

Conflict of interest: none

VS-6-4

HEV NAT screening of blood donors: as much as necessary or as much as possible?

Dreier J., Vollmer T., Knabbe C.
Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany

Background: The risk and importance of transfusion-transmitted hepatitis E virus (TT-HEV) infections by contaminated blood products is currently a controversial discussed topic in transfusion medicine. The infectious dose, in particular, remains an unknown quantity. In the year 2015, the German Advisory Committee on Blood (Arbeitskreis Blut) recommended a NAT sensitivity of 100 IU HEV RNA/ml (per single donation), but at present it is not clear whether this sensitivity is necessary at all. Therefore, the question of a general HEV NAT screening of blood donors and with which analytical sensitivity is raised.
Methods: We systematically review the presently known cases of TT-HEV infections and available routine NAT-screening assays. The review of the literature revealed a significant variation regarding the infectious dose causing hepatitis E.
Results: In the systematic case review analysis, all components with a viral load greater than 5.00E+04 IU caused infection (definitive infectios dose). The lowest infectious dose resulting in TT-HEV infection observed in general was 7.05E+03 IU (minimal infectios dose).
Conclusions: Taken into account the current knowledge on the minimum required infectious dose, the infectious dose inevitably resulting in TT-HEV infection, and the analytical sensitivities of the screening methods, we extrapolated the detection probability of HEV-RNA positive blood donors using different test strategies (NAT assay, ID vs. minipool with different pool sizes). We also considered the amount of plasma in the different blood products and calculated the infectious doses needed to be detected. If the initially stated NAT sensitivity of 100 IU HEV RNA/ml per single donation is taken into account, not even ID testing is sufficient to detect the minimum viral load in the donor to avoid TT-HEV infections based on the currently known minimum infectious dose. Minipool HEV NAT screening (96 samples) of blood donations should be adequate as a routine screening assay to identify high viremic donors and will cover at least a large part of viremic phases.

Conflict of interest: none

VS-6-5

Details of the decision-making process used for implementing HEV NAT screening in Swiss blood donors

Niederhauser C., Gowland P., Widmer N., Fontana S., Stolz M.

Interregionale Blutspende SRK, Labordiagnostik, Bern, Switzerland

Introduction: Hepatitis E virus (HEV) has been recognized as a zoonotic disease since about 10 years in Europe. During the last years greater than 70 transfusion-transmitted cases have been reported indicating an importance of HEV also in blood transfusion. In 2016 the BTS SRC setup a working party of blood transfusion, infectious disease and transplantation specialists to work out a plan and future strategy for blood components.
Methods: Based on data generated in European countries and the seroprevalence data from more than 3,600 Swiss blood donors (Wantai HEV IgG EIA) the working party proposed in summer 2016 as a at that time a feasible risk reducing approach, a minipool screening in pools of 96 (sensitivity of about 2000 IU/ml) but not required for the release of blood components. Since then further HEV data have been collected, especially incidence data from more than 30,000 donors in different minipools formats (12,16 and 96 donations) with the cobas HEV RNA test on the cobas 8800 platform. In the meantime, the Swiss Federal Office of public Health (FOPH) declared that HEV RNA-positive patients had to be notified from 1st January 2018.
Results: From the 3,600 Swiss blood donors on average 20.4% were positive for HEV IgG antibodies. This seroprevalence ranged from 12.8 to 33.4% depending on the specific region. In addition, around 400 specified matched archive plasma samples (age, region, gender) from 1996, 2007 and 2016 were tested for HEV IgG and a statistically significant decrease in the HEV seroprevalence from 30.3% (1996) to 22.3% in 2016 was observed. A pilot study to generate HEV incidence data in Switzerland was performed in different minipools sizes. From 18,695 donations tested in minipools of 96 revealed no positive donation, whereas in 11,589 donations tested in pools of 16 three HEV RNA positive donations were identified and from 1"248 donations tested in minipools of 12 one positive donation was identified.
Discussion: Based on the accumulated data and the juristic responsibility for safe blood component the BTS SRC decided in June 2018 to implement HEV RNA screening for immunosuppressed patients as a mandatory test from 1st October 2018 for the release of blood components. However, there are still several points which still need to be clarified related to the implementation of this additional molecular screen. For instance: 1) The sensitivity limit for HEV RNA in the individual donation has not been defined; 2) Information on the severity of immunosuppression which will require HEV RNA tested blood components is lacking; 3) A confirmation algorithm for HEV RNA screening reactive donors has not been defined; and 4) The temporary deferral period for HEV positive donors has not been specified and lastly; 5) The description of a HEV specific patient and donor look-back procedure has not been proposed. It is imperative that all these points are clarified before or shortly after the implementation of the HEV RNA testing.

Conflict of interest: none

VS-6-6

Borna Disease Virus – the new kid on the block

Offergeld R.

Robert Koch-Institut, Berlin, Germany

Borna disease virus 1 (BoVD-1) is a neurotropic RNA virus of the family Bornaviridae which causes Borna disease in animals, especially in horses and sheep. The infection in animals can lead to meningoencephalitis resulting in paralysis and death often occurs within 1–5 weeks in the majority of animals. The proposed animal reservoir of BoDV-1 is the bicoloured white-toothed shrew (Crocidura leucodon) which is endemic in large parts of Germany.

In 2016 a cluster of acute encephalitis occurred in three recipients of solid organs from one donor (kidney and liver) with fatal outcome in two recipients (kidneys). The third recipient (liver) survived with severe long-term-sequelae. A research consortium conducted by the Friedrich-Loeffler-Institute and the Bernard Nocht-Institute identified BoDV-1 as the cause for this disease cluster and consecutively diagnostic test were established. The laboratory results confirmed BoDV-1 genome in both kidney transplant recipients by RT-qPCR and next-generation-sequencing. Immunohistochemistry and in-situ-hybridization confirmed the presence of BoDV-1 antigens and RNA. BoDV-1-specific seroconversion was detected in all patients with high antibody titres in the liver transplant recipient. Apart from the organ transplantation, no other commonalities between the donors or between the donor and the recipients were reported. The donor had no evidence of neurological disease prior to explantation.
In addition to the transplantation-associated cluster, three independent fatal cases of meningoencephalitis caused by BoDV1-infections were detected in 2017 and 2018 pointing to an infectious risk presumably independent from medical procedures. All additional cases were young adults with no known underlying conditions.
This is the first time that BoDV-1 transmissions through organ transplantation were reported. It has to be noted that BoVD-1 genome could not be detected in the blood of any of the recipients. Especially in the light of the sporadic cases, further investigations will be necessary to understand the epidemiology of the disease and the zoonotic pathway. To assess the safety of other substances of human origin including blood, further research is necessary. The first seroprevalence studies in potentially exposed adults and blood donors have been initiated. It is of utmost importance that all cases of BoDV disease are reported to the local health authorities according to the infection protection law in order to assess the extent of this newly established infectious disease.

Conflict of interest: none

VS-7
Gewebezubereitungen

VS-7-1

Stability of viral antibodies in postmortal blood samples

Herrlinger F.[1], Larscheid G.[2], Schroeter J.[1], Pruß A.[1], Hofmann J.[1]

[1]Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Berlin, Deutschland
[2]Vivantes Klinikm Neukölln, Fachbereich Pathologie, Berlin, Deutschland

Introduction: A screening of postmortal tissue donors for antibodies of hepatitis B virus, hepatitis C virus, human immunodeficiency virus and Treponema pallidum in the blood is mandatory. While the testing is well standardized in premortal blood samples there is still little known about stability of such antibodies in postmortal blood samples over the time. This lack of knowledge led to the regulation, that postmortal blood samples have to be obtained not later than 24 hours after death. Therefore a high number of potential tissue donors is lost.
Methods: Blood samples from postmortal tissue donors were obtain at the time of tissue donation and again after 12 ± 6 hours. Blood samples from the same donor before death were obtained from the hospital laboratory. All blood samples were analyzed for Varizella-Zoster antibodies, Ebstein-Barr virus and Human Adenovirus.
Results: Preliminary results show no differences of the antibody titers in the two postmortal blood samples.
Conclusion: The stability of viral antibodies seems not to be correlated with a 24 hours postmortal time frame.

Conflict of interest: none

VS-7-2

Acceptability of postmortal blood samples for antibody/ NAT testing – can quality be quantified?

Schulz T.[1], Pruß A.[2], Schroeter J.[1]

[1]Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Berlin, Deutschland
[2]Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Universitätsgewebebank, Berlin, Deutschland

Introduction: A screening of postmortal tissue donors for antibodies of hepatitis B virus, hepatitis C virus, human immunodeficiency virus and Treponema pallidum in the blood is mandatory. When premortal blood samples are not available, this testing has to be performed with postmortal blood samples. While antibody and NAT testing in postmortal blood samples often show false positive or invalid results, a preanalytical testing of the blood sample would be helpful.
Methods: Blood samples from postmortal tissue donors were obtain at the time of tissue donation. Blood samples from the same donor before death were obtained from the hospital laboratory. All blood samples were analyzed for free hemoglobin, protein content, whole immunoglobulin (IgG) and albumin.
Results: Preliminary results show no correlation of the measured parameters, the difference to the premortal blood samples and the postmortal time.
Conclusion: Since the quality of postmortal blood samples, especially the amount of free hemoglobin, does not correlate with the postmortal time, a fixed limit of a postmortal time frame for the use of such samples alone does not seem to be useful.

Conflict of interest: none

VS-7-3

Concepts for validation of microbiological testing for cell and tissue preparation with antibiotic content

Schroeter J.[1], Schulz E.[1], Pruß A.[2]

[1]Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Berlin, Deutschland
[2]Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Universitätsgewebebank, Berlin, Deutschland

Introduction: The microbiological testing of tissue and cell preparations is of high importance for the recipient»s safety. Therefore it has to be sensitive and reliable enough to minimize the risk of transmitting microorganisms.
Methods: Different techniques (e.g. resine treatment, filtration, dilution) for the inactivation of antibiotics in samples for microbiological testing are established.
Results: A protocol for the validation of microbiological testing based on the recommendations of the European Pharmacopoeia will be presented. Advantages and disadvantages of different inactivation strategies will be discussed.
Conclusion: To avoid false negative results in microbiological testing of antibiotic containing samples a careful selection of an inactivation technique and its profound validation are necessary.

Conflict of interest: none

VS-8
Hämostasiologie I

VS-8-1

Chloride intracellular channel 1 supports mechanisms related to thrombosis and angiogenesis

Knowles L.[1], Ampofo E.[2], Menger M.[2], Wolter C.[1], Niewald P.[1], Drawz A.[1], Eichler H.[1], Pilch J.[1]

[1]Universitätsklinikum des Saarlandes, Institut für Klinische Hämostaseologie und Transfusionsmedizin, Homburg/Saar, Germany
[2]Universitätsklinikum des Saarlandes, Institut für Klinisch-Experimentelle Chirurgie, Homburg/Saar, Germany

Introduction: Chloride intracellular channel 1 (CLIC1) has been shown to be involved in angiogenesis as well as platelet activation but the functional context of CLIC1 action remains largely unexplored. The objective of this study is to determine if CLIC1 supports cell adhesive processes that are relevant for endothelial proliferation and platelet thrombus formation.
Methods: Human umbilical venous endothelial cells (HUVEC) were probed for cell proliferation on plastic and cell invasion/survival after embedding in fibrin and transfection with siCLIC1. The subcellular localization of CLIC1 in HUVEC as well as platelets was analyzed with fluorescence microscopy following treatment with the synthetic CLIC1 inhibitor IAA94, which was also used to determine the effect of CLIC1 on integrin activation and platelet aggregation. In addition we measured CLIC1 cell surface expression by flow cytometry to assess platelet activation in platelet concentrates after 5 days of storage. The role of CLIC1 for thrombus formation *in vivo* was assessed by intravital fluorescence microscopy in a mouse dorsal skin fold chamber model.
Results: Knocking down CLIC1 in endothelial cells has significant anti-proliferative and pro-apoptotic effects, which are associated with the inability of cells to spread after attachment to the extracellular matrix. Critical to this process is the endothelial integrin αvβ3, which mediates recruitment of CLIC1 into newly formed lamellipodia and subsequent co-localization with F-actin. Inhibiting CLIC1 with siRNA or the synthetic CLIC1 inhibitor IAA94, on the other hand, reduced F-actin formation in nascent adhesions indicating that CLIC1 supports integrin β3-mediated cytoskeletal dynamics during endothelial cell attachment. In addition, co-localization of CLIC1 with F-actin was detected in lamellipodia of platelets, which expressed CLIC1 on their cell surface in an integrin-dependent manner. Inhibition of CLIC1 relocation to the platelet membrane with IAA94 hindered activation of integrin αIIbβ3. This in turn resulted in impaired platelet aggregation in vitro and delayed vaso-occlusion in a mouse model of photo-chemical thrombus formation in vivo. Conversely, we detected a significant increase of cell surface CLIC1 in 5 day old platelet concentrates suggesting increased platelet activation at the end of their shelf life.
Conclusion: CLIC1 cooperates with integrins during cell adhesion and as such mediates functions related to platelet homeostasis, thrombus formation, and angiogenesis.

Conflict of interest: none

VS-8-2

Platelet-dependent thrombin generation induced by ADP or activated factor X is not increased in myeloproliferative neoplasms

Berens C.[1], Rühl H.[1], Müller J.[1], Oldenburg J.[1], Brossart P.[2], Wolf D.[2], Pötzsch B.[1]

[1]University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany
[2]University Hospital Bonn, Department of Hematology, Oncology and Rheumatology, Bonn, Germany

Introduction: Myeloproliferative Neoplasms (MPN) are a group of proliferative disorders of hematopoietic stem cells and progenitor cells. They include the clinical entities Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Primary Myelofibrosis (PMF), which are associated with an increased risk of thrombotic complications. An increased sensitivity of platelets to adenosin diphosphate (ADP) has been suggested to contribute to a hypercoagulable state in MPN through increased thrombin generation. In this study we monitored platelet-dependent thrombin generation in MPN patients with an additional focus on prothrombin activation by the prothrombinase complex.
Methods: A total of 25 blood samples were obtained from patients with MPN (PV, n = 18; ET, n = 3; PMF, n = 4) and from 24 healthy blood donors that served as controls. In vitro thrombin generation in platelet-rich plasma (PRP) and platelet-poor plasma (PPP) was assessed using the Calibrated Automated Thrombogram (CAT) assay. To induce thrombin generation either ADP (1 µmol/L final concentration) or activated factor X (FXa, 10 ng/mL final concentration) were applied.
Results: In PPP of both MPN patients and healthy controls neither ADP nor FXa induced a significant thrombin generation. In PRP of MPN patients a higher ADP-induced peak thrombin concentration (Cpeak) was observed than in the healthy controls, with 122 ± 43 vs. 95 ± 26 nmol/L (mean ± standard deviation, p = 0.011). There was no statistically significant difference in the ADP-induced endogenous thrombin potential (ETP) in MPN patients (1602 ± 459 nmol/L·min) compared with the controls (1412 ± 353 nmol/L·min, p = 0.112). With 1491 ± 406 nmol/L·min vs. 1733 ± 371 nmol/L·min the FXa-induced ETP was significantly lower in PRP from MPN patients than in the control group (p = 0.034) while Cpeak did not differ (115 ± 38/L vs. 113 ± 27 nmol/L, p = 0.803).
Conclusion: The observation of an increased ADP-induced Cpeak in PRP from MPN patients is in accordance with previous findings. However, as this did not increase the amount of generated thrombin, the mechanisms that regulate thrombin formation appear to counteract a possibly increased ADP sensitivity of platelets in MPN. Our data indicates, furthermore, that the activity of the prothrombinase complex is not up-regulated by MPN-associated alterations of the resting platelet membrane.

Conflict of interest: none

VS-8-3

A new method to assess platelet activation and leukocyte-platelet aggregates in whole blood samples by flow cytometry for clinical studies

Hidiatov O.[1], Jouni R.[2], Marini I.[1], Straub A.[3], Bakchoul T.[2,1]

[1]Medical Faculty Tübingen, University Hospital Tübingen, Tübingen, Germany
[2]Center for Clinical Transfusion Medicine Tübingen, Tübingen, Germany
[3]University Department of Anesthesiology and Intensive Care Medicine Tübingen, Tübingen, Germany

Introduction: Platelet activation *in vivo* is considered to be associated with thrombosis and thrombocytopenia. For this reason, a sensitive and easy-to-use method to determine platelet activation in whole blood is necessary. The aim of this work was to establish and validate an easy and rapid laboratory test to clinically investigate platelet activation using whole blood samples.
Methods: Citrated whole blood from healthy donors (n = 5) was added to buffer, ADP or TRAP. Samples were gently mixed and incubated at 37°C for 20 minutes. After fixation (to avoid further activation), samples were stained for 30 minutes directly or after 3 days in order to examine the stability of the signal for platelet activation. The expression of P-Selectin (CD62P) and CD63 on CD41-positive cells was determined by flow cytometry. The aggregates of platelet-leukocyte were detected using anti-CD41 and anti-CD45-antibodies.
Results: Samples treated with ADP showed higher expression of CD62P and CD63 compared to buffer (mean of MFI [Mean florescence intensity]±SD: 1.15 ± 0.185 vs. 0.51 ± 0.15, p = 0.02 and 1.13 ±0.13 vs. 0.76 ± 0.18, p = 0.012, respectively) as well as after after incubation with TRAP (mean of MFI±SD: 4.72 ± 0.83 vs. 0.51 ± 0.15, p = 0.012 and 1.87 ± 0.21 vs. 0.76 ± 0.18, p = 0.04, respectively). Significant increase was also observed in leukocyte-platelet aggregates after activation with TRAP (mean MFI±SD: 1.40 ± 0.31 vs. 0.69 ± 0.29, p = 0.001). A slightly but not significant increase of CD45 signal was observed using ADP.
There was no difference in the cytometric signal measured directly or 3 days after fixation for platelet activation with TRAP (mean fold increase (FI) of CD62P: 6.0 vs. 4.9, p = 0.30, respectively, and CD63: 2.6 vs. 2.0, p = 0.51, respectively), or for leukocyte-platelet aggregates (mean FI of CD45: 2.1 vs. 1.7, p = 0.27, respectively). Similarly, platelet activation remained stable after 3 days of fixation when samples were treated with ADP (mean FI of CD62P: 2.0 vs. 1.4, p = 0.14, respectively, and CD63: 1.5 vs. 1.3, p = 0.43, respectively), or for leukocyte-platelet aggregates (mean FI of CD45: 1.3 vs. 1.3, p>0.99, respectively).
Conclusion: In this study, we introduce a robust and easy-to-do method to investigate platelet activation and leukocyte-platelet aggregates using whole blood samples. This method could be suitable for laboratory investigation of platelet activation in clinical studies.

Conflict of interest: none

VS-8-4

Heparin-independent platelet activation by sera from patients with assist heart devices

Hidiatov O.[1], Hammer S.[2], Nowak-Harnau S.[2], Straub A.[3], Bakchoul T.[1,2], Althaus K.[2]

[1]Medical Faculty Tübingen, University Hospital Tübingen, Tübingen, Germany
[2]Center for Clinical Transfusion Medicine Tübingen, Tübingen, Germany
[3]University Department of Anesthesiology and Intensive Care Medicine Tübingen, Tübingen, Germany

Introduction: PF4/Heparin antibodies are frequently found in patients undergoing cardiac surgery. Postoperative thrombocytopenia is commonly and stepwise approach of testing for PF4/Heparin antibodies should be performed only in patients who meet the clinical criteria for HIT. The diagnosis of HIT can only be confirmed by *in vitro* demonstration of heparin-dependent, platelet-activating anti-PF4/heparin antibodies. While in most cases functional assays are able to provide a clear result, spontaneous i.e. heparin-independent platelet activation may hamper a final conclusion. In these patients correct diagnosis is challenging.
Methods: Clinical and laboratory data from consecutive patients undergoing cardiac surgery or receiving heart assist devices (HADs) and in whom the diagnosis of HIT was suspected were analyzed. Patients were divided into 2 groups: I. with HAD (ECMO or ECLS) and II. without HAD-implementation. Inclusion criteria were a positive rapid immunoassay and an intermediate to high pretest probability of HIT (4Ts score ≥ 4). Sera from both groups were tested by the functional assay, HIPA (Heparin-Induced Platelet Activation).
Results: Data from 55 patients with assist devices and 451 intensive care unit patients who were admitted to our university hospital between March 2016 and June 2018 were nalayzed in this study. IgG Antibodies against PF4/Heparin-complexes were found in 18/55 (33%) patients with HAD (Group I, median OD: 0.9665, range 0.553–2.404) and in 89/451 (20%) patients without HAD (Group II, median OD: 0.9555, range 0.502–2.702). Heparin-dependent, platelet-activating antibodies were detectable in 5/55

(9%) and 8/451 (2%) patients, respectively. Interestingly, while no patient in group II showed indeterminate results in the HIPA, sera from 4/55 (7%) patients in group II caused heparin-independent platelet activation in the HIPA assay. Platelet activation was completely inhibited by mAb IV.3 indicating that platelet activation was caused by circulating immune complexes. Since HIT could not be excluded in those patients, alternative anticoagulation was recommended for the management of all four cases. A rapid recovery in platelet count was observed only in one patient, making the diagnosis of HIT very likely.
Conclusions: With a prevalence of 9%, HIT seems to be a frequent complication in patients undergoing HAD-implementation with negative outcomes. Moreover, our study indicates that platelet activation in HIPA test is frequently unspecific in this cohort. The use of an inhibition step by mAb IV.3 may help distinguishing antibody-mediated platelet activation from unspecific reaction in the functional assay.

Conflict of interest: none

VS-8-5
Hemophilia macrophages exhibit a defect in polarization

Knowles L.[1], Lessig D.[1], Bernard M.[1], Schwarz E.[2], Eichler H.[1], Pilch J.[1]

[1]Unversitätsklinikum des Saarlandes, Institut für Klinische Hämostaseologie und Transfusionsmedizin, Homburg/Saar, Germany
[2]Universität des Saarlandes, Biophysik, CIPMM, Homburg/Saar, Germany

Introduction: Macrophages are master regulators of inflammation and wound healing. As such they play an important role in hemophilia, which is commonly associated with delayed tissue regeneration and bleeding-induced joint inflammation. The objective of this study is to determine if macrophage function is deregulated in hemophilia and whether this affects the physiological balance of tissue regeneration and inflammation.
Methods: Monocytes from hemophilia patients and healthy individuals were probed for morphological features of macrophage differentiation using phase contrast and fluorescence microscopy following treatment with M-CSF or GM-CSF. The wound healing response was assessed by measuring clot infiltration and phagocytosis. Phenotypical changes were measured by flow cytometry and fluorescence microscopy. Cytokine levels in plasma were examined using the Proteome Profiler Human XL Cytokine Array Kit (R&D Systems).
Results: Macrophage polarization is deregulated in hemophilia in response to M-CSF and partially in response to GM-CSF. In addition, hemophilia macrophages exhibit an impairment of M-CSF-mediated functions involved in wound healing such as clot invasion and phagocytosis. These deficits correlate with reduced cell surface expression of the receptors for M-CSF and GM-CSF on hemophilia monocytes as well as a failure to express TNFα, CD163 and CD206 in hemophilia macrophages after treatment with M-CSF and GM-CSF, respectively. Interestingly, protein expression was regained with respect to CD163 and CD206 after embedding hemophilia monocytes in clotted plasma in the presence of M-CSF suggesting that a functioning coagulation system promotes the regenerative capacity of macrophages. Mimicking the functional deficits of hemophilia macrophages in normal macrophages was possible by treating monocytes with the cytokine leptin, which we found to be upregulated in hemophilia when we performed a comparative proteomics analysis of blood samples from hemophilia patients and healthy controls.
Conclusion: Our data indicate that M-CSF-mediated regenerative macrophage functions such as clot invasion and red blood cell phagocytosis are deregulated in hemophilia and that these changes are the result of elevated leptin levels in the blood circulation of hemophiliacs.

Conflict of interest: none

VS-8-6
Fibrin supports glioblastoma self-renewal

Knowles L.[1], Wolter C.[1], Urbschat S.[2], Ketter R.[2], Dürr N.[1], Burkhardt A.[1], Lessig D.[1], Eichler H.[1], Pilch J.[1]

[1]Unversitätsklinikum des Saarlandes, Institut für Klinische Hämostaseologie und Transfusionsmedizin, Homburg/Saar, Germany
[2]Universitätsklinik des Saarlandes, Klinik für Neurochirurgie, Homburg/Saar, Germany

Introduction: Glioblastoma is a highly aggressive brain tumor characterized by diffuse invasion and growth. Angiogenesis in glioblastomas is poorly organized and tends to be associated with tumor cell necrosis, hemorrhage and thrombosis. This, in turn, leads to the formation of a fibrin-rich matrix, which may provide important adhesive cues for glioblastoma growth and proliferation.
Methods: Glioblastoma cell lines and primary tumor cells from patients with glioblastoma were embedded in a 3-dimensional matrix of clotted plasma, fibrin or Matrigel™ and scored for invadopodia formation as well as proliferation using phase contrast microscopy. Glioblastoma cell adhesion was tested using non-tissue culture-treated 48-well plates coated with fibrinogen, fibronectin and Matrigel™. Tumor stem cells were generated by culturing glioblastoma cells in the presence of Neurocult™ media. Integrin expression was assessed by immunoblotting, fibronectin expression by quantitative PCR.
Results: Our experiments revealed that highly adhesive U87MG and U373MG glioblastoma cells invade and sprout after embedding in 3D matrices of clotted plasma and fibrin. The poorly adhesive U343MG cells, on the other hand, failed to invade and sprout in 3D blood clot even though they readily proliferate in conventional 2D culture plates. Sprouting of U87MG and U373MG cells was more extensive in fibrin than in matrigel™ and correlated with their ability to generate spheres in a clonogenic stem cell assay. Glioma sprouting in 3D depended on adhesive interactions between integrin β3 and fibronectin, which mediated invadopodia formation and subsequent proliferation in cooperation with the Rho GTPase Rac. This mechanism appears to be clinically relevant since freshly isolated tumor cells from patients with glioblastoma express substantially more integrin β3 and fibronectin than primary tumor cells from patients with astrocytoma grade 2 or grade 3 and, based on this, invade and proliferate in clotted plasma.
Conclusion: Our data show that clotted plasma, which is present in the fibrin-rich edema of the tumor extracellular matrix, strongly promotes glioblastoma infiltration and colonization. Moreover, they suggest that integrin β3-mediated adhesion in fibrin clots promotes the self-renewal of tumor-initiating cells through activation of Rac.

Conflict of interest: none

VS-8-7
Cold storages of platelet concentrates induces biomechanical changes and aggregate formation

Aurich K.[1], Palankar R.[1], Hartwich O.[1], Wesche J.[1], Greinacher A.[1], Otto O.[2]

[1]Universitätsmedizin Greifswald, Transfusionsmedizin, Greifswald, Germany
[2]Universität Greifswald, Zentrum für Innovationskompetenz - Humorale Immunreaktionen bei kardiovaskulären Erkrankungen, Greifswald, Germany

Introduction: Platelet concentrates (PC) are currently stored at room temperature (RT) for 4 days. PC storage at 4 °C is an attractive option to reduce bacterial proliferation, reveals no differences in standard quality tests for platelet metabolism, aggregation and activation, but platelet function is impaired in *in-vivo* assays. To understand this contrast, we analyzed in detail platelet cytoskeleton and aggregation behaviour on different surfaces of cold stored PC. For a deeper insight in platelet biomechanical properties, we introduce real-time deformability cytometry (RT-DC), as a label-free functional platelet assay based on cell mechanical properties. As RT-DC measurements can be carried out without-sample preparation based on intrinsic material properties only, we highlight the potential of cell mechanics as a label-free biomarker in platelet quality control.

Methods: Platelets from apheresis PC of 8 healthy donors containing 65% additive solution (SSP+, Macopharma, France) were stored either at RT or at 4 °C for 10 days. Samples were taken at days 1, 4, 7, and 10 after donation. All platelet samples underwent fluorescence microscopy with tubulin staining. Platelet adhesion was assessed on collagen, laminin, fibronectin, and fibrinogen. Real-time deformability cytometry is a hydrodynamic method for mechanical cell characterization, which is based on suspended cells pumped through a microfluidic channel. Cells are deformed by hydrodynamic shear forces without any physical contact between cell and constriction wall. While cells translocate the channel, a high-speed camera captures images of the steady-state deformation, which allows for an analysis based on intrinsic cell properties with a throughput of approximately 1,000 cells/s on-the-fly. RT-DC allows to carry out label-free assays within seconds and without extensive sample preparation.
Results: RT-DC enables for a clear differentiation between RT and 4 °C stored platelets. For cold storage conditions, deformation of platelets was reduced, while samples stored at RT show an elevated deformation (Figure 1). Platelet size decreased during cold storage (29.0 ± 2.2 µm2 RT- platelets vs 24.8 ± 1.5 µm2 4 °C-platelets at day 4; p<0.0047). The cytoskeleton of 4 °C stored platelets was assessed to be rapidly degraded as shown by tubulin morphology already after day 1. Most importantly, 4 °C- platelets form aggregates on all tested matrices starting between day 1 and day 4 of storage and constantly increasing thereafter. This strongly indicates that cold stored platelets might increase the risk for micro-thromboembolism.
Conclusion: Platelets stored at 4 °C for more than 3 days bear the risk for causing micro-thromboembolism. RT-DC allows capturing underlying alterations in the platelet cytoskeleton after day 1 based on high-throughput analysis of platelet biomechanical properties.

Conflict of interest: none

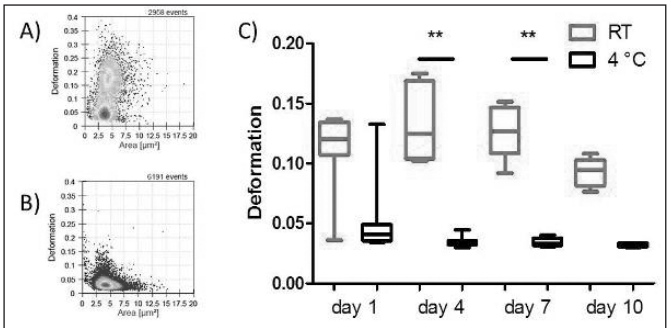

Figure 1. A) RT-DC deformation scatterplots of platelets stored at A) room temperature and B) 4 °C at storage day 1, C) deformation of platelets from stored PC (n=8)

Fig. 1.

VS-8-8

Cold storage of platelets in additive solution: the impact of residual plasma in apheresis platelet concentrates

Marini I.[1], Aurich K.[2], Jouni R.[3], Nowak-Harnau S.[3], Hartwich O.[2], Greinacher A.[2], Thiele T.[2], Bakchoul T.[1,3]

[1]Medical Faculty Tübingen, University Hospital Tübingen, Tübingen, Germany
[2]Institute of Immunology and Transfusion Medicine, University Medicine Greifswald, Greifswald, Germany
[3]Center for Clinical Transfusion Medicine Tübingen, Tübingen, Germany

Introduction: In the modern medicine platelet (PLT) transfusion is a well established therapy indispensable to prevent bleeding or hemorrhage. However, adverse reactions upon transfusion, especially transmission of bacterial infection due to room temperature (RT) storage, still remain the major limitation. For this reason, cold storage may represent a reasonable solution. In this study, we investigate the impact of different residual plasma contents in apheresis-derived platelet concentrates (APCs) stored at 4°C or RT on PLT functionality and survival.
Methods: 2 units of APCs from each healthy volunteer (n = 10) were collected and stored in plasma or in plasma additive solution (PAS) at different final plasma concentrations (100% [Plasma-APC], 35% [PAS-35-APC] or 20% [PAS-20-APC]) at 4°C and RT. After 7 days of storage, PLTs from APCs were stained with Annexin V and the phosphatidylserine expression was measured by flow cytometry (FC). The impact of different residual plasma content and storage conditions on the survival of human PLTs was analyzed using a NOD/SCID mouse model. PLT activation was determined measuring the expression of CD62P and CD63 after storage by FC. In addition, PLT function was investigated performing an aggregation assay using a 4-channel-aggregometer and collagen as inductor. Freshly isolated PLTs were always used as control.
Results: In this study, we found that after storage at 4°C PLTs express more of the apoptosis marker phosphatidylserine compared to RT (% positive events mean±SEM: 4°C vs. RT PAS-35-APC 9 ± 1 vs. 7 ± 1, p = 0.033 respectively; PAS-20-APC 22 ± 4 vs.17 ± 5, p = 0.317 respectively) in a residual plasma content independent manner. This was correlated with an accelerated PLT clearance after transfusion in a humanized NOD/SCID mouse model (survival of Plasma-APC stored at RT was considered as 1.0; 4°C vs. RT PAS-35-APC 1.05 ± 0.02 vs. 0.63 ± 0.16, p = 0.04 respectively; PAS-20-APC 0.58 ± 0.05 vs. 0.34 ± 0.05, p = 0.01 respectively). Furthermore, cold storage was associated with an increased response to activators. Of note, delta granule related functions, such as ADP-mediated aggregation and CD63 release, were better preserved at 4°C especially in 100% plasma (fold increase of CD63 after cold storage mean±SEM: plasma-APC vs. PAS-35-APC 3.88 ± 0.65 vs. 2.71 ± 0.27, p = 0.145 respectively; plasma-APC vs. PAS-20-APC, 4.75 ± 1.18 vs. 2.75 ± 0.75, p = 0.045 respectively). In addition, we performed an extended study to assess cold-stored platelet concentrates produced under standard care GMP conditions and we found that platelet function was better compared to those stored at room temperature (% maximal aggregation after collagen, mean±SEM: 4°C vs. RT 67 ± 6 vs. 22 ± 5, p
Conclusion: Our study provides additional insight into the *in vitro* haemostatic function and *in vivo* survival of cold-stored PLTs and suggests that cold storage of PLTs in PAS may become a suitable alternative to the current standard of care.

Conflict of interest: none

VS-8-9

Cold storage of platelet concentrates reduces the release of biological response modifiers: Potential mechanism in transfusion-associated adverse events in neonates

Waubert de Puiseau M.[1], Sciesielski L. K.[1], Meyer O.[1,2], Badur C. A.[1], Schönfeld H.[3], Tauber R.[3], Pruß A.[2], Dame C.[1]

[1]Charité - Universitätsmedizin Berlin, Klinik für Neonatologie, Berlin, Germany
[2]Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Berlin, Germany
[3]Charité - Universitätsmedizin Berlin, Institut für Laboratoriumsmedizin, Klinische Chemie und Pathobiochemie, Berlin, Germany

Introduction: Particularly in very preterm infants, platelet and plasma transfusions are repetitively discussed as risk factor for disorders such as necrotizing enterocolitis (ta-NEC) and lung injury (TRALI). We hypothesized that inflammatory proteins that are expressed differently in adults than in neonates could be relevant for the pathophysiology of transfusion-associated adverse events. This study aimed to investigate whether the (I) storage time, (II) preparation method, and (III) storage temperature have an impact on the putative release of such inflammatory proteins.
Methods: Pool- and apheresis PCs from clinical routine were stored with agitation for 7 days in room temperature (RT) or cold storage (CS; 4 °C; n = 6 per condition). Inflammatory mediators (DKK-1, CCL5 alias RANTES, TGFβ1, TSP-1) were measured in PC supernatant, lysate and corresponding plasma through ELISA. General inflammatory markers (IL-6 and CRP) were determined in PC supernatants. Platelet count, mean platelet volume (MPV), immature platelet fraction (IPF), pH, light transmission aggregometry, were assessed.

Results: In PC supernatants, we observed a significant increase in the concentrations of DKK-1, CCL5, TGFβ1 and TSP-1, both during the standard (up to four days) and extended (up to seven days) storage times. The concentrations of DKK-1, CCL5, TGFβ1 and TSP-1 increased up to two-fold more in RT PCs than in CS PCs, both during standard and extended storage. The concentration of DKK-1 was also significantly higher in PPCs than in APCs. The frequency of detectable CRP concentrations was significantly higher in PPCs than APCs and in RT PCs than in CS PCs.
Conclusion: In summary, this *in vitro* study shows that the release of certain inflammatory mediators is lower in CS APCs. This could possibly be a new approach to reduce transfusion-associated adverse events in neonates.

Conflict of interest: none

VS-9
Gentherapie – Joint session DG-GT

VS-9-1

Virus-specific T cells from stem cell, family and third party T cell donors: Patient monitoring, donor selection and GMP-compliant manufacturing

Maecker-Kolhoff B.[1], Tischer S.[2], Heuft H.-G.[2], Goudeva L.[2], Martens J.[2], Placzko S.[2], Verboom M.[2], Arseniev L.[3], Priesner C.[3], Köhl U.[3], Blasczyk R.[2], Eiz-Vesper B.[2]

[1]Medizinische Hochschule Hannover, Pädiatrische Hämatologie und Onkologie, Hannover, Deutschland
[2]Medizinische Hochschule Hannover, Institut für Transfusionsmedizin, Hannover, Deutschland
[3]Medizinische Hochschule Hannover, Institut für Zelltherapien, Hannover, Deutschland

Intensive immunosuppressive therapy puts patients before and after hematopoietic stem cell (HSCT) or solid organ transplantation (SOT) at risk of opportunistic infections. Among those, infection with and reactivation of cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV6), adenovirus (ADV) as well as polyoma virus BK (BKV) are frequent and associated with significant morbidity and mortality. The shortcomings of conventional therapies have increased the interest in antiviral T-cell transfer. The efficacy and clinical outcome can be improved by a rapid recruitment of a suitable T-cell donor and an established method for fast manufacturing of antiviral T cells.
A registry (alloCELL) for unrelated T-cell donors was established, which currently records >200 HLA-typed donors extensively screened for their antiviral T-cell repertoire. The alloCELL lab further established comprehensive protocols to consider clinical requirements of patients at high risk or with failed conventional therapy. The manufacturing license was obtained for generating clinical-grade mono- and multivirus-specific T-cell products using the IFN-g Cytokine Capture System and CliniMACS Prodigy device. T-cell donors were defined as eligible if ≥0.03% specific IFN-γ+ T cells are detectable. A related haploidentical or at least 3/6 HLA-A/B/DR-matched alloCELL donor is recommended, if the stem cell donor is not eligible.
T-cell frequencies in transplanted patients were determined. 98 out of 170 patients without detectable antiviral T-cells and response to conventional antiviral treatment were assigned to receive specific T-cell products. For those 160 donors were tested: 49 family, 38 stem cell, 73 alloCELL donors. 53 (58%) patients received products specific for CMV, ADV, EBV and BKV alone or in combination from related (36%) or alloCELL (64%) third-party donors. For patients in need of an unrelated third party donor we were able to find a suitable donor and provide the clinical grade T-cell product in less than 1.5 weeks after request with an HLA compatibility >5/10. T cells applied were monitored in patient blood to determine frequency, chimerism and T-cell receptor repertoire. Patients received antiviral donor T-cells without significant side effects and in 80% antiviral T cells became detectable after T-cell transfer.

Conflict of interest: none

VS-10
Blutspende und Weiterverarbeitung

VS-10-1

Well-being and return rate of first time whole blood donors

Jansen P.[1], Sümnig A.[1], Greffin K.[2], Esefeld M.[1], Greinacher A.[1]

[1]Institut für Immunologie und Transfusionsmedizin, Abteilung Transfusionsmedizin, Greifswald, Germany
[2]Institut für Psychologie, Greifswald, Germany

Background: Previous studies observed a transient increase in well-being in about one-third of regular donors after blood donation. In addition personal contact with donors after their donation seems to increase return rates. We were interested, whether changes in well-being and/or personal contact after the first donation impact return rates of first time donors (FTDs).
Study design and methods: FTDs were randomized into a questionnaire group (QG) in which questionnaires assessing the well-being (mood, vigilance, agitation) had to be filled in or into a control group (CG) without any intervention. The QG had to complete the same questionnaire three times at the day of the first donation with an additional personal contact to the donation staff and four times after donation spread over eight weeks with additional study-fact-finding calls. Return rates of participants were followed for 12 months.
Results: 115 FTDs participated in the CG and 120 in the QG. Changes in well-being after the first blood donation had minimal impact on the return rates. In contrast, contacting FTDs after their first blood donation had a major impact on the return rate of male donors (58.3% vs 89.2%; $p = 0.001$), 70.3% returned at least twice compared to 40.0% of the CG ($p = 0.003$). Females showed no significant difference in return rates between the CG and QG ($p = 0.32$).
Conclusion: The well-being of FTDs has no influence on their return rate. The intervention of research with FTDs after their first donation resulted in an increased return rate in male but not in female donors. The pronounced difference in the impact of this intervention between male and female donors requires further studies.

Conflict of interest: none

VS-10-2

Impact of whole blood donation on hypertension-induced changes in the proteome of human platelets

Esefeld M.[1,2], Steil L.[2], Sümnig A.[1], Thiele T.[1], Dhople V.[2], Hammer E.[2], Völker U.[2], Greinacher A.[1]

[1]Institut für Immunologie und Transfusionsmedizin, Transfusionsmedizin, Greifswald, Germany
[2]Interfakultäres Institut für Genetik und Funktionelle Genomforschung, Funktionelle Genomforschung, Greifswald, Germany

Introduction: Proteomics provides a global perspective of the protein composition of cells, including post-translational modifications and degradation and thus also reveal disease associated modifications in the protein profile. Previously, enhanced degradation of cytoskeletal proteins was observed in platelets of hypertonic rats, but the changes were reversible by experimental blood withdrawals[1]. Extending these studies, we now performed identification of N-termini and quantification of degraded proteins[2] to reveal if hypertension-related changes can be found in human platelets too, and if those changes are reversible after blood donation.

Methods: Platelets from 11 hypertensive and 13 normotensive donors were obtained by two blood withdrawals, corresponding to an initial blood donation (Day 0, 500 ml) and a second blood donation (Day 3, 100 ml) three days later. After sample preparation, the peptides and proteins were dimethylated using formaldehyde prior to tryptic digestion. A fraction of the samples was withdrawn (preTAILS) before the peptides generated by tryptic-digestion were bound to a synthetic polymer. The filtration of the polymer-peptide mixture provided an eluate highly enriched with naturally or chemically modified N-termini (TAILS), which was then analyzed and quantified by mass spectrometry.
Results: Overall, it was possible to identify 11721 peptides corresponding to 2555 proteins. The conventional shotgun-like analyses (preTAILS) unraveled a stable general proteome of platelets after blood donation. However, the TAILS analyses provided a total of 276 peptides with twofold changed abundance after blood donation, corresponding to 152 up- and 124 downregulated peptides, in the group of hypertonic donors but not in normotensive donors. A subgroup of cytoskeletal proteins (e.g. Talin-1, Actin, Tubulin-beta 1 and Myosin-9) showed more pronounced degradation in samples of hypertonic donors before blood donation. The intensities of those processed proteins decreased after the blood donation, indicating that blood donation can indeed reduce protein degradation.
Conclusion: This study provides for the first time data on blood donation induced changes in the proteome of human platelets. Notably, the hypertension-induced changes of some cytoskeletal proteins are reversible by a whole blood donation.

Figure 1

Platelets were collected from 24 blood donors on Day 0 and Day 3. Samples were prepared accordingly to the TAILS protocol[2]. Dimethylated peptides were enriched through negative selection step by reductive bounding of a synthetic polymer and N-termini of tryptic-generated peptides. TAILS analyses unraveled the N-termini of platelet proteins. Samples without polymer-mediated N- terminal enrichment (preTAILS) comprised the global proteome of platelets related to the blood donation.

References

1. Gebhard, S. et al.: Journal of hypertension 29;2011:2126–2137.
2. Kleifeld, O. et al.: Nature biotechnology 28;2010:281–288.

Conflict of interest: none

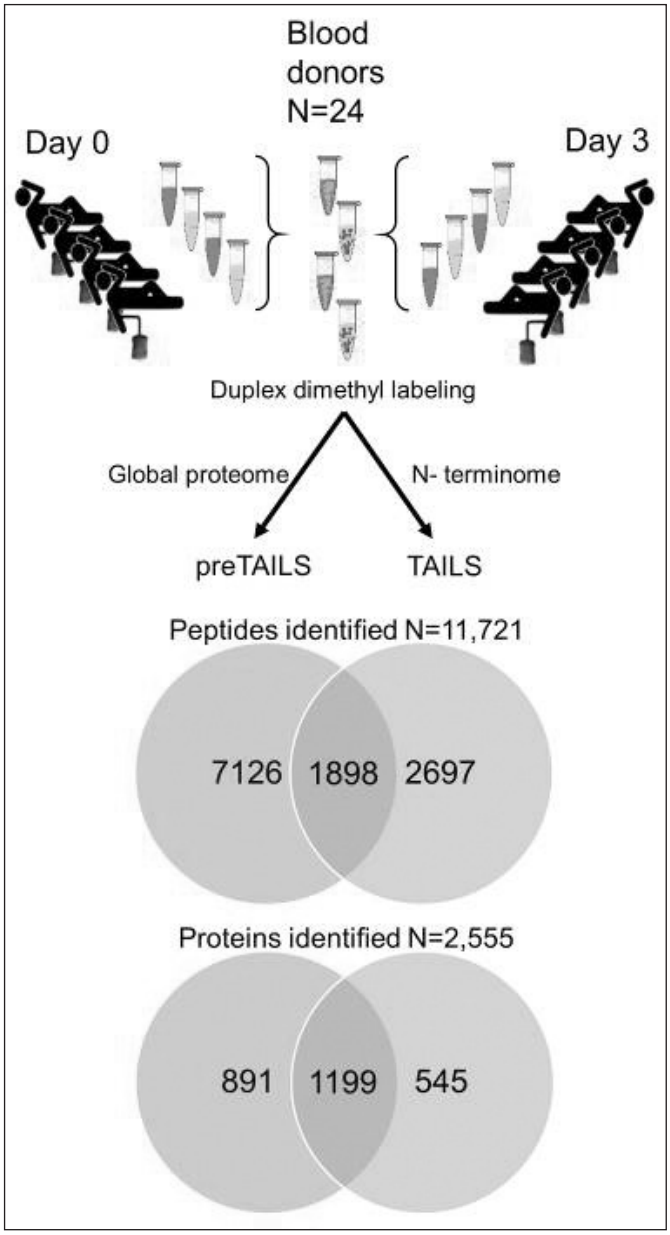

Fig. 1.

VS-10-3

Attitudes towards monetary and non-monetary incentives for blood donation in Germany – results of the Eurobarometer study

Weidmann C.[1], Klüter H.[2], Müller-Steinhardt M.[2]

[1]Hochschule Furtwangen, Angewandte Gesundheitswissenschaften, Furtwangen, Germany
[2]Institut für Transfusionsmedizin und Immunologie DRK-Blutspendedienst Baden-Württemberg - Hessen gGmbH Medizinische Fakultät Mannheim der Universität Heidelberg, Mannheim, Germany

Introduction: Incentives may help blood establishments to optimize recruitment and retention strategies in times of increasing demand. Although monetary and non-monetary incentives for blood donation have been discussed and criticised extensively in the past decades, empirical research on this topic remains limited. The aim of this study was to describe attitudes of German active donors, non-donors and inactive donors towards incentives for blood donation.

Methods: We present results of a secondary analysis of the German part of the Eurobarometer program (n = 1,532), which is a nationally representative survey in all member states of the European Union. In 2014, participants had to judge eight potential incentives for blood donation as acceptable or not. The incentives were refreshments (e.g. coffee), physical check-ups (e.g. blood pressure), free testing, free medical treatment, non-cash items (e.g. first aid kits), monetary travel reimbursements, cash amounts additionally to reimbursements, and time off work.
We describe the attitudes towards incentives among active donors, non-donors and inactive donors and chi-square statistics were used to test for significant differences. In a multivariate logistic regression model, we estimate the probability that participants judge travel reimbursements or cash amounts as acceptable to describe those participants that may be recruited by offering monetary incentives.
Results: Among the participants, 28.6% reported that they had donated in the past and would be prepared to donate in the future. Those donors were classified as active donors and were compared with participants that either had not donated (non-donors, 64.0%) or were not prepared to donate in the future (inactive donors, 7.4%).
Physical check-ups (51.9%), refreshments (50.0%) and free testing (38.3%) showed the highest acceptance by the participants. However, the acceptance was significantly higher among active donors than among non-donors or inactive donors. Travel reimbursements and free medical treatments were rated as acceptable by 28.6% and 25.3%, respectively. The lowest acceptance appeared for time off work (17.8%), non-cash items (13.9%) and cash amounts beyond reimbursement (11.8%). A positive judgment of travel reimbursements or cash amounts was found among men (OR 1.53), participants aged 25 to 39 years (OR 2.04), urban dwellers (OR 1.77), students (OR 2.36), and among participants that had problems paying their bills during the last year (OR 1.71).
Conclusion: The German participants prefer those incentives that are usually offered by blood establishments: refreshments, physical check-ups and und free testing. Only a minority, especially male students from urban areas, accept travel reimbursements or cash amounts as an incentive. Free medical treatment for blood donors may be an alternative to payments and further studies are needed to assess those treatments that are relevant for blood donors.

Conflict of interest: none

VS-10-4

Alloimmunization against HLA and HNA in female platelet apheresis donors – A single center experience

Nowak-Harnau S.[1], Althaus K.[1], Siebert S.[1], Lyshy F.[1], Enkel S.[1], Bakchoul T.[1,2]

[1]Center for Clinical Transfusion Medicine Tübingen, Tübingen, Germany
[2]Medical Faculty Tübingen, University Hospital Tübingen, Tübingen, Germany

Objective: Antibodies against human neutrophil (HNA) and leukocyte (HLA) antigens can lead to transfusion-related acute lung injury (TRALI). The frequency of alloimmunization against HNA and HLA was found to be very high in female donors. Antibody screening is a potential approach to avoid TRALI. However, excluding donors with positive results may impact the availability of apheresis platelets.
Methods: We investigated the frequency of HNA and HLA antibodies in female donors who were selected to donate apheresis platelets for HLA-immunized patients. Sera were tested using a flow-cytometry-based granulocyte immunofluorescence test (Flow-GIFT), granulocyte agglutination test and an ELISA test for HLA-class I and II antibody screening.
Results: 283 female donors (median 39 years, range 18–65) were included of whom 53/283 (19%) had positive results. 18 donors tested positive for HLA class I antibodies, 18 for HLA class II antibodies and sera from 9 donors reacted with both HLA class I and II antigens. In the GAT assay 8 female donors tested positive and sera from 5 female donors caused indeterminate results. Of note, 15/20 (5%) of positively tested female donors had no pregnancy (median age 26 years, range 24–31). In this group, 6 donors tested positive for HLA class I antibodies, 4 for HLA class II antibodies and one for both HLA class I and II antibodies. In addition, three nulliparous donors showed positive results in the GAT assay. In total, 58/283 (20%) of screened female donors had to be deferred from donating platelet apheresis because of the results of antibody screening. 19,217 apheresis platelet concentrates were transfused after the implementation of this screening strategy and no fatale TRALI has been reported.
Conclusion: Although screening of female donors for HNA and HLA antibodies seems to be a safe strategy to avoid TRALI after transfusion of apheresis platelet concentrates, indeterminate test results and the presence of naturally occurring antibodies with no clinical significance are still major practical limitations of this approach.

Conflict of interest: none

VS-10-5

Effects of DINCH® as an alternative plasticizer in blood bags on blood product quality

Aurich K.[1], Wesche J.[1], Gebicka P. D.[1], Westphal A.[1], Fuhrmann J.[1], Selleng K.[1], Greinacher A.[1], Füssl F.[2], Reichenberg S.[2], Fagoo G.[3], Chatellier S.[3]

[1]Universitätsmedizin Greifswald, Transfusionsmedizin, Greifswald, Germany
[2]Maco Pharma International GmbH, Langen, Germany
[3]Maco Pharma S.A.S., Mouvaux, France

Introduction: Currently used di(2-ethylhexyl)phthalate plasticizers (DEHP) in blood bags may leach into the blood product. A possible toxicity in transfused patients is debated. The objective of this study was to evaluate an alternative plasticizer, di(isononyl) cyclohexane-1,2-dicarboxylate (DINCH®) for its use in systems for whole blood (WB) processing and the resulting products: plasma, red blood cell concentrates (RBCs) and platelet concentrates (PCs). We evaluated the products characteristics for each plasticizer processed after 4 hours and 22 hours.
Methods: In total WB of 108 healthy volunteers was collected into DEHP and DINCH systems (Maco Pharma, France). Within 4 hours or 22 hours WB was centrifuged (4000 g, 10 min) and separated in plasma, buffy coat, and RBC. All plasma units per study arm (n = 16) were shock frozen in liquid nitrogen and stored for 14 days. Samples were taken after thawing at day 1 and 7 and analyzed for coagulation parameters, triglycerides, protein levels and micro particles. 4 Buffy coats were pooled in PCs (n = 4) in DEHP or DINCH systems by addition of SSP+ (Maco Pharma, France) in each study arm. PCs were stored for 8 days at room temperature under agitation. Samples were taken at days 1, 3, 6, and 8 and analyzed regarding metabolism and platelet function. After addition of additive solution PAGGS-M or SAG-M, RBCs (n = 16) in each study arm were leukoreduced and analyzed for in vitro characteristics of metabolism, hemolysis and microparticles during storage at 4 °C at days 1, 14, 35, 42, 49. Additionally 12 RBCs were irradiated at day 10 with 30 Gy and samples were taken at days 1, 10 (directly after irradiation) and 20.
Results: Whole blood processing was not impaired using DINCH based blood bag systems. Plasma coagulation factor levels, triglycerides and protein levels were comparable between plasticizers, but showed lower levels in units with long period WB storage before processing (e.g. factor VIII activity at day 1 after thawing 109.2 ± 17.4% DEHP 4 h vs 35.9 ± 22.7% DEHP 22 h, $p>0.05$). PCs showed equivalent outcomes regarding platelet count, metabolism, hypotonic shock response, aggregation and activation response for both plasticizers and different manufacturing times. RBC analysis resulted in comparable results regarding metabolism, RBC count, hematocrit, hemoglobin and micro particles. Hemolysis was increased in short term pre-fractionation stored DINCH based bags compared to DEHP bags but remained below the critical cut off of 0.8%, was no difference between additive solutions (figure 1). Irradiation increased hemolysis in DEHP bags at a similar extent as in DINCH bags (0.19 ± 0.09% DEHP; 0.39 ± 0.34% DEHP irradiated vs 0.37 ± 0.09 DINCH, 0.61 ± 0.31% DINCH irradiated, day 20).
Conclusions: Blood products obtained and processed in DINCH based and DEHP blood containers demonstrate comparable quality. DINCH

based blood containers are promising candidate substitutes for DEHP blood containers.

Conflict of interest: none

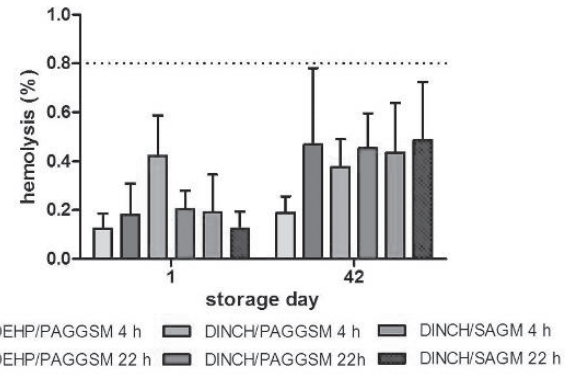

Figure 1. Hemolysis of RBC at storage days 1 and 42 in DEHP and DINCH based blood bags after whole blood processing after 4 or 22 hours.

Fig. 1.

VS-10-6

The plasticiser di-2-ethylhexylphthalate (DEHP) and red blood cells: a mechanism by which the DEHP can affect RBC shape

Melzak K. A.[1], Uhlig S.[2], Kirschhoefer F.[3], Brenner-Weiß G.[3], Bieback K.[1]

[1]Institute of Functional Interfaces, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germanydizin und Immunologie, Mannheim, Germany
[2]Institut für Transfusionsmedizin und Immunologie, Mannheim, Germany
[3]Institute of Functional Interfaces, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany, Karlsruhe, Germany

Introduction: The plasticiser di-2-ethylhexylphthalate (DEHP) is added to blood bags to keep the bags soft and flexible. The DEHP can leach out, and is known to accumulate in the red blood cell membrane over the course of the storage period. The DEHP improves the storage quality of the RBCs, decreasing the formation of vesicles and also the formation of echinocytes. The molecular mechanisms by which DEHP exerts these effects, are, however, not well characterised.
Methods: DEHP was suspended in aqueous buffer for addition to the RBCs, allowing us to look at the effects of the DEHP in isolation from other factors affecting cell morphology. Low numbers of RBCs were used to maintain a high ratio of DEHP to membrane lipid. The RBCs were observed on an adherent layer of poly(allylamine hydrochloride), using phase contrast microscopy. Control experiments showed that cells were stable on this surface over the period of observation. We were able to observe the cell shape changes caused by the DEHP, and were also able to observe dynamic processes that could be attributed to DEHP. The exposure of phosphatidylserine (PS) in the outer leaflet of the RBC membrane was assessed using annexin A5 in combination with flow cytometry. The increase in exposed PS was measured after incubation of RBCs with DEHP. The effect of DEHP on the PS exposure was also measured in the presence of vanadate, which inhibits the ATP-dependent active transport processes that maintain lipid asymmetry.
Results: DEHP increases the numbers of stomatocytes. The extent of this response to DEHP was shown to vary between donors. Flow cytometry assays showed that it was possible for the DEHP to affect the PS, on both the test samples and the positive controls and in the presence of vanadate. We also demonstrated that cells can lyse to produce ghosts without passing through an echinocyte stage, and that the ghosts can continue a slow stomatocyte formation; some cells were also seen to revert to smoother forms.
Conclusion: The formation of stomatocytes is associated with the expansion of the inner leaflet of the membrane relative to the outer leaflet. The possible mechanisms for this are selective extraction of lipids from the outer leaflet, selective insertion of the DEHP into the inner leaflet, activity as a synthetic scramblase (in which lipids from the inner and outer leaflets are mixed up), and via the translocase system that drives the lipid distribution. The first two possibilities listed are unlikely, due to the low solubility of DEHP in water and the lack of a positively charged group that could confer selectivity for the inner lipid leaflet; the DEHP does not appear to affect the translocase system, because the DEHP effects on PS were observed in the presence of vanadate and also on membrane ghosts. The stomatocyte formation therefore appears to be mediated by the action of DEHP as a synthetic scramblase, mixing up the lipids of the inner and outer membrane leaflets.

Conflict of interest: none

VS-10-7

Droplet digital PCR for quantification of residual leucocytes in red blood cell concentrates

Doescher A.[1], Müller T.[2]

[1]DRK Blutspendedienst NSTOB, F&E, Oldenburg, Germany
[2]DRK Blutspendedienst NSTOB, Springe, Germany

Background: Leucoreduction of blood products is controlled by the enumeration of residual white blood cells (rWBCs) to assure that the products meet national standards. Quality controls has to ensure, that residual white blood cells (rWBC) do not exceed < 1 x 106 cells per unit of red cells, platelets or plasma. In this context, flow-cytometric methods are accepted as gold standard with published sensitivities of approximately 0.1 WBC / µL. To overcome the limitation for storage of samples, PCR methods are an attractive alternative. We developed a digital droplet PCR (ddPCR) method as tool for determination of rWBCs in RBC units.
Material and Methods: Enumeration of rWBC by ddPCR was performed by amplification of *RHCE*-specific exon 3 and *SPEF1* sequences as internal control. Sensitivity of the assay was determined by dilution series. Routine samples from leucodepleted RBC (n = 150 units) were tested in parallel in flow-cytometry (LeucoCount) and ddPCR. In addition, after 7 days and 6 month of storage, part of the samples was tested again. For proof of reliability, samples from plasma and pooled platelet units were tested with ddPCR.
Results: Sensitivity of the new assay war comparable to flow-cytometry (0.4 WBC/µL). No discrepancies were observed by enumeration of rWBC using LeucoCount and ddPCR in parallel. DNA samples were stable up to six month without changes to initial rWBC count.
Measurement of residual WBC by ddPCR in samples from plasma and platelet concentrates provided also valid results in a pilot study.
Conclusion: Enumeration of rWBCs with ddPCR is an attractive alternative method for quality control of leuco-reduced blood products. Parameters like sensitivity, specificity and reproducibility are comparable to flow-cytometrie as gold standard. The great advantage of this new approach is the opportunity for long-time storage of samples and determination of rWBCs in a batch, for better comparison of results by bypassing inter-assay variations.

Conflict of interest: none

VS-11
Hämostaseologie II – Joint session GTH

VS-11-1

Plasma in the prophylaxis and treatment of coagulation disorders: Treating patients or laboratory values?

Rühl H.

University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Deutschland

Although transfusion of plasma in Germany has declined significantly since 2010, it remains one of the most frequently used blood component therapies. The most common indications for plasma are therapeutic use in the setting of acute dilutional or consumptive coagulopathy and prophylactic use in patients undergoing invasive procedures. Especially in the latter case, plasma transfusion therapy is often guided by abnormal laboratory values although there is a paucity of data regarding the efficacy of this approach. In addition to the efficacy of plasma transfusions, safety issues are also to be considered, as plasma transfusions are associated with the risk of adverse events including anaphylactic or allergic reactions, transfusion-related lung injury (TRALI) or transfusion-related immunomodulation. This talk provides an overview of the current evidence for plasma transfusion in therapeutic and prophylactic indications.

Conflict of interest: none

VS-11-2

The amount of the activated protein C (APC) response to in vivo thrombin formation contributes to the hypercoagulable state in factor V Leiden carriers

Rühl H., Winterhagen F. I., Berens C., Reda S., Müller J., Oldenburg J., Pötzsch B.

University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany

Background: Recently we have shown that thrombin generation and subsequent APC formation are increased in thrombosis-free (asymptomatic) carriers of the factor V Leiden mutation (FVL) after in vivo thrombin formation induced by recombinant activated factor VII (rFVIIa). Aim of the present study was to compare pro- and anticoagulant responses of symptomatic with asymptomatic FVL carriers after rFVIIa stressing.
Methods: The study population consisted of 12 heterozygous FVL carriers with a history of unprovoked venous thromboembolism (VTE+) and 13 without (VTE-), and a control group of 13 healthy non-FVL carriers. None of the subjects was under anticoagulant treatment. Blood samples were collected immediately before and during a period of 8 hours following injection of 15 µg/kg rFVIIa. Plasma levels of free thrombin and APC were quantified using oligonucleotide-based enzyme capture assays (OECAs). Prothrombin activation fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), and D-dimer were measured additionally. **Results:** Injections of rFVIIa were well-tolerated by all subjects and median D-dimer levels remained within the reference range in all three cohorts. Compared to baseline, plasma levels of F1+2 (median and interquartile range, IQR) increased significantly in all groups to peak levels 2 hours after rFVIIa injection, from 0.13 (0.09–0.21) to 0.18 (0.11–0.20) nmol/L ($p = 0.014$) in the controls, from 0.18 (0.13–0.21) to 0.24 (0.19–0.39) nmol/L ($p = 7 \times 10^{-4}$) in the VTE- group, and from 0.21 (0.13–0.30) to 0.24 (0.20–0.36) nmol/L ($p = 0.002$) in the VTE+ group. Median plasma concentrations of thrombin and TAT were below the respective limits of detection at baseline, and rFVIIa induced no statistically significant changes in all three cohorts. APC levels increased to peak levels 1 hour after rFVIIa injection, from 0.63 (0.54–1.16) to 2.94 (2.52–3.57) pmol/L ($p = 1.4 \times 10^{-4}$) in the controls, from 1.48 (1.23–2.05) to 7.98 (7.39–12.68) pmol/L ($p = 3 \times 10^{-5}$) in the VTE- group, and from 1.27 (1.00–1.52) to 5.28 (4.61–7.68) pmol/L ($p = 2.8 \times 10^{-5}$) in the VTE+ cohort. Compared with the controls, peak levels of APC were significantly higher in VTE- ($p = 3 \times 10^{-5}$) and VTE+ ($p = 6 \times 10^{-4}$). However, they were lower in VTE+ compared with VTE- ($p = 0.008$), while peak levels of F1+2 and TAT (2.95, 2.46–3.65 ng/mL in VTE-, 3.32, 2.59–4.10 ng/mL in VTE+ at 2 hours) did not differ significantly between these two cohorts.
Conclusion: The APC response to in vivo thrombin formation is significantly lower in symptomatic than in thrombosis-free FVL carriers. This implies that higher APC levels protect FVL carriers from thrombosis development. Further studies are warranted to identify the factors that modulate the APC response in FVL carriers.

Conflict of interest: none

VS-12
Infektionssicherheit II

VS-12-1

Efficient inactivation of transfusion-relevant bacteria species in platelet concentrates by UVC treatment

Gravemann U.[1], Tolksdorf F.[2], Handke W.[1], Müller T. H.[3], Seltsam A.[1]

[1]DRK-Blutspendedienst NSTOB, Forschung und Entwicklung, Springe, Germany
[2]Macopharma, Langen, Germany
[3]DRK-Blutspendedienst NSTOB, Springe, Germany

Introduction: The THERAFLEX UV-Platelets system (Macopharma) is a pathogen inactivation system for platelet concentrates (PCs) that uses UVC light only (wavelength: 254 nm) without the need of any additional photoactive compound. Inactivation efficiency has been shown for a broad range of viruses, bacteria, and protozoans. In previous studies with the first set of bacteria species of the WHO International Repository of Platelet Transfusion Relevant Bacteria Reference Strains a high inactivation capacity for clinically relevant bacteria was shown. Aim of the current study was to investigate the bacteria inactivation efficacy of the THERAFLEX UV-Platelets system for a number of strains which have recently been added to the WHO International Repository.
Methods: PCs were produced from buffy coats using the additive solution SSP+ (Macopharma) with a residual plasma content of 35%. For inactivation kinetics, PCs (n = 3) were spiked with bacteria (Enterobacter cloacae, Morganella morganii, Proteus mirabilis Pseudomonas fluorescens, Serratia marcescens, Staphylococcus aureus or Streptococcus bovis) to a final concentration of approx.10^6 colony forming units (CFU)/mL and irradiated with increasing doses until the standard UVC dose was achieved. Samples were taken for bacterial titer determination after each irradiation step. In order to show sterility after UVC treatment, two PCs were pooled and inoculated with bacteria to a final concentration of approximately 0.3 CFU/mL. Bacteria were allowed to grow for 6 h in the PCs at 22 ± 2 °C under agitation. After splitting, one PC remained untreated (growth control) while the other one was UVC-treated. After storage for seven days, samples were taken from both bags for sterility testing by BacTALERT (Biomerieux) and for determination of the bacterial titer in the untreated control units.
Results: Dose-dependent inactivation of bacteria in PCs by treatment using the THERAFLEX UV-Platelets system was shown. Mean $\log_{10}$ reduction factors ranged from 6 to 7 for seven different investigated bacteria species.
Spiked PCs (n = 12 for each bacteria species) were efficiently sterilized by UVC treatment (12 /12 tested). PCs treated by the THERAFLEX UV-Platelet system remained sterile during storage for 7 days, while bacteria grew to high titers of 10^6–10^9 CFU/mL in non-treated PCs.
Conclusions: The THERAFLEX UV-Platelets system efficiently inactivates a broad range of different bacteria species, including the WHO reference strains. PCs remained sterile over a storage period of 7 days. These

results suggest that the UVC-based pathogen inactivation technology can significantly improve the bacterial safety of platelet transfusions.

Conflict of interest: Ute Gravemann, Wiebke Handke, Thomas H. Müller and Axel Seltsam received project grants from the «Forschungsgemeinschaft der DRK-Blutspendedienste e.V." and work together with MacoPharma on the development of pathogen inactivation systems for blood products.

VS-12-2

The non-enveloped feline calicivirus, a model virus for hepatitis E virus, is efficiently inactivated by the UVC treatment

Gravemann U.[1], Handke W.[1], Tolksdorf F.[2], Müller T. H.[3], Seltsam A.[1]

[1]DRK-Blutspendedienst NSTOB, Forschung und Entwicklung, Springe, Germany
[2]Macopharma, Langen, Germany
[3]DRK-Blutspendedienst NSTOB, Springe, Germany

Introduction: Non-enveloped transfusion transmissible viruses such as hepatitis A virus (HAV) and hepatitis E virus (HEV) are resistant to many of the common virus inactivation procedures for blood products. It was shown in the past that HAV is inactivated (>4 logs) by the THERAFLEX UV-Platelets system (MacoPharma). For HEV, a reliable in-vitro infectivity system has not yet been established. In this study, we used the feline calicivirus (FCV), a non-enveloped single-stranded RNA virus, as model for inactivation of non-enveloped viruses, in order to assess the inactivation capacity of the UVC-based THERAFLEX UV-Platelets pathogen inactivation system for HEV-like viruses in platelet concentrates (PCs).
Methods: Plasma reduced PCs from buffy coats (35% plasma in additive solution SSP+, Macopharma) were spiked with virus suspension (10% v/v). PCs (n = 6, 350 mL) were then UVC-irradiated on the Macotronic UV machine (Macopharma) and samples were taken after spiking (load and hold sample) and after illumination with different light doses (0.05, 0.1, 0.15 and 0.2 (standard) J/cm^2)). The titre of FCV (strain FCV-2280, ATCC VR-2057) was determined as tissue culture infective dose ($TCID_{50}$) by endpoint titration in microtitre plate assays on feline kidney cell line CRFK (ATCC CCL-94).
Results: FCV was dose-dependently inactivated by the THERAFLEX-UV Platelets system. A titer of 5.5 ± 0.5 $\log_{10}$ $TCID_{50}$/mL was achieved in the PCs after spiking. At a UVC dose of 0.2 J/cm^2 the virus titer was reduced to 2.5 ± 0.5 $\log_{10}$ $TCID_{50}$/mL, resulting in a $\log_{10}$ reduction factor of 3.0 ± 0.2.
Conclusions: Treatment with the THERAFLEX UV-Platelets system is effective against FCV in PCs. In combination with previous data on inactivation of HAV, these results suggest a broad effectivity of this system against non-enveloped viruses.

Conflict of interest: Ute Gravemann, Wiebke Handke, Thomas H. Müller and Axel Seltsam received project grants from the «Forschungsgemeinschaft der DRK-Blutspendedienste e.V." and work together with MacoPharma on the development of pathogen inactivation systems for blood products.

VS-12-3

Pathogen Inactivation (PI) of plasma reduces factor I (Fibrinogen) and Factor VIII content in comparison to untreated plasma but did not impair clot formation and thrombin generation

Amato M.[1], Streif W.[2], Reif C.[2], Nussbaumer W.[1]

[1]Universitätsklink Innsbruck, Department für Transfusionsmedizin, Innsbruck, Austria
[2]Universitätsklink Innsbruck, Department für Kinder und Jugendheilkunde, Innsbruck, Austria

Background: Different techniques of pathogen inactivation(PI) are available for plasma. While increasing safety on one hand, additional manipulation followed by a delayed freezing of plasma, for some technologies combined with a pooling process of single units, may caus a loss of labile coagulation factors on the other hand. To understand, if PI may reduce therapeutic efficacy of frozen plasma, we compared the influence of PI techniques on factor I and factor VIII, clotting time, clot stability after 30 minutes (A30) and endogen thrombin potential (ETP) in comparison to untreated plasma.
Study design: We produced 6 pools of 5 plasma units each and split each pool into three arms: Control group (C), Methylene Blue treated group (MB) and Psoralen (S-59) treated group (P). All samples (treated and untreated) have been frozen after treatment. After one month at -40°C freezing, all samples were thawed and tested for factor I, factor VIII, clotting time, clot stability after 30 minutes and endogenous thrombin potential (ETP).
Results: Comparing C with PI treated groups, fibrinogen content in mg/dl was significantly lower in both PI treated arms (mean ± SD) 296 ± 19 *vs* 263 ± 15 *vs* 251 ± 13, C *vs* P *vs* MB, respectively, but within PI treated groups fibrinogen content of P group was significantly higher compared to MB group ($p< 0.05$). The same can be reported for factor VIII content in% between C and PI treated groups (84 ± 19 *vs* 58 ± 10 *vs* 67 ± 11), C *vs* P *vs* MB, respectively but comparing both PI treated groups MB showed significantly higher factor VIII levels ($p< 0,05$). Clotting time in sec (mean±SD) was not significantly different between all three groups (51 ± 3,27 vs 51,5 ± 3,86 vs 51,33 ± 1,89, C vs P vs MB), whereas treated groups show a significantly ($p< 0.05$) higher A30 (22,33 ± 1,25 vs 24 ± 1,00 vs 27 ± 1,63, C vs P vs MB). ETP in nmol/min was comparable and not significantly different between all groups (1336 ± 109,33 vs 1489,97 ± 432,57 vs 1355,83 ± 123,79, C vs P vs MB).
Conclusion: Additional treatment of plasma before freezing, usually done for increasing safety with pathogen inactivation technologies, causes not surprisingly a loss of coagulation factors compared with untreated plasma. As all groups consists of the same source material, results are comparable without individual variability of single coagulation factors. In contrast, missing significant differences for clotting time and ETP combined with a higher clotting amplitude after 30 minutes(A30) for inactivated plasma demonstrates that remaining coagulability of those plasma units is sufficient for clinical use and not significantly impaired compared with untreated plasma units.

Conflict of interest: none

VS-12-4

Occult hepatitis B virus infection in blood donors missed by standard hepatitis B screening

Ries J.[1], Frey B. M.[1], Gottschalk J.[2], Goslings D.[3], Glauser A.[4], Zbinden A.[5], Böni J.[5], Redli P.[5], Huzly D.[6], Valek A.[2], Hardegger K.[2]

[1]Zürcher Blutspende, Medizinischer Dienst, Zürich-Schlieren, Switzerland
[2]Zürcher Blutspende, Screening Labor, Zürich, Switzerland
[3]Zürcher Blutspende, Produktion, Zürich, Switzerland
[4]Zürcher Blutspende, Qualitätsmanagement, Zürich, Switzerland
[5]Universitiy Zurich, Medical Virology, Zurich, Switzerland
[6]Universitiy Hospital, Institute for Virology, Freiburg, Germany

Introduction: Screening of blood donations for the presence of HBsAg and HBV-DNA is current standard of technique and is used for selection of HBV negative blood donations. However, improvements of HBV-nucleic acid amplification testing (NAT) techniques (mini-pool (MP) versus individual donation (ID)) revealed donors with trace HBV viremia which were not recognized by previous screening protocols. Regularly, donors with trace viremia show antibodies to the hepatitis B core antigen (anti-HBc) suggesting an occult hepatitis B infection (OBI).
The aim of this quality assurance was to quantify donors with an occult hepatitis B infection in the blood donor collective and thereby assess the risk of releasing potentially infectious blood products with extremely low levels of HBV DNA.
Methods: During a one month period, all whole blood, platelet and plasma donors (new and multiple donors) were examined for the presence of anti-HBc and HBV surface antibodies (anti-HBs, Architect®, Abbott) in addition to standard HBV screening (HBV-ID-NAT, cobas® MPX, Roche

Diagnostics) and HBsAg EIA (Architect®, Abbott). Anti-HBc positive findings were confirmed by anti-HBc neutralisation test using recombinant HBcAg (Architect®, Abbott. J Clin Virol. 2011 Aug;51(4):283–4). Confirmed anti-HBc positive samples underwent ultracentrifugation (UC) and subsequent testing of HBV-ID-NAT to search for traces of HBV. Archived donor»s samples of confirmed anti-HBc positive donations were reassessed by HBV-ID-NAT.

Results: Of the 4923 donors tested, 78 (1.6%) were confirmed positive for anti-HBc. For 69 of these donors we had a fresh frozen plasma (FFP) unit for UC. **4/69** FFP units were HBV-PCR positive upon UC. **62/78** anti-HBc positive donors were reassessed using their archived samples. One donation previously declared HBV negative by MP-NAT turned out to be HBV-DNA positive by ID-NAT. Therefore, among the 78 confirmed anti-HBc positive donors, **5** donors with OBI were detected. This corresponds to 6.4% OBI donors among individuals with confirmed positive anti-HBc test. The anti-HBs titers of OBI donors varied between 80 and 1000 IU/l and were not different from non-viremic anti-HBc positive individuals.

Conclusions: The rate of donors who have ever had a HBV infection is low, making up 1.6% of the blood donors in the Zurich area. The number of donors with an occult HBV infection among anti-HBc confirmed positive donors is unexpectedly high at 6.4%. Improved screening protocols to efficiently exclude OBI donors from blood donation seem advisable, since transfusion-transmitted HBV infections by donors with OBI have been described (Lieshout-Krikke et al.: 2015, Transfusion).

Conflict of interest: Die zusätzlichen Screeninguntersuchungen (Testkits) auf Anti-HBc und Anti-HBs des Spenderkollektives wurden gesponsert von Abbott®.

VS-12-5

Prevalence of antibodies against hepatitis D virus (HDV) in blood donors

Juhl D., Thiessen U., Quandt T., Görg S., Hennig H.

Universitätsklinik Schleswig-Holstein, Transfusionsmedizin, Lübeck, Germany

Introduction: Infections with HDV occur only as co- or superinfection in line with a Hepatitis B virus (HBV) infection: successful HDV infection depends on the presence of HBV because HBsAg is an integral part of the surface of HDV and essential for its host cell entry. As HBV and HDV share transmission routes, HDV is probably also transmissible by transfusion. Data about the prevalence of HBV/HDV co-infections among blood donors in developed countries are lacking.

Methods: All blood donations of our institute, taken between the 1st January 2013 and the 31st December 2017, were tested for anti-HBc by Chemiluminescent microparticle immunoassay (CMIA, Abbott ARCHITECT, Abbott GmbH, Wiesbaden, Germany). Samples that tested repeteadly reactive in the CMIA and reactive in a second anti-HBc assay (VIDAS anti-HBc, Biomerieux, Marcy-l»Etoile, France or cobas 8000, Roche Diagnostics, Mannheim, Germany) were considered anti-HBc «true positive» (or «anti-HBc specificity confirmed»). To obtain data about the rate of donors with a past HDV infection, samples from donors with anti-HBc true positive results were tested for antibodies against HDV (anti-HDV) by a competitive ELISA (ETI-AB-DELTAK-2, DiaSorin, Sabeggia, Italy). Anti-HDV reactive results were confirmed to be reactive by repeat testing.

Results: 44,184 donors were tested for anti-HBc within the study period. 167 (0.38%) of these donors were anti-HBc true positive and 224 samples have been taken from them. In nine out of these 167 donors, additional HBsAg and HBV DNA were detectable. Of the 167 anti-HBc true positive donors, four could not be tested for anti-HDV due to lack of material. Two (1.2%) donors tested repeatedly reactive for anti-HDV. In one of these donors, the result could be confirmed by invest igation of a follow-up sample. In another five (3.0%) donors, an indeterminate result close to the cut-off of the assay was observed. No HBsAg and/or HBV DNA were detectable in these seven donors.

Conclusion: By investigation of anti-HDV, a low prevalence (1.2% of anti-HBc true positive donors, 0.005% of all donors) of HDV in blood donors was determined. However, antibodies against HDV are considered to be detectable only for a relative short period of a few years to months after infection was resolved. Thus, the rate of blood donors with a remote HBV/HDV infection is probably underestimated by anti-HDV testing. Also the results of five donors, in whom HDV-antibody testing yielded an indeterminate result, were possibly caused by a HBV/HDV infection, which was resolved a longer time ago. Consequent rejection of blood donors with all kinds of ongoing HBV infection also is appropriate to prevent transfusion transmitted HDV infections.

Conflict of interest: none

VS-13
Transplantationsimmunologie und Immungenetik – Joint session DGI

VS-13-1

Significance and future of HLA matching in solid organ transplantation

Lachmann N.

Charité-Universitätsmedizin Berlin, Inst. f. Transfusionsmedizin, Gewebetypisierung, Berlin, Deutschland

The significance of the human leukocyte antigens (HLA) as the major histocompatibility complex in humans has been established since the first kidney transplants have been performed between HLA non-identical individuals. The analyses of large kidney transplant register data revealed a direct correlation between HLA compatibility and increased transplant survival probability, decreased incidence of rejections and immunosuppression-associated comorbidities. Thus, HLA matching of kidney transplants and recipients at the time of transplantation is mandatory to prevent rejections due to preformed or de novo immunization against HLA. However, complete matching of HLA is not feasible in the context of solid organ transplantation due to the extraordinary polymorphism of the HLA genes and encoded proteins. This led to the establishment of the concept of HLA epitope matching over the last years as an alternative to the classical antigen matching to better predict histocompatibility. HLA epitope matching allows the objective quantification of immunogenic amino acid mismatches as the trigger of alloimmunization following organ transplantation. Thus, the new concept of HLA epitope matching opens up promising perspectives for the future of solid organ transplantation.

Conflict of interest: none

VS-13-2

Diagnostic applications of next generation sequencing in immunogenetics and transfusion medicine

Hirv K.

Zentrum für Humangenetik und Laboratoriumsdiagnostik, Martinsried, Deutschland

With the introduction of Next Generation Sequencing (NGS) technologies, extraordinary progress has been made in diagnostic applications in the field of human genetics. In the last ten years, improvements in accuracy, robustness and handling, combined with simultaneous reduction of DNA sequencing costs per base, have led to NGS becoming a widely used alternative to Sanger sequencing. The most relevant advantages of the technology are (i) the increased number of sequenced bases per run, (ii) parallel sequencing of different target regions, (iii) clonal sequencing of single DNA molecules, and (iv) massively parallel sequencing of hundreds of samples using sample specific indexing. Enrichment of genomic regions of interest is necessary for optimal use of sequencing capacities,

with amplicon-based and hybridization-based methods used. PCR amplification-based methods can be divided into short and long-range amplicon approaches. In the end, the method of choice is dependent on the number of genes/exons of interest, the expected read length, economic needs and other factors.
NGS is already a widely used method for HLA typing in the stem cell transplantation setting, providing unambiguous allelic resolution typing results for patients and their potential donors. In this case, HLA genes are amplified by long-range PCR. After fragmentation and index/adaptor ligation, paired-end sequencing of 2 × 150 bp reads is sufficient to obtain HLA haplotype information and results at an allelic level. Another common application is high throughput sequencing of registry donors. A short amplicon strategy is mainly used to simplify the workflow, increase the throughput and reduce costs. By using sequencing platforms with high data output, thousands of samples can by analyzed in every single sequencing run. Similar workflows might be feasible for blood group genotyping, to characterize blood donors and screen for donors with rare phenotypes.
In the field of immunogenetics, NGS is a valuable tool in the diagnostics of immunodeficiencies, autoinflammatory disorders and other rare diseases. As mutations in various genes may lead to similar clinical phenotypes, the multi-gene panel approach has advantages in comparison to SNP detection or single gene sequencing. Hybridization based target enrichment is preferentially used for target enrichment. Depending on the level of complexity, the number of targeted genes may vary from few genes (periodic fever syndromes) to one hundred or more genes (immunodeficiencies). Diagnostic pathways including standardized sequencing of a high number of genes can improve patients» quality of life by providing an early and accurate diagnosis. Furthermore, this strategy has proved to be economically beneficial, especially when compared with Sanger sequencing.

Conflict of interest: none

VS-13-3

HLA epitope matching of kidney transplants predicts antibody- and cell-mediated rejection

Lachmann N.[1], Staeck O.[2], Niemann M.[3], Budde K.[2], Spierings E.[4], Schönemann C.[1], Pruß A.[5]

[1]Charité-Universitätsmedizin Berlin, Inst. f. Transfusionsmedizin, Gewebetypisierung, Berlin, Germany
[2]Charité-Universitätsmedizin Berlin, Innere Medizin m.S. Nephrologie, Berlin, Germany
[3]PIRCHE AG, Berlin, Germany
[4]UMC Utrecht, Laboratory of Translational Immunology, Utrecht, Netherlands
[5]Charité-Universitätsmedizin Berlin, Inst. f. Transfusionsmedizin, Gewebebank, Berlin, Germany

Our previous study has demonstrated that epitope matching as performed by both the HLAMatchmaker and the Predicted Indirectly Recognizable HLA Epitope (PIRCHE) algorithm is an independent predictor for *de novo* donor-specific HLA antibodies (DSA). Here, we analyzed the correlation between HLA epitope matching and allograft rejection following kidney transplantation.
A total of 1,083 consecutive deceased and living kidney transplants performed between 1995 and 2015 were enrolled to the study cohort. All patients revealed having no DSA prior to transplantation as detected by solid-phase immunoassays. HLA epitope mismatches were determined by both the HLAMatchmaker and PIRCHE approach. Rejections were diagnosed according to Banff criteria.
During follow-up 63 (6%) patients developed antibody-mediated rejection (ABMR), which translates into an overall projected incidence of ABMR at 10-years post-transplant of 8%. T-cell-mediated rejection (TCMR) was observed for 226 (21%) patients during follow-up, which translates into an overall projected incidence of TCMR at 10-years post-transplant of 23%. There was a direct correlation between the degree of HLA epitope matching and the incidence of ABMR and TCMR. At 10 years of follow-up, patients with a HLAMatchmaker score <5 (n = 123), ≥5 to <18 (n = 173), ≥18 to <36 (n = 469) and ≥36 (n = 318) revealed a predicted incidence of ABMR and TCMR of 1% and 8%, 5% and 14%, 8% and 25% as well as 15% and 29%, respectively. Patients with a PIRCHE-II score <9 (n = 107), ≥9 to <35 (n = 149), ≥35 to <90 (n = 504) and ≥90 (n = 323) had a predicted incidence of ABMR and TCMR of 1% and 7%, 3% and 16%, 9% and 25% as well as 14% and 28%, respectively. In a multivariate analysis adjusted for HLA antigen matching, recipient and donor age the PIRCHE-II score was predictive for ABMR (HR 1.57 (95% CI 1.0–2.5), p = 0.05) but not TCMR (p = 0.1).
These findings confirm the predictive value of HLA epitope matching for renal allograft rejection.

Conflict of interest: Koautor MN arbeitet für Fa. PIRCHE AG, die Epitopmatching als Webservice anbieten.

VS-13-4

307 novel variants of HLA genes detected in German stem cell donor registry (DUS) and UCLA International Cell Exchange samples

Fischer J. C., Balz V., Enczmann J.

Medizinische Fakultät, Heinrich-Heine-Universität, Institut für Transplantationsdiagnostik und Zelltherapeutika, Düsseldorf, Germany

Introduction: High-throughput analysis of the HLA class I genes HLA-A, HLA-B, and HLA-C as well as the HLA class II genes HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DQB1, and HLA-DPB1 in samples of registered stem cell donors of the German Stem Cell Donor Registry Duesseldorf revealed 303 novel variants. In addition, 4 new variants were identified in well-defined samples obtained from the UCLA International Cell Exchange program. New alleles included 202 HLA class I (55 HLA-A, 69 HLA-B, 78 HLA-C), and 105 HLA class II (52 HLA-DPB1, 24 HLA-DQB1, 25 HLA-DRB1, 1 HLA-DRB3, 3 HLA-DRB4) variant alleles.
Methods: The variations were detected by amplicon-based NGS analysis of exon 2, exon 3 and exon 4 (HLA class I and HLA-DPB1) or exon2 and exon 3 (HLA-DQB1, HLA-DRB1, HLA-DRB3, and HLA-DRB4) including parts of the adjacent introns on genomic DNA. All variants of HLA class I genes were confirmed by NGS of full length genomic DNA including the respective 5′ and 3′ untranslated regions as well as by Sanger sequence analysis. Variants of HLA class II genes were either confirmed by repeated analysis or NGS of full length genomic DNA.
Results: We identified 222 intronic variants, non-synonymous nucleotide changes in 55 alleles, synonymous nucleotide changes in 27 alleles, 2 stop-mutations and 7 hybrid-alleles. 10 alleles carry exonic deletions or insertions resulting in frameshift of peptide translation. Four HLA class I alleles showed exonic as well as intronic changes. Several new alleles were found in multiple samples. Seven of the new alleles carry single nucleotide alterations in introns 1, 2, or 3 with changes potentially altering exon splicing. In case of four of these alleles we were able to perform mRNA analyses which revealed a splicing defect occurring from nucleotide changes 1 to 5 bases apart from the exon boundary.
Conclusion: Using amplicon based NGS analyses we were able to identify novel alleles of HLA class I and HLA class II genes. The majority of these new alleles carry single nucleotide alterations in introns 1, 2, 3, or 4. The verification of splicing defects as a result of nucleotide changes adjacent to the exon-intron-transition emphasizes the need to have a closer look at the intron sequences conterminous to the exons. In addition, exon 3 of HLA-DPB1, HLA-DRB1, HLA-DQB1 as well as exon 4 of HLA-DPB1 and HLA class I genes which are rarely characterized for screening purposes are frequently altered in this set of new alleles.

Conflict of interest: none

VS-14
Hämotherapie (Neighbour Day)

VS-14-1

Challenges and concepts for hemotherapy in bleeding emergencies

Selleng K.

Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Transfusionsmedizin, Greifswald, Deutschland

Bleeding emergencies can occur 1) during in-hospital treatment or 2) outside of the hospital. In the first case the patients´ constitution, laboratory results, blood type, red cell alloantibodies, bleeding cause are usually known and the bleeding situation is monitored and treated from the beginning. The second scenario is often more unclear. Blood loss, blood type, bleeding and transfusion history, intake of antiplatelet drugs or anticoagulants are unknown. The clinical situation seems to be life threatening and immediate treatment with different blood components is necessary. To improve the outcome of these patients several studies were performed and hemotherapy concepts further developed. Red blood cells of blood group O Rhesus D negative (RhD-) are still used in many centers as «universal» blood for transfusion of patients with unknown blood type to avoid anti-D alloimmunization. But in 80–85% of all these emergencies patients are Rhesus D positive (RhD+). This leads to unnecessary transfusion of RhD- blood to RhD+ recipients and increases the shortages of RhD- blood for RhD- patients. A survey in university hospitals in Germany showed that already 4–7% of RhD- patients has to be transfused with RhD+ blood because of RhD- blood shortages. Providing O RhD+ blood for emergency transfusions of patients with unknown blood type can safe up to 10% of RhD- red cell concentrate demand. It was shown that this concept results in an anti-D alloimmunization rate of 3–5% in this emergency transfusion population (Selleng K., Lancet Haematol 2017), which is not higher than the alloimmunization rate against other red cell antigens, but lower than the anti-D alloimmunization rate (up to 30%) after transfusion of a known RhD- patient.

Trauma studies indicated that early plasma transfusion with a transfusion ratio plasma/packed red cells of higher than 0.5 decreases mortality independently from coagulopathy (Brown LM., J Trauma 2011). Providing plasma within 8–10 min after request is challenging because of its frozen storage. Early plasma transfusion can only be realized by short term storage of thawed plasma. Stability of single clotting factors and global hemostasis were validated for up to 7 days and showed acceptable results to establish a liquid plasma bank. Storage of thawed plasma could lead to a higher wastage of plasma especially in smaller hospitals. Therefore the STAT study (Dunbar N. Transfusion 2017) evaluated the concept of issuing blood type A plasma for the beginning of transfusion in bleeding emergencies until freshly thawed blood group identical plasma is available. In patients receiving blood type incompatible plasma transfusions no clinical relevant transfusion reactions or hemolysis were observed.

Hemotherapy concepts to manage bleeding emergencies are in progress. Hospitals should reevaluate their standard procedures to improve resuscitation outcomes of bleeding emergencies and to optimize the use of blood as a limited resource.

References:

1. Selleng K, Jenichen G, Denker K, Selleng S, Müllejans B, Greinacher A. Emergency transfusion of patients with unknown blood type with blood group O Rhesus D positive red blood cell concentrates: a prospective, single-centre, observational study. Lancet Haematol. 2017;4(5):e218-e224.
2. Brown LM, Aro SO, Cohen MJ, Holcomb JB, Wade CE, Brasel KJ, et al.: A high fresh frozen plasma: packed red blood cell transfusion ratio decreases mortality in all massively transfused trauma patients regardless of admission international normalized ratio. JTrauma. 2011;71(2 Suppl 3):S358-S363.
3. Thiele T, Kellner S, Hron G, Wasner C, Nauck M, Zimmermann K, et al.: Storage of thawed plasma for a liquid plasma bank: impact of temperature and methylene blue pathogen inactivation. Transfusion. 2012;52(3):529–536.
4. Dunbar NM, Yazer MH, Biomedical Excellence for Safer Transfusion C, the SSI. Safety of the use of group A plasma in trauma: the STAT study. Transfusion. 2017;57(8):1879–1884.

Conflict of interest: Ohne Bezug zum vorliegenden Abstract erhielt K. Selleng Vortragshonorare und Reisekostenunterstützungen der Firma Aspen, Beraterhonorare und Reiskostenunterstützung durch die Firma Janssen Cilag, Forschungsunterstützung von Johnson & Johnson, Fortbildungsfinanzierung durch Novo Nordisk und Behring.

VS-14-2

Eculizumab (Anti-C5) in acute hemolytic transfusion reaction

Gathof B. S.

Uniklinik Köln (AöR), Zentrale Dienstleistungseinrichtung für Transfusionsmedizin/Blutspendezentrale, Köln, Deutschland

Patient misidentification with the consequence of erroneous blood transfusion is still the most prevalent preventable hazard in blood transfusion. In the latest German hemovigilance report for 2015 more than 20 cases have been reported to the PEI (1). Eculizumab, a C5- Inhibitor, has been successfully used for the prevention of serious hemolysis in case of incompatible transfusion (2).

Due to the potential side effects (loss of protection against meningitis due to complement inhibition) indication of Eculizumab is strictly limited to PNH and under strict surveillance. If it is used to prevent hemolysis in incompatible transfusionto prevent hemolysis, Eculizumab needs to be started within minutes to few hours as an «off label use» in an «individual healing attempt».

Several transfusion specialists have discussed ways to facilitate this life saving emergency measure. One attempt is to create SOPs for this «off label use» for centers were Eclulizumab is stored for its licensed use such as PNH. Due to regulatory restrictions the idea of a network with centers with an emergency supply of Eclulizumab could not be implemented up to now.

Several years after the «proof of principle», first notification of the use of Eculizumab in an erroneous transfusion (2) it is unknown how many patients have been treated with this potential life saving «individual healing attempt» and also their clinical course.

Despite elaborate quality management systems with various safety measures, erroneous transfusion is still not preventable in every case. Rapid availability of Eculizumab, which has been proven to prevent hemolysis not only in PNH but also in cases of incompatible transfusion, may help to save lives. Scientific societies, competent authorities and pharmaceutical industry should collaboratively find a solution for this question.

Literature

1. *https://www.pei.de/DE/arzneimittelsicherheit-vigilanz/haemovigilanz/haemovigilanzberichte/teaser-bericht-2015.html*
2. Weinstock C, Möhle R, Dorn C, Weisel K, Höchsmann B, Schrezenmeier H, Kanz L. Successful use of eculizumab for treatment of an acute hemolytic reaction after ABO-incompatible red blood cell transfusion. Transfusion. 2015 Mar;55(3):605–10

Conflict of interest: none

VS-14-3

Hemostatic resuscitation of bleeding – Norway

Hervig T.[1,2], Apelseth T.[3], Strandenes G.[2,3]

[1]University of Bergen, Dept. of Clinical Science, Bergen, Norwegen
[2]The Norwegian Armed Forces Joint Medical Services, Sessvollmoen, Norwegen
[3]Haukeland University Hospital, Dept. of Immunology and Transfusion Medicine, Bergen, Norwegen

Introduction: Hemostatic resuscitation of bleeding has changed dramatically in Norway during the last decade. Earlier, saline and colloid solutions were widely used – but now early treatment with blood components is considered essential. Whole blood, blood components, lyophilized plasma

and some blood products are used. However, there is no uniform agreement on the optimal treatment of bleeding yet.
Norway has an extensive national air ambulance system with different bases covering the whole country. Although this is a national service, the supply of blood components is dependent of the local hospital located near the base. As the transfusion policies vary, also the availability of blood components to the air ambulances vary. Norwegian freeze-dried plasma does not exist. Accordingly, German Lyoplas is used.
There is a national plan for trauma care in Norway. All hospitals taking care of trauma patients are obliged to have a massive transfusion protocol. This protocol does however not have to include platelets.
The Norwegian military does not have any blood bank and the transfusion system is not extensive, except for Special Forces. The military is therefore completely dependent of collaboration with civilian blood banks. There is not ongoing work to optimize this collaboration.
To improve the hemostatic resuscitation of bleeding, several research projects are ongoing. These include cold-stored platelets, donor performance studies, quality of whole blood during storage, pathogen reduction of whole blood and clinical evaluation of prehospital transfusion practice. Also, a new component as lyophilized platelets is in a clinical trial. In addition, there are plans for production of lyophilized plasma and cryopreserved platelets.
Methods: The prehospital and hospital transfusions policies are reviewed. The revised military transfusion policy is presented. Relevant research papers and information on ongoing projects are reviewed.
Results: Red cell concentrates and lyophilized plasma are available in the air ambulances. Whole blood is available at two air ambulance bases and at two civilian hospitals. In all civilian trauma hospitals, early access to blood components is required, although platelet concentrates are not always available. There is no uniform policy for use of tranexamic acid and fibrinogen concentrate. Whole blood is considered in many hospitals. In military settings, whole blood is the preferred blood component.
Conclusion: Hemostatic resuscitation of bleeding in Norway is now based on early access to blood components. The use of whole blood is increasing – but still controversial in civilian practice.

Conflict of interest: none

VS-14-4

Demography in Germany is a cause of the present decrease in the number of transfused red blood cell concentrates (RBC) and possibly of a future increase.

Brockmann C., Hagen C., Görg S.

Universitätsklinikum Schleswig-Holstein, Institut für Transfusionsmedizin, Lübeck, Germany

Background: In contrast to the total numbers of blood transfusions in Germany, the demography of individuals who receive transfusions is not well characterized.
Aims: We were especially interested to identifydemographic anddiagnosis relatedreasons for an observed decline in the number of transfused red blood cell concentrates (RBC).
Methods: In a retrospective analysis we analyzed data from our laboratory information system to characterize patients receiving RBCin the University Hospital of Schleswig-Holstein between 2002 and 2017. Additionally, we analyzed data from ourhospital information system regarding the Diagnosis Related Groups(DRG)and procedures (OPS) of our patients in the years from2009 to 2017.
Results: During the 16-yearperiod 164,404patients received a total number of 725.709RBCs. The total annual number of RBC units increased since 2004 (43.263 units) reaching a plateau from 2009 until 2013(49.000). Since then we observeda decline in RBC-transfusions with 42.787 units in 2016. Similarly, the number of transfused patients increased from 8065 in 2002 to 8716 in 2011 followed by a decrease reaching 7814 patients in 2016 with averyslight decrease of the average number of transfused RBC per case from 5,7 to 5,5 units.
We alsocompared the number of transfused RBCs in several age groups with population data of Germany and our province Schleswig-Holstein:The age group of 70–74 years demanded about 15,4% of all transfused RBC followed by the age group 75–79 (14,46%) and the age group 65–69 (13,02%). For the years 2012–2017we observe a similar slight increase in population data and transfused RBCs the age group 65–69, whereas in the age group 70–74 we observe a dramatic decrease in population as well as in the number of transfused RBCs. The number of transfused RBC units correlatesstrong with the number of persons in the age groups 65–69, 70–74 an 75–79. When we compared DRG-data we observed that the number of diagnosis prone to receive a RBC-transfusion is also dependent on the number of persons within the population.
Summary / Conclusions: Over the last years we observed a decline in thetotal number RBC transfusions whereas the number of transfused units per case was unchanged. Additionally,the number of diagnosis with a high RBC demand declined similarly. Here we demonstrate that the number of RBC transfusions in the University hospital of Schleswig-Holstein strongly correlateswith the number of persons in different age groups. We conclude that weak birthrates in Germany after world war second have a major influence on RBC consumption today, and that these demographic factors are predominantly responsible for a decline of blood transfusions within the University Hospital Schleswig-Holstein. We expect a future increase of the number of transfused units in a few years, when the persons of the increasing age group 65–69 reaches the age group 70–74.

Conflict of interest: none

VS-14-5

Changing patterns of red blood cell use by internal medicine departments in a tertiary-care university hospital

Zimmermann R.[1], Strobel J.[1], Orlemann T.[2], Ganslmayer M.[2], Hackstein H.[1], Ganslandt T.[3]

[1]University Hospital Erlangen, Department for Transfusion Medicine and Haemostaseology, Erlangen, Germany
[2]University Hospital Erlangen, Department for Internal Medicine 1, Gastroenterology, Erlangen, Germany
[3]Ruprecht-Karls-University Heidelberg, Department of Biomedical Informatics, Mannheim, Germany

Introduction: Since seven years, the transfusion medicine community, donation services, and authorities in Germany observe a surprisingly pronounced decline in the usage of red blood cell concentrates (RBCs). Ideas about the cause of this process are focusing on changing patterns of red blood cell use by departments for anaesthesiology and cardiac surgery. Altered patterns of red blood cell use by other hospital departments are not under consideration.
Material and Methods: The University Hospital Erlangen is a 1400-bed tertiary care hospital. Here, all relevant data for interpreting changing patterns in the use of RBCs are continuously collected since 2010. We are able to integrate the clinical data warehouse components of RBC recipients' personal data, German Diagnosis Related Group (G-DRG) system codes of inpatients, and RBC component data from the blood bank IT system. The obtained DRGs are associated with the data of the blood component consumption on an individual basis. The results can be grouped by Major Diagnostic Categories (MDCs) and by basic DRGs. Analyses can be performed throughout the whole hospital as well as in relation to individual departments.
Results: Between 2010 and 2017, we observed a 17-percent decline of the number of annually transfused RBCs. This decrease was almost exclusively restricted to inpatients whose diagnoses and procedures were grouped to the MDC 0 (i.e. the so-called Pre-MDC category). Surveys of individual departments show that our internal medicine ICU, which primarily treats patients with sepsis, gastrointestinal bleeding, and cirrhosis, is the department that has realized the largest percentage reduction in red blood cell consumption within our hospital between 2010 and 2017.
Conclusions: In the past, we could demonstrate that the pronounced decline in the usage of RBCs is almost exclusively restricted to intensive care

unit patients and to a lesser extent to stem cell transplant recipients. In the meantime, our ongoing studies on the causes of changes in red blood cell consumption have to our surprise revealed that our internal medicine ICU is significantly involved in causing the declining transfusion frequency of red blood cell products. Future investigations on the effects of so-called patient blood management systems should therefore be resolved from the inappropriate one-sided focus on anesthesiology and cardiac surgery.

Conflict of interest: none

VS-15
Pränatale Diagnostik und Therapie

VS-15-1

Overview of fetal RhD genotyping

Clausen F. B.

Copenhagen University Hospital, Clinical Immunology, Copenhagen, Denmark

Alloimmunization against the D antigen in pregnant RhD negative women is preventable using anti-D immune prophylaxis. A combination of antenatal and postnatal anti-D administration has dramatically reduced alloimmunization of RhD negative women from 16% to 0.3%, thus reducing the risk of hemolytic disease of the fetus and newborn.
During pregnancy, noninvasive prenatal testing (NIPT) of the fetal RhD genotype can be done based on analysis of cell-free fetal DNA (cffDNA) extracted from maternal plasma. Knowledge of the fetal RhD type can guide the use of antenatal prophylaxis, restricting prophylaxis to RhD negative women who carry an RhD positive fetus, and thus avoiding unnecessary treatment of RhD negative women who carry an RhD negative fetus.
Routine fetal RhD genotyping to guide targeted prophylaxis has been introduced as a national service in Denmark, the Netherlands, Finland, and Norway. Several other countries offer the service regionally, and other countries are in the process of national implementation. The performance of fetal RhD genotyping has proven highly reliable and robust, with reported sensitivities of approximately 99.9%. Because of the high performance of noninvasive fetal RhD genotyping, cord blood typing has been discontinued in Denmark and the Netherlands.
Overall challenges are low levels of cffDNA in maternal plasma which may cause false-negative results, and the complexity of the Rh blood group system which may cause, primarily, false-positive results.

Conflict of interest: none

VS-15-2

Rate and cases of false positive and false negative results of noninvasive determination of antenatal RHD status.

Jakobsen M. A.

Odense University Hospital, Clinical Immunology, Odense, Denmark

Since 2010, non-invasive prenatal RHD genotype test (NIP RHD) has been mandatory in Denmark before the administration of Rh immunoglobulin. The presence of fetal RHD is determined by real-time PCR targeting one or two coding regions in the *RHD* gene, a setup that does not discriminate between maternal and fetal targets. During 2011–2013, the accuracy of NIP *RHD* result was determined by correlation with serological RhD types of the neonates. In the region of Southern Denmark, a total of 1,618 pregnancies in 1,588 RhD negative women were included. The sensitive of the test was 99.6% whereas the specificity of the test was 98.5%. Compared to the neonate»s serological RhD type, 9/987 (0.9%) of positive NIP *RHD* results were false positive (FP), 4/582 (0.7%) of negative NIP *RHD* results were false negative (FN). False positive NIP *RHD* results were shown to be mainly caused by fetal or maternal RhD variants leading to a change in the standard procedure. In contrast, insufficient amount of cell free fetal DNA in the maternal plasma were likely the reason for false negative results. The cause of the low level of fetal DNA was explored focusing on the effect of the maternal BMI on the level of fetal DNA.

Conflict of interest: none

VS-15-3

Digital PCR for noninvasive prenatal diagnosis of blood group antigens from cell-free maternal plasma DNA

Eryilmaz M., Müller D., Rink G., Klüter H., Bugert P.

Institut für Transfusionsmedizin und Immunologie, Universität Heidelberg, Medizinische Fakultät Mannheim, DRK-Blutspendediens, Molekularbiologie, Mannheim, Germany

Background: Red blood cell antigens such as RhD or Kell as well as alloantigens on platelets (HPA) of the fetus can cause immunization of the antigen-negative mother. The formation of antibodies to fetal antigens can cause Morbus Haemolyticus Neonatorum (MHN) or Neonatal Alloimmune Thrombocytopenia (NAIT). For an early recognition and therapy, it is important to determine the fetal antigens in early stages of pregnancy. Invasive procedures (e.g. amniocentesis) are often conduced to obtain fetal cells but are at risk for the fetus. In non-invasive testing (NIPT) procedures maternal blood is used to extract cell-free DNA (cfDNA) from the plasma. Different methods, mostly based on RealTime-PCR, have been developed for the detection of fetal DNA in maternal cfDNA to determine the fetal antigens. Here, we present the development and evaluation of chip-based digital PCR (dPCR) methods for the specific and sensitive detection of RHD, RHCE, KEL and HPA alleles as well as for the detection of Y chromosomal markers and anonymous SNPs. RealTime-PCR and dPCR methods are based on same TaqMan probe chemistry in order to generate the fluorescence signals. However, dPCR is characterized by end-point detection of fluorescence and therefore more robust.
Methods: CfDNA was obtained from EDTA blood samples of healthy volunteers using a commercial kit (QIAamp Circulating Nucleic Acid Kit; Qiagen). All dPCR analyses were performed using a QuantStudio™ 3D system (Applied Biosystems). The PCR assays are based on sequence-specific TaqMan probes with FAM or VIC as fluorescent dye and a minor groove binding quencher. RHD assays with FAM probes for exon 3, 5, 7 and 10 were combined with a VIC probe for GAPDH. Further assays were used with diallelic probes for KEL1(FAM)/KEL2(VIC), HPA-1a(FAM)/HPA-1b(VIC), AMY(FAM)/AMX(VIC) and 10 SNPforID markers.
Results: Specificity of the assays was tested on cfDNA by typing of samples with known homozygous and heterozygous genotypes. Each test resulted in approx. 20,000 evaluable signals and up to 0.05 % of the signals were false positive. Sensitivity was tested in dilution series of heterozygous cfDNA (5 to 0.1 %) in excess of homozygous cfDNA (95 to 99.9 %). In all assays we were able to detect the rare allele even at the lowest concentration of 0.1% heterozygous cfDNA (0.05 % rare allele). Preliminary tests on plasma cfDNA form pregnant women revealed reliable results in early stages of pregnancy for RhD and Kell.
Conclusion: We were able to show high sensitivity and specificity for the dPCR methods that is superior to RealTime-PCR. The dPCR methods are highly applicable for NIPT of RHD, KEL, HPA and other antigens by using cfDNA of the mother. The clinical validation of the dPCR methods will be performed according to recommendations of the DGTI for «The validation of tests for the determination of the fetal RHD status from blood of RHD-negative women in pregnancy».

Conflict of interest: none

VS-15-4

Pitfalls in prenatal diagnosis of fetal *RHD*: Frequency of maternal RHD variants as cause for a false positive genotype of the fetus

Doescher A., Vogt C.

DRK Blutspendedienst NSTOB, F&E, Oldenburg, Germany

Background: Prediction of the fetal D antigen status by genotyping DNA from maternal plasma is a routinely used method. However, more than 400 variant *RHD* alleles are known and the presence of such allele may lead to incorrect antigen prediction: Usually, a RhD negative fetus is mistaken for RhD positive. Here, we present a retrospective analysis of 733 pregnancies with a fetus at risk due to an anti-D antibody of the mother and the occurrence of *RHD* variants.
Methods: Plasma was prepared according to Lo et al.: and free fetal DNA was extracted using 1 mL plasma and the MagnaPure Large Volume protocol (MagnaPure compact, Roche Diagnostics GmbH, Mannheim, Germany). DNA from maternal samples was isolated with the PureGeneÔ Kit according to the manufactors instructions (PureGene Blood Core Kit B, QIAgen, Hilden, Germany). Total DNA was amplified in a multiplex PCR with fluorogenic primers for *RHD* exons 2–7, 9 & 10; polymorphisms for D-weak type 1- 5, D-VII, D-HMi and *RHCE* polymorphisms for C, c, Cw, E, e. Capillary electrophoresis for size fractionation and fluorigeneic analysis was done in an ABI 310. In addition, *RHD* exons 1–10 including intron/exon border areas were investigated by direct taq cycle-sequencing using BigDye-terminators (v.1.1; Applied Biosystems, Foster City, USA) in an ABI 310.
Results: Out of 733 samples, 443 showed an *RHD* positive and 290 an *RHD* negative genotype. Five samples gave also *RHD* positive reactions in maternal DNA from leukocytes, indicating an *RHD*-positive genotype for the phenotype negative mother. Investigation of the molecular background revealed a weak D type 2, two hybrid genes (*RHD-CE (3–7)-D* and *RHD-CE(4–7)-D*) and one case with deletions in *RHD* exon 1. The reason for a non-expressed *RHD*-gene in one case remained unclear. No polymorphisms in the coding regions were detectable by sequencing of the *RHD* gene, in contrast, intron 8 showed a nucleotide change at position -31. Nevertheless, a correlation between IVS8–31 polymorphism and a negative phenotype could not be demonstrated yet.
Conclusions: The retrospective analysis of 733 pregnancies revealed five cases with positive *RHD* genotyping results for a phenotype negative mother (0.68%). Since maternal plasma contains both, maternal and fetal DNA, it is not possible to determine the origin of positive genotyping results. Although the frequency of *RHD* variants is low, we will continue to check the *RHD*-status of maternal cells in parallel to the examination of maternal plasma.

Conflict of interest: none

VS-15-5

High-dose immunoglobulins in pregnancies complicated by severe alloimmunisation to red blood cells

Mayer B.[1], Henrich W.[2], Weichert A.[2], Bein G.[3], Enzensberger C.[4], Axt-Fliedner R.[4], Salama A.[5]

[1]Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Berlin, Germany
[2]Charité - Universitätsmedizin Berlin, Klinik für Geburtsmedizin, Berlin, Germany
[3]Universitätsklinikum Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany
[4]Universitätsklinikum Gießen, Zentrum für Frauenheilkunde & Geburtshilfe, Schwerpunkt Pränataldiagnostik, Gießen, Germany
[5]Charité - Universitätsmedizin Berlin, Berlin, Germany

Background and objectives: Pregnancies complicated by red blood cell (RBC) immunization against Rhesus and Kell antigens are at increased risk of fetal anemia and potentially fetal loss. The standard treatment consists of intrauterine transfusion. However, this treatment is associated with relevant procedure related risks such as pregnancy loss and preterm delivery, especially if performed before 20 weeks of gestation.
We describe our experience with early commencement of antenatal treatment with high-dose i.v. immunoglobulins (IVIG) in highly alloimmunised pregnant women.
Patients and Methods: Until now, three women with high titer antibodies against D, D/C and K, respectively, were treated with IVIG (1g/kg/week). Treatment was started between 9 and 15 weeks of gestation. Two women had a history of recurrent fetal loss, and one of these women was treated during two subsequent pregnancies. In one pregnancy, this woman received only IVIG, and in the subsequent pregnancy, plasmapheresis was performed prior to IVIG administration. In addition, a fourth pregnant woman with anti-K and a history of fetal death is still under treatment.
All pregnancies were monitored by serial antibody titrations and Doppler measurements of fetal middle cerebral artery-peak systolic velocity (MCA-PSV) as a predictor of fetal anemia.
Results: Early commencement of IVIG resulted in prevention of intrauterine transfusion (IUT) in two pregnancies with anti-K and anti-D, respectively. Furthermore, a significant delay of anemia was achieved in the woman who was treated two times. Unfortunately, fetal death could not be prevented in one case with distinct fetal hydrops five hours after an IUT in gestational week 28. No signs of fetal anemia have yet been observed in the remaining fourth case.
Conclusions: Together with previously published cases, our data are highly indicative of a beneficial effect of antenatal therapy with IVIG in pregnancies affected by severe RBC immunization. Further prospective multicenter studies are required in this important field.

Conflict of interest: none

VS-15-6

The significance of ABO blood group compatibility between mother and child regarding incidence and severity of fetal and neonatal alloimmune thrombocytopenia.

Miserre L.[1], Wienzek-Lischka S.[1,2], Cooper N.[1,2], Sachs U. J.[1,2], Bein G.[1,2]

[1]Institut für klinische Immunologie und Transfusionsmedizin, Justus-Liebig-Universität Gießen, Gießen, Germany
[2]Deutsches Zentrum für feto-maternale Inkompatibilität (DZFI), Universitätsklinikum Giessen und Marburg, Gießen, Germany

Background: In fetal and neonatal alloimmune thrombocytopenia (FNAIT), maternal alloantibodies against fetal platelet antigens inherited from the father cause platelet degradation and interfere with vascular endothelial cells. The child presents with thrombocytopenia and intracranial hemorrhage (ICH) in about 10% of immunized mothers. Recently, an association between maternal ABO blood groups and the severity of FNAIT has been reported. Blood group A of the mother was associated with a higher risk of severe FNAIT compared to blood group O. In this study, we tested the genetic association of ABO blood group and ABO blood group incompatibility between mother and child with incidence and severity of FNAIT in a large cohort of 165 mother-child pairs.
Methods: Maternal and fetal ABO genotyping was performed with in-house TaqMan real time PCR assays to detect the major ABO alleles ABO*A1, ABO*A2, ABO*B, ABO*O.01, and ABO*O.02 in 165 mother-child pairs with proven FNAIT. Severe FNAIT was defined as neonatal platelet count less than 50 x 109/l and/or occurrence of ICH. Predicted ABO phenotype frequencies of cases were compared to ABO phenotype frequencies of 45295 first time blood donors. ABO blood groups of mothers were associated with neonatal platelet count and incidence of ICH. Severity of FNAIT was compared between ABO compatible and ABO incompatible pregnancies. Statistical analysis was undertaken with GraphPad Prism.
Results: There was no statistically significant difference in the distribution of ABO phenotypes among immunized women, neonates and controls. ABO incompatibility between mother and child was not associated with a lower incidence of FNAIT. The neonatal platelet count and the incidence of ICH were not associated with the maternal ABO blood group. ABO

incompatibility between mother and child was neither associated with the fetal platelet count nor with the occurrence of ICH.
Conclusion: In contrast to hemolytic disease of the fetus and newborn, where ABO incompatibility between mother and fetus reduces the risk of anti-RhD immunization, ABO incompatibility does not have a preventive effect on maternal immunization against human platelet antigens or on the development of severe FNAIT.

Conflict of interest: none

VS-15-7

Non-invasive risk-assessment and bleeding prophylaxis with IVIG in pregnant women with a history of fetal and neonatal alloimmune thrombocytopenia – a single center experience

Wienzek-Lischka S.[1], Krautwurst A.[1], Fröhner V.[1], Gattenlöhner S.[2], Bräuninger A.[2], Sawazki A.[3], Enzensberger C.[3], Axt-Fliedner R.[3], Santoso S.[1], Sachs U. J.[1], Bein G.[1]

[1]Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany
[2]Institut für Pathologie, Gießen, Germany
[3]Klinik für Gynäkologie und Geburtshilfe, Pränatalmedizin, Gießen, Germany

Introduction: In pregnant women with a history of fetal and neonatal alloimmune thrombocytopenia (FNAIT), prenatal interventions in subsequent pregnancies to prevent fetal bleeding are required if the fetus is at risk. Several invasive and non-invasive protocols have been published: amniocentesis for fetal genotyping, fetal blood sampling for the determination of fetal platelet count, intrauterine platelet transfusions, and weekly maternal i.v. immunoglobulin (IVIG) infusion with or without additional corticosteroid therapy. Here we report single center results of a complete non-invasive protocol
Methods: 12 pregnant women with proven FNAIT in history (11 cases Anti-HPA-1a, 1 case Anti-HPA-15a) were treated with IVIG (1g/kg/bw) every week without additional corticosteroid therapy. In case of paternal heterozygosity for the implicated HPA allele, potential fetal incompatibility was determined by assessing cell-free fetal DNA from maternal plasma and subsequent fetal genotyping using next-generation sequencing before starting IVIG administration. To identify potential IVIG-related hemolytic reactions or maternal pancytopenia, total blood count was assessed at every outpatient consultation. Furthermore, ultrasound examination of the fetus was performed weekly. Before administration of a new IVIG lot, isoagglutinin titers were determined. Side effects were prospectively documented. Outcome of index pregnancy and current pregnancy was compared.
Results: The treatment with IVIG was started at 20 weeks of gestational age (median). The average number of treatments was 15. Compared to the index pregnancy, platelet counts of the newborns were in all cases higher (185 G/L mean, range 39–335 G/L). No intracranial hemorrhage occurred (Index pregnancies: 1 case). In 2 newborns mild bleeding signs were observed and treated with single-dose HPA-antigen negative platelet concentrate. The analysis of side effects revealed as most common symptoms: headache (92%), followed by nausea and vomiting (42%), exanthema and chills (25%), hypertension (17%), and hypotension (8%). No severe hemolytic reaction was observed.
Conclusion: Among pregnant women with FNAIT history, the use of non-invasive fetal risk assessment and maternal IVIG resulted in favorite outcome of all newborns. Headache and nausea/vomiting were common side effects of IVIG treatment. Invasive diagnostic or therapeutic procedures in women with a history of FNAIT should be abandoned.

Conflict of interest: none

VS-16
Zell- und Gentherapie

VS-16-1

MHC Streptamer sorting to generate alloreactive KIR-2/3DL negative primary NK cell subsets in a KIR allele dependent subset pattern

Seidl C., Petra B., El Nahry Y., Hennecke J.H., Seifried E.

DRK Blutspendedienst Baden-Württemberg - Hessen, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt am Main, Germany

Introduction: Natural killer (NK) cells have been used in several clinical trials as adaptive immunotherapy after hematopoetic stem cells therapy. However, the low numbers of these cells in peripheral blood mononuclear cells (PBMC) with a range of 5–20%, have resulted in various approaches to preferentially expand primary NK cells from PBMC. While some clinical trials have used the addition of Interleukin 2 to co-stimulate the expansion of purified NK cells from allogeneic donors, recent studies have shown promising results in achieving in vitro expansion of NK cells to large numbers for adoptive immunotherapy. The graft versus leukaemia (GvL) effect is mediated by NK cells that solely express inhibitory killer cell immunoglobulin-like receptors (KIR) missing their corresponding HLA ligand in the patient (KIR-HLA mismatch).
Method: In the present study, we developed a technique for the isolation of patient specific alloreactive KIR+ donor-NK subsets at GMP-level by MHC Streptamers to be used for GMP conform adaptive immunotherapy. This techniques is based on KIR-ligand specific MHC Streptamers, with specific binding affinity to KIR2DL1, KIR2DL2/L3 or KIR3DL1 positive NK-cells (HLA-C*04:01-CMV, HLA-C*01:02-TIMP, HLA-B*57:01-HIV) respectively. Following negative isolation of NK cells from peripheral blood, functional activity was assessed by the CD107 degranulation assay against the HLA negative cell line L721.221 transfected with HLA-C1, HLA-C2 and HLA-Bw4 ligands for the respective KIR expressing NK cells. Furthermore, a sequencing strategy to define KIR allele specific binding of NK cells has been developed.
Results: Flow cytometry showed a mean staining of 83% forKIR2DL1+ NK cells, 71% for KIR2DL2/L3+ NK cells, and 63% for KIR3DL1+ NK cells. Following magnetic assisted cell sorting we obtained a mean purity of 89% KIR2DL1+and73% KIR2DL3+cells indicating a specific and reproductive ligation of MHC Streptamers to KIR on the NK cell surface. Importantly, sorted KIR+ NK cells showed lytic activity and higher degranulation activity against KIR-HLA matched target cells compared to bulk NK cells. Based on the degranulation assays, KIR allelic binding pattern can be defined.
Conclusion: This technology allows sorting of alloreactive KIR+ NK cell populations by negative isolation of all KIR-expressing NK cells that will be inhibited by the patient´s HLA and only select the alloreactive NK cell subset for direct adoptive cell therapy or *ex vivo* NK cell expansion. It could be also implemented to assess the NK cell cytotoxic potential based on allelic KIR differences.

Conflict of interest: none

VS-16-2

CliniMACS Plus versus CliniMACS Prodigy for the preparation of virus-specific T-cells: virus-specific and donor group related results

Heuft H. G.[1], Tischer S.[1], Aleksandrova K.[2], Priesner C.[2], Leise J.[2], Martens J.[1], Goudeva L.[1], Blasczyk R.[1], Maecker-Kolhoff B.[3], Eiz-Vepser B.[1]

[1]Medizinische Hochschule Hannover, Transfusionsmedizin, Hannover, Germany
[2]Medizinische Hochschule Hannover, Cellular Therapy Centre, Hannover, Germany
[3]Medizinische Hochschule Hannover, Pädiatrische Hämatologie und Onkologie, Hannover, Germany

Objective: To compare the quantity and composition of Interferon-γ (IFN-γ) secreting T-cell products after short term (4 h) exposition to polypeptide pools of different virus species (CMV: pp65; EBV: EBNA-1/EBV Select; ADV: ADV5 Hexon; BKV: LT/VP1, alone or combined) and enrichment by the semiautomated CliniMACS Plus (C-PL) or the recently introduced fully automated CliniMACS Prodigy (C-PR) device.
Patients and Methods: From 07/2014 to 04/2018, we performed 82 runs (1 [1–4] runs per patient) to obtain virus-specific T-cells for 73 symptomatic patients (C-PL, n = 41 [CMV, 17, EBV 13, ADV 9, BKV 1, multi-specific 1] vs C-PR, n = 41 [CMV 21, EBV 10, ADV 3, BKV 2, multi-specific 4]). Donor distribution for haploidentical or HLA-matched family (FD), HLA-matched stem cell (SCD) and unrelated haploidentical T-cell registry donors (alloCell, ATD) was very similar: FD 18/14; SCD 10/11, ATD 13/16 for C-PL vs C-PR. We performed flow cytometry analyses on WBC counts, viability and IFN-γ+ positive T-cells as well as for T-cell subsets for fresh (non-cryopreserved) machine products. After transfer T-cells were monitored in pa-tient blood to determine frequency, chimerism and T-cell receptor repertoire. **Results** (C-PL vs C-PR, medians, range before adjustment to patient-specific T-cell doses): Total WBCs were 6,4 (1,8–27,9)×10^6 vs 13,9 (2,1–32,7)×10^6; p≤0.001. The viability was 64 (31–77)% vs 75 (22–86)%. For analyses to specific virus species and donors, see Table 1/2. **Conclusions:** Irrespectively from the virus peptide exposition or the donor source the CliniMACS Prodigy yielded significantly more IFN-γ+ («virus-specific») T-cells. FD might be slightly inferior to other donor groups. Antiviral T-cells became detectable in 80% of the patients after transfer. Donor T-cells were well tolerated without any side effects.

Conflict of interest: none

Tab. 1. Device related virus-specific T-cell yields

Virus	C-PL, viable T-cells, IFN-γ+, ×10E6			C-PR, viable T-cells, IFN-γ+, ×10E6		
	CD3+	CD3+CD4+	CD3+CD8+	CD3+	CD3+CD4+	CD3+CD8+
CMV	1,6 (0,2-7,1)	1,0 (0,2-6,8)	0,4 (0,03-5,0)	4,9 (0,5-10,3)	4,5 (0,5-10,3)	0,9 (0,2-4,0)
EBV	0,7 (0,2-5,3)	0,7 (0,2-4,9)	0,2 (0,04-2,0)	2,7 (0,5-6,2)	2,4 (0,5-6,0)	0,6 (0,1-2,0)
ADV	0,7 (0,1-5,2)	0,5 (0,1-3,8)	0,1 (0,01-3,6)	4,2 (3,8-5,2)	3,7 (1,2-3,8)	0,4 (0,2-1,6)

Tab. 2. Device related donor-group-specific T-cell yields

Donors	C-PL, T-cells viable, IFN-γ+, ×10E6			C-PR, T-cells viable, IFN-γ+, ×10E6		
	CD3+	CD3+CD4+	CD3+CD8+	CD3+	CD3+CD4+	CD3+CD8+
FD	0,6	0,5	0,1	3,8	3,4	0,9
SCD	1,6	1,1	0,4	4,2	3,8	1,2
ATD	1,0	0,8	0,2	4,3	4,1	0,9

VS-16-3

Decentralized GMP-manufacturing of a dendritic cell-based vaccine for children with recurrent glioblastoma – The HIT-HGG Rez Immunovac Trial

Eichler H.[1], Graf N.[2], Rachor J.[3], Schlegel P. G.[3], Kramm C.[4], Eyrich M.[3]

[1]Universitätsklinikum des Saarlandes, Institut für Klinische Hämostaseologie und Transfusionsmedizin, Homburg/Saar, Germany
[2]Universitätsklinikum des Saarlandes, Klinik für Pädiatrische Onkologie und Hämatologie, Homburg/Saar, Germany
[3]Universitätskinderklinik, Pädiatrische Hämatologie/Onkologie, Würzburg, Germany
[4]Universitätsmedizin Göttingen, Klinik für Kinder- und Jugendmedizin, Abteilung für Pädiatrische Hämatologie und Onkologie, Göttingen, Germany

Background: High-grade gliomas in children are very rare malignancies with an incidence of 2 per 1 million children/year. Since the overall survival rate is poor despite multimodal treatment, innovative treatments such as therapeutic vaccines in combination with immunmodulating agents are currently investigated in clinical trials.
Trial design: For manufacturing of therapeutic vaccines, a GMP-compliant methodology was established in three academic GMP-facilities across Germany. Monocytes are collected by unstimulated apheresis; subsequently enriched via elutriation, differentiated *in vitro* with GM-CSF/IL-4 and finally matured with TNFα/IL-1ß. Prior to maturation, immature DCs are pulsed with a protein mixture derived from autologous glioma lysate. Prior to vaccination, metronomic cyclophosphamide (metrCyc) will be used to deplete regulatory T-cells (Treg). The DC-vaccine is injected intra-dermally four times in weekly intervals, either immediately following QC-testing and release of freshly prepared DCs or after freezing/thawing. The vaccination response will be boosted by intradermal application of glioma lysate if available.
Results: The manufacturing process was thoroughly validated and stringent, yet feasible release criteria could be established. Mature DCs showed a targeted migration towards CCL19/21 and were able to prime cytotoxic T cells. In a pilot phase, six pediatric and five adult patients with recurrent gliomas were treated as per protocol. All patients were vaccinated with at least 7 s. c. injections (4xDCs, 3xlysate). Upfront metrCyc was well tolerated and reduced Treg frequency temporarily by 48%. The vaccine induced tumor-specific IFNg T-cell responses in 9/10 analyzed patients, and an increase of the CNS-homing receptor VLA4 on CD8+ but not on CD4+ T cells. Despite a survival advantage during the first 12 months (6-month OS 100%), all but one adult WHO III° patient relapsed during follow-up.
Conclusion: The HIT-HGG Rez Immunovac study (Eudra-CT 2013–000419–26) represents the first multicenter trial with decentralized GMP-manufacturing of a DC vaccine. Recruitment started in spring 2018.

Conflict of interest: none

VS-16-4

Production of clinically relevant numbers of Megakaryocytes using microcarrier-assisted stirred bioreactors

Figueiredo C., Eicke D., Baigger A., Blasczyk R.

Hannover Medical School, Institute for Transfusion Medicine, Hannover, Germany

Introduction: In vitro produced Megakaryocytes (MKs) may serve as source to produce platelets (PLTs) *ex vivo* or *in vivo*. Previously, we have established a strategy to differentiate MKs from induced pluripotent stem cells in bioreactors. This study aimed at the large-scale production of MKs using microcarriers to increase the MK yield and to characterize their phenotype and functionality after irradiation as a method to decrease possible safety concerns associated to the iPSC origin.
Methods: IPSCs were cultured in an aggregate form in presence or absence of microcarriers using 50mL stirred flasks. Cells were differentiated into MKs using thrombopoietin, stem cell factor and interleukin-3 for a period of 22 days. Non- irradiated or irradiated iPSC-derived MKs were analysed for polyploidy, phenotype and proPLT production using flow cy-

tometry and fluorescence microscopy. Also, PLT-production was investigated *in vivo*. Non-irradiated or irradiated MKs were transfused to NOD/SCID/IL-2Rγc–/– mice and blood was analyzed for human PLTs.
Results: Differentiation of MKs in presence of microcarriers resulted in a 8-fold increase of MKs per iPSC in comparison to only aggregates. This resulted in mean of total MK harvest of $18.7 \pm 6.8 \times 10^7$ in microcarrier-assisted bioreactors in comparison to $4.9 \pm 1.3 \times 10^7$ MKs collected from bioreactors containing only aggregates. Interestingly, MKs produced in microcarrier-assisted bioreactors showed higher capacity of proPLT formation than MKs derived from only aggregates bioreactors. MK phenotype and DNA content was comparable between MKs derived from both types of bioreactors. Irradiation of MKs did not affect their phenotype and capability to form proPLTs or PLTs after transfusion into NOD/SCID/IL-2Rγc$^{-/-}$ mice.
Conclusion: The use of microcarriers in stirred bioreactors showed to significantly increase the yield of iPSC-derived MKs to clinically relevant numbers. Irradiation of MKs prior transfusion showed to be an efficient strategy to abrogate potential tumorigenic risks. This may facilitate the use of iPSC-derived MK for *ex vivo* production of PLTs, direct transfusion or for innovative MK-based regenerative therapies.

Conflict of interest: none

VS-16-5

A GMP-compliant protocol to manufacture *CCR5*-edited hematopoietic stem cells to treat HIV infection

Romito M.[1], Meyer E.[1], Turchiano G.[1], Hildenbeutel M.[1], Poddar S.[1], Haas S.[1], Gautron A. S.[2], Kok Y. L.[3], Rositzka J.[1], Mussolino C.[1], Smith J.[2], Schäfer R.[4], Metzner K.[3], Duchateau P.[2], Cathomen T.[1], Cornu T.[1]

[1]Universitätsklinikum Freiburg, Institut für Transfusionsmedizin und Gentherapie, Freiburg, Germany
[2]Cellectis S.A., Paris, France
[3]Universitätsspital Zürich, Zürich, Switzerland
[4]DRK Blutspendedienst, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt am Main, Germany

Targeted genome editing in hematopoietic cells enable new therapeutic interventions, especially for infectious diseases. We have developed a GMP-compliant protocol to manufacture *CCR5*-edited CD34+ hematopoietic stem and precursor cells (HSPCs) with the aim to cure patients suffering from chronic infection with human immunodeficiency virus type 1 (HIV-1). Disruption of the *CCR5* gene, which codes for the major HIV-1 co-receptor, in HSPCs will give rise to an HIV-resistant immune system after stem cell transplantation. Electroporation of CD34+ HSPCs with mRNA encoding TALEN designer nucleases targeting *CCR5* revealed up to 90% knockout of *CCR5* alleles in CD34+ HSPCs derived from cord blood or leukapheresate of mobilized donors. Deep sequencing confirmed the high gene editing frequency and revealed no substantial cleavage activity at any of the top 20 predicted TALEN off-target sites in HSPCs. Furthermore, *CCR5*-edited HSPCs maintained their capacity to differentiate into the various blood lineages *in vitro* and *in vivo*, and clonal analysis revealed bi-allelic *CCR5* disruption in more than 75% of stem cells. Potency and safety of *CCR5*-targeting TALENs was further demonstrated in CCR5 expressing CD4+ T cells. Proliferation capacity and biological function of the cells was preserved after CCR5 knockout. Moreover, the lymphocytes showed significantly reduced CCR5 expression and became resistant to infection with the R5-tropic HIV1(JR-FL) virus. In summary, our developed protocol enables highly efficient and GMP-compliant knockout of the *CCR5* locus in hematopoietic stem cells, so forming the basis for a phase I/II clinical study.

Conflict of interest: T.Ca. is a consultant for TRACR Hematology. His research efforts are supported by Cellectis S.A., Casebia Therapeutics, and Miltenyi Biotec.

VS-16-6

Generation of chimeric antigen receptors (CARs) based on peptide-selective monoclonal antibodies with unique TCR-like properties to control Epstein-Barr virus-associated tumors

Dragon, A.[1], Lahrberg, J.[1], Nerreter T.[2], Tischer S.[1,3], Riese S.[1], Bak S.[1], Ziegler A.[4], Blasczyk R.[1,3], Maecker-Kolhoff B.[3,5], Hudesec M.[2], Eiz-Vesper B.[1,3]

[1]Institute for Transfusion Medicine, Hannover Medical School (MHH), Hannover, Germany
[2] Department of Internal Medicine II, University of Wuerzburg, Würzburg, Deutschland
[3] Integrated Research and Treatment Center (IFB-Tx), Hannover Medical School (MHH), Hannover, Deutschland
[4] Ziegler Biosolutions, Waldshut-Tiengen, Deutschland
[5] Department of Pediatric Hematology and Oncology, Hannover Medical School (MHH), Hannover, Deutschland

Introduction: Immunosuppressive therapy or T-cell depletion in stem cell and solid organ transplant patients can cause uncontrolled growth of Epstein-Barr virus (EBV)-infected B cells, resulting in post-transplant lymphoproliferative diseases (PTLDs). The two main immunotherapeutic strategies to control EBV-associated tumors are (1) treatment with monoclonal antibodies (mAbs) against CD20 and (2) transfer of EBV-specific cytotoxic T lymphocytes (EBV-CTLs), but these have still limitations.
The mAb TÜ165 binds to EBV-infected human B cells (B-LCLs) via HLA-B*35:01 presenting an EBNA3C-derived peptide. EBNA3C is expressed in type III latency associated with PTLD. The study should answer the question whether a chimeric antigen receptor (CAR) based on a TÜ165-derived scFv-fragment can recognize an EBV target antigen in a T-cell receptor (TCR)-like manner and if CAR expressing T cells are able to effectively control infection and eliminate virus-infected malignant B cells.
Methods: A 2nd generation CAR was designed by cloning of TÜ165 scFv into a CAR backbone with CD3ζ and 4–1BB signaling domains. Since optimal distance between effector cell and target cell epitope is critical for recognition, short (12AA) and long (229AA) extracellular spacer domains were tested (TÜCAR_short, TÜCAR_long). CD3+ T cells were lentiviral transduced, expanded for 8 days, followed by sorting of TÜCAR+ T cells. Functionality of engineered T cells in response to target cell recognition was determined using a newly established outgrowth assay and activation marker expression (CD25, CD69, CD137 and CD154). Specific lysis of target cells after day 14 and 21 in a co-culture of TÜCAR+ T cells with peptide-loaded HLA-B*35:01+K562 cells or autologous B-LCLs was determined.
Results: Isolated CD3+ T cells were lentiviral transduced with a mean efficacy of 19% flowing the sorting of expanded TÜCAR+ T cells with a purity of more than 90%. Enhanced expression of the specific activation marker on TÜCAR+ T cells was detected in response to specific target cell recognition. Moreover, cells expressing TÜCAR_short constructs were capable to specifically target and lyse HLA*B35-EBNA3C peptide-loaded target cells. Consistent with this data, live-cell imaging using live-cell microscopy confirmed the capacity of TÜCAR+ effector cells to specifically recognize peptide-loaded target cells leading to an effective target cell killing.
Conclusion: Our results provide first evidence that CARs recognizing peptide/MHC complexes in a TCR-like manner enable engineered T cells to effectively respond to target cells expressing EBV-restricted, PTLD-associated antigens.

Conflict of interest: none

VS-16-7

Selective effects of mTOR inhibitor sirolimus on naïve and memory T cells extending its applicable range beyond immunosuppression

Bak S.[1], Tischer S.[1,2], Dragon A.[1], Ravens S.[3], Pape L.[4], Koenecke C.[5], Oelke M.[6], Blasczyk R.[1,2], Maecker-Kolhoff B.[2,7], Eiz-Vesper B.[1,2]

[1]Institute for Transfusion Medicine, Hannover Medical School (MHH), Hannover, Germany
[2]Integrated Research and Treatment Center (IFB-Tx), Hannover Medical School (MHH), Hannover, Germany
[3]Institute of Immunology, Hannover Medical School (MHH), Hannover, Germany
4Department of Pediatric Nephrology, Hannover Medical School (MHH), Hannover, Germany
[5]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School (MHH), Hannover, Germany
[6]Department of Pathology, John Hopkins School of Medicine, Baltimore, MD, United States
[7]Department of Pediatric Hematology and Oncology, Hannover Medical School (MHH), Hannover, Germany

Introduction: Cytomegalovirus (CMV) infection and reactivation remains one of the most important complications in transplantation medicine, particularly in patients undergoing intense immunosuppression (IS). Recent observations indicated that patients treated with mTOR inhibitor, e.g. sirolimus, may exhibit favorable outcomes of CMV infection following transplantation. This study was designed to elucidate this effect through investigation of the role of mTORC1 signaling in CMV-specific cytotoxic CD8+ T cells (CTLs).
Methods: CD8+ T cells were stimulated with artificial antigen-presenting cells (aAPCs) loaded with CMVpp65 peptide. The effect of sirolimus on the proliferative capacity, phenotype and functionality of CTLs was determined. Furthermore, we applied next-generation sequencing (NGS) to monitor dynamics of TCR repertoires under the influence of sirolimus as well as detection of signaling pathways and expression of target and effector molecules was assessed.
Results: Despite the inhibited expansion, sirolimus induced strong T-cell activation, had no effect on the effector memory phenotype and significantly increased antigen-specific effector T-cell response. Key elements of T-cell activation and function such as (1) dynamics of TCR repertoires, (2) phosphorylation of kinases and proteins, and (3) expression of miRNAs and genes were differently affected under sirolimus treatment, indicating their influence in the improved functionality.
Conclusion: In contrast to expectations, we showed improved functional qualities of CMV-CTLs exposed to sirolimus. Modulating the environmental cues during CTL formation by IL-2R driven STAT-5 signaling under mTORC1 inhibition allows the fine-tuning of CTL programming to promote antiviral T-cell response with stable dynamics of TCR repertoires. This study provides help for further individualization of IS therapy, indicating a potential benefit of sirolimus in patients with elevated risk of CMV infection.

Conflict of interest: Text

VS-16-8

Flow-cytometric analysis of HLA disparities as a highly efficient tool to detect chimerism in antigen-specific T-cell populations after non-identical transplantation and adoptive T-cell transfer

Tischer S.[1,2], Schultze-Florey R.[2,3], Kuehnau W.[4], Blasczyk R.[1,2], Maecker-Kolhoff B.[2,3], Eiz-Vesper B.[1,2]

[1]Institute for Transfusion Medicine, Hannover Medical School (MHH), Hannover, Germany
[2]Integrated Research and Treatment Center (IFB-Tx), Hannover Medical School (MHH), Hannover, Germany
[3]Department of Pediatric Hematology and Oncology, Hannover Medical School (MHH), Hannover, Germany
[4]Institute for Human Genetics, Hannover Medical School (MHH), Hannover, Germany

Intoduction: Chimerism analysis after HLA-mismatched stem cell transplantation (SCT) represents an essential diagnostic tool in monitoring of patients to initiate fast clinical decisions about treatment modification needed for an effective therapy. In particular, flow-cytometric analysis using monoclonal anti-HLA antibodies (mAbs) offers an attractive approach for the detection of the chimerism status in single cell populations in transplanted patients.
Methods: We established a flow-cytometric protocol providing a stable and feasible platform for monitoring chimerism in antigen-specific T cells. Flow-cytometric chimerism analysis of antigen-specific T cells was validated by using the human cytomegalovirus phosphoprotein 65 (CMVpp65) as viral target and the anti-HLA (IgM) mAbs A1/11/26+ and A2. Donors were selected from alloCELL registry (*www.alloCELL.com*) according to their HLA type, CMV serology and antiviral T-cells frequencies. PBMCs from HLA-A01+/02- (donor 1, D1) and A01-/02+ (donor 2, D2) donors were shortly restimulated with CMVpp65 peptide pool and mixed at a ratio of 1:1 and 10:1, followed by magnetic isolation of IFN-γ secreting CMVpp65-specific T cells using cytokine secretion assay. Isolated IFN-γ+ T cells were stained with anti-HLA-A1 and A2 mABs respectively and T-cell surface marker to examine frequencies of the HLA donor-specific antiviral T cells. Enriched IFN-γ+ T cells were further analyzed for chimerism by STR PCR.
Results: A similar distribution of HLA-A01+ (D1) and HLA-A02+ (D2) antiviral T cells was detected before and after enrichment for both ratios by anti-HLA-A1 and A2 mAbs, respectively. In the enriched 1:1 fraction, 61% of A02+ cells (D2) within IFN-g+/CD3+ cells were detected, while 13% were positive for A02 at ratio 10:1 (D1:D2). Analysis by STR PCR showed similar HLA distributions (ratio 1:1: 70% A02+; 10:1: 30% A02+). Comparable results between flow cytometry and PCR for CMV-specific T cells could be confirmed in additional HLA-A01/A02 mismatched donor pairs.
Conclusion: The increasing application of HLA-mismatched transplantations and HLA-partial matched T-cell products requires fast and consistent determinations of cell chimerism. Flow-cytometric chimerism analysis using anti-HLA antibodies represents a fast and highly sensitive technique. It allows the analysis of dynamic changes in the single cell subsets. The sensitivity was proven to be comparable to the STR PCR chimerism analysis. Moreover, the flow cytometric approach can be combined with techniques of functional single cell identification such as cytokine secretion, as a powerful tool for monitoring of antigen-specific cells after allogeneic SCT and adoptive T-cell transfer.

Conflict of interest: none

VS-16-9

Invisible organs made by genetic engineering to turn off MHC prior to allogeneic transplantation prevent a pro-inflammatory cytokine response in the recipient

Figueiredo C.[1], *Carvalho-Oliveira M.*[1], *Chen-Wacker C.*[1], *Höffler K.*[2], *Yuzefovych Y.*[1], *Pogozhykh O.*[1], *Haverich A.*[2], *Warnecke G.*[2], *Blasczyk R.*[1]

[1]Hannover Medical School, Institute for Transfusion Medicine, Hannover, Germany
[2]Hannover Medical School, Department of Cardiac-, Thoracic-, Transplantation- and Vascular Surgery, Hannover, Germany

Introduction: HLA and minor antigen mismatches are the main causes of allograft rejection and graft failure. Previously, we have shown in mice and rats that MHC silenced cells and tissues are protected against immune rejection. We also demonstrated in porcine lungs that SLA expression can be turned off in a complex vascularized organ.
Methods: In this study we evaluated the effect of MHC I silencing prior to allogeneic lung transplantation (Tx) in an established porcine Tx model by monitoring the cytokine response during the first 12 weeks after Tx with immunosuppression given only in the first 4 weeks. SLA I was permanently silenced during normothermic ex vivo perfusion with lentiviral vectors encoding short hairpin RNAs targeting β2m. A lentivirally transduced non-specific shRNA was used in the control lung Tx group. NanoLuc was used as a reporter gene in both groups. In each transplant experiment both donor lungs were genetically engineered with one lung being transplanted and the other lung used for quality control. Levels of β2m mRNA and SLA were quantified by RT-PCR and flow cytometry. SLA downregulation of the endothelial cells was designed to reach a level of 70%. Cytokines were monitored weekly after Tx using multiplex technology.
Results: Already 1h after Tx the serum levels of IL-1beta, IL-6 and IL-8 increased significantly in all animals by up to 0.263, 1.370 and 0.497 pg/ml, respectively. On POD 1, the cytokine secretion in the SLA silenced group decreased to pre-transplant levels whereas those of the control group remained significantly elevated ($p<0.01$). On POD 14, levels of IL-12 increased significantly by up to 0.286 pg/ml in the control group whereas it remained at pre-transplant levels in the SLA silenced lung recipients. In addition, levels of IL-2, IL-10 and TNF-α increased exclusively in animals with SLA expressing lungs while it was undetectable in animals receiving SLA silenced lungs. Due to severe rejection none of the control group animals reached the end of the monitoring period whereas recipients of the SLA silenced lungs could be monitored over the entire period.
Conclusion: These data strongly indicate that grafts silenced for MHC I expression are immunologically invisible and may successfully combat the burden of rejection and immunosuppression.

Conflict of interest: none

VS-17
Immunity

VS-17-1

Clinical determinants of red cell alloimmunization

Evers D.

Radboudumc, dept. of hematology, Nijmegen, Niederlande

Transfusion of red cells causes exposure to foreign antigens and potentiates alloantibody formation. The understanding of what factors dictate alloimmunization has recently expanded.
Red cell alloantibody formation requires exposure to an immunogenic alloantigen. In this regard, red cell alloimmunization incidences in a general multiply-transfused Caucasian patient population reaches 8%. Alloimmunizations to E, K, Jka and c are the most prevalent, explained by population-based antigen frequencies and their high immunogenicity.
Available evidence supports the view of a «responder population», being determined by clinical, genetic and environmental conditions which affect the capability of a recipient´s immune system to mount a significant adaptive immune response upon alloantigen exposure. With regard to clinical conditions, inflammation, functioning of the spleen, and treatment-associated immunosuppression are important determinants of a red cell alloimmune response in humans. In murine models, experimentally induced inflammation induced a shift from a splenic macrophage based to a dendritic cell based consumption of transfused red blood cells, promoting CD4+ T-cell proliferation and ultimately red cell alloimmune responses. In line, pro-inflammatory conditions related to sickle cell disease as well as febrile reactions to donor platelets have been reported to enhance alloimmunization in humans. In a large-scale, multicenter case-control study, we recently observed an association between a patient»s inflammatory condition at the time of red cell transfusion and the subsequent development of red cell alloantibodies. In detail, a severe bacterial or viral infection significantly increased red cell alloimmunization incidences. Contrary but corroborating murine LPS-models, bacteremia with gram-negative pathogens was associated with a 2-fold reduced alloimmunization incidence. Next, both animal and human studies have identified the spleen and its resident immune cells to be pivotal in alloantibody production against red cell antigens. At least applying to the non-hemoglobinopathy patient population, alloimmunization seems a highly unlikely event following splenectomy. Finally, a strong protective role has been identified for general immunosuppressive agents, but more specifically for dose-intensive chemo-immunotherapy received by patients with acute (myeloid or lymphoblastic) leukemia, mature lymphomas, and patients undergoing hematopoietic cell transplantation.
Taken together, both exposure to high immunogenic foreign red cell antigens as well as conditions affecting the recipient»s immune system modulate alloimmunization risks. A thorough identification of all relevant red cell alloimmunization determinants may hopefully enable the establishment of an accurate alloimmunization prediction model. Such a model could tailor current matching strategies, targeting eradication of transfusion-induced alloimmunization and its clinical consequences.

Conflict of interest: none

VS-17-2

Anti-PF4/Polyanion antibody induced autoimmune HIT: A new mechanism of autoimmunity

Greinacher A.

University Medicine Greifswald, Institute of Immunology and Transfusion Medicine, Greifswald, Deutschland

Background: Platelets contribute to innate immunity. We recently introduced the concept that hereby platelet factor 4 (PF4) plays an important role. Antibodies recognizing a neoepitope on PF4/polyanion (P) complexes opsonize bacteria *via* binding to PF4 bound to bacterial surface polyanions. When this bacterial host defense mechanism is misdirected to PF4/heparin complexes on platelets and monocytes, the prothrombotic adverse drug reaction heparin-induced thrombocytopenia (HIT) occurs.
HIT as a model for autoimmunity: HIT occurs when anti-PF4/P-antibodies activate PF4/heparin-coated platelets; while autoimmune-HIT occurs in the absence of heparin treatment.
Material and Methods: We assessed binding characteristics of different anti-PF4/polyanion antibodies by single molecule force spectroscopy.
Results: Anti-PF4/P-antibodies activating platelets only in the presence of polyanions show binding forces to PF4/P-complexes <60pN; antibodies activating platelets in the absence of polyanions (autoimmune-HIT antibodies) show much higher binding forces ~100pN. Most interestingly, these anti-PF4-autoantibodies substitute for heparin. They bind to PF4 alone and thereby induce the same change in the conformation of PF4 as heparin does. This induces exposure of the HIT neo-epitope, and re-

cruits physiologic, polyanion-dependent anti-PF4/P-antibodies into the autoimmune-HIT process, resulting in massive, heparin-independent platelet activation. Antibody-mediated changes in endogenous proteins triggering binding of otherwise non-pathogenic antibodies may also be important for other antibody-mediated autoimmune disorders.
Conclusion: We show that PF4/P antibodies mediate an innate bacterial defense mechanism and provide a hypothesis why recent infections are often linked with autoimmune disorders.

Conflict of interest: none

VS-17-3

Autoantibody mediated glycan modification: potential impact on platelet lifespan in autoimmune thrombocytopenia

Zlamal J.[1], *Marini I.*[1], *Jouni R.*[2], *Rigoni F.*[1], *Bakchoul T.*[1,2]

[1]Medical Faculty Tübingen, University Hospital Tübingen, Tübingen, Germany
[2]Center for Clinical Transfusion Medicine Tübingen, Tübingen, Germany

Introduction: Immune thrombocytopenia (ITP) is a bleeding disorder caused by autoantibodies (AAbs) that recognize glycoproteins (GPs) expressed on platelet (PLT) surface. A recent study identified an Fc-independent PLT clearance by murine PLT-reactive antibodies. Anti-GPIb antibodies were shown to be able to induce cleavage of sialic acid (desialylation) of GPs leading to PLT binding to the hepatic Ashwell-Morell-Receptor (AMR) and subsequent destruction. In this study, we investigate the impact of AAbs from ITP patients on the glycan pattern of PLTs and the consequent effect on their survival *in vivo*.
Methods: The glycan modification of GPs induced by AAbs from ITP sera was analyzed using lectin binding assay (LBA). In LBA two lectins; Ricinus communis agluttinin (RCA) and Erythrina cristagalli lectin (ECL) that bind to galactose and N-Acetyllactosamin, respectively, were used. After incubation of PLTs with ITP or healthy donor sera the glycan changes were analyzed using flow cytometry. The impact of glycan pattern changes on PLT life-span was investigated using the NOD/SCID mouse model.
Results: A total of 16 sera from ITP patients were investigated. Different modifications of glycan pattern expression were observed after incubation with ITP sera in the LBA. 9/16 ITP sera induced a significant increase in RCA binding compared to sera from healthy donors: (median fold increase (FI): 3.69, range: 1.69–13.61). 8/16 sera induced increased binding of ECL (median FI: 1.94, range: 1.56–5.47). The impact of PLT desialylation was confirmed *in vivo* using the NOD/SCID mouse model. The injection of ITP AAbs resulted in accelerated clearance of human PLTs from the mouse circulation. The destruction of human PLTs by ITP AAbs was reduced but not completely inhibited by a specific neuraminidase inhibitor that prevents glycan changes on PLT surface (survival of human PLTs after 5h: 29%, range 22–40% vs. 48%, range 41–53%, p = 0.014, respectively).
Conclusion: Our investigations suggest that ITP AAbs are able to induce changes in glycan pattern on PLT surface. The mechanism of antibody-mediated modification of glycan pattern seems to contribute to PLT destruction *in vivo*.

Conflict of interest: none

VS-18
Hämostaseologie III

VS-18-1

Von Willebrand Factor: from DNA to the circulating factor VIII/VWF-complex

Schneppenheim R.[1], *Schneppenheim S.*[2]

[1]Universitätsklinikum Hamburg-Eppendorf, Klinik für Pädiatrische Hämatologie und Onkologie, Hamburg, Deutschland
[2]AK Altona, Medilys Laboratory mbH, Hamburg, Deutschland

Von Willebrand Factor (VWF) is the largest circulating blood protein with manifold functions in hemostasis. The primary translation product of 2813 amino acids in endothelial cells and megakaryocytes consists of a signal peptide, a pro-peptide and the mature VWF-monomer. Two of them dimerize in the ER at their carboxyterminal CK-domains. Further polymerisation of dimers up to giant VWF multimers, is catalyzed by the VWF pro-peptide, which exhibits disulfide isomerase activity, but no other known functions. Mature VWF possesses different binding sites for collagen in the vessel wall, platelet glycoproteins and Factor VIII. This allows initiation of primary hemostasis by platelet adhesion and aggregation at the injured vessel wall and concurrently the essential local concentration of coagulation FVIII. As a mechano-responsive protein, VWF function in primary hemostasis is shear-dependent, and plays its most important role in capillaries and in the arterial circulation. While circulating as a globular protein in the normal blood stream, VWF unfolds after adhesion at a vessel lesion under shear conditions to a long protein string, thereby exposing its specific functional binding domains. Concurrently, unfolding of the VWF-A2-domain by shear forces leads to exposition of the proteolytic cleavage site for the VWF specific protease ADAMTS13, thereby regulating VWF functional activity and preventing thromboembolic events, like TTP. Absence of VWF defines severe von Willebrand disease (VWD) type 3 with concurrent severe reduction of FVIII, whereas deficiency of individual VWF functions correlates with VWD type 2, which can be subdivided (2A, 2B, 2M, 2N) with respect to the particular compromised function. The role of VWF and its protease ADAMTS13 in cardiac and cerebral vascular events is currently subject of intensive research.

Conflict of interest: R. Schneppenheim:Mitglied in Advisory Boards (Fa. SHIRE, Fa. CSL Behring) Molekulargenetische Referenzdiagnostik für Zulassungsstudien (VWF, ADAMTS13; Fa. SHIRE)

VS-19
Molecular Typing (Neighbour Day)

VS-19-1

Routine genotyping of blood donors

Rieneck K.

Rigshospitalet, Blodbank, Kopenhagen, Dänemark

A large well characterized donor population is important in order to be able to provide compatible blood, especially under circumstances where recipients have antibodies against one or more blood group antigens.
The typing of blood donors has been performed by serotyping for many years, but based on extensive knowledge of the underlying genotypes and the relationship between phenotype and genotype as well as availability of reliable high throughput platforms for performing genotyping, it has become feasible to shift from serotyping to routine genotyping of blood donors.
We report the initial results of establishing routine blood donor genotyping using the KASP (kompetitive allele specific PCR) technology as well

as a description of the procedures established for safe application of this technology.

Conflict of interest: none

VS-19-2

Towards a regional registry of fully typed blood donors: molecular typing of blood group, platelet and granulocyte antigens

Portegys J., Rink G., Klüter H., Bugert P.

Institut für Transfusionsmedizin und Immunologie, Molekularbiologie, Mannheim, Germany

Background: Besides ABO and RH a number of additional blood group antigens have to be considered for the blood supply especially of immunized or chronically transfused patients. The same is true for platelet antigens (HPA) and neutrophil antigens (HNA) for patients receiving platelet or granulocyte concentrates. Here, we describe the molecular screening for a number of blood group, HPA and HNA antigens. Based on the screening results we build up a regional blood donor registry to provide extended matched blood products on demand.
Methods: We developed and validated TaqMan-PCR and PCR-SSP methods for 21 blood group defining SNPs, 5 SNPs defining platelet alloantigens (HPA-1, -2, -3, -5, -15) and 7 SNPs defining granulocyte antigens (HNA-1, -3, -4, -5). All data were statistically evaluated regarding genotype distribution and allele frequencies were compared to ExAC data.
Results: Blood donors of our institute were enrolled in the genotyping study since June 2016 irrespective of age, sex or ABO/Rh blood group. Using TaqMan-PCR more than 2,000 donors were screened for 37 blood group, 10 HPA and 10 HNA antigens. The screening results were confirmed by PCR-SSP methods for selected numbers of samples. The allele frequencies were similar to non-Finnish Europeans in the ExAC database except for the S, HPA-3b and HNA-4b antigens with significantly lower prevalence in our cohort. We identified 71 donors with rare blood types such as Lu(a+b-), Kp(a+b-), Fy(a-b-), Yt(a-b+), Co(a-b+) or Vel-. All genotype data will be implemented into a searchable donor registry of our blood service.
Conclusion: Molecular screening for rare blood types by using TaqMan-PCR is an adequate method that can be easily scaled to high throughput screening for additional blood groups. The knowledge of the antigen status of blood donors is a prerequisite to establish a donor registry for the supply of antigen-matched blood supply to critical patients.

Conflict of interest: none

VS-19-3

Genotyping of blood donors using Next Generation Sequencing

Weinstock C.[1], Mytilineos J.[1], Portegys J.[2], Bugert P.[2], Schrezenmeier H.[1], Fürst D.[1]

[1]DRK-Blutspendedienst Baden-Württemberg - Hessen, Institut für Klinische Transfusionsmedizin und Immungenetik Ulm, Universität, Ulm, Germany
[2]DRK-Blutspendedienst Baden-Württemberg - Hessen, Mannheim, und Institut für Transfusionsmedizin und Immunologie, Universität Heidelberg, Medizinische Fakultät Mannheim, Mannheim, Germany

Background: Extended phaenotyping of blood donors is expensive and time consuming. Next generation sequencing (NGS) techniques enable the testing of many blood group genes and polymorphisms of a large number of donors in parallel. NGS is well established for typing the human leukocyte antigens of stem cell donors, it may also be used for cost-effective typing of blood donors.
Methods: The MiSeq system (Illumina) enables typing of 768 donors in parallel. We developed an amplicon-based targeted sequencing panel with 33 primer pairs for amplification of the genomic regions encompassing 65 polymorphic sites of the blood group systems MNS, KEL, FY, JK, LU, YT, DO, CO, DI, SC, LW, IN, JR, LAN, and VEL. In addition, two sequences within exon 1 and exon 5, respectively, of the *RHCE* gene were included in order to screen for *RHCE* variants. 5 multiplex polymerase chain reactions (PCR) were generated, each containing up to 7 primer pairs. The resulting amplicons were extended for indices, which allow donor identification and adapters by a second PCR. The programme R/Bioconductor pipeline was employed for data analysis. It calls the blood group defining base(s) at the specified position in each target sequence and reports the genotype.
Results: 768 DNA samples were typed in parallel, coming to 25,344 PCR amplicons during the first level of the sequencing process. 80 of these reactions (0.3%) failed or gave low reads (final number of sequenced templates = coverage) for data analysis. For data analysis, the cut-off for the number of amplicons required for a reliable allele call was set to 15. A typical run, however, results in a coverage of more than 100 per target.
570 of these samples had been typed earlier by a taqman genotyping assay. Comparing the results, 23 discrepant allele calls were found. 10 discrepant results were caused by poor amplification of the sequence of the *DO* gene. Adjustment of the primer concentration in the first PCR improved the results in subsequent runs. Coverages near the cut-off were the reason for one false *LU*, *FY* and *YT* allele, respectively. Discrepant *VEL* genotypes and 1 discrepant *YT* genotype were found to be correctly typed by the new NGS method. 1 discrepant *FY* genotype was shown by Sanger sequencing to be caused by a new allele, which was not recognised by NGS and was typed falsely as *FY*02* by the taqman method.
Summary/conclusion: The Illumina technique allows parallel typing of 768 blood donors. The newly developed test system was found to be suitable, reliable, and cost effective. In the present setup the panel tests for frequent alleles (e.g. in the systems MNS, KEL, FY, or JK) and also screens for rare alleles or null alleles (e.g. in the systems IN, VEL, JR, LAN), but it easily can be customised by addition of new alleles or removal of unwanted alleles.

Conflict of interest: none

VS-19-4

CD36 variation in Arabs is associated with a higher rate of platelet CD36 deficiency

Flesch B. K.[1], Steitz M.[2], Janson A.[2], Ochmann O.[2], Opitz A.[2]

[1]DRK Blutspendedienst West, Bad Kreuznach, Germany
[2]DRK Blutspendedienst Rheinland-Pfalz und Saarland, Bad Kreuznach, Germany

Introduction: The CD36 (GPIV=Naka) isoantigen is expressed on platelets and monocytes. Lack of CD36 can present as type I deficiency with neither platelets nor monocytes expressing CD36 and type II deficiency with only platelets lacking GPIV. Only individuals with type I deficiency can develop CD36 isoantibodies than have been reported in cases of fetal/neonatal alloimmune thrombocytopenia (F/NAIT) and platelet refractoriness. In Caucasians a deficiency on either cell type is virtually unknown whereas in African and Eastern Asian populations between 3 and 10% show a negative expression at least on platelets. Little is known about the distribution of CD36 in Arabs.
Methods: The CD36 phenotype was determined by flow cytometry on platelets of 772 blood donors and volunteers originating from Syria, 132 from other Arabian countries like Iraq and Libanon and 147 donors from Iran. In the case of a CD36 negative platelet phenotype genomic DNA sequencing followed including amplification of the 14 *CD36* exons including short flanking intron sequences and the promoter region. Cycle sequencing was performed with the Big Dye Terminator v3.1 chemistry (ABI, Weiterstadt, Germany) followed by electrophoretic separation in an ABI Prism 310 DNA analyser.
Results: A total of 25 platelet samples (2.4%) showed a CD36 negative phenotype in flow cytometry and another eight samples (0.8%) had borderline MFI values. In 26/28 samples a wide variety of different known or new *CD36* mutations were detected, many of them inducing a premature stop or frame shift. In some cases however, only one chromosome was affected, so that the molecular reason for the CD36 negative phenotype

on platelets in these cases could not be finally elucidated. Three of the CD36 negative Syrian probands were female, but none had developed a CD36 isoantibody.
Conclusion: Further studies on the molecular background of the CD36 negative phenotype and the type of deficiency in Arabs have to follow. Especially cDNA sequencing in samples derived from platelets and monocytes could enlighten differential transcription in those cases with only heterozygous mutations and add insights into the clinical significance of the findings.

Conflict of interest: Es bestehen keine Interessenkonflikte. Alle Autoren sind Mitarbeiter des DRK Blutspendedienst Rheinland-Pfalz und Saarland, bzw. DRK Blutspendedienst West.

VS-19-5

The *CD177*787A>T* polymorphism and regulation of transcription account for differential expression in HNA-2 deficient and HNA-2 positive individuals

Flesch B. K.[1], Nogués N.[2], Canals C.[2], Bugert P.[3], Schönbacher M.[4], Huiskes E.[5], Porcelijn L.[5], Höglund P.[6], Ratcliffe P.[6], Kerchrom H.[7], Bayat B.[8], Reil A.[9]

[1]DRK Blutspendedienst West, Bad Kreuznach, Germany
[2]Banc de Sang i Teixits, Barcelona, Spain
[3]DRK Blutspendedienst Baden-Württemberg Hessen, Mannheim, Germany
[4]Medizinische Universität Wien, Wien, Austria
[5]Sanquin, Amsterdam, Netherlands
[6]Karolinska University Hospital, Stockholm, Sweden
[7]MNH, Nantes, France
[8]Justus-Liebig-Universität Gießen, Gießen, Germany
[9]DRK Blutspendedienst West, Hagen, Germany

Introduction: The human neutrophil antigen 2 (HNA-2) bears some unique characteristics. Most individuals show two neutrophil subsets with one proportion being HNA-2 negative. A complete deficiency (HNA-2null) in 2–3% of Caucasians enables the formation of maternal isoantibodies that can induce immune neonatal neutropenia. A *CD177*787A>T* (rs879198465) substitution that induces a premature stop causes HNA-2 deficiency and also is thought to be associated with the percentage of HNA-2 positive neutrophils (Li Y et al.: 2015, Bayat B et al.: 2016). But not all tested individuals matched this observation. Recently, a monoallelic expression was added (Eulenberg-Gustavus C et al.: 2017). To gain further insights into the molecular basis of the HNA-2null phenotype and the differential neutrophil subsets, a multi-center comprehensive study has been undertaken.
Methods: A total of 64 samples with HNA-2 negative or questionable phenotype from 9 centers, including HNA-2null blood donors and immunized women with anti-HNA-2 antibodies as well as 31 HNA-2 positive blood donors were analyzed in this study. All samples were phenotyped by validated granulocyte serology methods and genotyped based on exon 7 gDNA sequencing applying the long range PCR protocol of Bayat B et al.: in order to discriminate the *CD177* gene from the shorter *CD177P* pseudo gene. Additionally, cDNA isolated from peripheral blood or isolated neutrophils was sequenced.
Results: A total of 29/40 HNA-2null donors were homozygous for the CD177**787A>T* mutation, seven were heterozygous and four homozygously carried the *787AA* wild type (WT). 14/16 immunized HNA-2 negative women carried the homozygous mutation whereas two were heterozygous. From eight **787T* homozygous mothers also samples of their affected neonates were available. Six babies were heterozygous as expected whereas four only carried the WT type without the maternal haplotype. In twin babies this was due to an oocyte donation, but in the two remaining cases no explanation could be found. HNA-2 positive donors with a positive neutrophil subset of more than 40% preferentially exclusively carried the WT, whereas samples with a lower proportion of positive neutrophils predominantly were heterozygous. Sequencing of cDNA in 29/30 individuals only demonstrated one haplotype, even if the gDNA analysis demonstrated heterozygosity (9/30). In the latter cases only the wild type allele was detected on the cDNA level which fits well to a monoallelic expression.
Conclusion: Most HNA-null individuals carry the *CD177*787A>T* mutation, but some cases remain unresolved. The percentage of HNA-2 positive neutrophils seems to be influenced by both, monoallelic expression and the *CD177*787* polymorphism but the regulatory elements are still unknown. Additional investigations to comprehensively explain the HNA-2 pheno- and genotypes as well as transcription need to be done.

Conflict of interest: none

VS-19-6

Blood group MNSs, U negativity in black Africans is molecularly defined by two prevalent *GYPB* deletions

Gassner C.[1], Denomme G. A.[2], Portmann C.[1], Meyer S.[1], Trost N.[1], Jungauer C.[3], Just B.[4], Storry J.[5], Forster M.[6], Franke A.[6], Frey B. M.[7]

[1]Blood Transfusion Service Zurich, Molecular Diagnostics and Research & Development, Zürich-Schlieren, Switzerland
[2]Blood Research Institute, Versiti, Inc., Milwaukee, WI, United States
[3]Austrian Red Cross, Blood Service for Vienna, Lower Austria and Burgenland, Wien, Austria
[4]German Red Cross Blood Donation Service West, Hagen, Germany
[5]Department of Laboratory, Hematology & Transfusion Medicine, Lund, Sweden
[6]Christian Albrechts University of Kiel, Institute of Clinical Molecular Biology, Kiel, Germany
[7]Blood Transfusion Service Zurich, Head Office, Zurich-Schlieren, Switzerland

Introduction: The U antigen (MNS5) was originally described in 1953 and was characterized as a high-frequency-antigen that is absent in 1.2% of African Americans (Wiener, JAMA, 1953). In 1954, the association with the MNS blood group system and concurrent S-s- phenotype became evident (Greenwalt, PNAS, 1954), and was later postulated to be caused by a homozygous *GYPB* deletion (Huang, Blood, 1987). This study aimed for an exact molecular definition of *GYPB* deletions, causative of recessive negativity in phenotype S-s-U- Black Africans, in order to allow for their positive molecular detection.
Methods: Bioinformatical analysis of the 1000 Human Genomes (1000G) data revealed several hits for two distinct ~100 kb and one hit each for a ~32 kb and ~18 kb *GYPB* deletion, all identified among Black Africans. Sanger sequencing of gap-PCRs bridging these deletions in predefined S-s-U- samples revealed the deletions» exact molecular positions and were used to design specific diagnostic PCRs using sequence-specific priming (PCR-SSP). Subsequent validation genotyping was performed in 24 samples of known S-s-U- phenotype, including 2 1000G samples of the Coriell Human Genetic Cell Repository, showing the ~32 and ~18 kb deletions.
Results: One 110.24 kb deletion stretched from 4.96 kb 5" of the *GYPB* start codon until 8.51 kb 5" of the *GYPE* start codon. The 103.26 kb deletion started 16.42 kb 3" of the *GYPA* stop codon and ended 4.58 kb 3" of the *GYPB* stop codon. Both deletions encompassed the whole *GYPB* gene and involved paralogous intergenic sequences. Of 24 validation samples, 13 genotyped as *GYPB*05N(del110kb)* homozygotes, 7 *GYPB*05N(del110kb)/(del103kb)* heterozygotes, one *GYPB*05N(del103kb)* homozygote and 3 were heterozygous for *GYPB*05N(del110kb)* and a yet undefined, second parental *GYPB*-deletion. The originally expected ~32 kb deletion in one Coriell sample, was in fact a *GYPB*05N(del110kb)/(del103kb)* heterozygote, rather than representing a unique deletion. The ~18 kb deletion was only observed in the other Coriell sample. Of 48 S-s-U- haplotypes with a presumptive *GYPB* deletion analysed, 35 (72.9%) were *GYPB*05N(del110kb)*, 9(18.8%) were *GYPB*05N(del103kb)*, and 3(6.3%) remained unresolved. Overall haplotype-frequency was estimated to be 11.0%, consistent with the 1.2% S-s-U- phenotype frequency reported in Black Africans.
Conclusions: This study describes three causal *GYPB* deletions underlying the S-s-U- phenotype, and genotyping assays for the molecular detection of such carriers This allows for unequivocal results and correct phenotype predictions of all genotypes involved, when performed si-

multaneously with classical genotyping for S, s, and Uvar alleles. With an observed 1.2% S−s−U− phenotype frequency, hemizygous involvement of such *GYPB* deletions may be expected in about 19.5% of all Black Americans. Recent reports of *GYPB* deletional haplotypes are further supportive of our independent findings (Leffler, Science, 2017).

Conflict of interest: C.Gassner acts as a consultant to InnoTrain GmbH, Kronberg i.T. Blood Transfusion Service Zurich and Christoph Gassner have filed a patent on the topic of GYPB deletional haplotypes and their moleculare detection in the context of MNSs blood group S-s-U- negativity.

VS-19-7

Phenotype and exome NGS analysis of a case of severe congenital anemia

Dziegiel M. H.[1], *Hansen A. T.*[1], *Lausen B.*[2], *Rieneck K.*[1]

[1]Copenhagen University Hospital, Department of Clinical Immunology, Copenhagen, Dänemark
[2]Copenhagen University Hospital, Department of Pediatrics and Adolescent Medicine, Copenhagen, Deutschland

Introduction: We report on a case of congenital anemia in a male infant. Transfusion was necessary from the first days of life and was regularly repeated every 6th week throughout the first 5 years. Intensive examination of infant and mother did not reveal antibodies that could explain the anemia. Other attempts to elucidate the cause were inconclusive or negative. Specifically, EMA for cytoskeletal variants was negative or inconclusive, probably due to interference from donor erythrocytes.
Methods & Results: At the age of 3 year we examined erythrocytes in a flow cytometric assay of osmotic resistance. A significant deviation from normal was observed, corresponding to severely reduced osmotic resistance. The mother, father, a healthy brother, and the boy (extended trio) were examined by exome sequencing by NGS. This showed that the boy was compound heterozygotic with two variants in the erythrocytic spectrin alpha 1 (*SPTA1*) gene, a very rare missense mutation, and a novel stop mutation.
Conclusion: The combined phenotypic and genetic analyses strongly suggest that compound heterozygosity in the *SPTA1* gene underlies the clinical findings. Splenectomy at the age of 5 eliminated the requirement for transfusion.

Conflict of interest: none

PS-1A
Hämatopoetische Stammzellen

PS-1A-2

Dual-needle stem cell apheresis: technical incidents and side-effects

Flommersfeld S.[1], Marschall R.[1], Bein G.[2], Sachs U. J.[1]

[1]University Hospital Giessen and Marburg, Transfusion Medicine, Marburg, Germany
[2]University Hospital Giessen and Marburg, Institute for Clinical Immunology and Transfusion Medicine, Gießen, Germany

Background: Peripheral blood progenitor cells (PBPC) are the most common stem cell source for autologous and allogeneic transplantation. In Marburg, stem cell collections are performed with Spectra apheresis devices (COBE Spectra from 2010 to 2015 and Spectra Optia from 2015 onwards; from Terumo BCT, Garching, Germany). Both devices are dual-needle systems with continuous blood flow and citrate as anticoagulant. Here, we report on technical incidents and side effects in patients and donors.
Material and Methods: Apheresis protocols of 316 autologous and 105 allogenic procedures in 275 patients and 93 donors were analysed retrospectively for the period between November 2010 and April 2018.
Results: In the autologous setting, 294/316 procedures were performed via peripheral vein access using a 18 G catheter; 27 patients (10%) required change of access site during the procedure (range, 1 to 5 changes per patient). 12/316 patients (4%) were primarily scheduled for central venous access (CVA) after inspection of their peripheral access sites. In addition, 5 patients with initial peripheral access were switched to CVA because no sufficient flow could be established.
In allogenic donors, all 105 procedures could be performed via peripheral access; 6 donors (6%) required change of peripheral access during the procedure (range, 1 to 3 changes).
Collection was significantly faster with CVA (177 vs. 219 min; p = 0.008), while pre CD34-count was not significantly different between these two groups.
Clinical side effects were observed in 63/316 autologous procedures (20%) and included citrate reactions (n = 5), vasovagal reactions and/or nausea (n = 15), pain (n = 35), and agitation (n = 8). Red blood cells or platelet transfusions were required in 11 patients. Technical incidents led to an early termination in n = 4 procedures (venous access = 2, apheresis system failure = 2), one procedure could not be initiated because no flow could be established over the CVA.
In the allogeneic setting, side effects were recorded in 50/105 procedures (48%) and included citrate reactions (n = 15), vasovagal reactions and/or nausea (n = 7), pain (n = 20), and agitation (n = 8). Technical incidents led to an early termination in n = 8 procedures (citrate reaction = 1, venous access = 2, apheresis system failure = 5). Aggregates in the collection system were recorded in 11/105 allogeneic procedures.
Conclusions: Collection of PBSC can be reliably performed via peripheral vein access in the majority of patients (95%) and in all allogeneic donors. Occasionally, a periprocedural change of the venous access site may be necessary. Early termination of the collection because of the access site is a rare event (0.9%) and not more frequent than early termination because of apheresis system failures (1.6%). Clinical side effects during stem cell collection are mild and easily manageable.

Conflict of interest: Alle Autoren sind wissenschaftliche Angestellte des Landes Hessen, es bestehen keine Interessenkonflikte in Bezug auf diese Arbeit.

PS-1A-3

Red blood cell depletion of bone marrow: comparison of two cell separators and first experience with small volume grafts

Rosskopf K.[1], Sipurzynski S.[1], Posch U.[1], Schwinger W.[2], Torreiter P.[1], Kovacic K.[1], Schlenke P.[1]

[1]Med. Uni. Graz, Univ. Klinik für Blutgruppenserologie und Transfusionsmedizin, Graz, Austria
[2]Med. Uni. Graz, Pädiatrische Hämatoonkologie, Graz, Austria

Red blood cell (RBC) depletion of bone marrow is advisable in ABO major incompatible transplantation and – in the autologous setting – before cryopreservation of the bone marrow (BM) graft. We report about our experience with two cell separators, the Cobe Spectra and the Spectra Optia, and present first data of red blood cell depletion of small volume bone marrow.
In the first period 11 BM grafts (8 allogeneic, 3 autologous) were RBC depleted with Cobe Spectra (CS; 1999–2009) and in the second period 11 BM grafts (8 allogeneic, 3 autologous) were RBC depleted with Spectra Optia (SO; 2013–2017) including 3 small volume (SV) grafts (all autologous) with the addition of third party packed red cells so that the RBC volume surpassed the limit of 125 ml for cell separation. Recovery rates for mononuclear cells (MNC), CD34+ cells and the volume of the remaining RBC were calculated for the RBC depleted BM. Data are presented as median (range).
The total volume of the BM before manipulation and after RBC depletion was 1,43L (0,77–2,35) and 0,13L (0,11–0,30) for CS, 1,21L (0,47–2,09) and 0,15L (0,08–0,24) for OS, 0,49l (0,47–0,68) and 0,11L (0,10–0,11) for SV. The recovery of MNC in the RBC depleted graft was 91% (40–128) for CS, 83% (51–123) for SO and 80% (51–85) for SV. The recovery of CD34+ cells was 113% (59–177) for CS, 96% (79–111) for SO and 88% (82–101) for SV. Remaining RBC volume was 7ml (4–15) for CS, 7ml (4–17) for SO and 5ml (4–8) for SV, respectively.
The results show feasibility and safety of RBC depletion with both devices regarding cell recovery and RBC reduction. There were no significant differences between the two cell separators regarding MNC recovery and CD34 recovery, respectivly. The loss of CD34+ cells in the three runs of small volume grafts were acceptable to achieve a transplantation dose in each case.

Conflict of interest: none

PS-1A-4

Frequency and spectrum of bacterial contamination of autologous cord blood units

Strasser E.[1], May C.[1], Kunz B.[2], Hackstein H.[1]

[1]Universitätsklinikum Erlangen, Transfusionsmedizinische und Hämostaseologische Abteilung, Erlangen, Germany
[2]Universitätsklinikum Erlangen, Institut für klinische Mikrobiologie, Immunologie und Hygiene, Erlangen, Germany

Introduction: A retrospective analysis was performed to analyze the frequency of bacterial contamination, as well as the specificity of gram positive and gram negative bacteria in autologous cord blood (CB) samples screened for antibiotic sensitivity.
Methods: 6,075 autologous cord blood samples were analyzed from the original CB bag and after leucocyte preparation (Rubinstein method). As method of sterility testing BACTEC Standard Aerob/F and Anaerob/F medium was used and handled as prescribed by the supplier (Becton Dickinson). 0.5 ml of CB suspension was added to the BACTEC media. Aerobic and anaerobic testing was performed by the Institute of Clinical Microbiology, Immunology and Hygiene, University Clinic of Erlangen, by use of the BACTEC FX device for cultivation. In case of bacterial detec-

tion, bacterial subcultures were performed and analyzed by MALDI-TOF mass spectrometry. Finally, the sensitivity of antibiotic chemotherapy was tested automatically by the VITEK 2 XL device (BioMerieux).
Results: Overall, the frequency of contamination was 6.68%, 406 positive tested CB samples (n = 6,075). Sixty-eight different bacterial strains were identified by MALDI-TOF mass spectrometry. The most frequently found bacterial species were coagulase-negative Staphylococcus aureus (114/6,075; 1.88%), followed by Escherichia coli (51/6,075; 0.84%), Enterococcus faecalis (47/6,075; 0.77%), Propionibacterum acnes (28/6,075; 0.46%), Bacterioides uniformis (26/6,075; 0.42%), and Corynebacterium sp. (21/6,075; 0.34%). 65.8% of CB samples were contaminated with gram-positive bacteria, 41.6% of CB samples contained gram-negative bacteria. For 517 of 526 detected microbes (98.3%) antibiotic sensitivity testing methods (antibiogram) were performed and the classification of CSLI criteria was applied (results: sensible, intermediate, resistant). Thus, antibiotic chemotherapy was evaluated for the different identified bacteria in CB units.
Conclusion: CB units show a low frequency of bacterial contamination between 3% and 6% and an balanced composition of gram-positive and gram-negative bacteria which are partially sensitive to antibiotic chemotherapy. The use of contaminated CB samples in cellular research needs validation of efficient antibiotic protocols.

Conflict of interest: none

PS-1A-5

Epigenetic mechanisms control of hematopoietic lineage differentiation at the erythroid/megakaryocytic branching

Lausen J., Herkt S., Kuvardina O., Meyer A., Schneider L., Bonig H., Seifried E.

DRK- Blutspendedienst, Institute for Transfusion Medicine and Immunohematology, Frankfurt am Main, Germany

Background: During hematopoiesis lineage fate decisions are decisive for a balanced supply of functional blood cells. Transcription factors and associated epigenetic processes control differentiation of distinct blood cell types. The knowledge of molecular mechanisms, which drive hematopoiesis can provide means to influence cell type specification to produce defined hematopoietic cell types such as erythrocytes in cell culture.
Methods: We are studying the role of hematopoietic transcription factors and downstream epigenetic during hematopoiesis. For this we employ use a combination of proteomics and transcriptomics using CD34+ progenitor cells. We are studying epigenetic alterations during differentiation using chromatin immunoprecipitation. We utilize differentiation assays to define the role of transcription factors and their epigenetic cofactors.
Results: We have unravelled a gene regulatory network of transcription factors, microRNAs and epigenetic cofactors, which drives erythroid differentiation. Changes within this network alter differentiation. Manipulation of epigenetic cofactor-function increases erythroid differentiation by triggering erythroid gene expression. This can also be achieved by using small molecule inhibitors, which target the epigenetic landscape at master genes.
Conclusion: Our data show that manipulation of a gene regulatory epigenetic network alters the balance between megakaryocytic and erythroid differentiation. This way *in vitro* differentiation of erythrocytes or megakaryocytes can specifically be enhanced.

Conflict of interest: none

PS-1A-6

Follow-up outcomes after transplantation of unrelated single cord blood units provided by DKMS cord blood bank

Lüdemann J., Wobus A., Aurich A. C., Raab S., Sauer A., Schmidt A. H., Platz A.

DKMS Nabelschnurblutbank, Dresden, Germany

Introduction: To date umbilical cord blood (CB) is used for treatment of more than 80 different diseases. The number of CB stem cells is limited due to the low blood volume and therefore mainly used in pediatrics. In total DKMS Cord Blood Bank released 95 cord blood units (CBU) to children and adults. Here we summarize outcome data of cord blood transplantation (CBT), e. g. engraftment, graft versus host disease (GvHD) and survival of unrelated single cord blood units transplanted from January 2010 to March 2018.
Methods: Follow-up (FU) data were collected by Eurocord Registry. All CBUs requested in the selected period (n = 89) were used in allogeneic CBT. This analysis focuses on single transplants with available FU data (n = 51) and provides information of appearance of acute GvHD as one of the major complications of allogeneic stem cell transplantation. The data for neutrophil and platelet engraftment following CBT were analyzed as well. Probability of survival was estimated by Kaplan-Meier method.
Results: Overall 87% of unrelated single CBT were used for children with a median age of 1 year (0–16) and 13% were performed in adult patients with a median age of 54 years (27–61 y). Indications were hematological diseases (57%), immune system (25%) and metabolic diseases (16%). One CBU was requested for treatment of neurodegenerative disease. The ratio between CBT for treatment of malignant and non-malignant diseases is balanced (n = 25 vs. n = 26). With a range of 1.0 to 70.9 months the median FU is 9.0 months. Among the 51 evaluated patients 48 showed sustainable neutrophils engraftment within 43 days (median 20 d). Two patients died before engraftment. The median time to platelet engraftment (n = 39) was 37 days (16–92 d). Nine patients died before platelet engraftment. Data referring to acute GvHD are available for all 51 patients including 31 without a reaction or grade I. 20 patients experienced acute GvHD grade ≥II, among them 7 showed grade ≥III. For patients with malignant diseases the probability of survival at median FU (6.5 months) is 55% and for patients with non-malignant diseases at median FU (13.5 months) is 76%.
Conclusion: The field of application in malignant and non-malignant diseases is wide-ranging and does not only include hematological diseases. Our data confirm that CB is an alternative source for hematopoietic stem cells with a low incidence of high grade acute GvHD. The time of neutrophil and platelet engraftment was in line with published data. Patients treated with CB to cure non-malignant diseases showed higher probability of survival than patients suffering from malignant diseases. In summary no deaths and severe adverse events related to the transplanted CBU had been recorded.

Conflict of interest: none

PS-1A-7

Influence of ABO-incompatibility on the outcome of stem cell transplantation – a retrospective stuy on children and young adults

Witt V., Pichler H.

St. Anna Kinderspital, pädiatrische Apherese, Gewebebank, Wien, Austria

In hematopoietic stem cell transplantation (HSCT) hematopoietic stem cells of variable donors and sources are administered to patients with the aim of repopulating or replacing the hematopoietic system. The main criteria of finding a suitable donor is whether they have matching HLA-antigens with the patient. Other factors such as age, sex, number of parities, CMV-serostatus and ABO-compatibility are only secondary. ABO-incompatibility is present in approximately 50% of all HSCTs but it is vastly unclear to what extent it affects the outcome.

In this study 573 cases of allogenic HSCTs performed at the St. Anna Kinderspital in Vienna between 1980 and 2016 were retrospectively analysed to check if ABO-incompatibility has an influence the outcome. A wide range of patients differing in age (mean age 8,9 years, range 0–26 years), underlying disease (leukaemia, non-malignant disease, solid tumour and lymphoproliferative disease), stem cell graft source (bone marrow, peripheral blood stem cells and umbilical blood) and the type of donor were included. 294 (51,3%) of the analysed cases were ABO-compatible whereas 113 (19,7%) were major-, 114 (19,9%) minor- and 52 (9,1%) bidirectionally incompatible. In univariate as well as multivariate analysis ABO-incompatibility had no influence on the overall survival. A significant effect on the non-relapse mortality (NRM), the rate of successful engraftment, the rate of rejections, the overall transfusion requirements, the engraftment of thrombocytes, the incidence of acute or chronic graft-versus-host disease (GvHD) or the risk of having a positive antiglobulin test post-HSCT could not be observed either. However, some of the analyses (NRM, engraftment, transfusion requirements) showed a trend towards a possible influence. Therefore, it cannot be excluded that an actual influence was underestimated due to an insufficient number of included cases or the applied statistical methods. Additional larger multicentre studies and meta-analysis are required to further investigate if ABO-incompatibility leads to an increased mortality or morbidity. This is the first study including solely paediatric patients. Children are different from adults in several aspects. Thus, it is important that particular attention is paid to this group of patients in future studies.

Conflict of interest: none

PS-1A-8

Is the minimal residual disease (MRD) in HPC apheresis products in patients with neuroblastoma of importance for the prognosis – A retrospective data analysis.

Witt V., Pichler H.

St. Anna Kinderspital, pädiatrische Apherese, Gewebebank, Wien, Austria

Background: The question arises whether residual neuroblastoma cells in autologous stem cell grafts increase the risk of relapse when being reinfused. Apheresis products, as well as bone marrow and peripheral blood, are analyzed to detect residual neuroblastoma cells, but the clinical significance of this minimal residual disease (MRD) and the impact on the outcome remain controversial.
Aim: In the following thesis the contamination status in peripheral stem cell grafts (=apheresis products) and in the corresponding bone marrow samples at the end of induction will be investigated and the impact of MRD detection in apheresis products on the survival will be evaluated.
Design: Retrospective data analysis.
Participants and methods: In total 228 apheresis products from 181 children with high-risk neuroblastoma were analyzed for minimal residual disease detection at the CCRI from 2000 to 2017. Correlations between contamination of apheresis products and corresponding bone marrow samples were investigated. Survival probability depending on the MRD status was calculated by using Kaplan-Meier survival analysis.
Results: Frequency of positive detected apheresis products was low with 12% (13/113 patients or 17/145 samples). Tumor cells were detected in apheresis and in the corresponding bone marrow in 12% (12/100) whereas 49% (49/100) showed negativity in both. In 39% (39/100) tumor cells were detected in the bone marrow but not in the corresponding apheresis. 0% (0/100) showed positivity in the apheresis and negativity in the bone marrow. Therefore only 23,5% of all positive bone marrow samples at the end of induction showed positivity in the apheresis product too and 76,5% were still negative despite detected neuroblastoma cells in the corresponding bone marrow. There is no significant difference in survival between patients with a positive or patients with a negative apheresis product ($p = 0,656$) whereas bone marrow contamination significantly correlated with the survival ($p = 0,007$). Overall survival of patients observed in this analysis was 43,3% for female and 44,5% for male patients with significant differences within the age groups.
Conclusion: Because tumor cell contamination rates of apheresis products are much lower than contamination rates of the corresponding bone marrow samples, apheresis procedure may work as a kind of purging or there might not is a directly exchange of the bone marrow and the peripheral blood at the time point of apheresis as it is known from haematological tumor spread.

Conflict of interest: none

PS-1A-9

Effect of graft quality parameters on outcome in autologous HPC transplantation assessed by Poisson and binomial logistic regression: role of a novel luminometric stem cell assay

Trost S.[1], Ricard I.[2], Ostermann H.[3], Humpe A.[1], Wagner B.[1]

[1]University Hospital, Ludwig-Maximilians Universität München, Department of Transfusion Medicine, Cell Therapeutics and Hemostasteology, München, Germany
[2]Ludwig-Maximilians Universität München, Institute for Medical Information Processing, Biometry and Epidemiology, München, Germany
[3]University Hospital, Ludwig-Maximilians Universität München, Clinic of Internal Medicine III, München, Germany

Introduction: The quality of hematopoietic progenitor cell (HPC grafts is routinely determined by non-functional and functional assays. Colony-forming unit (CFU) assays in cytokine-supplemented semisolid media have been used as a standard for measuring HPC proliferation potential. Microscopic scoring is a source of investigator-dependent bias thus hampering standardization. Luminometry allows for quantification of cellular ATP. It has been suggested as an alternative tool to investigate the proliferation potential of HPCs in grafts.
Methods: 132 cryopreserved autologous HPC-As from 109 patients with multiple myeloma, NHL or other malignancies receiving high dose therapy (HDT)/autologous HPC rescue were analysed.
20 -250 CD34+ HPCs/well were inoculated in liquid media supplemented by various hematopoietic cytokines and incubated for 7 days at 37°C and 5% CO2. ATP was determined after cell lysis by readout in a microplate luminometer. The results of this HALO (Hematopoietic/Hemotoxicity Assay via Luminescence Output) method were compared to CFU-GM and BFU/CFU-E as assessed with semisolid media after 14 days´ culture, and CD34+ quantification by flow cytometry. Number of chemotherapies, number of harvests, CD34+ counts at apheresis, blood counts (BC) before HDT and diagnosis (independent variables) were analysed for their effect on the dependent variables hematopoietic reconstitution (WBC >1/nl, PLT >20/nl), transfusions (RBCs, PCs) and length of in-hospital stay (LOS) by Poisson regression and binomial logistic regression.
Results: Luminometric HALO readouts were consistently dependent on the number of input CD34+ cells. Between the 4 different media, stimulating growth of HALO-CFU-GM, -CFU-MK, -BFU-E and -CFU-GEMM, respectively, values were highly correlated. This was in contrast to a rather low correlation ($R< 0.6$) with other graft quality parameters.
Poisson regression revealed consistent effects of diagnosis and Hb in BC before HDT on RBCs and PCs transfused, as well as diagnosis alone on LOS. Binomial logistic regression showed similar results: fast WBC reconstitution (< 10d) could not be predicted reliably, but favourable MK reconstitution (<11d), transfusion need (< 2RBCs, <3PCs) and LOS (<24d) were affected by diagnosis. Hb in BC before HDT was predictive for few RBC transfusions as well as short LOS. The graft quality parameters CD34+ HPCs and ATP measured with HALO-GEMM significantly affected WBC and MK reconstitution in univariate analysis only.
Conclusion: The luminometric assay yielded consistent results for measuring HPC function. But like all parameters of graft quality no significant effect on outcome after HDT/HPC rescue could be detected in multivar-

iate regression analysis which was in contrast to Hb in BC before HDT, a marker of restored hematopoiesis in patients.

Conflict of interest: none

PS-1B
Zelltherapie

PS-1B-1

Successful treatment of refractory chronic graft versus host disease with mini photopheresis

Hackstein H.[1], Amoros J. V.[2], Bein G.[3], Woessmann W.[4]

[1]Universitätsklinikum Erlangen, Transfusionsmedizin und Hämostaseologie, Erlangen, Germany
[2]Universitätsklinikum Valencia, Valencia, Spain
[3]Universitätsklinikum Gießen, Gießen, Germany
[4]Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany

Background: Recently we have reported results on the use of mini extracorporeal photopheresis (mini-ECP) for the treatment of acute graft versus host disease (GVHD) but the efficacy of mini-ECP in chronic GVHD is unknown. Here we present clinical data on the activity of mini-ECP for the treatment of children with therapy-refractory chronic GVHD. Safety and efficacy of mini-ECP for the long-term treatment of chronic GVHD (cGVHD) have not been described.
Study Design and Methods: A retrospective analysis of mini-ECP-treatments for children and adolescents with cGVHD was performed. The mini-ECP with 100–200 ml of whole blood was used to treat 14 patients. The median age at start of treatment was 7 years (range, 1–17 years), the median body weight 20kg (range, 8–53kg). A total of 703 mini-ECP treatments were performed. The median number of treatments per patient was 35 (range, 8–129), the median treatment duration 11 months (range, 1.4–28.5).
Results: Mini-ECP was well tolerated. A total of four adverse events occurred in three patients. Two of them were related to the ECP procedure. Complete or partial responses were observed for 10 patients. Steroids could be discontinued in 7 patients and tapered in further three. Responses were seen in skin, mouth, gastrointestinal and ocular involvement.
Conclusion: Mini-ECP represents a less invasive ECP alternative for low body weight patients with cGVHD and apheresis contraindications.

Conflict of interest: Member of the scientific advisory board of Macopharma until July 2017

PS-1B-2

Effects of IL1B on mesenchymal stromal cells in an *in vitro* trauma model

Amann E. M.[1,2], Rojewski M.[1,2], Groß A.[3], Kestler H. A.[3], Kalbitz M.[4], Huber-Lang M.[5], Brenner R.[6], Schrezenmeier H.[1,2]

[1]Universitätsklinikum Ulm, Institut für Transfusionsmedizin, Ulm, Germany
[2]Deutsches Rotes Kreuz Blutspendedienst Baden-Württemberg - Hessen, Institut für Klinische Transfusionsmedizin und Immungenetik, Ulm, Germany
[3]Universität Ulm, Institut für Medizinische Systembiologie, Ulm, Germany
[4]Universitätsklinikum Ulm, Klinik für Unfall-, Hand-, Plastische- und Wiederherstellungschirurgie, Ulm, Germany
[5]Universitätsklinikum Ulm, Institut für Klinische und Experimentelle Trauma-Immunologie, Ulm, Germany
[6]Universitätsklinikum Ulm, Sektion Biochemie der Gelenks- und Bindegewebserkrankungen, Ulm, Germany

Background: Polytrauma (PT) is a life-threatening disease and a major global burden of injury. Mesenchymal stromal cells (MSC) might be a therapeutic option for PT patients. MSC home to the site of injury, replace damaged tissue and secrete active molecules to accelerate healing. We hypothesised that priming of MSC with damage or inflammation markers can alter their functional properties. Therefore, we investigated the effect of a mixture of factors, playing a role in the early phase of PT or acute respiratory distress syndrome, on MSC which were expanded according GMP-standardized protocols.
Methods:MSC were incubated with a PT cocktail consisting of IL1B (300pg/ml, 10ng/ml), IL6 (300pg/ml, 10ng/ml), C3a (500ng/ml), C5a (10ng/ml, 100ng/ml), HMGB1 (10ng/ml, 60ng/ml), THBD (100ng/ml, 300ng/ml) and THPO (150ng/ml, 450ng/ml) in physiological (PTCL) and supra-physiological (PTCH) concentrations for different times to analyse the effect on proliferation, migration and cytokine secretion of MSC. CyQuant proliferation assay, transwell migration assay, RNA sequencing, ELISA and Multiplex assays of cell culture supernatant were performed. Classification of up- or downregulation of gene expression by RNA sequencing was based on stringent criteria (>3fold difference; false detection rate <0.001).
Results: We did not observe significant effects of PTCH, PTCL or IL1B (10ng/ml) stimulation on proliferation and migration of MSC compared to unstimulated MSC. Stimulation of MSC with PTCH, PTCL or IL1B led to significant up- or downregulation of 470, 183 and 469 genes compared to unstimulated MSC (at 6h). The intersection of differentially expressed genes in these groups was very high (92% overlap with regard to the PTCL group; 6h). Differentially expressed genes of the intersection of the PTCH group and IL1B group 6h and/or 24h after stimulation were assigned to signalling pathways. The 30 most frequently involved signalling pathways include IL1B-associated pathways like NF-kappa B signalling pathway, MAPK signalling pathway, cytokine-cytokine receptor interaction and TNF signalling pathway. The intersection of differentially expressed genes in the PTCH group and the IL1B group contained C3a, IL1B, IL6, CXCL1, CCL2, CXCL8, FGF2, MMP1, MMP10, IL1RN and TNFAIP6. Stimulation of MSC with PTCH, PTCHL and IL1B significantly altered cytokine expression profile (at protein level) of MSC time-dependently. In general, IL1B stimulation and PTCH stimulation induced secretion of IL6, CXCL1, CXCL8, VEGFA, MMP1, MMP2 and MMP10 in a similar way.
Conclusion: Stimulation of MSC with a high concentration of IL1B did not alter proliferation or migration at any measured time in this experimental setting. RNA sequencing as well as cytokine secretion profile of MSC revealed that IL1B mimics the effect of a more complex PT cocktail. Therefore, IL1B stimulation of MSC might be a valid *in vitro* model to investigate the role of MSC in trauma and to prime MSC prior to therapeutic use.

Conflict of interest: none

PS-1B-3

Role of platelets in the pathophysiology of cancer revisited

Fabricius H.

Privat, Esgrus, Germany

Introduction: Platelets facilitate tumour cell proliferation and metastasis in a very essential manner. Circulating tumour are known to acquire a coat of platelets which is essential for successful metastatic seeding. Platelets are upon contact with a tumour cell rapidly activated and release mediators and substances of importance, not only for coagulation but also for signalling in cell proliferation.
Results: Cell culture experiments demonstrate that almost all tumour cell lines and normal primary cells kept in vitro, need a serum supplement to the culture medium in order to proliferate. If this supplement is left out, cells will stop growing. The majority will perish. Platelet free plasma cannot promote cell growth.
Contrastingly, tumour cell lines, which are subjected to a short daily contact with platelets, will proliferate continuously, even without any serum supplement in their culture medium. Serum is the liquid end product of coagulation and contains signal substances released from platelets.
Conclusion: Above facts lead to the conclusion, that

a) Tumour cell lines in vitro are unable to sustain growth without external supply of signal substances which promote cell signalling. This observation contradicts one fundamental Hallmark of Cancer.
b) Cells committed to proliferation must possess a mechanism to access these signal substances in vivo.
We have demonstrated, that proliferating tumour cells in vitro possess platelet receptors and that primary cells in vitro also develop such receptors upon entering the cell cycle. These receptors gradually disappear when the primary cell lines end their log phase of cell growth. Platelets are upon docking rapidly activated, releasing theit contents in close vicinity to appropriate RTK´s on the cell surface. Tumour cells can also absorb the docking platelet.
Also, we have found a class of substances, able to prevent platelets from ligating the receptor expressed on proliferating cells. Heparins belong to this class. This observation might explain the beneficial effects of Heparins observed in Oncology and give hints about a new class of therapeutics, directed towards metastasis.

Conflict of interest: Die Versuche zu den vorliegenden Ergebnissen wurden vom Autor selbst finanziert und durchgeführt. Interessenkonflikte liegen nicht vor.

PS-1B-4

A HeLa-Cell line based method to assess bioactivity of platelet lysate as a supplement for culturing media

Lotfi R.[1,2], Geier T.[2], Winkelmann M.[1], Schrezenmeier H.[1,2]

[1]Universitätsklinikum Ulm, Institut für Transfusionsmedizin, Ulm, Germany
[2]DRK Blutspendedienst Baden-Württemberg-Hessen, Gewebe und Zellbank IKT Ulm, Ulm, Germany

Introduction: In order to avoid the transmission of diseases from animal to human being, regulatory competent authorities request replacement of xenogenic material used during the process of production of medicinal products. For culturing mesenchymal stromal cells, for instance, human platelet lysate (HPL) has been identified as a suitable supplement for culturing media in order to replace FBS. However controls are requested to quarantee functional activity of the PL. This can also help to monitor and to mimize batch-to-batch variability. Several properties of PL might be used for this purpose. As we and others previously demonstrated, PL contains a variety of growth factors. However, the whole range of biological activity of PL for ex-expansion of (stem) cells in the GMP-setting can not be attributed to a single or a few growth factors. Therefore we propose to measure proliferation of an immortalized cell line as a robust, standardized, functional quality control.
Methods: Outdated platelet concentrates (PC) were irradiated with 30 Gy and frozen at < 30 C (by controlled freezing) within 7 days following blood donation. Frozen PCs selected for HPL production were thawed for 1 hour at 37°C. Thus lysed platelets were kept for 8 to 24 hours at 4°C. Released soluble factors from lysed platelets were obtained by 10 minute centrifugation at about 5000 x g, in order to remove cell debris which would otherwise interfere with the subsequent sterile filtration step.
Activity of HPL was measured by assessing the proliferation of HeLa cells cultured in DMEM (containing 1 IE Heparin/ml and Pen/Strep) supplemented with 5, 10, or 20% HPL. HeLa cells were seeded at 0.5, 1, or 2 x103/cm2 and incubated for 3, or 4 days. FCS at 10% was used as positive control.
Manual counting, CyQuant® assay, and FACS analysis of CFSE stained Hela cells were applied to estimate proliferation.
Results: All three applied methods confirmed the activity of HPL which was equal or superior to 10% FCS in terms of supporting HeLa proliferation. CyQuant® assay was the most straight-forward method with low intra-assay variability. Comparing different culturing conditions (i.e. PL-concentration, incubation length, and seeded cell number) the condition with 5% PL, 3 days of incubation, and 2 × 103cells/cm2 showed the lowest intra-assay coefficient of variation of 12%. Comparison of HPL-driven proliferation of HeLa and other cell types, e.g. MSC, will be presented.
Conclusions: Depending on the infrastructure of the lab all three methods are suitable for assessing HPL activity within 3 days of incubation and CyQuant® assay being a robust, standardized and straight-forward method for quality control of HPL batches intended to be used in a GMP-setting.

Conflict of interest: none

PS-1B-5

Human mesenchymal stromal cells inhibit platelet activation and aggregation by CD73-converted adenosine

Bieback K.[1], Netsch P.[1], Elvers-Hornung S.[1], Uhlig S.[1], Klüter H.[1], Huck V.[2], Kirschhoefer F.[3], Brenner-Weiß G.[3], Janetzko K.[4], Solz H.[5], Wuchter P.[1], Bugert P.[1]

[1]Institut für Transfusionsmedizin und Immunologie, Mannheim, Germany
[2]Center for Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany 4 Experimental Dermatology, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany, Hamburg, Germany
[3]Institute of Functional Interfaces, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany, Karlsruhe, Germany
[4]Institute for Clinical Chemistry, Medical Faculty Mannheim, Heidelberg University, Germany, Mannheim, Germany
[5]Mannheim Clinic for Plastic Surgery, Mannheim, Germany, Mannheim, Germany

Introduction: Mesenchymal stromal cells (MSCs) are promising candidates for cell therapy. Clinical application is considered safe. Thromboembolism and instant blood-mediated inflammatory reactions (IBMIR) have been reported as minor side effects suggesting that MSC infusion may affect hemostasis. Previous studies focusing on plasmatic coagulation described both pro- and anticoagulatory activities of MSCs. We now analyzed whether MSCs can promote or inhibit platelet activation.
Methods: Effects of MSCs and MSC supernatant on platelet activation and function were studied using flow cytometry and further platelet function analyses. MSCs from bone marrow (BM), lipoaspirate (LA) and cord blood (CB) were compared to human umbilical vein endothelial cells (HUVECs) or HeLa tumor cells as inhibitory or activating cells, respectively.
Results: BM- and LA-MSCs inhibited activation and aggregation of stimulated platelets independent of the agonist used. This inhibitory effect was confirmed in diagnostic point-of-care platelet function analyses in platelet-rich-plasma and whole blood. Using inhibitors of the CD39-CD73-adenosine axis, we observed that adenosine produced by CD73 ectonucleotidase activity was largely responsible for the LA- and BM-MSCs platelet inhibitory action. With CB-MSCs, batch-dependent responses were obvious, with some batches exerting inhibition and others lacking this effect.
Conclusion: We demonstrate that MSCs can, dependent on their tissue origin, inhibit platelet activation involving adenosine produced from AMP by CD73 ectonucleotidase activity. This is of importance for safety and risk/benefit assessment regarding MSCs from different tissue sources. It may help to explain the tissue protective mode of action of MSCs. The adenosinergic pathway emerges as key mechanism by which MSCs exert hemostatic and immunomodulatory functions.

Conflict of interest: none

PS-1B-6

Manufacturing of mesenchymal stromal cells for treatment of mild to moderate osteoarthritis in the clinical trial ADIPOA2

Rojewski M.[1,2], Lotfi R.[1,2], Fleury S.[3], Nöth U.[4], Jorgensen C.[5], Barry F.[6], Schrezenmeier H.[1,2]

[1]Universitätsklinikum Ulm, Institut für Transfusionsmedizin, Ulm, Germany
[2]Deutsches Rotes Kreuz Blutspendedienst Baden-Württemberg - Hessen, Institut für Klinische Transfusionsmedizin und Immungenetik Ulm, Ulm, Germany
[3]Établissement français du sang Occitanie, Institut Universitaire du Cancer – Oncopôle, Toulouse, France
[4]Evangelisches Waldkrankenhaus Spandau, Orthopädie und Unfallchirurgie, Berlin, Germany
[5]Centre Hospitalier Universitaire de Montpellier, Montpellier, France
[6]National University of Ireland, Regenerative Medicine Institute, Galway, Ireland

Introduction: Osteoarthritis (OA) is the 11th highest contributor of global disability. Replacement of the joint often remains the only possibility to overcome immobility, causing high costs for hospitalization and rehabilitation therapy. The use of adipose derived mesenchymal stromal cells (ASC) may offer an innovative regenerative therapy.
Methods: Patients with mild to moderate OA (Kallgren-Lawrence-Scale 2–3) were included in the ongoing placebo-controlled, double blinded, randomized clinical trial ADIPOA2 using three doses (placebo, 2×10^6 and 10×10^6 ASC in 5% HSA/saline solution, 50 patients per arm). Lipoaspirate was shipped at 5 ± 3°C to the manufacturing centre. No antibiotics were used in the manufacturing process. Stromal vascular fraction (SVF) was isolated (cleanroom class A in B) by collagenase digestion for 30 minutes at 37°C. SVF was seeded at a density of 4000 cells/cm² on 2 chamber CellStacks in MEMalpha, supplemented with 5% platelet lysate (IKT Ulm). After 1 day, non-adherent cells were removed. A second medium exchange was performed after 4 ± 1 days and ASC of passage 0 (P0) were harvested at day 6 ± 1, reseeded at a density of 2000 ASC/cm² and cultured for another 7 ± 1 days with one additional medium exchange. Passage 1 cells (P1) were harvested and shipped at 5 ± 3°C in sterile packed 10 ml syringes for inter-articular injection.
Results: Until May 2018, the manufacturing centre Ulm received 21 lipoaspirates, but only 9 ATMP could be delivered, since the cell count of 2 SVF was out of specification and 9 lipoaspirates and one culture were tested positive for bacterial contamination (*Staphylococcus epidermidis*, *Staphylococcus caprae*, *Staphylococcus saccharolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus lugdunensis, Propionibacter acnes*, *Massilia spp.*). Despite SVF was negative in 8 cases with microbial contamination of the lipoaspirate, cell culture was stopped. A volume of 7 ± 19 ml was processed 27 ± 2 hours after aspiration to isolate $22 \pm 13 \times 10^6$ SVF with a viability of 82 ± 8%. Doubling time was 28 ± 4 hours for P0 and 32 ± 4 hours for P1. After 6.5 ± 0.8 population doublings in P0 and 4.3 ± 0.8 population doublings in P1, the viability of P0 and P1 cells was 99 ± 1% and 98 ± 1% when harvested at a density of $22 \pm 11 \times 10^3$ and $47 \pm 29 \times 10^3$ cells/cm². The total harvest of P1 cells was $126 \pm 95 \times 10^6$ cells. The calculated yield was $1.7 \pm 0.9 \times 10^6$ ASCP0 and $44.7 \pm 31.1 \times 10^6$ ASCP1 per ml lipoaspirate. Clonogenicity (CFU-F/10^6 cells) was $55 \pm 19 \times 10^3$ for SVF, $330 \pm 168 \times 10^3$ for P0 and $205 \pm 98 \times 10^3$ for P1. Identity as determined by flow cytometry was within the defined specifications. The number of delivered products was: 9 placebos, 7 doses of 2×10^6 and 2 doses of 10×10^6 ASC.
Conclusion: ASC production for the clinical trial ADIPOA2 was successful, as far as lipoaspirate passed microbial testing and allowed the harvest of up to 298×10^6 ASC. Implementation of further corrective actions to reduce contamination remains a key challange.

Conflict of interest: none

PS-1B-7

GMP-compliant generation of human granzyme B+ regulatory B cells for the therapy of graft-versus-host disease

Mangold C.[1], Felsen A.[1], Kurz L.[1], Trzaska T.[1], Reinhardt P.[1,2], Schrezenmeier H.[1,2], Fabricius D.[3], Schilbach K.[4], Jahrsdörfer B.[1,2]

[1]Department of Transfusion Medicine, Ulm University, Ulm, Germany
[2]Institute for Clinical Transfusion Medicine and Immunogenetics, German Red Cross Blood Transfusion Service Baden-Württemberg – Hessen, University Hospital Ulm, Ulm, Germany
[3]Department of Pediatrics, Ulm University, Ulm, Germany
[4]Department of General Pediatrics, Oncology/Hematology, Eberhard-Karls University Tübingen, Tübingen, Germany

Granzyme B (GrB)-secreting regulatory B cells (Breg) suppress T cell proliferation by GrB-mediated degradation of the T cell receptor and are involved in various pathologies. Their exploitation as novel cell-therapeutic agents may therefore represent a promising approach for the treatment of *graft-versus-host disease* (GvHD). Recently, we developed a cocktail consisting of IL-21 and antibodies against the human B cell receptor, allowing for easy *ex-vivo* induction of GrB+ Breg from peripheral B cells isolated from whole blood. In our current study, we now used a GMP-compliant positive selection kit to directly isolate CD19+ B cells from leukapheresis products collected from eight unstimulated healthy donors. Subsequently, we tested the isolated B cells in terms of their potential to differentiate into Breg. On average, we were able to isolate 56.5 × 106 B cells from ~6.6 × 108 total PBMC. Purity was 99.4%, viability was 98.1%. Extrapolated to the size of a full leukapheresis product, the generation of >600 × 106 Breg is possible. After 48 hours of stimulation, an average of 64.7% of B cells showed the typical GrB+ phenotype. Of note, these B cells maintained their Breg phenotype for up to another 72 hours after the end of stimulation. In conclusion, our findings demonstrate that GMP-compliant generation of induced Breg is feasible. Our results pave the way for further development of Breg as novel cell-therapeutic agent. A first pilot study on the effect of GrB+ Breg on GvHD in a humanized mouse model will be starting in June 2018. Initial results will be discussed on the conference.

Conflict of interest: none

PS-1B-8

Development of a GMP-compliant two-step maturation process for the generation of plasmacytoid dendritic cells as anti-tumor vaccine

Kurz L.[1], Smith N.[2], Fecher T.[1], Dimitriou E.[1], Trzaska T.[1], Felsen A.[1], Reinhardt P.[1,3], Schuler P.[4], Münch J.[2], Hoffmann T.[4], Schrezenmeier H.[1,3], Fabricius D.[5], Jahrsdörfer B.[1,3]

[1]Department of Transfusion Medicine, Ulm University, Ulm, Germany
[2]Institute for Molecular Virology, Ulm University, Ulm, Germany
[3]Institute for Clinical Transfusion Medicine and Immunogenetics, German Red Cross Blood Transfusion Service Baden-Württemberg – Hessen, University Hospital Ulm, Ulm, Germany
[4]Department of Otolaryngology, Ulm University, Ulm, Germany
[5]Department of Pediatrics, Ulm University, Ulm, Germany

Allogeneic plasmacytoid dendritic cells (pDC) from partly HLA-matched healthy donors may represent a promising alternative to conventional DC as anti-tumor vaccine. Recently, GMP-compliant isolation of pDC precursors from peripheral blood became possible, so that their clinical application appears within reach. In our current study, we tested a GMP-compliant positive selection kit to isolate BDCA-4+ pDC from leukapheresis products collected from 26 unstimulated healthy donors. After isolation, pDC precursors can be activated and matured within 24–48 hours. On average, we retrieved 4.4 × 106 pDC from ~1.9 × 109 total PBMC. Purity was 95.9%, viability 94.5%. Extrapolated to the size of a full leukapheresis product, it is possible to isolate >20 × 106 viable pDC precursors. After pulsing pDC with K562 lysate, followed by TLR-stimulation, we tested the capacity of pDC to generate cytotoxic T cells via CFSE staining, in-

tracellular IFN-g staining, and a Europium-based cytotoxicity assay. We observed that maturation of pDC consists of a biphasic activation process (**Fig. 1**). During phase 1, immature pDC expressed high levels of proteases and few costimulatory molecules. Confocal microscopy demonstrated that only in this phase pDC can take up antigens from K562 lysates. In phase 2, antigen-loaded pDC matured by TLR ligands downmodulated proteases, but upregulated costimulatory molecules and MHC/peptide complexes. Mature pDC induced IFN-g expression and proliferation of allogeneic CD8+ cytotoxic T cells. Moreover, after expansion in the presence of K562 lysate-pulsed pDC, cytotoxic T cells were able to kill K562 cells. Our study indicates that GMP-compliant generation of pDC as allogeneic anti-tumor vaccine is feasible.

Conflict of interest: none

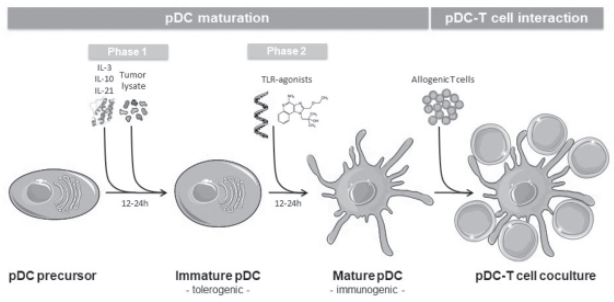

Figure 1. Concept for the two-step maturation of plasmacytoid dendritic cell precursors.
Activation of pDC precursors occurs in a biphasic process. During the first phase, pDC precursors take up antigens and develop an immature phenotype in the presence of various cytokines including IL-3, IL-10 and IL-21. This phenotype also involves the production of proteases including the serine protease GrB, which may support antigen uptake and processing. In the second phase, pDC are matured in response to TLR ligands. Now, GrB is downmodulated, while co-stimulatory molecules and MHC/peptide complexes are upregulated, resulting in highly immunogenic and mature antigen-presenting cells. The whole maturation process requires between 24 - 48 hours. Abbreviations: GrB = Granzyme B, MHC = Major Histocompatibility Complex, TLR = Toll-like receptor.

Fig. 1.

PS-1B-9

Comparison of human BM-MSC isolated with a GMP-compatible non-woven fabric filter device vs. density gradient centrifugation

Spohn G., Witte A. S., Vogel A., Seifried E., Schäfer R.

German Red Cross Blood Service Baden-Württemberg – Hessen gGmbH, Institute for Transfusion Medicine and Immunohematology, Frankfurt am Main, Germany

Introduction: Mesenchymal stem/stromal cells (MSC) receive increasing attention with respect to their therapeutic applicability. Current protocols for the isolation of MSC from bone marrow (BM) mainly include density gradient centrifugation (DGC), i.e. an open system. In 2010 Ito et al.: first described a GMP-compatible filter device to isolate BM-derived MSC. The aim of our work was to compare phenotypic and functional characteristics of MSC isolated with this device to MSC isolated with DGC.
Methods: Human bone marrow samples (40–48 mL each) from 5 healthy donors were analyzed. Each sample was divided equally followed by processing one half with the filter device (CellEffic BM; Kaneka Corporation) and the other half with DGC.
CFU-F: equal volumes of the harvested cell suspensions were plated into 6 cm dishes in duplicates; colony forming units fibroblasts (CFU-F) were stained with crystal violet and counted.
Immunophenotype: cells were stained at room temperature for 20 minutes with antibodies targeting CD45, CD34, CD19, CD14, HLA-DR, HLA-ABC, CD29, CD44, CD59, CD73, CD90, CD105, CD140b, CD140a, MSCA-1, CD271, CD146, CD119, and CD106, comprising 3 multicolor panels. 7-AAD or fixable viability stain were used to exclude dead cells. Cells were assayed using a BD LSRFortessa flow cytometer.
Differentiation potential: in vitro osteogenic, adipogenic, and chondrogenic differentiation was induced and evaluated according to standard protocols.
Immunomodulatory potential: MSC were plated in 24-well plates (8 × 10E4 MSC/well) 24h prior to co-culture with PBMC from healthy donors. PBMC were labelled with CellTrace Violet (ThermoFisher Scientific) according to manufacturer»s instructions and added to MSC (4 × 10E5/well) followed by stimulation with PHA (10 µg/mL). After 5 days, proliferation of stimulated PBMC alone versus PBMC in co-culture with MSC was assessed by flow cytometry in technical duplicates. Each MSC donor was analyzed by co-culture assays with PBMC from at least 2 donors.
In vitro wound healing: in vitro wound healing/migration was assessed using an in vitro wound scratch assay visualized by the IncuCyte® Live-Cell Imaging System. MSC plated at 10E4 cells/well in 96-well plates were allowed to attach overnight, wounded and monitored for wound closure.
Angiogenesis: MSC conditioned medium was tested for in vitro induction of endothelial cells tube formation in the IncuCyte® Live-Cell Imaging System.
Results: The yield of CFU-F per mL of BM was significantly higher (mean 15-fold) in the MSC isolated with the device compared to those isolated by DGC.
We did not observe differences of population doubling times, immunophenotype and tri-lineage differentiation potential of MSC from both groups.
The immunomodulatory potential showed donor-to-donor variabilities without differences between fabric filter-isolated and DGC-isolated MSC. The results from the wound closure assays and the tube formation assays did not differ with respect to the isolation method.
Conclusion: Isolation of MSC using a GMP-compatible fabric filter closed system device resulted in higher yield of CFU-F, giving rise to MSC with similar phenotype and functional characteristics as MSC isolated by DGC.

Conflict of interest: none

PS-2A
Blutspende

PS-2A-1

A questionnaire-based survey of blood donors refraining from further donations

Pruszak J., Ikert A., Umhau M.

Institut für Transfusionsmedizin und Gentherapie, Universitätsklinikum Freiburg, Freiburg, Germany

Introduction: Blood donor recruitment requires a considerable amount of marketing efforts. While the explicit aim is to recruit and retain donors for multiple, ideally long-term regular donations, a number of first-time donors do not show up for a second donation. Even among repeatedly active voluntary blood donors, a substantial fraction does not return for further donations. The goal of the present study was to assess underlying reasons for not returning for another blood donation.
Methods: To this end, all blood donors not returning within a period of 12 months were contacted by e-mail with the request to anonymously fill an online questionnaire. Over the course of a 5 year period donors who had not shown up within 12 months after their last donations were questioned regarding reasons for refraining from further visits due to healthand procedure-related issues as well as logistics associated with donating blood.
Results: Of the 3000 donors included in the study (17.4% first-time donors, 55.1% regular donors with ≤ 10 donations, 27.5% regular donors with >10 donations) 646 individuals participated in the survey (21.5%). The main reasons for abstaining from further donations were as follows: unwanted side effects of the donation (33.5%), other health problems (48.2%), avoidance of venipuncture (30.4%), time (62.7%) and waiting-time associated reasons (50.4%), feeling of inadequate appreciation (15.8%) and unpleasant atmosphere (19.7%), material factors (monetary reimbursement (22.2%) and refreshments (28.9%)) as well as logistic

factors (opening hours (49.4%) and change of residence (37.3%)). Thus, keeping low response rate and participation bias in mind, the following primary reasons for refraining from further donations could be deduced: health reasons, logistic factors, material/monetary aspects.
Conclusion: Although certain factors such as underlying health issues and change of residence are not amenable to outside influence, rates of continued donation could be potentially improved by facilitating access (opening hours), reducing waiting time and enhancing efficiency and ensuring sufficient emotional reward. In order to improve rates of continued donation, addressing these aforementioned factors might prove particularly beneficial.

Conflict of interest: none

PS-2A-2

Online survey on blood donating-related adverse reactions, Germany, April – September 2018

Pötzsch K., Ruppert-Seipp G., Schönefeld S., Fiedler S. A., Hoffelner M., Stoll H., Ruhaltinger D., Oberle D., Heiden M., Funk M.

Paul-Ehrlich-Institut, Sicherheit von Arzneimitteln und Medizinprodukten, Langen, Germany

Introduction: Whereas up to the late 1980ies, research had primarily focused on the safety of blood recipients, in the past three decades, blood and plasma donor safety has gained more and more attention. Hitherto, in Germany, the reporting obligation is limited to serious adverse reactions. This is why a comprehensive safety profile of blood and plasma donators is still missing. To obtain a detailed overview of adverse reactions following blood and plasma donations, the Paul-Ehrlich-Institut, the national competent authority for hemovigilance in Germany, initiated an investigation in blood and plasma donors.
Methods: An online survey is being conducted in Germany from April through September 2018 (see *www.pei.de/spendesicherheit*). It is open for every blood and plasma donor in Germany.
To protect privacy, only basic demographics, i.e. age group and gender, are requested. Participants are asked to report type and year of donation, whether and, if yes, what kind of reactions they experienced, time to symptoms onset, and whether they had to undergo medical treatment in an outpatient / inpatient setting. In addition, they are asked to assess the severity (non-serious / serious) of the experienced adverse reaction as well as the quality of informed consent.
For qualitative variables, absolute and relative frequencies will be calculated. For quantitative variables, medians, minimums and maximums will be computed. In addition, stratification by age group and type of donation will be performed. With the scope of a sensitivity analysis, the risk of recall bias will be assessed. The statistical analysis will be conducted using the SAS version 9.4 (SAS Institute, Cary, NC, United States).
Results: The survey is still ongoing. First results are expected to be available in September 2018.
Conclusion: To our knowledge, this is the first survey on non-serious and serious adverse donation-related reactions conducted in Germany. We expect to obtain new insights and to gain novel conclusions with respect to blood and plasma donor safety. Finally, the findings may help improving the safety of blood donation.
Acknowledgement: We would like to thank all participating blood and plasma donors for their active support.

Conflict of interest: none

PS-2A-3

BluStar.NRW – A project for typing refugees and migrants as potential blood and stem cell donors in North Rhine-Westfalia

Lenz V.[1], Wagner B.[1], Baumgart C.[1], Kordelas L.[2,3], Jiménez Klingberg C.[3], Gebhardt K.[3], Reimer T.[4], Zeiler T.[4], Siebert M.[5], Enczmann J.[6], Balz V.[6], Fischer J. C.[6], Horn P.[1]

[1]Universitätsmedizin Essen, Institut für Transfusionsmedizin, Essen, Germany
[2]Universitätsmedizin Essen, Klinik für Knochenmarktransplantation, Essen, Germany
[3]Westdeutsche SpenderZentrale, Ratingen, Germany
[4]DRK-Blutspendedienst West gGmbH, Breitscheid, Germany
[5]Caritasverband für die Stadt Essen e.V., Integration und Migration, Essen, Germany
6Heinrich-Heine-Universität, Medizinische Fakultät und Universitätsklinikum, Institut für Transplantationsdiagnostik und Zelltherapeutika (ITZ), Düsseldorf, Germany

Introduction: In 2016, 18.6 million people with a migration background have been living in Germany. The majority of those descend from regions where the distribution pattern of blood group antigens in the population differs considerably from the predominant one in the German population. Sufficient supply of these individuals with red blood cell (RBC) and platelet concentrates (TC) will continue to be a major challenge in the future, as blood donors with compatible blood group antigens are dramatically underrepresented in the local donor pools. This project was initiated to recruit new donors with a migration background for blood donation and to increase the number of blood stem cell donors among this group.
Methods: So far, more than 600 blood donors with a migration background have been recruited for a blood cell donation in this project. Serological RBC phenotyping included AB0, Rh(CcDEe), Kk, Fy(ab), Jk(ab), Lu(ab), M, N, S, s.
RBC genotyping using Next Generation Sequencing is currently being established and will additionally include those antigens with the most frequent distribution pattern differences between migrant and resident populations according to literature.
Results: An initial evaluation of the data revealed a very similar distribution of blood groups compared to the current blood donor population in North Rhine-Westphalia. After all, a number of ten Fy(a-b-) donors were found, which corresponds to a percentage of 1.6%.
Conclusion: Blood donors with rare blood groups (e.g. null types such as Fy(a-b-)), are being sought for adequate medical care of people with a migration background, thus enabling a better compatible supply of RBC. The technological development of blood group determination by Next Generation Sequencing will significantly improve the supply for all blood transfusion recipients in Germany.

Conflict of interest: This project is funded by the European Regional Development Fund 2014-2020 (ERDF) and the European Union.

PS-2A-4

GUILTY AS CHARGED The role of guilt in private donation decisions for stigmatized causes in terms of donating blood: An experimental study

Möller D.[1], Müller-Steinhardt M.[2], Klüter H.[2], Edinger-Schons L. M.[1], Blatt I.[1]

[1]Universität Mannheim, Corporate Social Responsibility, Mannheim, Germany
[2]DRK Blutspendedienst Baden-Württemberg - Hessen, Institut für Transfusionsmedizin und Immunologie, Mannheim, Germany

Background: Most studies on stigmatization in donation decisions deal with monetary donations and choose scenario or qualitative research designs. The present study aims to close this research gap by collecting quantitative data from whole blood donors to measure attributions of guilt. The following hypotheses (H1-H3) structured the investigation:
H1: An individuals willingness to donate blood will vary depending on whether illnesses are described as genetically caused, behaviorally caused, or described without cause.

H2: Genetically caused illnesses lead to the highest willingness to donate blood whereas behaviorally caused illnesses lead to the lowest willingness to donate.
H3: Genetically caused illnesses lead to a lower perceived degree of guilt whereas illnesses caused by misbehavior lead to a higher perceived degree of guilt.
Methods: Through a paper-and-pencil survey 213 whole blood donors were confronted with the decision how much (on a 7-point Likert scale) they would be willing to donate blood for patients suffering from lung cancer, diabetes, and HIV. Each illness was ascribed to be either behaviorally caused (treatment group 1), genetically/accidentally caused (treatment group 2), or without mention of the cause (control group). A between-subjects design where each participant is exposed randomly to only one treatment was chosen. 71 participants were in treatment group 1, 70 participants in treatment group 2 and 72 participants were in the control group.
Results: The results of a one-way independent ANOVA showed that there is a significant effect of the way illnesses are caused on the willingness to donate blood ($F(2, 202) = 18.497$, $p < .001$). Bonferroni post-hoc tests showed that the willingness to donate blood is significantly higher for patients with genetically caused diseases (= 6.61, = 0.96) versus patients with behaviorally caused diseases (= 5.38, = 0.20).
Testing the perceived degree of guilt linked to the cause of the affected people, the ANOVA showed a significant effect of the degree of guilt on the willingness to donate blood ($F(2, 197) = 122.759$, $p < .001$). Illnesses which are described as behaviorally caused evoke a higher perceived degree of guilt (= 4.51, = 0.13) than illnesses which cannot be related to any misbehavior in advance (= 1.65, = 0.10).
Conclusion: Within a field experiment, attribution theory was tested through blood donation behavior for the first time. The data showed that all hypotheses were supported. Hence, there is a major impact of the way illnesses are caused and the attributed degree of guilt on the willingness to donate blood. It is of huge interest for *all blood donor services* to gain knowledge about donation behaviors in order to ensure an adequate blood supply. Therefore, additional studies will be required to further investigate what factors impact on the decision to donate blood.

Conflict of interest: none

PS-2A-5

Blood donors with disposition for hemochromatosis – efficient prevention and gift for others in need

Klasen G., Mrotzek M., Störmer M., Gathof B.
Uniklinik Köln, Transfusionsmedizin, Köln, Germany

Introduction: Hemochromatosis is the most prevalent genetic disposition (2–5/1000) in Caucasian populations. The genetically determined increased iron absorption can lead to excessive iron accumulation with its respective consequences. Iron depletion by phlebotomy is the treatment of choice; (therapeutic) low normal iron levels (ferritin < 100 µg/l) are the target. Due to increased awareness for hemochromatosis in the medical profession and increased laboratory testing in the population persons with disposition for hemochromatosis are detected at an earlier stage of disease or even as asymptomatic carriers. Regular blood donation can help to maintain low iron levels and support the blood supply. If therapeutic goals are maintained hemochromatosis patients even have a better life expectancy than the «normal» population due to decreased mortality related to cardiovascular disease. This study was to evaluate the results of the special program for donors with determination for hemochromatosis at our institute in 2017 and 2018.
Methods: The data of donors with disposition for hemochromatosis at our institute for phlebotomies and iron storage parameter were evaluated.
Results: Since 1998 more than 120 hemochromatosis donors have been cared at our institute. Weekly phlebotomies for iron depletion are performed until normal levels for iron storage parameters are reached. With each consecutive blood donation iron storage parameters are determined. The respective donation is only accepted for transfusion to patients if iron storage parameters are within normal range. During 2017 und 2018 70 patients with hemochromatosis have been treated with phlebotomies or blood donation. 41 patients show low normal iron levels (
Conclusion: Blood donation can help persons with genetic determination for hemochromatosis to maintain the recommended low iron levels. The donation as «gift» for patients in need can help to sustain the motivation for this long term prevention, which has been shown to increase survival even compared to the «normal» population.

Conflict of interest: none

PS-2A-6

Iron store of repeat plasma and platelet donors

Pfeiffer H., Hechler J., Dullinger K., Hackstein H., Achenbach S.
Universitätsklinikum Erlangen, Transfusionsmedizin, Erlangen, Germany

Introduction: Repeat apheresis donation (plasma as well as platelets) causes a noticeable loss of whole blood: routine blood tests with every donation as well as residual blood within the used apheresis set. And while the effect of blood loss on donor iron stores has been widely researched for whole blood donations, fewer and especially contradictory results exist for apheresis donors. We therefore analyzed the blood samples of apheresis donors at our department to investigate the relationship between repeat apheresis donation and iron store depletion.
Methods: A retrospective analysis of the donor blood samples (Department of Transfusion medicine, University Hospital Erlangen) of the past 11 years (n = 56.749) was performed. Serum ferritin and hemoglobin were used to detect iron deficiency, comparing repeat apheresis donors with whole blood donors, while first-time donors served as a control group. To investigate the impact of the frequency of blood donation, this frequency was calculated for every single donor (for the whole period of 11 years as well as for each individual year) and correlation and regression analysis between frequency and iron parameters was performed. Additionally, subgroup analysis according to the donation frequency was performed. A special group were long-time repeat donors, whose changes in ferritin-values were analyzed in comparison to the first-ever ferritin value before the first donation.
Results: Apheresis donors have significantly lower ferritin and hemoglobin values compared to the control group. This is more noticeable in males than in females. Interestingly, there are much more iron-depleted females in the control group than there are iron-depleted males. The correlation and regression analysis showed a significant relationship between donation frequency and iron-deficiency in males, but not in females, with the subgroup analysis providing further back-up. The analysis of the long-time repeat donors showed that the relative ferritin value dropped more in males than in females. When comparing iron-depleted long-time donors, females tend to be iron-depleted much more often even before the first donation.
Conclusion: A noticeable effect of (repeat) apheresis donation on the iron store of the blood donor was shown. The high percentage of iron-deficient donors, especially women, showed the special importance of this investigation. The very small correlation between donation frequency and iron depletion for females is most likely due to the fact that women tend to be iron-deficient even before the first donation, as can be seen in the control group. Because of a very high variance in donation frequency, our calculation model was rather vague. Therefore, the effect of donation frequency on the donors iron store is most likely higher than we have shown.

Conflict of interest: none

PS-2B
Herstellung und Qualitätskontrolle

PS-2B-1

Validation of red blood cell concentrates processed with a new four-fold whole blood system (CQ41575, Fresenius)

Kahlenberg F.[1], Zimmermann A.[1], Henning J.[2], Thierbach J.[3], Baumann-Baretti B.[1]

[1]Haema AG, Qualitätskontrolle, Leipzig, Germany
[2]Haema AG, Medizin, Leipzig, Germany
[3]Haema AG, Verarbeitung/Vertrieb, Leipzig, Germany

Introduction: The currently used CQ41470 whole blood system (Fresenius) will be replaced by a new whole blood system CQ41575 (Fresenius). The new system differs in use of integrated filter. The previous whole blood universal filter is replaced by a whole blood flex universal filter. The film material itself is unchanged. Therefore it can be assumed that the product quality, the freezing process of the plasma or the label adhesion will be impaired.
A study was conducted to evaluate the storage quality of processed RBCs. In order to ensure a constant quality after processing and after expiration of shelf life, quality control parameters were determined up to day 49. Results were compared with quality control data of the CQ41470 whole blood system.
Methods: On three consecutive days, a total of fourteen whole blood samples were collected, centrifuged and separated using the CQ41575 whole blood system in accordance with the applicable manufacturing instructions. Weekly quality control samples were tested and results compared to CQ41470.
Results: The mean hemoglobin content in all preparations was 63.0 g/TE. The rates of hemolysis do not differ within the two systems. At the end of the shelf life (day 49), it was 0.32%. The potassium concentration increased during the storage (CQ41575) from 1.52 mmol/l (day 1) to 57.48 mmol/l on day 49. The same trend was seen in the other system. The pH slightly decreased during storage. ATP content also slightly decreased in both systems (e.g. CQ41575 on day 1: 4.65 µmol/gHb to 2.10 µmol/gHb on day 49). Concentration of glucose decreased from 30.55 mmol/l (day 1) to 13.89 mmol/l (day 49). In comparison, concentration of lactate increased from 4.57 mmol/l to 35.30 mmol/l at the end of the storage. There was also the same trend in the CQ41470 whole blood system for the two parameters of metabolism. After 49 days of storage, all tested units were negative in bacterial cultures.
In comparison the filtration time is considerable lower with the new filter system CQ41575.
Conclusion: Red blood cell concentrates processed using the CQ 41575 Fresenius whole blood systems retained adequate *in vitro* function and storage quality for up to 49 days and met the criteria of the «Council of Europe Recommendation N .R(95) 15" as well as the German guidelines for RBC`s.

Conflict of interest: none

PS-2B-2

A new technology for the production of blood components exploiting modular combinable blood bags and a sterile cold-connector-system

Madla W.[1], Suck G.[1], Jungk H.[2], Heil U.[2], Tryankowski R.[2], Zeiler T.[3]

[1]DRK BSD West, Herstellung, Hagen, Germany
[2]DRK BSD West, Herstellung, Bad Kreuznach, Germany
[3]DRK BSD West, GF, Ratingen, Germany

Introduction: Blood components are generally produced in complex ready-for-use whole blood (WB) bag systems consisting of multiple components and integrated filters in top-top or top-bottom-configuration. It was the goal of the project to reduce complexity of the bag systems used in blood donations and to enable versatility in the course of WB unit manufacturing, including filtration, by means of instantaneous, effortless, and stable connection of distinct blood bag modules.
Methods: A luer lock connection device SC1 was developed to interlink in a stable and sterile manner two complementary connector-parts, integrated into the tubing of different blood bags. Tests were performed with a top-bottom two-component system (donation/plasma bag) connectable to a second two-component system containing a filter (transfer/red blood cell (RBC) bag). After centrifugation the WB units were connected to the respective modules. A conventional blood bag set for RBC inline filtration was used as control. For each test two identical WB units were generated through pairwise pooling and subsequent separation. The identical pairs served as test and control samples, respectively. Product quality data, esp. sterility, were obtained for the processed test blood components and compared to the respective control samples.
Results: The integrity of the SC1-system was proven in a microbial stress test according to validation guidelines. Furthermore, the connector was successfully used throughout all processing steps without failure. Most importantly, no leakages were observed. In-process control parameters for separation, filtration and filling amounts of the manufactured blood products were comparable between test and control samples and compliant with specifications. This was also the case for the RBC-quality parameters, including HCT, Hb/unit, RBC-indices, contaminating leukocyte-content, ATP, potassium and haemolysis rates. In addition, there were no statistically significant differences between quality data of test and control pooled platelet concentrates (filling amounts, contaminating leukocyte-content, platelet content, pH, collagen induced platelet aggregation) and of plasma samples. Microbiological testing results were negative in all cases.
Conclusion: Handling, mechanical resilience and robustness of combinable modular bag systems connected with the sterile connector SC1 fulfilled all experimental requirements. Furthermore, pairwise comparison of quality data obtained from blood components, which were conventionally produced, with those processed in a modular bag system, did not reveal any significant differences. Usage of only at least a single primary bag for blood donation in combination with secondary blood-bag modules enables simplifying steps like centrifugation of the units, especially in view of automation endeavours. Moreover, such a modular system allows for choosing production lines relatively late in the process of WB productions.

Conflict of interest: none

PS-2B-3

Development and implementation of an automated labelling- and sorting machine in a national blood donation service

Madla W.[1], Suck G.[1], Kischnick T.[1], Grolle A.[1], Ljubetic N.[2], Kettrup R.[3], Kremser M.[4], Steinke W.[3], Demmer U.[1], Zeiler T.[5]

[1]DRK BSD West, Herstellung, Hagen, Germany
[2]DRK BSD West, IT, Ratingen, Germany
[3]DRK BSD West, IT, Münster, Germany
[4]DRK BSD West, IT, Hagen, Germany
[5]DRK BSD West, GF, Ratingen, Germany

Introduction: Manufacturing of blood products relies to a large extent on manual processing with only few semi-automated or automated functions, still to date. It was the objective of the project to streamline labour-intensive, time-consuming and monotonic processing steps. A high-throughput automated plant, involving high-precision-robots operating on a conveyer belt, was established.
Methods: The requirements for automation of weighing, labelling and sorting processes of blood products were defined, based on a risk analysis, in product and functional requirements documents and implemented by a plant builder. The Good Manufacturing Practice compliant plant-prototype was built according to given plant-layouts in a defined project schedule. This included assembly of plant-elements, build-up of host-communication-modules, bidirectional connection of the IT-plant-control to the

host-IT-blood bank system (BAS), validation, qualification (DQ, IQ, OQ, PQ) and approval by the authorities.
Results: The plant is laid out to weigh, label and sort units of incoming unprocessed whole blood, red blood cell concentrates, plasma and optionally platelet-concentrates. The plant comprises a conveyer belt, four camera-scanners for barcode- and product-type recognition, a balance, one labelling robot and two sorting robots, three label printer and twelve sorting places. The BAS and the IT-plant-control (SPS-control type Beckhoff) communicate via TCP/IP-protocols. The host system processes the print- and sorting commands. The exchange of a data-telegram requires 14 ms. Products are placed manually on the convey belt, which is adjusted to a speed of 30 m/min. Three different product-specific types of label are placed on the respective bags with an accuracy of ± 1 mm. Digital photographs are taken of the labels and archived. Products are sorted into designated Euronorm-boxes according to pre-defined BAS-criteria. The quantity of bags per box is thereby defined for each product-type. Products are lifted up by vacuum-suction-pads equipped with pneumatic grip-plates and stacked in the boxes in defined batches. The performance reaches processing of approximately 700 products/h and is aimed to perform ca. 20 h/day.
Conclusion: In the course of establishing the prototype-plant extensive obstacles, concerning mechanics, materials or IT, were solved in the past 2,5 years. As a result, the plant possesses the intrinsic capacity to allow automated high-throughput and high-precision blood-product-processing. Manual labelling and sorting processes requiring high level of staffing are consequently rendered obsolete. The plant is planned as an integral part of the central blood production centre Hagen / BSD West, which is laid out to process 3500–4000 whole blood products/day by end of 2018. It paves the way for a future of smart production in blood donation service establishments.

Conflict of interest: none

PS-2B-4

Validation of gamma-irradiation 50 Gy indicators for intraoperative blood salvage (MAT)

Posset M.[1], Ahrens N.[1], Johnson C.[1], Seyfried T.[2]

[1]Uniklinikum Regensburg, Institut für Klinische Chemie, Laboratoriumsmedizin und Transfusionsmedizin, Blutbank, Regensburg, Germany
[2]Uniklinikum Regensburg, Klinik für Anästhesiologie, Regensburg, Germany

Introduction: In tumour surgery, there is a risk that red blood cells (RBC) salvaged through the use of an intraoperative blood salvage device (MAT) are contaminated with radiation-tolerant tumour cells. To prevent transfusion-associated graft-versus-host disease, allogeneic blood products are gamma-irradiated with 25 Gy to eliminate donor leukocytes. In comparison, tumour cells require gamma-irradiation with 50 Gy to ensure complete inactivation. Colourmetric indicators for 50 Gy radiation, such as the RC-Scale indicators, have recently become available. These indicators are designed to turn light blue following exposure to 25 Gy and dark blue on exposure to 50 Gy irradiation. Here, we present the results of our validation of this new generation of irradiation indicator.
Methods: Discarded RBC units were labelled with RC-Scale indicators (On Point indicators GmbH, Austria) and irradiated separately with between 10 to 60 Gy using an IBL437C, CIS bio international. Adherence of the indicators to blood product bags and label surfaces was evaluated. To assess the colourmetric response of indicators, the indicator field region was scanned before and after irradiation as were the 25 Gy and 50 Gy calibration fields.
Results: The indicators adhered to all bag and label surfaces tested. The colour of both the 25 Gy and 50 Gy calibration fields was robust to radiation treatment and displayed only minor, non-significant fading following irritation. Increasing radiation dosage (10 to 60 Gy) resulted in a linear increase in blue staining within the indicator field, with a clear distinction between 25 Gy and 50 Gy.
Conclusion: RC-Scale indicators displayed the adherence properties required. Furthermore, indicators were able to distinguish between 25 Gy and 50 Gy gamma-irradiation treatments. Thus, these indicators potentially provide an appropriate means of indicating the inactivation of radiation-tolerant tumour cells present in MAT salvaged blood recovered during tumour surgery.

Conflict of interest: none

PS-2B-5

Quality of blood components irradiated by X-rays to avoid transfusion-associated Graft-versus-Host disease

Arlt N.[1], Rothe R.[1], Dreweck C.[2], Tilmann M.[2], Kronstein-Wiedemann R.[3], Karl R.[2], Tonn T.[3], Moog R.[1]

[1]DRK Blutspendedienst Nord-Ost, Institut Cottbus, Cottbus, Germany
[2]DRK Blutspendedienst Nord-Ost, Institut Berlin, Berlin, Germany
[3]DRK Blutspendedienst Nord-Ost, Institut Dresden, Dresden, Germany

Introduction: Transfusion-associated Graft-versus-Host Disease (TA-GvHD) can be avoided by irradiation of blood components. ϒ-irradiation is the current gold standard of irradiation devices. In the present study the quality of blood components irradiated with X-rays was investigated.
Methods: Packed red blood cells (PRBCs) and platelets (PLTs) were irradiated using the RadSource RS 3400 device (RadSource, Buford, USA). The device uses a single X-ray source and rotates individual canisters around this X-ray source. Mean irradiation dose was 30 Gy and the irradiation time was 280 seconds.
PRBCs were suspended in PAGGS-M or SAG-M additive solutions. Studied PLTs were whole blood derived pooled platelet concentrates (P-PCs) or collected by apheresis (A-PCs).
Aliquots were drawn after irradiation and at defined days until the expiry date of the blood components. Blood counts were measured electronically (CellDyn Ruby, Abbott, Wiesbaden) and pH was measured on a gas blood analyser (ABL 80, Radiometer, Copenhagen). Leukocyte contamination of blood components and PLT activation (CD 62) were analysed by flow cytometry (FC 500, Beckman Coulter, Krefeld). Citrate, free hemoglobin and potassium were analysed on an automated analyser (AU480, Beckman Coulter, Krefeld).
All units were tested for sterility at the end of storage time using BactAlert technology. To analyse T-cell stimulation buffy coats of 3 donors were irradiated for two different time periods either with X-rays or with ϒ-irradiation. T-cell proliferation was tested by using mixed lymphocyte reaction or by a non-radioactive proliferation assay.
Results: The average volume of PAGGS-M PRBCs was 305 ± 31 mL (n = 15) and of SAG-M PRBCs 302 ± 16 mL (n = 15) at the day of irradiation. Mean Hb was 60.6 ± 10.3 g per PAGGS-M unit and 60.3 ± 5.6 g per SAG-M unit, resp. All PRBCs were effectively leukocyte-reduced and the WBC contamination was less than 1 x 10^6 WBC per unit. Potassium levels and the hemolysis rate over the storage time are shown in figure 1.
The mean volume of P-PCs was 272 ± 11 mL (n = 15) and of A-PCs 237 ± 4 mL (n = 15), resp. Mean PLT content of P-PC was 3.32 ± 0.39 and of A-PC was 2.88 ± 0.28 x 10^{11} per unit, resp. The citrate level of P-PC was 2.79 ± 0.16 and of A-PCs 3.04 ± 0.21 g/L, resp. CD 62 and pH are shown in figure 2. All tested PRBC and PC units were sterile at the expiry date.
Conclusion: Quality parameters of X-ray irradiated PRBCs and PLTs met current national and European guidelines. X-ray and ϒ-irradiation were equivalent in affecting lymphocyte function thereby avoiding TA-GvHD.

Conflict of interest: none

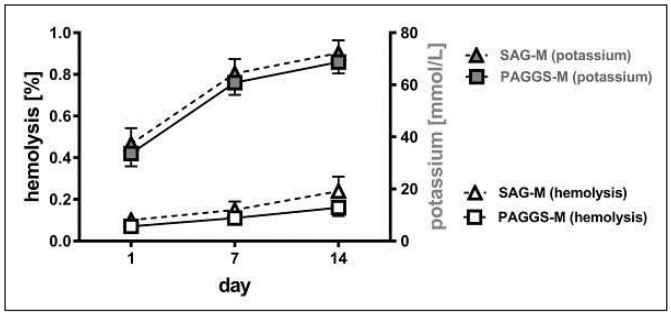

Fig. 1

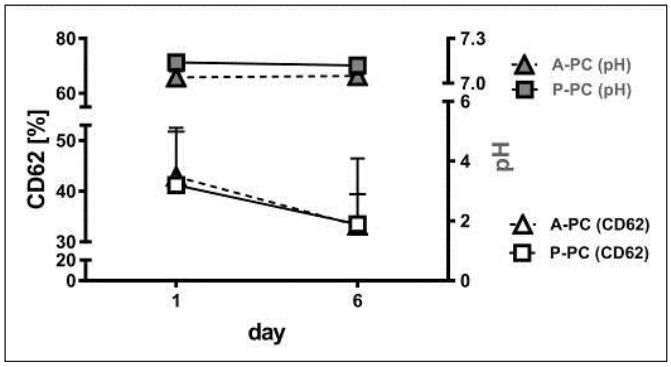

Fig. 2.

PS-2B-6

Influence of sex and lipid metabolism on hemolysis of stored red blood cells

Hofmann M., Niklas N., Suessner S., Havlicek-Poetscher M.

Red Cross Blood Transfusion Service of Upper Austria, Immunohematology and quality control, Linz, Austria

Background: Various studies previously investigated the association of donor-related variables and red blood cell (RBC) hemolysis during storage. Some reported higher hemolysis in male RBCs compared to female RBCs and that lipaemic plasma promotes hemolysis of RBCs (Kanias et al, Tarasev et al). In this study, we focused on sex differences and the effect of body-mass-index (BMI) and hyperlipidemia at time of donation on hemolysis rate in stored RBCs.
Methods: We collected data of expired (after 42 days of storage) stored RBCs between January 2010 and December 2017 in our department. The hemolysis rate at the end of storage was calculated. Blood donor characteristics and cholesterol level were obtained within the blood donor screening at time of donation. Based on these data we selected 48 additionally blood donations for testing of triglycerides, low-density lipids (LDL) und high-density lipids (HDL). Stored RBCs were tested over 42 days of storage for hemolysis rate.
Results: In total we included 1,673 expired stored RBCs from 1,033 male and 640 female blood donors where mean±sd age was 39.97 ± 12.7. Hemolysis rate in the tested products ranged from 0.015–1.31%, mean±sd 0.19 ± 0.1%. We could confirm a significant difference between male and female donors. RBC from male donors showed a mean hemolysis rate at 0.196% and female donors at 0.177%. BMI itself showed a significant difference in male (BMI 26.41 ± 3.7) and female (BMI 24.95 ± 4.0), despite there was a similar correlation with hemolysis in both male and female donors (r = 0.148). Regarding hemolysis rate and age, male donors tend to an increase of hemolysis at higher age, whereas in female donors this effect was slightly visible but not significant. Cholesterol was tested in a subset of 147 male and 99 female donors with a positive correlation in male donors (r = 0.180; 211.22 ± 35.64 mg/dl), whereas in female donors a negative but not significant trend was found (r=-0.060; 215.39 ± 39.99 mg/dl). Further investigations of 48 stored RBCs showed correlation of triglycerides and hemolysis in male (r = 0.699), but not in female donors (r = 0.215). Regarding HDL levels and hemolysis, male and female donors tend to a decrease of hemolysis at higher HDL levels.
Conclusion: Sex differences not only influence hemolysis rate of RBCs, but also factors like hyperlipidemia that promote hemolysis. Our focus on gender-dependent relationships of lipid metabolism and its effect on RBC hemolysis confirms differences of RBC viability in male and female. This tendency indicates that HDL could help to reduce hemolysis, while LDL and triglycerides promote hemolysis in RBCs and should be more included in donor screening and selection of cellular blood products.

Conflict of interest: none

PS-2B-7

GMP-compliant detection of leucocyte apoptosis in extracorporeal photopheresis

Hähnel V.[1], Dormann F.[1], Kronenberg K.[2], Hutchinson J.[2], Ahrens N.[1]

[1]Universitätsklinikum Regensburg, Transfusionsmedizin, Regensburg, Germany
[2]Universitätsklinikum Regensburg, Experimentelle Chirurgie, Regensburg, Germany

Introduction: Extracorporeal photopheresis (ECP) is a therapy applied to patients with autoimmune diseases, cutaneous T-cell lymphomas and graft-versus-host-disease, which induces complex immunological effects. The induction of apoptosis of lymphocytes by treatment with 8-methoxypsoralene (8-MOP) and UVA-irradiation is well known and an accepted potency assay for ECP. However, GMP-compliant validation of this functional assay has not been attempted before.
Methods: Leucocytes were collected by apheresis with Spectra Optia, Terumo BCT and UV-irradiated in presence of 8-MOP with the UVA PIT system, Med Tech Solution GmbH. Samples were taken before the addition of 8-MOP and after UV-irradiation and incubated in TexMACS GMP medium, Miltenyi, (1 x 10^6 each) at 37°C, 5% CO_2 for up to 72 h. Apoptosis of $CD3^+$ cells and $CD33^+$ monocytes was detected with the Navios Ex and Navios, Beckman Coulter, by staining with Annexin V and 7-AAD. Cut-offs were set by using isotype controls or fluorescence minus one samples (FMO). We used Camptothecin-incubated samples as positive control. Testing procedures and acceptance criteria were set in advance.
Results: In this GMP-compliant validation accuracy, intra-assay and inter-assay precision and stability of samples were verified. Accuracy of the gating strategy was shown with isotype controls and FMO for CD3, CD33, CD14 as well as for Annexin V and 7-AAD with an unspecificity threshold of 5%. Precision was calculated with CD3 as it was the most robust parameter. The intra-assay precision coefficient of variation (CV) was 3.46–9.50% (≤ 15%, in-specification). The inter-assay precision showed comparability between Navios and Navios Ex (median CV 1.01–6.02%) as well as between staff members with CV of 0.29–10.77% and deviations of 0.55–7.68%. This was in-specification for both CV (≤ 15%) and deviation (≤ 20%). Preanalytical stability of T-cells and monocytes from sampling to the beginning of preparation showed that short-time storage up to 2 h on ice was possible. There was no analytical stability after staining, samples should therefore be measured immediately.
Conclusion: Flow cytometry is a suitable technique to detect apoptosis. This functional assay was implemented as a potency assay for ECP. The results of this validation showed that this assay is GMP-compliant, and that reliability concerning accuracy and precision could be quantified on an acceptable level. Pre-analytical stability was compatible with on-site analysis, and analytical stability indicated that this assay was not suited for batch mode analysis.

Conflict of interest: none

PS-2B-8

APD-mediated responsiveness of platelets from stored whole blood or from apheresis-derived platelet concentrates

Kößler J., Schwarz M., Etzel J., Weber K., Kößler A., Böck M., Kobsar A.

Universitätsklinikum Würzburg, Institut für Klinische Transfusionsmedizin und Hämotherapie, Würzburg, Germany

Background: Tampered platelet integrity occurring during manufacturing and storage of platelet concentrates is a known phenomenon called storage lesion. The deterioration of ADP-mediated platelet responsiveness has been evaluated in apheresis-derived platelet concentrates (APC), but is less investigated in the milieu of whole blood (WB).
However, for the preparation of buffy-coat PCs or for experimentation in research, citrated whole blood stored for different time periods is frequently used. Therefore, the intention of this study was to compare ADP-mediated responsiveness of stored platelets in both milieus.
Methods: WB units (500 mL, with 70 mL CPD, in PVC bags from Haemonetics) and APC (2.5 × 10^11 platelets in 250 mL of plasma, Trima Accel system, Terumo BCT) were collected from healthy voluntary donors and stored at 22°C under continuous agitation for 5 days. On day 0, 2 and 5 samples were drawn under sterile conditions. Using platelet-rich-plasma, 10 µM ADP induced light transmission aggregometry was performed. Purinergic receptor expression was determined by flow cytometry. P2Y12 receptor activity was assessed by the flow cytometric PLT VASP/P2Y12 Kit as platelet reactivity index (PRI). In washed platelets, P2Y1 and P2Y12 activity was analyzed by calcium flux-induced fluorescence in Fluo-4AM loaded platelets after selective stimulation.
Results: ADP-induced aggregation was abrogated in APC samples on day 2, whereas it was maintained partially in WB samples even on day 5. Purinergic receptor expression, basal or stimulated with TRAP-6, remained unaffected throughout the storage period for platelets in both milieus. PRI levels remained stable for 5 days and were similar in WB and APC samples. P2X1-dependent calcium-induced fluorescence levels were slightly reduced after apheresis but stable and comparable to WB under continued storage. P2Y1-dependent calcium-induced fluorescence deteriorated during the storage period, more accelerated in APC compared to WB samples.
Conclusion: Indicated by induced aggregation, ADP-mediated responsiveness decreases more rapidly in the milieu of APC compared to WB during storage. Especially, the progressive decline of the P2Y1 activity contributes to reduced ADP-mediated platelet integrity, whereas the function of the P2X1 and P2Y12 receptors is substantially preserved.

Conflict of interest: none

PS-2B-9

In vitro function of double dose apheresis platelet components suspended in 40% plasma/60% PAS after photochemical treatment using the new dual storage set designed with alternative plastics

Kahlenberg F.[1], Zimmermann A.[1], Thierbach J.[2], Henning J.[3], Baumann-Baretti B.[1]

[1]Haema AG, Qualitätskontrolle, Leipzig, Germany
[2]Haema AG, Verarbeitung/Vertrieb, Leipzig, Germany
[3]Haema AG, Medizin, Leipzig, Germany

Introduction: Although the risk of viral transfusion-transmitted infections (TTI) from recognized pathogens is low today, bacterial contamination and emerging pathogens remain a threat to transfusion safety. The INTERCEPT™ Blood System was developed to prevent TTI by inactivating pathogens utilizing amotosalen in combination with UVA light. The dual storage (DS) set has been designed with alternative plastics (non-DEHP material) to treat also double dose platelet donations. This study evaluated whether the residual amotosalen levels and *in vitro* function of pathogen inactivated platelets processed using the new DS set were functional over 7 days storage.
Methods: Six double dose platelet donations from normal volunteer donors were collected using Trima 6.0 automated blood collection system (Terumo, BCT). The pathogen reduction of the products was performed using the INTERCEPT DS-Set INT2504B. In order to ensure a constant quality after processing and after expiration of shelf life, quality control parameters for active substance content and platelet function were determined on day 1, 5 and 7.
Results: Platelets treated with amotosalen in the new system had *in vitro* function comparable to previous studies. The pH (22°C) was 6.94 ± 0.07 (d1) to 6.98 ± 0.05 (d7). The level of pO_2 was stable, while the level of pCO_2 dropped consistently during storage (pCO_2 treated platelets 32.00 ± 4.86 mmHg [d1] to 18.17 ± 1.94 mmHg [d7]). The concentration of HCO_{3-} decreased during storage (6.33 ± 0.52 mmol/l [d1] to 3.83 ± 0.75 mmol/l [d7]).
The response to hypotonic stress decreased from 101% [d1] to 84% [d7]. Platelet units showed an expression of activation marker CD62 of 29% at d1 and 16% at d7. Platelets maintained their ability to be activated by ADP. After 7 days of storage, all tested units were negative in bacterial cultures.
Conclusion: The collected results of measured parameters does not differ from the results of previous studies. Pathogen inactivated platelets processed using the DS-Set INT2504B consisting of alternative plastics (non-DEHP material) retained adequate *in vitro* function for up to 5 days and met the criteria of the «Council of Europe Recommendation N°.R(95) 15" as well as the German guidelines.

Conflict of interest: none

PS-2B-10

Effects of shear stress on platelet function and integrity

Fiedler S. A., Junker A. C., Seitz R., Salge-Bartels U.

Paul-Ehrlich-Institut, Hämatologie/Transfusionsmedizin, Langen, Germany

Introduction: The short lifespan of platelets and their lack of robustness call for special measures in handling and storage. Yet, about 20–30% of transfused platelet concentrates fail to sustainably increase platelet increment. Therefore, application of a meaningful test might help to identify those concentrates which do not possess adequate constitutive capacity. We hypothesize that stress exposure can reveal frail and non-performing platelets. Shear forces were applied to estimate platelet functional integrity.
Methods: Platelet rich plasma (PRP) was obtained from single donor apheresis and pooled platelet concentrates after three days of storage. Suspensions of 3 × 108 platelets/mL were perfused through channels of collagen-coated µ-slides (ibidi™, Munich, shear rate 1700 per sec) for 4 min at 37°C with or without 5 µmol/L thrombin receptor activator peptide (TRAP6). Adherent platelets were visualized microscopically after staining with CD41-Fluorescein isothiocyanate (FITC). Markers of platelet activation as P-selectin and phosphatidylserine (PS) surface expression, soluble P-selectin and the release of chemokines CXCL7 and RANTES (Regulated And Normal T cell Expressed and Secreted) were determined by flow cytometry (FCM) and ELISA, respectively. Collagen and ADP-stimulated aggregation was assessed by light transmission aggregometry, thrombelastometry (ROTEM™, tem international, Munich) was applied to measure clot formation and firmness after recalcification.
Result: Platelet adhesion to collagen surfaces was found to some extent under static and shear stress conditions and was moderately increased in the presence of TRAP6. Only single samples showed an excessive response. PS expression was low (< 2%) in platelets and microvesicles (MV) (< 6%) regardless of shear forces. P-selectin expression was in the range of 5% to 15% in general, but in three of 19 samples the initial low P-selectin levels were markedly elevated after shear stress. Plasma concentrations of RANTES and CXCL7 were increased after shear stress in all samples, but to a different extent. In aggregation assays a loss of platelet responsiveness to ADP and collagen was observed for 4 of 20 samples. While MV shedding analysed by FCM did not show significant differences, thrombelastometry performed with platelet-depleted plasma showed that only after

shear stress the MV fraction was not able to essentially trigger coagulation as evident in the decreased velocity in clot formation and firmness.
Conclusion: Exposure of platelets to shear stress at an intensity similar to conditions in the arterial system caused a particular pattern of response and revealed a deviant behaviour for discrete samples. This kind of challenge could be helpful to identify platelets in a vulnerable status.

Conflict of interest: none

PS-2B-11

Assessment of the impact from pathogen inactivation of platelet concentrates on quality, production processes and costs two years after routine implementation

Rosskopf K., Helmberg W., Schlenke P.

Med. Uni. Graz, Univ. Klinik für Blutgruppenserologie und Transfusionsmedizin, Graz, Austria

Pathogen inactivation (PI) of double platelet concentrates made of eight buffy coats (BC-PC) and of apheresis PC (A-PC) with the INTERCEPT™ Blood System (IBS) was implemented at our centre in 2016 after a validation phase and a cost-benefit assessment. We present our experience after two years of routine PI, comparing with a «historic control» before PI regarding cost-benefit.
To increase the production and cost efficacy we produce PC made of 8 small-volume buffy coats and using a INTERCEPT platelet processing set with dual storage containers and one illumination bag for treatment. An annual revalidation of the process and routine quality controls were done on both BC-PC and A-PC. PI allowed to raise shelf life of PC from five to seven days in Austria. During the PI period little adjustments of the component separators were necessary to stay within the processing specifications for both IBS and PC. Regarding economical aspects for both products a waterfall analysis was undertaken which includes costs for PI and savings such as bacterial screening, gamma irradiation, lower scrap rates, lower outdating rates and slightly higher amounts of whole blood plasma by reducing the BC volume. Labor costs were not included as no additional manpower was necessary.
BC-PC before and after PI implementation had a platelet yield (mean±sd) of 2,5 ± 0,4 vs. 2,5 ± 0,3 x10E11 whereas A-PC had 2,7 ± 0,5 vs. 3,0 ± 0,7 x10E11. The limit of <2 × 10E11 Plt was not met for BC-PC in 8,1% vs. 3,4%, and for A-PC in 4,9% vs. 1,4%, respectively. The rate of expired products was reduced by 50% for the BC-PC (17,6% vs 8,7%) and by 20% for the A-PC (4,7% vs 3,8%). Scrap rates also decreased markedly by optimizing the process flow (2,7% vs. 0,9% for A-PC and 7,3% vs. 2,8% for BC-PC). PC adverse reactions has also decreased by one third. The waterfall analysis showed that the production cost for BC-PC after PI-implementation decreased with 9 € whereas for A-PC it increased with 38 €.
The implementation of PI for PC leads to reconsidering our manufacturing processes in producing A-PC as well as BC-PC, resulting in optimization of production, lower scrap rates and other advantages. Routine experience learns that the production of double-dose BC-PC increases the production efficacy and decreases the cost impact of PI. Our cost-benefit assessment before the implementation of PI could be confirmed by reviewing the costs and other aspects in a waterfall analysis two years after implementation. Additionally, the increase in blood safety for patients receiving PCs has to be taken into account.

Conflict of interest: none

PS-2B-12

Differences in the consistency of plasma obtained from whole blood and apheresis under consideration of plasma proteins

Suessner S.[1], Hofer M.[2], Angermayr K.[1], Havlicek-Poetscher M.[1]

[1]Red Cross Blood Transfusion Service of Upper Austria, Immunohematology and quality control, Linz, Austria
[2]FH Gesundheitsberufe OÖ GmbH, Linz, Austria

Background: Human plasma is used as a raw material to produce therapeutic plasma products. It is collected from single donors via whole blood donation or apheresis. As there are differences in the processing as well as in the interval for donation, the target of this study was to evaluate the content of several proteins for this two groups of plasma products.
Methods: To show the differences in both plasma products, 121 whole blood and 99 apheresis plasma units were collected and analysed in the Blood Transfusion Service of Upper Austria. Clotting Factor VIII and Fibrinogen were measured with the BCS XP system (Siemens), Immunoglobulin G (IgG) and Total Protein with the Architect System (Abbott). The results were statistically analysed by using IBM SPSSv23.
Results: The results showed significant differences ($p<0.001$) between both collection methods in consideration of the measured parameters. FVIII and Fibrinogen showed significantly higher levels in plasma units collected by apheresis than whole blood, which differed from that with significant higher levels of IgG and Total Protein as apheresis plasma units (both $p<0.001$). There were no relatable gender specific differences nor was there any difference between blood groups, expect for the factor VIII, which was significantly lower in blood group 0 than in A, B or AB.
Conclusion: These two collection and preparation methods differ greatly and significantly through the levels of the measured plasma proteins. The analysis of those parameters could be used as additional quality control criteria. The Clotting Factor VIII is more unstable then both fibrinogen and IgG.

Conflict of interest: none

PS-2B-13

Development and validation of an extraction method for microplastics from biological materials

Monteleone A., Fath A., Wenzel F.

Hochschule Furtwangen, Villingen-Schwenningen, Germany

Introduction: Since the beginning of industrial production in 1950, plastic production has continued to grow strongly worldwide and is now at 322 million tonnes in the year 2015. From these very high production volumes ever larger quantities are found in the environment. There the plastics degradate to microplasticity and spread ubiquitously in the world. The present work deals with the possible uptake of microplastic particles in human organisms. For the detection of these plastic particles, an extraction method was developed and validated.
Materials and Methods: Biological materials consist of human blood (healthy volunteers, n = 5) and different tissues of pigs and cattles. Various lysis solutions were tested for degradation efficiency of biological material and for effects on the plastics. The mass loss, surfaces and structure variations as well as the physicochemical spectrum of the material were observed after treatment by atomic force (AFM) and electron microscopy (EM) and Fourier transform infrared spectrometry (FTIR).
Results: The different plastic types as polyamide (PA), polycarbonate (PC), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC) could be clearly differentiated and identified by FTIR. Regarding the surface control, especially PVC showed detectable alterations: After extraction an irregular surface structure caused by protuberances or bubbles could be observed. However, instead of these alterations an equivalent count of plastic particles was found in correlation to the applied plastic amount (recovery rate overall was 99,35 ± 0,43%).

Conclusion: The applied method can be used for plastic extractions from human or animal tissues without remarkable effects on the plastics.

Conflict of interest: none

PS-3A
Immunhämatologie

PS-3A-1

DaraEx: a new approach to solve the interference of Daratumumab in the indirect antiglobulin test

Lejon Crottet S.[1], Lobsiger S.[2], Hustinx H.[1]

[1]Interregionale Blutspende SRK AG, Immunhämatologie, Bern, Switzerland
[2]Medi, Zentrum für medizinische Bildung, Biomedizinische Analytik, Bern, Switzerland

Background: In 2016 Daratumumab (DARA) was approved in Europe as a third-line treatment for patients with multiple myeloma (MM). DARA is a human monoclonal anti-CD38 antibody that binds to the glycoprotein CD38, which is highly expressed on the surface of MM cells. CD38 is also expressed on other cells, such as erythrocytes, and thus causes pan reactions when DARA containing plasma (DARA-pla) is used in the indirect antiglobulin test (IAT). In order to eliminate this pan reactivity test cells can be treated with dithiothreitol (DTT). This reducing agent disrupts the disulphide bonds in the CD38 molecule, thereby denaturing the protein. DTT also destroys important blood group antigens such as KEL1, KEL3, DO1 and DO2. A new reagent, DaraEx (Inno-Tran), inhibits the reactions between DARA and the test cells without negatively influencing other blood group antigens. The aim of this work was to evaluate the performance of DaraEx
Methods: DARA-pla were used in the IAT with untreated (UT), 5 mM DTT treated (DTT-T) and DaraEx treated (DaraEx-T) cells according to manufacturer»s instructions. IAT with DTT-T cells was performed in IAT/tube, whereas IAT with UT and DaraEx-T cell was performed in antiglobulin gel cards (BioRad). A titration of DARA-pla was also performed. In addition DARA-pla was mixed 1:1 with antibodies of known specificity and analysed by IAT using the UT, DTT-T and DaraEx-T cells. Furthermore, the stability of DaraEx-T cells was evaluated
Results: During the initial experiments DaraEx-T cells exhibited in IAT weak reactions with DARA-pla in comparison to DTT-T cells which showed negative reactions. The incubation with DaraEx was initially performed on an Eppendorf thermomixer at 600 rpm, according to manufacturer» instructions. When the agitation method was changed to a Sysmex rolling mixer, these weak reactions could be mostly eliminated. Titration studies of DARA-pla showed titres ranging from 4 to 4000. IAT with DaraEx-T cells were able to detect very weak anti-K antibodies whereas IAT with DTT-T could not. Control of the K antigen on DTT-T cells revealed that with 5 mM DTT the antigen was not totally eliminated. Other antibodies with specificities such as anti-D, anti-Fy(a), anti-Fy(b) and strong anti-K could be detected by both methods, although the reactions were slightly weaker with DTT-T cells. DaraEx-T cells are stable for at least a week
Conclusion: The incubation step during treatment with DaraEx was showed to be crucial as incubation with insufficient agitation caused an incomplete inhibition of the pan reactivity. Both DaraEx-T and DTT-T cells could detect antibodies, although only DaraEx-T cells could detect very weak anti-K antibodies. The weaker reaction observed with DTT-T cells was not only due to the DTT-T, but also the less sensitive method (e.g. tube technique). DaraEx is an expensive reagent that mostly eliminates the interference of DARA and a more sensitive method for antibody detection compared to DTT-T cells.

Conflict of interest: none

PS-3A-2

Validation of Grifols antisera for routine red blood cell phenotyping by comparative analysis

Gleich-Nagel T.[1], Corisello C.[1], Huber-Marcantonio D.[1], Canellini G.[1], Niederhauser C.[2]

[1]Interregionale Blutspende SRK Bern, Unité de médecine transfusionnelle, Lausanne, Switzerland
[2]Interregionale Blutspende SRK Bern, Labordiagnostik, Bern, Switzerland

Background: The field of immunohematology is directly linked to a patient»s wellbeing. Suitable blood products for transfusion purposes must be chosen carefully since it directly affects the outcome of patient treatments and survival. Extended phenotyping of red blood cells is particular important in chronically transfusion e.g. for patients with sickle cell anemia or in patients with warm autoantibodies. To guarantee a reliable phenotyping result we use Biorad antisera on a routine basis. Since phenotyping is a crucial technique to guarantee the delivery of patient compatible blood, we decided to validate an additional method to avoid the delay of transfusion because of material failure. Consequently, we performed comparative analysis with the routinely used Biorad antisera and the newly obtained Grifols antisera.
Material/Methods: For the comparative analysis, we phenotyped red blood cells with Grifols regencies in comparison to the already validated Biorad antisera (Biorad, Biorad Seraclone, Biorad Diaclon). To determine the robustness of the test, we used selected Grifols antisera to find a time window for the most reliable result interpretation. For all experiments, donor erythrocytes of a known phenotype were used. The obtained results where statistically evaluated by the Mann-Whitney test using Graphpad Prism software.
Results: We obtained no false negative or positive results using Grifols regencies. Furthermore, we observed a significant stronger reaction intensity for the antigens Cw, s, Leb, and Fyb with Grifols antisera, while only the antigen M reacted stronger when using Biorad regencies. For further qualification of the Grifols antisera, we performed tests for the robustness by comparing the reaction intensity at different time points during 60min after the final centrifugation step. We could not observe any intensity variation of more than 2+ or changes to a false positive or negative result.
Discussion: Extended phenotyping of red blood cells is an important step to develop transfusion strategies. Therefore, during the validation process of Grifols regencies it was a prerequisite to ensure reliable phenotyping results. The aforementioned results confirmed that Grifols antisera indeed can be used in routine red blood cell phenotyping without any restriction. In fact, the routinely used Biorad regencies showed only for the M-antigen a higher sensitivity. For the test of robustness, we considered variations of more than 2+ and changes to a false positive or negative result as exclusion criteria. Since, none of these criteria were met we concluded that phenotyping results can be safely read within one hour after the last centrifugation step. Consequently, based on the obtained results we validated Grifols antisera for routine red blood cell phenotyping in our laboratory.

Conflict of interest: none

PS-3A-3

Autoanti-LW^a appears as anti-D at first sight

Song Y. L.[1], Glaus S.[1], Sirgurdadottir S.[2], Gassner C.[2], Frey B. M.[1], Engström C.[1]

[1]Blutspende Zürich SRK, Immunhämatologie, Zürich, Switzerland
[2]Blutspende Zürich SRK, Molekularbiologie, Zürich, Switzerland

Introduction: Although LW and D antigens are located on different proteins, LW glycoprotein requires an interaction with Rhesus (Rh) proteins to exhibit its expression. RhD positive red blood cells (RBC) express LW^a more strongly than RhD negative cells. Hence, allo-/autoanti-LW may be mistaken for allo-/autoanti-D. Fortunately, and in contrast to Rh antigens, LW antigens are denatured when treated with dithiothreitol (DTT). We report a case of a 61-year-old woman with autoanti-LW^a, in need of

several peri- and postoperative transfusions, due to a septical infection after implantation and several revisions of a knee replacement surgery. Previously, she had received multiple RhD positive transfusions and already formed an anti-E.
Methods: Standard serological techniques for antibody detection and differentiation were applied (gel-card and tube test; BioRad, Cressier, CH). Rhesus pheno- and genotype were analyzed serologically (Erytra®, Grifols, Duedingen, CH) and molecularly using PCR-SSP (inno-train GmbH, Kronberg i. T., D). Additionally, molecular LW antigen status was confirmed by PCR-SSP (in-house).
Amplicons for RHD sequencing were prepared in-house and analyzed externally (Microsynth, Balgach,CH).
Results: The serological analysis presented an O CcD.ee (R1r), K- phenotype. Except the already known anti-E, the antibody differentiation revealed an antibody, weakly reactive with all RhD positive RBC in indirect antiglobulin test and on papainized cells. IgG coating could be shown in the direct antiglobulin test with six times washed patient's erythrocytes. The autocontrol was positive and the eluate showed negative to weak reactions with RhD positive RBC. Sequencing of the RHD gene affirmed the absence of any mutation possibly explaining the presence of an alloanti-D. However, there were no reactions with DTT-treated RhD positive and RhD negative RBC which confirmed the specificity of an anti-LW. In addition, the genotyping showed the predicted phenotype LW(a+b-), suggesting the diagnosis of an autoanti-LW^a.
Conclusions: Before all diagnostic procedures were finalized, the patient was transfused with 16 O rr RBC products not to overlook an alloanti-D. Summarizing, still presenting autoanti-LW^a, but not showing any signs of hemolysis, we recommended continuing transfusions with RhDpositive blood of compatible phenotype. This specifically, since autoantibodies are usually considered irrelevant for transfusion management. Our approach was proven right, upon re-surgery after 3 months and transfusion of 10 O R1r RBC products, followed by 4 OR1r RBC products two months later, both times without any hemolytic transfusion reaction. Besides, in our case all crossmatches of the transfused blood products were negative. Only 2 of the initially 12 tested O R1r products showed slightly positive crossmatches due to the autoanti-LW^a. These were not selected for transfusion, since sufficient compatible erythrocyte concentrates were available.

Conflict of interest: none

PS-3A-4

MDMULTICARD® – A fast and reliable new member for the immunohematology toolbox

Zorbas A.[1], Rizzi G.[1], Engström C.[1], Caesar A.[2], Schwind P.[2], Gassner C.[3], Song Y. L.[1], Frey B. M.[1]

[1]Blutspende Zürich SRK, Immunhämatologie, Zürich, Switzerland
[2]Medion Grifols Diagnostics, Duedingen, Switzerland
[3]Blutspende Zürich SRK, Molekularbiologie, Zürich, Switzerland

Data have already been presented at ISBT 2018, Toronto.
Introduction: The MDmulticard® Basic Extended Phenotype (Grifols, Duedingen, CH) was launched in September 2016 and allows simultaneous typing for Jk^a, Jk^b, Fy^a, Fy^b, S, s antigens using lateral flow technique. In order to implement the MDmulticard® as an additional analytic platform we examined samples taken from patients suffering from clinical conditions known to hamper serological red blood cell (RBC) antigen typing.
Methods: A series of 63 cases were assessed by MDmulticard® including samples of patients with warm and cold autoimmune hemolytic anemia (AIHA, n = 11), hemoglobinopathies (n = 7), paraproteinemia (n = 5), under treatment with daratumumab (n = 6) and samples of neonates (n = 4) and of pregnant women (n = 2) as well as samples of healthy blood donors (n = 14, three with known Fy^x). The results were compared with standard test methods, either serologically (Erytra®, Grifols, Duedingen, CH or BioRad, Cressier, CH) or by molecular typing using PCR SSP (inno-train GmbH, Kronberg i. T., D).
Results: The MDmulticard® was easy to handle and provided rapid results (in average 9 minutes from test start) making the method suitable for emergency applications. Overall the results were confirmed by alternative methods or known pre-values. Two of known Fy^a negative samples showed false positive reactions by MDmulticard® due to the patients' strongly positive DAT (3+ and 4+).
One sample delivered a weak Jk^b positive result by MDmulticard® although the patient was known to be Jk^b negative by PCR. Clinical evaluation revealed recent transfusion of Jk^b positive RBC concentrates. In two IgM-DAT positive samples, the predicted phenotype by PCR could accurately diagnosed by MDmulticard® only upon washing the patient's RBCs with NaCl 0.9%. Another sample from a patient with severe cold AIHA needed to be washed with warm NaCl 0.9%.
Conclusions: MDmulticard® allows reliable RBC typing even of DAT positive samples MDmulticard® may be applied to samples of patients suffering from clinical conditions such as sickle cell disease, AIHA or paraproteinemia impairing standard serological typing. In pre-transfused patients or such with a strongly positive DAT, the distinct positive reaction by MDmulticard® allows differentiating between false positive reactions and inherited antigen positive RBC. For emergency situations, the MDmulticard® proves to provide rapid and reliable antigen typing which allows phenotype compatible RBC transfusion.

Conflict of interest: Die MDMulticard wurde dem BSD SRK Zürich während der Testperiode von Medion Grifols Diagnostics kostenfrei zur Verfügung gestellt.

PS-3A-5

Comparison of clinically significant antigens detected by lateral-flow-method and PCR in samples of patients with autoantibodies having a positive direct antiglobulin test (DAT), recently transfused patients and newborns

Hoeller K., Suessner S., Unterberger E., Juengling G.

Red Cross Blood Transfusion Service of Upper Austria, Immunohematology and quality control, Linz, Austria

Background: Patients with autoantibodies having a positive direct antiglobulin test (DAT) as well as recently transfused patients who need blood transfusion in case of emergency need testing of clinically significant blood antigens. Molecular techniques such as PCR have become a crucial tool but are on the one hand expensive and on the other hand time consuming. Serological typing of blood group antigens have in case of column agglutination technique limitations as far as DAT positive and recently transfused patients are concerned.
Methods: Patient Blood samples with positive DAT (≥ 2) and thereof some which were recently transfused as well as newborn samples were tested with the MDmulticard Basic Extended Phenotype and genotyped and the results compared.
Results: In case of positive DAT two out of 11 samples were tested falsely Fya positive. In case of recently transfused patients one out of three samples was tested falsely positive, the other two were obviously recently transfused. Newborn samples were all detected correctly.
Conclusion: For typing patients with a positive DAT as well as confirming weak phenotypes such a Fyx the MDmulticard Basic Extended Phenotype may be very useful in case of emergency during weekend or night. Furthermore, with the very low sample volume of 25 µl required it is also adequate for the typing of newborns.

Conflict of interest: none

PS-3A-6

Automated alloantibody titration on the NEO Iris

Gellerer A., Preuß E., Spigarelli M. G.

Immucor Medizinische Diagnostik GmbH, Research & Development, Dreieich, Germany

Introduction: Titration for clinically significant red cell antibodies is frequently used as a semi-quantitative method to predict the risk of hemolytic disease of the fetus and newborn (HDFN) in pregnant women or for donor/patient characterization in incompatible blood transfusions and organ transplantation. Conventional manual tube test and column agglutination technique (gel) are most commonly used methods in the field. These manual assays are labor intensive and prone to errors making it difficult to obtain fully reproducible results for clinical applications such as monitoring the production of IgG antibodies during pregnancy or to determine the time course of antibody concentration change. Therefore, the need for an automated, more standardized method remains high. Currently, Immucor is developing a fully automated IgG antibody titration for the NEO Iris™ platform based on the patented Capture® technology. The aim of the study was to compare the performance of the fully automated IgG titration assay using solid phase (Capture®) technology to manual column agglutination technique (gel) and manual tube test (IAT). Additionally, titer reproducibility within the same red cell reagent lot was assessed.
Methods: Donor samples with different known alloantibodies as well as the 2nd WHO international standard for anti-D immunoglobulin were tested using a Capture® based prototype assay including automated titration of the samples on the NEO Iris™. Titer results were compared to a manually prepared doubling dilution series tested on a Capture® based prototype assay, on manual gel card (Coombs anti-IgG) with and without DTT and with indirect agglutination tube test.
Both donor and clinical samples were tested at various time points on the automated titration prototype assay on the NEO Iris™ using the same red cell reagent lot to test reproducibility of titer results.
Results: The titer results of the Capture® based assay using automated dilution of the samples are very consistent to the titer results of the manually diluted samples on the same assay type on NEO Iris™ and on gel card with and without DTT treatment (all titers within two doubling dilutions). IAT shows the highest variations compared to gel and Capture®. Reagent stability studies demonstrated high reproducibility of titer results for donor and clinical specimens.
Conclusion: Immucor»s alloantibody titration prototype assays on the NEO Iris™ provides a fully automated, standardized solution to generate reproducible and consistent titer results for a wide range of clinically significant red cell IgG antibodies.

Conflict of interest: none

PS-3A-7

Autoimmune hemolytic anemia in pregnancy caused by autoanti-Jk(a) with novel described mutation of the JK*A (Q21R) in Exon 1 of the SLC14A1 gene

Strathmann K.[1], Winzen D.[2], Najjari L.[3], Doescher A.[4], Wessiepe M.[1], Hutschenreuter G.[1]

[1]Transfusion Medicine, University Hospital RWTH Aachen, Aachen, Germany
[2]Gynecology and Obstetrics office, Zülpich, Germany
[3]Clinic for Gynecology and Obstetrics, University Hospital RWTH Aachen, Aachen, Germany
[4]German Red Cross Blood Service NSTOB, Oldenburg, Germany

Introduction: We report a case of a warm antibody autoimmune hemolytic anemia in a 32-year old female patient in the first pregnancy. The degree of hemolysis in the context of autoimmune hemolytic anemia in pregnancy may be very variable interindividual. In the context of maternal autoimmune hemolytic anemia in the literature fetal courses are described in a range from clinical unremarkable to transfusion-requiring courses.
Methods: Blood specimens (EDTA) from the patient were tested. ABO and rhesus antigens, antibody screening and antibody identification and antibody titre were performed by using the gel card technique and RBC-bound antibodies acid elution kit was used (bio-rad, Munich, Germany). Genotyping was done with allele specific PCR (Rh-Type, BAG, Lich, Germany). For sequencing ABIprism 310 (Applied Biosystems, Darmstadt, Germany) was applied.
Results: In this case in maternal plasma a autoantibody auto-anti-Jk (a) could be detected. The direct coombstest for IgG and C3d was strong reactive and a panreactive bound antibody was detectable in acid elution. In a external laboratory initially the anti-Jk(a) specifity was diagnosed as a alloantibody because serological antigen typing was described as Jk(a)-negative. However, a genotype JK*A/ JK*B could be detected in a SSP-PCR. In the DNA sequencing analysis (DRK NSTOB), a previously unknown mutation in Exon 1 in SCLC14A1 gene at position Q21R with an exchange of glutamine to asparagine could be detected. In a sequencing at the cDNA level, the localization of this mutation could be confirmed on the JK*A allele as JK*A(Q21R)/B.
The patient had hemoglobine values of 8.2–10.8 g/dl during pregnancy. She received an oral iron substitution and prednisolone therapy with 10 mg/d. In the 27 week of gestation an antibody titre of 2 had been detected (ICT-IgG). The haptoglobine level was < 10 mg/dl and the LDH was 456 U/l (norm < 250 U/l). *In the course of pregnancy follow-up, there was no clinical or sonographic evidence of anemia in fetus. The hemoglobin concentration was 19.6 g/dl at birth. The clinical course in the newborn was unremarkable. Unfortunately, no material from the newborn was available for a direct coombs test.*
Conclusion: In this case of warm antibody autoimmune hemolytic anemia, a hitherto undescribed mutation in the SCLC14A1 gene had been detected. The definition of whether it is an allo- or autoantibody is relevant for the assessment of a risk for the occurrence of hemolytic disease of the newborn and to choose the right therapy for the mother. There are described mutations in the near of JK*A(Q21R) which are associated with a decreased Jk(a) antigen expression. In such cases, a close interdisciplinary cooperation between the supervising gynecologists in practice and in the clinic, the physician of transfusion medicine and the laboratory for molecular blood group diagnostics is particularly important for the safe supply of the mother and the child.

Conflict of interest: none

PS-3A-8

Casereport: Autoanti-D causing acute warm autoimmune hemolysis

Song Y. L.[1], Zorbas A.[1], Siciliano D.[2], Gassner C.[3], Frey B. M.[1], Engström C.[1]

[1]Blutspende Zürich SRK, Immunhämatologie, Zürich, Switzerland
[2]Tucare, Onokolgie und Hämatologie, Bülach, Switzerland
[3]Blutspende Zürich SRK, Molekularbiologie, Zürich, Switzerland

Introduction: We report a case of a 68 year-old woman who developed a warm autoimmune hemolytic anemia (AIHA) caused by an autoantibody with anti-D specificity requiring transfusions.
Two weeks after suffering from gastroenteritis of unknown cause, the patient was hospitalized because of symptomatic hemolytic anemia. Laboratory tests revealed hemoglobin of 6.2g/dl, elevated lactate dehydrogenase, reticulocytes and bilirubin as well as decreased haptoglobin. Differential diagnostics excluded any primary hematological disorder such as MDS, CLL, NHL or PNH. The patient denied having taken any medications or nutrient additives in the recent past.
Methods: Standard serological methods for antibody detection and specification were applied (gel-card and tube test; BioRad, Cressier, CH). The Rhesus (Rh) pheno- and genotype were assessed serologically (Wadiana®, Grifols, Duedingen, CH) and by molecular typing using PCR-SSP (in-no-train GmbH, Kronberg i. T, D and in-house).
Results: Rhesus phenotype was D+, C+/c+, E+/e+ (R1R2). Direct antiglobulin test (DAT) was positive for IgG1.The patient's serum reacted with

all RhD positive panel cells in indirect antiglobulin test (IAT) and on papain treated cells. The eluate of patient's red blood cells reacted the same way as her serum. The autocontrol was positive. Maintained reactions with Dithiothreitol(DTT)-treated RhD positive RBC ruled out anti-LW mimicking anti-D specificity. Initial anti-D-titer was very high (1:16384). Additional genotyping revealed RhD homozygosity (*RHD*01/RHD*01*) and excluded the most prevalent RhD variants, further supporting the presence of an autoanti-D.
At follow-up examinations, DAT and antibody detection were negative. by routine techniques. However, DAT became weakly positive for IgG after incubation with patient's serum and there were weakly reactions with some papain treated R2R2 cells in IAT. Autocontrol was positive, but the eluate was entirely negative.
Conclusion: Only few cases of AIHA caused by autoanti-D have been published so far. In our laboratory, whenever autoanti-D is suspected certain investigations must be performed not to overlook an alloantibody. The overall results confirmed autoanti-D causing the hemolysis. Autoantibodies are generally considered irrelevant for transfusion management but become crucial when causing acute hemolysis. Fortunately, autoanti-D can easily be taken into account by choosing RhD negative RBC for transfusion. In our case, the patient was initially transfused with three RhD negative RBC products showing an increase of the hemoglobin value up to 9.8g/dl. Shortly thereafter, when steroid therapy was started and performed over five months, no more blood transfusion was required and the patient improved completely.

Conflict of interest: none

PS-3A-9

Severe fetal haemolytic anaemia due to anti-c immunization following heterologous ovum donation: A case report

Güsmer C.[1], Eschenburg M.[2], Blohm M.[3], Peine S.[1], Goebel M.[1]

[1]UKE Hamburg, Institut für Transfusionsmedizin, Hamburg, Germany
[2]UKE Hamburg, Klinik für Geburtshilfe und Pränatalmedizin, Hamburg, Germany
[3]UKE Hamburg, Sektion Neonatologie und Pädiatrische Intensivmedizin, Hamburg, Germany

Introduction: Risk for haemolytic disease of the newborn (HDN) is increased in pregnancies resulting from assisted reproductive technology (ART) with heterologous ovum donation. Supply with suitable RBC is maybe difficult. Therefore, considering suitable rhesus subtypes of mother and ovum donator should be implemented routinely.
Methods: A 47-year-old fourth gravida first para was referred to our university perinatal centre in 27+6 weeks of gestation. Her blood type was 0 Rh pos. (CCD.ee) and screening showed an anti-c antibody titre of 1:512. The blood types of the ovum donor and the child's father were both 0 Rh pos. without genotype information. Analysis of the fetal blood revealed a 0 Rh neg (ccddee) genotype and anaemia with 6g/dl.
Results: Regardless to the ccddee genotype of the child, intrauterine and postnatal blood transfusions (exchange transfusion immediately after birth and twice RBC transfusion during hospitalization) were performed with O Rh pos. (CCD.ee) erythrocytes. Compatible cc blood would have been eliminated due to the high anti-c-titer. Due to rupture of membranes a caesarean section was performed 30+2 weeks of gestation and after intensive paediatric support the child was discharged in good conditions two months later.
Anti-c-titer in child decreased from 8 after birth, 2 after six weeks to 1 after 2 month. As expected no alloimmunisation of the newborn after IUT with Rh pos. RBC was seen in control at 6 month after birth (1).
Conclusion: Due to the antibody we had to leave our standard procedure with 0 Rh neg. RBC for intrauterine and postnatal transfusion. Anti-c-titer in child decreased in 2 month and haemolysis parameter normalized. ART conceived pregnancies without rhesus genotype matching may result in serious immunization with necessity of immense perinatal efforts to optimize the child»s outcome.
We are convinced that this case will help to become more vigilant for possible complications that are associated with the increasing amount of ART initiated pregnancies that we have to face today.

Reference:

1. Red blood cell alloimmunization in neonates, Türkmen et al, Transfusion 2017 Nov; 57(11):2720–2726.

Conflict of interest: none

PS-3A-11

Altered immunity of the stem cell niche in MDS – contribution of microRNAs

Kindermann A.[1], Heidel F.[2], Kielstein H.[1], Quandt D.[1]

[1]MLU Halle, Institut für Anatomie und Zellbiologie, Halle (Saale), Germany
[2]Friedrich-Schiller Universität Jena, Institut für Hämatologie und Internistische Onkologie, Jena, Germany

Myelodysplastic syndromes (MDS) are clonal hematopoietic neoplasia characterized by diverse peripheral cytopenia caused by defective hematopoiesis and a variable tendency to evolve into acute myeloid leukemia. MicroRNAs are known to be tight regulators of normal hematopoiesis but also to be dysregulated in various types of cancer and therefore are targets of current therapeutic approaches. Differential expression of microRNAs in MDS patient»s cellular components of bone marrow and plasma are identified. Many of those microRNAs, among them miR-34a are well established for other tumor entities but the molecular mechanism of action remains elusive in MDS. In particular the affiliation of altered microRNAs to different cell types of the stem cell niche is unknown. The stem cell niche is a complex cellular and multifactorial space that is among others influenced by cells of the innate and adaptive immunity, such as T cells.
To identify the impact of microRNAs to the disease pathology we made use of different hematopoetic precursor cell lines, bone marrow material of patients and healthy controls and T cells derived from healthy donors of the local blood bank.
Constitutive and cytokine induced alterations in microRNA profiles of hematopoetic cell lines such as MDS-L, THP-1 and HL-60, were investigated. MicroRNA 15a, 34a, and 324–5p are expressed constitutively with varying levels but no alteration upon IFNgamma, TNFalpha or IL-1beta were detected. The same cytokines led to differential expression of MHC molecules as well as costimulatory molecules of the B7 and the TNF receptor family.
Allogeneic tumor-T cell co-culture assays with precursor cell lines and healthy T cells were used to study the impact of mir34a. By the use of Viromer transfection we were able to overexpress miR-34a using mimics in primary human T cells and malignant precursor cell lines as determined by stem loop qPCR. Tumor-T cell co-culture assays with altered miR-34a expression on the tumor site did not alter T cell proliferation neither T cell activation marker expression. We identified a high frequencies of T cells in the bone marrow samples of MDS patients, established a differential cell sorting strategy for uncommitted stem cells and T cell and were able to isolate high quality RNA from as low as 100 cells.
MiR-34a seems not to contribute primarily to T cell-tumor communication in MDS. The role of miR34a for stem cell survival and differentiation and the impact of other differential expressed microRNAs for the pathogenesis of MDS will be explored.

Conflict of interest: none

PS-3A-12

Bombay phenotype in a German patient with short stature, mental retardation and seizures

Cooper N.[1], Möller A.[1], Wienzek-Lischka S.[1], Hackstein H.[2], Wagner F.[3], Sachs U. J.[1], Bein G.[1]

[1]Justus Liebig Universität Giessen, Institut für Transfusionsmedizin und Immunologie, Gießen, Germany
[2]Universitätsklinikum Erlangen, Transfusionsmedizin, Erlangen, Germany
[3]DRK-Blutspendedienst NSTOB gemeinnützige GmbH, Springe, Germany

Introduction: The Bombay phenotype (Oh) results from a lack of membrane glycoprotein H from the surface of red blood cells (RBC). Individuals develop anti-H antibodies, but have no other clinical symptoms. Bombay phenotype with clinical symptoms has been described in few patients with leukocyte adhesion deficiency type II (LAD II). Mutations in SLC35C1, which encodes GDP-fucose transporter 1 (GFTP), can lead to defects in the fucosylation of different surface proteins, resulting in immunodeficiency, mental retardation, dysmorphic features, and growth retardation.
Case report: We describe a 22 year old German male patient with periodontitis, admitted to the University Hospital for dental treatment under general anaesthesia because of severe mental retardation. The patient presented with growth retardation (140 cm, 35 kg), unusual facial appearance, and chronic seizures. In pre-operative routine, his RBCs were phenotyped as group O, and pan-reactive antibodies were detected both in saline and indirect Coomb»s test. There was no history of blood transfusion. Additional phenotyping with Anti-H lectin and a monoclonal Anti-H antibody revealed blood group Bombay (Oh). Lewis blood group antigens Le(a) and Le(b) were not expressed (non-secretor phenotype). His parents were non-relatives. Together with the clinical features, although recurrent infections were not reported, the patient received the preliminary diagnosis of LAD type II. Long-range PCR products covering the complete genomic sequence of the SLC35C1 gene were sequencend (Illumina, MiSeq), revealing two triplet deletions of the SLC35C1 gene encoding GFTP: maternal allel: c.247_249delGTG, and paternal allel: c.177_179delTAA.
Conclusion: We report a patient with Bombay phenotype with clinical symptoms related to mutations of the SLC35C1 gene. Anti-H lectin or monoclonal Anti-H antibodies are simple tools which allow rapid detection of this phenotype in patients with pan-reactive antibodies in saline and indirect Coomb»s test.

Conflict of interest: none

PS-3A-13

Immunophenotypic changes of human lamina propria myeloid cells in acute inflammation

Paparella M.[1], Kurzhals S.[2], Heidtmann A.[2], Lasitschka F.[3], Al-Saeedi M.[4], Wabnitz G.[2], Giese T.[2], Meuer S.[2], Schröder-Braunstein J.[2]

[1]DRK Blutspendedienst BaWü-Hessen, IKTZ Heidelberg, Heidelberg, Germany
[2]Universitätsklinikum Heidelberg, Institut für Immunologie, Heidelberg, Germany
[3]Universitätsklinikum Heidelberg, Institut für Pathologie, Heidelberg, Germany
[4]Universitätsklinikum Heidelberg, Chirurgische Klinik, Heidelberg, Germany

Introduction: The human colonic lamina propria (LP) harbors a vast amount of resident myeloid cells. Under homeostatic conditions, they exist in a hyporesponsive state, but are nonetheless able to mount an effective immune response when necessary. The molecular mechanisms initiating their activation are still poorly understood. We recently described a human intestinal organ culture model («Loss of Epithelial Layer» (LEL) model) which allows studying the onset of an inflammatory response in resident LP immune cells. In this model, the depletion of the epithelial cells leads to the activation of resident LP immune cells as well as their emigration out of the LP. Notably, the LEL model reflects many features of intestinal inflammation in vivo as shown by a comparison of global gene expression profiles of the LEL model with those of inflamed mucosa of IBD patients. Here, we aimed to immunophenotype myeloid cell subsets in the human colonic LP under homeostatic and inflammatory conditions employing the LEL model.
Methods: Human colonic mucosa was obtained from patients undergoing surgery. One part of the mucosa was rapidly digested to obtain LP myeloid cells in their resting state. The rest of the mucosa was depleted of epithelial cells by EDTA treatment and cultured in medium for 12 hours, resulting in the emigration of activated myeloid cells out of the LP. Harvested colonic cells as well as Ficoll-purified autologous peripheral blood mononuclear cells (PBMCs) were then analyzed using a multicolor flow-cytometry panel based on an antibody panel introduced by the HIP-C (Human Immunophenotyping Consortium).
Results: We were able to identify several myeloid cell populations in the colonic LP, including macrophages (MOs), plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs), both CD33+ and CD33-. Interestingly, among emigrated cells we identified myeloid-derived suppressor cells (MDSCs). In line with their activation in the LEL model, emigrated MOs and mDCs upregulated CD40, CD80, CD86 and HLA-DR compared to their resting counterparts. At the same time, both cell populations showed reduced expression or lacked the innate response receptors CD11b, CD14, CD16. Unlike PBMCs, activated intestinal MOs and mDCs expressed CD127 and CCR7 as well as high levels of TSLPR. The latter receptor was expressed on all activated myeloid populations, including MDSCs, but was not detectable on resting intestinal myeloid cells.
Conclusion: There is evidence that the TSLP-TSLPR axis plays an important role in the resolution of inflammation and wound healing. The significant upregulation of TSLPR on LP myeloid cells could therefore represent an important mechanism to counteract excessive early intestinal inflammation and thereby keeping it locally limited.

Conflict of interest: none

PS-3A-14

Eryptosis and delayed haemolysis in a patient with pre-existing anti-D and anti-C alloantibodies after an emergency transfusion of RhD-positive RBCs

Yürek S., Bartolmäs T., Mayer B., Pruß A.

Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Berlin, Germany

Introduction: In emergencies, transfusion of RhD-positive red blood cells (RBCs) to a priory unknown RhD-negative patient with Anti-D antibodies may sometimes be inevitable. Consequently, delayed haemolytic transfusion reaction often occurs due to IgG-mediated phagocytosis (extravascular haemolysis). Additionally, eryptosis, the suicidal death of RBCs, may occur in cases where strong reactive antibodies are involved.
Methods: A RhD-negative patient (age 64) with cardiovascular disease received two units of RhD-positive RBCs in an emergency after gastrointestinal bleeding. Afterwards, alloantibodies against D and C were detected in the pre-transfusion blood sample.
The patient was subsequently investigated for serological and laboratory signs of haemolysis using standard techniques. Additionally, to examine for eryptosis, patient»s EDTA sample was analysed for phosphatidylserine (PS) surface exposure by treatment with phycoerythrin (PE)-labelled Annexin V. Cell-associated fluorescence was measured using a MACSQuant flow cytometer.
Results: A strong anti-D and weak anti-C antibody were detected in the patient»s pre-transfusion sample. Three days later, the reactivity of anti-D was considerably reduced in the indirect antiglobulin test (IAT) due to adsorption of the antibody onto the transfused RhD-positive RBC. Consequently, direct antiglobulin test (DAT) and eluate reacted strongly, indicating a massive binding of anti-D to the transfused RBCs. After 3 months, anti-D and anti-C were still reacting strongly in the patient»s plasma and also in DAT/eluate. Laboratory parameters (lactate dehydrogenase, bilirubin, free haemoglobin, haptoglobin) showed considerable delayed haemolysis starting from about 4 days after incompatible transfusion and the patient subsequently required the transfusion of several

RhD-negative RBCs. In addition, fluorcytometric analysis revealed an increased level of PS+ RBC, indicating an eryptosis following the incompatible RBC-transfusion.
Conclusion: Delayed haemolysis after incompatible transfusion in patients with alloantibodies not only be due to antibody-mediated destruction and clearance of red blood cells (RBCs) by phagocytosis, but also by eryptosis. Eryptotic RBCs are usually removed quickly from circulation by macrophages, but may be detectable when phagocytosis is saturated.

Conflict of interest: none

PS-3B
Hämotherapie und Patienten-Blut-Management

PS-3B-1

Prevention of hemolysis and platelet dysfunctions in autologous surgery blood by a novel turbulence controlled suction system

Budde H., Riggert J., Vormfelde S., Tirilomis T., Friedrich M.
Universitätsmedizin Göttingen, Göttingen, Germany

Introduction: Suction and salvage of blood represents an important part of surgery. Especially the retransfusion of autologous blood is a central aspect of patient blood management. High hemolysis rates and platelet dysfunctions due to turbulences in the blood suction system are major problems for blood retransfusion. The aim of our study was to analyze a novel surgery blood suction system with an automatic control setup for prevention of turbulences in the blood flow.
Methods: We compared the turbulence controlled suction system (TCSS) with both, a conventional suction system and untreated control blood in an *in vitro* test setup. Blood cell counts, hemolysis levels according to free hemoglobin (fHb) and platelet functions were measured to determine the integrity of the three blood fractions.
Results: The conventional suction system was associated with a strong increase of the fHb levels. In contrast erythrocyte integrity was almost completely preserved when using the turbulence control system. We obtained similar results regarding the platelet function. Expression of platelet glycoproteins such as GP IIb/IIIa and p-selectin native or after stimulation with ADP were markedly impaired by the conventional system but not by the TCSS. In addition platelet aggregometry revealed significant platelet dysfunctions in conventional suction blood, but less aggregation impairments were present in blood samples from the TCSS.
Conclusion: Our findings from the *in vitro* test-setup show major improvements in red blood cell integrity as well as platelet function of suction blood when using the TCSS, compared to the conventional suction system. These results indicate significant functional benefits for the retransfusion of autologous blood. Testing the TCSS in surgery should be performed for clinical evaluation.

Conflict of interest: Martin Friedrich is the inventor of the TCSS and all included features. The TCSS is patented and the patent holder is the University Medical Center Göttingen (PCT/EP/2011/006330; US 9,402,937 B2; Aug 2, 2016). H. Budde, S. Vormfelde, T. Tirilomis and J. Riggert reported no financial interests or potential conflicts of interest. Disclosure of received funding: The authors declare that they have not received funding for this work.

PS-3B-2

Patient Blood Management (PBM) – From the «WHO Bench» to the bedside – Implementation of a simple PBM program in a high volume orthopaedic hospital

Stögbauer R.
Orthopädische Klinik Markgröningen, Anästhesie, Markgröningen, Germany

Introduction: Anaemia and allogeneic blood transfusions in surgical patients are associated with poor outcomes (1)(2). Patient blood management (PBM) has been developed as an evidence-based clinical tool, by which clinicians can optimise preoperative anaemia, manage peri-operative bleeding, avoid unnecessary blood transfusion and improve patient outcome.
Although the World Health Organization has endorsed PBM in 2011 (3), many hospitals still seek guidance with the implementation of PBM in clinical routine.
Many barriers and challenges limit translation of PBM guidelines into clinical practice, in particular due to lack of knowledge, interdisciplinary commitment, resources and concerns.
The succesfull implementation of a PBM program can become challenging especially in the daily routine of a high volume orthopaedic clinic.
Methods: We describe the details of a simple and succesfull 2 years PBM implemantation strategy with four cornerstones:
Treatment of preoperative anaemia
Application of tranexamic acid
Implementation of an hospital-wide transfusion trigger
Introduction of a single unit blood transfusion policy
Results: Preoperative anaemia was found in 23% of patients with elective primary total hip and knee replacement surgery.
Intravenous iron substitution for iron deficienca anaemia could be performed in 50% of anaemic patients.
The transfusion rate of patients with primary total hip replacement surgery (THR: n = 750/year) and total knee replacement surgery (TKR: n = 700/year) decreased after implementation oft the PBM program from 10% to 2,1% for THR and from 10% to 2,6% for TKR.
The overall hospital number of red blood cell transfusions decreased from 1.700 to 800/year.
Conclusions: The implementation of a simple peri-operative blood management program has reduced the post-operative RBC transfusion rate following primary elective hip and knee replacement surgery and halved the overall hospital number of red cell transfusions.

References:

1. Musallam KM, Tamim HM, Richards T, Spahn DR, Rosendaal FR, Habbal A et al.: Preoperative anaemia and postoperative outcomes in non-cardiac surgery: a retrospective cohort study. Lancet 2011; 378 (9800): 1396–1407.
2. Acheson AG, Brookes MJ, Spahn DR. Effects of Allogeneic Red Blood Cell Transfusions on Clinical Outcomes in Patients Undergoing Colorectal Cancer Surgery: A Systematic Review and Meta-Analysis. Annals of Surgery. 2012;256(2):235–244.
3. WHO Global Forum for Blood Safety: Patient Blood Management. 14–15 March 2011, Dubai, United Arab Emirates.

Conflict of interest: none

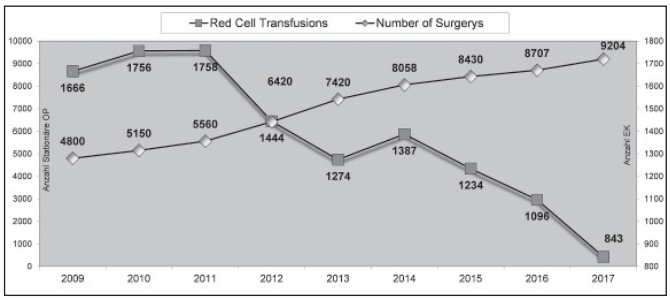

Fig. 1.

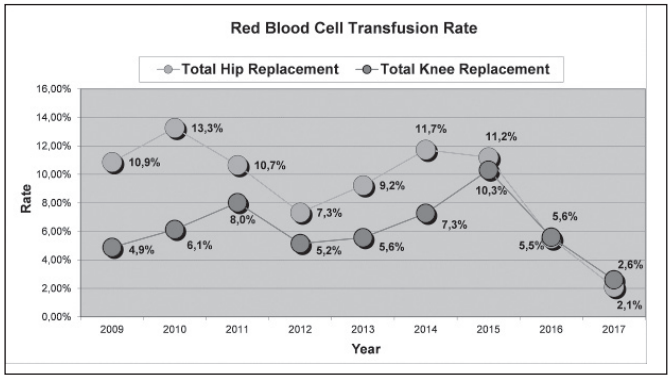

Fig. 2.

PS-3B-3

Anemia in patients with chronic kidney disease – analysis of a population based sample and evaluation of therapeutic consequences

Weckmann G.[1], Haase A.[1], Zimak C.[2], Chenot J. F.[1], Stracke S.[3,4], Spallek J.[5], Angelow A.[1]

[1]University Medicine Greifswald, Institute for Community Medicine, Department of General Practice, Greifswald, Germany
[2]University Medicine Greifswald, Institute for Community Medicine, Department SHIP-KEF, Greifswald, Germany
[3]University Medicine Greifswald, Universitätsmedizin Greifswald, Bereich Nephrologie, Dialyse und Hochdruckkrankheiten, Greifswald, Germany
[4]KFH Kidney Center, Greifswald, Germany
[5]Brandenburg University of Technology Cottbus–Senftenberg, Faculty 4: Social Work, Health Care and Music, Senftenberg, Germany

Introduction: Anemia is highly prevalent among patients with chronic kidney disease (CKD) and is associated with poor outcome. Mechanisms underlying anemia of CKD are diminished production of erythropoietin, dysfunctional iron metabolism and shortened erythrocyte survival, especially in advanced CKD stages. Anemia has been mentioned by some clinical practice guidelines (CPG) as a criterion for referral to specialist nephrological services. When treating anemia of CKD, blood transfusion should be avoided in patients who may need kidney transplantation in the future.
The objective of this study was to evaluate Hb values and anemia in different CKD stages in the general population and to determine which therapeutic consequences warrant referral according to recommendations from international CPG on anemia in CKD.
Methods: Data of the population based cohort Study of Health in Pomerania (SHIP-2) were analyzed to estimate the proportion of subjects who meet the criteria for anemia and CKD. A systematic review of clinical practice guidelines for management of anemia in CKD patients issued between 2012 and 2017 was performed and evaluated to identify relevant therapeutic recommendations. This study was funded by the KFH Foundation for Preventative Medicine (KFH Stiftung Präventivmedizin).
Results: Data of 2328 subjects from SHIP-2 (53% female; median age 57, SD 14) were analyzed. 9% of subjects had reduced kidney function with eGFR<60ml/min/1.73m² and 48% of CKD patients were anemic. Median hemoglobin levels decreased with kidney function, from 13.9 g/dl (range 5.8–18.5) in subjects without kidney dysfunction to 13.0 (9.2–16.8) g/dl in stage G3a and 12.4 (8.9–16.3) in stage G3b, stabilizing in stages G4 (13.0; 10.4–14.7) and G5 (12.6; 11.4–13.5). Most patients in stages G4 and all patients in stage G5 had been referred to nephrologists. Recommendations for management of anemia across guidelines included avoiding transfusion of cellular blood products, Erythropoiesis-Stimulating Agents (ESA) if intravenous iron has insufficient effect and therapeutic Hb targets of <12 g/dl.
Conclusions: Hemoglobin levels decreased with kidney function in early CKD stages, increasing again from stage G4. This may reflect more intensive treatment of suboptimal Hb-levels, as nephrologist referral has typically occurred at this stage. Because the small proportion of patients with stage G4–5 CKD, results should be interpreted with caution in this group. Because of the higher prevalence of relevant anemia in advanced CKD, referral can benefit patients needing treatment for this condition, as treatment with intravenous iron and ESA can help avoiding blood transfusion in severely anemic patients.

Conflict of interest: none

PS-3B-4

A new patient- and surgery-specific blood ordering algorithm (psBOA) predicting intraoperative demand for packed red blood cells (PRBCs)

Hamann P.[1], Humpe A.[1], Wittmann G.[1], Zwißler B.[2], Rehm M.[2]

[1]Klinik für Anaesthesiologie der Universität München, Abteilung für Transfusionsmedizin, Zelltherapeutika und Hämostaseologie, München, Germany
[2]Klinik für Anaesthesiologie der Universität München, München, Germany

Introduction: Frequently, a large proportion of the packed red blood cells (PRBCs) ordered and prepared for surgical interventions is not transfused. To reduce the unnecessary efforts and the associated costs, both related to the preoperatively testing of PRBCs, a new patient- and surgery-specific Blood Ordering Algorithm (psBOA) PS-3B-4developed.
Methods: The data of 19,776 surgical interventions performed at a University Hospital from January 1, 2012 to December 31, 2015, were included in this retrospective study. Thereby 25 different types of surgical intervention were analysed.
The number of tested PRBCs, sent to the operating room (OR) or stored in the blood depot was predicted by different statistical methods considering the patient-specific variables: hemoglobin concentration before surgery, patient´s age, sex, body surface area, health status according to the American Society of Anesthesiologists physical status classification, and the urgency of the surgical procedure.
The number of PRBCs, which should be preoperatively tested and then delivered to the OR was calculated by a specialized regression analysis. In order to avoid overordering of PRBCs due to the predictions of the regression-model, the number of PRBC, sent to the OR, was limited to 2 PRBCs. The number of PRBCs, preoperatively tested and stored in the blood depot, was calculated on the basis of two parameters: the 85% percentile of PRBCs transfused between 2012 and 2015 and the number of PRBCs calculated by the regression-model. Accuracy of psBOA was evaluated for every surgical procedure by the comparison of the predicted and the transfused PRBCs. The results of the psBOA were compared to the predictions of the traditional Maximum Surgical Blood Ordering Schedule (MSBOS) of the University Hospital. The potential economic savings by introducing the psBOA were calculated.
Results: The analysis of the 25 different types of surgical procedures revealed, that the predictions of the psBOA met the demand of 90.43% (range: 84.51%-96.61%) of the analysed surgical procedures. Introducing the psBOA in our clinic could reduce the preparation of 6,990 PRBCs per year, leading to an economic saving of 105,000 Euro per year.
Conclusion: The new type of a psBOA, including the surgical procedure and six different patient specific variables, showed convincing results, which are superior to the results of the traditional blood ordering schedule of the University Hospital. In this context, the determined deviation between the transfused and predicted PRBCs has to be seen in comparison to the results of the previous blood ordering schedule. Additionally, the comparison with literature data regarding the blood ordering systems MSBOS and Surgical Blood Ordering Equation showed a satisfying accuracy of the BOA.

Conflict of interest: none

PS-4A
Infektionssicherheit von Blutprodukten

PS-4A-1

Routine testing of blood donor infectious disease markers using Siemens Health Care ADVIA Centaur XP

Hiller J.[1], Polywka S.[2], Lütghehetmann M.[2], Peine S.[1], Denzer U.[1]

[1]Universitätsklinikum Hamburg Eppendorf, Institut für Transfusionsmedizin, Hamburg, Germany
[2]Universitätklinikum Hamburg Eppendorf, Institut für Med. Mikrobiologie, Virologie und Hygiene, Hamburg, Germany

Introduction: We report on a five years experience using the Siemens Health Care ADVIA Centaur XP platform for routine testing of blood donor infectious disease markers at the University Hospital Hamburg Eppendorf.
Methods: In 2011 decision was taken to install a fully integrated laboratory platform for clinical chemistry and infectious disease markers testing patients and blood donors.
Public bidding was won by Siemens Health Care offering the ADVIA Centaur XP for infectious disease testing.
GMP compliant design qualification (DQ) and installation qualification (IQ) was initiated considering all components of the automation (bulk sorter, input-/output module, tube transport, centrifuge, analysers, sample storage). When it turned out that main laboratory room was not big enough for an integrated concept the analyser for donor testing was installed in a separate location to be operated in manual, front loading mode. GMP qualification was therefore limited to the ADVIA Centaur analyser (DQ/IG/OQ/PQ) thus significantly reducing complexity. A second identical analyser connected to the automation and routinely used for patient samples was qualified to serve as a backup system for donor testing to be used in manual front loading mode in case of problems with the primary machine.
Results: Since this was the first implementation in a GMP environment most procedures for initial IQ as well as for requalification after service and repairs had to be newly developed together with Siemens Health care. A change control procedure was initiated to keep track of all necessary changes prior to implementation including the analyser-middleware-LIS connection. After approval of the local authority serological routine testing for anti-HIV, HBsAg, anti-HCV, anti-HBc and Syphilis was started in May 2013.
Handling and performance of analyser and tests did not create any problems, test duration for five screening parameters of 70–200 samples per day was satisfactory.
Positive samples were retested and subjected to confirmatory testing. Over a five year period 138.814 donations (whole blood, platelet-, plasma and stem cell apheresis) from 18.105 donors where tested. Specificity was high with an overall rate of false positive and indeterminate results for anti-HIV (0,28%), HBsAg (0,13%), anti-HCV (0,38%), and Syphilis (0,15%). Only anti-HBc had slightly higher rates of false positive results (1,13%) which proved to be partially lot-dependent.
By exclusion of false positive donors loss of donations by false positive results could be kept below 0,3%.
Analyser performance was persistent, except for an increase in downtime events after four years. Thus maintenance measures had to be increased.
Hemovigilance information gave no evidence of false negative donor tests or windows period misses.
Conclusion: The Siemens Health Care ADVIA Centaur XP system is suitable for infectious disease testing of blood donors with an adequate test sensitivity and specificity.

Conflict of interest: none

PS-4A-2

Evaluation of Abbott Alinity s immunoassay analyzer for blood donor screening

Hourfar K., Schröder F., Gubbe K., Frank K., Reinhardt F., Karl A., Tonn T., Seifried E., Schmidt M.

DRK Blutspendedienst Institut Frankfurt, Frankfurt am Main, Germany

Background: German Red Cross blood donor service Frankfurt is using the Abbott PRISM chemiluminescence analyzers for blood screening for about two decades. Although performance of the PRISM-assays for HBs-Antigen, HIV combo-, HCV- and HBV-Core- antibodies is still state-of-the-art, the instrument itself has several disadvantages, such as frequent technical problems, high amount of waste, lack of automated re-testing procedures for initially reactive samples etc. Therefore we are planning to change to an up-to-date test system, which helps us to improve automation of blood screening to a next level.
Aims: As one of the first laboratories worldwide we received the new Abbott Alinity s analyzer for implementation into routine screening. The diagnostic specificity was evaluated in a head-to-head performace between the Alinity s analyzer and the PRISM analyzer by screening of first-time donors.
Methods: Samples were tested on both instruments in accordance to the instruction to use and after passing a qualifiedtraining with certificate by the manufacturer. We evaluated performance of the Alinity s by testing multiple time donors in comparison to the predecessor Abbott PRISM. Additionally we evaluated the diagnostic specificity was tested by testing a total number of 8080 first- time donors on both systems.
Results: Among 1603 multiple-time donors each one repeatedly reactive sample for HIV, HCV and HBsAg, but not confirmed by supplementary testing, have been observed with Alinity s. With PRISM there were two false reactive results among the same number of samples.
From November 2017 to February 2018 a total of 8011 first-time donors were tested on both systems. In total 18 repeat reactive samples for HCV (2 confirmed positive), 11 repeat reactive samples for HIV (none confirmed positive) and 13 HBsAg repeat reactive samples (9 confirmed reactive) were found on Alinity s.
Based on these data specificity of the Alinity s is 99,80% for HCV, 99,86% for HIV and 99,95% for HBsAg, respectively, and therefore in our hands slightly lower than stated by the manufacturer. Interestingly 10 out 16 false reactive samples on Alinity were also repeatedly reactive on PRISM.
Summary / Conclusions: When changing a serological screening system to a new instrument specificity of the new tests is always a concern. A high rate of repeatedly reactive samples is associated with a loss of blood donors, which are excluded from blood donor population as well as high cost for confirmatory testing. Abbott Alinity in our laboratory showed comparable specificity to Abbott PRISM. Major benefits of the new system are the ease of use, the low quantity of waste and the reduced hands-on time for maintenance and operation. Regarding workflow, handling and work-alone-time Alinity s could be a huge step ahead compared to Abbott PRISM. However with our instrument the frequency of technical problems could be lower.

Conflict of interest: none

PS-4A-3

Examination of blood products positive tested for HBV, HCV, HIV, HAV, and Parvovirus B19 in routine NAT Screening before discarding

Driesel G., Zimmermann A., Kahlenberg F., Baumann-Baretti B.

Haema AG, Leipzig, Germany

Introduction: Examination of blood donations to detect HBV, HCV, and HIV-1 by serological and nucleic acid amplification techniques (NAT) is mandatory in Germany. Most blood donation services also use NAT methods for detection of HAV and Parvovirus B19. For serological and NAT tests aliquots of the donations from predonation bags were used. If a

donation is positive for one or more of the viral agents tested the donation must be discarded.
Aim: The aim of the study was the proof of viral nucleic acids in all blood products before discarding in order to compare the viral load in the sample tubes and the products, not only in source or recovered plasma but also in red blood cell preparations.
Method: The cobas® MPX-test and the cobas® DPX-test on the cobas® 6800 instrument (Roche) were used for detection of viral nucleic acids in whole blood and plasma donations. The MPX-test is a qualitative multiplex real-time RT-PCR-test for detection of HBV, HCV, HIV-1, and HIV-2, whereas the DPX-test is a duplex real-time RT-PCR-test for qualitative detection of HAV-RNA, and quantitative detection of Parvovirus B19 DNA. NAT testing was carried out in pools of 96 donations. NAT-positive pools were resolved by retesting raw and column subpools with the same methods. NAT-positive single donations were retested using the MPX- or rather the DPX-test to confirm the proof of the appropriate viral nucleic acid. To quantify HBV-DNA, HCV-RNA, and HIV-1-RNA in a donation a standard curve against the corresponding WHO standard was constructed.
Not only NAT positive source plasma or recovered plasma fraction from whole blood donations were tested but also the additive solutions in the red cell donation bags.
Results: Between January 1st 2017 and up to day in 120 donations viral DNA or rather RNA had been found. Most of them were positive for Parvovirus B19 DNA as shown in the table.

Tab. 1.

Virus	HAV	HBV	HCV	HIV-1	ParvoB19
No. of NAT positive donations	1	8	11	4	96

In all but one cases viral nucleic acids were detected both in the sample tubes and the products. It is remarkably that viral nucleic acids are detectable not only in plasma but also in the additive solutions. But in all cases, especially in products containing HBV, HCV and HIV-1, the viral load in these additive solutions were a hundred times lower as in the sample tube and in the corresponding recovered plasma fraction. In one case the HCV viral load was too low to detect viral RNA in the red cell bag.
Conclusion: Using the cobas® MPX- and DPX-test it is possible to detect viral RNA or DNA not only in blood plasma but also in the additive solutions of the red blood cell preparation. But using both tests outside the specification of the manufacturer as for these storage solutions the detected viral loads are mostly one hundredth lower in comparison to the corresponding plasma fraction.

Conflict of interest: none

PS-4A-4

A safe screening algorithm for individual Nucleic Acid Testing (ID-NAT): 10 years of experience in a low endemic country

Niederhauser C., Gowland P., Stolz M.

Interregionale Blutspende SRK, Labordiagnostik, Bern, Switzerland

Introduction: A highly sensitive and specific nucleic acid test (NAT) for the blood-borne viruses HIV, HCV and HBV is essential for the safety of blood components. Since more than two decades NAT screening of blood donations has become standard in developed countries but only a handful of countries worldwide perform ID-NAT. With this powerful technique the confirmation of initial NAT reactive samples has become a challenge. Different algorithms are currently in use to eliminate false reactive results. To determine whether our algorithm using repeat testing of initial reactive (IR) samples in duplicate runs is a safe strategy, especially in low endemic countries, to discriminate between «false» and «true» reactive results. Data from a 10 year experience of ID-NAT were extensively analysed when follow-up data was available.
Methods: From July 2007 until December 2014 the Procleix Ultrio assay on a Tigris system and from January 2015 until December 2017 the cobas MPX on a cobas 8800 platform was used for ID-NAT screening. All initial reactive samples were subjected to repeat testing in duplicate independent runs. Only when both tests remained negative the products were released. Donor data from the last 10 years were investigated retrospectively looking for the re-occurrence of a reactive result in a follow-up sample. Only those donors with at least an x+1 donation result were included for the confirmation of a false reactive result.
Results: From the 1'830'657 donations tested 2'454 samples were initial reactive (0.13% IR). Only 229 of these samples were repeat reactive (RR: 18 HIV, 61 HCV, 150 HBV). 2'223 donations were non repeat reactive (0.12% nonRR). Of these 2'223 IR but nonRR donations follow-up data were available from 1'267 donors (57%) for further analysis. All except one of these donors were ID-NAT negative in all of the follow-up samples. The one exception was from a donor who acquired a fresh HBV infection 10 years after the initial reactive donation (in the x+28 donation) and subsequently seroconverted. Subsequent serological tests from all succeeding donations (X+1, X+2, etc.) were negative in all the other cases proving no seroconversion took place post to the initial reactive ID-NAT result.
Conclusions: The algorithm to deal with initial reactive ID-NAT donations using duplicate repeat testing is very safe and cost effective in low endemic countries. There is no unnecessary destruction of blood products, no counselling of false reactive donors and there is no need to add further complexity to the screening algorithm.

Conflict of interest: none

PS-4A-5

Risk factors for HCV infections among blood donors – more attention to young heterosexual males is needed

Preußel K., Offergeld R.

Robert Koch-Institut, Abt. für Infektionsepidemiologie, Berlin, Germany

Background: Mandatory HCV NAT testing increased blood safety remarkably by shortening the infectious window period to less than 7 days. HCV infections among blood donors declined constantly during the last decade in Germany. However, recent infections with HCV are still a serious threat for blood safety. The constant high proportion of NAT-only HCV infections among repeat donors shows that identification of potentially infectious donors remains a challenge. Our investigation of demographic characteristics and probable transmission risks with special attention to NAT positive donors may support an effective donor selection and deferral process and enable the identification of populations for targeted donor education.
Methods: HCV infections of blood donors in Germany are notifiable to the national donor vigilance system. We analyzed donor characteristics of HCV infected donors between 2006 and 2016 with respect to their NAT testing result. HCV infections with positive NAT (NAT+) result were classified as active infection. Risk factors for infection of donors with active infection were compared with risk factors of RNA negative donors (NAT-). We performed logistic regression analysis to identify independent risk factors for active HCV infections.
Results: Between 2006 and 2016 2,102 NAT positive among 4,252 confirmed HCV infections were reported (702 donations were not tested by NAT). Repeat donors (RD) were more frequently affected by active HCV infection than first time donors (FTD) (348/549 vs. 1,754/2,997; $p = 0.03$. NAT+ donors are remarkably younger than NAT-: 50% NAT+ are younger than 35 years compared to 39% NAT-. Two thirds of the NAT+ are male. There is no difference between whole blood (59%) and apheresis donations (61%) regarding the proportion of active infections.
Infection risks were reported for 658 (19%) NAT tested donors (and 217 non-tested donors). Heterosexual risk factors were significantly more often disclosed by NAT+ compared to NAT- donors (34% vs. 24%, $p<0.01$), as well as imprisonment (6% vs. 2%, $p<0.01$). In contrast occupational exposure (11% vs. 5%, $p = 0.01$) and former blood transfusions (22%

vs. 11%, p<0.01) were more frequent among NAT-. Considering adjustment for age, sex and donor status heterosexual risk contacts (OR = 1.5, p = 0.02), and imprisonment (OR = 2.8, p = 0.04) could be identified as independent risk factors for active HCV infections among blood donors.
Conclusion: Enhancing the donor education about risk exposures for young heterosexual males and facilitation of accurate capture of heterosexual risk contacts by skilled personal in confidential donation environments could especially prevent HCV window period donations.

Conflict of interest: none

PS-4A-6

Syphilis on the rise – focusing on a re-emerging infection in the blood donor population

Offergeld R., Preußel K.

Robert Koch-Institut, Berlin, Germany

Background: Syphilis is caused by the transfusion transmissible spirochaete, *Treponema pallidum*. Universal testing of blood donors played an important role in the reduction of transfusion-transmitted syphilis making it a very rare event. Still, the rising number of reported syphilis cases in the general population led us to a deeper analysis of the incident syphilis cases in the repeat blood donor (RD) population in Germany from 2006–2016.
Methods: Donor vigilance is based on mandatory reporting. Reports include the number of donors and donations stratified for sex, age, type of donation and type of donor. Confirmed positive results additionally include donation history, residential area and transmission category. We performed Pearson $\chi 2$ tests to test for differences in distribution with respect to sex. P-values < 0.05 were considered significant.
Results: In the 11-year surveillance period, 1,250 syphilis infections (4.8/100,000 RD) were identified. In every year, male donors were significantly more affected (p<0.05). Between 2006 and 2010, the trend of reported syphilis infections per 100,000 population was stable. Since 2010, the syphilis infections have increased in the group of male RD from 5.7 to 8.6/100,000 and from 1.8 to 2.6/100,000 among female RD. Between 2006 and 2016, the proportion of cases increased in the three younger age groups (18–24, 25–34, 35–44 years) with the 25–34 year old being most affected group (7,6/100,000 RD). Infections rates remained stable in the age groups 45 years and older. The proportion of confidential self-exclusion (CSE) among syphilis positive RD per year varied in the observation period between 0.8% and 5.7%. Information on transmission category was available for 23.8% of cases. The predominant transmission category was heterosexual transmission in 50% and MSM in 14%. Surgery in Germany was noted as transmission category in 14% of cases and 16% claimed tattoos, piercings, occupational or household contacts as their mode of transmission.
Conclusion: Incident syphilis infections are to date the most frequent detected infection in routine blood donor screening. Although the most affected risk population for syphilis infections (active MSM) is deferred from donating, we identified an increasing trend of syphilis infections in the RD population which is parallel to the trend in the general population. Information on transmission categories was incomplete. Heterosexual transmission was indicated most often as expected for a sexually transmitted infection. Unexpectedly, for 30% of syphilis positive donors an unlikely transmission category was reported. In order to specifically identify risk behavior among blood donors, reporting of transmission categories should improve as transmission categories differ from the general population. This would enable targeted donor information and evidence based modification of donor selection criteria.

Conflict of interest: none

PS-4A-7

Hemovigilance: actual HEV-screening in blood donors of apheresis platelets for highly immunosuppressed patients

Wittmann G.[1], Buhmann R.[1], Möhnle P.[1], Wagner B.[1], Eberle J.[1,2], Humpe A.[1]

[1]Klinikum der Universität München, Abt. für Transfusionsmedizin, Zelltherapeutika und Hämostaseologie, München, Deutschland
[2]Universität München, Max von Pettenkofer-Institut, Lehrstuhl Virologie, München, Deutschland

Background: HEV is an emerging risk in blood transfusion for immunocompromised patients. HEV-infections transmitted by blood transfusion came into focus since the last four years. At the moment the Paul-Ehrlich-Institute is planning new additional requirements for HEV-testing of blood donors according to Section 63 Graduated Plan of the German Medicinal Product Acts (The Drug law). Among these medicolegal settings we evaluated the HEV prevalence of our blood donors of apheresis platelets for immunocompromised patients.
Methods: All blood donors of platelet apheresis concentrates in our institution in the first quarter of 2018 were enrolled after informed consent for testing of infectious hepatitis E with blood donations between April 2018 and May 2018 (n = 220). Finally in 219 blood donors we performed HEV-testing for anti-HEV IgG and IgM with the recomWell HEV IgG IgM test (Mikrogen Diagnostik, Neuried, Germany) and by positivity in IgM testing subsequent HEV-NAT of HEV. Currently all HEV anti-IgG and anti-IgM tested blood donors are evaluated for seroconversion in HEV for the last 2 years beginning in January 2016. If seroconversion or positive HEV-NAT is confirmed, we will perform look-backs according to the German Transfusion Act.
Results: Of all enrolled blood donors (n = 220) we could evaluate 219 donors. From these 17.8% (n = 39) were HEV anti-IgG positive, 2.7% (n = 6) HEV anti-IgG and anti-IgM positive and 0.5% (n = 1) anti-IgM positive only. None of these donors had a positive HEV-NAT (see table). The analysis of seroconversion in the last two years using our retain samples and subsequently look-backs in patients transfused with platelet apheresis concentrates of donors in seroconversion period are in progress.
Conclusion: In concordance with previous published results for Germany we found a HEV seroprevalence of 17.8%. We found no infectious blood donor at time of donation. In our opinion HEV seropositive donors eventually should be tested for seroconversion and look-backs in transfusion recipients in the seroconversion period are desirable.

Conflict of interest: none

Tab. 1.

Table			
Donors	female	male	Age (median, range)
219	86	133	38.5 [18;66]
Anti-IgG only			
39	17	22	39,0 [22;60]
Anti-IgG and Anti-IgM			
6	5	1	26,5 [23;58]
Anti-IgM only			
1	0	1	39

PS-4A-8

Hepatitis E Virus blood donor screening: comparison of minipool versus individual NAT testing

Vollmer T., Knabbe C., Dreier J.

Herz- und Diabeteszentrum NRW, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany

Introduction: The question of a general HEV-NAT screening of blood products is currently controversy discussed. A summarized assessment

of the current situation in 11 European countries described the situation as «a shift to screening» but different countries have chosen different approaches [1]. One central question is the need of individual NAT screening (ID) versus minipool NAT screening (MP) approaches to identify all relevant viremias in blood donors. In the present study, we compare the findings of ID-NAT versus MP-NAT in pools of 96 samples.

Methods: From November 2017 to January 2018, a total of 10,141 allogenic blood donations from 7,650 individual German blood donors were screened for the presence of HEV RNA using a MP-NAT in pools of 96 samples (in house, RealStar HEV RT-PCR Kit, Altona Diagnostic Technologies (ADT), Hamburg, Germany). In comparison, MP were also analysed on the fully automated cobas 6800 platform (Roche Diagnostics, Mannheim, Germany). Furthermore, all samples included in MP were screened with ID-NAT on the cobas 6800 platform. The presence of HEV-specific IgM and IgG antibodies in HEV RNA positive samples was determined using the anti-HEV IgM/IgG ELISA (Euroimmun, Luebeck). HEV RNA concentrations were quantified using the first WHO international Standard for hepatitis E Virus RNA for NAT-based assays.

Results: Parallel screening of MP (n = 122, 96 samples per MP) with the in house method and on the cobas 6800 detected seven reactive pools. After pool resolution, a total of nine HEV RNA positive donations were identified by the in house detection method, whereas 17 HEV RNA positive donations were identified by ID-NAT on the cobas 6800 platform. This resulted in a prevalence of 1:1.449 donations (0.07%) for MP-NAT screening and 1:597 donations (0.17%) for ID-NAT screening. The lowest viral load detected by MP-NAT was <25 IU/ml IU/ml, all ID-NAT only positive donations had viral loads <25 IU/ml. Acute HEV infection was confirmed either by HEV RNA detection in a secondary sample or by evidence of seroconversion.

Conclusions: The incidence of HEV infection was approximately 50% higher if ID-NAT was used compared to MP-NAT. However, viral loads were below 25 IU/ml and will most likely not result in transfusion-transmitted HEV infection (TTI) taken into account the currently known infectious dose of 5.0E+04 IU inevitable resulting in TTI [2]. The clinical relevance of and need of identification of these low level HEV positive donors still require further investigation.

1. Domanović D, Tedder R, Blümel J, et. al.: Hepatitis E and blood donation safety in selected european countries: A shift to screening? Eurosurveillance 2017;22:30514.
2. Dreier J, Knabbe C, Vollmer T: Transfusion-transmitted Hepatitis E: NAT screening of blood donations and infectious dose. Frontiers in medicine 2018;5:5.

Conflict of interest: none

PS-4A-9

High Seroprevalence of Parvovirus B19 in blood donors of the Zurich region

Gottschalk J., Glauser A., Hadegger K., Frey B. M.

Blutspende Zürich, Screeninglabor, Schlieren, Switzerland

Background: Transmission of Parvovirus B19 (PB19) by blood transfusion is life-threatening for immuno-compromised patients. Immunity against PB19 infection in recipients of blood products may protect from transfusion-transmitted PB19 (ttPB19) infection. For Switzerland, data on PB19 prevalence in blood donors (BD) are lacking.

Objectives: Seroprevalence of anti-PB19 antibodies in BD was determined in a representative fraction of BD of the Zurich area. Testing for PB19 DNA in BD's EDTA-plasma was routinely assessed by PCR in pools of 96. IgG positive donors were further analysed for age group and provenience of donor (rural versus urban).

Methods: Archived samples of donations provided between 01.03.2017 and 11.10.2017 were chosen randomly and tested retrospectively for anti-PB19 IgG against capsid protein VP1 and VP2 using a commercial test (RecomWell ELISA, Mikrogen GmbH, Neuried, Germany) according to the recommendations of the manufacturer. PCR for PB19 was performed on cobas 6800 in a duplex format with HAV (DPx, Roche) in pools of 96. DNA titre of 10^2 IU/ml was considered «DNA positive» and the respective pool was resolved by single sample testing to identify the PB19-DNA positive donation. Resulting DNA titres of PB19 in a single sample had a minimal concentration of 10^4 IU/ml. IgG data were correlated with PB19-NAT data of the respective donations extracted from donor»s data file. The software SPSS Statistics 17.0 was used for descriptive analysis of the data.

Results: Of 1549 donors tested for anti-PB19 IgG 1197 (77.3%) were found positive. There is a significant increase of IgG-positive donors with increasing age (< 30years: 72.4%, > 60 years: 82.3%) and there seems to be a higher IgG positive rate in rural donors as compared to donors in urban area (82.3% versus 78.6%, resp.). Routine pool testing for PB 19 DNA was negative for all BD»s assessed in the study, suggesting that healthy BDs carrying PB19 virus titer >106 IU/ml are rarely expected.

Conclusions: Parvovirus B19 seroprevalence of donors in the Zürich area is rather high and increasing with age of BD. Even higher rates of PB19-IgG positive BD are possibly found in rural areas. From such a high rate of PB19 positive BD»s can be inferred, that most of the Swiss patients will also be PB19 IgG positive. Receiving blood products with low titres of Parvovirus B19, Swiss patients should therefore be protected from infection. As a precautious measure however, we recommend to use «PB19 DNA negative» blood products for immune compromised patients and during pregnancy. Seroprevalence in Switzerland seems to be in accordance with other European countries. As expected, positive serology increases steadily with age group. There is no overt reason for a higher seroprevalence in rural areas.

Conflict of interest: Die Studie wurde unterstützt durch die Firma Mikrogen GMbH, Neuried, Deutschland, indem die Testkits für die Studie verbilligt bezogen werden konnten. Die Einreichung des Abstracts erfolgt unabhängig von der Firma Mikrogen, die auch keinen Einblick in die Daten und auch keinen Einfluss auf deren Auswertung hatte. Es besteht keine geschäftliche Beziehung zwischen Mikrogen und der Blutspende Zürich und es ist auch keine solche Beziehung geplant. Es bestand und besteht kein Interessenkonflikt.

PS-4A-10

Plasma-reduced single donor apheresis platelet concentrates manufactured under routine conditions for the Capture trial: A one year experience

Doescher A.[1], Baume H.[2], Petershofen E.[2], Schenck P.[2], Gebauer W.[2], Pohler P.[3], Seltsam A.[3]

[1]DRK Blutspendedienst NSTOB, F&E, Oldenburg, Germany
[2]DRK Blutspendedienst NSTOB, Oldenburg, Germany
[3]DRK Blutspendedienst NSTOB, Springe, Germany

Iintroduction: Treatment of apheresis platelet concentrates (PCs) with UVC may enhance transfusion safety of platelets with respect to contamination with pathogens. For use in a clinical trial (CAPTURE; EudraCT No.: 2015–001035–20) single donor apheresis platelet concentrates were produced under routine conditions. Here we present the quality data of untreated, UVC-treated and y-irradiated platelet units.

Methods: 405 PCs were prepared from single donors with standard operation procedures (Amicus) using SSP+ (Macopharma,Mouvaux; France) as additive solution from February 2017 to February 2018. UVC-PCs were treated with UVC within six hours after preparation using the THERAFLEX UV-Platelets system (Macopharma); y-PCs were y-irradiated with a minimum of 25 Gy; and control PCs (AP-PCs) were left untreated. Sampling for quality control parameters was done on day of preparation (n = 44) and at the end of shelf life (AP-PCs: n = 35, UVC-PCs: n = 44; y-PCs: n = 22). The following parameters were examined on day 0: PC volume, platelet concentrations, plasma content, residual erythrocyte and leucocyte counts. Determination of platelet concentration, pH, swirling and sterility testing was done at the end of shelf life.

Results: Mean volumes were 344 ± 16.9mL in AP-PCs, 355 ± 8.0mL in γ-PCs and 344 ± 14.9mL in UVC-PCs with platelet counts of 3.1 ± 0.3/unit, 3.2 ± 0.3/unit and 3.0 ± 0,2/unit, respectively. Residual plasma concentration ranged between 30% and 39%. Residual erythrocyte and leucocyte counts met the standard specifications for PC products in Germany.

At the end of shelf life, the pH value of UVC-PCs (7.27 ±0.05) was comparable to γ-PCs (7.31 ±0.08) and AP-PCs (7.29 ±0.09). Tests for bacterial contamination were negative for all tested PCs.
Conclusions: Quality control data demonstrate that plasma-reduced UVC-treated apheresis PCs meet the standard specifications for PC products in Germany. No differences in quality control were observed between AP-PCs, γ-PCs and UVC-PCs. The safety and efficacy of UVC-treated PCs is being evaluated in CAPTURE trial.

Conflict of interest: none

PS-4A-11

Comparison of UVC-treaded versus GAMMA-irradiated and untreaded plasma reduced platelet concentrates produced from buffy coats under routine condintions

Karatas M. E.[1], *Brixner V.*[1], *Dombos S.*[1], *Haase S.*[1], *Schmidt M.*[1], *Tolksdorf F.*[2], *Pohler P.*[3], *Seltsam A.*[3], *Seifried E.*[1]

[1]German Red Cross Blood Donor Service Baden-Württemberg-Hessen gGmbH, Frankfurt am Main, Germany
[2]MacoPharma International GmbH, Langen, Germany
[3]German Red Cross Blood Service NSTOB, Springe, Germany

Background: Reducing bacterial and viral contamination by the use of pathogen reduction technology may decrease the risk of transfusion transmitted infection by blood product transfusion. We established the manufacturing of UVC-treated platelet concentrates (PCs) from buffy coats under routine conditions.
This study was conducted to evaluate possible *in vitro* effects of the THERAFLEX UV-Platelets treatment on pooled PCs in comparison to untreated and gamma-irradiated PCs.
Methods: 367 leukocyte reduced and plasma reduced PCs were prepared from five buffy coats using SSP+ additive solution (Macopharma, Mouvaux, France) and stored for 7 days in the storage bag of the THERAFLEX UV-Platelets kit under routine conditions.
Three different platelet products were manufactured: PCs treated with the THERAFLEX UV-Platelets system (Macopharma) within 6 h after pooling, gamma-irradiated (30 Gy) PCs and untreated PCs.
To compare the quality of these products, we analyzed product volume, residual erythrocytes, residual leukocytes, platelet content, total protein concentration, pH and sterility. For statistical analysis the Kruskal-Wallis-test was applied, and p-values <0.05 were considered statistically significant.
Results: The quality control parameters showed no significant differences between UVC-treated and gamma-irradiated or untreated PC for platelet content, residual erythrocytes and total protein concentration, but demonstrate slight but statistically significant differences in volume ($p < 0.0001$), residual leukocytes ($p = 0.0194$) and pH value ($p < 0.0001$). All data are shown in the table below. Tests for bacterial contamination were negative for all tested PCs.
Conclusion: The collected data showed that plasma reduced, UVC treated pooled PCs meet the quality standards for PC products according to the German Guidelines. However, safety, tolerance and efficacy of PCs manufactured by the THERAFLEX-UV Platelets system are currently evaluated in a clinical study.

Conflict of interest: none

Quality Parameter	Untreated PC n=151	Gamma-irradiated PC n=86	UVC-treated PC n=130
Volume [mL]	PP 360±9	PP 360±11	PP 355±10
pH	EOS 7.4±0.1	EOS 7.4±0.1	EOS 7.3±0.1
Platelet content [x10^{11}/unit]	EOS 3.09±0.35	EOS 3.15±0.4	EOS 3.15±0.46
Total Protein concentration [g/L]	PP 22.3±1.6	PP 22.7±1.5	PP 22.6±1.5
Residual erythrocytes [x10^{9}/unit]	PP 0.83±0.32	PP 0.85±0.31	PP 0.86±0.45
Residual leucocytes [x10^{6}/unit]	PP 0.04±0.06	PP 0.05±0.06	PP 0.07±0.11

PP = post production (Day 2)
EOS = end of storage (Day 4-6)

Fig. 1.

PS-4A-12

Emerging viruses MERS coronavirus, SARS coronavirus and Ebolavirus are efficiently inactivated in human plasma by MB/light treatment

Handke W.[1], *Eickmann M.*[2], *Gravemann U.*[1], *Reichenberg S.*[3], *Müller T. H.*[4], *Seltsam A.*[1]

[1]DRK-Blutspendedienst NSTOB, Forschung und Entwicklung, Springe, Germany
[2]Universität Marburg, Institut für Virologie, Marburg, Germany
[3]Macopharma, Langen, Germany
[4]DRK-Blutspendedienst NSTOB, Springe, Germany

Introduction: Emerging viruses like Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV) and Ebola virus (EBOV) have been identified as potential threats to blood safety. This study aimed to investigate the efficacy of the THERAFLEX MB Plasma system to inactivate these viruses in human plasma. The THERAFLEX MB Plasma system (Macopharma) uses methylene blue (MB) in combination with visible light for reduction of pathogen infectivity in plasma.
Methods: Leukodepleted plasma was prepared from whole blood using standard blood banking technology. Plasma units (n = 2 for each virus) were spiked with virus suspension (10% v/v). MB/light treatment was done according to the manufacturer»s instructions using the Macotronic B2 illumination device. Samples were taken after spiking (load and hold sample) and after illumination with different light doses (30, 60, 90 and 120 (standard) J/cm^2)). The titer of MERS-CoV (strain HCoV-EMC, Ron A. Fouchier), SARS-CoV (strain Frankfurt 1) and Zaire EBOV (strain Mayinga-76) was determined as tissue culture infective dose ($TCID_{50}$) by endpoint titration on Vero E6 cells (ATCC CCL-22).
Results: After spiking, a virus titer of 5.9–6.1 (MERS-CoV), 5.4–5.6 (SARS-CoV) or 6.9–7.0 (EBOV) $\log_{10}$ $TCID_{50}$/mL was achieved in the plasma units. Already with low light doses of 30–60 J/cm^2 viruses were inactivated down to the detection limit of the system, resulting in log10 reduction factors of ≥3.3 for MERS-CoV, ≥3.1 for SARS-CoV and ≥4.7 for EBOV.
Conclusions: Our results demonstrate that the THERAFLEX MB-Plasma procedure is an effective technology to inactivate various emerging viruses in contaminated plasma.

Conflict of interest: Ute Gravemann, Wiebke Handke, Thomas H. Müller and Axel Seltsam work together with Macopharma on the development of pathogen inactivation systems for blood products.

PS-4A-13

Emerging viruses MERS coronavirus, SARS coronavirus and Ebolavirus are efficiently inactivated in platelet concentrates by UVC treatment

Gravemann U.[1], Eickmann M.[2], Handke W.[1], Tolksdorf F.[3], Müller T. H.[4], Seltsam A.[1]

[1]DRK-Blutspendedienst NSTOB, Forschung und Entwicklung, Springe, Germany
[2]Universität Marburg, Institut für Virologie, Marburg, Germany
[3]Macopharma, Langen, Germany
[4]DRK-Blutspendedienst NSTOB, Springe, Germany

Introduction: Emerging viruses like Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV) and Ebola virus (EBOV) have been identified as potential threats to blood safety. This study aimed to investigate the efficacy of the THERAFLEX UV-Platelets system to inactivate these viruses in platelet concentrates (PCs). The THERAFLEX UV-Platelets system (Macopharma) uses UVC light only without the need of any additional photoactive compound.
Methods: Plasma reduced PCs from 5 BCs (35% plasma in additive solution SSP+) were spiked with virus suspension (10% v/v). PCs (n = 2 for each virus, 375 mL) were then UVC-irradiated on the Macotronic UV machine (Macopharma) and samples were taken after spiking (load and hold sample) and after illumination with different light doses (0.05, 0.1, 0.15 and 0.2 (standard) J/cm^2)). The titer of MERS-CoV (strain HCoV-EMC, Ron A. Fouchier), SARS-CoV (strain Frankfurt 1) and Zaire EBOV (strain Mayinga-76) was determined as tissue culture infective dose ($TCID_{50}$) by endpoint titration on Vero E6 cells (ATCC CCL-22).
Results: The results of the infectivity assay demonstrated that UVC irradiation dose-dependently inactivated the different viruses. After spiking, a virus titer of 6.4 (MERS-CoV), 5.8–6.0 (SARS-CoV) or 6.8–7.0 (EBOV) $\log_{10}$ $TCID_{50}$/mL was achieved in the PCs. At a UVC dose of 0.15 J/cm^2 and higher the three viruses were inactivated down to the detection limit of the system, resulting in $\log_{10}$ reduction factors of ≥3.7 for MERS-CoV, ≥3.4 for SARS-CoV and ≥4.5 for EBOV.
Conclusions: Our results demonstrate that the THERAFLEX UV-Platelets procedure is an effective technology to inactivate various emerging viruses in contaminated PCs.

Conflict of interest: Ute Gravemann, Wiebke Handke, Thomas H. Müller and Axel Seltsam received project grants from the «Forschungsgemeinschaft der DRK-Blutspendedienste e.V." and work together with MacoPharma on the development of pathogen inactivation systems for blood products.

PS-4A-14

No Induction of S-303/Glutathione specific antibodies in a randomized, controlled phase III study to evaluate pathogen-inactivated red blood cells in Thalassemia Major patients (SPARC)

Geisen C.[1], Aydinok Y.[2], Origa R.[3], Piga A.[4], Brixner V.[5], Mufti N.[6], Erickson A.[6], Ernst C.[6], North A.[6], Corash L.[6], Seifried E.[5], Benjamin R.[6]

[1]DRK Blutspendedienst Baden-Württemberg - Hessen gemeinnützige GmbH, Abteilung für Immunhämatologie, Hämostaseologie und Molekulare Diagnostik, Frankfurt am Main, Germany
[2]Ege University, Izmir, Turkey
3University of Cagliari, Cagliari, Italy
[4]University of Torino, San Luigi Gonzaga Hospital, Torino, Italy
[5]DRK Blutspendedienst Baden-Württemberg - Hessen, Frankfurt am Main, Germany
[6]Cerus Corporation, Concord, CA, United States

Introduction: Thalassemia major outcomes are markedly improved with chronic RBC transfusion combined with iron chelation therapy, but treatment carries a lifetime risk of transfusion transmitted infections. The INTERCEPT Blood System for RBC (Cerus Corporation, Concord, CA) is an investigational device used ex vivo to prepare pathogen-inactivated RBC components for transfusion (S-303/glutathione (GSH)-treated RBC). Treatment results in broad spectrum inactivation of viruses, bacteria, protozoa and donor leukocytes. In a prior study using First Generation S-303/GSH-treated RBC, low titer non-hemolytic antibodies were detected in two subjects after study transfusions. The S-303/GSH process has been reformulated to decrease RBC surface labelling with S-303 using a Second Generation process. We aimed to evaluate the use of S-303/GSH-treated RBC as screening reagents in a gel column assay during a randomized, controlled study to evaluate Second Generation S-303/GSH-treated RBC in Thalassemia major patients.
Methods: A three-cell RBC screening panel and a six-cell confirmatory panel were generated with treatment of selected RBC units with either the First Generation process (high S-303 adducts: 0.2 mM S-303 with 2 mM GSH) or the Second Generation process (low S-303 adducts: 0.2 mM S-303 with 20 mM GSH), or left untreated (Control). Cell labelling was assessed by flow cytometry and gel column centrifugation (ID-Card, BioRad) using plasma and RBC volumes according to the manufacturer»s recommendations. A monoclonal acridine-specific antibody served as positive control. 10,721 general hospitalized patients and 998 chronically transfused patients were screened for natural S-303-specific antibodies. Subsequently, the assay was used during a randomized, controlled, double-blind, non-inferiority, two-period, two-treatment, crossover study to evaluate S-303/GSH-treated RBC in Thalassemia major subjects (SPARC).
Results: Screening of general hospitalized patients and chronically transfused patients not previously exposed to S-303/GSH-treated RBC, revealed 17 patients (0.1 and 0.5%, respectively) with natural, low titer (2–32) IgM and/or IgG (non-IgG1 or IgG3 isotype) antibodies with (14) acridine or (3) non-acridine inhibitable specificity. In the SPARC study, 81 subjects were transfused with 1007 S-303/GSH-treated and 999 Control RBC study products at hospitals in Italy (n = 14) and Turkey (N = 67). Subjects received means of 12.6 (3–18) units of Test and 12.6 (6–18) of Control RBC over 6 transfusion episodes in each period, with a mean transfusion interval of 19.4 (Test) and 19.5 (Control) days. No treatment emergent RBC alloantibodies or S-303 specific antibodies were detected during the study.
Conclusion: S-303 labelled RBC are sensitive and specific reagents for the detection of S-303 specific antibodies. In Thalassemia major patients on a chronic transfusion regimen, multiple transfusions with S-303/GSH-treated RBC did not evoke S-303-specific antibodies.

Conflict of interest: Cerus Corporation, CA, USA hat die wissenschaftliche Untersuchungen im Rahmen der Studie finanziert.

PS-4B
Hämostaseologie

PS-4B-1

Protac-based measurement of protein C (PC) activity levels corresponds to thrombin-thrombomodulin-induced generation of activated PC (APC) in hereditary PC deficiency

Reda S., Witkowski J., Rühl H., Müller J., Oldenburg J., Pötzsch B.

University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany

Introduction: Commonly applied assays for PC activity measurement utilize Protac, a PC activator isolated from the venom of Agkistrodon contortrix. The Protac-based mode of PC activation differs from endothelial cell-dependent PC activation through the thrombin-(FIIa)-thrombomodulin (TM) complex. Therefore, PC activity levels measured with Protac-based assays might not capture the true enzyme function in patients with mutations in the gene for PC (PROC). In this study we developed and evaluated an assay format in which both modes of PC activation can be applied.
Methods: Citrated plasma samples were prepared for measurement by addition of aprotinin and the peptide Gly-Pro-Arg-Pro to prevent inactivation of formed APC and to suppress fibrin polymerization, respectively.

Either Protac, or a solution containing FIIa, TM, and CaCl2 was added to initiate PC activation. The formed APC in the reaction mixture was quantified using an oligonucleotide-based enzyme capture assay (OECA) which utilizes the DNA-aptamer HS02-G52 to immobilize APC. Calibrators consisting of a dilution series of normal citrated plasma in PC deficient plasma were processed simultaneously with the samples. Using the Protac-based assay and the FIIa-TM-based assay we determined PC activity levels in 20 healthy individuals and 20 patients with different PC deficiency causing mutations. In addition, PC activity levels were measured using a commercially available test performed on a coagulation analyzer.
Results: The Protac-based assay demonstrated 8.2% interassay variation and 4.6% intraassay variation while the FIIa-TM-based assay variant demonstrated 11.2% interassay variation and 6.1% intraassay variation. In healthy individuals, PC activity levels ranged between 70% and 102% using the Protac-based approach and between 63% and 92% in the FIIa-TM-based assay. The results of the Protac-based assay and the FIIa-TM-based assay correlated well with PC activity level measurements of the automated coagulation analyzer, with r = 0.92 and r = 0.91, respectively. No PC deficient patient showed PC activity levels within the normal range in any of the three assays.
Conclusion: Discrepancies between kinetics of Protac-induced and FIIa-TM-induced APC generation are not a frequent finding in patients with loss of function mutations in the PROC gene. Further studies are needed to investigate a potential advantage of the FIIa-TM-based assay in the detection of PC mutations that alter its Protac-binding functions.

Conflict of interest: The authors do not have a financial interest/arrangement or affiliation that could be perceived as a real or apparent conflict of interest in the context of the subject of this abstract.

PS-4B-2

Two successful pregnancies by treatment with fibrinogen and low-molecular-weight heparin in a woman with dysfibrinogenemia and recurrent pregnancy loss

Zimmermann R.[1], Peisl J.[2], Geisen C.[3], Oppelt P. G.[4], Achenbach S.[1], Hackstein H.[1]

[1]University Hospital Erlangen, Department for Transfusion Medicine and Haemostaseology, Erlangen, Germany
[2]Frauenärzte im Netz, Bamberg, Germany
[3]Goethe University, Institute for Transfusion Medicine and Immunohaematology, Frankfurt am Main, Germany
[4]University Hospital Erlangen, Department of Obstetrics and Gynecology, Erlangen, Germany

Introduction: Dysfibrinogenemia may result in a tendency for bleeding or thrombosis as well as obstetric complications. The obstetric complications of dysfibrinogenemia include early pregnancy loss, hemorrhage, placental abruption, and thrombosis. There are only a few case reports with treatment suggestions for pregnancies in affected women.
Case report: We report on a meanwhile 34-year-old woman who had experienced four times an early abortion between 2013 and 2015. Presenting in our outpatient clinic the patient reported bruising, but on no other bleeding events and no thromboembolic events. A grandfather had died because of pulmonary embolism while suffering from lung cancer. Laboratory examinations revealed a decreased Quick value, an increased thrombin time, a largely increased reptilase time, and fibrinogen concentrations of 0.67 g/l (Clauss method) and 3.87 g/l (immunoturbidimetry). The patient was diagnosed with dysfibrinogenemia. Molecular biological examination revealed the mutation c.103C>T (p.Arg35Cys) in Exon 2 of the FGA-Gen. Shortly later, the fifth pregnancy was diagnosed. Immediately, the patient was treated with a low-molecular heparin using the recommended prophylaxis dose for enhanced thromboembolic risk. Concurrently, the patient received a substitution of fibrinogen concentrate twice weekly (2 x 2 g per week at the beginning, dose adjustment to achieve a fibrinogen concentration of 1.00 g/l). While this was performed, the fifth pregnancy remained uneventful. The child was born six weeks before the calculated delivery time. In 2017, a sixth pregnancy occurred, which was just as successfully accompanied by the same treatment combination. Currently, mother and both childs are doing well.
Conclusions: Poor pregnancy outcomes were reported in many cases if functionally active fibrinogen levels were less than 0.6 g/L (Clauss method). After first-time identification of a woman suffering from dysfibrinogenemia without an obvious bleeding tendency and without thromboembolic events in her history, it is best-practice to substitute fibrinogen and to applicate low-molecular-weight heparins simultaneously to prevent bleeding, thrombosis, and pregnancy loss as well.

Conflict of interest: none

PS-4B-3

Next generation viscoelasticity assays in cardiothoracic surgery: Feasibility of the TEG6s system

Erdös G.[1], Schloer H.[1], Eberle B.[1], Nagler M.[2,3]

[1]Inselspital, Bern University Hospital, Department of Anesthesiology and Pain Medicine, Bern, Switzerland
[2]Inselspital, Bern University Hospital, Universitätsklinik für Hämatologie und Hämatologisches Zentrallabor, Bern, Switzerland
[3]Universität Bern, Department for BioMedical Research, Bern, Switzerland

Introduction: Viscoelastic near-patient assays of global hemostasis have been found useful and cost-effective in perioperative settings. Shortcomings of current systems include substantial labor-intensity, user-dependent reproducibility, relatively large sample volumes, sensitivity to ambient vibration and limited comparability between techniques and devices. We aimed to assess feasibility of a new, resonance-based viscoelastic whole blood methodology (TEG®6s) in cardiac surgery with cardiopulmonary bypass (CPB) and to compare the parameters this system produces with the ROTEM® delta system and standard coagulation tests.
Methods: In a prospective evaluation study, twenty-three consecutive cardiac surgery patients underwent hemostasis management according to current guidelines, using the ROTEM® delta system and standard coagulation tests. Blood samples were collected prior to CPB before anesthetic induction (pre-CPB), during CPB on rewarming (CPB), and 10 minutes after heparin reversal with protamine (post-CPB). ROTEM and standard coagulation test results were compared with TEG®6s parameters, which were concurrently determined using its multi-channel microfluidic cartridge system.
Results: TEG®6s provided quantifiable results pre-CPB and post-CPB, but only R (clotting time) of CKH (kaolin with heparinase) was measurable during CPB (full heparinization). Spearmans correlation coefficient (r_s) was 0.78 for fibrinogen levels and MA CFF (functional fibrinogen). Correlation of several TEG®6s parameters was good (0.77 to 0.91) with MCF FIBTEM, and poor (<0.56) with prothrombin time and activated partial thromboplastin time (<0.44). r_s with platelet count was moderate (0.70, MA CK; 0.73, MA CRT). Accuracy of MA CFF for detection of fibrinogen deficiency < 1.5 g/L was high (ROC-AUC 0.93).
Conclusions: The TEG®6s system, which is based on resonance viscoelastic methodology, appears to be feasible for POC hemostasis assessment in cardiac surgery. Its correlations with standard coagulation parameters are quite similar to those of ROTEM® and there is good diagnostic accuracy for fibrinogen levels lower than 1.5 g/L. During full heparinization, TEG®6s testing is limited to R measurement. Larger studies are needed to assess superiority over other POC systems.

Conflict of interest: none

PS-4B-4

A rare novel missense mutation in ITGB3 gene causing autosomal dominant Glanzmann Thrombasthenia characterized by giant platelets

Ivaskevicius V., Biswas A., Pezeshkpoor B., Pötzsch B., Pavlova A., Oldenburg J.

Institut für experimentelle Hämatologie und Transfusionsmedizin, Bonn, Germany

Introduction: The platelet integrin αIIbβ3 is essential for platelet aggregation. A complete loss of this integrin expression usually cause autosomal recessive Glanzmann thrombasthenia. Rarely, *ITGB3* gene mutations may cause autosomal dominant macrothrombocytopenia. Those patients may develop severe mucocuteneous bleeding. Here we report a 4-generation pedigree including seven individuals affected by autosomal dominant macrothrombocytopenia associated with impaired function.
Methods: Blood count, platelet aggregation, flow cytometry analysis (FACS) with monoclonal antibodies and light microscopy were performed by standard procedures. Genomic DNA of affected individuals and controls were screened with next generation sequencing (NGS) on the Illumina platform. The crystal structures and models corresponding to the platelet integrin αIIbβ3 have been investigated *in silico* in order to explain the autosomal dominant effect on a structure functional level.
Results: Recurrent epistaxis was a predominant clinical sign in the affected persons. Administration of antifibrinolytics was sufficient to stop bleedings. Surgical interventions have been performed usually without platelet concentrates. Affected individuals (five males and two females, age: 3–85) showed decreased platelet counts (17–85 × 10e9/l). Thrombocytes size was found enlarged (forward scatter in patients: 143–168 and in controls 46–53). Platelet aggregation was severely reduced or absent with ADP (5–10 µM), Adrenalin (5 µM), arachidonic acid, and collagen (20 µg/ml) while agglutination induced by ristocetin (0,5–1,2 mg) was normal or mildly reduced. FACS analysis showed normal expression of glycoprotein Ib/IX, glycoprotein IIb/IIIa, and α-membrane glycoproteins. NGS analysis showed a novel heterozygous missense mutation (new nomenclature: c.[2213T>G];[=], p.(Leu738Arg), old nomenclature: Leu712Arg) in exon 14 of *ITGB3* gene. In silico analysis suggests a gain of function mechanism brought about by formation of non-native contacts in the presence of substituted positively charged arginine residue. Interestingly, the p.Leu738Arg mutation was not detected in two further family members with regular platelet count and function.
Conclusion: We identified a novel missense mutation causing autosomal dominant Glanzmann Thrombasthenia. This novel heterozygous integrin β3 missense mutation might contribute to thrombocytopenia most likely through gain-of-function mechanisms.

Conflict of interest: none

PS-4B-5

Testing for alloantibodies in patients on Factor VIII replacement therapy using functional Bethesda and immunoassay

Pekrul I.[1], Bidlingmaier C.[2], Kurnik K.[2], Krebs H.[3], Schleef M.[3], Spannagl M.[1]

[1]Department of Transfusion Medicine, Cell Therapeutics and Hemostaseology, Hemostaseology and Hemophilia Care, München, Germany
[2]Dr. v. Hauner Children's Hospital, LMU Munich, Department of Pediatric Hemostaseology, München, Germany
[3]Sonnen-Gesundheitszentrum, München, Germany

Introduction: The primary aim in the treatment of patients with hemophilia A is the prevention of bleeds with factor VIII (FVIII) replacement therapy. One major adverse event of factor VIII replacement therapy is the development of alloantibodies (FVIII inhibitors). These antibodies may show a neutralizing effect on FVIII activity, resulting in an inadequate recovery after intravenous FVIII application and an increase in bleeding episodes. In hemophilia care the repetitive examination of the patients´ inhibitor status is mandatory. The functional Bethesda assay has a broad acceptance as a standard procedure, although it is difficult in performance, standardization and costly. Immunoassays for detection of Factor VIII antibodies have been introduced. Data from a nationwide cohort study in the US suggests to replace the costly clotting assay by an immunoassay using recombinant Factor VIII as antigen.
Methods: FVIII inhibitor testing was performed with dilutional FVIII clotting assay (Technoclone, Factor VIII Inhibitor Kit™) and anti-FVIII immunoassay (Hyphen Biomed, Zymutest™ anti-FVIII IgG mono strip). We examined 312 blood samples obtained from 200 paediatric and adult patients with haemophilia A on FVIII replacement therapy.
Conclusion: Discordant results were only detected in the Bethesda assay negative results (<0,4 BU). We found a considerable number of borderline reactive samples (29%, 91/312) and 8,3% (26/312) samples positive in the immunoassay. The clinical relevance of the immunoassay positive and functional Bethesda assay negative samples needs to be further investigated.

Conflict of interest: none

	anti-IgG FVIII positive > 0,3 OD	anti-IgG FVIII 0,15 < greyzone < 0,3 OD	anti-IgG FVIII negative ≤ 0,15
BU > 1	6 (1,65 median OD)	0	0
BU ≥ 0,4 - 1	0	4 (0,2 median OD)	0
BU < 0,4	26 (0,36 median OD)	91 (0,21 median OD)	184 (0,1 median OD)

Median OD	0,42	0,2	0,1
Min. OD	0,3	0,15	0,04
Max. OD	2,1	0,29	0,147
No. Patients	25	56	119
No. Samples	**32**	**95**	**184**

*OD = optical density

Tabel 1 Interpretation of the immunoassay results on basis of the manufacturer´s instruction (positive > 0,3 OD, 0,15 < greyzone < 0,3 OD, negative ≤ 0,15 OD). Interpretation of the Betheda assay results on basis of laboratory standards. The table shows the number of samples after test interpretation for both assays and the median optical density of the immunoassay (round brackets).

Fig. 1.

PS-5A
Immunhämatologische Genetik

PS-5A-1

An *ABO*A101c.182insG (fs)* Mutation Induces an A_{weak} Phenotype

Flesch B. K.[1], Kreklau P.[2], Ibrahim Y.[2], Stenzel A.[2]

[1]DRK Blutspendedienst West, Bad Kreuznach, Germany
[2]DRK Blutspendedienst West, Ratingen, Germany

Introduction: A- and B-transferases specifically transfer carbohydrates to the H-antigen resulting in the blood groups A and B. The alleles *A* and *B* at the *ABO* locus on chromosome 9q34.1-q34.2 code for the respective transferases whereas the *O* allele does not induce an active enzyme. Genetic variation of the *ABO* alleles may impair the enzymatic activity and transfer of the carbohydrates resulting in markedly reduced or even completely abolished A or B blood group expression. We describe the case of a mutation of the *ABO*A101* reference allele that induces a strongly diminished blood group A expression.
Methods: AB0 blood groups were determined by standard automated routine serology (IH-500, BioRad, Munich, Germany). Molecular typing was performed by commercial PCR-SSP (ABO Type Variant, BAG Healthcare, Lich, Germany). Genomic DNA sequencing covered amplification of the seven *ABO* exons including short flanking intron sequences

and the promoter region using published primer sequences followed by the electrophoretic separation in an ABI Prism 310 sequencer.
Results: Routine AB0 blood group determination of a female patient attracted attention by a markedly reduced agglutination of her RBC with Anti-A (+), negative reactivity with Anti-B and a negative DAT. The reverse grouping detected a strongly reactive anti-B (++++) but no reactivity with A1, A2 and 0 test cells. Commercial PCR-SSP clearly indicated the genotype *O1vA1* predicting the phenotype A1. DNA sequencing detected two alleles that can be assigned to an *ABO*O1v* (*261delG* plus **O2* specific variation) and a mutated *ABO*A101* allele with an insert of a G at position 182 (*ABO*182insG (fs)*). The variation induces a frame shift and a premature stop at codon 81 leading to an impaired α1,3-N-acetyl-D-galactosaminyl-transferase activity. The phenotype was identified as A_{weak} or Aw. The mutation was submitted to GenBank.
Conclusion: This is another case of a new *ABO* mutation that induces an A_{weak} phenotype. DNA sequencing enables the determination of rare mutations that are not included in commercial genotyping assays.

Conflict of interest: Bei keinem der Autoren besteht ein Interessenkonflikt. Alle Autoren sind Mitarbeiter des DRK Blutspendedienst West.

PS-5A-2

Sequencing of a patient with an IN(LU) phenotype reveals a new *KLF1* Allele

Stettler J., Lejon Crottet S., Hustinx H., Niederhauser C., Henny C.

Interregionale Blutspende SRK AG, F&E Blutgruppendiagnostik, Bern, Switzerland

Background: The In(Lu) phenotype is caused by mutations in the Krüppel-like factor gene *KLF1* on chromosome 19. Expression of the Lutheran (Lu) antigens depends on the level of functional KLF1 activity. The protein KLF1 is an erythroid-specific transcription factor crucial for erythropoiesis regulating the expression of several erythroid genes such as beta globin. The expression of the Lu antigens in the In(Lu) phenotype is strongly reduced and Lu antigens can often only be detected by adsorption-elution analysis. Additionally other antigens such as P1, In and AnWj are also suppressed. Several alleles, most as a result of single nucleotide polymorphisms (SNPs), have been reported. Usually these alleles are present in a heterozygous state. Homozygosity or compound heterozygosity for non-functional *KLF1* alleles was long believed not to be compatible with life, but one case of compound heterozygosity has been reported, leading to severe nonspherocytic hemolytic anemia and kernicterus. The sample of a patient was referred to our reference laboratory for investigation due to an In(Lu) phenotype.
Methods: Phenotyping on ID/IAT-cards (Bio-Rad) was done using anti-Lu(a) and anti-Lu(b) polyclonal antibodies (in-house). The adsorption-elution analysis was performed using a polyclonal anti-Lu(b) antibody. For *LU* genotyping an in-house sequence specific primer (SSP)-PCR method was applied. The sample was further characterized by exon sequencing including flanking intronic regions of the *KLF1* gene using published and in-house primers for amplification and sequencing.
Results: Standard phenotyping of the sample confirmed the Lu(a) and Lu(b) negativity. The SSP-PCR genotyping method showed that the patient was positive for nucleotide (nt.) 230G (*LU*02*) and the presence of nt.230A (*LU*01*) could be excluded. Sequencing of *KLF1* disclosed mutations c.304T>C and c.636C>G in a heterozygous state. Both could be assigned to the same allele by allele-specific sequencing. Whereas c.304T>C (p.S102P) is a common polymorphism having no influence on the expression of Lu antigens itself, the mutation c.636C>G leads to a premature stop codon at amino acid 212 (Y212Ter) in front of the C-terminal zinc finger DNA-binding domains. To the best of our knowledge the variant *KLF1*304C,636G* has not been reported previously. The adsorption-elution test with a polyclonal anti-Lu(b) gave a very weak positive result, which is in accordance with the In(Lu) phenotype.
Summary/conclusions: Here we present a patient with an In(Lu) phenotype, resulting from a new *KLF1* null allele, *KLF1*304C,636G* in heterozygous state. In case of need of a transfusion the patient would be transfused with Lu(b) positive blood as long as no antibody is present according to the Swiss guidelines.

Conflict of interest: none

PS-5A-3

A pregnant woman with rare –D–/–D– phenotype and anti-Rh17 (Anti-Hr0)

Torreiter P.[1], Kovacic K.[1], Macher S.[1], Moritz M.[1], Sebauer K.[1], Körmöczi G.[2], Polin H.[3], Wagner T.[1]

[1]LKH Univ.-Klinikum Graz, Universitätsklinik für Blutgruppenserologie und Transfusionsmedizin, Graz, Austria
[2]Medizinische Universität Wien, Universitätsklinik für Blutgruppenserologie und Transfusionsmedizin, Wien, Austria
[3]Österreiches Rotes Kreuz, Landesverband Oberösterreich, Blutzentrale Linz, Linz, Austria

Background: The management of pregnancies with rare anti-Rh17 is difficult. Little is known about the nature of this antibody, which reacts with red cells of all common Rh phenotypes. Here we report a case of a 23-year-old pregnant homozygote –D– woman (gravida 4, para 2, one spontaneous miscarriage) with anti Rh17.
Methods: Blood group testing was performed using human and monoclonal antibodies (gel cards). Antibody screening, antibody differentiation, direct antiglobulin test (DAT) and antibody titer were carried out by routine gel technology (LISS/Coombs, papaine and NaCl gel cards with bromeline milieu). Furthermore, we performed adsorption and elution. Moreover, genotyping was processed by PCR-SSP and DNA sequencing.
Results: The blood group of the pregnant woman was typed O, C-c-D+E-e- and K-. CDE PCR-SSP showed comparatively weak bands for antigens c and E. Consequently, DNA sequencing was performed, revealing no changes in exon 1–10 as well as no polymorphisms in any intron within the RHD gene. The RHCE sequencing resulted in the conclusion she is having an RHCE-D hybrid, the genotype RHCE-D(3–9)-CE which leads to a null allele.
The DAT was negative, antibody screening and differentiation first appeared as a combination of anti-C and anti-e. However, with additional adsorption and elution method, a highly reactive anti-RH17 allo-antibody was determined.
The pregnancy was closely monitored by analysing the antibody titer and multiple sonographic assessments of the middle cerebral artery peak systolic velocity of the foetus. For the potential case of a foetal haemolytic complication due to the anti-RH17 we would have produced a red blood cell concentrate from the antigen negative pregnant woman for intrauterine transfusion of the foetus. For the planned caesarean section we provided a cryopreserved –D–/–D– red blood cell unit for the mother.
The baby was born at 38+3 weeks of gestation without complication. The newborn girl was typed O pos. –D–/CDe. The DAT of the cord blood was positive with a titer of 1:8. No additional therapeutic treatment of the newborn was necessary.
Conclusion: Persons with red blood cells lacking expression of any formation of CcEe probably develop allo-antibodies against Rh17 after transfusion, transplantation or pregnancy. In our case the allo-anti-Rh17 is considered as to be benign as it did not induce a haemolytic disease of the foetus or the newborn. Nevertheless, we were prepared for potential transfusion requirements – at the one hand for the child during pregnancy and after birth, on the other hand for a potential bleeding complication of the mother during or after the caesarean section. Summing up, the –D–/–D– patient with allo-anti-Rh17 and her child were successfully treated.

Conflict of interest: none

PS-5A-4

Presence of a RHD (147delA) mutation allele associated with an abnormal high allo-anti-D titer

Kovacic K.[1], *Torreiter P.*[1], *Matzhold E. M.*[1], *Sebauer K.*[1], *Polin H.*[2], *Körmöczi G.*[3], *Wagner T.*[1]

[1]LKH Univ.-Klinikum Graz, Universitätsklinik für Blutgruppenserologie und Transfusionsmedizin, Graz, Austria
[2]Österreiches Rotes Kreuz, Landesverband Oberösterreich, Blutzentrale Linz, Linz, Austria
[3]Medizinische Universität Wien, Universitätsklinik für Blutgruppenserologie und Transfusionsmedizin, Wien, Austria

Background: An obviously Rh D neg female patient with an anti-D of unknown origin was subsequently typed rare RHD (147delA) allele. This allele, causing a frameshift mutation, which could permit the production of trace amounts of functional protein, was described as phenotype RH DEL. An anti-D alloantibody development as shown in this constellation is very unlikely and was so far not described.
Methods: A 27-year-old, nulligravida, nullipara patient, trying to conceive, was sent to us for immunohematological testing. We performed blood group typing with human and monoclonal antibodies in gel cards. Although tested O Rh neg, SSP-PCR and DNA sequencing were performed. An adsorption-elution method was used to serologically confirm the DEL antigen, using 11 different anti-D antibodies including the patient´s own antibody. Furthermore, Rh D antigen site density and mRNA analysis were performed (results pending). Antibody screening and antibody differentiation were performed in three different milieus (LISS/Coombs, bromelin and papain) using gel cards.
Results: Serological blood typing methods showed the patient was O Rh neg Ccddee, but molecular analysis revealed a RHD (147delA) mutation allele, being expressed as a very weak D antigen (DEL). DEL antigen was serologically confirmed using an adsorption-elution method. Incubation of patient´s erythrocytes with 11 different anti-D antibodies (9 of clonal and 2 of human origin) gave positive eluate reactions with only some of the clonal anti-D antibodies and was negative with the patient´s own antibody, excluding an auto-anti-D antibody.
The latter antibody was observed with a titer of 1/4.000 in gel cards. The regular follow-up titer analysis in the following months showed a constant progressing trend, the highest anti-D titer being 1/1.000.000. A possible anti-LWa antibody was excluded, using O Rh neg and O Rh pos cord blood erythrocytes. The family analysis showed that her brother shares the same mutation, but without anti-D antibody development.
Conclusion: We report a DEL phenotype with a high titer allo anti-D antibody. Due to selective positive reactions with different clonal anti-D antibodies we conclude that some of the epitopes of D antigen on patient´s erythrocytes are missing and that this specific DEL is actually a form of a D-partial. The past medical history and current anamnestic data which could explain the observed development of an allo-anti-D (transfusions, transplantations, underwent or ongoing pregnancy, unusual blood transfer practice) was negative, so the underlying cause for the anti-D antibody development was most likely an unnoticed miscarriage. Since the woman is currently not being pregnant, the ongoing rapid and constant anti-D antibody titer rise still remains unclear.

Conflict of interest: none

PS-5A-5

Defining the DIVa/weak D type 4 cluster recombination breakpoint in DIII type 5 using thousand genome project phase 3 data

Wagner F.

DRK Blutspendedienst NSTOB, Institut Springe, Springe, Germany

Background: For many years, analysis of *RHD* alleles focused on the exome. Recently, whole genome-based approaches gained importance. The «thousand genome project» (TGP, *http://www.internationalgenome.org/*) is a publicly available dataset of more than 1000 genomes from different populations.
Methods: Data of 2504 genomes were obtained from the TGP phase 3 dataset using a perl script. Single nucleotide polymorphism (SNP) data were evaluated in the region GRCh38 25142465 to 25460151 (GRCh38) covering about 100,000 bp on both sides of the *RHD* gene. Known allele data were collated from the Human RhesusBase (HRB; *www.rhesusbase.info*). For SNP located in exons, frequency in TGP was compared to current knowledge. For alleles observed repeatedly, SNP data including intronic SNP were compared to those of «standard RHD» focusing on SNPs almost exclusively present or strongly overrepresented in carriers of the allele.
Results: TGP listed 38 missense and nonsense mutation s (HRB 352), only 3 of which (N135S; S248T and Y311S) were not represented in HRB. Both HRB and TGP listed 22 silent mutations, 11 were present in both datasets. Frequency data for L62F, E233Q, Y311S and T379M seemed to be unreliable, as homozygotes were strongly underrepresented for L62F and Y311S and frequencies predicted for Europeans were considerably higher than expected. No *RHD* negative or C homozygous genome was represented. The most frequent alleles were RHD*08N.01 (*RHD*psi, n = 11), RHD*04.01 (DIVa), RHD*03.04 (DIII type 4) and RHD*03.01 (DIII type 5), RHD*10.03 (DAU-3; DAU-0 not included due to artefacts) and RHD*09.03.01 (weak D Type 4.0). RHD*08N.01 was strictly linked to 30 non-coding SNP stretching from *RHD* IVS1 to the downstream Rhesus Box (DRB), RHD*04.01 to 12 (IVS3 to DRB); RHD*09.03.01 to 6 (IVS9 to DRB), RHD*10.03 to 1 in IVS3. RHD*03.01 showed no strictly linked SNPs; however, 100 homozygous SNP occurred in all 4 RHD*03.01 samples but less than 40 samples overall. The status of these SNPs was compared to other samples carrying L62F, A137V, N152T, T201R, F223V, 819G>A, RHD*09.03.01 and RHD*04.01. RHD*03.01 -associated SNP in the region 25275003 to 25293530 were present in a large proportion of samples homozygous for L62F, A137V or N152T including RHD*04.01 alleles, while those in the region 25296627 to 25306148 were shared by samples carrying T201R, F223V or 819G>A including RHD*09.03.01. SNPs in the region 25307491 to 25322788 showed association with both types of alleles but not DIVa.
Conclusion: Data from TGP are heavily affected by artefacts and may misinterpret large deletions or hybrid genes. Still, the association of many intronic SNP reveals the evolutionary distance of alleles of the DIVa and weak D type 4 clusters from standard RHD. DIII type 5 obviously occurred by a recombination between these clusters with a breakpoint in IVS3 between 25293530 and 25296627 (c.486+2739 to c.486+5836).

Conflict of interest: Arbeitnehmeranteil an Patenten bzgl. Molekularbiologie von RH

PS-5A-6

Validation report of simultaneous *RHD, RHCE, KEL, JK, FY, GYPA/B, DO, LU, YT, DI, VEL, CO, KN* and *ABO* genotyping

König S.[1], *Helmig S.*[1], *Gassner C.*[2], *Frey B. M.*[2], *Scholz S.*[1]

[1]inno-train Diagnostik GmbH, Kronberg, Germany
[2]Blutspende Zürich, Zürich, Switzerland

Introduction: Blood group compatibility between patient and donor is the key to a safe transfusion. Standard red blood cell (RBC) typing is performed by serological means but the method comes to its limits in cases of pre-transfusion, the existence of alloantibodies or inaccurate results due to certain antigen variants. Molecular RBC typing can circumvent these problems due to analysis of the DNA sequences coding for the antigens. In this study we analyzed serologically pre-typed samples with two rapid molecular methods.
Methods: DNA samples of 26 individuals with pre-existent blood group serotypes (serotyping for ABO, RhD, RhCE, K/k, JK, Fy, MN and Ss done by standard techniques) were genotyped for *RHD, RHCE, KEL, JK, FY, GYPA/B, DO, LU, YT, DI, VEL, CO* and *KN* with a combined PCR-SSP based TaqMan Probe 96 well assay (RBC-FluoGene vERYfy eXtend, in-

no-train Diagnostik GmbH). The kit detects more than 100 blood group specificities, including RHD DELs, D weak, D neg, categories and partial Ds. 23 of these 26 samples were also typed for ABO (RBC-FluoGene ABO Basic, inno-train Diagnostik GmbH). The samples were analyzed by two methods: end point fluorescence and real-time PCR. The results were automatically evaluated by software.

Results: With respect to the antigen pairs encoded by the blood group systems JK, Fy, MN and Ss, all zygosities were tested. Above mentioned 26 samples further included samples with rare antigens, such as 1 Kk, 1 Kp(a+b+), 5 Yt(a+b+), 1 Vel+/Vel-, 1 Kn(a+b+) and 1 Co(a+b+). All of the analyzed samples revealed 100% concordance between serological and molecular typing, done in this study. Both molecular methods, end point PCR and real-time PCR, agreed to 100%.

Conclusion: The study shows that molecular RBC typing methods represent a suitable addition to serology in terms of hands-on and assay time as well as resolution. In this case, only two assays are required for parallel molecular testing of ABO, RhD, RhCE, Kell, Kidd, Duffy, MNS, Dombrock, Lutheran, Cartwright, Diego, Vel, Colton and Knops. Low manual hands-on-time and fully automated result evaluation with assay time of 90 minutes for end point PCR and 70 minutes for real-time PCR provide a beneficial opportunity for molecular blood group testing.

Conflict of interest: Ich bin in Teilzeit bei der inno-train Diagnostik GmbH beschäftigt.

PS-5A-7

Molecular blood group typing by means of MALDI-TOF MS

Just B.[1], Reil A.[1], Deitenbeck R.[1], Flesch B. K.[1,2]

[1]DRK-Blutspendedienst West, Hagen, Germany
[2]DRK-Blutspendedienst Rheinland-Pfalz und Saarland, Bad Kreuznach, Germany

Introduction: Antibodies often lead to critical supply problems due to the inadequate availability of antigen-negative preparations – especially in case of antibodies against high frequency antigens (HFA). High throughput methods (in-house procedures or commercial test systems in molecular blood group typing) are available besides routine serological testing. In our laboratory, a method using MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry, Agena, Hamburg) was successfully established. After our first experiences with this system in 2012 to 2013, we now report our experience with more than 11.000 donors until 2016.

Methods: Repeat donors of blood group 0 (rarely A) CCD.ee, ccD.EE as well as ccddee and K neg. were tested [without preliminary investigation of Vel, Lu(b), Kp(b), Co(a) und Yt(a)]. The examination of each donor was limited to a single multiplex approach with as many polymorphisms as possible (the approach contains the alleles Vel, Kp(ab), Lu(ab), Lu(8,14), Yt(ab), Co(ab), CoDef(601delG), Do(ab), Fy(ab), Jk(ab), MNSs). In the beginning of our investigations, negative molecular results were confirmed by a CE-marked PCR-test supplemented by a serological control of a subsequent donation and in few cases additionally confirmed by the International Reference Laboratory (IBGRL) in Bristol, UK.

Results: Positive and negative results (genetically as well as serologically pre-tested samples) were confirmed in the course of validation. Among 11.448 donors, a total of 90 «rare donors» could be detected by genotyping. Testing of Vel was included since 2014. In the meantime 59 of 90 «rare donors» [Co(a-) n = 29, Yt(a-) n = 35, Lu(b-) n = 13, Vel- n = 7, Lu8- n = 6 – and n = 0 for Kp(b-) and CoDef.] could be confirmed serologically. To evaluate the improvement of testing Do(a/b), Fy(a/b), Jk(a/b), and MNSs we compared the number of several combinations of phenotyped and genotyped donors. For example, 19 phenotyped donors with blood group 0 RhD-neg. ccddee K-, Fy(b-), Jk(a-), s- (the incidence of Fy(b-), Jk(a-) and s- is about 1:200) donated blood during the last 6 years (partially phenotying was performed many years ago). Applying molecular testing additional 26 donors of this rare combination could be found.

Conclusion: MALDI-TOF MS is suitable for extended blood group genotyping (especially of antigens encoded by a single nucleotide polymorphism (SNP) like many HFA and other antigens important in routine supply). Our results correspond well with other genotyping as well as phenotyping procedures. An extended typing of blood donors could improve the supply of patients with antibodies against HFA and also in cases of mixtures of typical antibodies with acceptable costs. In the future, further investigations by means of MALDI-TOF MS are intended to improve the rapid availability of rare donations.

Conflict of interest: none

PS-5A-8

Evaluation of diagnostic tests for platelet allo-antigen HPA-1a and HPA-5b phenotyping

Schwab C.[1], Saint-Cyr M.[2], Rink G.[1], Schawaller M.[3], Merieux Y.[2], Bugert P.[1]

[1]Institut für Transfusionsmedizin und Immunologie, Molekularbiologie, Mannheim, Germany
[2]Etablissement Français du Sang Auvergne-Rhône-Alpes, Lyon, France
[3]Davos Diagnostics, Davos, Switzerland

Background: The human platelet alloantigens (HPA) are located on glycoproteins on the platelet surface and can induce alloimmune reactions as a common cause of neonatal alloimmune thrombocytopenia (NAIT) or refractoriness after platelet transfusion. The HPA-1a and HPA-5b antigens are the most relevant antigens. The antigens are defined by single nucleotide polymorphisms (SNPs) in the glycoprotein genes and the antigen status can be determined by genotyping the SNPs. However, genotyping is time consuming and costly depending on the method and sample throughput. Here, we tested the reliability of the evanescent biosensor technology (EVA) for the rapid phenotyping of the HPA-1a and HPA-5b antigens.

Methods: HPA-1a and HPA-5b phenotyping was performed on EDTA blood samples from 336 blood donors using EVA typing assays and the biosensor system (Davos Diagnostics; Davos, Switzerland). For genotyping HPA-1 and HPA-5 we used validated PCR-SSP methods and assays with gene-specific primers and fluorescent allele-specific TaqMan probes.

Results: HPA-1a phenotyping was positive for all samples with HPA-1aa (n = 244; EVA value 807 ± 167 U/sec) and HPA-1ab (n = 82; 542 ± 110 U/sec) genotypes. All samples (n = 10) with negative EVA values had the HPA-1bb genotype. HPA-5b phenotyping was negative for all HPA-5aa genotypes (n = 267) and positive for the HPA-5ab (n = 66; 83 ± 22 U/sec) and HPA-5bb (n = 3; 118 ± 25 U/sec) genotypes. EVA values from heterozygotes were significantly lower compared to HPA-1a or HPA-5b homozygotes ($p<0.0001$ each). However, discrimination between the two genotypes was not possible on the basis of the EVA values.

Conclusion: EVA is a reliable method for rapid phenotyping of the clinically relevant HPA-1a and HPA-5b platelet antigens. The test can be performed from only 10 µl of fresh or frozen blood samples. Phenotyping of 4 samples on one EVA chip could be completed within 10 minutes (3 minutes hands on time).

Conflict of interest: Manfred Schawaller ist Mitarbeiter des Testherstellers (Davos Diagnostics). Für alle anderen Autoren bestehen keine Interessenskonflikte.

PS-5B-1

A family of HLA DQA1*01Q alleles?

Binder T. M. C.[1], Krammes L.[2], Rabbi S. N. I.[3], Planelles D.[4], Balas A.[5], Kelsch R.[6], Schäfer M.[2], Peter W.[2]

[1]UKE Hamburg, Transfusionsmedizin, HLA-Labor, Hamburg, Germany
[2]Stefan-Morsch-Stiftung, HLA Laboratory, Birkenfeld, Germany
[3]Labaid Diagnotics, Phamacogenetics Laboratory, Dhaka, Bangladesh
[4]Blood Bank, HLA Laboratory, Valencia, Spain
[5]Blood Bank, HLA Laboratory, Madrid, Spain
[6]University Clinics of Münster, HLA Laboratory, Münster, Germany

Background: On the occasion of HLA-typing of a patient with Multiple Myeloma and his brothers and sisters, we found a new DQA1*01:01-like allele in 3 of his 5 siblings. OneLambda rSSO typing (Luminex) together with the analysis software HLA Fusion gave as DQA1 typing results: DQA-1*01:07Q and *03. Because the new DQA1 allele should be part of the DRB1*01:01~DQA1*01:01~DQB1*05:01 haplotype and DQA1*01:07Q (most similar to DQA1*01:04) is normally part of the DRB1*14:01P~DQA1*01:04/DQA1*01:07Q~DQB1*05:03 haplotype, we were not sure, if these are the right results. In Valencia, and later in Munster, we found the DQA1*01:13 allele (most similar to DQA1*01:02 ?), which should be part of the DRB1*15:01~DQA1*01:02/DQA1*01:13~DQB1*06:02 haplotype.

Methods: To complete the genomic sequences of these DQA1 alleles, we developed a workflow based on long range PCR (LR-PCR) and next generation sequencing (NGS). Therefore we designed different HLA locus and/or allele specific LR-PCRs and sequenced the generated amplicons on a MiSeq platform (Illumina). The subsequent NGS data evaluation was performed with two different HLA software tools (Omixon Twin, Omixon and NGSengine, GenDx). The phased sequence alignment according to the individual single nucleotide variants (SNVs) pattern present ended up with allele-specific contigs. The final alignment of these contigs was done with AliView (Muscle) and BioEdit (ClustalW) software along with published IMGT/HLA database DQA1*01 sequences.

Results: The full-length sequence analysis of the DQA1*01:01Q*new* unraveled a quite high similarity to the DQA1*01:01 allele. As in DQA-1*01:07Q and DQA1*01:13 we found in the new allele a single nucleotide exchange (C → T) in the first position of codon 79 resulting in an Arginine → Cysteine exchange in exon 2. This supplementary Cysteine seems to be the cause of the questionable (Q) expression of these 3 alleles. The full-length sequence analysis of the DQA1*01:13 unraveled a quite high similarity to the DQA1*01:02 allele, as expected. DQA1*01:01Qnew respectively DQA1*01:13 differ from their parent alleles DQA1*01:01 respectively DQA1*01:02 in one nucleotide exchange (and one amino acid exchange in codon 79), only.

Conclusions: LR-PCR and NGS including phased sequence analysis revealed the unambiguous full-length sequence of DQA1*01:01Q*new* and DQA1*01:13 alleles. Now we are looking for DQA1*01: 03Q*new* and DQA1*01:05Q*new*. Are all these DQA1 alleles (together with their equivalent DQB1) expressed on cell surface or not?

Conflict of interest: Es bestehen keine Interessenkonflikte. Ich bin angestellter Co-Director (EFI) im HLA-Labor des UKE Hamburg.

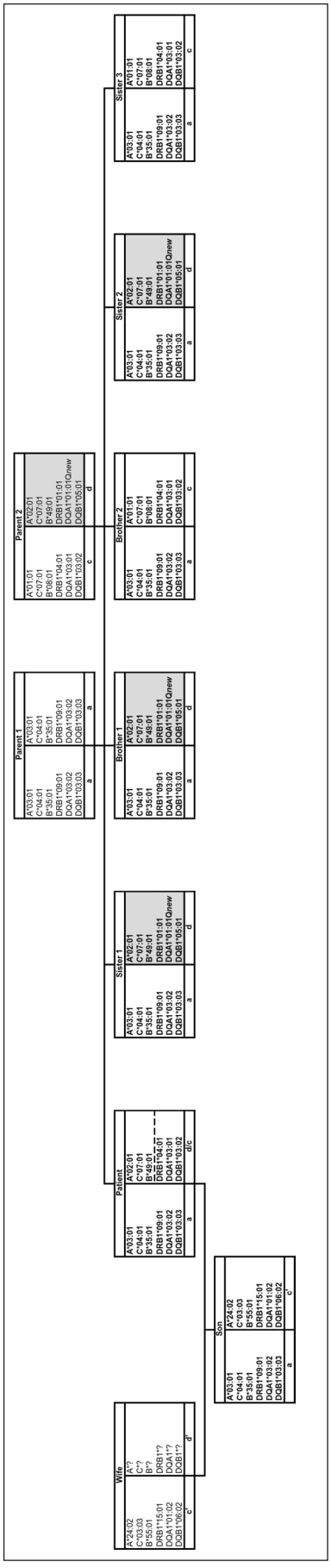

Fig. 1.

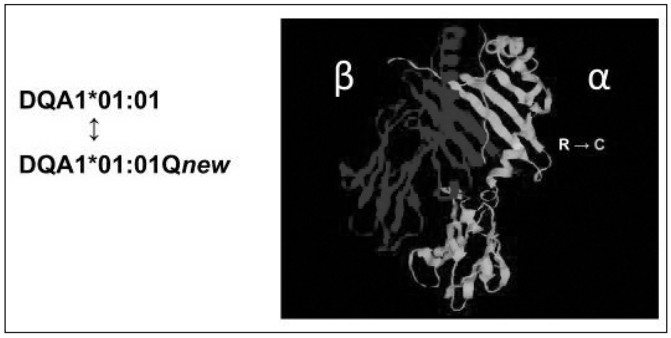

Fig. 2.

PS-5B-2

Case report: Detection of an allel-specific de novo donor specific HLA-DQB antibody in a 73-old female patient after kidney transplantation

Dick A.[1], Kauke T.[1], Renders L.[2], Humpe A.[1]

[1]Klinikum der Universität München, Abteilung f. Transfusionsmedizin, Zelltherapeutika und Haemostaseologie, München, Germany
[2]Klinikum Rechts der Isar, II.Medizinische Klinik und Poliklinik, München, Germany

Introduction: Requests for analysing patient´s sera for donorspecific HLA-Antibodies (DSA) after solid organ transplantation have become an integral part of daily routine in an HLA-laboratory. But in case of multiple HLA-antibodies in patient´s sera the specification of HLA-antibodies might be challenging especially if the donor is only typed on a low resolution level. We hereinafter report a case clearly demonstrating the advantages of medium resolution typing of donor and recipient enabeling a rapid and reliable post transplantation diagnosis. **Methods:** In December 2017 we implemented the LinkSēq™ SABR typing kit (Linkage Biosciences, Thermo Fisher Scientific) for typing of cadaveric donors during on call duty. Within two hours the donor is medium resolution typed for HLA-A, B, C, DRB1, DQA1, DQB1, DPA1 und DPB1 based on a rapid melt curve SSP technology on a real-time PCR instrument. For HLA-antibody specification the Luminex Single Antigen bead technology is used (OneLambda, Thermo Fisher Scientific). The patient, a 73 years old woman, had been typed by Luminex SSO medium resolution technology (OneLambda, Thermo Fisher Scientific) before entering the active waiting list. In January 2018 she was transplanted without any preformed HLA-Antibodies with a kidney of a 79 years old donor (HLA-A/B/DR mismatches: 2/2/1) who had been typed with the recently implemented LinkSēq™ SABR typing kit.
Results: Routine screening of patient´s serum for DSA was negative three weeks after transplantation. But 4 months after kidney transplantation laboratory and medical parameters indicated a severe graft dysfunction potentially on the basis of a humoral immune reaction. Focussing on HLA-class II the recipient was transplanted with an assumed HLA-DR15,-DQ6 compatible donor. A new analysis for DSA at that time revealed a de novo allel-specific antibody against HLA-DQB1*06:01 with the recipient´s HLA-typing being DRB1*04:01, 15:01, DQB1*03:01, 06:02 and the donor´s HLA-typing being HLA-DRB1*11:01, 15:02, DQB1*03:01, 06:01. In addition to the allel-specific DSA further de novo DSA were detectable.
Conclusion: In summary the implementation of the medium resolution LinkSēq™ SABR typing kit for donor´s typing allowed us to close the gap between finding allel-specific HLA-antibodies and classifying them as donorspecific.

Conflict of interest: none

PS-5B-3

Pre- and posttransplant peripheral blood donor-specific interferon-gamma enzyme-linked immune spot assay and de novo donorspecific alloantibodies in lung and kidney transplant recipients

Pan M.[1], Dick A.[2], Kunze S.[2], Stangl M.[3], Habicht A.[4], Fertmann J.[1], Hatz R.[1], Guba M.[3], Humpe A.[2], Kauke T.[2,1]

[1]Klinikum der Universität München, Thoraxchirurgie, München, Germany
[2]Klinikum der Universität München, Transfusionsmedizin, Zelltherapeutika und Hämostaseologie, München, Germany
[3]Klinikum der Universität München, Allgemein, Viszeral und Transplantationschirurgie, München, Germany
[4]Klinikum der Universität München, Transplantationszentrum, München, Germany

Introduction: Long-term outcomes following kidney transplantation is currently estimated at only about 11 years. In lung transplant recipients the half-lives are much lower with 6 years after transplantation. So early posttransplant identification of patients at highest risk of late graft loss could allow targeted therapies. Detection of donor-specific alloantibodies (DSA) is a well-established non-invasive method to assume humoral immune response. The aim of our study was to investigate whether quantifying pre- and posttransplant donorspecific T-cells by IFN-g ELISpot non-invasively detects T-cell mediated rejection and predicts development of de novo DSA.
Methods: In a prospective study we evaluated 17 kidney and 18 lung transplant recipients transplanted between September 2017 and January 2018. All patients displayed no evidence of immunization before transplantation and received an ABO compatible organ. Pre and 1 months posttransplant we performed a donorspecific IFN-g ELISpot with peripheral blood lymphocytes. We prospectively screened all patients before and after transplantation (1, 3, 6, 12 months and annually post TX) for the presence of DSA. Biopsies were only conducted on demand in case of graft dysfunction. The patients were regularly controlled in our transplantation center.
Results: Interestingly we found elevated INF-g spots in patients on the waiting list for lung as well as kidney transplantation. We defined a cut-off of >25 spots for positivity. 15/18 (84%) lung (mean 83 spots) and 7/17 (42%) kidney (mean 48 spots) patients were positive before transplantation. One month after transplantation the INF-g spots decreased in both groups (KTX: 83 vs 60 spots; LTX: 48 vs 26 spots) and increased in the later course (KTX: 83 vs 60 vs 110 spots; LTX: 48 vs 26 vs 69 spots). More lung transplant recipients remained positive for IFN-g ELISpot than kidney transplant patients (72% vs 36%). One out of 17 (5%) kidney and 4 out of 18 (22%) lung transplant patients developed de novo DSA during the first month. Biopsy proven rejection could not be associated to elevated IFN-g spots for both groups.
Conclusion: Both before and after transplantation elevated IFN-g spots were detected in lung and kidney transplant patients. Intensified immunosuppression early after transplantation reduces the allo T-cell reactivity as expected. Up to now due to the small amount of samples we were not able to correlate the INF-g spots to the development of de novo DSA.

Conflict of interest: none

PS-5C
Präparative und Therapeutische Apherese

PS-5C-1

IgE immunoadsorption in patients with severe atopic dermatitis: a pilot study

Lindlbauer N.[1], *Meyersburg D.*[2], *Mayer G.*[1], *Laimer M.*[2], *Bauer J. W.*[2], *Rohde E.*[1], *Grabmer C.*[1]

[1]Paracelsus Medizinische Privatuniversität Salzburg, UI für Transfusionsmedizin, Salzburg, Austria
[2]Paracelsus Medizinische Privatuniversität Salzburg, UK für Dermatologie, Salzburg, Austria

Background: Atopic dermatitis (AD) is a chronic, inflammatory condition of the skin that includes symptoms like erythema, scaling and pruritus in affected areas. Some patients with severe AD show high serum IgE levels. Therefore IgE might be one pathogenic mechanism in AD, still bearing in mind that also further complex immune dysregulation play an important role in this disease. Recently published data showed promising results for the treatment of AD patients with IgE Immunoadsorption (IA). The purpose of this pilot study was to determine the clinical efficacy of IgE depletion with the newly developed single-use IgE immunoadsorber column *IgEnio*® (FreseniusMedical Care, Bad Homburg, Germany) in severe AD with high serum IgE levels.
Methods: Five patients with severe AD were enrolled in this study. Inclusion criteria were severe clinical signs of AD (SCORAD ≥ 50) and elevated IgE levels (> 750 kU/l). All patients received three cycles of IA on a monthly regimen. The first cycle consisted of three treatments on consecutive days, followed by two cycles on two consecutive days. The twofold plasma volume of the patient was separated by centrifugation with an apheresis device (Spectra Optia, Terumo BCT, Lakewood, CO, USA). Efficacy of IA was determined by immunoglobulin levels of IgE before, during and up to 6 months after termination of treatment and of IgG, IgM, IgA during IA, respectively. Two experienced dermatologists, using the SCORAD index, evaluated the clinical improvement.
Results: IA was well tolerated in all patients and during a total of 28 procedures only mild adverse events were recorded. Our results revealed a significant decrease of IgE levels in all studied patients. Although a rebound phenomenon of IgE levels, following a saw tooth manner, after each IA cycle occurred in all patients. In total, IA selectively depleted 80,6% of IgE until the end of each treatment cycle. The average SCORAD index from all patients showed a significant reduction until the end of the study period. However the clinical effect was notably stronger in patients with extremely high IgE levels (> 5.000kU/l), compared to those with only moderately elevated serum IgE. We noticed a re-increase of the SCORAD index 6 months after the start of the treatment.
Conclusion: In conclusion, we observed that IgE IA using the newly developed column *IgEnio*® is a safe and well-tolerated therapy for patients with severe AD. Although IgE levels rose to initial values after each cycle, we detected a significant clinical amelioration for several months. As the long-term effects seems to be limited to 6 months, a quarterly to biannual repetition of the IA therapy might be an effective long-term treatment strategy for patients with highly elevated IgE levels. However, further studies are necessary to determine an optimized treatment protocol.

Conflict of interest: none

PS-5C-2

Prerequisites for appropriate vascular access in therapeutic apheresis: validation of peripheral (in vivo) and central vein access (ex vivo, NuPort)

Rox J. M.[1], *Wenzel F.*[2], *Fischer J. C.*[1]

[1]Medizinische Fakultät, Heinrich-Heine-Universität, Institut für Transplantationsdiagnostik und Zelltherapeutika, Düsseldorf, Germany
[2]Universität Furtwangen, Fakultät Medical and Life Sciences, Villingen-Schwenningen, Germany

Introduction: Appropriate vascular access is critical for successful therapeutic apheresis procedures. Thus, upfront assignment of cubital vein status enabling stable blood flow conditions is of prognostic value. Here, we compared cubital vein diameter with reachable mean flow rates. In addition we investigated a recently introduced port system, which allows high pressure infusions, but was never validated in an apheresis setting using whole blood before.
Methods: In 16 patients (diagnostic leukapheresis) and 31 donors (unrelated PBPC) the diameter of the cubital vein was determined by ultrasonic investigation, while congesting with a blood pressure cuff at 40mmHG before starting the apheresis procedure (cMNC, Spectra OPTIA (Terumo BCT)). 16G venous indwelling cannulas (Vasofix grey, inner diameter 1.7mm, Braun) were used for inlet access. Flow rates were calculated from processed blood divided by processing time. In addition a single lumen (NuPort **CTP**- 009CP, id 1.6 mm) as well as a double lumen port (**C**-Port-**DL,** id 1.18 mm) (PHS Medical GmbH) were validated ex vivo. Ports were punctured with 16G or 18G non coring needles (Norfolk Inc.). For simulation 4 non-process-able whole blood donations were pooled and held at 37°C. This 2l whole blood was connected with the inlet and/or return lines of the apheresis system (cMNC, Spectra Optia). Displayed inlet, return pressures, and any alarms were recorded. Starting at 30ml/min the flow rate was increased by 5ml/min every two minutes up to 142ml/min, when possible.
Results: Vein diameter ranged from 3.2 to 6.2mm (mean 4.3 ± 0.7), inlet flow rate from 46 to 110ml/min (mean 77 ± 16). Higher diameter allowed higher inlet flow rates (inlet flow [ml/min] = 17.8 x diameter [mm], r2 = 0.48). Using the CTP port and the 16G needle ex vivo, 142ml/min could be achieved. Occasional alarms started at 130ml/min. Inlet pressure correlated directly with the flow rate (displayed pressure [mmHg] = –1.56 x inlet flow, r2 = 0.96) Using the CTP and the 18G needle rates up to 125ml/min could be achieved, first alarms occurring at 115ml/min (pressure = -1.76 x inlet flow, r2 = 0.94). On the return side 142ml/min could be reached without any alarms (350mmHg return line pressure). Using the dual lumen CDL and 18G needles for inlet and return up to 70ml/min could be realized, occasional alarms starting at 65 ml/min (pressure = –3.09 x inlet flow, r2 = 0.97). Return line pressure at 70ml/min was than 292 mm HG. No leaks, damage, or occlusions were observed.
Conclusion: Inlet flow rates strongly depend on the diameter of the punctured vein, and sufficient conditions seems not achievable with diameters below 3mm. Catheter port systems are an option, especially in patients undergoing multiple sessions. At the time being Spectra OPTIA procedures are only dual needle procedures – here the double lumen C-Port DL is a valuable option. The achievable flow rates with the single lumen CTP-port are depended on the size of the puncture needle.

Conflict of interest: none

PS-5C-3

Immunoadsorption as adjunctive treatment in patients with acquired hemophilia

Esteves Pereira M.[1,2], *Nagler M.*[1], *Bocksrucker C.*[1], *Mansouri Taleghani B.*[1], *Daskalakis M.*[1]

[1]Inselspital, Universitätsklinik für Hämatologie und Hämatologisches Zentrallabor, Bern, Switzerland
[2]University of Bern, Department for BioMedical Research, Bern, Switzerland

Background: Aquired hemophilia is a rare but life-threatening disease with substantial therapeutic challenges. Immunoadsorption is suggested as an adjunctive treatment to reduce the antibody levels rapidly and support the initial treatment. The clinical data are however limited to case reports and small case series.
Aim: We report about the clinical course of all patients treated with immunoadsorption in our institution.
Methods: In a retrospective design, we identified, retrieved, and summarized all patients with acquired hemophilia and treated with immunoadsorption since implementation. Patients were identified by diagnosis codes and electronic patient charts as well as laboratory records were used for data collection. Determination of factor VIII was done using Pathromtin® SL (Siemens Healthcare) and inhibitor titer was conducted using the Bethesda method.
Results: Seven patients were identified between 2003 and 2018. Median age was 77 years (range: 68 to 86), 14% were female (n = 1). Indication for immunoadsorption was high inhibitor titer (n = 4; 57%) and/or severe bleeding. Triggering cause was not evident (idiopathic) in 6 patients (86%), and Wegener granulomatosis in 1 patient (14%). Factor VIII was below 1% in 5/7 patients (71%). Median inhibitor titer was 21.6 BU/ml (range 6.7 to 214). Additional treatments were high-dose steroids (n = 7; 100%), recombinant factor VIIa (n = 5; 71%), cyclophosphamide (n = 6; 86%), rituximab (n = 1, 14%), intravenous immunoglobulins (n = 2; 28%), activated prothromplex concentrate (n = 1, 14%), and high-dose factor VIII (n = 1, 14%). Columns for immunoadsorption being used were Immunosorba (Protein-Al Ligand) in 6/7 patients (86%) and GlobAffin Adsorber (Peptid-GAM-Ligand) in 1 patient. The median number of immunoadsorption cycles was 5 (range 4 to 7).
Bleeding was stopped in 5/7 patients (71%). No recovery of factor VIII was recorded in 2/7 patients (28%), and only partial recovery (>30%) was observed in one patient (14%). Within a follow-up of 12 months, three patients died due to different causes (one patient lost to follow-up).
Conclusions: Our results suggest that immunoadsorption supported antibody clearance and factor VIII recovery in the majority of patients. Larger registry studies might seek to establish which patients benefit most.

Conflict of interest: none

PS-5D
Qualitätssicherung

PS-5D-1

Implementation of an electronic change control module at DRK blood donation services Baden-Württemberg-Hessen and Nord-Ost

Kramer D.[1], *Scharnagl Y.*[1], *Spyrantis A.*[2], *Capalbo G.*[2], *Gubbe K.*[1], *Schmidt M.*[2]

[1]DRK Blutspendedienst Nord-Ost gGmbH, QM, Plauen, Germany
[2]DRK Blutspendedienst Baden-Württemberg-Hessen gGmbH, QM, Frankfurt am Main, Germany

Introduction: The DRK blood donation services Baden-Württemberg-Hessen and Nord-Ost dedicated themselves to a constantly high level of quality in all divisions. To guarantee the quality, change control procedures (CCs) are initiated within GDL- and Non-GDL-divisions (GDL = German Drug Law). Furthermore, the certification according DIN EN ISO 9001 and the accreditation according DIN EN ISO 15189 and 17025 require documented information to monitor changes and Annex 15 of the EU GMP guideline «Qualification and Validation» requires GMP-compliant (prospective) qualifications/validations and a risk-based consideration of changes.
Especially blood donation services, that consist of several institutes, have a great challenge in CCs because all responsible persons (management, divisional director, head of department, head of quality control, head of production and qualified persons) must be involved in general changes (more than 2 institutes are included).
Methods: A fully electronic CC module within the quality management software Saperion Pharao was introduced in 2015 in both blood donation services. The validation was performed within a test environment (Saperion Pharao validation module). About 50 different test runs with different change scenarios and involved persons (project managers, quality assurance managers, responsible persons) were carried out.
Results: Since the implementation of the electronic CC module in 2015, changes accross different departments and different company sites are finished faster, more uncomplicated and formally correct. All responsible persons are actively involved (including qualified persons, validation managers, IT etc.). A prompt authorisation by the responsible persons and a formal guidance of the entire procedure by quality management is ensured.
Furthermore, the complete CC including all attachments is electronically archived in Saperion, therefore available at all times in all company sites and useful as template for similar changes. Prospective qualifications/validations could be monitored by the electronic time stamp from uploading the attachments. The high acceptance of the system is reflected by the strongly increased number of general CCs (2011–2014: 65, 2015–2017: 211).
Conclusion: With the implementation of the electronic CC module, all GMP and standard requirements as well as additional internal requirements were realized:
All responsible persons are involved in CCs especially in general changes.
The classification of changes occurs automatically based on the risk analysis (FMEA).
The entire CC is electronically available (including all attachments as PDF).
GMP-compliant qualifications/validations are guaranteed.
Consequently, the electronic CCs are more transparent than the former paper system and the overall workload could be reduced due to the easily achievable general CCs.

Conflict of interest: none

PS-5D-2

A new and simple Eurocode based labeling system for the implementation of the SEC in small tissue banks

Pruß A.[1], *Müller H.*[2], *Hiller J.*[3], *Klerke J.*[4], *Stüpmann K.*[5], *Kardoeus J.*[6], *Schroeter B.*[1], *Knels R.*[7]

Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Berlin, Germany
[2]IMP Computersysteme, Berlin, Germany
[3]Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany
[4]DGFG, Hannover, Germany
[5]DRK-BSD Mecklenburg-Vorpommern, Rostock, Germany
[6]Universitätsklinik Münster, Münster, Germany
[7]MVZ Labor Limbach Dresden, Dresden, Germany

Introduction: The Directive 2015/565/EC on the coding of human tissue and tissue preparations provides the mandatory use of the Single European Code (SEC) to ensure traceability from the donor to the recipient and vice versa in the Member States of the EU. In scientific cooperation with Eurocode IBLS eV, the «Eurocode Labeling Program for Tissue Banks»

has been developed by the OSM group/IMP computer systems to facilitate the introduction of the SEC in Germany on 29.04.2017.
Methods: In addition to current label requirements, the program offers a number of other simple and creative functions: Collection of donor data, Recording time of donation, Type of donation, Automatic assignment of laboratory number, Manual entry / barcode scan of the laboratory number, Printing of laboratory labels.
Results: The Eurocode labeling program is adapted to the individual tissue types in order to optimally integrate them into the respective workflows. At present it is available for the labeling of femoral head and cornea transplants. Adjustments to other tissues will follow. With regard to introduction and training, video tutorials are available that comprehensively explain the individual sections. Course 1: Overview, Course 2: Recording and Labeling in one step, Course 3: Recording of the donation, Course 4: Labeling the tissue preparations, Course 5: Information functions.
Conclusion: After an evaluation phase in the Tissue Bank of the Charité, the Eurocode program for small tissue banks can be recommended. It generates labels with the required SEC with small effort and offers additional functions who are relevant to pharmaceutical tissue products. The program is available via Eurocode IBLS (headquarters@eurocode .org).

Conflict of interest: none

PS-5D-3

Establishment of a bidirectional IT system for the synchronised management of transfusion therapies and human blood and plasma derived drug products in a multi-site hospital

Hoyer M.

DRK gemeinnützige Krankenhausgesellschaft Thüringen Brandenburg mbH, Labor, Bad Frankenhausen, Germany

Background: The standardisation and digitalisation of transfusion therapies and its drug product management is a precondition to overcome the paper-based documentation, which is nearly impossible to monitor in a multi-site company of 4 hospitals with distances up to 273km that are provided with drug products by 3 different pharmacys and 2 blood donation centres. Although this is a fundamental requirement for an enhanced patient safety, a flexible deployment of the staff at each ward or hospital site, thoroughly accounting as well as internal service charges and financial controlling. Therefore a bidirectional laboratory information (LIS)-ward communication (TC) system was implemented to digitally map the entire process.
Methods: All master data (MD) for immunhaematological analyses provided by the hospital and blood donor centre laboratories as well as for all drug products derived from human blood and plasma were fed into the LIS. The product MD included information about manufacturer, supplier, product code or pharmaceutical central number, batch, expiry, delivery day, permitted stock locations and accounting codes.
While the patient data were transferred from the medical information system, the MD of the staff composed of name, personnel number and access authorization was stored in the LIS. A LIS affiliated TC was used for orders, result reports, monitoring of critical events and patient related drug product management. The TC included also a transfusion tracking module, which captured therapy relevant data while recording the staff logins as digital signature and was also linked back to the LIS to create a complete look back and transfusion protocol. The contents of the TC tracking protocols were defined by MD of the LIS to ensure uniformity. The documentation was set to trigger the product status change in the LIS depot module to replace the dispatch notes for the laboratory and pharmacy. The staff was trained in mixed user groups, had a 24/7 remote and a 3 day on site start support. The staff was provided with badges including their TC login as barcode label serving as credential.
Results: The initial training for the digital modules was attended by 46% of the staff. On site support was demanded for debriefing of the new organization under local conditions. An experienced handling was achieved within two weeks. It was possible to track each drug product and evaluate sufficiency and quality of the therapy protocols subjected to patient and staff. System errors occurred due to lack of communication about gathering products from the depots, because a subsequent release registration interfered the on time transfusion tracking.
Conclusion & Perspectives: A synchronized and controllable transfusion therapy tracking, result monitoring, drug product management and performance of different statistical evaluations were achieved. To lower the error rate of the transfusion tracking module, additional modules and improvements were enquired of the LIS supplier.

Conflict of interest: none

PS-5D-4

Metabolomic investigation of human blood products using Raman-Trapping Microscopy

Kronstein-Wiedemann R.[1], Arlt N.[2], Moog R.[2], Schütze K.[3], Tonn T.[1,2,4]

[1]Medical Faculty Carl Gustav Carus, TU Dresden, Experimental Transfusion Medicine, Dresden, Germany
[2]German Red Cross Blood Donation Service North East, Institute Cottbus, Cottbus, Germany
[3]CellTool, Bernried, Germany
[4]German Red Cross Blood Donation Service North East, Institute for Transfusion Medicine, Dresden, Germany

Background: In recent years, the safety of blood transfusion has shifted focus to detection of bacterial contamination in platelet concentrates as well as to the stability and functionality of blood components during storage in an attempt to make transfusion safer and better. Currently, it is not possible to test all blood products for sterility or integrity of the cellular components using the available methods for sterility release, which requires an incubation time for up to seven days. This is significant when considering how platelet concentrates have a limited shelf life of 5 days, forcing patients to receive outdated preparations while using the commercial microbial detection systems. The aim of the present study is to use Raman spectroscopy to develop a minimally invasive, innovative point-of-care technology for a faster, simpler and highly reliable quality control analysis of blood products.
Methods: Raman spectroscopy is based on detection of light inelastically scattered by molecules and shifted to the red (long-wave) range. The photons, which are changed in wavelength, are subsequently detected by a spectrograph. As all the molecules within a cell contribute to the Raman spectrum, the spectral sum is as characteristic as a fingerprint. In this project we want to generate Raman profiles of red blood cells and platelet concentrates during the normal storage process. The identified Raman parameters are a quality feature for blood products with regard to aging/functionality.
Results: We were able to show that both erythrocytes and thrombocytes have their own Raman profile, which will allow the identification of residual leukocytes in the preparation since the spectra of leukocytes should be different when compared to erythrocytes and thrombocytes. Quality control studies of platelet concentrates during storage showed the expected decrease in platelet activation capacity as well as the correlation in metabolic consumption by decrease of glucose and citrate and increase in lactate and lactate dehydrogenase (LDH). At once an area (1000–1100 cm-1) was identified in the Raman spectrum that defines the age of the cells.
Conclusions: The use of Raman spectroscopy may lead to essential improvements in the field of transfusion medicine. For the first time, the opportunity opens up to ensure a functional and quality control analysis of blood products immediately before transfusion, thus minimizing contamination risks. The project is funding by the BMBF.

Conflict of interest: none

Transfusion Medicine and Hemotherapy

Author Index

Transfus Med Hemother 2018;45(suppl 1):1–90

A

Achenbach S. PS-4B-2, PS-2A-6
Ackermann M. VS-3-5
Ahrens N. VS-1-1, VS-1-3, PS-2B-4, PS-2B-7
Aleksandrova K. VS-16-2
Al-Saeedi M. PS-3A-13
Althaus K. VS-4-8, VS-8-4, VS-10-4
Amann E. PS-1B-2
Amato M. VS-12-3
Amoros J. PS-1B-1
Ampofo E. VS-8-1
Angelow A. PS-3B-3
Angermayr K. PS-2B-12
Apelseth T. VS-14-3
Aranko K. PL-3-2
Arlt N. PS-2B-5, PS-5D-4
Arseniev L. VS-9-1
Aurich A. PS-1A-6
Aurich K. VS-8-7, VS-8-8, VS-10-5
Axt-Fliedner R. VS-15-5, VS-15-7
Aydinok Y. PS-4A-14

B

Badur C. VS-8-9
Baier M. PL-5-2
Baigger A. VS-16-4
Bak S. VS-16-6 ,VS-16-7
Bakchoul T. VS-4-4, VS-4-8, VS-8-3, VS-8-4, VS-8-8, VS-10-4, VS-17-3
Balas A. PS-5B-1
Balola A. VS-4-6
Balz V. VS-13-4, PS-2A-3
Barry F. PS-1B-6
Bartolmäs T. VS-4-6, PS-3A-14
Bauer J. PS-5C-1
Baumann-Baretti B. PS-2B-1, PS-2B-9, PS-4A-3
Baume H. PS-4A-10
Baumgart C. PS-2A-3
Bayat B. VS-19-5
Beekes M. PL-5-2
Bein G. VS-15-5, VS-15-6, VS-15-7, PS-1A-2, PS-1B-1, PS-3A-12
Bekeredjian-Ding I. VS-6-3
Benjamin R. PS-4A-14
Berens C. VS-8-2, VS-11-2
Bernard M. VS-8-5
Bernecker C. VS-3-5
Berry J. VS-4-3
Bidlingmaier C. PS-4B-5
Bieback K. VS-10-6, PS-1B-5
Binda M. VS-4-3
Binder T. PS-5B-1
Biswas A. PS-4B-4
Blasczyk R. VS-9-1, VS-16-2, VS-16-4, VS-16-6, VS-16-7, VS-16-8, VS-16-9
Blatt I. PS-2A-4
Blohm M. PS-3A-9
Böck M. PS-2B-8
Bocksrucker C. PS-5C-3
Böni J. VS-12-4
Bonig H. PS-1A-5
Bräuninger A. VS-15-7
Brenner R. PS-1B-2
Brenner-Weiß G. VS-10-6, PS-1B-5
Brixner V. PS-4A-11, PS-4A-14
Brockmann C. VS-14-4
Brossart P. VS-8-2
Budde H. PS-3B-1
Budde K. VS-13-3
Bugert P. VS-15-3, VS-19-2, VS-19-3, VS-19-5, PS-1B-5, PS-5A-8
Buhmann R. PS-4A-7
Burkhardt A. VS-8-6

C

Caesar A. PS-3A-4
Canals C. VS-19-5
Canellini G. PS-3A-2
Capalbo G. PS-5D-1
Carvalho-Oliveira M. VS-16-9
Cathomen T. PL-2-2, VS-16-5
Chatellier S. VS-10-5
Chenot J. PS-3B-3
Chen-Wacker C. VS-16-9
Chen-Wichmann L. VS-3-3
Chocholi I. VS-4-2
Clausen F. B. VS-15-1
Cooper N. VS-15-6, PS-3A-12
Corash L. PS-4A-14
Corisello C. PS-3A-2
Cornu T. VS-16-5
Dame C. VS-8-9
Daskalakis M. PS-5C-3
Deitenbeck R. PS-5A-7
Demmer U. PS-2B-3
Denomme G. VS-19-6
Denzer U. PS-4A-1
Dhople V. VS-10-2
Dick A. PS-5B-2, PS-5B-3
Diehlmann A. VS-3-2
Dimitriou E. PS-1B-8
Doescher A. VS-10-7, VS-15-4, PS-3A-7, PS-4A-10
Dombos S. PS-4A-11
Dormann F. PS-2B-7
Dorn I. VS-3-5
Dragon A. VS-16-6, VS-16-7
Drawz A. VS-8-1
Dreier J. VS-6-4, PS-4A-8
Dreweck C. VS-2-3, PS-2B-5
Driesel G. PS-4A-3
Duchateau P. VS-16-5
Dullinger K. PS-2A-6
Dürr N. VS-8-6
Dziegiel M. H. VS-19-7

E

Eberhardt M. VS-5-4
Eberle B. PS-4B-3
Eberle J. PS-4A-7
Edinger-Schons L. PS-2A-4
Eichler H. VS-8-1, VS-8-5, VS-8-6, VS-16-3
Eicke D. VS-16-4
Eickmann M. PS-4A-12, PS-4A-13
Eiz-Vepser B. VS-16-2
Eiz-Vesper B. VS-9-1, VS-16-6, VS-16-7, VS-16-8
El Nahry Y. VS-16-1
Elvers-Hornung S. PS-1B-5
Enczmann J. VS-13-4, PS-2A-3
Engström C. PS-3A-3, PS-3A-4, PS-3A-8
Enkel S. VS-4-5, VS-10-4,
Enzensberger C. VS-15-5, VS-15-7
Erdös G. PS-4B-3

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

Accessible online at:
www.karger.com/tmh

Erickson A. PS-4A-14
Ernst C. PS-4A-14
Eryilmaz M. VS-15-3
Eschenburg M. PS-3A-9
Esefeld M. VS-10-1 ,VS-10-2
Eske-Pogodda K. VS-4-4
Esteves Pereira M. PS-5C-3
Etzel J. PS-2B-8
Evers D. VS-17-1
Eyrich M. VS-16-3

F

Fabricius D. PS-1B-7, PS-1B-8
Fabricius H. PS-1B-3
Fagoo G. VS-10-5
Fath A. PS-2B-13
Favaloro V. VS-4-3
Fecher T. PS-1B-8
Felsen A. PS-1B-7, PS-1B-8
Fertmann J. PS-5B-3
Fiedler S. PS-2A-2, PS-2B-10
Figueiredo C. VS-16-4, VS-16-9
Fischer J. VS-13-4, PS-2A-3, PS-5C-2
Fleck E. VS-4-7
Flesch B. VS-19-4, VS-19-5, PS-5A-1, PS-5A-7
Fleury S. PS-1B-6
Flommersfeld S. PS-1A-2
Fontana S. VS-6-5
Forster M. VS-19-6
Frank K. VS-2-2, PS-4A-2
Franke A. VS-19-6
Frey B. VS-12-4, VS-19-6, PS-3A-3, PS-3A-4, PS-3A-8, PS-4A-9, PS-5A-6
Friedrich M. PS-3B-1
Fröhner V. VS-15-7
Fuhrmann J. VS-10-5
Funk M. PS-2A-2
Fürst D. VS-19-3
Füssl F. VS-10-5

G

Ganslandt T. VS-14-5
Ganslmayer M. VS-14-5
Gassner C. VS-19-6, PS-3A-3, PS-3A-4, PS-3A-8, PS-5A-6
Gathof B. VS-6-1, PS-2A-5
Gattenlöhner S. VS-15-7
Gautron A. VS-16-5
Gebauer W. PS-4A-10
Gebhardt K. PS-2A-3
Gebicka P. VS-4-1, VS-10-5
Geier T. PS-1B-4
Geisen C. VS-4-7, PS-4A-14, PS-4B-2
Gellerer A. PS-3A-6
Giese T. PS-3A-13
Glaus S. PS-3A-3
Glauser A. VS-12-4, PS-4A-9
Gleich-Nagel T. PS-3A-2
Goebel M. PS-3A-9
Görg S. VS-12-5, VS-14-4
Goslings D. VS-12-4
Gottschalk J. VS-12-4, PS-4A-9
Gottwald E. VS-3-2
Goudeva L. VS-9-1, VS-16-2
Gowland P. VS-6-5, PS-4A-4
Grabmer C. PS-5C-1
Graf N. VS-16-3
Gravemann U. VS-12-1, VS-12-2, PS-4A-12, PS-4A-13
Greffin K. VS-10-1
Greinacher A. VS-8-7, VS-8-8, VS-10-1, VS-10-2, VS-10-5, VS-17-2
Grolle A. PS-2B-3
Groß A. PS-1B-2
Gruber M. VS-1-3
Guba M. PS-5B-3
Gubbe K. VS-2-2, PS-4A-2, PS-5D-1
Güsmer C. PS-3A-9

H

Haas S. VS-16-5
Haase A. PS-3B-3
Haase S. PS-4A-11
Habicht A. PS-5B-3
Hackstein H. VS-1-2, VS-14-5, PS-1A-4, PS-1B-1, PS-2A-6, PS-3A-12, PS-4B-2
Hadegger K. PS-4A-9
Hagen C. VS-14-4
Hähnel V. PS-2B-7
Hamann P. PS-3B-4
Hammer E. VS-10-2
Hammer S. VS-8-4
Handke W. VS-12-1, VS-12-2, PS-4A-12, PS-4A-13
Hansen A. T. VS-19-7
Hardegger K. VS-12-4
Hartwich O. VS-8-7, VS-8-8
Hatz R. PS-5B-3
Haverich A. VS-16-9
Havlicek-Poetscher M. PS-2B-6, PS-2B-12
Hechler J. PS-2A-6
Heidel F. PS-3A-11
Heiden M. PS-2A-2
Heidtmann A. PS-3A-13
Heil U. PS-2B-2
Helmberg W. PS-2B-11
Helmig S. PS-5A-6
Hendig D. VS-5-4
Hennecke J. VS-16-1
Hennig H. VS-12-5
Henning J. PS-2B-1, PS-2B-9
Henny C. PS-5A-2
Henrich W. VS-15-5
Herkt S. PS-1A-5
Herrlinger F. VS-7-1
Hervig T. VS-14-3
Herziger A. VS-4-6
Heuft H. VS-9-1, VS-16-2
Heuser J. VS-5-4
Hidiatov O. VS-8-3, VS-8-4
Hildenbeutel M. VS-16-5
Hiller J. PS-4A-1, PS-5D-2
Hinse D. VS-5-4
Hirv K. VS-13-2
Hoeller K. PS-3A-5
Hofer M. PS-2B-12
Hoffelner M. PS-2A-2
Höffler K. VS-16-9
Hoffmann T. PS-1B-8
Hofmann M. PS-2B-6
Hofmann J. VS-7-1
Höglund P. VS-19-5
Hölig K. VS-4-2
Homburg K. PL-4-1
Horn P. PS-2A-3
Hourfar K. VS-2-2, VS-6-2, PS-4A-2
Hoyer M. PS-5D-3
Huber-Lang M. PS-1B-2
Huber-Marcantonio D. PS-3A-2
Huck V. PS-1B-5
Hudecek M. VS-16-6
Huiskes E. VS-19-5
Humpe A. VS-3-3, PS-1A-9, PS-3B-4, PS-4A-7, PS-5B-2, PS-5B-3
Hustinx H. PS-3A-1, PS-5A-2
Hutchinson J. VS-1-1, PS-2B-7
Hutschenreuter G. PS-3A-7
Huzly D. VS-12-4

I

Ibrahim Y. PS-5A-1
Ikert A. PS-2A-1
Ivaskevicius V. PS-4B-4

J

Jahrsdörfer B. PS-1B-7, PS-1B-8
Jakobsen M. A. VS-15-2
Janetzko K. PS-1B-5
Jansen P. VS-10-1
Janson A. VS-19-4
Jelkmann W. PL-3-1
Jiménez Klingberg C. PS-2A-3
Johnson C. PS-2B-4
Jorgensen C. PS-1B-6
Jouni R. VS-8-3, VS-8-8, VS-17-3
Juengling G. PS-3A-5
Juhl D. ZF-2-1, VS-12-5
Jungauer C. VS-19-6
Jungk H. PS-2B-2
Junker A. PS-2B-10
Just B. VS-19-6, PS-5A-7

K

Kahlenberg F. PS-2B-1, PS-2B-9, PS-4A-3
Kalbitz M. PS-1B-2
Karatas M. PS-4A-11
Kardoeus J. PS-5D-2
Karl A. VS-2-2, PS-4A-2
Karl R. VS-2-3, PS-2B-5
Katalinic A. PL-1-1
Kauke T. PS-5B-2, PS-5B-3
Kelsch R. PS-5B-1
Kerchrom H. VS-19-5
Frank, K. VS-2-4
Kestler H. PS-1B-2
Ketter R. VS-8-6
Kettrup R. PS-2B-3
Kielstein H. PS-3A-11
Kiessig S. VS-5-2, VS-5-5
Kindermann A. PS-3A-11
Kirschhoefer F. VS-10-6, PS-1B-5
Kischnick T. PS-2B-3
Kjaer M. VS-4-7
Kjeldsen-Kragh J. VS-4-7
Klasen G. PS-2A-5
Klerke J. PS-5D-2
Klump H. VS-3-3
Klüter H. VS-3-2, VS-10-3, VS-15-3, VS-19-2, PS-1B-5, PS-2A-4
Knabbe C. VS-5-4, VS-6-3, PS-4A-8
Knels R. PS-5D-2
Knowles L. VS-8-1, VS-8-5, VS-8-6
Kobsar A. PS-2B-8
Koenecke C. VS-16-7
Köhl U. VS-9-1
Kok Y. VS-16-5
König S. PS-5A-6
Koo S. VS-1-3
Kordelas L. PS-2A-3
Körmöczi G. PS-5A-3, PS-5A-4
Kößler A. PS-2B-8
Kößler J. PS-2B-8
Kovacic K. PS-1A-3, PS-5A-3, PS-5A-4
Kramer D. PS-5D-1
Kramm C. VS-16-3
Krammes L. PS-5B-1
Krautwurst A. VS-15-7
Krebs H. PS-4B-5
Kreklau P. PS-5A-1
Kremser M. PS-2B-3
Kronenberg K. PS-2B-7
Kronstein-Wiedemann R. VS-3-3, PS-2B-5, PS-5D-4
Krull M. VS-5-3
Krut O. VS-6-3
Kuehnau W. VS-16-8
Kuhn S. PS-2B-8
Kunz B. PS-1A-4
Kunze S. PS-5B-3
Kurnik K. PS-4B-5
Kurz L. PS-1B-7, PS-1B-8
Kurzhals S. PS-3A-13
Kuvardina O. PS-1A-5

L

Lachmann N. VS-3-5
Lachmann N. VS-13-1, VS-13-3
Lahrberg J. VS-16-6
Laimer M. PS-5C-1
Larscheid G. VS-7-1
Lasitschka F. PS-3A-13
Lausen J. PS-1A-5
Lausen B. VS-19-7
Leise J. VS-16-2
Lejon Crottet S. PS-3A-1, PS-5A-2
Lenz V. PS-2A-3
Lessig D. VS-8-5, VS-8-6
Lindlbauer N. PS-5C-1
Lindner R. VS-1-3
Ljubetic N. PS-2B-3
Lobsiger S. PS-3A-1
Lotfi R. PS-1B-6,
Lüdemann J. PS-1A-6
Lütghehetmann M. PS-4A-1
Lyshy F. VS-10-4

M

Macher S. PS-5A-3
Madla W. PS-2B-2, PS-2B-3
Maecker-Kolhoff B. VS-9-1, VS-16-2, VS-16-6, VS-16-8, VS-16-7
Mangold C. PS-1B-7
Mansouri Taleghani B. PS-5C-3
Marini I. VS-8-3,VS-4-8, VS-8-8, VS-17-3
Marschall R. PS-1A-2
Martens J. VS-9-1, VS-16-2
Matzhold E. PS-5A-4
May C. PS-1A-4
Mayer B. VS-4-6, VS-15-5, PS-3A-14
Mayer G. PS-5C-1
Melzak K. VS-10-6
Menger M. VS-8-1
Merieux Y. PS-5A-8
Metzner K. VS-16-5
Meuer S. PS-3A-13
Meybohm P. PL-3-2
Meyer A. PS-1A-5
Meyer E. VS-16-5
Meyer O. VS-8-9
Meyer S. VS-19-6
Meyersburg D. PS-5C-1
Michel G. VS-1-2
Milanov P. VS-3-3
Miserre L. VS-15-6
Möhnle P. PS-4A-7
Möller A. PS-3A-12
Möller D. PS-2A-4
Monteleone A. PS-2B-13
Moog R. PS-2B-5, PS-5D-4
Moritz M. PS-5A-3
Mrotzek M. PS-2A-5
Mufti N. PS-4A-14
Müller D. VS-15-3
Müller H. PS-5D-2
Müller J. VS-11-2, PS-4B-1, VS-8-2
Müller T. VS-10-7, VS-12-1, VS-12-2, PS-4A-12, PS-4A-13
Müller M. PL-3-2
Müller-Steinhardt M. VS-10-3, PS-2A-4

Münch J. PS-1B-8
Mussolino C. VS-16-5
Mytilineos J. VS-19-3

N

Nagler M. PS-4B-3, PS-5C-3
Najjari L. PS-3A-7
Nerreter T. VS-16-6
Netsch P. PS-1B-5
Niederhauser C. VS-6-4, PS-3A-2, PS-4A-4, PS-5A-2
Niemann M. VS-13-3
Nies C. VS-3-2
Niewald P. VS-8-1
Niklas N. PS-2B-6
Nogués N. VS-19-5
North A. PS-4A-14
Nöth U. PS-1B-6
Nowak-Harnau S. VS-4-5, VS-8-4, VS-8-8, VS-10-4
Nussbaumer W. VS-12-3

O

Oberle D. PS-2A-2
Ochmann O. VS-19-4
Oelke M. VS-16-7
Offergeld R. PL-5-2, VS-6-5, PS-4A-5, PS-4A-6
Offner R. VS-1-3
Oldenburg J. VS-8-2, VS-11-2, PS-4B-1, PS-4B-4
Opitz A. VS-19-4
Oppelt P. PS-4B-2
Origa R. PS-4A-14
Orlemann T. VS-14-5
Ostermann H. PS-1A-9
Otto O. VS-8-7

P

Palankar R. VS-8-7
Pan M. PS-5B-3
Paparella M. PS-3A-13
Pape L. VS-16-7
Pasini E. VS-3-3
Pavlova A. PS-4B-4
Peine S. PS-3A-9, PS-4A-1
Peisl J. PS-4B-2
Pekrul I. PS-4B-5
Peter W. PS-5B-1
Petershofen E. PS-4A-10
Petra B. VS-16-1
Pezeshkpoor B. PS-4B-4
Pfaehler S. VS-1-3
Pfeiffer H. PS-2A-6
Pichler H. PS-1A-7, PS-1A-8
Piel N. VS-4-3
Piga A. PS-4A-14
Pilch J. VS-8-1, VS-8-5, VS-8-6
Pirschtat N. VS-3-3
Placzko S. VS-9-1
Planelles D. PS-5B-1
Platz A. PS-1A-6
Poddar S. VS-16-5
Pogozhykh O. VS-16-9
Pohler P. PS-4A-10, PS-4A-11
Polin H. PS-5A-3, PS-5A-4
Polywka S. PS-4A-1
Porcelijn L. VS-19-5
Portegys J. VS-19-2, VS-19-3
Portmann C. VS-19-6
Posch U. PS-1A-3
Posset M. PS-2B-4
Pötzsch B. VS-8-2, VS-11-2, PS-4B-1, PS-4B-4
Pötzsch K. PS-2A-2
Prax M. VS-6-3
Preuß E. PS-3A-6
Preußel K. PS-4A-5, PS-4A-6
Priesner C. VS-9-1, VS-16-2
Pritzkuleit R. PL-1-1
Pruß A. VS-7-1, VS-7-2, VS-7-3, VS-8-9, VS-13-3, PS-3A-14, PS-5D-2
Pruszak J. PS-2A-1

Q

Quandt D. PS-3A-11
Quandt T. VS-12-5

R

Raab S. PS-1A-6
Rabbi S. PS-5B-1
Rachor J. VS-16-3
Ratcliffe P. VS-19-5
Ravens S. VS-16-7
Reda S. VS-11-2, PS-4B-1
Redli P. VS-12-4
Rehm M. PS-3B-4
Reichenberg S. VS-10-5, PS-4A-12
Reif C. VS-12-3
Reil A. VS-19-5, PS-5A-7
Reimer T. PS-2A-3
Reinhardt F. VS-2-1, VS-2-2, VS-2-3, VS-2-4, PS-4A-2
Reinhardt P. PS-1B-7, PS-1B-8
Renders L. PS-5B-2
Ricard I. PS-1A-9
Rieneck K. VS-19-1, VS-19-7
Ries J. VS-12-4
Riese S. VS-16-6
Riggert J. PS-3B-1
Rigoni F. VS-4-8, VS-17-3
Rink G. VS-15-3, VS-19-2, PS-5A-8
Rizzi G. PS-3A-4
Rohde E. PS-5C-1
Rojewski M. PS-1B-2, PS-1B-6
Romito M. VS-16-5
Rositzka J. VS-16-5
Rosner A. VS-4-2
Rössig C. PL-2-1
Rosskopf K. PS-1A-3, PS-2B-11
Roth W. K. PL-5-1
Rothe R. PS-2B-5
Rox J. PS-5C-2
Ruhaltinger D. PS-2A-2
Rühl H. VS-8-2, VS-11-1, VS-11-2, PS-4B-1
Ruppert-Seipp G. PS-2A-2

S

Sachs U. VS-15-6, VS-15-7, PS-1A-2, PS-3A-12
Saffrich R. VS-3-2
Saint-Cyr M. PS-5A-8
Salama A. VS-4-6, VS-15-5
Salge-Bartels U. PS-2B-10
Santoso S. VS-15-7
Sauer A. PS-1A-6
Sawazki A. VS-15-7
Schäfer M. PS-5B-1
Schäfer R. VS-16-5, PS-1B-9
Scharnagl Y. PS-5D-1
Schawaller M. PS-5A-8
Schenck P. PS-4A-10
Schilbach K. PS-1B-7
Schleef M. PS-4B-5
Schlegel P. VS-16-3
Schlenke P. VS-3-5, PS-1A-3, PS-2B-11
Schloer H. PS-4B-3
Schmauder A. VS-5-4
Schmidt A. PS-1A-6

Schmidt M. VS-2-2, VS-6-2, PS-4A-2, PS-4A-11, PS-5D-1
Schneider L. PS-1A-5
Schneppenheim R. VS-18-1
Schneppenheim S. VS-18-1
Scholz S. PS-5A-6
Schönbacher M. VS-19-5
Schönefeld S. PS-2A-2
Schönemann C. VS-13-3
Schönfeld H. VS-8-9
Schrezenmeier H. VS-19-3, PS-1B-2, PS-1B-4, PS-1B-6, PS-1B-7, PS-1B-8
Schröder F. VS-2-2, PS-4A-2
Schröder-Braunstein J. PS-3A-13
Schroeter B. PS-5D-2
Schroeter J. VS-7-1, VS-7-2, VS-7-3
Schuler P. PS-1B-8
Schultze-Florey R. VS-16-8
Schulz E. VS-7-3
Schulz T. VS-7-2
Schütze K. PS-5D-4
Schwab C. PS-5A-8
Schwab L. VS-1-2
Schwarz E. VS-8-5
Schwarz M. PS-2B-8
Schwind P. VS-4-3, PS-3A-4
Schwinger W. PS-1A-3
Sciesielski L. VS-8-9
Sebauer K. PS-5A-3, PS-5A-4
Seidl C. VS-16-1
Seifried E. PL-3-2, VS-2-2, VS-6-2, VS-16-1, PS-1A-5, PS-1B-9, PS-4A-2, PS-4A-11, PS-4A-14
Seitz R. PS-2B-10
Selleng K. VS-4-1, VS-10-5, VS-14-1
Seltsam A. VS-12-1, VS-12-2, PS-4A-10, PS-4A-11, PS-4A-12, PS-4A-13
Setzer S. VS-3-3
Seyfried T. PS-2B-4
Shvartsman M. VS-3-3
Siciliano D. PS-3A-8
Siebert M. PS-2A-3
Siebert S. VS-10-4
Sipurzynski S. PS-1A-3
Sirgurdadottir S. PS-3A-3
Smith J. VS-16-5
Smith N. PS-1B-8
Soliman S. VS-3-3
Solz H. PS-1B-5
Song Y. PS-3A-3, PS-3A-4, PS-3A-8
Spallek J. PS-3B-3
Spannagl M. PS-4B-5
Spierings E. VS-13-3
Spigarelli M. PS-3A-6
Spohn G. PS-1B-9
Spyrantis A. PS-5D-1
Staeck O. VS-13-3
Stangl M. PS-5B-3
Steil L. VS-10-2
Steinke W. VS-2-1, PS-2B-3
Steitz M. VS-19-4
Stenzel A. PS-5A-1
Stettler J. PS-5A-2
Stögbauer R. PS-3B-2
Stoll H. PS-2A-2
Stolz M. VS-6-5, PS-4A-4
Storch H. VS-5-1
Störmer M. VS-6-1, PS-2A-5
Storry J. VS-19-6
Stracke S. PS-3B-3
Strandenes G. VS-14-3
Strasser E. PS-1A-4
Strathmann K. PS-3A-7
Straub A. VS-8-3, VS-8-4
Streif W. VS-12-3
Strobel J. VS-14-5
Stüpmann K. PS-5D-2
Suck G. PS-2B-2, PS-2B-3
Suessner S. PS-2B-6, PS-2B-12, PS-3A-5
Sümnig A. VS-10-1, VS-10-2

T

Tauber R. VS-8-9
Thiele T. VS-4-1, VS-8-8, VS-10-2
Thierbach J. PS-2B-1, PS-2B-9
Thiessen U. VS-12-5
Tilmann M. VS-2-3, PS-2B-5
Tirilomis T. PS-3B-1
Tischer S. VS-9-1, VS-16-2, VS-16-6, VS-16-7, VS-16-8
Titlestad K. PL-4-2
Tolksdorf F. VS-12-1, VS-12-2, PS-4A-11, PS-4A-13
Tonn T. VS-2-2, VS-3-4, PS-2B-5, PS-4A-2, PS-5D-4
Torreiter P. PS-1A-3, PS-5A-3, PS-5A-4
Trost N. VS-19-6
Trost S. PS-1A-9
Tryankowski R. PS-2B-2
Trzaska T. PS-1B-7, PS-1B-8
Turchiano G. VS-16-5

U

Uhlig S. VS-10-6, PS-1B-5
Ulrich E. VS-5-2
Umhau M. PS-2A-1
Unterberger E. PS-3A-5
Urbschat S. VS-8-6

V

Valek A. VS-12-4
Van Remoortel H. PL-3-2
Vandekerckhove P. PL-3-2
Verboom M. VS-9-1
Vetlesen A. VS-4-7
Vogel A. PS-1B-9
Vogt C. VS-15-4
Völker U. VS-10-2
Vollmer T. VS-6-4, PS-4A-8
Vormfelde S. PS-3B-1

W

Wabnitz G. PS-3A-13
Wagner B. PS-4A-7
Wagner B. PS-2A-3
Wagner F. ZF-3-1, PS-3A-12, PS-5A-5
Wagner T. PS-5A-3, PS-5A-4
Warnecke G. VS-16-9
Wasmund E. VS-4-4
Waubert de Puiseau M. VS-8-9
Weber K. PS-2B-8
Weckmann G. PS-3B-3
Weichert A. VS-15-5
Weidmann C. VS-10-3
Weinstock C. VS-19-3
Wenzel F. PS-2B-13, PS-5C-2
Werle E. VS-4-4
Wesche J. VS-4-1, VS-8-7, VS-10-5
Wessiepe M. PS-3A-7
Westphal A. VS-10-5
Wichmann C. VS-3-3
Widmer N. VS-6-5
Wienzek-Lischka S. VS-15-6, VS-15-7, PS-3A-12
Windisch R. VS-3-3
Winkelmann M. PS-1B-4
Winterhagen F. VS-11-2
Winzen D. PS-3A-7
Witkowski J. PS-4B-1
Witt V. PS-1A-7, PS-1A-8
Witte A. PS-1B-9

Wittmann G. PS-3B-4, PS-4A-7
Wobus A. PS-1A-6
Woessmann W. PS-1B-1
Wolf D. VS-8-2
Wolter C. VS-8-1, VS-8-6
Wuchter P. VS-3-2, PS-1B-5

Y

Yürek S. PS-3A-14
Yuzefovych Y. VS-16-9

Z

Zaehres H. VS-3-5
Zbinden A. VS-12-4
Zeiler T. ZF-2-2, PS-2B-2, PS-2A-3, PS-2B-3
Ziebart J. VS-4-4
Ziegler A. VS-16-6
Zimak C. PS-3B-3
Zimmermann A. PS-2B-1, PS-2B-9, PS-4A-3,
Zimmermann R. VS-14-5, PS-4B-2,
Ziyaie-Hochstrat O. VS-5-3
Zlamal J. VS-4-8, VS-17-3,
Zorbas A. PS-3A-4, PS-3A-8
Zwißler B. PS-3B-4