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

24.–27. Oktober 2017, Köln

ABSTRACTS

Gast-Herausgeberin

Birgit Gathof, Köln
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Vorträge

V01

Immunhämatologie Teil 1

V01-2

Targeted next generation sequencing is a universal tool for non-invasive diagnosis of fetal platelet antigens in fetal and neonatal alloimmune thrombocytopenia

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Background: In pregnant women with a history of fetal and neonatal alloimmune thrombocytopenia (FNAIT), fetal human platelet antigen (HPA) genotyping is required to determine whether the fetus is at risk and whether prenatal interventions to prevent fetal bleeding are required. Methods for non-invasive prenatal diagnosis of fetal HPA antigens have not been published with the exception of fetal HPA-1a.

Methods: Cell-free DNA was isolated from plasma of 21 pregnant women with different gestational age. Polymorphic regions of ITGB3 (HPA-1), ITGA2B (HPA-3), ITGA2 (HPA-5), CD109 (HPA-15), RHD, RHCE, KEL, DARc, SLCA4A1, GYP A, GYPB, SRY, and 14 autosomal SNPs were massively parallel sequenced by means of semiconductor technology (Ion Torrent, life technologies). HPA-genotypes of the pregnant women and newborns were determined using an in-house TaqMan assay to verify the not-invasive prenatal diagnosis.

Results: In all cases, massively parallel sequencing of polymorphic regions coding for common blood group antigens, SRY, and of anonymous SNPs allowed quantification of the fractional fetal DNA concentration. Non-maternal sequences of ITGB3 (HPA-1), ITGA2B (HPA-3), ITGA2 (HPA-5) or CD109 (HPA-15) were correctly determined in all pregnancies with a fraction of cell free fetal DNA that reached the pre-defined cut-off value of 4% (n = 17). All results were verified by confirmatory typing after birth. In four pregnancies, the fetal fraction was below 4% and thus follow-up testing was recommended.

Conclusion: Targeted massively parallel sequencing of plasma cell-free DNA from pregnant woman with a history of FNAIT can avoid invasive methods to determine fetal HPA-genotypes. We propose this method also for non-invasive detection of other fetal blood group polymorphisms that are frequently in hemolytic disease of the newborn.
ent antibody subtypes reacted either with antigenic determinants residing on β3 subunit (1. type), αβ3 complex (2. type) or αvβ3 complex (3. type). Interestingly, only the 3. type of anti-HPA-1a antibody could induce endothelial dysfunction by induction of cell death (termed anokisia) and by inhibiting angiogenesis process when compared to other two subtypes. Hence, significant amount of this anti-HPA-1a type was detected in severe FNAIT cases with intracranial bleeding (ICH) (Santoso et al, 2016). Based on this observation, specific identification of the 3. type of anti-HPA-1a is mandatory to predict the development of ICH in fetuses with severe FNAIT. However, the specific identification of this antibody type is hampered by the presence of the 1. type of anti-HPA-1a antibody in the most FNAIT sera that can also react with endothelial cells.

In this study, we established a rapid method for the depletion of the 1. type (anti-β3 antibody) using a novel recombinant biotinylated β3 monomer (αβ3) protein produced in HEK293 transfected cells. Two well-characterized murine moabs (clones AP3 and 23C6) react with β3 subunit (representing the 1. type) or with αvβ3 complex (representing the 3. type), respectively, were selected to evaluate the specificity of our test approach. In addition, human moab against HPA-1a (clone 26.4) was tested. After incubation with χ3, antibodies bound to χ3 protein were removed using streptavidin-coupled magnetic beads. Subsequently the supernatants were tested by flow cytometry using endothelial cell line (EaHy hy926) as target. Significant high depletion (>86%) of moab AP3 was observed. In contrast, no significant absorption (<5%) of moab 23C6 was detected. Interestingly, moab 26.4 could be completely absorbed by αβ3 indicating that this anti-HPA-1a moab does not recognize compound epitopes formed by αvβ3 heterodimer, but reacts solely with epitopes residing on β3 subunit. This result could be confirmed by the use of χ3 transfected HEK293 cells. By flow cytometry, moab 26.4 showed positive reaction was with χ3, but not with αvβ3 expressing cells. Furthermore, antigen capture assay was performed using EaHy hy926 cells and moab AP3 as capture antibody to analyze the capability of χ3 to deplete human moab 26.4. In accordance to our flow cytometry result, significant high absorption of moab 26.4 with αβ3 was observed.

Taking together, our results indicate that recombinant αβ3 monomer is suitable tool to separate anti-β3 and anti-αvβ3 subtype of HPA-1a antibodies. By this approach, the identification of different anti-HPA-1a subtypes in sera of FNAIT cases is anticipated.

V02-2
Prospective study on the efficacy of different tools to implement patient blood management

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Introduction: Implementation of patient blood management (PBM) is one goal of the World health organization (WHO). We defined PBM as avoidance of unnecessary red blood cell (RBC) transfusions in in-hospital patients based on current evidence and guidelines.

Methods: We implemented PBM at the University Medicine Greifswald in a three phase prospective study with 4 evaluation periods of 3 months each: phase (I) (07, 08, 09–2014); baseline, before PBM implementation, without any intervention; phase (II) (07, 08, 09–2015): after systematic education and training of all physicians and all departments; phase (III) (01, 02, 03–2016):after additional change of the pretransfusion ordering form with request of the pretransfusion haemoglobin (Hb)-value and colour visualized recommended Hb-transfusion triggers; phase (IV) (01, 02, 03–2017): poststudy stabilization phase: quarterly feedback of pretransfusion Hb triggers by the transfusion medicine department and request of a written statement from the departments transfusion officer (Transfusionsbeauftragter) for justification of all transfusions with pretransfusion Hb >10 g/dl. Departments with < 20 RBC transfusions per month and paediatrics were excluded. Resources: For the implementation phase (I–III) 0.5 study nurse for 12 month, 0.5 physician for 18 months, and 0.2 medical documentation officer for 18 months were needed. Since the end of phase III, 1 person working day/month is required for maintenance of the project. Primary endpoint was the change in pretransfusion Hb-values; secondary endpoint was the change in transfused RBCs; safety endpoints were the in-hospital documented adverse events acute coronary syndromes, mortality and mean in-hospital stay of all patients.

Results: During the study periods 6112 (I), 6090 (II) and 6100 (III) patients were treated. Median pretransfusion Hb-values decreased from 7.7 g/dl (I), over 7.4 g/dl (II), to 7.3 g/dl (III) and stabilized at 7.3 g/dl (IV).
Number of transfused RBCs decreased from 3007 (I) to 2834 (II), 2103 (III), 2092 (IV) (I vs. III; p < 0.001). Decrease of mean pretransfusion Hb-values and reduction of RBC transfusions were observed in medical as well as in surgical patients. Between study period (I) and (III) no increase in acute coronary syndromes or in mortality occurred (p > 0.3). The effects obtained until study period (III) were maintained during the stabilization period (IV).

Conclusion: By the combination of systematic education, change of the pretransfusion ordering form, and regular feedback of pretransfusion Hb-values, RBC transfusion demand decreased by 30% both in medical and in surgical patients, without an increase of severe adverse events.

V02-3 Analysis of the red blood cell transfusion demand over 10 years in a German federal state

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Introduction: More than 60% of red blood cell concentrates are transfused to patients >60 years. Despite a population increase of this age group due to the demographic change, the transfusion demand decreased during a 10-years prospective study. Here we describe the changes in transfusion demand depending on characteristics of patients and hospitals.

Material and Methods: Age, gender, and patient classification of each patient were obtained for all transfused red blood cell concentrates (RBCs) in all hospitals in Mecklenburg-Western Pomerania in the years 2005, 2010, and 2015. The hospitals were categorized by the number of beds in 2015 in small (<400), medium size (400–< 700), and large hospitals (≥700 beds).

Results: Over the 10-years study period RBC demand decreased by 12,864 RBCs (~13.5%), although the population in the age group >60 years increased by 15.7%. In small hospitals 5,351 less RBCs (~17.7%) were transfused, in medium size hospitals 2,595 RBCs (~18.6%), and in large hospitals 4,918 RBCs (~9.6%). In all hospitals transfusion demand in surgical patients decreased, while it increased for medical patients in medium size hospitals (~10.4%) and for critically-ill patients in large hospitals (~9.8%) (Fig.1). The reduction in transfusion demand was not distributed equally between age groups and sex. 60% of the reduction in transfusion demand was achieved in patients over 60 years, both when measured as absolute reduction (~7,658 RBCs, –11.2%) or relative reduction per 1,000 population in the respective age group (~36.6/1,000 population, ~23.3%). The reduction in the absolute number of transfused RBCs was much more pronounced in females compared to males in surgical (~25.8% vs. –9.2%) and in medical patients, without an increase of severe adverse events.

Conclusion: The reduction in transfusion demand differs by size of hospitals, patient category, patients’ age groups, and gender of transfused patients. Any model for projection of the transfusion demand has to include these parameters and monitoring of the transfusion demand should not be restricted to large hospitals.

V02-4 Effects of red blood cell transfusion in patients with surgical intervention for pancreatic cancer

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Introduction: It is not clear whether red blood cell transfusions (RBC-Tx) have a direct detrimental effect on surgical transfusion recipients or whether RBC-Tx act as an indicator revealing clinical conditions for bad outcome. We retrospectively investigated outcome in 499 patients with and without RBC needs who had undergone surgery for pancreatic cancer (PC) at Hannover Medical School from 2000–2015.

Materials and Methods: 350 out of 499 PC patients (70%) received a median of 3 (1–94) RBC-Tx. The other 149 PC patients (30%) did not require any RBCs. Both groups were analyzed in depth for 93 clinical parameters including demography (n = 10), pre-surgical antiagulation treatment (4), blood counts (3), blood products used (7), disease progression and tumor extent (25), surgical intervention (11), outcome (15) and others (18). Statistical evaluation comprehended x2 and Wilcoxon Rank sum test for comparison of variables between the two groups and univariate logistic and Cox regression analyses for evaluation of factors contributing to RBC-Tx and outcome.

Results (medians): Patients with RBC-Tx had a longer stay in ICU postoperatively (2 vs 1 days; p ≤ 0.001) as well as in hospital (25 vs 21 days; p ≤ 0.001), a higher hospital mortality (8% vs 3%; p ≤ 0.03), and lower long-term survival (18.6 vs 25.0 months; p ≤ 0.001) and overall survival rates (17% vs 26%; p ≤ 0.02). For clinical factors with significant influence on RBC-Tx and survival in univariate regression analyses, see table.

Conclusion: Periampullary carcinoma, usually earlier detected than other types of PC, symptoms of tumor progression and difficult tumor sites resulting in insufficient tumor resection and revision surgery (for repeated tumor resection as well as for hemorrhage) significantly influenced the need for RBC-Tx and outcome.

<table>
<thead>
<tr>
<th>Tab. 1, RBC-Tx and Outcome in PC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Pamp CA</td>
<td>0.60 (0.38-0.96)</td>
</tr>
<tr>
<td>Vase infiit</td>
<td>2.36 (1.39-4.18)</td>
</tr>
<tr>
<td>Utrate pos</td>
<td>1.16 (1.04-1.29)</td>
</tr>
<tr>
<td>R1 reset</td>
<td>3.26 (1.35-7.84)</td>
</tr>
<tr>
<td>Reves res</td>
<td>1.73 (1.15-2.54)</td>
</tr>
</tbody>
</table>

Legend: Pamp (preoperative antiagulation), Vase infiit (vascular infarction), Utrate pos (urea rate postoperatively), R1 (first readmission), Reves res (revised readmission).

V02-3 Analysis of the red blood cell transfusion demand over 10 years in a German federal state

V02-4 Effects of red blood cell transfusion in patients with surgical intervention for pancreatic cancer

Fig. 1. Absolute number of transfused RBCs by hospital category depending on the number of beds.
Implementation of the electronic documentation of prescription and application of blood products in a university hospital

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Introduction: A number of advantages is associated with computerized physician order entry: patients and clinicians benefit from faster available electronic results. Errors are reduced by clearly legible orders and signatures, documentation quality improves, and case-specific billing options arise. Here, we share our experiences.

Methods: Our hospital information system is ORBIS. The laboratory information management system of our blood bank is PC-Blut/PC-LIMS. The intensive care units use ICM for clinical documentation. For defining the specific needs, over a hundred discussions with physicians, nurses, management experts, and with several software corporations were carried out and documented. Programing and testing were followed by extensive user training and tutoring.

Results: The electronic documentation system was introduced in two steps. Step 1 (order entry) was implemented gradually over several months to ensure that users become acquainted with it. An alternative (paper) option still remains available for emergencies. Electronic orders are prepared by nurses and signed by a physician in ORBIS. The orders are available in PC-LIMS immediately thereafter. If no laboratory testing is needed, the prescribed blood products such as clotting factor concentrates will be delivered immediately. If lab testing is required, the sample tube has to be accompanied by an order printout with a handwritten signature of the person who collected the blood sample. Results on blood groups and other tests – except crossmatches – are exported to ORBIS after validation. They are permanently stored in the patient’s case history.

Conclusion: Patient safety and documentation quality can be improved by an electronic order entry and transfusion documentation system. Enough man power is needed for planning, training, and tutoring.

V02-2

Large-scale production of induced pluripotent stem cells-derived megakaryocytes in bioreactors

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Introduction: Platelet (PLT) transfusion is crucial to prevent severe bleeding complications in thrombocytopenic patients due to haematological diseases or intensive disease treatment. PLT transfusion is limited by the lack of donors, short shelf life of PLTs and the risk of contaminations. Here, in vitro produced Megakaryocytes (MKs) and Platelets (PLTs) represent an alternative to PLT donation. While several attempts were performed to upscale the production of PLTs from CD34+ cell-derived MKs, so far less efforts have been made to upscale MK production. Hence, this...
study aimed at the establishment of the large-scale differentiation of MKs from induced pluripotent stem cells (iPSCs) using stirred bioreactors.

Methods: iPSCs were seeded in 50 ml spinner flasks to form aggregates under agitation. Aggregates were differentiated into MKs using TPO under xeno-free conditions for a period of 22 days. iPSC-derived MKs were analysed for DNA content, phenotype, PLT production in vitro using flow cytometry and fluorescence microscopy. Their capacity of producing PLTs was investigated in vivo using a mouse model. Here, non-irradiated or irradiated MKs were transfused to NOD/SCID/IL-2Rγc−/− mice and blood was drawn and analysed for human PLTs.

Results: Differentiation rates of MKs harvested from the stirred bioreactor increased throughout the differentiation until day 22 to 41.9 ± 11.5% CD41+CD42a+CD61+ and 24.8 ± 9.88% CD41+42b+ cells. During the next 3–4 days of further cultivation the harvested cells increased the marker expression to 62.3 ± 4.9% and 39.98 ± 9.8%, respectively. Means of 48.7 ± 12.9 x10⁶ MKs per 100 ml were achieved in the bioreactor. The harvested MKs show to be polyclonal and 35.0 ± 16.2% of the MKs exhibit a DNA content ≤8n. Both non-treated and irradiated MKs showed proPLT formation and the produced PLTs were able to adhere to fibrinogen-coated surfaces. Human PLTs were detectable in murine blood 1 h after transfusion of irradiated or non-treated iPSC-derived MKs at similar frequencies suggesting that irradiation do not affect the capacity of MKs to produce PLTs in the mouse circulation.

Conclusion: We present a time and cost-efficient method for the large-scale production of iPSC-derived MKs under xeno-free conditions. In vitro generated MKs might be either used for large-scale in vitro production of PLTs or may be considered for direct transfusion to treat thrombocyto-penia.

V03-3

Droplet digital™ PCR to monitor platelet engraftment after stem cell transplantation

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Introduction: Reliable and sensitive detection of platelet engraftment after haematopoietic stem cell transplantation is essential for optimization of both transplantation regimen and platelet support.

Methods: Patients (n = 25) with haemato-oncological disorders were included in the feasibility study to compare the time to engraftment based on EBMT definition (no transfusion for 3 days and platelet >20 × 10⁹/L) to the detection of ≥10⁹ x 10⁹ PLTs/L derived from the transplant. Mitochondrial markers were genotyped in samples from the patient, stem cell- and platelet donors. After transplantation, each patient was exclusively treated with platelet units from donors selected on the basis of their mitochondrial markers to share identical markers with the pre-transplantation patient and 2) to provide at least one mismatch with the stem cell donor. For quantification of platelets, daily blood samples from the patients were collected on days 7–20 after transplantation. Platelet rich plasma was collected on days 7–20 after transplantation. Platelet rich plasma was prepared from 3 mL EDTA blood and diluted to approximately 5×10⁶ PLTs/ml, for automated extraction of mitochondrial DNA (Magna Pure compact®, Roche, Germany). Droplet digital PCR (ddPCR) was performed using the QX200 system (Bio-Rad, Germany). Amplification of sequences from blood group gene RHCE followed each extraction to exclude contamination by DNA from leucocytes.

Results: In 7/25 cases at least 2 markers were available to quantify transplant-derived platelets. According to the EBMT definition, 7 patients had no platelet engraftment until day 20 and the other patients (n = 18) required a median of 16 (13–20) days for engraftment. Engraftment based on ddPCR was achieved after a median of 12 days (8–17; n = 24), 1 patient had no engraftment until day 20. The mean difference between EBMT vs ddPCR time to engraftment was 5 ± 4 days (n = 18).

Conclusion: The simplicity of the ddPCR allows to complete the quantification of the different platelet populations within three hours. Our results demonstrate adequate reliability and sensitivity of ddPCR for direct monitoring of platelet engraftment after stem cell transplantation. Platelet engraftment was detected by this approach 3–5 days earlier than by conventional blood cell counting.

V03-4

Excellent response, low TRM and good survival in patients with therapy-refractory aGVHD after treatment with potency-defined doses of MSCs generated from a serum-free MSC-bank of pooled BM-MNCs from multiple healthy donors

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Introduction: For almost 20 years, individual cases and small series have suggested beneficial effects of mesenchymal stroma cells (MSC) on the course of acute graft-versus-host disease (aGVHD). However, several recent trials or case series, including the commercial MSC product Prochymal and reports from the Finnish, Brazilian and Dutch groups failed to demonstrate any therapeutic effect whatsoever. Meanwhile, we generated a novel, proprietary off-the-shelf MSC product with high in-vitro suppressive capacity and maximal inter-batch consistency (Kuci et al. Haematologica 2016). A «hospital exemption» issued by the Paul-Ehrlich-Institut (A.11748.01.1) licenses routine clinical use of MSC-FFM for steroid refractory aGVHD.

Patients and Methods: Using these standardized MSC products 67 patients were treated between 12/2014 and 12/2016. One third were female, transplant was for leukemia (73%) or non-malignant diseases. Median age was 10 (range: 0.5–55) years. The cohort contains all possible donor-patient constellations and all stem cell sources. aGVHD was mostly severe, with grade III (40%) or IV (54%). Patients who had failed up to seven lines of immune suppressive drugs received at weekly intervals up to four infusions of 1–2 × 10⁶/kg MSC-FFM. Response was defined as complete (CR, complete resolution of all signs of GvHD), partial (PR, GvHD reduction by at least one grade according to the Glucksberg criteria), or none (NR) at day 28 after first MSC transfusion.

Results: By day +28, 32% of patients had achieved CR, 49% PR (overall response = 81%), with 15.5% NR, and 3.5% no data available. At the last follow up of GvHD, 54% patients were in CR, 30% in PR, 15% in NR (1% n.a.). The predicted one-year non-relapse and relapse mortality rates were 28 ± 2% and 2 ± 2%, respectively, for a one-year overall survival rate (OS) of 70 ± 6%. Patients with «simple» steroid-refractory vs. therapy-resistant aGVHD had an OS probability at one year of 76 ± 13% and 68 ± 7%, respectively, thus dramatically in excess of expected for patients with such severe aGVHD. Clinical response did not differ between children (<16y, n = 47) and adolescents/adults (>16 years, n = 20), although overall survival in children trended towards higher values.

Conclusion: Treatment with the unique MSC product MSC-FFM offers children and adults an excellent chance to overcome treatment resistant and steroid refractory acute GvHD. A pivotal multi-national phase III trial is underway to support a European marketing authorization application.
Fibrin supports a stem cell phenotype in glioma

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Abstracts

Introduction: High-grade gliomas are brain tumors that are characterized by diffuse growth and resistance to most if not all therapies. Typically, these aggressive tumors possess high angiogenic activity, which causes vascular leakage and fibrin-rich edema. The aim of this study is to determine if fibrin preserves the proliferative and invasive capacity of glioma stem cells.

Methods: Glioblastoma cell lines U87MG, U373MG and U343MG where embedded in a 3-dimensional matrix of clotted plasma, fibrin or matrigel™ and scored for proliferation as well as invasion using phase contrast microscopy. To define adhesive interaction, tumor cells were transfected with siRNA against fibronectin and integrins. Stem cell properties were investigated by maintaining suspended or clot-embedded tumor cells in Neurocult stem cell media. Primary tumor cells freshly isolated from patients with high-grade glioma were used to substantiate the main findings.

Results: To study glioma invasion, we embedded 3 different glioblastoma cell lines with varying degrees of invasiveness in clotted plasma, fibrin and matrigel™. The two most invasive glioblastoma cell line, U87MG and U373MG, expressed high amounts of fibronectin and integrin β3 while the poorly invasive U343MG cells only expressed low levels of these adhesion factors. Knocking down either of the two proteins, fibronectin or integrin β3, significantly reduced invasion of U87MG cells and effectively halted their proliferation in 3D-fibrin. Knocking down fibronectin also diminished the capacity of U87MG cells to generate neurospheres suggesting that the interaction between fibronectin and integrin β3 is relevant for the maintenance of glioma stem cells. Moreover, glioma stem cells grew significantly better in fibrin than in matrigel™ indicating that clotting generates a permissive extracellular matrix for tumor-initiating glioma cells. This preference of fibrin over matrigel™ was reiterated by primary tumor cells from patients with high grade glioma, which at the same time strongly overexpressed fibronectin.

Conclusion: Our data show that fibrin, which is present in the edema of the tumor extracellular matrix, strongly promotes invasion as well as colonization of high-grade glioma. Mechanistically, we found that this process is promoted by the interaction of fibronectin with integrin β3, which appear to support a cancer stem-like subpopulation of glioma cells.

A new FUT1*c.787insA mutation induces the Bombay (O,) phenotype

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Background: The A-, B-, H- phenotype on red blood cells (RBC) is caused by mutations of the FUT1 gene that can abolish the alpha 1,2 fucosyltransferase activity. The Bombay (Oh) phenotype is characterized by missing H-substance on RBC and soluble H-substance within saliva and plasma and requires further mutations of the FUT2 gene, which regulates the secretor function. This very rare phenotype has been first described in individuals from Southern Asia, but also cases from other regions have been reported. We describe the Bombay phenotype in a woman of Turkish origin, living in Southern Germany whose blood had been submitted for testing by a fertility center because of abnormal immunohaematological results. The serological findings were typical for a Bombay blood group, with an A, B and H negative phenotype, strongly reactive A, B and O cells in the reverse grouping, strong reactivity with each panel cell in antibody screening and differentiation, and a negative DAT. The molecular basis of the Bombay (Oh) phenotype of the patient should be determined by DNA sequencing.
Methods: The coding regions of both FUT1 and FUT2 genes including short flanking sequences of the untranslated regions were amplified in a gene specific manner by use of published primer sequences. 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. Determined DNA sequences were aligned to published reference sequences.

Results: DNA sequencing of the patient’s sample demonstrated a new FUT1 c.787insA mutation which induces a frame shift and a premature stop at codon 269 instead of codon 365. The mutated gene induced a loss of function of the fucosyltransferase with the consequence that no H substance could be synthesized on the patient’s red blood cells. Furthermore, a number of already known mutations of the FUT2 gene induced the same genotype and thus prevented secretion of H into the plasma: 171A > G (silent), 216C > T (silent), 428G > A (Ttpr13Stop), 739G > A, 960A > G. The combination of mutations of both genes induced the Bombay (Oh) phenotype of the patient. The new FUT1*787insA mutation was submitted to GenBank under the accession number KY593920.

Conclusions: The new FUT1 c.787insA mutation in combination with a FUT2 c.428G>T mutation induced the Bombay (Oh) phenotype in a patient of Turkish origin. As this is our 5th case of the Bombay (Oh) phenotype within 3 years in patients or donors of non-middle European origin we suppose that blood groups which are assumed as very rare may be observed more frequently by a big blood service than hitherto suspected.

V04-4
Knock-out of multiple classical HLA class II beta chains by one-shot CRISPR-Cas9 mediated genome editing
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The class II Human Leukocyte Antigens (HLA-II) HLA-DR, DQ and DP are heterodimers formed by the association of polymorphic alpha and beta chains co-expressed on a variety of immune cell subsets as well as certain tumors. The possibility of modifying any cell type of interest to express only a single HLA-II antigen is desirable for addressing many questions in immunology and transplantation and could be of potential clinical use in cell therapy. Here we have used CRISPR-Cas9 genome editing to comprehensively knock-out HLA-II beta genes in the B cell line MGAR (HLA-DRB1*15:01, DRB5*01:01, DQB1*06:02, DPB1*04:01). In order to target the different genes simultaneously, we designed a single guide RNA spanning a conserved region in exon 2 of the HLA-II beta genes. In-silico analysis suggested that neither HLA-II alpha chains nor the processing machinery components DM and DO were targeted. After lentiviral vector transduction encoding the sgRNA, Cas9 and GFP, MGAR cells were sorted for GFP+/HLA-DR- expression and found to be negative also for HLA-DQ and DP, with median fluorescence intensity identical to the isotype control, while expression of HLA class I remained at wild type (WT) levels. HLA-DP expression could be rescued to WT levels by electroporation of the mRNA encoding HLA-DPB1*04:01. This fully restored recognition by HLA-DPB1*04:01 specific alloreactive T cells in CD137 upregulation assays (40.3%, 11.4%, 43.4% T cells responding to MGAR WT, sgRNA-edited and rescued, respectively). Our results show that CRISPR-Cas9 mediated knock-out of multiple HLA-II beta genes is feasible in BLCL and that expression can be rescued functionally by transfection of a full-length HLA-DP beta chain. This is a potentially attractive new tool for the flexible generation of HLA-II knock-out cells with a variety of possible experimental and clinical applications.

V04-5
Human neutrophil antigen-1c (HNA-1c): a more effective FcgRIIIb receptor on neutrophils?
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The FcgRIIIb (CD16) is a low-affinity receptor of IgG, which is exclusively expressed on neutrophils and is essential in many effectors functions of immune system including phagocytosis, antibody-dependent cellular cytotoxicity (ADCC) and release of inflammatory mediators. Currently, four FcgRIIIb allotypes are found carrying HNA-1a, -1b, -1c and -1d alloantigenic determinants. Population studies showed high prevalence of HNA-1c among African (23–31%) when compared to Caucasian (around 5%) and Asian (around 1%) populations. Molecular biological analysis showed that amino acid change at position 78 (Ala>Asp) is responsible for the formation of HNA-1c. However, little is known about the exact function of HNA-1c allform.

In this study, we developed stable transfected FcgRIIIb cell lines expressing HNA-1a, -1b and -1c and soluble recombinant HNA-1a, -1b and -1c proteins to dissect the function of HNA-1c allform. Our results showed that HEK293 cells expressing HNA-1c exhibited higher affinity toward IgG when compared to HNA-1a and HNA-1b expressing cells. This interaction was blocked by inhibitory moab 3B8, but not by non-inhibitory moab DJ130c against FcgRIIIb. Similar results were obtained by solid phase ELISA using soluble recombinant HNA-1 proteins. Furthermore, real-time analysis of protein-protein interaction by Surface Plasmon Resonance (SPR) showed higher binding affinity of HNA-1c toward IgG in comparison to HNA-1a and HNA-1b allforms (KD 7.24E-05M versus 1.15E-05M). Analysis of the 3D-structure showed the unique position of the amino acid change at position 78 and thus the unique function of HNA-1c.

In summary, we showed that HNA-1c alloform of FcgRIIIb exhibits high binding affinity to IgG when compared to HNA-1a and -1b allforms. Consequently, antibody bound to HNA-1c alloform can cause high emission of ROS in neutrophils; a mechanism, which may responsible for the effective clearance of malaria-infected erythrocytes. The question whether the HNA-1c alloform represents genetic risk/protective factor in other diseases is intriguing.
Abstracts

V05
Hämostaseologie Teil 1

V05-1
Imbalance between wound healing and inflammation in hemophilia
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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 isolated from hemophilia patients and healthy individuals were treated either with M-CSF or GM-CSF and probed for morphological features of macrophage differentiation using phase contrast and fluorescence microscopy. Wound infiltration of macrophages was determined by probing the formation of invasive podosomes in clotted plasma. The capacity of macrophages to phagocytose was assessed by measuring the uptake of fluorescent-labeled latex beads and red blood cells. Expression of CSF1-R, GM-CSF-R, CCL2, CD11a, CD11b and Tie2 was measured by flow cytometry. In addition, we assessed expression of the hemoglobin receptor CD163 by flow cytometry and fluorescence microscopy.

Results: Morphological analysis of macrophages from hemophilia patients revealed a significant defect in cell polarization and filopodia formation, which ultimately led to an impairment of regenerative macrophage functions such as clot infiltration and red blood cell phagocytosis. These functional deficits could at least be partially explained by a reduced expression of the receptor tyrosine kinase Tie2 on hemophilia monocytes. Expression of CSF1-R, GM-CSF-R, CCL2, CD11a, and CD11b on the other hand was largely maintained. In addition to Tie2, hemophilia monocytes also failed to express the hemoglobin-receptor CD163, which is known to be a critical mediator of regenerative macrophage functions after blood-injured. Significantly, CD163 expression was recovered when we embryed hemophilia monocytes in clotted plasma from healthy individuals suggesting that a reconstituted clotting function can contribute to a normalization of macrophage function in hemophilia.

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 functional deficits correlate with reduced expression of the receptor tyrosine kinase Tie2 on hemophilia monocytes and the hemoglobin receptor CD163 on hemophilia macrophages. Given the central role of red blood cells and their heme components in promoting hemophilic arthropathy, we speculate that these changes are associated with increased joint inflammation and that a reconstituted clotting system can contribute to a normalization of the macrophage-dependent wound healing response following blood-induced joint injury.

V05-2
In-vivo assessment of the anticoagulant countermeasure in asymptomatic factor V Leiden carriers by monitoring of rFVIIa-induced generation of activated protein C (APC)
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Introduction: Only a minority of patients with spontaneous venous thromboembolism (VTE) exhibit laboratory risk factors. Monitoring a standardized and limited activation of coagulation in patients without established risk factors might help to identify prothrombotic phenotypes in VTE. Promising biomarkers to monitor subclinical changes of the hemostatic activation status are plasma levels of active enzymes including thrombin and APC. Aim of this study was to characterize the prothrombotic phenotype in factor V Leiden carriers (FVL) as a prerequisite for the identification of novel prothrombotic mechanisms.

Methods: Subclinical activation of coagulation was induced by i.v. injection of recombinant activated factor VII (rFVIIa, 15 µg/kg) into 12 asymptomatic FVL (10 heterozygous, het.) and 12 healthy wild type carriers (WT), and monitored by repeatedly measuring plasma levels of thrombin and APC during an 8 h lasting follow-up period using oligonucleotide-based enzyme capture assays (OECA). In addition, prothrombin fragment F1+2 (F1+2), thrombin-antithrombin complex, plasmin-a2-antiplasmin complex, soluble fibrin monomer, and D-dimer were determined.

Results: Administration of rFVIIa was well tolerated, and its pharmacokinetics showed an expected course in all subjects. At baseline, median APC plasma levels were 92 (IQR: 71–120) pg/ml in het. FVL and 35 (28–67) pg/ml in WT. Following rFVIIa injection, a significant (p < 0.05) elevation of APC levels from baseline was observed between t = 0.5–5h in both cohorts. Peaks of 604 (445–743) pg/ml (p = 2 × 10–4) in het. FVL and 164 (139–194) pg/ml (p = 3 × 10–4) in WT were observed at t=1h. In addition to higher peaks (p = 4 × 10–5) in het. FVL than in WT, the area under the APC generation curve (AUC) was greater (p = 3 × 10–6) with 2619 (2143–2976) vs. 770 (630–968) pg/ml x h. Interestingly, the AUC of both homozygous FVL (2819 and 3394 pg/ml x h) lay within the range of het. FVL. Among the other biomarkers, only F1+2 showed an increase from baseline (177, 104–197 pmol/l in het. FVL; 125, 87–188 pmol/l in WT) that became statistically significant (p = 0.03, p = 0.04) at t = 2h (205, 172–242 pmol/l; 179, 111–200 pmol/l). All other biomarkers remained unchanged, and no statistically significant differences were observed between FVL and WT.

Conclusion: Increased thrombin generation rates are counterbalanced by increased APC generation rates in clinically asymptomatic FVL after subclinical activation of coagulation. Moreover, the data presented demonstrate that subclinical activation of coagulation by rFVIIa combined with serial APC testing is a new diagnostic tool to measure the functionality of the anticoagulant APC pathway in vivo.
Updated results from a dose-escalation study in adults with severe or moderate-severe hemophilia B treated with AMT-060 (AAV5-hFIX) gene therapy: up to 1.5 years follow-up

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Background: Gene transfer for hemophilia offers the potential to convert the disease from a severe to a mild phenotype with a single treatment. AMT-060 consists of an AAV5 vector containing a codon-optimized wild-type hFIX gene under control of a liver-specific promoter.

Aim: This phase 1/2 study investigates the safety and efficacy of AMT-060 at 2 dose levels in adults with moderate-severe or severe hemophilia B.

Methods: Multi-national, open-label, dose-escalating study in patients (pts) with factor IX (FIX) activity ≤2% of normal, and a severe bleeding phenotype (prophylactic exogenous FIX; or on-demand exogenous FIX, plus ≥4 bleeds/year or hemophilic arthropathy). Pts received either 5 × 1012 gc/kg (n = 5) or 2 × 1013 gc/kg (n = 5) of AMT-060 iv. Efficacy assessments include endogenous FIX activity (measured ≥10 days after use of exogenous FIX); reduction of exogenous FIX use; and annualized spontaneous bleeding rates. Safety assessments include treatment related adverse events, immunological and inflammatory biomarkers.

Results: There were no screening failures due to AAV5 antibodies. Mean FIX activity in the lower dose cohort was 5.2% (min-max: 3.0–6.8%; n = 4; 1 patient remaining on prophylaxis excluded) during 1 year of follow-up, and 6.9% (min-max: 3.1–12.7%; n = 5) in the higher dose cohort during 26 weeks follow-up. Eight of 9 pts on FIX prophylaxis discontinued use after AMT-060 infusion. Follow-up to 1.5 years will be presented, with annualized reduction of exogenous FIX use and spontaneous bleeding rates. Mild, temporary elevations in ALT were observed in 3 pts (pts) with factor IX (FIX) activity ≤2% of normal, and a severe bleed phenotype (prophylactic exogenous FIX; or on-demand exogenous FIX, plus ≥4 bleeds/year or hemophilic arthropathy). Pts received either 5 × 1012 gc/kg (n = 5) or 2 × 1013 gc/kg (n = 5) of AMT-060 iv. Efficacy assessments include endogenous FIX activity (measured ≥10 days after use of exogenous FIX); reduction of exogenous FIX use; and annualized spontaneous bleeding rates. Safety assessments include treatment related adverse events, immunological and inflammatory biomarkers.

Conclusions: Patients continue to show sustained clinical benefit and endogenous FIX activity with no T-cell activation ≥1 year after a single infusion of AMT-060.

Antithrombin activity is overestimated in patients under rivaroxaban and apixaban when measured using a Factor Xa-based assay

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Introduction: Congenital antithrombin (AT) deficiency is associated with a high risk for thromboembolic disease. AT activity is therefore routinely determined in clinical settings concerning coagulation disorders, often in patients under anticoagulant (AC) medication. Functionally active AT can be quantified by chromogenic substrate assays utilizing the inhibition rates of thrombin or activated factor X (FXa). Several studies have found AT activity assays to be influenced by AC drugs, but this has not been evaluated in a real-world scenario so far. The aim of our study was to investigate the effects of FXa-inhibiting drugs on two commonly used AT activity assays, either based on inhibition of thrombin or FXa.

Methods: The study population comprised 646 consecutive outpatients (412 females) with a mean age (range) of 43 (1–89) years, who were referred to our centre between 1st January and 31st March 2017. Patients taking edoxaban (n = 15), dabigatran (n = 2), or receiving fondaparinux (n = 8) were excluded due to the small size of these subgroups. Patients with mutations in the SERPINC1 gene (n = 13) were not included in the further analysis. 714 samples were available for statistical analysis. 159 patients were under rivaroxaban (n = 87) or apixaban (n = 72), a total of 76 patients received low molecular weight heparin (LMWH). AT activity was measured by two commercially available assays based on thrombin inhibition (Berichrome AT III, Siemens Healthcare, Germany) or FXa inhibition (Innovance Antithrombin, Siemens Healthcare). AC drug levels were quantified by anti-FXa testing using AC specific calibrators.

Results: AT activities measured by the thrombin-based assay (ATFIIa) and the FXa-based assay (ATFXa) were 98.4 ± 11.5% (mean ± SD) and 106.5 ± 14.0%, respectively. Linear regression analysis demonstrated coefficients of determination between ATFIIa and ATFXa of R² = 0.76 for patients taking edoxaban (n = 8) were excluded due to the small size of these subgroups. Patients with mutations in the SERPINC1 gene (n = 13) were not included in the further analysis. 714 samples were available for statistical analysis. 159 patients were under rivaroxaban (n = 87) or apixaban (n = 72), a total of 76 patients received low molecular weight heparin (LMWH). AT activity was measured by two commercially available assays based on thrombin inhibition (Berichrome AT III, Siemens Healthcare, Germany) or FXa inhibition (Innovance Antithrombin, Siemens Healthcare). AC drug levels were quantified by anti-FXa testing using AC specific calibrators.

Results: AT activities measured by the thrombin-based assay (ATFIIa) and the FXa-based assay (ATFXa) were 98.4 ± 11.5% (mean ± SD) and 106.5 ± 14.0%, respectively. Linear regression analysis demonstrated coefficients of determination between ATFIIa and ATFXa of R² = 0.76 for patients without FXa inhibitors or heparin, R² = 0.28 for patients under rivaroxaban, R² = 0.54 for patients under apixaban, and R² = 0.79 for
patients under LMWH. Bland-Altman analysis revealed limits of agreement of +6.6/%15.8%, +8.9/%51.1%, +3.8/%42.5%, and +4.1/%14.4%, respectively. A correlation between ATFXa and plasma levels of rivaroxaban (R2 = 0.68) and apixaban (R2 = 0.50) was observed while ATFIIa was not affected (R2 = 0.07 and R2 = 0.02, respectively). Neither assay was influenced by LMWH (R2 = 0.01). Thus, the higher variability in the rivaroxaban and apixaban subgroups can be explained by the effect of these FXa inhibitors on FXa-based AT activity tests.

**Conclusion:** Measurement of AT activity using a FXa-inhibition based method overestimates AT activity in patients receiving FXa inhibitors. Our results indicate that an inherited AT deficiency might be missed, if a thrombophilia investigation is conducted during AC therapy. Therefore, thrombin-based tests should be used for AT activity assessment in patients treated with FXa inhibitors.

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**V06**

Hämostaseologie Teil 2

**V06-1**

**Validation of cold stored apheresis and pooled platelet concentrates in additive solution**

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**Introduction:** Platelet concentrates (PC) may be stored at 2–6°C in additive solution to reduce the risk of bacterial growth and to allow extended storage. We have shown that this approach is feasible and does not impair in vitro platelet function compared to storage at room temperature (RT). In order to validate our findings for different platelet products, we compared systematically platelet function and metabolism in PCs produced by apheresis (APC) or by pooled buffy coats (PPC) stored at 2–6°C or RT.

**Methods:** Twelve APC and splitted PPC were stored under agitation either at RT (n = 6) or at 2–6 °C (n = 6) for 10 days in additive solution (SSP+, Macopharma) with a residual plasma content of ~35%. Samples were taken after 0, 1, 4, 7 and 10 days. Platelet metabolism was determined by pH, glucose consumption, lactate production, hypotonic shock response (HSR). Aggregation ability with collagen (2 and 8 µg/mL), ristocetin (1.5 µg/mL) and ADP (80 µM) was determined by light transmission aggregometry. Activation status and activation ability by flow cytometry (CD62P-, CD63-, PAC1- and annexin V- expression) before and after the addition of thrombin receptor activating peptide 6 (TRAP-6) were performed to analyze platelet function.

**Results:** Cold storage of either APC or PPC did not alter platelet metabolism. HSR did not differ for PPC (p = 0.250), but was better maintained for APC after 7 days storage at RT compared to cold storage: 59.9 ± 14.3% vs 16.9 ± 8.5%, p < 0.0001. At day 7, 8 µg/mL collagen resulted in better aggregation response of APC platelets stored in the cold compared to RT (67.1 ± 21.2% vs. 22.3 ± 18.8%, p < 0.0001), addition of 2 µg/mL collagen, ristocetin and ADP showed similar aggregation responses for RT and cold stored APC and PPC, respectively. Similarly, expression of platelet activation markers did not differ between PC storage conditions.

**Conclusion:** Cold storage of APCs and PPCs in additive solution did not alter platelet metabolism and maintained in vitro platelet function comparable to RT storage. Thus, cold storage is feasible in APCs and PPCs. This opens up the perspective to test cold storage of PCs in a Phase I clinical study.

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**V06-2**

**Is platelet function testing superior to platelet count?**

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**Introduction:** Bleeding therapy with platelet concentrates (PC) is routinely guided by the platelet count. However, doubts are increasing as to the suitability of the platelet count for reflection of the haemostatic platelet capacity. We used cardiac surgery as a model to compare platelet count with platelet function.

**Patients and Methods:** We prospectively collected data from 3.214 cardio surgery patients. Impedance aggregometry (Multiplate®, ADPtest, TRAPtest) was used to assess platelet function based on stimulation with thrombin receptor activating peptide (TRAP) or adenosine diphosphate (ADP). Conventional routine testing was used for the assessment of platelet count. The testing was performed at incision and shortly before administration of protamine. We compared subgroups according to surgery types: bypass-, single valve-, combined bypass and valve-, multiple valves-, aorta surgery, heart transplantation (HTX), left ventricular assist device implantation (LVAD). Red blood cell concentrate (RBC) transfusion rates were put into relation with aggregation findings.

**Results:** Sequential testing at incision and before protamine application (n = 665 single valve surgery patients) shows the platelet count to decrease in 95% of patients. Despite this platelet count decrease, 46.8% and 32.3% of patients demonstrate an increase in aggregation capacity as assessed by TRAP and ADP induced aggregometry, respectively. Differences in the aggregation development during surgery are found depending on the type of surgery. The most significant finding is aggregation capacities to decrease in patients with aorta surgery, HTX or LVAD as compared to patients with bypass or valve surgery (p < 0.001). This difference holds true after correction for the platelet count by pooling patients with equal platelet counts. The number of RBC transfused until 24hrs postoperatively is correlated inversely with the TRAP induced aggregation capacity at pre-protonamine testing. The figure shows results for patients with platelet counts between 80–100/nl (n = 214):

![Fig. 1. Correlation of RBC transfusion with TRAP induced aggregation.](image-url)
Abstracts

V06-3
In vitro heparin-independent platelet activation: a further diagnostic challenge in patients undergoing extracorporeal circulation in the intensive care setting
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Introduction: Thrombocytopenia is commonly observed in patients during treatment with extracorporeal circulation (ECC) including extracorporeal membrane oxygenation and extracorporeal life support. A relatively high incidence of heparin induced thrombocytopenia (HIT) has been reported in this patient cohort. The diagnosis of HIT is based on clinical criteria (a fall in platelet count >50% between days 5 to 14 after start of heparin with or without thrombosis) and confirmed by in vitro demonstration of heparin-dependent, platelet-activating anti-PF4/heparin antibodies. However, as most patients show a characteristic decrease of the platelet count after ECC implementation, functional assays such as the Heparin-Induced Platelet Activation (HIPA) assay are indispensable for a reliable diagnosis.

Methods: This case-control study assessed data of patients who were admitted to the intensive care unit of our university hospital between March 2016 and April 2017 and in whom HIT was suspected. Inclusion criteria were positive result in an immunoassay for IgG antibodies against PF4/heparin complexes (optical density (OD) >0.5) and an intermediate to high pretest probability of HIT (4T score ≥4). Patients were divided into 2 groups: I. patients who received ECMO or ECLS and II. without ECC-implementation. Sera from both groups were tested by HIPA using low and high heparin concentrations to investigate heparin-dependency of the reactions. Heparin-independent reactions were further investigated using an Fcg-receptor-IIA blocking monoclonal antibody (mAb IV.3).

Results: 8 of 18 patients with 4T score ≥4 (median: 5, range 4–6) and positive IgG immunoassay (median OD: 1.099, range 0.396–3.060) needed supportive therapy with an assist device. In this group, sera from 3 out of 8 patients tested negative and 2 showed positive results in HIPA. In contrast, sera from 3 of these patients induced heparin-independent platelet activation in HIPA. 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, alternative anticoagulation was recommended for the management of all 3 patients. A rapid recovery in platelet count was observed in one patient, making the diagnosis of HIT very likely.

Conclusions: HIT has been suggested as a frequent complication in patients undergoing ECC treatment with negative outcomes. However, most studies had retrospective designs with functional tests done after first line laboratory tests. Moreover, testing for the heparin-dependency of platelet activation was not routinely performed. In our current study, we directly employed the functional assay HIPA in the diagnostic algorithm for HIT and investigated heparin- and Fcg-receptor-IIA-dependency of platelet activation. Our data show that sera from patients with ECC-devices could cause unspecific platelet activation which may impair the specificity of the functional assay.

V06-4
Anti-teicoplanin antibodies as a cause of a false positive HIPA test
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Background: Heparin-induced thrombocytopenia (HIT) is the most frequent drug induced immune-thrombocytopenia. Nearly all patients with HIT test positive for anti-PF4/heparin IgG antibodies also rarely antibodies with other specificity than PF4/heparin have been described. Due to the low specificity of anti-PF4/heparin antibody tests for clinically relevant antibodies, and for recognition of HIT antibodies with rare specificities, the diagnosis of HIT is confirmed by functional assays, namely the heparin-induced platelet activation test (HIPA). The platelet count nadir in HIT is typically >20,000/µL. Other drug-dependent immune thrombocytopenias (DITP) are much less frequent. The platelet count nadir in DITP is typically <10,000/µL in patients are at risk of bleeding not of thrombosis.

In this report, we present a patient with teicoplanin induced DITP, whose antibodies caused a false positive result in the HIPA.

Case report: A 62-year-old woman underwent lobectomy after diagnosis of lung cancer. Due to limited lung function she was treated with extracorporeal membrane oxygenation (ECMO) postoperatively. During her intensive care stay she developed severe thrombocytopenia (<10GPT/l), which was initially suspected to be caused by HIT. The PF4/heparin ELA was negative but the HIPA test showed a typical positive reaction with platelet activation at low heparin concentration, which was inhibited by high heparin concentration. Without the addition of heparin (buffer reaction) the HIPA was negative. Platelet activation was also inhibited by the monoclonal antibody IV.3, proving Fc receptor-dependency. First we ruled out an effect of HLA class I antibodies (which were also present in the patient’s serum) on the HIPA test by using HLA-compatible washed platelets. Due to the negative ELA test and the very low platelet count we further investigated for DITP and identified teicoplanin-dependent anti-platelet antibodies.

Discussion: We describe the first case of a drug-dependent antilatelet antibody, which activates platelets Fc-receptor-dependent in the presence of low concentrations of heparin which is inhibited by high concentrations of heparin, thereby mimicking the typical laboratory features of HIT. DITP should be considered in patients with a negative PF4/heparin ELA but the positive HIPA test, especially if the clinical presentation is not typical for HIT, e.g. platelet counts < 20,000/µL.
Abstracts

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Abstracts

V07
Blutspender Management

V07-2
The role of social media for blood donor motivation and recruitment

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Introduction: Recruitment of whole blood donors is a daily challenge for blood donation services and many centers implement strategies to motivate first time and regular donors. As the use of social media platforms among the younger population has increased substantially, we implemented communication via social media platforms to motivate repeat and to recruit new donors. Here, we report a survey among donors of our blood donation facility to identify the impact of different media and resources for donor motivation from the donors’ perspective.

Method: During five consecutive weeks (May–June 2017), all consecutive whole blood donors of the blood donation facility of the University Medicine Greifswald were asked to participate. The survey consists of a one-page questionnaire including the demographic items sex, age, and number of prior donations and 14 potential motivators for blood donation. Social media included the items «Facebook» and «Jodel» (German social network), and together with other motivators such as «I do not need further motivation; I come anyway», followed by 27.2% (mean age 28.7 years) who were «motivated by post card reminders of our facility. Social media anyway», followed by 27.2% (mean age 28.7 years) who were «motivated by social media».

Results: Results of real time coagulation assessment in line with standardised clinical judgement allowed to identify 12.9% of patients without any need for specific haemotherapy. The other patients were treated with one or more of the haemostatically active compounds (desmopressin, platelet concentrate, fibrinogen concentrate, factor XII concentrate, prothrombin complex concentrate, recombinant activated factor VII) were established. By now, we assessed 3214 patients undergoing cardiac surgery.

Conclusion: Social media have become the second most important motivator to recruit first time donors, beside relatives and friends who are by far the main motivators for first time donors. For repeat donors, social media play a less important role because most of them stated that they do not need extra motivations to donate. However, the impact of social media will likely increase over time also in repeat donors. Social media will become increasingly important for transfusion Services.

V07-3
The introduction of the new German Blood Donor Questionnaire impacts on donor deferral rates, a 12 months observation

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Background: As the implementation of a new national German blood donor questionnaire was proposed to improve donor and recipient safety and was recommended by the Arbeitskreis Blut as the responsible commission of experts, a slightly modified version was introduced by the German Red Cross Blood Donor Service Baden-Württemberg – Hessen starting with calendar week (cw) 23 in June 2016. The objective of this study was to describe donor deferral rates after the implementation of the new German Blood Donor Questionnaire.

Methods: We compared deferral/exclusion rates of whole blood donors at the German Red Cross Blood Donor Service Baden-Württemberg – Hessen before (June 2015–May 2016, n = 654,700) and after (June 2016–May 2017, n = 627,663) the introduction of the new blood donor questionnaire. In a subanalysis (cw23–45 2016 versus cw23–45 2015), a detailed analysis of deferral/exclusion rates was performed considering seasonal variations with respect to collection site (mobile vs. fixed), sex, donor status (first-time vs. repeat), age, and the frequencies of sexual risk behaviour and other reasons for deferral.

Results: During the initial time period cw23–45, we observed a statistically significant increase (p < 0.001) of the overall deferral/exclusion rate from 8.3 to 10.5%, irrespective of the type of collection site (fixed: from 14.1 to 16.0%; mobile: 7.9 to 10.1%), sex (females: 10.8 to 13.1%; males: 6.5 to 8.5%), donor status (first-time donors: 24.3 to 30.2%; repeat donors: 7.4 to 8.7%) or age (< 25 years: 14.1 to 18.8%; 65–68 years: 6.0 to 6.8%; > 25 years: 8.3 to 10.5%). Besides confidential self-exclusion also increased from 0.10 to 0.13% (p = 0.001). Interestingly confidential self-exclusion also increased from 0.10 to 0.13% (p = 0.001). Besides sexual risk behaviour, various medical reasons could be identified to explain this increase. While the overall deferral/exclusion rate for whole blood donors returned to initial values within 12 months (10.5 to 8.3%) in May 2017, it remained elevated for first-time donors (30.2 to 31.0%).

Conclusions: The new blood donor questionnaire resulted in a transiently increased overall deferral/exclusion rate for all whole blood donors. However, in first-time donors, the deferral rate remained elevated after 12 months. Thus the impact on future blood supply must be considered.
carefully as increased deferral rates in first-time donors may impact substantially on future recruitment strategies. In addition, further studies will be required to clarify the subsequent decline of the overall deferral rate in all whole blood donors.

V07-4

Time series analysis for blood demand forecast

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Introduction: Erythrocyte concentrates (ECs) are a scarce resource. Wastage of ECs is both an ethical as well as a financial problem. Since donors have a mandatory pause between donations, wasted ECs prohibit future blood donations from the same person for several months. Depending on the blood bank analyzed, the total number of wasted ECs is suggested to be between 0.5% and 5%. Outdated ECs are responsible for approx. 2/3 of total EC wastage. Currently, many blood banks utilize inventory stock management systems calculating average consumption or use empirical, non-adaptive methods, which do not take temporal changes in blood demand into account. Therefore, an inventory stock management system utilizing forecasting methods could improve efficiency of EC stock management as well as increase stock when higher demand is required.

Methods: We performed our analyses using data from the Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Vienna, Austria. We evaluated different algorithms developed for time series analysis, including autoregressive, integrated moving-average (ARIMA) models and exponential smoothing models as well as neural network analysis and regression models. The algorithms were provided with the amount of ECs utilized on each day between 2004 and 2016. Additionally, temporal context information was provided to the model when applicable. Predictions were performed for 3, 5, 7, 10 and 14 days into the future, defined as different periods in the year 2016. Data were compared to the real demand of ECs at that time. Safety buffers were defined as both confidence intervals (80% and 95%) as well as fixed EC stock limits.

Results: All of the evaluated models outperformed both the empirical blood demand model as well as the utilization of average consumption calculations. The inclusion of temporal context information led to an increase in prediction accuracy. Without safety buffers, the models tended to underestimate the blood demand in specific situations (e.g., days with unusually high blood demand). This effect could be greatly reduced by including safety buffers to account for unforeseen exceptional blood demand.

Conclusion: The use of forecasting models to predict blood demand is an improvement over the use of inventory stock management systems based on averaging blood consumption or non-adaptive, empirical models. By using forecasting methods, the amount of outdated ECs could be reduced, therefore ensuring less EC wastage and subsequently higher blood availability. By including safety buffers into the calculations, forecasting models additionally increase the safety of EC stock management systems. Especially small to medium-sized blood banks, hospitals with transfusion departments as well as institutions with an inventory stock specifically designed to fit short time periods could increase their ratio of transfused to produced/ordered ECs.
Blood donors infected with HIV or HCV 2006–2016 – the impact of NAT and confidential self-exclusion on transfusion safety

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The introduction of mandatory HCV and HIV NAT shortened the infectious window period to less than 10 days and was an important measure to increase blood safety. However, recent infections with HIV or HCV are still a serious threat for blood safety. The proportion of very recent infections with non-detectable antibodies in blood donors related to donor characteristics and the use of confidential self-exclusion (CSE) was investigated further to identify risks and challenges in donor information and selection.

HIV and HCV infections of blood donors in Germany are notifiable to the donor vigilance system which is hosted by the Robert Koch Institute. We analyzed the proportion of very recent HIV or HCV infections between 2006 and 2016 in Germany based on the identification of confirmed cases with positive NAT (NATonly) and lack of seroconversion. HCV infections with positive NAT result were classified as active infection. Infections were related to donor age and type of donor (first-time, FTD, vs. repeat donor, RD). Additionally, the use of confidential self-exclusion (CSU) was investigated.

Between 2006 and 2016, 977 confirmed HIV infections and 4,252 HCV infections (2,102 active HCV infections) were reported. In the 11 year observation period, confirmed HIV infections among FTD and RD declined from 76.4 to 44.9/100,000 cases of FTD and 2.6 to 1.4/100,000 cases among RD. HIV infections dropped in FTD from 6.2 to 3.6/100,000. In contrast, the proportion of HIV infections among RD remained stable over time (2.0/100,000 RD). Very recent infections for HIV and HCV were mainly found in RD: 5.3% of HIV infections and 26.4% of active HCV infections among RD were NAT-only compared to 1.4% and 0.6%, respectively, on FTD with no decreasing trend over time. Donors up to 24 years contributed more than one third of active HCV infections with 12% NAT-only findings. In case of HIV one third of infections were found in the age group 25–34 years but the share of NAT-only was highest for donors older than 54 years (6.4%).

CSU was only used by a minority of infected donors: 33 (3.4%) of donors infected with HIV and 27 donors (1.3%) with active HCV infection blocked their donation. Only one HIV NATonly and three HCV NATonly donations were self-excluded by the donor.

Despite the declining numbers of HCV-infected FTD and RD and HIV infected FTD in the past decade, the proportion of HIV positive RD is critical. In addition, a constant proportion of especially young RD donors with very recent (NATonly) HIV- and HCV-infections donated. These infections could threaten the high level of blood safety in Germany despite excellent testing. Only a small proportion of infected donors used the CSU indicating room for improvement in terms of donor information and selection. Donor vigilance therefore needs to be complemented by the CSU indicating room for improvement in terms of donor information and selection.

V08
Zelltherapie Teil 2

Decreasing the immunogenicity of the lung: silencing MHC expression in the lung during normothermic ex vivo perfusion

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Introduction: Lung transplantation is a successful therapy, however it is hampered by development of chronic rejection and opportunistic infections. Disparities at the HLA loci are the main cause for rejection and the need for immunosuppression.

Methods: We evaluated the capacity to silence MHC class I and II expression in the porcine lung. Lenti vectors encoding for short hairpin RNAs targeting β2-microglobulin (shβ2m) or the class II transactivator (shCIITA) were produced to target SLA class I or class II, respectively, and to express NanoLuc as reporter gene. Lungs were connected to an ex vivo lung perfusion system and perfused for 2 h with the shRNAs encoding vectors. After perfusion, tissue of different lung regions was enzymatically digested and endothelial cells (ECs) were isolated. ECs were cultured and analysed for NanoLuc expression in bioluminescence assays. Transcript levels of β2-microglobulin, CIITA or SLA-DR in ECs were measured by real-time PCR. Tissue histological analyses were performed.

Results: Expression of NanoLuc was already detectable 24 h after perfusion in all regions of the lungs. ECs of lungs perfused with shβ2m encoding vectors showed a downregulation of β2-microglobulin by up to 80%. Similarly, lungs perfused with the vector encoding for shCIITA showed a knockdown of CIITA, SLA-DR and SLA-DQ by up to 70%. In presence of IFN-γ, ECs from non-perfused lungs were able to up-regulate CIITA expression up to 15-fold. In contrast, levels of CIITA transcripts remained unaffected or even decreased upon IFN-γ stimulation on ECs of lungs perfused with shCIITA-encoding vectors. Vector integration was detected in the cell genome, which is essential to achieve a permanent MHC suppression. The integrity of the pulmonary tissue remained unaffected.

Conclusion: This method allowed the permanent genetic modification of the lung endothelium. The permanent MHC downregulation decreases the organ’s immunogenicity and creates a status of immunological inviability. This approach may represent a promising strategy to combat the burden of rejection and immunosuppression.

Live cell imaging of the immune synapse of CAR-expressing NK-92 cells and breast cancer cells as a tool to evaluate combination therapies

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Introduction: Recently it has been shown that the expression of tumor antigen-specific chimeric antigen receptors (CARs) or Fc receptors (FcRs) in natural killer (NK) cells is capable of overcoming tumor cell resistance in some – but not all – cases. With regard to NK cells, several signals at the immunological synapse between tumor and effector cell orchestrate the cytotoxicity by release of lytic enzymes. In order to further optimize tumor antigen-directed NK cell therapies, we aim to analyze the immunological synapse between effector and cancer cells, and to identify signals mandatory for (CAR-/FcR-) NK cell activation as indicated by polarization of lytic granules at the site of tumor/effector cell synapse.
Methods: NK-92 cells and NK-92 cells genetically engineered to express an ErbB2-specific CAR (NK-92/5.28.z) or a high affinity Fc receptor (FcR-NK-92) were analyzed in a 2 h europium cytotoxicity assay with regard to their cytotoxicity towards ErbB2-positive (MDA-MB-453, SKOV-3) and ErbB2-negative (MDA-MB-468) breast cancer cells. FcR-NK-92 were co-incubated with an ErbB2-specific mAb, Herceptin®. Live cell imaging was performed by staining NK-92 lytic granules with LysoTracker Red DND-99. Target cells were stained with Cell Mask Deep Red plasma membrane stain and dead cells were discriminated by SYTOX Blue. Cells were imaged over 10 h by an Olympus spinning disk confocal microscope.

Results: While parental NK-92 failed to kill breast cancer cell lines irrespective of the ErbB2 expression, addition of a tumor-specific antibody fragment, by either CAR or mAb/FcR, resulted in highly effective killing of ErbB2-positive MDA-MB-453 (86% by NK-92/5.28.z, 43% by FcR-NK-92). Live cell imaging showed polarization of lytic granules at the site of the immunological synapse when antibody and target binding were mediated through CAR or mAb/FcR signaling. Interestingly, in the case of absence of antibody/antigen-mediated signaling, polarization could not be observed in any case despite cell-cell contact.

Conclusion: Our data suggest that cell adhesion molecules required for cell-cell contact and initial steps of immunological synapse formation are present on NK-92 resistant tumor cells. However, further NK cell activation steps are prevented in the absence of additional CAR or mAb-mediated activation by mechanisms that need to be further clarified. Using the polarization of granules as a read out system, we now aim to further analyze the immunological synapse and the impact of PD-1/PD-L1 immune-checkpoint blocking mAb and/or induced upregulation of NKG2D ligands by metronomic chemotherapy on the effectiveness of NK cell killing.

V08-4

Novel immunomodulatory function of plasmacytoid dendritic cells during gram-negative bacterial pneumonia

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Background: Plasmacytoid dendritic cells (pDC) play various roles during innate and adaptive immune responses. Especially regarding viral infections pDC are well-investigated in many studies because of their ability to produce large amounts of anti-viral interferon-alpha. In contrast, their function during bacterial infections is unknown. Therefore, we investigated pDCs immunoregulatory role in vivo in a Klebsiella-pneumonia model by using a systemic conditional pDC depletion model.

Methods: Using BDC2-A-DTR mice, pDC were depleted by application of Diphtheria toxin in a sublethal pneumonia model with Klebsiella pneumoniae (ATCC 43816) over 14 days. Samples of pDC-depleted and 0.9% Diphtheria toxin in a sublethal pneumonia model with Klebsiella pneumoniae were performed by staining NK-92 lytic granules with LysoTracker Red DND-99. Target cells were stained with Cell Mask Deep Red plasma membrane stain and dead cells were discriminated by SYTOX Blue. Cells were imaged over 10 h by an Olympus spinning disk confocal microscope.

Results: In pDC depleted mice we found increased granulocytosis in blood whereas in bronchoalveolar lavage it was decreased on day 2. Interestingly, we observed a lower mortality and a generally better general condition (measured in scores) in the acute and post-acute pneumonia phase on day 2 and 5 in pDC depleted mice. Whereas the condition of control mice improved until day 14, the scores of pDC depleted mice stayed at the same level. In addition, in pDC depleted mice we found a significant increase of FoxP3 T regulatory cells on day 2 and 5 and a significantly reduced IL-17A and IL-22 production on day 2. Bacterial load showed no significant differences between groups.

Conclusion: Our experiments revealed a novel immunomodulatory function of pDC during bacterial pneumonia in mice enhancing inflammation in the beginning of the infection and possibly attenuating it later on. Further in vivo and in vitro experiments are required to dissect the molecular mechanism of this novel immunoregulatory pDC functions during bacterial infection.

V09-5

Mini photopheresis for refractory chronic graft-versus-host disease in children and adolescents

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Background: Extracorporeal photopheresis (ECP) has been established for the treatment of graft-versus-host disease (GVHD). Our mini ECP technique (mini-ECP) allows for treatment of patients with GVHD and contraindications for classical ECP or low body weight. However, safety and efficacy of its application for the long-term treatment of chronic GVHD (cGVHD) have not been described.

Study design and Methods: A retrospective analysis of 703 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 6 years (1–17 years), the median body weight 20 kg (8–53 kg). The median number of treatments per patient was 35 (8–129), the median treatment duration 11 months (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. The clinical response could be evaluated in 13 patients. 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 and clinically effective ECP alternative for low body weight patients with cGVHD and apheresis contraindications.

V09 Blutsicherheit

Evidence that cross-organizational preparedness plans are required to strengthen structures for an efficient blood supply in emergency situations

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Introduction: At Paul-Ehrlich-Institut (PEI), the interdisciplinary working group «Safety of blood and blood products (BSG)», headed by the Section Transfusion Medicine, coordinates the evaluation and initiates measures for handling of ad hoc events that might have a general impact on quality and safety of blood components. An interdisciplinary team of PEI experts is able to rapidly assess the influence that deviations from established processes for collection, processing and testing of blood donations might have on blood supply. We here summarize the events analyzed by the BSG in 1999–2016. We exemplarily highlight the significance of flexible structures for collection, processing and testing of blood donations including procedures for marketing authorization (MA) and post-MA that are sufficiently robust under routine conditions as well as sufficiently adaptable to situations that may result in shortages of blood supply.

Methodological approach: Triggered by reported events, members of the BSG ascertain the severity of the events, develop standardized procedures, the BSG analyzes the severity of the events, develops standardized procedures, and notifies concerned parties.

Abstracts

Transfus Med Hemother 2017;44(suppl 1):2–87
Abstracts

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Results: From 1999–2016, 58 events were assessed by the BSG. The majority was related to in-vitro-diagnostics (IVD). We demonstrate the impact of events on blood supply taking advantage of scenario analysis. The assessment indicates that preventive measures could have stabilized procedures efficiently upon occurrence of events leading to a disturbance of established workflows.

Conclusion: Events reviewed by the PEI-BSG indicate that blood establishments should further strengthen their capacity to rapidly implement cross-organizational emergency preparedness plans – such as required by §3 Transfusion Act (TFG) – in order to respond to conditions enforcing workflows that deviate considerably from routine procedures as established at the level of single institutions. Established structures to assure availability, safety and quality of blood and blood products and their flexible adaption to health care emergencies contribute substantially to efficient health care systems. As a way to address similar challenges in Africa, the German Ministry of Health has started the Global Health Programme (GHP) which includes the project Availability, Safety and Quality of Blood and Blood Products – Support to implement a regulatory structure and its adaption to an emergency situation in partner countries, being implemented at the Section Transfusion Medicine at the PEI. We expect relevance of outcomes of this project also for advancing preparedness structures for blood supply in Germany.

V09-3
Anti-HBc screening – is it worth the effort? Results of a 10-year surveillance covering 50 million donations in Germany

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In 2006 mandatory anti-HBc-testing was introduced in addition to HBsAg-screening to increase blood safety for HBV for all non-virus-inactivated blood products. This measure is able to reduce transfusion-associated HBV infections caused by subclinically persistent HBsAg-negative HBV-infections as well as virus mutations escaping the HBsAg-screening assays.

The national advisory board recommended surveillance of anti-HBc reactive and HBsAg negative blood and plasma donations. We present the results of the surveillance since the introduction of anti-HBc screening in a 10-year observation period (2006–2015). All blood establishments were invited to submit data on HBsAg-negative and repeatedly anti-HBc reactive donations electronically to the Robert Koch Institute. Reports included demographic parameters, NAT-results (ID and pool), anti-HBs-titer and information on test assays used.

In the observation period, 60% of all blood establishments in Germany participated in the anti-HBc surveillance collecting 49,823,143 donation in (69.2% of all donations). 388 per 100,000 donations were HBsAg negative and anti-HBc reactive. Of these, 163 donations were ID-HBV-NAT positive (84 per 100,000 anti-HBc reactive/HBsAg negative donations). The proportion of NAT-negative HBsAg negative/anti-HBc reactive donations with an anti-HBs titer below 100 IU/L was 7,445 per 100,000, and with a negative anti-HBs titer 16,267 per 100,000 donations. The proportion of anti-HBc reactive/HBsAg negative blood and plasma donations declined over the 10-year period from 0.66% in 2006 to 0.11% in 2015.

On the basis of a 10-year surveillance of almost 50 million donations, we found 163 ID-HBV-NAT positive donations, which would have not been detected without anti-HBc screening and would have likely led to transfusion transmitted HBV infections. Additionally, considerably high numbers of anti-HBc positive and HBsAg negative donations with Anti-HBs-titer below 100 IU/L and negative HBV-NAT results were reported – indicating possibly infectious donors with chronic HBV infections. After anti-HBc screening was implemented and the established donor population was completely screened, the additional testing only lead to a moderate donor loss. Therefore, anti-HBc screening is currently warranted to increase blood safety with respect to HBV. Further studies are necessary to evaluate the cost effectiveness and the impact on the residual risk estimation in comparison to other potential actions like mandatory ID-NAT or pathogen inactivation.

V09-4
Reducing blood-borne HEV transmission: experience with routine HEV-RNA pool screening at a university blood center

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Introduction: The rate of HEV-RNA positive donors in Germany in recent studies varies from 0.022% to 0.147%, but general HEV testing of blood products has not been implemented. The clinical relevance of blood-borne HEV transmission is still under debate.

Methods: After a preliminary testing phase in September 2016, from October 2016 all blood donations at the University Hospital Hamburg Eppendorf were routinely screened for HEV-RNA prior to their release. Pools of donations were tested using the new Roche cobas 6800 HEV-PCR assay (pool size 24, calc. sensitivity per single donation: 446 IE/ml). Reactive pools were tested individually to identify HEV-RNA positive donors. For identified donors additional testing was performed: retained samples of preceding donations and follow up samples were tested by single HEV-PCR. Donors were temporarily deferred from further donations allowing reentry four months after a HEV-RNA positive follow up sample.

Results: Utilizing the pooled donor samples prepared for HIV- and HCV-PCR testing was convenient, test handling was easy with a running time below 2 hours. The pool size of 24 donations kept costs moderate. Until May 2017 18,737 donations were tested, 23 (0.123% or 1:815) HEV-RNA positive donors were identified (D1 to D23). Virus load ranged from low (< 1,000 IU/ml: n = 14), to medium (1,000–10,000 IU/ml: n = 5) and high (>10,000 IU/ml, n = 4). 21/23 positive donors were healthy and asymptomatic, showing no elevation of ALT. Two donors presented with acute self-limiting hepatitis. D4: HEV-RNA 11,200,000 IU/ml, ALT 192 U/ml (ALT max. 1287 U/l); D6: HEV-RNA 461,000 IU/ml, ALT 50 U/ml (ALT max. 955 U/l).

HEV-RNA in donors persisted up to 19 weeks. For identified donors with preceding donations we tested retained samples by single HEV-PCR. One positive apheresis donor had three HEV-RNA positive preceding donations which had not been detected since HEV-PCR was not implemented at the time. Three HEV-RNA positive whole blood donors had preceding donations that revealed HEV-RNA in single PCR that had not been detected by routine pool testing (760–4,500 IU/ml).

Conclusion: Rate of HEV-RNA positive donations was higher than in most German studies. Pool testing considerably reduced the risk of HEV-exposition for all recipients as 60 HEV-RNA positive blood products could be prevented from transfusion. Pool size of 24 however resulted in lower sensitivity, leaving three donations (8 blood products) with low virus load undetected. It needs to be discussed, if this presents an acceptable proportion. Since prolonged viremia (>4 months) can be observed, a single HEV-positive blood donor can cause HEV exposition for multiple patients. This should especially be considered for apheresis donors with short donation intervals.

To evaluate the cost benefit ratio of different testing regimes further studies are needed also investigating the relationship of virus load and infectivity of blood products.
The THERAFLEX UV-Platelets technology efficiently inactivates transfusion-relevant bacteria species in concentrated platelet concentrates

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Introduction: The THERAFLEX UV-Platelets system (Macopharma) is a UVC-based pathogen inactivation system for platelet concentrates (PCs). Inactivation efficiency has been shown for a broad range of viruses, bacteria, and protozoans. Previous studies with the first set of bacteria species of the WHO International Repository of Platelet Transfusion Relevant Bacterial Reference Strains revealed a high inactivation capacity for clinically relevant bacteria. Aim of the current study was to investigate the bacteria inactivation efficacy of the THERAFLEX UV-Platelets system for Enterobacter cloacae, Pseudomonas fluorescens, Staphylococcus aureus and Streptococcus bovis which have recently been added to the WHO International Repository.

Methods: PCs were produced from 5 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 to a final concentration of approx. 10^6 colony forming units (CFU)/mL and irradiated with increasing doses until the full UVC dose was achieved. Samples were taken for the bacterial titer determination after each irradiation step. For sterilization studies, 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: Bacteria in PCs were inactivated in a dose-dependent manner by treatment using the THERAFLEX UV-Platelets system. Mean log10 reduction factors ranged from 6 to 7 for Enterobacter cloacae (6.3 ± 0.6, PEI-B-P-43), Pseudomonas fluorescens (7.1 ± 0.4, PEI-B-P-77), Staphylococcus aureus (6.6 ± 0.4, PEI-B-P-63), and Streptococcus bovis (7.0 ± 0.3, PEI-B-P-61). PCs (n = 12 for each species) spiked with these different bacteria species were efficiently sterilized (12 out of 12). Treated PCs remained sterile during storage for 7 days, while bacteria in non-treated PCs grew to high titers of 10^8–10^10 CFU/mL.

Conclusions: The THERAFLEX UV-Platelets system efficiently inactivates a broad range of different bacteria species, including for WHO reference strains. Sterility is maintained over a storage period of 7 days. These results suggest that the UVC-based pathogen inactivation technology will significantly improve the bacterial safety of platelet transfusions.

Tab. 1. Rate of regeneration of TP and IgG

<table>
<thead>
<tr>
<th>parameters</th>
<th>male (n = 1795)</th>
<th>female (n = 2070)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean IgG-Regeneration per day</td>
<td>0.033 [n = 4020]</td>
<td>0.04 [n = 3245]</td>
</tr>
<tr>
<td>mean TP-Regeneration per day</td>
<td>0.211 [n = 3245]</td>
<td>0.217 [n = 4392]</td>
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Conclusion: The calculated daily rate of regeneration per donor for TP and IgG measured facilitates a prediction of the individual recovery time and allows a specific determination by when a donor qualifies again for donation. Consequently an optimal plasma donation interval can be found for the total donor population reviewed. Introducing such methodology may lead into significant reduction of the donor deferral rate for TP and IgG. Additionally the risk for donors will be reduced and the operations of the collection center positively impacted not only as of lower cost of collection but increased overall donor satisfaction as of lower deferral probability. Therefore acceptable predictive values for daily regeneration of TP and IgG shall be established and continuously evaluated.

A singly center study on the long term impact of plasmapheresis intensity on IgG and total protein levels in regular plasma donors

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Introduction: The safety of long-term intensive donor plasmapheresis remains a controversial issue. Frequent plasmapheresis donors may develop reduced levels of total protein and/or immunoglobulins. The influence of more moderate long-term plasmapheresis on plasma proteins and IgG concentration is not well documented.

Methods: In this single center study, IgG concentrations and total protein levels from 2,893 donors were analyzed over a 30-month period. Body-weight adjusted plasma collection was performed using the Apache plasmapheresis system. Total serum protein and immunoglobulin G (IgG) were determined at baseline and at every 5th donation. Donors with IgG < 6 g/L or total protein < 60 g/L were put on hold for 50 days.

Results: 23,361 plasmapheresis were performed between January 2015 and May 2017. Decreased levels of IgG or total protein levels were ob-
served at least once in 237/2,893 (8%) donors with a median age of 32 years (18–64 years). Donors were divided into two groups depending on the number of dropouts during the study period: Group I: < 3 dropouts and group II: > 3 dropouts. In the first group, n = 42 patients (14 females and 28 males) had dropouts due to low levels of total protein (n = 13), IgG (n = 16), or both (n = 13). In this group moderate plasmapheresis was performed (estimated interval between apheresis median 6.3 days, range 4–341 days). In group II, 26 donors (11 females and 15 males) had more than three dropouts on 76 occasions during the study period. 12, 9 and 2 donors had 3, 4 or 5 dropouts, respectively, due to low levels of total protein (n = 13), IgG (n = 36), or both (n = 50). In this group, plasmapheresis was performed at a similar interval compared to group I (estimated interval between apheresis median 6.4 days, range 3–90 days).

**Conclusion:** Our study indicates that the number of donor dropouts is not necessarily related to the interval of plasma donation. Further studies are required to analyze why donors disqualify from intensive plasmapheresis programmes.

**V10-4**  
**Use of central venous catheters in peripheral stem cell apheresis**  
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**Introduction:** In donors undergoing a peripheral stem cell (PBSC) apheresis there is not always an adequate peripheral venous access possible, especially regarding that the amount of collected CD34+ cells is dependent on the processed volume. In these cases a central venous catheter (CVC) is required. Therefore in this study we compare and evaluate the effects of the different venous access on the procedural parameters in PBSC apheresis.

**Material and Methods:** 192 PBSC donations (allogenic n = 37, autologous n = 155) were examined using either peripheral access 16 Gauge catheters (Vasofix, Fa. Braun, Germany) or central access 14 Gauge catheters (Certofox Duo, Fa. Braun). Blood pressure and heart rate were measured, and procedural parameters like blood flow were calculated.

**Results:** The use of central venous catheters resulted in a significant increased blood flow in autologous (79 ± 21 mL/min) (peripheral) vs. 96 ± 18 mL/min (central) PBSC donors. In contrast, the procedural time was significantly reduced, as example in allogenic PBSC donors from 170 ± 32 min (peripheral) to 156 ± 27 min (central). Additionally, an elevation of the mean arterial blood pressure (MAP) resulted in an increase in procedural blood flow (67 ± 19 mL/min (MAP < 100 mmHg) vs. 76 ± 20 mL/min (MAP > 100 mmHg)).

**Conclusion:** The use of central venous catheters showed a significant increase in procedural blood flow and a decrease in time duration of the apheresis resulting in comparable procedural volumes between peripheral and central catheters. In peripheral venous catheters a tendency to an elevated collection efficiency could be observed. Additionally, procedural blood flow was dependent on the mean arterial pressure.

**V10-5**  
**Alloimmunization against D and other Rhesus antigens after platelet transfusion**  
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**Background:** Platelet concentrates (PC) contain residual contaminating red blood cells (RBC), being considerably higher in pooled platelet concentrates prepared from buffy coats of whole blood donations (BC-P) than in PC obtained from apheresis procedures (AP-PC). Frequencies of D alloimmunization after transfusing BC-PC are reported to be higher (up to 18.7%). However, there are limited data about PC-induced alloimmunization against other Rhesus (Rh) antigens than D.

**Methods:** We retrospectively determined the rate of Rh alloimmunization induced by C, c, E, e, and D incompatible PC transfusions detected at our institution (Inselspital Bern, Switzerland) during 08/2015–11/2016. In line with our national (Swiss) standards (a) the serologic Rh blood grouping of all donors was performed at least twice and confirmed by molecular methods, and (b) the quality controls of PC included their RBC contamination, and (c) all PC are mandatory pathogen inactivated using the Intercept® method. The transfusion history of all patients being identified with a newly detected Rh-alloimmunization was analysed for Rh incompatible RBC and/or PC transfusions within the last 3 months.

**Results:** From 08/2015 to 11/2016 a total of 2620 AP-PC and 3136 BC-PC were transfused. In 9 patients that had received PC transfusions we identified a total of 11 newly acquired Rh-antibodies: 5x anti-D (2/5 possibly being a booster-effect), 2x anti-c, 3x anti-E and 1x anti-f (details in table 1). These 9 patients received a total of 130 PC (70 BC-PC, 60 AP-PC): 3 patients received BC-PC only (2/3 only 1 unit and 1/3 totally 6 units), one patient received AP-PC only (1 unit), whereas 5 patients received both BC-PC and AP-PC: 10/1, 1/1, 24/20, 2/10 and 25/21 BC-PC/AP-PC. Quality control of BC-PC and AP-PC showed a mean (range) of 0.304 (0.152–1.662) and 0.014 (0.003–0.080) respectively x109 red cells per litre. Eight of the 9 patients received RBC-transfusions (total number 121, range 3–41). All RBC units were antigen-negative for the respectively identified allo-antibodies. For further details see table 1.

**Tab. 1. Demography, Rh antibodies, history of patients**

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>Newly detected Rh antibody</th>
<th>Date of detection</th>
<th>Previously known antibodies</th>
<th>Date of Last transfusion</th>
<th>Antigen+/- total Units of RBC</th>
<th>Antigen+/- total Units of BC-PC</th>
<th>Antigen+/- total Units of AP-PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>63 / m</td>
<td>Anti-f</td>
<td>24.08.2015</td>
<td>none</td>
<td>21.08.2015</td>
<td>0/1 (f- pos)</td>
<td>0/1 (f- pos)</td>
<td>0/1 (f- pos)</td>
</tr>
<tr>
<td>80 / f</td>
<td>Anti-D (Booster)</td>
<td>08.10.2015</td>
<td>none</td>
<td>10.09.2015</td>
<td>1/1 (D- pos)</td>
<td>0/1 (D- pos)</td>
<td>0/1 (D- pos)</td>
</tr>
<tr>
<td>73 / f</td>
<td>Anti-c/E</td>
<td>10.11.2015</td>
<td>none</td>
<td>19.10.2015</td>
<td>10/10 (c-pos)</td>
<td>5/7 (c-pos)</td>
<td>1/17 (E- pos)</td>
</tr>
<tr>
<td>53 / m</td>
<td>Anti-D</td>
<td>15.12.2015</td>
<td>none</td>
<td>01.09.2015</td>
<td>1/1 (D- pos)</td>
<td>0/1 (D- pos)</td>
<td>0/1 (D- pos)</td>
</tr>
<tr>
<td>71 / f</td>
<td>Anti-D</td>
<td>22.05.2016</td>
<td>none</td>
<td>08.05.2016</td>
<td>1/1 (D- pos)</td>
<td>0/1 (D- pos)</td>
<td>0/1 (D- pos)</td>
</tr>
<tr>
<td>78 / f</td>
<td>Anti-D (Booster)</td>
<td>03.06.2016</td>
<td>Anti-K and Anti-C (b)</td>
<td>10.05.2016</td>
<td>5/6 (D- pos)</td>
<td>0/ (D- pos)</td>
<td>0/ (D- pos)</td>
</tr>
<tr>
<td>64 / m</td>
<td>Anti-c/E</td>
<td>03.06.2016</td>
<td>none</td>
<td>31.05.2016</td>
<td>24/24 (c-pos)</td>
<td>na/20 (c-pos)</td>
<td>17/24 (E-pos)</td>
</tr>
<tr>
<td>61 / f</td>
<td>Anti-E</td>
<td>23.08.2016</td>
<td>none</td>
<td>19.08.2016</td>
<td>0/1 (E- pos)</td>
<td>1/1 (E- pos)</td>
<td>1/1 (E- pos)</td>
</tr>
<tr>
<td>56 / m</td>
<td>Anti-E</td>
<td>19.11.2016</td>
<td>none</td>
<td>19.11.2016</td>
<td>18/25 (E-pos)</td>
<td>6/21 (E-pos)</td>
<td>0/1 (E- pos)</td>
</tr>
</tbody>
</table>

**Conclusion:** PC transfusions may not only induce Rh D allo-immunization, but also immunization against further Rh antigens such as anti-c, anti-E, and anti-f. The risk seems to be higher in case of BC-PC transfusions as compared to AP-PC. Our study results may be taken into account for future specific recommendations of PC transfusion with respect to their Rh compatibility (e.g. transfusing females of childbearing age) and/or may have impact on the prescribed limit of RBC contamination of PC.
Abstracts eingeladener Referenten

S01
Better-Aging und Regenerative Medizin

S01-2
Large volume of custom made PRP: it is possible without blood loss

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Introduction: PRP has shown to be a useful adjunct to fat for enhancing the take of lipofilling. With commercial available kits, the amount of platelet rich plasma (PRP) which can be obtained from 10ml whole blood varies between 1–2 m. Therefore this option seems still limited for small aesthetic procedures as 20% of PRP are necessary to effectively enrich the transferred fat. For breast augmentation, however, 200–350ml of fat per side are necessary; and the production of enough PRP for such fat-volumes would be associated with a great blood loss (400–700ml for two breasts) which is unacceptable for the patients.

We found a way to produce large volume custom made PRP at any desired concentration without noteworthy blood loss.

Patients and Methods: Ten female patients with breast cancer were enrolled in this pilot study. They were scheduled for breast reconstruction with autologous fat transfer (lipofilling) after partial breast removal. PRP production was done via apheresis using the Amicus™ cell separator (Frei senius-Kabi company) on the day before surgery. 100 ml of PRP with a platelet concentration of about 1:7 compared to pre-values was collected without any additive solution media within 30–45 minutes.

Results: The collection of PRP was well tolerated in all females. The amount of transferred fat ranged between 90 ml and 320 ml, the volume added of PRP was a fifth, respectively. No postoperative complications occurred, namely no infection, lump formation etc. All patients were photographed the day before surgery, 10 days, 1 month and three months thereafter.

Conclusion: Breast reconstruction after cancer surgery by PRP enriched lipofilling is feasible without much inconvenience for the patients. PRP collection is simpler and faster as an alternative option – enrichment of fat with adipose-derived stem cells (ADSCs). A drawback is the possible cancerogenic capacity of both, PRP and ADSCs in patients at risk. Further studies are warranted to clarify this.

S02
Zell- und Gewebebanken

S02-2
Fecal microbiota transfer

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In recent years, the number of studies on fecal microbiota transfer (FMT) – sometimes called stool transplantation – has increased significantly. The starting point for many of the research projects was a small randomized study on the treatment of recurrent Clostridium difficile infection (rCDI), in which FMT via duodenal tube was superior to vancomycin [1]. Previously, the efficacy and good tolerability of this therapy had already been shown in a high number of case reports. FMT appears to be effective by restoring a diverse intestinal microbiome, which is usually disturbed in the case of rCDI. By now, many clinics and doctors worldwide are offering FMT for rCDI and are studying the efficacy of FMT in other indications, such as inflammatory bowel diseases.

However, the implementation protocols differ in many respects, for example, with regard to material preparation and administration via duodenoscopy, coloscopy or application as capsules [2]. The donor testing and stool storage differ and are to date not regulated in Germany. In some countries newly established stool banks exist – quite similar to blood banks [3–5]. And this despite the difficulties to apply existing regulations for therapeutic agents or transplantations on FMT – with the major component being a complex biosystem and with huge differences in the composition by individual donor.

In Germany, we are now striving to find a solution as well in order to offer FMT as a standard treatment for rCDI (and not only a “individueller Heilverfahren”) and in order to conduct clinical trials on our own.

References:

S02-4
Tissue banking

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The transplantation of tissues is widely used in orthopedics, traumatology and reconstruction after extensive tumor resections as well as in ophthalmology and cardiovascular surgery. Of particular interest are the new lamellar techniques in keratoplasty and the use of massive cancellous blocks in periacetabular osteotomies. One main risk after transplantation is the potential transmission of clinically relevant microorganisms like viruses, bacteria or fungi. Tissue banks has therefore established a safety concept for the manufacture of musculoskeletal tissue transplants, which contains donor selection with general and tissue-specific criteria, laboratory testing following the standards of blood donation services, tissue procurement including reverent reconstruction, tissue processing under clean room conditions and quality assurance. Furthermore, for some tissues inactivation procedures were developed to protect the biological properties of the tissue and to guarantee a high microbiological security as well. Quality assurance in accordance with German AMWV and TPG-tissue regulation ensures the lawful work of Tissue Banks. A logistical challenge is the introduction of the Single European Code, which is mandatory in Europe from April 2017 onwards.
Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare disease leading to severe bleeding complications of the fetus/newborn in approximately 1 in 10,000 pregnancies. The disease is caused by maternal alloantibodies against fetal platelet antigens (HPA) inherited from the father. These antibodies will cross the placenta and cause thrombocytopenia and/or disturbance of the endothelial integrity leading to intracranial hemorrhage (ICH). This review summarizes the antenatal as well as postnatal diagnostic and therapeutic options in FNAIT. Since antenatal screening programs have not been implemented so far, FNAIT cases are usually detected at birth. Administration of compatible platelets to the newborn in cases of active bleeding or severe thrombocytopenia is the treatment of choice. In case matched platelets are not available, random platelets should be administered initially. Subsequent pregnancies require diagnostic and prophylactic interventions. Non-invasive fetal HPA genotyping is required if the father is heterozygous for the implicated HPA antigen to determine whether the fetus is at risk. Based on risk stratification, first-line antenatal management in FNAIT is weekly IVIG (1 g/kg/bw) starting early in the second trimester. In conclusion, the current FNAIT management strategies in subsequent pregnancies completely avoid all invasive procedures, e.g. amniocentesis, fetal blood sampling, and intrauterine transfusions. The analysis of anti-HPA antibody sub-specificity has potential in prediction of ICH risk and may allow risk-adapted IVIG prophylaxis in the near future.

Changes in quality of life after unrelated bone marrow donation are dependent on donor sex but not on volume harvested

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Introduction: Hematopoietic stem cells can be procured either by bone marrow (BM) aspiration or peripheral blood stem cell (PBSC) collection methods. The goal of this longitudinal investigation was to describe health related quality of life (HRQoL) experiences after unrelated allogeneic BM donation.

Donors and Methods: Unrelated donors were offered to participate in a HRQoL survey (SF36/SF12 questionnaire) between 2002 and 2016. Time points where donors were asked were: donor clearance (WU), morning before donation, 12 hours after donation, 4 weeks after and later on a yearly base. The SF36/ SF12 questionnaire delivers scales for bodily pain (BP), general health (GH), mental health (MH), physical functioning (PF), vitality (VT), emotional role (RE), social functioning (SF), role physical (RP). Scores can be summarized into a mental (MCS) and a physical (PCS) component score, based on a principal component analysis of the factors mentioned above. Scores are normalized on age and sex based on the actual German standard survey. Thus, a score of 50 represents normal values, a score of 60 a value one SD above the norm.

Results: Out of 315 donors 175 (55%) participated in this survey (113 men (36.5y ± 8.6) and 62 women (37.6y ± 8.7)), answering at least one of the SF36/SF12 HRQoL surveys. In all donors maximum hospital stay was one day. The most recent 90 donors (45 responder) were treated in a day surgery setting. Mean collected bone marrow volume was 896 ± 306 ml, with more volume collected in males. At WU men showed lower MCS (48.3 vs 56.5) while higher PCS (53.9 vs 45.2) than women. Before donation donors seem to be healthier than the normal population (PCS 54.2 ± 4.8, MCS 51.4 ± 6.9) without sex related differences. Directly after donation men were more mentally impressed (MCS 61.7 vs 58.4) while feeling more handicapped due to lower PF (13.7 vs 18.9). 4 weeks after donation and later on women report better BP (60.1 up to 65.0 after 2y) than men (58.0 up to 60.4 after 2y). Assessment after 1year revealed that almost all scales normalized to values at WU in our donors. To our surprise, bone marrow volume taken had no impact on the analyzed HRQoL scores, even when analysis was stratified for sex.

Conclusion: BM collection impacts HRQoL only temporally. In this temporary impacts men showed different pattern compared to women: women are physically less affected by the donation, men showed more overwhelming euphoria shortly after BM collection.
Transfus Med Hemother 2017;44(suppl 1):2–87 Abstracts

PS01
Joint IHN-DGTI: Hemovigilance

PS01-1
International cooperation for haemovigilance

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Haemovigilance is defined by the International Haemovigilance Network (IHN) as "A set of surveillance procedures covering the whole transfusion chain (from the collection of blood and its components to the follow-up of recipients), intended to collect and assess information on unexpected or undesirable effects resulting from the therapeutic use of labile blood products, and to prevent their occurrence or recurrence." Definitions of most major adverse reactions have been published by the International Society of Blood Transfusion (ISBT) and IHN, and guidance on establishing and sustaining haemovigilance systems is available from the World Health Organization and other sources. Many countries in the world have, or are in the process of developing, haemovigilance systems. The frameworks, scope and content vary internationally. For example: does it involve voluntary or mandatory participation? Does the system collect donor, product and recipient events [true 'vein to vein'], or just recipient adverse reactions? Are all events included or just serious ones? Are all reports analysed, or only confirmed cases? Is the analysis performed by an independent expert group, or by the reporting Institution? These differences have important influences on the operations of, and outputs from, the haemovigilance system, and should be taken into account when analysing the data and reading the reports. However, there is no 'right' or 'wrong' way to 'do' haemovigilance — what is more important is that the system is conceived to work with the health system in the particular country.

Most established haemovigilance systems publish reports which are publicly available to inform health authorities, clinicians, blood services, researchers, scientists, manufacturers, and the wider community. Most are also willing to share information on their processes, tools, and results, with the intention of strengthening systems and providing data to inform readers and for international benchmarking purposes. Individuals interested in haemovigilance are welcome to join ISBT's working party on haemovigilance, and haemovigilance systems are welcome to join IHN, to share experience and participate in international collaborative activities including education, data sharing and analysis, and benchmarking.

PS01-2
Serious adverse transfusion reactions – Reporting rates and effect of risk minimization measures

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Introduction: Annual reports of serious transfusion reactions and events based on Section 16 TFG and/or Section 63i AMG enable the Paul-Ehrlich-Institut (PEI) to monitor the safety of blood components.

Methods: Based on the number of reported reactions such as transfusion transmitted bacterial infection (TTB), viral infection (TTVI) and immune mediated transfusion related acute lung injury (TRALI) and the number of distributed blood components annual reporting rates were calculated. After implementation of risk minimization measures reporting rates related to pre- and post-implementation periods (1997–2012) were compared.

In addition the annual frequencies of transfusion transmitted HEV infections, incorrect RBC transfusions and donor reactions during a 4 year reporting period have been documented without implemented risk minimization measures.

Results: A significant reduction was registered following the introduction of HCV NAT testing (from 1:0.6 to 1:83.16 million units administered), the implementation of donor screening for antibodies to hepatitis B core antigen (from 1:2.90 to 1:10.70 million units), the risk minimization measures for TRALI (from 1:0.094 to 1:2.42 million fresh frozen plasma (FFP) units) and the limitation of shelf life for platelet concentrates (from 1:0.088 to 1:0.19 million PC units). No significant risk reduction was recorded after introduction of HIV NAT pool-testing and the implementation of pre-donation sampling.

From 2012 to 2015 the number of HEV transmissions varied between 1 and 5 cases per year and the reporting rate of incorrect RBC transfusions increased from 1.5 to 13.5 cases per million administered units. During the same period the PEI received also an increased number of donor reactions. These reports mostly involved local reactions but some also referred to systemic reactions e.g. haemolysis, embolism and infiltration, which mainly occurred associated with apheresis donation.

Conclusion: Based on haemovigilance data, a significant benefit has been demonstrated for four out of six implemented risk minimization measures. Calculation of reporting rates seems to be a valuable tool in monitoring the effectiveness of safety measures. With regard to an increasing number of adverse reactions coming into focus future potential risk minimization measures may be discussed.

PS01-3
Emerging infections and blood safety

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Western blood transfusion services face a wide range of infectious threats to blood safety, due to the popularity of travelling abroad, and the emergence (or recognition) of new infections at home. Fortunately most of the infections involved are rare or short-lived among blood donors, and the consequences for infected recipients often are limited. Nevertheless infectious threats deserve constant monitoring, and evaluation regarding the necessity of appropriate interventions.

Outbreaks of arbovirus infections occur on a global scale. Travelling blood donors run the risk of acquiring West Nile fever, zika, dengue, chikungunya or even yellow fever abroad. Except for chikungunya, these infections (probably including yellow fever virus infections in Brazil) often are asymptomatic, and blood donors unknowingly may be viremic while donating blood, shortly after returning home. Strikingly the consequences of transfusion-transmitted dengue, zika and chikungunya virus, even in immunosuppressed patients, seem very mild. Transmission of WNV or YFV can be fatal. Because arbovirus-viremia in infected blood donors is short lived, prevention is simple: donors returning from arbovirus-affected areas should be deferred for 4 weeks after their return.

Imported arbovirus infections may spark secondary outbreaks in Europe, which has repeatedly been reported in the Var department in France. Endogenous West Nile virus infections occur in several areas of southern Europe, necessitating the screening of local blood donations for presence of WNV RNA. The boundary of the WNV endemic area in Europe seems to spread slowly to the north. Also the area of TBE may extend: recently in the Netherlands TBE virus was found in ticks, and 2 patients seem to have acquired TBE in Holland. Usutuvirus is increasingly found in birds in Germany and Holland. A German blood donor was found to be viremic for usutuvirus.

Regarding zoonotic infections VCDJ and HEV deserve attention. One might assume that the mad cow disease problem is behind us and that preventive donor deferral measures can be relaxed. Unfortunately the British Appendix-III study produced complex results, possibly suggesting that dietary exposure to the VCDJ agent in the UK started earlier and lasted longer than assumed. In addition, recently a new case of VCDJ occurred, in a person heterozygous for methionine/valine at codon 129 of the prion protein gene, which could indicate the start of a second wave of human cases. Zoonotic infections with pig-borne hepatitis E virus genotype 3 are
frequent in parts of Europe. Fortunately in most persons HEV gt3 infection is asymptomatic, but in immune-suppressed patients chronic hepatitis E can develop, sometimes causing rapid onset of cirrhosis. Should blood components be made HEV safe, while at the same time transmission of HEV via other sources -such as food- continue? In Ireland, the UK, and the Netherlands the screening of each blood donation for HEV RNA has been implemented recently. It seems logical that, as a next step, the governments involved pay more attention to the real sources of HEV.

PS03
Optimal Use 2: Plasma, PLT

PS03-1
Optimal use of platelets
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Platelet transfusion continues to be the mainstay of the treatment for patients suffering from quantitative and qualitative platelet disorders. In recent years significant changes have been introduced in its preparation, such as the utilization of platelet additive solutions or the use of pathogen inactivation (PI) techniques to prevent the main infectious risk for the recipient of a platelet transfusion, bacterial sepsis. Currently, two methods of PI for platelets are licensed in the European Union. The first one available (Intercept®, Terumo BCT, Belgium) is based on riboflavin and illumination with ultraviolet light. One randomized controlled clinical trial has been published that have looked at the impact of the technology on different aspects of platelet transfusion outcomes. The second one introduced (Mirasol®, Cerus, the Netherlands) is based in the combination of amotosalen and ultraviolet A light. Several randomized controlled clinical trials have been published that have looked at the impact of the technology on different aspects of platelet transfusion outcomes. The second one introduced (Mirasol®, Terumo BCT, Belgium) is based on riboflavin and illumination with ultraviolet light. One randomized controlled clinical trial has been published and there is another one on-going. Currently there is another technology under investigation which uses ultraviolet light and strong agitation (Theraflex UV-Platelets, Macopharma, France). Fortunately, randomized controlled clinical trials have been published that has helped us to guide the use of platelet transfusion in questions such as the dose, the transfusion threshold and even the strategy, i.e. prophylaxis or therapeutic. In this presentation, the available evidence will be reviewed.

PS03-2
Optimal use of plasma
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Plasma, the acellular portion of blood, is made into multiple blood components and fractions. For the basic transfused blood components, plasma and cryoprecipitate, indications continue to evolve. In 2010, Roback and colleagues published a systematic review of the indications for plasma and found few. Plasma is indicated for the replacement of factors for which specific factor concentrates do not exist and for large volume plasma replacement as in plasma exchange for thrombotic thrombocytopenia purpura. The utility of plasma in trauma resuscitation was strongly suggested, but the evidence was felt weak. Since that time, wider understanding of cell-based coagulation and broad experience with hemorrhage control resuscitation suggests that plasma, used in a 1:1 unit ratio with red blood cells, is the ideal resuscitation fluid for massive uncontrolled hemorrhage. We have level 1 evidence that such use is safe, and strong level 3 evidence that it is effective. Beyond trauma resuscitation, plasma is important in other massive hemorrhage situations. The volume of bleeding and mortality in cardiac surgery and liver transplantation is greater when 5 units of red cells are given without equivalent amounts of plasma. Cryoprecipitate is largely used for fibrinogen replacement, but at least in vitro, the blood component is more effective for treating hyperfibrinolysis than purified fibrinogen concentrate alone. Retrospective data suggest excess mortality in trauma and obstetric hemorrhage when the fibrinogen concentration is less than 2g/L. Minimum plasma concentrations for coagulation factors in non-hemorrhage situations are not rigorously defined, but experience with hemophilias suggests that they are low.

PS03-3
Reducing blood bank sample errors using electronic patient identification
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Errors leading to ABO mistransfusion can occur at essentially every step in the blood collection and transfusion process. In Germany, bedside pretransfusion agglutination testing essentially eliminates the risk of accidentally transfusing an ABO incompatible red blood cell unit. Even bedside testing, however, cannot prevent hemolytic reactions due to non-ABO antibody that may result from a variety of pretransfusion errors. Wrong Blood in Tube (WBIT) errors made during blood bank sample collection represent a distinct pre-analytic source of preventable error. A multi-Institutional, international study reported by the Biomedical Excellence for Safer Transfusion (BEST) Collaborative in 2003 showed that WBIT errors occurred at a median corrected rate of 1 per 1986 samples drawn. A follow-up study performed across sites in the U.K. found a median corrected rate of WBIT of 1 per 1303 blood bank samples, with high degree of variation in policy and practice across sites.2 In recent years, electronic patient identification systems designed to reduce phlebotomy errors have been implemented at many healthcare facilities. The impact of these systems on patient safety has not been systematically evaluated. This presentation will discuss a new BEST study aimed at measuring the safety benefit provided by using electronic patient identification systems such as bar code scanning versus conventional manual patient identification at the time of blood bank sample collection.

References:
Acquired haemophilia (AH) is a rare autoimmune bleeding disorder caused by neutralising antibodies against coagulation factor VIII, occurring in persons without a previous bleeding history. Patients present with various bleeding patterns, for example large skin haematomata, bleeding into muscles, soft tissue and mucous membranes together with a prolonged aPTT, caused by a reduced factor VIII activity. Diagnosis is confirmed by measuring the antibody with the Bethesda assay or its Ni- jmegen modification. A limitation for quantification of the antibody is a high inter-laboratory variation of test results. An alternative method is an anti-FVIII IgG ELISA, which is not influenced by the type 2 kinetics of the antibody and can provide additional prognostic information.

In approximately 40% of cases AH is associated to an underlying condition like other autoimmune diseases, malignant diseases or pregnancy. The primary goals of management are bleeding control and eradication of the inhibitor. In patients with high titre inhibitors and FVIII activity < 1% bleeding control can be achieved with bypassing agents: recombinant activated factor VII (rFVIIa), activated prothrombin-complex-concentrate (aPCC) and recombinant porcine factor VIII (rpFVIII). In patients with low titre inhibitors and measurable residual factor VIII activity high doses of factor VIII might overcome the inhibitor and be effective for bleeding treatment. Invasive procedures should be avoided if possible.

Different immunosuppressive regimens have been used to eradicate the inhibitor, resulting in remission of disease in 60–80% of patients over a period of days to months. Immunosuppression (IS) is associated with frequent adverse events, including infections as a leading cause of death. First line treatment consists of steroids alone or in combination with cyclophosphamide. Addition of cyclophosphamide upfront can shorten time to remission but has no significant impact on survival. For second line treatment or if cyclophosphamide is contraindicated, the CD 20-antibody rituximab or other immunosuppressive agents can be used.

The GTH-AH 01/2010 study, a multicentre, prospective non-interventional study, in which patients have been treated with IS following a uniform consensus protocol, demonstrated that partial remission was achieved less frequently, after a longer time, and with a lower rate in patients with a baseline factor VIII activity < 1%.

Despite these treatment options AH is a disease with a high mortality of 26–43%, caused by infections, bleeding and the underlying disease. The GTH-AH 01/2010 study demonstrated that prognostic factors for poor overall survival are a baseline factor VIII activity of < 1%, underlying malignant disease and a WHO performance status of > 2.

In summary, AH is a serious, mostly unexpected autoimmune disease with still high mortality and morbidity caused by bleeding and infections as major complications of disease and treatment.
projects provide the financial backbone for our cell therapy activities. We currently dedicate most of our clinical development activity to immunotherapies and regenerative medicine. As to the former, most cater to specific immunological problems of patients during and after stem cell transplantation, such as antigen-specific and allo-depleted donor T-cell products, supposed to provide GvHD-free adoptive T-cell immunity, cytokine-induced killer cells for minimal residual disease or overt relapse of leukemia, or mesenchymal stroma cells against GvHD. Two distinct programs for manufacturing of CAR-modified T-cells for B-cell malignancies are ongoing and together with a CAR-modified NK cell line for glioblastoma which is currently entering clinical trials represent our portfolio of anti-cancer immunotherapies. In the field of regenerative medicine, products for cardiovascular regeneration after MI, diabetic peripheral angiopathy, and bone augmentation for high-risk fractures are being explored. Examples and clinical snippets for each product group will be presented.

PS05-3
Invisible organs
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Modern transplantation medicine has made significant progress within the last decades due to a better immunological understanding of rejection and advances in immunosuppression. However, the severe side effects of long-term, typically lifelong, immunosuppression and the shortage of donor organs remain the major restrictions in transplantation. The idea behind all research to improve transplant outcome has always been the modification of the recipient’s immune system to ideally induce a specific tolerance towards the donor’s graft. In fact, the immunological blindness of the recipient towards the donor’s graft is achieved by a general reduction of the immune system’s competence and represents a major burden for transplant patients. The idea of invisible organs is an entirely different approach to solve the problem: instead of inducing an immunological blindness of the recipient’s immune system an immunological invisibility of the donor’s organ is created. This is achieved by genetically engineering the transplant to eliminate the organ’s immunogenicity defined by the gene products of the major histocompatibility complex (MHC) and presented allogeneic peptides referred to as minor histocompatibility antigens (mHag). Silencing of MHC genes in an entire organ is possible by genetically replacing the gene products of the donor’s organ is created. This is achieved by genetically engineering the transplant to eliminate the organ’s immunogenicity defined by the gene products of the major histocompatibility complex (MHC) and presented allogeneic peptides referred to as minor histocompatibility antigens (mHag). Silencing of MHC genes in an entire organ is achieved during ex vivo perfusion by lentiviral transduction of short hairpin RNAs (shRNA) under the control of an epigenetically stable promoter targeting essential structures and regulators of MHC proteins. Importantly, MHC silencing also prevents the presentation of minor histocompatibility antigens, which usually are not possible to match between donor and recipient, but which trigger potent immune responses and graft rejection. Eliminating the targets of cellular and humoral rejection camouflages the graft by making it immunologically invisible for rejecting T cells and MHC antibodies. This allows keeping the recipient’s immune system fully functional and capable to combat infections and cancer. This approach has the potential of avoiding rejection without the need of relevant immunosuppression, thereby increasing transplant survival, life quality and organ availability. Preclinical in vivo studies show a clear survival advantage of these engineered tissues. Thus, genetic ex vivo engineering of transplants are capable of creating immunologically invisible organs which have the potential to sustainably eliminate the burden of organ rejection and immunosuppression.
Purpose: Autologous serum (AS) eye drops offer a potential treatment alternative for non-healing corneal epithelial defects. In corneal epithelial cell cultures, fetal bovine serum (FBS) is often used to support the growth of the cells. Our aim was to study and compare the dose-dependent effects of human serum (HS) and FBS on HCEC migration, proliferation and viability in vitro, and to determine FGFb, HGF, KGF and TGF-β1 concentration in the supernatant of keratocytes following incubation with HS.

Methods: AS was prepared from 13 patients according to the regulations of the LIONS Cornea Bank Saar-Lor-Lux, Trier/Westfäl. HCECs were firstly cultured in DMEM/F12 with 5% FBS, 0.5% DMSO, 10 ng/mL human epidermal growth factor, 1% insulin-transferrin-selenium, until reaching confluence, then were incubated in serum media which was consisting of DMEM/F12 supplemented by 5%, 10%, 15% or 30% AS or FBS for 24 hours. Thereafter, HCEC viability was analyzed using Cell Proliferation Kit XTT, HCEC migration using wound healing assay and HCEC proliferation by the cell proliferation ELISA BrdU (colorimetric) kit. Primary human keratocytes were isolated from human corneoscleral rings using collagenase A (1mg/ml) (n = 1) and were cultured in DMEM/Ham’s culture medium with 10% fetal bovine serum (FBS). Keratocyte cultures were incubated in 15 or 30% HS and FGFb, HGF, KGF and TGF-β1 concentration was determined by ELISA from the supernatant of the culture after 24 hours.

Results: HCEC viability was the highest at 30% AS or 15% FBS and the lowest at 10% AS or 30% FBS application. HCEC migration was the quickest through 30% AS or 30% FBS and the slowest through 5% AS or 5% FBS concentrations. Proliferation was the most increased through 15% AS or 5% FBS and the least increased through 30% AS or 30% FBS concentrations. HCEC viability at 10% and 15% AS was significantly worse (P = 0.001, P = 0.023) compared to baseline and significantly better at 15% FBS (P = 0.003) concentrations. HCEC migration was significantly worse (P ≤ 0.007) and HCEC proliferation significantly better (P < 0.001) in all concentration groups compared to baseline.

HGF concentration was, for both HS concentrations, significantly higher in the supernatant of keratocytes, than in HS controls (without keratocytes) following 24 hours (p < 0.01). FGFb concentration was significantly increased in 30% HS with keratocytes compared to 30% HS without keratocytes after 24 hours (p < 0.01). TGF-β1 and KGF concentrations remained unchanged through keratocytes.

Conclusions: HCEC viability is most increased through 30% AS or 15% FBS, migration through 30% AS or 30% FBS and proliferation through 15% AS or 5% FBS. In addition, AS better supports HCECs viability and migration than FBS. HGF and FGFb concentrations increase in the supernatant of keratocytes, 24 hours after incubation with human serum. These concentration changes may play a role in wound healing of epithelial defects.

Abstracts

JS03

Joint Session DOG-DGTI: Autologe Serum Augentropfen

JS03-2

Effect of human serum (HS) on human epithelial cells (HCEC) and keratocytes

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JS04

Joint Session ISBT WP-Cellular Therapy: Platelet Lysates

JS04-1

Production and quality control of platelet lysates worldwide – an approach towards GMP-grade manufacturing

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Platelets are essential not only for primary haemostasis but also for endothelial integrity and wound healing. They abundantly store growth factors, cytokines and chemokines for supporting tissue regeneration after injury. For regenerative medicine two principles of platelet preparation have been developed predominantly, depending on different scopes of application: Platelet rich plasma (PRP) is applied locally or topically for clinical tissue repair, whereas human platelet lysate (HPL) is used as a highly efficient animal-serum-free medium supplement for in vitro cell culture, replacing fetal bovine serum, in a growing number of applications. For both types of platelet preparation the critical variables for product quality besides donor selection are concentration of platelets, leukocytes and fibrin, as well as composition of growth factors and other plasma proteins. Although the usual starting material are platelet concentrates from healthy blood donors, there exist divergent protocols for platelet enrichment, storage, lysis and HPL production due to in-house manufacturing at blood centers or industrial production. Notably, the significance of ABO blood groups and isoagglutinines as well as the impact of gamma irradiation or pathogen inactivation of platelet concentrates on HPL quality and functionality is still unclear. At present it is not possible to provide common recommendations for a standardization of HPL production, also due to a lack of systematic lab-to-lab comparison. As soon as HPL is used as a ‘raw/ancillary material’ for manufacturing of cell based medicinal products, the national and international regulatory requirements have to be considered. For GMP-grade manufacture of HPL a qualification program needs to be established including risk-assessment and performance testing. Product specification and release criteria have to be defined in addition. For the future, continuous efforts will pave the way to a standardized product for manufacture of selected cell products.

JS04-2

Human platelet lysate as cell culture supplement

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It is well known that thrombocytes can contribute to wound healing processes, not only by initiating the coagulation cascade but also by secreting a plethora of soluble factors. Platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) for instance mediate pro-inflammatory processes, PDGF and transforming growth factor-beta (TGF-b) stimulate fibroblast proliferation, fibroblast growth factor FGF and VEGF induce collagen synthesis and wound retraction, prior to epithelial growth factor (EGF) promoting epithelisation. Besides these few mentioned, platelets store many more factors in their lysosomes, dense and alpha-granules. Based on these, there have been early attempt to utilize platelet preparations, most commonly as platelet-rich-plasma in a variety of clinical indications beyond the classical use of thrombocytes. Phase III and IV clinical trials were undertaken for instance in orthopaedics, odontology, ophthalmology and dermatology. Systematic Cochrane reviews however so far were not able to provide sufficient evidence supporting clinical efficacy. Since the 80s, platelet-derived factors have been tested as cell culture supplements, first for cell lines. However, with the rapid progress in the cell therapy field and associated increasing regulatory demands, human plate-
Commercial production and new developments in platelet lysates including pathogen inactivation and prion removal

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Academic research work, typically conducted using single-donor or small-pool platelet concentrates as starting materials, has now convincingly established that human platelet lysates (hPL) can reliably substitute for fetal bovine serum (FBS) as supplement of growth media used for expanding cells in vitro cultures. This demonstration has been proven using mesenchymal stromal cells originating from various tissues, as well as for several differentiated cells. As such, hPL, due to its human origin, is emerging as the new “gold standard” for xeno-free clinical-grade growth medium supplement. hPL provide an immunological and infectious safety profile objectively superior to that of FBS, while also allowing good cell growth productivity. hPL is now available from blood establishments in some countries as well as commercial suppliers. These hPL are typically prepared by pooling at least 40 to 50 expired platelet concentrates (corresponding to up to approximately 250 donors), as pooling contributes to inter-lots consistency in quality parameters and performance. However, it is felt that one still needs to better understand how donors demographics [such as age], use of platelet additive solution, production parameters [such as methods for (a) inducing platelet lysis or activation, or (b) removing fibrinogen] can impact hPL specifications [such as protein and growth factor content] and capacity to expand certain types of cells. With regards to viral safety, recent publications have reported on the characteristics and cell-promoting effectiveness of hPL prepared from pathogen-reduced platelet concentrates [using sporalen/UVA or UV treatments] or subjected to dedicated batch inactivation [solvent/detergent] or removal [adsorption] procedures of viruses and prions. Data suggest that these additional virus safety measures do not alter the capacity to use hPL as growth medium supplement. These results reinforce the belief that hPL will be playing an ever increasing role as xeno-free adjunct component in clinical applications of human cell propagation and regenerative medicine.

Pathogen Inactivation and function of platelets and red cells

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The treatment of blood components with pathogen inactivation (PI) technologies represents a balance between increased blood safety and alteration to the component that appears as a reduction in quality when assessed by laboratory or clinical investigations. Damage to the components occurs either from the effects of the ultraviolet (UV) radiation itself, or from chemical byproducts generated from compounds added to the units prior to UV exposure. Gaining a detailed understanding of the changes induced by PI treatments is an essential step in developing strategies to minimize the negative effects of PI on blood components. PI technologies are designed to interfere with a pathogen’s ability to replicate, an essential feature of establishing infection, by disrupting nucleic acid structure. They have a similar effect on nucleic acids in blood cells, disrupting DNA and RNA in leukocytes, platelets and reticulocytes to varying degrees. In addition, reactive oxygen species may be produced that cause biochemical changes beyond nucleic acid alteration. As treated cells are living, they respond to the PI treatments in varying ways. Although our understanding is incomplete, the largest volume of data exists for platelet concentrates. PI treatment accelerates the development of platelet storage lesions and, in general, these changes are indistinguishable from those which occur over the platelet storage period under standard blood banking conditions. Platelet RNA species are significantly altered by PI treatment as would be expected from the use of these non-specific approaches, and protein synthesis is markedly reduced. Platelets actively respond to PI treatment as evidenced by activation of signal transduction and apoptotic pathways even without full protein synthetic capacity. One result of these biochemical changes is the increased release of platelet microvesicles. PI treatment also has direct effects on proteins. Some of the proteins are among those for which plasma is transfused, including some coagulation factors. Although different treatments have varying effects on individual types of proteins, all PI cause some damage to proteins. Red blood cells have been least studied, in large part because they have been the most recent target of PI technology development. It is clear that PI treatments will also cause changes in RBCs although it appears that the degree varies considerably between the two technologies currently in late stage development. Some strategies to maintain RBC quality after UV exposure are being explored including modified storage solutions and reduction of oxygen levels. Although PI technologies for the treatment of all types of components are able to be used in their current form without apparent detriment to patients, the measurable reduction in product quality seen with these first generation products argues for continuing effort to improve PI technologies.

Epidemiologic data of blood donors in Germany

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Blood donor surveillance is an essential part of the hemovigilance system. Active blood donor surveillance for HIV, HCV, HBV and syphilis infections has been mandatory in Germany since 1999. All blood establishments report confirmed positive results for HIV, HCV, HBV and syphilis and number of donors and donations stratified for age group, sex, type of donor (first time donor (FTD), repeat donor (RD)) and type of donation (whole blood, plasmapheresis and cytapheresis). Data from the EDQM report on the collection, testing and use of blood and blood components in Europe allows for international comparisons. Infections rates for HCV and HBV have declined between 1999 and 2016 in first time (FTD) and repeat donors (RD). HIV infection rates in FTD did not change significantly over time, the HIV incidence rate increased between 2007 (1.6/100,000 RD) and 2012 (3.3/100,000 RD) but dropped again to 2.0/100,000 RD in 2016 indicating fluctuation. While prevalent syphilis infections rose slightly in FTD over time, incident syphilis infections in RD increased in the past 10 years with a peak in 2015. Male donors are significantly more often diagnosed with any of the screened infections. In the past five years, HIV infections rates were 3.9 to 9.0 fold higher...
and syphilis infection rates were 1.6 to 8.4 fold higher in men than in women. Syphilis infections in female RD increased in the past five years. Comparing infections rates between Germany, Belgium and the Netherlands, HIV rates in RD were comparable in all states. HBV rates in RD were similar in Belgium and Germany. Rates for HCV in FTD and RD were highest in Germany and the described rise in incident syphilis infections was also not observed in the neighbouring states. Both Belgium and the Netherlands have a donor population of approximately 10% of the number of donors in Germany and therefore infection rates might be subject to greater fluctuation. Still, reasons for changes in infection rates over time and differences in states with a similar epidemiology need further investigation and warrant additional safety measures: Motivation for donation should be analyzed carefully and groups with higher infections risks should be addressed directly. There is also potential for optimized donor selection, e.g. by using standardized and validated questionnaires. In addition, routine surveillance as well as sentinel studies and second generation surveillance in the general population may help to understand changes in infection patterns and should be included in a risk analysis. This can be achieved by interacting closely with the competent authorities and the National Public Health Institutes.

Information on the spread of infections in different donor populations helps to assess transfusion risks and is also needed for modelling of transfusion risks of established and new or emerging infections.

ND02-3
Donor Health Care

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Donor selection and vigilance: Donor Health Care is getting more and more attention and the scope is changing. One of these changes is integrating several kinds of donations in research and education; blood, cells, tissues and organs. Another change is increasing attention for donor vigilance, especially long term effects such as depletion of iron. An European consortia is collaborating in a European granted project; Donor Health Care, http://www.donorhealthcare.org. This educational program offers lessons and tools to improve the quality of professionals working in Donor Health Care. The innovation of this program is integrating all SoHO (blood, cells, tissues and organs). Selection criteria for unrelated and related donors can be regulated by different professional organisations. Although we should have the same safety margins for all donor, this is not the case and there are different levels of donor risks we consider as acceptable. We have a low risk, in case of blood donation on one hand, and clearly a higher risk in living organ donation on the other hand. The ISBT, AABB, HNH and endorsed by the EBA has published ‘Standards for Surveillance of Complications Related to Blood Donation’. This a huge step towards international cooperation and interaction. With respect to attention of all donors of SoHO (blood, cells, tissues and organs), the establishment of www.NotifyLibrary.org is of great importance for both scientific and educational goals.

The ultimate decision making: The global tendency is to endeavour 100% voluntary non-remunerated donations. This is promoted by the International Society of Blood Transfusion (ISBT) and the World Health Organisation (WHO). The WHO promotes to reach this by 2020. This is important to have the right, in terms of safety, donor for the recipient. But reality can influences choices in certain countries facing bigger problems with war, disasters or clean water supply epidemics of infectious diseases. The right donor for transfusion or transplantation only exist it the right donor is known (tested) and available. Comparable with the different risks we expose our donor to (blood donation versus living organ donation), we accept different risks for the recipients dependent of the availability of SoHO and the life expectancy of the recipients (compassionate need). The right donor for the right recipient depends on policies, cultural-economic aspects, knowledge and means for donor and patient care. We are strict and regulated on one hand, but criteria are ‘negotiable’ in critical need for patient care. The basis of the best care is always education and exchange of knowledge!

ND02-4
Use of medication and blood product quality

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During blood donor screening, assessment of medication use is usually one of the aspects in the donor questionnaire. Not much is known about the relation between use of medication and the effect on blood product quality. Two studies to investigate the effect of medication and/or underlying disease on blood product quality will be discussed.

1. Effect of pain relievers

Buffy coats (BC) from donors using pain medication with an inhibitory effect on platelet function, like aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), are often excluded for platelet production. However, if the medication really affect the quality of platelets is unknown. We performed a study in which we analyzed the in vitro quality during storage of BC-derived platelet concentrate (PC), obtained from donations by donors who used aspirin or NSAIDs up to 4 days prior to donation. In total 18 PC from donors using different pain medication were used. On Day 8 all groups showed comparable pH and changes in platelet content. Minor negative effects on aggregation or thromboelastography were measured on Day 1, but these differences disappeared during storage. Main storage properties of PC prepared from NSAID-BC were comparable with PC from normal controls, especially PC from donors who used ibuprofen showed little or no deviations. We conclude that discarding BC from donors using pain medication has to be re-evaluated, also because the minor effects will be diluted in pooled buffy-coat derived PC.

2. Effect of Type 2 Diabetes (T2D)

In most countries, donors with oral medication like metformin for treatment of T2D are accepted as donors, as long as not using insulin. It is unknown if their blood components have a similar quality as from healthy donors. Previously we showed that donors could be classified as having platelets with good, average or poor storage properties [Bontekoe, Transfusion 2017; doi: 10.1111/trf.14238]. A main difference between ‘good’ and ‘poor’ storage properties involved metabolic activity, resulting in increased glycosylation and faster decline of pH during storage of ‘poor’ PC. This might be caused by a different functionality of the mitochondria and donors with a history of ‘poor’ PCs are more likely to have Metabolic Syndrome or T2D. We hypothesized that PC from donors diagnosed with T2D, but eligible for donation, would show an increased tendency to be qualified as ‘poor’. We studied in vitro characteristics of PC from a group of 12 donors diagnosed with T2D (8/12 using metformin, 3/12 without medication), compared to a group of age and gender matched normal donors. Donors with T2D had a higher mean BMI and higher HbA1c than controls. On Day 8, the average in vitro quality was comparable in both groups. PCs from donors with T2D who did not use insulin and fulfilled donor criteria, were comparable with PCs from age and sex matched controls. The policy of accepting donors with diagnosed T2D, either with or without oral medication, seems to be appropriate.
Hematopoietic stem cell lineage commitment

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Multipotent hematopoietic stem cells (HSCs) are responsible for the lifetime production of all blood and immune cells. In the classical model of hematopoiesis, blood formation is believed to occur through stepwise progression of hematopoietic stem cells following a tree-like hierarchy of oligo-, bi-, and unipotent progenitors. However, this model is based on experimental approaches unable to describe how individual HSCs and their progeny enter lineage commitment during steady-state hematopoiesis. To establish a comprehensive model of human hematopoiesis, we have developed single-cell approaches that integrate single-cell RNA-sequencing with flow cytometric and functional lineage potency data. This allows us to reconstruct developmental trajectories and to gain a detailed view on lineage commitment of individual HSCs into all major branches of human haematopoiesis.

We found that individual HSCs do not pass through discrete intermediate progenitor cell stages. In contrast, HSC lineage commitment occurs in a gradual manner best described by a continuous Waddington landscape with initially flat but progressively deepening valleys. Our data determine a detailed model of developmental trajectories within this landscape and demonstrate that distinct gene expression modules operate in a combinatory manner to control stemness, early lineage priming and the subsequent progression into all major branches of hematopoiesis. These results establish the concept of a developmental continuum, which can replace the `differentiation tree’ as a comprehensive model of human steady-state haematopoiesis and provide a basis for the understanding of hematopoietic malignancies.

Quacks and charlatans: untested stem cell therapies

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Unproven or untested stem cell therapies are commercial treatment programs with stem cells which have not been tested for safety and efficacy in clinical trials. Thus, they do not have approval by regulatory agencies. However, depending on the nature of the cell preparation and the country where the treatment takes place, such authorization may legally not be necessary for direct-to-consumer (DTC) marketing, i.e. independent of health care professionals or insurances. Often there is no detailed information on the cells or their derivatives, the method of application and the intended mode of action. Nevertheless, hundreds of clinics, practices and other facilities around the world offer stem cell-based products and therapies for the treatment of incurable or difficult-to-treat diseases. Treatment is performed both on an outpatient – and a stationary-based and often in combination with longer psychotherapy sessions. The clinics are located not only in countries with lax regulations such as China and Mexico, but also in the US and Central Europe. A study from the year 2016 has identified more than 400 independent websites with offers for stem cell therapies worldwide. In addition to hundreds of clinics in the US, there were also more than 10 offers in Germany. These often change websites without a detailed description of the method of treatment, which makes a legal classification and prosecution difficult for the regulatory authorities. Alleged patient recommendations as well as treatment sites in unnamed third countries are common. Moreover, all these offers praise the regenerative potential of stem cells and are directed at patients in often desperate situations. In my presentation, I will summarize the range of untested stem cell therapies and refer to informational offers for interested and affected persons.

CMV by NAT, of which 32 donations were tested positive (0.02%). Higher yield of NAT-positive donations can be explained by higher input volume of analyzed plasma. CMV contamination of investigated blood products, such as platelets, erythrocyte concentrates and fresh frozen plasma, could be proved by PCR analysis. Despite leucocyte-reduction Cytomegalovirus could be detected in blood products. CMV-NAT screening is a strategy to identify viraemic donors and consequently contaminated blood products.

References:
1 Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie) Bundesanzeiger Nr. 101a.
2 Roth et al.: Vox Sang 99;2010;suppl 1:276.

POG02-4
Donor safety under the individualized plasma donation program IPS – an interim analysis
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The aim of this study is to investigate the donor safety of donors who donate plasma according to an individualized plasma donor program (IPS) in comparison to donors, who donate according to the guidelines currently valid in Germany.

Materials and Methods: Each donor can participate in this study; the time of participation per donor is 3 years and can extended up to 3 times for 3 years respectively. 760, 820 and 860 mL plasma per donation may be donated depending on the body weight of the donor (50–59 kg: 60–69.9 kg and > 70 kg). Depending on the Immunoglobulin G (IgG) concentration in the donor’s blood before taking up the donor activity (6–7.99 g/L; 8–9.99 g/L and >10 g/L) the donor can donate plasma up to 1 time per 14 days, once a week or twice a week (verum group). Donors of the control group of this study donate (plasma volume and donation frequency) according to the conditions of the current german hemotherapies guideline. All unwanted events/serious incidents (UE/SI) occurring during the donation and between and after plasma donations were recorded by the physician and entered by the study monitor into a database and were evaluated statistically. Each event is evaluated according to severity from 1 (light) to 5 (fatal), as in the causality with the plasma donation from 1 (no causality) to 4 (causality assured). The recorded events are subdivided into «Circulation and fall in blood pressure», «venipuncture», «technical problems», «citrate induced reactions», «medical problems» and «others». The incidences of these categories were calculated, a trend analysis from 2011 to 2015/2016 was performed and the calculated incidences between verum and control group were compared.

Results: Since 9/2007 16925 donors have participated in the IPS study. On Dec.31, 2016, 5978 donors were registered as active IPS donors and 1 394 825 donations have been performed under IPS. A total of 28 323 donors of this study donate (plasma volume and donation frequency) according to the guidelines of the current german hemotherapies guideline. The overall incidence of all UE/SI from 2011 up to 2015 was 2.28; 1.89; 1.86; 1.91; 1.96 and 2.03 events per 100 donations. The incidence rate of severe (grade 3 to 5) events was 0.023 vs 0.026 (verum vs. control) as at 12/31/2015 and didn’t differ statistically.

Conclusion: An individualized donation program according to IPS is concerning donor safety just as safe as plasma donation according to the actual hemotherapies guideline.

PO1-1
Quality of life of German stem cell donors – a prospective analysis

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Introduction: Every year over 5000 people in Germany donate peripheral blood stem cells for unrelated patients all over the world. Such stem cell donation is associated with physical and psychological stress before and after donation for the donor. Until now there are only few studies, which investigate the mental health of donors before and after stem cell donation. The results of this study can lead to a better estimation and evaluation of the need for the psychological support of unrelated donors.

Methods: This study investigates 360 unrelated donors for peripheral blood stem cells in two collection centres in Germany. The donors got several psychological questionnaires at three different time points (2 weeks before donation, 4 weeks after donation, 6 months after donation). The drop out was 16% (59 donors). The rate of non-compliance was 36% (129 donors), these donors did not answer at the second or third time point.

Results: The quality of life over three different time points was measured with the WHOQOL-Bref. This questionnaire records the quality of life and the well-being of the donors in different domains. Here the physical domain, which measures the self-assessed physical health, was significantly decreased over three time points (X²(2) = 14.392, p = 0.001; d= 0.31). The psychological domain, which measures the self-assessed psychological well-being, revealed no significant change of the psychological well-being of the donors over three time points (X²(2) = 2.875, p = 0.237; d= 0.22).

Conclusion: The results of this study indicate that the stem cell donation has an influence on the quality of life and well-being of the donors, where-by the physical restraints outweigh the psychological restraints of the donors after stem cell donation. This was shown in significantly decreased self-assessed physical health 4 weeks and 6 months after stem cell donation. This effect seems to be not clinically relevant, because of the small effect size (d= 0.31). However, the most important finding of this study is that the psychological quality of life of stem cell donors after donation is not influenced at a measurable level.

PO1-2
Blood donors with disposition for hemochromatosis – Efficient Prevention and gift for others in need

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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 ug/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
Abstracts

- Blood Donor Questionnaire

- Conclusion: Donors from the German Red Cross reported a high satisfaction with the new German Blood Donor Questionnaire and the risk of dropout due to dissatisfaction with the questionnaire seems to be very low. However, from the donors' perspective, the scope of the questionnaire and the questions about sexual risk behaviour could still be improved.

Competing interests: None

References: [1] Blood donation can help persons with genetic determination to improve donor selection. The aim of this study was to describe donor satisfaction regarding different aspects of the new German Blood Donor Questionnaire and to compare the results with donor satisfaction with the first version of the questionnaire.

Methods: A random number of 7,500 blood donors, donating at the German Red Cross Blood Service Baden-Württemberg – Hessian in August 2016, were asked to rate their satisfaction with four different aspects of the German Blood Donor Questionnaire. On a 5-point Likert scale ranging from 'very dissatisfied' to 'very satisfied' donors rated format and layout of the questionnaire, clarity of the questions, the scope of the questionnaire and questions about sexual risk behaviour. Donors with a score of four or five were considered to be satisfied. Chi-square statistic was used to test for significant differences between men and women, experienced and non-experienced donors and different educational level. The results were compared with a previous survey on donor satisfaction with a previous version of the questionnaire.

Results: A total of 2,526 (34.7%) completed questionnaires were returned by the donors. The majority of the donors were satisfied with format and layout of the questionnaire (78.7%) and the clarity of the questions (81.0%). The satisfaction with both other aspects was lower: 53.5% of the donors were satisfied with the scope of the questionnaire and 55.6% with the questions about sexual risk behaviour. A high proportion of dissatisfied donors appeared among men, experienced donors and high-educated donors. However, only 3.8% of the respondents reported that they might reduce further donations due to dissatisfaction with the new German Blood Donor Questionnaire.

Donor satisfaction with every aspect was significantly higher than in a previous survey on donor satisfaction with the first version of the questionnaire from 2011. Satisfaction with format and layout increased by +6%, satisfaction with the clarity of the questions by +8.5%, satisfaction with the scope of the questionnaire by 14.0%, and satisfaction with the questions about sexual risk behaviour by 11.3%.

Conclusion: Donors from the German Red Cross reported a high satisfaction with the new German Blood Donor Questionnaire and the risk of dropout due to dissatisfaction with the questionnaire seems to be very low. However, from the donors' perspective, the scope of the questionnaire and the questions about sexual risk behaviour could still be improved.

How satisfied are blood donors with the new German Blood Donor Questionnaire? Results of a Blood Donor Survey

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Introduction: Recently, a new German Blood Donor Questionnaire has been developed to establish a nationwide questionnaire standard and to improve donor selection. The aim of this study was to describe donor satisfaction regarding different aspects of the new German Blood Donor Questionnaire and to compare the results with donor satisfaction with a previous version of the questionnaire.

Methods: The data of donors with disposition for hemochromatosis at our institute for number of donations / phlebotomies and iron storage parameters were evaluated.

Results: Since 1998 more than 70 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 the normal range. This resulted in more than 600 blood donations besides numerous phlebotomies. 35 of presently 58 active donors not only maintain normal but low iron storage levels (ferritin < 100 ug/L).

Conclusion: The 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.

Trending of IgG and TP values depending on the frequency of the plasma donations

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Introduction: In addition to the basic donation appropriateness, the donation frequency in plasma donations depends to a large extent on the donor IgG & TP concentrations. The question in the routine procedure is whether a high-frequency donation frequency has a short-term or long-term negative effect on the above values. Our goal was a retrospective comparison between 2 different donation frequencies and their effects (with a special focus on the IgG value).

Method: A comparison of new qualified donors of the year 2016 with at least 30 donations from 8 locations was made. The local pool was made up of 30 sp./centre (donation volume according to body weight: 1. group with donation interval of 3 to 7 days and 2. group of 8 to 14 days, on each 5th donation protein values were collected. The mean values, as well as the individual values were compared.

Results: The comparison of the individual IgG and GE concentration as well as the mean value of all 8 pools resulted in an analogous course. It was found that the IgG & TP concentration in both groups was significantly reduced up to the 15th donation. Between the 15th and 30th donations, both groups remained at a relatively constant low level above a mean of 8.5 ± 1.5 g / L. After the 30th donation the IgG value increases. The GE showed an analogous behavior. The ‘regeneration’ despite plasma donations reaches in the first group no longer the original starting value, in contrast to the second group. However, the overall regeneration effect is not significant.

Discussion/Conclusion: It is known that periodic plasmapheresis lead to a sudden drop in the IgG value. In our study, this has occurred in both groups. In the middle and long term, however, this is apparently being compensated. The assumption is that the humoral immune system quickly adjusts itself ‘to the regular IgG loss’. The results can be interpreted as meaning that a periodic donation (2 donations / week) is possible according to the guidelines in the routine. The phenomenon of the ‘regeneration effect’ must be researched further in the future.

Ageing society – ageing donors. Developments in demography and donation activity of blood donors in Germany

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Background: The population in Germany is ageing and facing a negative natural population balance. Due to these demographic changes, the number of individuals who are eligible to donate blood, reached its lowest value in 2012 but has increased slightly since. Detailed knowledge of the demographic profile and donation behavior of donor populations are essential to secure an adequate blood supply.

Methods: Surveillance data from the national donor vigilance reporting system and population data from the federal states statistical offices were used to assess changes in demography and donation frequencies of whole
blood donors and the percentage of donors among the general population over a 10 year period.

**Results:** From 2006 to 2015, the general population aged 18 to 68 decreased by 2%. At the same time, the number of first time and repeat whole blood donors dropped by 21% and 8%, respectively. In 2015, 3.7% of the population eligible to donate was active as repeat whole blood donors (2006: 4.0%). The highest proportion of donors was observed among those aged 18 to 24 (4.5%). As the age structure of whole blood repeat donors roughly resembled that of the general population, the greatest number of donors were 45 to 54 years of age in 2015 (35 to 44 in 2006). While in 2006 58% of all whole blood donations came from new and repeat donors younger than 45 of age, these donors only contributed to 48% of the entire whole blood donations in 2015. As observed in previous years, the number of donations per year (1.9 on average) increased with age in both males and females: from 1.8 and 1.5 respectively among the youngest donors (18 to 24 years) to 2.3 and 1.9 for donors 55 years of age and older.

**Conclusion:** Parallel to the receding number of potential blood donors in Germany in the past 10 years we observed a disproportionate decrease in the number of whole blood donors and donations. Haemovigilance data from the competent authority showed that the number of RBC transfusions declined by 17% in the 10 year observation period. In the same period, patient blood management (PBM) was introduced in many hospitals and contributed to a more restrictive use of blood and blood products. Even though currently we observe a balance between donations and transfusions, blood establishment and authorities have to carefully observe the impact of the demographic changes in Germany in order to implement adequate measures in good times to maintain a sufficient supply.

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**P01-7**

**No tissue transplantation without donation – the crux of consent: tissue transplantation is an important element of modern medicine**

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**Introduction:** Up to 100 000 cornea transplants are saving and restoring eyesight for patients without other medical options each year worldwide. An artificial alternative has yet to be found. Amnion membrane can be used to cover orthopaedologic injuries, promote healing and reduce scarring, because of its anti-inflammatory, anti-microbial and antiangiogenic properties. Skin transplants restore the natural barrier function for large-scale defects such as severe burns or chronic wounds, helping to prevent infections and dehydration and supporting thermoregulation.

Human heart valves are life-saving therapy options with better flow properties than mechanical ones, meaning less need for anti-coagulation and, thus, a lower bleeding risk. Furthermore, they are less immunogenic than their xenogenic alternatives. New decellularization techniques even enable them to grow along with very young recipients, reducing the need of re-operations. Homograft transplants of blood vessels are often used in emergency operations to replace an infected artificial graft. Bone, ligaments, tendons and cartilage allografts are a valid option for a wide variety of orthopaedologic problems, e.g. for Achilles tendon injuries or in knee surgery to preserve joint mobility, deep cartilage deiscencs or restoration after tumor resection.

**Task:** Some of these tissues can be donated by living donors (e.g. amnion, femur heads, heart valves coming from ‘domino hearts’), most can only be procured from either brain dead patients following organ donation or from cadaveric donors. Apart from logistic challenges of narrow time frames, suitable procurement facilities and highly trained personnel, the biggest obstacle for routine treatment of patients with tissue transplant is the shortage of available tissues.

**Results:** Raising awareness in and giving more information about tissue transplantation to both medical staff and the general public is one of the key issues to higher consent rates. The questions of tissue donation itself should more often be raised with relatives of suitable donors. Establishment of workflow diagrams and special training programs for hospital personnel can help to reduce reluctance to approach bereaved relatives. Alternatively, a tissue donation program with trained, in-house coordinators could be installed to relieve the doctors and nurses from this additional task.

Families should be encouraged to speak about organ and tissue donation. A known will to donate has been shown to be very beneficial to consent rates. Information campaigns have been shown to favor a positive attitude towards donation, reducing anxieties, prejudice and common misconceptions (e.g. too old or too sick to donate).

**Conclusion:** Currently, the potential for tissue donation and greater transplant availability is not used to full potential. But this situation could be significantly improved by joint effort of different parts of the medical system.
**Gruppe 2**

**Blutkomponenten**

P02-1

**Cryopreservation of autologous hematopoietic stem cells by uncontrolled freezing leads to excellent bone marrow regeneration after transplantation**

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**Introduction:** Controlled rate freezing and storage in vapor phase nitrogen are used by the most transplantation centers for cryopreservation and storage of peripheral blood hematopoietic stem cells (PBSC). However, this procedure requires extensive and quick handling of PBSC and expensive equipment. In contrast, uncontrolled freezing on dry ice is easy to handle and cost effective. In this study, we analyzed 32 autologous PBSC transplants that underwent uncontrolled freezing on dry ice, storage of PBSC at -80°C for 24h and vapor phase liquid nitrogen until transplantation.

**Methods:** We obtained 32 autologous PBSC of 22 patients (age 48.5 ± 20.6 years) by apheresis with a Spectra Optia machine (Terumo BCT, Eschborn, Germany). After volume reduction by centrifugation (Roto Silenta RS, Hettich, Tuttingen, Germany) and addition of the cryopreservation solution (autologous plasma and final 10% dimethyl sulfoxide; DMSO) cryopreserved PBSC products were stored immediately on dry ice. After 20 min storage on dry ice, the products were first transferred to a -80°C freezer and after 24 h to vapor phase nitrogen tanks. Each cryopreservation process was accompanied by a product dummy of the same volume for monitoring temperature curves during freezing process. We determined colony forming units (CFU) before freezing and after 4 weeks storage in vapor phase nitrogen and regeneration data of 13 patients.

**Results:** We analyzed the freezing rates of 32 accompanying dummy products starting from room temperature to -80°C and observed a relatively consistent mean diminishment of -1.2°C per minute. The number of cryopreserved products stored on dry ice has only minor effects on temperature decreasing rates. CFU before freezing and 4 weeks after storage in vapor phase nitrogen were 7.15 ± 4.81 x10^4/ kg body weight and 6.54 ± 5.71 x10^4/ kg body weight, respectively. Mean regeneration time of blood cells after 13 PBSC autologous transplantations was > 1,000/ leu 6.54 ± 5.71 x10^4/ kg body weight, respectively. Mean regeneration time of blood cells after 13 PBSC autologous transplantations was > 1,000/ leu

**Conclusion:** Cryopreservation by uncontrolled freezing on dry ice results in a stable temperature decrease and leads to sufficient regeneration of blood cells after transplantation.

P02-2

**Validation of red blood cell concentrates divided into satellite units in two different systems (VQX0001XU Macopharma, PB-4TR150MBS Terumo) and different volumes (70 ml and 120 ml, group A: 70 ml Macopharma, group B: 70 ml Terumo, group C: 120 ml Macopharma, group D: 120 ml Terumo). Before splitting all RBC’s were irradiated by Biobeam 8000 with a median dose of 30 Gy.

**Results:** The mean hemoglobin content in group A was 11.7 g/TE, in group B 12.7 g/TE for group C 22.5 g/TE and for group D in 23.6 g/TE, respectively. The rates of hemolysis differed slightly within the various groups. At the end of the shelf life (day 28), it was 0.2% for group A, 0.16% for group B, 0.17% for group C and 0.15% for group D. The potassium concentration increased during the storage (group A) from 31.07 mmol/l (day 14) to 68.35 mmol/l on day 28. This trend was the same in all four groups. The pH slightly decreased during storage. ATP content also slightly decreased in all groups (e.g. group A on day 14: 5.17 µmol/gHB to 4.48 µmol/gHB on day 28). Concentration of glucose decreased from 26.44 mmol/l (d14, group A) to 20.31 mmol/l (d28, group A). In comparison, concentration of lactate increased in group A from 16.87 mmol/l to 27.58 mmol/l at the end of the storage. This was the same trend in all four groups for these two parameters of metabolism.

**Summary/Conclusion:** Irradiated divided RBC’s in both volumes processed using the Macopharma and Terumo systems retained adequate in vitro function and storage quality for up to 28 days and met the criteria of the «Council of Europe Recommendation N°R(95)15» as well as the German guidelines for RBC’s.

P02-3

**Production of inline filtered autologous whole blood using the blood bag system FDG7500LC (Macopharma)**

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**Introduction:** Autologous whole blood donations are collected and re-transfused in case of planned surgery. Another indication is a rare blood group or multiple blood group antibodies. The Haema AG used for the production of autologous whole blood preparations the blood bag system DDG7500LC with leukocyte filter LXT1, CPDA-1 (Macopharma). As this blood bag system is no longer available, a study was performed to evaluate the use of the new blood bag system FDG7500LC with leukocyte filter LXT (Macopharma).

**Methods:** 6 whole blood donations from normal volunteer donors were collected. The donations were stored on cooling plates at room temperature and filtrated within 22 hours after collection. Standard hematologic and biochemical variables were evaluated the day after donation as well as on day 7, 14, 21, 28, 35 and at the end of shelf life on day 42.

**Results:** The hemoglobin content of autologous whole blood was 64.18 ± 6.68 g/TE one day after donation. The rate of hemolysis (d1: 0.02 ± 0.01% and d42: 0.22 ± 0.09%), lactate (d1: 6.80 ± 1.62 mmol/l and d42: 25.14 ± 25.52 mmol/l and potassium (d1: 3.26 ± 2.26 mmol/l) increased, whereas glucose (d1: 23.58 ± 0.60 mmol/l and d42: 12.31 ± 0.56 mmol/l) and ATP (d1: 7.85 ± 7.15 µmol/l and d42: 28.78 ± 7.11 µmol/l) and pH (d1: 6.87 ± 0.04 and d42: 6.48 ± 0.04) decreased in all tested units over 42 days. Levels of coagulation factors fibrinogen, II, V, VIII and XI were determined as well. All coagulation factors except fibrinogen showed a slight decrease during storage. The units were tested...
for bacterial contamination; negative results were obtained after 7 days in culture.

**Conclusion:** The results of analyzed hematologic and biochemical variables measured for autologous whole blood using the blood bag system FGD7500LC with leukocyte filter LXT (Macopharma) were comparable to other performed studies and met the criteria of the German guidelines.

**P02-4**

**Umbilical cord blood: factors influencing content, viability and potency of hematopoietic progenitor cells**

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**Introduction:** The advantages of using cord blood derived hematopoietic progenitor cells for the treatment of leukemia and other hematological malignancies are now well recognized. Advantageous factors are the side-effect-free collection, lower risk of rejection compared to transplantation of bone marrow and peripheral blood progenitors and fast availability.

The benefit of treatment with cord blood units (CBU) is primarily influenced by the content of viable progenitor cells and their potency (proliferative potential).

The aim of the study was monitoring of potential influence factors (i.e. gestational age at delivery, type of delivery, collection-to-processing time) on quality and quantity of hematopoietic progenitor cells from umbilical cord blood.

**Methods:** About 1500 fresh samples from red cell and plasma depleted cord blood units (after processing), were analyzed with BD FACSCalibur (BD, enumeration of viable CD34+ and viable CD45+ cells by flow cytometry), Sysmex XS-800i (Sysmex GmbH, white blood cell count, WBC) and cultivated on semi-solid methylcellulose-based medium for in vitro detection and quantification of hematopoietic progenitor cells in a colony forming unit (CFU) assay (MethoCult™, STEMCELL Technologies Inc.).

**Results:** Increased gestational age was associated with a lower content of viable CD34+ cells in cord blood. In a similar way, the CFU count (CFU-GM + GEMM) slightly decreased with increasing gestational age at delivery.

There was no influence of mode of delivery (cesarean section / vaginal delivery) on the CD34+ cell count and the proliferative potential of progenitor cells.

The collection-to-processing time, in the timeframe of 45h, did not influence the count of CFUs and the count and viability of CD34+ cells. A slight impact on viability of CD45+ cells was detected. CBU containing 25 to 100 GPl/l WBC (post processing) have a CD34+ cell percentage between 0.2 and 3%. There is a strong correlation of CD34+ cell count with count of CFU. Furthermore there is also a correlation of WBC and TNC (total nucleated cell count) with CD34+ cell count. Furthermore there is also a correlation of CD45+ cells and CFU count. The strong correlation between the count of viable CD34+ cells and count of CFU shows that these assays are well suited to evaluate the quality of cord blood units.

**Conclusion:** The energy transferred by the UFT100 does not deteriorate FFP quality, compared to a conventional system. Some factor activities rather seem to be preserved by the new technique. Thawing time was much shorter in the UFT100. However, at least two UFT100 are needed in the blood bank to be able to provide FFP for patients both fast and sufficiently.

**P02-6**

**Storage of platelets: the effect of additive solution and plasma concentration in vitro quality parameters**

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**Introduction:** Platelet additive solutions (PASs) are becoming increasingly popular for storage of platelets, and PAS is steadily replacing plasma as the storage medium of platelets. All PASs require some residual plasma
to maintain PLT quality and functionality. The residual plasma concentration in recovered, pooled platelets normally is around 30–35%. However, there is an increasing interest in PASs that can be used after further reduction of residual plasma content to around 15–20%. The aim was to compare in vitro quality of platelets during storage in PAS-C/30% plasma, PAS-E/30% plasma and PAS-E/20% plasma.

**Methods:** After overnight storage, whole blood was centrifuged and separated in plasma, buffy coat and red cell suspensions. For platelet concentrates (PCs) with 30% plasma, the standard separation program was used. For PCs with 20% plasma, a separation program designed to get more plasma from a donation was used. Platelet concentrates were made from 5 buffy coats and 280 mL PAS-C or PAS-E. A total of 6 PCs were made in PAS-C/30% plasma and PAS-E/30% plasma, 10 PCs were made in PAS-E/20% plasma. PCs were stored on a flat bed agitator at room temperature. PCs were sampled at day 2, 6 and 8 and analyzed for hematologic, metabolic and morphologic parameters, activation (CD62P), apoptosis (PS exposure) and release of cytokines.

**Results:** All PCs fulfilled the requirements for platelet count and concentration. Plasma concentrations, calculated from total protein content, were slightly lower than intended in all series (28–28% in 30% series and 15–19% in 20% series). As compared to PAS-C/30% plasma, storage PCs in PAS-E with 30% or 20% plasma resulted in better maintenance of platelet in vitro quality as measured by better maintenance of morphological properties, lower glycolytic activity as measured by less production of lactate, less reduction in pH, lower activation (CD62P+) and less apoptosis (Annexin V+ cells), and lower release of cytokines. Storage in PAS-E with lower plasma concentration had only minimal effects on the in vitro quality, most noteworthy a slightly increased glycolytic activity. Together with the lower starting concentration of glucose, this resulted in complete glucose deprivation at the end of storage (day 8) in some PCs.

**Discussion:** As compared to PAS-C/30% plasma, storage of PCs in PAS-E with 30% or 20% plasma resulted in better maintenance of platelet in vitro quality. Storage of PCs in PAS-E/20% plasma was in no in vitro aspect inferior to storage in PAS-C/30% plasma. The results indicate that PAS-E allows for the production of recovered, pooled platelets with lower plasma content.

**Conclusion:** The described filtration protocol did not induce platelet activation and preserved, or improved, the quality of PC. The 40 µm filter was effective in removing aggregates which would otherwise pass through the pore filter size (200 µm) present in transfusion equipment. The applied method could be performed rapidly (10 min) and was cost-effective; thus enabling release of aggregate-containing apheresis platelet products that would otherwise have to be discarded, thereby reducing the need to purchase replacement platelet concentrate from other blood donation centers.

**P02-8 Double red blood cell apheresis – comparison of two apheresis systems**

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**Introduction:** The procedure of red blood cell (RBC) apheresis offers the advantage of collecting 2 units of RBCs from one donation. There are different devices available varying mainly in the filtration process. Here, we compare the in vitro characteristics of RBC units collected with the Fenwal Alyx and the Haemonetics MCS+.

**Methods:** RBC apheresis of normal volunteer donors was performed with the MCS+ (Haemonetics) and compared to RBC apheresis processed with Alyx (Fresenius). As additive solution saline-adrenaline-glucose-mannitol (SAG-M) in both procedures was used. Measurements of standard hematologic and biochemical variables including hemoglobin, glucose and lactate were performed the day after donation as well as on day 7, 21, 28, 35 and at the end of shelf life on day 42.

**Results:** The mean hemoglobin content of RBC units collected with MCS+ was 53.45 ± 1.11 g/TE one day after donation and comparable to the mean hemoglobin content of RBC units collected with Alyx (52.20 ± 1.70 g/TE). Hematocrit ranged from 0.52 to 0.56 l/l in MCS+ collected RBC units and from 0.52 to 0.60 l/l in Alyx collected RBC units. While the rate of hemolysis, lactate and potassium increased, glucose, ATP and pH decreased during storage over 42 days. The performed analyses were except of the rate of hemolysis comparable for the two products. Hemolysis ranged from 0.05% on day 1 to 0.21% on day 42 in MCS+ collected RBC units, the results for Alyx collected RBC units were higher. All tested units were negative in bacterial cultures. 

**Conclusion:** Both MCS+ and Alyx collected RBC products showed acceptable quality. All measured hematologic and biochemical variables were comparable for the two products and to other performed studies and met the criteria of the German guidelines. However, Alyx collected RBC products showed higher hemolysis during storage. A potential cause might be the filtration performed by pressure instead gravity.

**P02-9 Single-donor platelet concentrates from whole blood donations with collection times between 12 and 15 minutes**

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**Introduction:** According to European guidelines, the collection time of whole blood is limited to 15 minutes. In addition, donations with collection times between 12 and 15 minutes should not be used for preparation of platelet (PLT) concentrates (PCs) due to excessive platelet activation. It seems justified to re-evaluate the quality of PLTs from 12–15 min donations, because new generations of collection systems and mixers were introduced, including a more efficient needle. The aim was to investigate the in vitro quality of PCs prepared from 12–15 min buffy coats (BCs), both to prevent unnecessary discarding of BC, as well as to simplify the blood bank process.
Methods: Single-donor PCs (sPCs, n = 6) were prepared from one 12–15 min BCs and 60 mL of autologous plasma in a 600 mL PVC-DEHP container. As a reference, sPCs from donations with collection times of less than 12 min were prepared (n = 5). In addition, pooled PCs were prepared from 5 BCs, of which at least 4 BCs were from 12–15 min donations (n = 5). After pooling of the BCs, 300 mL PAS-E was added and a standard pooling set with a PVC-BTCH storage container was used for storage of PCs. All PCs were stored for 8 days at 22 ± 2°C and sampled at regular intervals for determination of the in vitro quality. Aggregation tests were performed with Chronolog (ADP or collagen) and Multiplate (arachidonic acid) aggregometers. Thromboelastography (TEG), using kaolin as an activator, was applied for assessment of the overall clotting capacity. Values are shown as mean±SD.

Results: Volume (67 ± 5 vs. 66 ± 16 mL) and platelet content (74 ± 11 vs. 71 ± 15 ± 109) were similar in the 12–15 min and the < 12 minutes groups. At the end of storage, both groups showed comparable in vitro quality (Day 8, pH(37°C): 6.84 ± 0.16 vs 6.83 ± 0.17, other data not shown). No differences in aggregation response after stimulation with arachidonic acid, ADP or collagen were measured. TEG parameters in both groups were also comparable. The five-donor PCs fulfilled all requirements of the European guidelines. On Day 8, PLTs showed low CD62P expression (17 ± 2%) and phosphatidylserine exposure (Annexin V binding, 9 ± 2%). Hypotonic shock response of platelets was comparable with historical data.

Conclusion: Single-donor PCs in plasma, as well as pooled 5-donor PCs in PAS-E prepared from 12–15 min whole blood donations, had a normal composition and showed good in vitro quality during 8 day storage. To substantiate that the exclusion of 12–15 min donations for PC preparation could be stopped, further studies will be performed.

P02-12
Evaluation of donor characteristics of lipemic whole blood donations

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Introduction: Several studies on the impact of lipemic plasma on the quality of blood products indicate that lipemic plasma has an adverse effect on the quality of platelets and red blood cells (RBC) during storage. As a follow-up of those studies, a possible relationship between donor characteristic and the occurrence of lipemic plasma was examined. The aim of this study was to expand the knowledge of donor characteristics in relation to the quality of blood products.

Methods: From whole blood (WB) donations with lipemic plasma, RBC concentrates in SAGM and Annexin V positive cells, indicating no activation or induction of apoptosis. pH was not influenced by simulated transfusion. Due to the dilution effect, glucose and lactate concentrations were slightly lower after simulated transfusion.

Conclusion: Warming and simulated transfusion of PCs under high pressure via a bone needle has no negative effect on the in vitro quality parameters of platelets. Transfusion of warmed PCs via an in vitro eventive transfusion via a bone needle is not expected to have a negative effect on the in vivo functionality of platelets. It is recommended to study the in vivo effects in a limited clinical study.

P02-11
Transfusion under pressure of warmed platelet concentrates via a bone needle

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Introduction: In case of big traumas, an intraosseous entrance via a bone needle is combined with a fast flow fluid warmer to quickly administer infusion fluids, including blood products, under pressure. This is done because veins of trauma patients are often not suitable for infusion of fluids. Suppliers of pump and needles describe the possible transfusion of blood products, but this is mainly limited to plasma and red cells. There is no information available concerning transfusion of platelets under pressure via a bone needle, and therefore our aim was to investigate the effects of warming and administration of a platelet concentrate (PC) under pressure via a bone needle on the in vitro quality of platelets.

Methods: Pools of 5 BCs and 280 mL of platelet additive solution-C (PAS-C) were used to produce PCs (n = 5). PCs were stored on a flat-bed agitator in a temperature-controlled cabinet at 22 ± 2°C for 4–7 days. To mimic hospital conditions, PCs were warmed using a blood warmer and «transfused» via a bone needle to a transfer bag. On the PCs, a pressure of 300 mm Hg was applied. Using clamps, a flow velocity of 90–120 mL/min was realized. Platelet quality before and after pressurized simulated transfusion was determined by means of various in vitro parameters.

Results: Due to priming of the transfusion disposable with saline, the PCs were diluted 10–30%, resulting in a significantly increased PC volume and decreased platelet concentration after simulated transfusion. Because of loss of platelets in the disposable set, also the total number of platelets was decreased after simulated transfusion. The PCs still fulfilled the requirements for platelet concentration (0.8–1.6×1011/L) and number (>250×109/unit). Simulated transfusion had no effect on the percentages of CD62P and Annexin V positive cells, indicating no activation or induction of apoptosis. Due to the dilution effect, glucose and lactate concentrations were slightly lower after simulated transfusion.

Conclusion: Warming and simulated transfusion of PCs under high pressure via a bone needle has no negative effect on the in vitro quality parameters of platelets. Transfusion of warmed PCs via an intraosseous entrance via a bone needle is not expected to have a negative effect on the in vivo functionality of platelets. It is recommended to study the in vivo effects in a limited clinical study.

P02-10
Mini buffy coat preparation with a fully automatic whole blood component separator for extracorporeal photopheresis

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Introduction: Mini buffy coat (BC) photopheresis was first developed to facilitate treatment of small children suffering from graft-versus-host disease. The number of reinforced white blood cells (WBCs) required to achieve a clinical response in extracorporeal photopheresis (ECP) is still unknown. Depending on the apheresis system used for WBC collection, apheresis products show a wide range of product yield with a minimum mononuclear cell (MNC) content of 1.0 × 109. Therefore mini BC photopheresis might also be a therapeutic option for adult patients.

In this study we used a fully automatic whole blood component separator to develop a mini ECP technique that allows preparation of the MNC-rich BC fraction for treating adult patients.

Methods: Whole blood (WB, 450 ± 45 mL) of 30 randomly selected healthy volunteer blood donors was collected into a bag system containing citrate phosphate dextrose. WB units were centrifuged at 1,803g and WBCs were separated with a WB component separator device (MACOPRESS Smart, MacoPharma, Mouvaux, France). After separation of the BC the final product was automatically prepared by resuspending the BC in autologous plasma. Volume of final products after resuspension was 300 mL RBC, WBC and MNC counts as well as hemoglobin and Hct levels were measured after BC preparation using an automated cell counter.

Results: Mean WBC count (x109/µL) of final products was 5.5 ± 0.4 and resulted in a final WBC content per product (x109) of 1.7 ± 0.1. Total MNC content (x109) of final products was 1.0 ± 0.1. Hct (%) after separation was 2.6 ± 0.1 and therefore reached the threshold of < 3% that allows efficient irradiation.

Conclusion: MNC yield of BCs from healthy blood donors separated with the MACOPRESS smart device was within the range of apheresis product yields of the conventional ECP. Therefore this method might be a cost-saving alternative for ECP therapy in patients unsuitable for apheresis.
Transfusion of long-term stored red blood cells (RBCs) is an established procedure in clinical practice. However, long-term storage may lead to the release of free heme, which can have pro-inflammatory effects of cell-free hemoglobin and its derivative heme, and have been described as the 'storage lesion'. More recently, pro-oxidant and pro-inflammatory effects of cell-free hemoglobin and its derivative heme, have been described as the 'storage lesion'. The current observed lower efficiency of Gelafundin in granulocytes collection is due to its use at similar concentrations usually used for Hespan. However, the current in vitro findings indicate that increased levels of Gelafundin result in an improved sedimentation efficacy that can be comparable to Hespan or even better when 3 fold MFG is used. Collectively, these data show that increased concentrations of MFG is an alternative to HES.

**References:**


**Conclusion:** In vitro comparison of MFG and HES in comparison to HES.

**Methods:** Blood obtained from healthy donors (n = 14) using citrate tubes (1:10) combined with different sedimentation agents [Hespan, MFG, Venofundin (VFN, 130 kDa), Voluven (Volu, 130 kDa)] at different concentrations and loaded in equal volume on western green sedimentation tubes. ESR of the different groups was recorded up to four hours.

**Results:** Hespan, MFG, Volu, and VFN had significantly different ESR with HES being the most effective (HES: 49 ± 5.5 mm/h, MFG: 27 ± 4.2 mm/h, Volu: 10 ± 1.2 mm/h, and VFN: 15 ± 2.9 mm/h). Additional tests showed that increasing the concentrations of MFG and VFN by 2 fold resulted in almost double increased ESR in the ESR of both agents (MFG: 43 ± 1.1 mm/h and VFN: 29 ± 7.1 mm/h). While only 2 fold MFG resulted in an ESR comparable to that of HES (43 ± 1.1 vs 49 ± 5.5 mm/h), 3 fold MFG was even superior to HES (MFG: 59 ± 3.7 mm/h, HES: 49 ± 5.5 mm/h, p < 0.001).

**Conclusion:** The current observed lower efficiency of Gelafundin in granulocytes collection is due to its use at similar concentrations usually used for Hespan. However, the current in vitro findings indicate that increased levels of Gelafundin result in an improved sedimentation efficacy that can be comparable to Hespan or even better when 3 fold MFG is used. Collectively, these data show that increased concentrations of MFG is an alternative to HES.

**Group 3**

**Blutsicherheit**

**P03-1**

**How to estimate CD3+ T-cell yield in unstimulated lymphocyte apheresis based on peripheral CD3+ T-cell concentrations**

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**Introduction:** Lymphocyte yield in unstimulated lymphocyte apheresis of healthy voluntary donors is often estimated by a thumb rule. A yield of 100×107 mononuclear cells is anticipated for every liter of total blood volume (TBV) processed. About half of these cells are CD3+ T-cells.

**Methods:** Blood obtained from healthy donors (n = 14) using citrate tubes (1:10) combined with different sedimentation agents [Hespan, MFG, Venofundin (VFN, 130 kDa), Voluven (Volu, 130 kDa)] at different concentrations and loaded in equal volume on western green sedimentation tubes. ESR of the different groups was recorded up to four hours.

**Results:** Hespan, MFG, Volu, and VFN had significantly different ESR with HES being the most effective (HES: 49 ± 5.5 mm/h, MFG: 27 ± 4.2 mm/h, Volu: 10 ± 1.2 mm/h, and VFN: 15 ± 2.9 mm/h). Additional tests showed that increasing the concentrations of MFG and VFN by 2 fold resulted in almost double increased ESR in the ESR of both agents (MFG: 43 ± 1.1 mm/h and VFN: 29 ± 7.1 mm/h). While only 2 fold MFG resulted in an ESR comparable to that of HES (43 ± 1.1 vs 49 ± 5.5 mm/h), 3 fold MFG was even superior to HES (MFG: 59 ± 3.7 mm/h, HES: 49 ± 5.5 mm/h, p < 0.001).

**Conclusion:** The current observed lower efficiency of Gelafundin in granulocytes collection is due to its use at similar concentrations usually used for Hespan. However, the current in vitro findings indicate that increased levels of Gelafundin result in an improved sedimentation efficacy that can be comparable to Hespan or even better when 3 fold MFG is used. Collectively, these data show that increased concentrations of MFG is an alternative to HES.
To improve the accuracy of prediction we investigated whether a relevant correlation factor between lymphocyte count before apheresis and CD3+ T-cell yield in the apheresis unit as well whether a correlation factor between CD3+ T-cell count and CD3+ T-cell yield in the apheresis unit exists.

**Methods:** We retrospectively reviewed donor and product records of all unstimulated lymphocyte apheresis that took place between March and June 2017 at our collection center in Cologne (42 donors and 42 collections). All collections were performed with the Spectra Optia® using the Continuous Mononuclear Cell Collection (CMNC) protocol. A chart trend line formula was determined for calculation of absolute CD3+ T-cell yield per TBV (in 10^8) processed based on lymphocyte (/µl) or based on CD3+ T-cell count (/µl). For feasibility reasons the formula was simplified and afterwards tested for graphic and statistic correlation (correlation coefficient and p-value).

**Results:** The trend line formula was simplified to a factor of 0.005 to be multiplied with the lymphocyte count to predict the CD3+ T-cell yield per liter TBV processed. Furthermore, the trend line formula simplified to a factor of 0.007 to be multiplied with the CD3+ T-cell count to predict the CD3+ T-cell yield per liter TBV processed. No statistical significant difference was noted between the two different sets of values: Comparison of CD3+ T-cell yield measured and calculated based on lymphocyte count revealed a p-value of 0.2 and a correlation coefficient (cc) of 0.77. Comparison of CD3+ T-cell yield measured and calculated based on CD3+T-cell count revealed p-value of 0.68 and a cc of 0.79.

**Conclusion:** Here we present a simple calculation factor to predict CD3+ T-cell yield per liter TBV collected with the Spectra Optia® based on CD3+ T-cell count or lymphocyte count before apheresis. The calculated values seem to be relevantly correlated with measured outcome yield. Further validation by prospective testing is planned and evaluation for feasibility in patients is warranted.

![Fig. 1.](image1)

**Fig. 1.**

**Fig. 2.**

**P03-2**

**Anti-CD36 antibodies impair the barrier of microvascular endothelial cells: mechanism of hydrops and TRALI?**

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Anti-CD36 isoantibodies (also known as anti-Nakα) developed by CD36 deficient individuals (CD36null) are the most platelet antibodies found in Asian populations. These isoantibodies play an important role on the mechanism of platelet transfusion refractoriness (PTR), fetal neonatal alloimmune thrombocytopenia (FNAIT) as well as in transfusion related acute lung injury (TRALI). In addition to platelets, CD36 is also found on cell surface of monocytes, macrophages, erythroid precursors and endothelial cells. Recent study from our group showed that platelet alloantibodies against Human Platelet Antigen-1a (HPA-1a) can interact with αvβ3 integrin (also known as vitronectin receptor) expressed predominantly on endothelial surface leading to endothelial dysfunction; a mechanism that is associated with the development of intracranial hemorrhage (ICH) of fetus with severe FNAIT (Santoso et al, 2016).

In this study, the effect of anti-CD36 isoantibodies purified from patient’s serum samples on endothelial functions was investigated. Treatment of human macrovascular endothelial cells (HUVEC) with anti-CD36 antibodies caused significant increase of albumin flux through endothelial monolayer. Interestingly, this phenomenon was not observed with anti-HPA-1a antibodies. However, anti-CD36 mediated permeability was more pronounced when human microvascular endothelial cells (HMVEC) were investigated. This is in accordance with our flow cytometry analysis demonstrating that HMVEC express higher CD36 surface density in comparison to HUVEC. Furthermore, the influence of anti-CD36 isoantibodies on endothelial apoptosis and angiogenesis was measured by caspase3/7 activation and tube formation assay, respectively. Anti-CD36 isoantibodies caused significant increase of albumin flux through endothelial monolayer. Interestingly, this phenomenon was not observed with anti-HPA-1a antibodies. However, anti-CD36 mediated permeability was more pronounced when human microvascular endothelial cells (HMEC) were investigated. This is in accordance with our flow cytometry analysis demonstrating that HMVEC express higher CD36 surface density in comparison to HUVEC. Furthermore, the influence of anti-CD36 isoantibodies on endothelial apoptosis and angiogenesis was measured by caspase3/7 activation and tube formation assay, respectively. Anti-CD36 isoantibodies induced apoptosis and impaired tube formation of HMVEC, but not HUVEC. In comparison to anti-HPA-1a antibodies, however, the effect was significantly lower.
In conclusion, our observation indicates that anti-CD36 antibodies can impair the function of microvascular rather than macrovascular endothelial cells. In contrast to anti-HPA-1a, anti-CD36 antibodies affect predominantly endothelial permeability more than vessel formation. Our finding suggests a direct involvement of anti-CD36 antibodies in endothelial barrier disruption, which appears to constitute an important pathway in the mechanism of immune-mediated TRALI as well as on the development of fetal hydrops that are frequently observed in severe FNAIT mediated by anti-CD36 antibodies. However, the question whether recruited and/or resident CD36 expressing blood cells (monocytes and macrophages) may reinforce this pathomechanism is intriguing.

P03-3

Effective and potent treatment of active Bleeding with INTERCEPT pathogen-inactivated platelets

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Introduction: Platelet concentrates (PC) pathogen-inactivated using the INTERCEPT™ Blood System (IBS; Cerus Corporation, Concord, CA) have been shown to be comparably efficacious to conventional platelets for the treatment of hematologic/oncology (H/O) patients. As these patients are mostly transfused to prevent rather than to treat active bleeding, concern has been raised whether such platelets would also be effective in the treatment of acutely hemorrhagic patients. This is especially important in the case of massively bleeding patients. We investigated component use and clinical outcomes after massive transfusion (MT) in patients transfused with IBS-PC. Here, we evaluated whether the routine implementation of INTERCEPT treated platelets was associated with an increase in component usage, in-hospital mortality or delayed patient discharge of patients who received massive transfusion (MT).

Methods: INTERCEPT for PC was introduced in our medical center where we performed a retrospective cohort analysis comparing component use, in-hospital mortality and length-of-stay after MT (receiving >10 RBC within 1 day and at least 1 PC), for two periods (21 months each) before and after implementation.

Results: From 306 patients with MT (74% males and 99% adults), 93 had liver transplant; 97 cardiac or vascular surgery and 51 trauma. No differences in demographics were observed between the treatment cohorts. PC, RBC and plasma use on the day and within 7 days of the MT event was unchanged post-IBS implementation (Mean pre-/post-IBS was 16.4 vs. 16.2, p = 0.78 for RBC; 3 vs. 3.3, p = 0.33 for PC; 24.2 vs. 23.5, p = 0.75 for plasma units). The mean RBC:PC:plasma ratio on the MT day was close to 1:1:1 in both periods (1.0 : 0.9 : 1.2 vs. 1.0 : 1.0 : 1.3), except for liver transplants with MT who received more plasma components. In-hospital mortality and median time to discharge did not change (pre-/post-implementation was 27.6% vs. 24.0%, p = 0.51; 27 vs. 23 days, p = 0.37, respectively), except for cardiac and vascular surgery patients who were discharged earlier after IBS implementation.

Conclusion: Introduction of IBS-PC did not adversely affect clinical outcomes in MT patients in terms of blood product usage, in-hospital mortality, and length of stay for a range of clinical indications for platelet transfusion support in the MT indication. These data are consistent with recently reported study results from multiple hospitals in France and Belgium. The INTERCEPT Blood System can be safely introduced with no major adverse risk of bleeding or increased component use and in addition strongly reduces the risk of blood borne pathogen transmission.

P03-4

Improved blood safety for TT-HCMV by the implementation of NAT, first routine data

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Introduction: As universal leukocyte-reduction of blood products is able to reduce the residual risk for transfusion transmitted HCMV infection to 1–3%. A residual risk exists as free viruses in plasma especially in the early infection period can be transmitted by blood products. Testing by NAT is able to detect free HCMV in the early stage of infection. We summarize here the first results of testing for HCMV by NAT in our blood donation services.

Methods: Starting from October 2016 all whole blood and thrombapheresis donations has been screened for HCMV DNA which was performed with Roche Cobas CMV on Roche Cobas 6800/8800 instrument. The analytical sensitivity (95% LOD) of this mini-pool NAT system (maximum mini-pool size 96 donations per pool) is 1678 IU/ml. All PCR reactive samples were tested for HCMV antibodies by means of ABBOTT ARCHITECT CMV IgG and ABBOTT ARCHITECT CMV IgM. Follow up samples were tested with quantitative ID NAT which has an analytical sensitivity of 34,5 IU/ml.

Results: HCMV antibody screening of whole blood and thrombapheresis donations was replaced by NAT based HCMV testing in October 2016. Since then about 400.000 donations were screened. 53 donations were found to be HCMV NAT positive (rate of 1 in 7500), 42 of it were antibody positive and 11 NAT only positive.

Three follow-up whole blood donations were given by one IgM positive but IgG negative donor between October 2016 and March 2017 to evaluate the course of viral load over time.

Whole blood donations were processed into different blood products (leukocyte-reduced RBC, leukocyte-reduced pool PLC and frozen recovered plasma). At each production step samples were taken (unfiltrated RBC,uffy coat and non-frozen recovered plasma, end products) and tested by means of quantitative HCMV ID PCR. Seven weeks after the initial donation HCMV DNA could be detected in all fractions but a decrease of HCMV in the final products was detected. Two weeks later HCMV DNA was only found at a lower level in whole blood,uffy coat and plasma (not filtered) but not in unfiltered RBC, leukocyte-reduced RBC and leukocyte-reduced pool PLC. Five months after the initial donation no HCMV DNA could be detected in all fractions.

Conclusion: NAT screening further reduce the residual risk of TT-HCMV infections. We were able to show the decreasing trend of viral load during the production process of leukocyte-filtrated blood products by means of HCMV NAT.

P03-5

Activated endothelial cells are capable to acquire soluble NB1/PR3 complex from plasma: mechanism of anti-HNA-2 mediated immune TRALI?

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CD177/PR3 complex exclusively found on neutrophils was identified as a heterophilic binding partner for platelet/endothelial adhesion molecule-1 (PECAM-1) which is abundantly expressed on endothelial cells. This molecular crosstalk is important for neutrophil diapedesis. Some individuals (<5%) fail to express CD177 on neutrophils and may develop anti-CD177 (anti-HNA-2) antibodies when challenged with CD177 positive blood. It has been reported that transfusion of plasma containing anti-HNA-2 can
induce transfusion-related acute lung injury (TRALI). Besides on neutrophils, significant amounts of soluble CD177 were found in plasma of healthy individuals. In this study, we asked the question whether soluble CD177/PR3 complex (sCD177/PR3) in plasma can bind to the endothelium and serve thereby as a direct target for anti-HNA-2 or anti-PR3 antibodies leading to endothelial dysfunction. Immunoprecipitation with anti-CD177 coated beads and subsequent analysis with anti-PR3 monoclonal antibodies (moabs 7D8 and D3) demonstrated the existence of sCD177/PR3 complex in plasma. By quantitative ELISA, 3−15 ng/ml of sCD177/PR3 could be measured in plasma of different individuals, rising significantly after stimulation with G-CSF (2.1 fold). Analysis by surface plasmon resonance (SPR) technology showed that PECAM-1 could interact with purified sCD177/PR3 complex, but not with recombinant CD177 alone (KD: 4.98E-08 M). Interestingly, incubation of LPS pre-treated endothelial cells (HUVECs) with sCD177/PR3 converted HUVECs into CD177/PR3 positive cells, as shown by flow cytometry. Immunoprecipitation of CD177/PR3 positive HUVECs with anti-CD177 led to co-precipitation of PECAM-1 indicating that sCD177/PR3 complex is also able to interact heterotopical with PECAM-1 expressed on endothelial cell surface. After stimulation with anti-HNA-2, only CD177/PR3 positive, but not CD177/PR3 negative HUVECs were able to produce reactive oxygen species (ROS). This antibody binding significantly increased the influx of fluorescein labelled albumin through a CD177/PR3 positive HUVEC monolayer and induced endothelial cell apoptosis as measured by activation of caspase 3/7 activity. Similar results were observed with anti-PR3 antibodies. In conclusion, our data suggest that endothelial cells in inflammatory conditions are capable to acquire soluble CD177/PR3 from plasma through specific interaction with endothelial PECAM-1. Interaction of anti-HNA-2 with CD177/PR3 complex bound onto endothelial cells can lead to endothelial barrier dysfunction. This phenomenon may play an important role in the mechanism of anti-HNA-2 mediated TRALI.

P03-6
Two and a half year experience with routine hepatitis E virus NAT blood donor screening – can we afford a little more safety?

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Background: Hepatitis E virus (HEV) infection is recognized as an undiagnosed disease with asymptomatic infections often occurring in blood donors of various European countries. However, the clinical relevance of transfusion-associated HEV infection is still insufficiently understood, connected to an ongoing debate on the importance of timely introduction of HEV screening of blood donors and the impact of blood safety. We decided to routinely test all our blood products for HEV instead of testing of a targeted screening strategy for at risk patients. In the present, we present our experience of the last two and a half year showing the successful implementation of a 100% screening of therapeutic blood products for HEV RNA.

Methods: Routine screening for HEV RNA was introduced in January 2015 in our blood transfusion service to identify viremic donors. From January 2015 to May 2017, 235,524 donations from 52,143 individual donors (whole blood, platelets) were routinely screened for the presence of HEV RNA using the RealStar HEV RT-PCR assay (Altona Diagnostics GmbH). Nucleic acids were extracted from 4.8 ml plasma using the Chemagen MSM-1 extractor (Viral sk, Perkin Elmer Chemagen GmbH). The 95% LOD of the assay was determined to 4.66 IU/ml (447 IU/ml per single donation). The presence of HEV-specific IgM and IgG antibodies 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. All HEV RNA positive donors were deferred from donation for 3 months. Follow-up samples were tested for the presence of HEV RNA and HEV-specific antibodies. Genotyping was performed by sequencing of the hypervariable region (HVr) and ORF1.

Results: In total, 166 HEV RNA positive donors were identified. The month-dependent incidence ranges from 1 out of 611 to 1 out of 3613 blood donations with a peak in June in 2015 and 2016. The cumulative incidence of HEV RNA positive donors over the testing period of 29 month was determined to be 0.0032% in our cohort of blood donors. 36 donors showed reactive IgM and/or IgG antibody titers (IgM+/IgG−, IgM+/IgG+, IgM-/IgG−, the other 130 donors were HEV NAT-only positive. Follow-up samples were currently available for 87 donors and all donors present IgG seroconversion. Anti-HEV IgG reactivity indicating a previous infection was found in 12 donors (7.23%). Genotyping revealed genotype 3 in all cases.

Conclusions: Routine NAT testing of all blood products was comparatively simple and cost-effective since screening was additionally performed to our routine procedure for HAV, HBV, HCV, HIV and Parvovirus B19 in a pool size of 96 samples with substantially lower efforts than selective screening of blood donors for at risk patients. This study further confirmed that HEV infection is common in German blood donors. Consequently, we afford a little more safety of blood products.

P03-7
 Influence of the temperature on the quality and virus inactivation capacity of methylene-blue treated plasma using the theraflex mb-plasma system

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Introduction: Photodynamic treatment using methylene blue (MB) and visible light is in routine use for pathogen reduction of human plasma in different countries. Temperature conditions of either environment or human plasma during production might vary between production sites. Aim of the study was to investigate the influence of different temperature conditions on the quality and virus inactivation capacity of the THERAFLEX MB-plasma procedure (Macopharma).

Methods: For quality investigations, plasma (n = 3) was pooled and split and either stored at room temperature (reference) or stored at 5°C and 30°C (test) for a minimum of 2h to ensure equilibration to the desired temperature. Plasma unit (n = 8 for each temperature) was connected to the MB bag system including a leukocyte depletion filter (Plasmalex) and a filter for the removal of MB and its photoproducts (Blueflex). Treatment was done according to the manufacturer’s instructions on the Macotronic B2 device (Macopharma) with a light dose of 120 J/cm2. Samples were taken at different process steps to determine the activity of plasma factors and the concentrations of MB and photoproducts. For virus inactivation kinetics, plasma units (n = 3 for each temperature and virus species) were spiked with virus suspension (10%) of three different virus species and MB/light treated. Samples were taken, after different dose steps, and virus titers were determined by endpoint titration.

Results: MB/light treatment affected the activity of most of the investigated coagulation proteins and inhibitors. The highest decreases in reference conditions were detected for Factor VIII (−17.7%, 22°C) and Fibrinogen (−14.4%, 22°C). There was a trend towards a higher reduction of plasma factor activities with increasing plasma temperatures, albeit differences were statistically not significant, except for Factor VIII (p = 0.044). However, plasma temperature significantly influenced the solubility of the MB pill, the concentrations of MB and photoproducts after illumination and the virus inactivation capacity. Solubility of the MB pill was visibly reduced at 5°C. Degradation of MB during illumination intensified with increasing temperature, resulting in augmented formation of photoproducts (mainly Azure B) at 30°C. However, the filtration efficacy of the Blueflex
P03-8

Development of a mitochondrial DNA multiplex real-time polymerase chain reaction assay for quality control of pathogen inactivation of platelets with UVC light

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Introduction: Several ultraviolet (UV) light-based pathogen inactivation (PI) technologies for platelet products have been developed or are under development. Upon implementation of PI technologies, quality control measures are required to ensure consistent efficacy of UV illumination process. Previous reports showed that amotosalen/UV A and riboflavin/UV-based PI technologies induce modifications of the platelet-derived mitochondrial DNA (mtDNA) that can be detected by polymerase chain reaction (PCR) inhibition assays. Aim of the study was to develop a multiplex real-time PCR for detection of UVC-induced mtDNA modifications as a quality control for PI of platelets by the THERAFLEX UV-Platelets system.

Methods: Based on a binucleotide frequency analysis of the mtDNA genome, a multiplex real-time PCR assay was developed to simultaneously amplify short (143 bp) and long (794 bp)-amplons from a template DNA prepared using a platelet count adjusted DNA extraction method. Multiplex real-time PCR assay performance was evaluated on apheresis and buffy coat-derived UVC-treated and untreated plasma-reduced platelet concentrates (PCs) and challenged by using PCs with volumes, platelet counts and plasma contents at the upper and lower limits of the specifications defined for the THERAFLEX UV-Platelets system.

Results: PI of platelets using UVC light resulted in significant inhibition of PCR amplification of long-amplon mtDNA targets relative to untreated products. Amplification of short-amplon mtDNA targets was not affected by treatment. Evaluation of more than 100 blinded platelet samples from routine-like production resulted in prediction of UVC treatment status with 100% accuracy.

Conclusion: A differential sized amplon real-time PCR assay of mtDNA effectively detects nucleic acid damage induced by UVC illumination of platelets and could be used as an informative quality indicator of PI by the THERAFLEX UV-Platelets system.

References

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Background: There is a growing demand for plasma derived medicinal products (PDMPs) globally, driven by the use of polyclonal immunoglobulins. Plasma manufacturers need highly efficient systems to cope with the workload of screening large numbers of samples collected by plasmapheresis. To support these labs, Abbott developed the Alinity s System, a high-throughput immuno-analyzer generating up to 600 tests/h with extended throughput equivalency.

Objective: To prove equivalent performance of Alinity s assays for HBsAg, Anti-HCV and Syphilis antibodies with the new Alinity s System.

Methods: Surplus samples from plasmapheresis donors in Germany, the Netherlands and the United States were tested between 3 and 7 days after the initial draw date prior to and after centrifugation. The number of initial and repeat reactive specimens and the mean S/CO value for all negative results were compared between the two conditions.

Results: No difference in the number of initial and repeat reactive results nor mean S/CO value was observed when testing samples for antibodies against HCV (N = 3,336) or Syphilis (N = 3,348). One out of 3,364 samples was found initially reactive for HBsAg after centrifugation while the non-centrifuged specimen was below the cut-off. A difference of 0.01 in the mean S/CO values of the samples tested with or without centrifugation was observed for HBsAg. No such S/CO difference was observed for Alinity s
HIV Ag/Ab Combo when looking at the distribution of the mean S/CO values, however a slight difference in the number of total reactive specimens was observed. Three of the 3,350 HIV samples had qualitative differences upon centrifugation. One sample transitioned from only initially reactive to repeatedly reactive and 2 samples transitioned from repeat reactive to non-reactive. In total, three out of 13,298 specimens were qualitatively different for all assays when tested with and without centrifugation.

**Conclusion:** To support a highly efficient workflow in labs testing large numbers of fresh plasmapheresis samples, no prior centrifugation is needed to run samples in the Alinity s screening assays for HBsAg, HIV Ab/Ab Combo, Anti-HCV and Syphilis.

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**P03-11 Rapid bacterial detection system prevents severe bacterial infection**

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**Background:** Bacterial contamination of blood components is still a major challenge in Transfusion Medicine especially for platelet concentrates which are stored at room temperature. To improve blood safety the maximum shelf life was reduced in Germany for platelets from 5 days to 4 days. In addition rapid bacterial screening methods were developed (NAT and Bactiflow) to implement a generic bacteria screening 48h to 72h after blood donation.

**Aims:** We implemented a mini-pool bacterial screening system (Bactiflow) for platelets with a maximum pool size of 10 platelet components per pool. Platelets were released without bacterial testing on day 1–2. 48h after blood donations samples from platelet concentrates were collected, pooled and tested for bacteria contaminations. Shelf life of all screened platelets with a negative test result will be extended to 5 days.

**Results:** Between May 2016 and May 2017 36,097 platelets were screened for bacterial contaminations. In Total 5 platelets were identified with bacterial contaminations (Klebsiella pneumoniae, Staphylococcus epidermidis, Escherichia coli and 2x Staphylococcus aureus). Growth kinetics demonstrated rapid bacteria growing.

**Conclusions:** Compared to the culture methods, bacterial rapid detection methods like Bactiflow or NAT are characterized by a high clinical efficiency rate. Bacterially contaminated platelet concentrates could be identified and discarded before release of platelet components. Recalls as described by culture methods with the negative-to-date concept are not necessary. The introduction of rapid bacterial screening methods leads to an improvement to supply hospitals with platelet concentrates by extending the shelf life to 5 days, and in addition increases blood safety. Therefore, our blood donor service performs on a voluntary basis a test strategy to screen all available platelet concentrates from day 3 onwards on bacterial contaminations which is a major step to improve blood safety.

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**P03-12 In vitro assessment of plasma-reduced single donor apheresis platelet concentrates: comparison of UVC-treated, y-irradiated and untreated Platelet units**

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**Introduction:** Treatment of apheresis platelet concentrates (PCs) with UVC may enhance transfusion safety of platelets with respect to contamination with pathogens. In vitro quality of UVC-irradiated PCs (UVC-PCs) was compared with that of γ-irradiated PCs (γ-PCs) and untreated PCs (AP-PCs) under routine production conditions.

**Methods:** 18 PCs were prepared from single donors with standard operation procedures (Amicus) using SSP+ (Macopharma, Mouvaux, France) as additive solution and divided into three groups (n = 6 each): UVC-PCs were treated with UVC within six hours after preparation using the THERAFLEX UV-Platelets system (Macopharma); y-PCs were γ-irradiated with a minimum of 25 Gy; and AP-PCs were left untreated. Sampling for quality control parameters was done on day of preparation (day 0) and after six days of storage (day 6). The following parameters were examined on day 0: PC volume, platelet concentrations, residual erythrocyte and leucocyte counts, pH, swirling and sCD62 (with and without thrombin-receptor activating peptide [TRAP]/collagen activation); measurements on day 6 were: platelet concentration, pH, swirling, sCD62 and sterility testing.

**Results:** Mean volumes were 333 ± 5.4 mL in AP-PCs, 341 ± 7.1 mL in γ-PCs and 329 ± 6.0 mL in UVC-PCs with platelet counts of 3.6 ± 0.5/unit, 3.8 ± 0.3/unit and 3.9 ± 0.3/unit, respectively. Residual plasma concentration ranged between 31% and 38%. Residual erythrocytes and leucocytes met the standard specifications for PC products in Germany. At the end of shelf life, the pH value of UVC-PCs was significantly lower (7.01 ± 0.05) compared to γ-PCs (7.18 ± 0.04) and AP-PCs (7.17 ± 0.05). No differences were detected for sCD62 expression between the three PC types with and without TRAP/collagen activation. Tests for bacterial contamination were negative for all tested PCs.

**Conclusions:** Study results demonstrate that plasma-reduced UVC-treated apheresis PCs meet the standard specifications for PC products in Germany. The significantly lower pH value at end of storage may be attributable to a higher metabolic activity in UVC-PCs. The safety and efficacy of UVC-treated PCs is being evaluated in a clinical study.

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**P03-13 Antibiotics – a current problem of microbiological testing in tissue banking**

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**Introduction:** The microbiological safety of tissue preparation is of great importance. A major problem is the antibiotic content in the test sample, which influences the result of the microbiological test. To investigate the inhibitory effect, RESEP (ALCHIMIA, Padova, Italy), a new medical device for the removal of antibiotics, was validated according to the European Pharmacopoeia (chapter 2.6.1) using the automatic BACTEC system (Becton Dickinson, Franklin Lakes, New Jersey, USA).

**Methods:** Selected aspects of our validation studies of microbiological testing of the antibiotic-containing medium used for cornea organ culture and cardiovascular tissue preservation with different kind of blood culture bottles will be presented.

**Results:** All tested microorganisms could be detected to 100% in the RESEP groups. In comparison to the positive controls no significant delay of the time to detection was found. The control groups without RESEP treatment showed lower detection rates and false negative results to some extent.

**Conclusion:** Microbiological testing of tissue preparations remains a critical aspect in tissue banking. A careful selection of microbiological tests used and their validation is necessary to reduce the risk of false negative test results. The RESEP systems seems to be a promising approach to improve microbiological testing.
Background: Before blood donors are qualified for blood donations, they have to answer a donor questionnaire. In 2016 a new uniform national donor questionnaire was introduced in our blood donor service. The new donor questionnaire was improved regarding intensive questions to potential sexual transmitted infections due to new sexual partners within the last four months.

Aims: All donors who answered one question regarding sexual risk behavior (new sexual partner, sexual partner with AIDS or hepatitis, paid sexual partner, bi-sexual partner or homo-sexual partner) with «YES» were deferred for blood donation for four months. In a case control study, we analyzed the incidence rate for HBV, HCV, and HIV-1 for first time donors who answered the question with «YES» with first time donors who answered the question with «NO».

Results: Between February and May 2017, 2,476 and 20,762 first time donors (case versus control group) were analyzed, respectively. After standardisation to 100,000 donors, the incident rates were significantly higher in the case group for HBsAg (case group 162 versus 82 control group), HBV NAT (case group 121 versus 67 control group) and HCV NAT (case group 81 versus 43 control group). No differences were observed for anti-HBc, HIV combo and HIV NAT.

Conclusions: The new uniform donor questionnaire focussed on sexual risk behavior by new partners. Based on the first 4 months of our case control study HBV and HCV were detected significantly higher in the case group in first time donors with sexual risk behavior. This demonstrates that a uniform donor questionnaire is able to improve blood safety to defer donors with increased infectious risks for a period of 4 months and changing the sexual partner itself is an infectious risk especially for HBV and HCV infections.

Cytomegalovirus serostatus of registered volunteer allogeneic stem cell donors: implications for donor search

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Introduction: Cytomegalovirus (CMV) seroprevalence varies strongly from country to country ranging from 30% to over 90%. Exposure to young children and sexual activity are thought to be the main factors for CMV transmission in adulthood. Infection with CMV causes usually minimal if any clinical symptoms in immunocompetent individuals. In immunodeficient individuals CMV infection or reactivation can cause severe outcomes and even death. Therefore, CMV seronegative recipients of allogeneic hematopoietic stem cell donations should preferably receive a transplant from a CMV seronegative donor. In seropositive recipients CMV seropositive donors are preferred for allogeneic hematopoietic stem cell transplants. As soon as a CMV seropositive donor is needed this selection diminishes the available donor pool.

Conclusion: The analysis of CMV seroprevalence in a population of volunteer stem cell donors confirms that age and female sex are associated with CMV seropositivity. A history of pregnancy is not sufficient to explain the observed differences between male and females alone. Serocconversion seems to occur not only in young adulthood. Those young males are the preferred donors for hematopoietic stem cell transplants. As soon as a CMV seropositive donor is needed this selection diminishes the available donor pool.

P03-16

Experiences with the NAT testing for Parvovirus B19 (B19V) and Hepatitis A virus (HAV) in a five years period

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Introduction: B19V as well as HAV can be transmitted by transfusion, but the incidence of infections with each of these viruses in our donor population over a longer period is unknown.

Methods: All blood donations of our institute taken between the 1st January 2012 and the 31st December 2016 were screened for B19V DNA and HAV RNA once a week using the cobas Taqscreen DPX test (Roche diagnostics, Mannheim, Germany) in minipools up to 96 donations before clearance of fresh frozen plasma. Cellular blood components were cleared without prior B19V DNA or HAV RNA screening. Minipools were resolved if they tested reactive for HAV RNA or reactive for B19V DNA with a DNA concentration of at least 10,000 IU B19V DNA/ml plasma relating to the single donation.

Results: 265,125 donations were tested for HAV RNA and B19V DNA within the study period. None of the donations tested positive for HAV RNA. 25 (0.009%) donations had B19V DNA in a concentration of at least 10,000 IU/ml, compatible with a recently acquired infection. In 2012, 2013 and 2014, incidence of B19V infection was almost identical with four to five infections per year. The incidence decreased in 2015 (one infection/year) and reached a peak in 2016 (ten infections/year).

Conclusion: As we found no HAV RNA-positive donation by screening a quarter of a million donations, taking an HAV RNA-positive donation seems to be a very rare event. Thus, HAV is of minor relevance as a transfusion-transmitted virus in our donor population. HAV RNA screening can be maintained before the clearance of plasma for fractionation but is not necessary for cellular blood components. Also a B19V DNA-positive donation is seldom, but occurs, with a varying frequency in different years. However, due to the often minor clinical relevance of transfusion-transmitted B19V-infection, there is also no urgent need for a general B19V DNA-screening of cellular blood components.

Feasibility test of double doses pathogen-reduced platelet components by Intercept™ Blood System after separation from whole blood by Reveso® Automated Blood Processing System

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Introduction: Reveso® Automated Blood Processing System was recently chosen for blood component preparation in Västmanland and Dalarna counties in the Uppsala region of Sweden. It was also decided to continue...
the implementation of pathogen reduction (PR) of platelet units by Intercept™ Blood System using the dual-storage (DS) processing set. The aim of this study was to evaluate if the pool of 8 interim platelet units (IPU) processed by Reveos® system met the input requirements for the Intercept™ DS processing set, and if the results from quality controls of the PR-platelet units were in accordance with the local and EU guidelines.

Methods: Reveos® system processes 4 units of whole blood collections in a single run. Whole blood is separated into one red cell unit, one plasma unit, one IPU containing platelets in approximately 25 mL plasma, and one bag containing leukocytes. Eight ABO-identical IPUs were pooled so that the total number of platelets in the pool was within 5.5–7.5×10¹¹ according to the platelet yield index. After addition of 250 mL additive solution (SSP+), the pooled IPUs were stored in room temperature for two hours, of which the second hour on an agitator, before filtration for leukocyte reduction. Intercept™ treatment was then applied using DS set according to the method described by the manufacturer.

Results: During validation period, a total number of 26 Intercept™-treated platelet units prepared from 13 pools of IPUs were measured for platelet counts which showed 2.2 ± 0.1 × 10¹¹/unit. The first 14 units were also controlled for pH, lactate, glucose, pO₂ and pCO₂ at day 2, 5 and 7 of storage. The mean pH value was 6.9 on day 2 and 7.0 on day 5 and 7 of storage. Lactate concentration increased from 4.7 to 8.3 and 11.1 mmol/L and glucose content decreased from 6.0 to 3.9 and 2.6 mmol/L from day 1 to day 5 and 7, respectively. Over the 7 days of storage, the mean pO₂ level was 16.0, 18.7 and 16.6 kPa and the mean pCO₂ level was 4.1, 2.3 and 2.1 kPa on day 2, 5 and 7, respectively. After routine implementation of the method, quality controls during the following 8 months showed mean platelet count of 2.5 ± 0.3 × 10¹¹/unit (n = 343). In 54 patients with mainly hematological disorders, the measurement of the platelet increment showed platelet counts of 17 ± 17 × 10⁹/L before and 31 ± 23 × 10⁹/L after one hour of transfusion of one PR-platelet unit, respectively. There was no report of any adverse reaction to transfusion of PR-platelet units.

Conclusion: Production of pool of 8 IPUs processed in Reveos® for Intercept™ treatment of double dose platelet components is feasible. All pools produced in this study met the requirements for the Intercept™ treatment. The results from the quality controls of the units were in accordance with the local and EU guidelines. Analyses of the in vitro parameters showed that PR-platelet units were suitable for transfusion up to 7 days of storage.
but not split. Usually, whole blood (WB) was either separated on the day of collection (day 0) and plasma was stored overnight (o/n) for pooling and pathogen inactivation on day 1 or WB was stored o/n and separation, pooling and inactivation were done on day 1. Following parameters were assessed: Time until ready for freezing, plasma loss because of exceeding the 18.5h limit or missing the 630–650mL range. Factor (F) VIII content based on routine QC data.

**Results:** 3,300 Pi-FFPs were successfully made from RP (86% pools of 5) and 327 from SP. SP 5 failed meeting the 18.5h limit. Range of time to freezing was 3.1–11.7 h for SP frozen on day 0 (54% of SP) and 13.9–18.3 h if frozen on day 1. Range of time for RP frozen on day 0 (1%) was 7.4–13.1 h and 14.4–18.5 h if frozen on day 1 (99%). No plasma was lost because of coming under 630mL. Loss due to exceeding 650mL was 1.6% of total plasma pooled (pools of 3: 12.8% loss; pools of 5: 0.5%; pools of 6: 11.3%; filtration loss not included). F VIII specification (≥ 0.5 IU/mL) was met at 100%.

**Conclusion:** Meeting the limit of 20h from donation until end of freezing is not a problem in routine. Hence, INTERCEPT allows reducing manufacturing time of FFP from ≥4 months to <1 day compared to quarantine storage. This tremendously increases the possibility to react on fluctuations in demand. Plasma loss can be minimized by generating as many pools of 5 as possible since their splits are closest to 650mL.

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**P03-20**

**Platelet function and pathogen inactivation data obtained using the INTERCEPT triple storage set for platelet Production**

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**Introduction:** The INTERCEPTTM Blood System for platelets (PLT) offers three disposable sets for the treatment of small (2.5–6.0 × 10¹¹) large volume (2.5–7.0 × 10¹¹) or double dose (2.5–7.0 × 10¹¹ /7.1–8.0 × 10¹¹) PLT from Buffy coat pools or apheresis collections. A new INTERCEPT Triple Storage Set (ITS) was designed to process apheresis PLT components (PC) in PAS with doses of 6.0 to 12.0×10¹¹ PLT in 420 to 650 mL. PLT function and pathogen inactivation (PI) efficacy were evaluated.

**Methods:** Apheresis PC were collected in PAS-3 (for in vitro function assays and PI evaluation). One study was performed at the nominal dose (9.2–10.0 × 10¹¹ PLT), volume (558–629 mL) in 65% PAS/35% plasma using single donor apheresis collections. Two studies were performed to evaluate the high dose and high volume condition (9.7–11.8 × 10¹¹ PLT in 593–659 mL) using either single or pooled donations. Input PCs (n = 20) were treated with the ITS by the end of Day 1 post collection; the incubation time in the Compound Adsorption Device (CAD) container ranged from 4 to 16 hours resulting in a total of 60 units. Day 5 and 7 post-donation PC were evaluated for in vitro PLT function. PI was evaluated for extreme conditions combining the lowest amotosalen concentration (135 µM) with the highest PLT concentration (47% plasma/53% PAS at a final volume of ~630 mL for a dose of 9–12×10¹¹ PLT). Control (Pre-UV A) and Test (Post-UV A) samples were assessed for bacteria growth by culture and viral infectivity by plaque assay. The Log reduction expressed as the difference between the Log10 titers in Control and Test samples is reported.

**Results:** Apheresis PC in PAS-3 manufactured with the ITS set had acceptable in vitro function over 7 Day storage. All INTERCEPT treated PCs had pH (22°C) ≥6.2. Platelet dose and volume recovery post-treatment ranged from 82% to 99% and 88% to 92%, respectively. The inactivation potential for representative viruses (bovine viral diarrhea, bluetongue and adenovirus-5) and bacteria (E. coli, K. pneumonia and S. aureus) were evaluated. The Log10 reductions obtained were: for the viruses: >5.6, >5.9 and >5.8, and for the bacteria >7.0, 6.7 and >7.7, respectively. The results compare well with the CE mark label claims for the same pathogens. This indicates that efficacy of inactivation is not affected significantly, even in the extreme limits of the TS range for volumes and PLT count.

**Conclusions:** Pathogen reduced PLT components processed using the INTERCEPT TS maintained acceptable in vitro quality through 7 days of storage while high levels of viral and bacterial reduction were achieved. Inactivation of pathogens in PLT concentrates treated with the INTERCEPT TS kit is similar to that achieved under nominal conditions, even at extreme ranges of volume, plasma content and platelet dose.

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**P03-21**

**Productivity and cost optimization of double dose pathogen inactivated platelets**

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**Introduction:** The INTERCEPT Blood System (IBS, Cerus) utilizes a photochemical treatment with amotosalen and UVA to inactivate pathogens and leucocytes in platelet concentrates and plasma. The Dual Storage (DS) set allows the treatment of a double dose (DD) of platelets. The Blood Center of Aalborg between October 2012 and October 2014 first period (P1) prepared platelet concentrates (PC) from pools of 4 buffy coats (BC) with Taci automated platform (Terumo BCT). After a decision to implement INTERCEPT, the production method was adapted to prepare DD PC from pools of 7 BC, first with Taci (Nov. 2014 to Jan. 2016 – P2) then with a manual pooling set PC (Macopharma) (Feb. to Dec. 2016 – P3). A variation was introduced from Jan. to May 2017 (P4) with use of the I-Platelet Pooling Set (IPP – Kansuk) specially designed for DD PC and incorporating a Sepacell™ PLK5 (Asahi Kasei) leukodepletion filter. QC data, productivity and cost were compared.

**Methods:** BC prepared on Macopress separators (Macopharma) had the following average specifications during P1: volume 62 mL, Hct 50%, plt count 1 × 10¹¹. For subsequent periods this was adapted to: volume 45 mL, Hct 35%, plt count 1.1 × 10¹¹. Single dose (SD) PC were produced on Taci from pools of 4 BC with 300 mL SS+ (Macopharma) during P1. DD PC produced in P2 from 7 BC on Taci or in P3 and P4 with manual pooling sets and 280 mL SS+ were photochemically treated with INTERCEPT DS sets to yield two pathogen inactivated PC.

**Results:** Production during P1 (4 BC, Taci) was 250 ± 20 SD PC with 54 ± 22 irradiated PC per month. During P2 (7 BC, Taci, IBS), it was reduced to 142 ± 13 DD PC (equivalent to 284 ± 26 SD PC) without need for irradiated products. It remained relatively stable during P3 and P4 (7 BC, manual separation, IBS) with respectively 144 ± 16 DD PC and 124 ± 13 DD PC per month. Platelet content per PC was 293 ± 43 × 10⁹ (n = 258), 266 ± 40 × 10⁹ (n = 158), 272 ± 39 × 10⁹ (n = 92) and 242 ± 29 × 10⁹ (n = 47) for the 4 periods so the impact of shifting from SD to DD and treating with IBS was a reduction of 9 to 17% of the platelet content per PC. WBC outliers (>1 × 10⁶ / PC) were 0.4% during P1, 2.4% during P2, 5.4% during P3 and 0% during P4. The production cost per platelet dose when implementing INTERCEPT increased by 16% in P2 but decreased by 13% when adopting manual methods.

**Summary/Conclusions:** The production of DD BC Platelets with INTERCEPT was successfully implemented. BC characteristics were adapted (reduction of volume and hematocrit) and platelet production process optimized to obtain two doses of platelets from 7 BC instead of one dose from 4 BC. Use of the IPP set reduced the WBC outliers. The monthly production of PC became more efficient when implementing the DD method with IBS (43%). Gamma irradiation of PC was stopped. The adoption of the DD BC production method with a manual pooling process is less expensive despite the addition of a pathogen inactivation process.
P04-1
Identification of a rare para-bombay FUT1 mutation in an apparent ABO Bel phenotype
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Introduction: Discrepan sorological findings in ABO blood group typing not only may be the result of underlying ABO gene variants. In rare cases also variants of fucosyltransferases 1 (FUT1: H) and 2 (FUT2: Secretor) which encode enzymes crucial for ABH blood group antigen synthesis may be causative for aberrant ABO phenotypes. In an Austrian patient of Far Eastern origin a suspected Bel phenotype finally was identified to carry a rare non-functional mutation in the FUT1 gene, previously described as a para-Bombay phenotype in Taiwan.

Material and Methods: ABO blood groups were determined with standard serologic and gel matrix techniques (MicroTyping system, DiaMed ID, Cressier, Switzerland). Adsorption-elution studies of the patient’s RBCs were carried out using polyclonal anti-A and anti-B (Biostest, Dreieich, Germany) and acid elution (DiaCidel, DiaMed). Crossmatch with group O and B RBCs was performed in antiglobulin and neutral gel cards (DiaMed). Sequence analyses of the ABO gene and regulatory regions as well as FUT1 and FUT2 were done (AB 3500, Big Dye Terminator V 3.1 life technologies).

Results: Monoclonal antigen typing indicated blood group O, but typing with polyclonal human anti-A and anti-B showed weak reactions (+/+ and +++, respectively), and a negative result with anti-A. In reverse blood group typing at ambient temperature, the serum was strongly reactive with A1 RBCs, 2+ reactive with A2, and negative with O cells. Adsorption-elution studies showed negative results with anti-A, but positive reactions with anti-B, suggesting a Bel phenotype. The indirect antiglobulin crossmatch of the patient’s plasma was positive with one of three blood group B RBC samples, and generally negative with group O RBCs. By sequencing analysis the alleles B114 and O01 were detected. FUT1 gene showed a deletion of one of three AG repeats (547–552 delAG) in the coding region of the gene, a rare frameshift mutation found in individuals with the para-Bombay phenotype in Taiwan. FUT2 was homozygous for the functional Se37 allele, indicating secretor status.

Conclusion: The detected mutation in FUT1 abolishes or at least greatly reduces the activity of the corresponding α(1,2)fucosyltransferase synthesizing H type 1 antigen in red cells, while functional FUT2 alleles are present, indicating a para-Bombay phenotype. As these individuals usually retain some H antigen on RBCs and only weak anti-H activity of the RBCs is observed, the para-Bombay phenotype of this apparently Bel typed patient nearly would have remained unidentified. When transfusion is indicated, group O RBCs appear to be compatible for this patient.

P04-2
Sensitive, rapid and reliable detection of PNH clones by a new flow cytometric method based on a combination of the leukocyte antigen CD157 and fluorescence-labelled modified aerolysin (FLAER)
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare life-threatening disease due to acquired mutations of the PIGA gene in hematopoietic progenitor cells which cause reduced/absent expression of GPI-anchored proteins, including key complement regulators CD55 and CD59. Clinical key features are intravascular hemolysis, thrombophelia and cytopenia. PNH must be considered as differential diagnosis if hemolysis and/or thrombophelia of unknown origin are present. Eculizumab inhibits formation of the terminal membrane attack complex and can significantly reduce symptoms and improves quality of life and survival in PNH. Thus, a disease-modifying targeted therapy is available and it is necessary that accurate, early diagnosis of PNH is made. This is based on analysis of GPI-anchored proteins on blood cells by FACS. Cytometry guidelines recommend multicolor flow cytometry using various GPI-anchored proteins (e.g. CD16, CD24, CD66b on granulocytes (Gr); CD14, CD48 on monocytes (Mo); CD48, CD52 on lymphocytes (ly)) There are some shortcomings of these methods: broad antibody panel and different tubes required, activation dependent expression of some markers. CD157 is also a GPI-anchored marker stably expressed on Gr and Mo. FLAER is a modified bacterial toxin binding directly to the GPI-anchor.

Method: New FACS protocol based on CD157/FLAER double staining for detection of PNH Gr, Mo and lymphocytes with independent staining with other GPI-markers (e.g. CD14, CD16, CD24, CD48, CD52, CD66b).

Results: Mixture of GPI-deficient and non-deficient Gr (in 20% steps from 0% to 100%) demonstrated linearity of results (r² > 0.99 for CD157 on Gr and Mo). Correlations of results obtained with FLAER and CD157 were high (r² = 0.94, regression line 0.98x + 0.44 for Gr; r² = 0.98, regression line 1.09x + 0.61 for Mo). These correlations were much higher than among traditional markers. Background levels of negative cells in normal samples were low, allowing to establish a low cut-off of C157/FLAER cells: 0.01 for Gr and 0.06 for Mo. In a series of 1113 FACS analyses (only first analysis per patient, no follow up) we could detect a significant PNH Gr population in 261 (23.5%) and Mo population in 224 analyses (20%) with a mean clone size of 27.8% (Gr) and 32.4% (Mo) and a correlation between granulocyte and monocyte clone size (r² 0.84; Gr clone size = 1,0096-fold Mo clone size + 2.63; p < 0.0001). All patients with GPI-deficient Mo had GPI-deficient Gr (n = 224), whereas in 37 pts. in our series the clone could be detected in Gr only – and not in Mo. Majority of pts. had small PNH clones (in 50% of pts. < 7.5% PNH Gr). Correlation of clone size in Gr and Mo with red cell clone size will be presented.

Conclusion: Measurement of GPI-deficient Gr and Mo with FLAER/CD157 double staining allows sensitive, rapid and reliable detection of PNH cells. This should facilitate an adequate use of FACS in suspected PNH (hemolysis and/or thrombophelia of unknown cause, cytopenia).

P04-3
Evidence for carbohydrate epitope sharing between pneumococcal polysaccharides and blood group ABO antigens after immunization with Pneumo 23 Vaccine
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Hypothesis: Blood group antibodies are natural antibodies that develop in life in response to cross-reactive environmental antigens in the absence of antigen encounter. Even later in life structural similarities in saccharide composition between environmental antigens such as bacterial polysaccharides and blood group ABO antigens could lead to changes in serum levels, IgM/IgG isotype, and affinity maturation of blood group anti-A/B antibodies. We addressed the question whether immunization with pneumococcal polysaccharide (PnP) vaccine Pneumo 23 Vaccine «Pasteur Merieux» (Pn23) could have such an effect in patients with type 1 diabetes mellitus (DM1), an autoimmune disease where an aberrant immune response to microbial antigens likely plays a role.

Methods: Anti-PnP IgM and IgG responses were determined by ELISA, and the DiaMed-ID Micro Typing System was used to screen anti-A/B antibody titer before and after Pn23 immunization in 28 healthy individuals and 16 patients with DM1. In addition, surface plasmon resonance (SPR)
technology using the Biacore® device and a synthetic blood group A/B tri-
saccharide as the antigen was applied to investigate IgM and IgG anti-A/B
antibodies and to measure antibody binding dynamics.

Results: All healthy individuals and DM 1 patients responded with an-
ti-PnP IgM and IgG antibody production 4–6 weeks after Pn23 immu-
nization, while no increase in blood group anti-A/B antibody titer was
observed when measured by the DiaMed-ID Micro Typing System. Inter-
estingly, isotype-specific testing by SPR technology revealed an increase in
blood group anti-A/B IgG, but not IgM, following Pn23 immunization in
both patients and controls. No change in binding characteristics of blood
group anti-A/B antibodies could be detected following Pn23 vaccination,
supporting the assumption of an increase in IgG antibody titer with no or
very little affinity maturation.

Conclusion: The study provides evidence for epitope sharing between
pneumococcal polysaccharides and blood group ABO antigens, which leads to a booster of blood group anti-A/B antibodies of the IgG isotype
after Pn23 immunization in healthy individuals. Manifest autoimmunity
such as present in DM1 patients has no additional effect on the cross-react-
tive antibody response against pneumococcal polysaccharides and blood
group antigens.

P04-4

A new genetic background of Jr negative blood group
phenotype caused by the ABCG2*R147W allele

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Background: The JR blood group system (ISBT number 032) is defined
by the ABCG2 gene that encodes an ATP-dependent transport protein
with broad substrate specificity. The Jra antigen is located on the ABCG2
protein and is a high prevalence antigen in all populations. Numerous null
alleles, i.e. either nonsense or frame-shift mutations, have been reported
leading to the Jr negative phenotype. One fatal case of hemolytic disease
of the fetus and newborn caused by anti-Jra was described in the litera-
ture. We report on a Turkish family with a Jr negative male index indivi-
dual who developed an alloanti-Jra. Sequencing all exons of the ABCG2
gene revealed homozygosity for a missense mutation.

Methods: The antibody identification was performed in the indirect anti-
globulin test (IAT) in the gel technique using untreated and papain treat-
ed red cells. The Jr antigen of the patient’s twin sister (MA2), brother
(MA3) and father (MA4) were serologically tested with a monoclonal
anti-Jra serum and two different sources of polyclonal anti-Jra from the
SCARF exchange program. The family members were also tested for com-
patibility with the patient’s (MA1) serum in IAT. The ABCG2 exons were
sequenced in all four individuals as described before. A PCR-SSP method
was established for the 439C>T mutation.

Results: The patient’s serum showed positive reactions with all tests cells
of several antibody identification panels and with multiple red cell nega-
tive for a number of high frequency antigens. Three different samples of
Jra negative red cells were compatible with his serum. The phenotype of the
patient’s twin sister (MA2) was Jr negative, his brother (MA3) and
father (MA4) were Jr positive. The patient himself could not be tested
because of a positive direct antiglobulin test. The eluate derived from the
patient’s red cells was negative. By DNA sequence analysis of all ABCG2
exons we found a homozygous C to T exchange in exon 4 at position c.439
in MA1 and MA2, whereas, MA4 was heterozygous. These genotypes and
the homozygous c.439C (wild type) genotype of MA3 could be confirmed
by PCR-SSP. Molecular screening by PCR-SSP for the ABCG2*439T allele
of 10,058 blood donors from Southwestern Germany revealed 3 heterozy-
gous donors (allele frequency 0.00015). The c.439C>T mutation is listed in
the dbSNP (rs372192400) and ExAC databases with a frequency for the
439T allele of 0.00007 in the European population.

Conclusion: We describe the first null allele of the ABCG2 gene caused
neither by a nonsense nor a frame-shift mutation. The 439C>T missense
mutation found in a family of a patient with Jra negative phenotype and an
alloanti-Jra results in a single amino acid exchange (Arg147Trp).

P04-5

A new device for simultaneously typing Fy+, Fy−, Jk+, Jk−, S, and
s was superior to the tube method

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Introduction: Serological typing of blood group antigens is done by
haemagglutination. For many antigens monoclonal IgM-antisera are avail-
able, which allow direct agglutination of antigen positive cells. For
other antigens, including Fya, only IgG antisera exist requiring an indirect
antiglobulin test. The MDmulticard Basic Extended Phenotype is a new
device for simultaneously typing red cells for Fya, Fyb, Jka, Jkb, S, and s. In
a beta study we tested practicability and reliability in laboratory routine.

Methods: Blood samples from 45 donors and 40 patients were collected.
The patient samples included 10 samples with a reactive direct antiglob-
ulin test (DAT) and 5 cord bloods. All patients had not been transfused
for 3 months prior to sampling. The samples were phaenotyped by the tube
method for Fya, Fyb, Jka, Jkb, S, and s. The donors had been phaenotyped
or/genotyped at earlier occasions. For comparison, all samples were
phaenotyped with the multicard. In brief: the blood was washed once with
saline, then 1 drop of saline blood was mixed with 8 drops of Diluent
F. Two drops of the resulting suspension were added to the application
zone of the multicard. After 30 seconds, 6 drops of Diluent F were added to
the application zone. After 4 minutes, another 6 drops of Diluent F were
added to the application zone. The results were read and recorded
8.5 minutes after application of the suspension to the card. The results of
both methods were compared. In case of discrepant results the samples
were genotyped for FY*A, FY*B, FY*X, Jk*A, Jk*B, S and s using
commercially available PCR kits. In addition, all samples with a reactive
DAT were genotyped.

Results: The test procedure was easy to perform and the results were
available after a few minutes. 510 antigens were typed of which 5 results
were discrepant: The samples from two patients with a reactive DAT were
typed Fy(a+)/by the tube method and Fy(a-) by the multicard. Genotyping
showed that both lacked the FY*A allele and confirmed the results of the
multicard. One patient, one newborn, and one donor were typed Fy(b−)
by the tube method but Fy(b+) by the multicard. Genotyping of the three
samples revealed the FY*X allele which causes a weak Fy phaenotype.
Genotyping again confirmed the results of the multicard.

Conclusions: The performance of the multicard was superior to the stan-
dard tube method in two ways: Weak Fyb phaenotypes were tested nega-
tive by the tube method, but were easily detected by the multicard. For
Fya only IgG antisera are available, requiring the indirect antiglobulin test
for phaenotyping. Two samples with a reactive DAT gave false positive
results in the tube method but were correctly typed as Fya negative with
the multicard.

In this study the new device gave reliable results, was easy to handle,
and was faster than genotyping. The MDmulticard demonstrated its usability
for extended phaenotyping of patients with, e.g., warm reactive autoanti-
bodies or sickle cell disease.
Cold agglutinins: haemolysis caused by antibody and efficacy of complement inhibitors assessed by a sensitive flow cytometric assay

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Background: The complement inhibitor ecuizumab has been used successfully in the treatment of paroxysmal nocturnal hemoglobinuria (PNH) and aHUS. A preliminary patient study points to a certain therapeutic potential of ecuizumab in cold agglutinin disease (CAD). To obtain more information about the potential effectiveness of complement inhibitors in CAD, we investigated by in vitro experiments complement activation by cold agglutinins and the effectiveness of different complement inhibitors.

Methods: Complement activation by antibody was tested by a sensitive assay based on the haemolysis of paroxysmal nocturnal hemoglobinuria (PNH) III RBCs which lack the complement inhibitors CD59 and CD55. We selected 7 samples with cold agglutinins showing titres from 32 to 512 with the serologic characteristics of anti-I. RBCs of patients with a PNH III clone > 20% were incubated with cold agglutinins for 20 min at the highest temperature still showing agglutination, assessed for each antibody. Haemolysis was developed at 37°C with normal human serum of blood group AB with or without the addition of complement inhibitors. Not haemolysed PNH III RBCs were counted by flow cytometry using PE-labelled anti-CD59.

Results: All cold agglutinins caused strong complement mediated haemolysis of human PNH RBCs. Haemolysis was dependent on the dose of antibody used for sensitization and was predominantly mediated via the classical pathway as indicated by the effectivity of factor B deficient serum to develop haemolysis. Complete inhibition of haemolysis could be achieved by ecuizumab when PNH-RBCs were coated with low titer cold agglutinins. However, haemolysis could not be stopped completely when the PNH RBCs were sensitized with high amounts of antibody. The combination of ecuizumab with the complement inhibitors CR1CCP1–3 or MiniFH caused more efficient inhibition than by each inhibitor alone.

Conclusion: The DECADE clinical trial included 13 patients with CAD; a positive effect of ecuizumab therapy based on LDH levels and on the required transfusion support was reported (Röth et al. Blood 2015;126:127). Our sensitive test for complement activation by antibodies allowed to prove the complement activating potential of cold agglutinins and to demonstrate the inhibitory potential of complement inhibitors including ecuizumab in vitro. Our recent report (Harder et al., Blood 2017;129:970–980) indicates that ecuizumab was able to stop moderate alloantibody mediated haemolysis; it also decreased strong haemolysis but residual haemolysis remained, a finding which also applies to the action of cold agglutinins. A promising subject for further investigations is the observed additive effect of different complement inhibitors in interfering with antibody mediated complement activation.

Performance of MDmulticard® Basic Extended Phenotype in laboratory routine

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Background: Extended antigen-matching can reduce the risk of alloimmunization in chronically transfused patients. However, current serological methods for extended phenotyping are time consuming and are associated with limitations, e.g. in patients having a positive DAT. Furthermore, molecular methods are complex, expensive and not universally available.

With the new MDmulticard® Basic Extended Phenotype (Medion Grolfis Diagnostics), typing for Jk, K, Fy, S and antigens can be performed simultaneously and fast using lateral flow technology. Aim of this study was to compare the performance of MDmulticard® to standard laboratory methods and to evaluate its use for laboratory routine.

Patients and Methods: Unselected samples of blood donors (n = 45), patients (n = 23) and newborns (n = 5) were tested using MDmulticard® Basic Extended Phenotype and the results were compared to gel column agglutination and solid phase technology, respectively.

In addition, 11 DAT positive samples of patients with known autoantibodies (DAT ≥ 2+) and 12 mixed-field samples from patients who had been previously transfused were investigated. All DAT positive and mixed-field samples were analyzed after DNA-extraction by PCR-SSP technology (BAG, Lich, Germany).

Results: Testing of donor, patient and neonatal specimens with the MDmulticard® Basic Extend Phenotype was consistent with the expected results obtained by routine laboratory method in all 73 samples. Regarding
DAT-positive samples, there was a very high concordance between the results of the MDmulticard® and the phenotype deduced from the genotype of the samples, albeit there were very weak (false positive) reactions in three of the Fya-negative samples. Performance of phenotyping of samples from pre-transfused patients with mix-field reactions was comparable to those of gel column agglutination. However, both serological methods overestimated positive antigens as shown by results predicted by genotyping.

During evaluation but also after introduction in daily laboratory routine, extended phenotyping with MDmulticard® was also found to be very useful in identifying rare phenotypes (e.g. S-α, Jk(a-b-)) leading to prompt identification of the corresponding antibody specificity.

**Conclusions:** Extended Phenotyping using MDmulticard® is easy, rapid and reliable in laboratory routine for various sample types. Furthermore, it could be used in patients with positive DAT, which is a clear advantage.

**P04-9**

**Relevance of a positive DAT with respect to the detection of a delayed serological transfusion reaction due to red blood cell alloantibodies**

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**Introduction:** The direct antiglobulin test (DAT) is used in pre-transfusion samples to trace immunoglobulin (IgG) or complement on the surface of red blood cells (RBC). In recently transfused patients a positive DAT may be a first sign of a delayed serological transfusion reaction. DAT work up using elution procedures and highly sensitive antibody detection methods as indirect antiglobulin tests with enzyme treated test cells may identify novel alloantibodies not detected in routine antibody screening tests. In order to investigate the diagnostic efficiency of the work up of a positive DAT we analyzed in a retrospective study 159,719 blood samples in 34,100 different patients (age above 28 days) covering a 4-year period.

**Methods:** In all samples a polyspecific DAT was performed using a gel card assay (BioRad, Switzerland). If positive, monospecific DAT (anti-IgG, anti-C3d) were obtained. In patients with newly identified positive DAT with ant-IgG or a significant increase of the agglutination strength with anti-IgG and a recent RBC transfusion within the prior 28 days a DAT work up including elution (BAG, Germany) and highly sensitive antibody detection methods such as IAT using enzyme treated test cells was performed.

**Results:** 29,942 (19%) samples showed a positive polyspecific DAT. 85% were positive only with anti-IgG, 7.3% with both anti-IgG and anti-C3d and 6.5% with anti-C3d, respectively. Following our internal algorithm 2,866 (9.6%) samples were further investigated. In 40 samples DAT work up revealed 44 novel RBC alloantibodies not identified in routine antibody screening tests. The rate of informative positive DAT work up thus was 1.4%. Most frequent novel alloantibodies were anti-E (14) and anti-Jk(a) (11). In 33 (98%) of these agglutination strength with anti-IgG was equal or below 2+. In 26 of 40 the interval of a recent putative incompatibility between 15 and 21 days and in 2 (7.7%) more than 21 days, respectively.

**Conclusion:** In 21 (81%) the interval was between 1 and 14 days, in 3 (11.5%) between 15 and 21 days and in 2 (7.7%) more than 21 days, respectively.

**P04-10**

**The novel missense mutation AQP1*c.517G>C leads to the Colton_null phenotype in a patient with an alloanti-Co3**

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**Background:** The Colton blood group system (CO, ISBT 015) consists of the commonly known Coa and Cob antigens and the high prevalence antigens Co3 (Conull) and Co4. Antibodies to Co3 usually occur in patients whose red blood cells are negative for all Co antigens. The Co system is encoded by the aquaporin-1 (AQP1) gene on chromosome 7p14.3 with 4 exons spanning approximately 12 kbp genomic DNA. The missense variant c.134C>T (p.A45V) represents the molecular basis of the Coa and Cob antigens. Different AQP1 missense or frame shift mutations were described as null alleles leading to the Conull phenotype. The clinical significance of alloanti-Co3 is documented by mild hemolytic transfusion reactions and mild to severe hemolytic disease of the fetus and the newborn (HDFN). We describe a novel variant of Conull phenotype found in a pregnant woman of oriental origin whose serum contained anti-Co3.

**Methods:** The antibody identification was performed in the indirect antiglobulin test (IAT) in the gel technique using untreated and papain treated cells. The Coa and Cob antigens of the patient were tested with commercial polyclonal sera in the IAT; the Co3 antigen was tested with two different sources of polyclonal anti-Co3 from the SCARF exchange program. The AQP1 exons were sequenced and a PCR-SSP method was established for the novel mutation according to a previously described protocol.

**Results:** The phenotype of the patient was Coa-b- and Co3-. Her serum showed positive reactions with all tests cells of several antibody identification panels and with multiple red cells negative for a number of high frequency antigens. One available sample of Co3-red cells was negative. In combination with the patient’s phenotype an alloanti-Co3 was identified. AQP1 gene sequencing of the patient showed a homozygous nucleotide exchange c.517G>C (p.Gly173Arg) in exon 2. By using PCR-SSP the sequencing result was confirmed. Screening of 10,608 blood donors for the AQP1*517C allele by PCR-SSP was negative. The mutation was not listed in the dbsNP and ExAC databases.

**Conclusion:** We describe the novel AQP1 variant c.517G>C (p.Gly173Arg) in a patient with the Conull phenotype and alloanti-Co3. Remarkably, there is an AQP1*c.517G>T (p.Gly173Cys) mutation listed in both databases (rs766229713) with a very low frequency of 0.00013 only in the South Asian population. However, the Co phenotype of the c.517G>T mutation is not known. From our data we conclude for the AQP1*c.517G>C mutation a very low frequency or even a private mutation in the patient’s family. Homozygous individuals must be unique.

**P04-11**

**Identification of alloanti-Sc1 in a patient with Glanzmann thrombasthenia**

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**Background:** In 2016 a 52 year old Caucasian woman was admitted to our medical center due to gastrointestinal bleedings. She suffered from Glanzmann thrombasthenia requiring repeated transfusions of red cell concentrates in her history. Adverse transfusion-related reactions have been described.

**Methods:** In a pregnant woman of oriental origin whose serum contained anti-Co3. The antibody identification was performed in the indirect antiglobulin test (IAT) using enzyme treated test cells was performed.

**Results:** The antibody identification was performed in the indirect antiglobulin test (IAT) using enzyme treated test cells was performed.

**Conclusion:** In 21 (81%) the interval was between 1 and 14 days, in 3 (11.5%) between 15 and 21 days and in 2 (7.7%) more than 21 days, respectively.
not been reported at any time. The current pre-transfusion diagnostics showed an alloreactive antibody to an unclear high frequency antigen.

**Methods:** ABO, Rh and Kell blood group typing, antibody screening and identification tests, compatibility testing and direct antiglobulin test (DAT) were performed with column agglutination technique (BioRad Diagnostica, Munich, Germany). For antibody identification, the Identification RedCell Special Panel (DRK-BSD, Baden-Baden, Germany) was additionally used. Neutralization test was performed by using a recombinant Sc1 protein (rBGA Sc1, Imusyn, Hannover, Germany). Molecular typing was performed by using an in-house SSP-PCR.

**Results:** The antibody screening, the antibody identification tests and the compatibility testing showed panagglutination with 2+ positive reactions in the indirect antiglobulin test. The autocontrol and the DAT were negative. A special panel containing a number of red cells negative for high frequency antigens showed a negative reaction with one cell which was negative for the Yka antigen and heterozygous for the Sc2 antigen (Sc1,2). Anti-Yk\(^a\) alloantibody was ruled out with several Yk\(^b\) negative cells which were positive with the patient’s plasma. After plasma neutralization with the recombinant Sc1 protein the antibody screening test was negative. In addition, there was no agglutination with one Sc1 negative and one Sc3 negative cell indicating the presence of a Sc1 antibody in the patient’s plasma. The molecular typing of the patient revealed the homozygous genotype Sc\(^{00}\).

**Conclusion:** We report on a female Caucasian patient who developed an anti-Sc1 alloantibody after numerous transfusions in the past. The Sc1 negative phenotype is very rare in the Caucasian population and severe hemolytic transfusion reactions have not been reported for alloanti-Sc1. Accordingly in our patient, there was no hemolysis although serologically incompatible transfusions had to be performed. Since the patient required several transfusions after developing alloanti-Sc1 the Sc1 rBGA was a helpful tool to rule out further alloantibodies.

**Results:** The automated ABO titration assays demonstrate a very high reproducibility across different NEO Iris™ instruments and different time points. 180 titer results were analyzed for each IgM and IgG assay on A1, A2 and B Referencells. On average, 77% of all assays show the same titer, 23% differ by one doubling dilution and only 0.1% differs by two doubling dilutions. High consistency between NEO Iris™ and Galileo NEO\(^b\) was demonstrated. 672 valid test results were used for the analysis obtained from the different assays tested on three NEO Iris™ instruments and one Galileo NEO\(^b\) instrument. For all assays, 62% of test results achieved the same titer, 37% were within one doubling dilution and 0.7% were demonstrated at two doubling dilutions.

**Conclusion:** Fully automated ABO titration assays offer an excellent reproducibility and repeatability across several NEO Iris™ instruments and high consistency between two different types of automated platforms. Immucor provides a complete and highly consistent IgM and IgG ABO titration assay set which may help to overcome currently labor intensive and less precise manual techniques.

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**P04-12 Consistent automated ABO antibody titrations on the NEO Iris™ platform – reproducibility and repeatability**

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**Introduction:** IgM and IgG ABO antibody titration is clinically important in several clinical areas including: ABO-incompatible stem cell and solid organ transplantation, blood group O donor characterization for transfusion as well as ABO-incompatible pregnancies. The most common methods for the determination of ABO antibody titers, like conventional tube test (CTT) and column agglutination technique (CAT), are time consuming, poor in accuracy and reproducibility, and are subjective in respect to interpretation of the titer end point. Immucor’s Capture\(^b\) based fully automated ABO titration assays were validated for use on the Galileo NEO\(^b\) to overcome the weaknesses of manual techniques. Immucor now developed a new automated platform, the NEO Iris™.

The aim of the study was to evaluate the performance of fully automated IgM and IgG ABO titration assays regarding reproducibility and repeatability on the NEO Iris™ platform. Consistency of ABO titration titer results between the Galileo NEO\(^b\) and NEO Iris™ was also investigated.

**Methods:** For reproducibility and repeatability of fully automated ABO titration assays, donor plasma was tested and evaluated with low titer (1/1 to 1/128) IgM and IgG as well as high titer IgG (1/16 to 1/4096) configuration. Two donor specimens per assay were tested in triplicates in the morning and afternoon on five non-consecutive days within 21 days on three NEO Iris™ instruments resulting in 180 titer results per assay. To verify titer consistency, different fresh or frozen blood specimen types, such as donor, pregnancy and transplant, as well as different tube types (CPDA, EDTA, and serum) were tested utilizing the automated ABO titration range assays on the NEO Iris™ versus Galileo NEO\(^b\).

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**P04-13 Third generation sequencing: an easy way for internal controls in non-invasive prenatal genotyping**

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**Introduction:** Non-invasive prenatal genotyping in maternal blood samples may become the state of the art method. It critically depends on a valid control for free fetal DNA (fDNA) applicable for female fetus or in the absence of a paternal sample. Assays of fetal blood groups from maternal plasma largely base on quantitative real time PCR. Alternative methods are multiplex PCR with capillary electrophoresis and next generation sequencing. Nanopore sequencing as a novel approach measures the change of an ionic current while molecules pass the nanopores. The change identifies the passing molecule, e.g. DNA or RNA. We tested this technology for its use in non-invasive prenatal RHD genotyping.

**Methods:** Commercial kits from Oxford Technologies for a) rapid 1D sequencing and b) 1D exon sequencing were used. Samples from 1ml maternal plasma were used for DNA extraction with MagnaPure Compact. For 1D exon sequencing a pre-PCR step was performed for amplification of RHD specific sequences and up to 12 SNP’s. The use of barcode sequences was tested to differentiate between maternal DNA from leucocytes and fDNA. Extraction of generated fast5-files was performed with porefleet script (N. Loman, A. Quinlan) and identification of sequences was done with Geneious (Biomatters Ltd) and MacVector (MacVector Inc.) software.

**Results:** Both sequencing kits (rapid 1D, 1D exon) are suitable to examine small DNA amounts (> 0.5 ng). Determination of fDNA requires a pre-PCR step to accumulate enough starting material for nanopore sequencing. Addition of barcoding sequences allows to examine up to 12 samples in parallel.

**Conclusion:** Nanopore sequencing introduces a cost-effective alternative to analyze fetal RHD. Barcoding of samples offers the direct comparison of maternal and fetal DNA in the same run. Thus, negative results in fetal blood group determination can be validated with SNPs as a positive control for the presence of fetal DNA.
Abstracts

P04-14

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

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Background: The RHD, Kell and other blood group antigens of the fetus expressed on red blood cells as well as alloantigens on platelets can cause immunization of the antigen-negative mother. Prenatal determination of the fetal antigens is important for the early recognition and therapy of Morbus Haemolyticus Neonatorum (MHN) and Neonatal Alloimmune Thrombocytopenia (NAIT). The use of cell-free plasma DNA from the mother and methods based on RealTime-PCR represent a common strategy in the noninvasive prenatal diagnosis. We present first data on the development and evaluation of chip-based digital PCR (dPCR) for the specific and quantitative detection of RHD, RHCE, KEL and HPA alleles.

Methods: Cell-free plasma DNA 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. Furthermore, assays were used with diadeic probes like: KEL1(FAM)/KEL2(VIC), HPA1a(FAM)/HPA1b(VIC), AMY1(FAM)/AMX1(VIC) and 10 SNPs from the SNPforID panel.

Results: Specificity was tested on regular genomic DNA from leukocytes by typing of samples with known homozygous and heterozygous genotypes. False positive signals were observed in a range of 0 to 0.2%. In dilution series of heterozygous DNA (1 to 10%) in excess of homozygous DNA (90 to 99%) we found a high sensitivity (< 1%) for the detection of the rare allele. Preliminary results regarding specificity and sensitivity of cell-free plasma DNA indicated highly promising results for the identification of minor allele quantities.

Conclusion: RealTime-PCR and dPCR are based on the same TaqMan probe chemistry to generate fluorescence signals by amplification. However, dPCR with the end-point detection of fluorescence is more robust and efficiency of amplification can be neglected. The novel dPCR is highly applicable for specific and sensitive prenatal determination of RHD, KEL, HPA and other antigens of the fetus by using cell-free plasma DNA of the mother.

P04-15

Prospective evaluation of two different red blood cell antibody column agglutination methods

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Background: Red blood cell (RBC) antibodies can be tested by several sensitive methods that have been demonstrated to have comparable performance in retrospective studies. Performance in prospective studies is not sufficiently known.

Patients and Methods: Glas-bead based (Ortho Clinical Diagnostics) and gelcard column agglutination (Biorad) was prospectively compared with patient samples for antibody screening (group A). In addition, differentiation was compared with stored samples that were known to contain RBC antibodies (group B) using untreated and ficin-treated Panel C-cells (Ortho) or papain-treated cells (Biorad) according to manufacturer’s instructions.

Results: In group A, 24 of 1000 samples were found to contain antibodies. Of these, three sera were only reactive by gelcard agglutination (numbers and blood group specificity: 2 Rh, 1 warm-reactive-autoantibody (WAK)).

In addition, three samples, were only reactive by glas-bead agglutination (1 K, 1 Lu, and 1 WAK).

In group B, 164 sera with known antibodies were tested. Of these, 29 antibodies (11 mostly weakly reactive papain-only Rh, 2 Jk, 3 M, 2 Le, 6 Lu, 5 WAK) in 27 sera were only detected by gelcard agglutination. Further 9 sera contained antibodies that were only detectable by glas-bead based agglutination (1 weakly reactive ficin-only Rh, 2 K, 2 Jk, 1 P1, 1 N, 1 Lu, 1 WAK). Two sera contained antibody mixtures that neither of the systems detected completely (E and Lu and WAK). Of note, anti-Lu(a) was reactive in antibody differentiation batches one and two with 7 of 7 and with 1 of 8 of the samples, respectively.

Conclusion: This prospective study demonstrates equal performance for both antibody screening systems. Specificity was excellent for both systems with no unspecific reactions in group A. Antibody differentiation sensitivity appeared higher using the gelcard system. However, Panel A and B differentiation cells of the glas bead system were not used in this study. In addition, there was a considerable interference by batch-to-batch variation that could be demonstrated for anti-Lu(a).

P04-16

Anti-Fyε – a challenge in transfusion medicine

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Introduction: The Duffy (FY) blood group system consists of 6 antigens. Fy3 and Fy5 are expressed on red blood cells (RBC) of all Duffy phenotypes apart from Fy(a-b-), whereas Fy5 is not present on Rhnull cells even if there is a FY gene. This phenomenon suggests that Fy5 is a composite phenotype.

We report a case of anti-Fyε in a 24-year-old African, gravid 2, para 0, with transfusion dependent sickle cell disease. The patient’s red cells presented Fy(a-b-) phenotype with homozygous c.1-67T>C mutation of FYB allele. In the past, the patient received multiple transfusions of FYB positive RBC without sequelae except forming anti-E.

Methods: Standard serological methods for antibody detection and specification were used (gel-card and tube test; BioRad, Cressier, CH). The Kel, Kidd, Duffy and MNS blood group systems were analyzed by molecular typing with PCR-SSP (inno-train GmbH, Kronberg i. T, D). Monococyte monolayer assay (MMA) was carried out to assess the likely clinical significance of RBC antibodies. Paternal serological typing was performed (Erytra4, Grifols, Duedingen, CH) in order to predict the antigen profile of the fetus.

Results: The indirect antiglobulin test showed weakly reactive anti-Fy5, which was positive with all test cells, including papainized cells, except Fy(a-b-) cells and Rhnull Fy(a+b+) cells. Anti-E was only reactive on enzyme treated RBC. Based on molecular typing, the patient’s predicted phenotype was R0r, K-k+, Fy(a-b-), Jk(a-b+). Paternal antigen profile showed incompatibility in the FY and JK blood group systems, namely Fy(a-b+) and I(a-b-). The MMA of anti-Fy5 on O rr, Fy(a+b+) RBC revealed 0.7% reactive monocytes.

Currently we have 4 registered C-, E-, Fy(a-b-), Jk(a-b+), Fy(a-b+) cells and Rhnull Fy(a+b+) cells. Anti-E was only reactive on blood group O donors in Switzerland. In total 6 fully antigen compatible RBC products were transfused during pregnancy and further 2 peripartum when a semi-elective cesarean section was performed because of looming cardiac decompensation in 34th week of pregnancy. Serological phenotype of the newborn was R0r, K-, Fy(a-b-). Therefore the father must be most likely heterozygote for the c.1-67T>C mutation of the FYB allele. Hence, the newborn presented a negative direct antiglobulin test and no clinical signs of HDFN.
Conclusions: So far, only few cases with anti-Fy5 have been reported and its clinical relevance is obscure. In our case, the request of several compatible RBC in order to maintain hemoglobin level during pregnancy and for cesarean section was challenging. Based on in vitro data (MMA) we recommend transfusing Fyb positive RBC if no Fya(-b-) units were available. By doing so, one has to keep in mind the potential risk of delayed hemolysis due to boosting of anti-Fy5. According to postnatal assessment it is not possible to make any statement about diaplacental transmission of anti-Fy5, as the inherited FY genotype was preventing HDFN.

**P04-17**

Extracorporeal photopheresis does not induce drug-dependent antibodies to 8-methoxypsoralene

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Background: Extracorporeal photopheresis is an immunomodulatory treatment that is used for cutaneous T cell lymphomas to enhance the patients’ tumour immunity. The treatment consists of the application of 8-methoxypsoralen (8-MOP) to autologous leukaphereses that is re-transfused after UV illumination in intervals of one or several weeks. Drug application in intervals is known to cause immunization-like symptoms e.g. for rifampicin including provocation of drug-dependent red cell antibodies (ddab). Though side effects are rarely known for ECP, nothing is known about the potential occurrence of ddab to 8-MOP.

Patients and Methods: Sera from six patients on long-term ECP treatment were investigated for ddab of the immune complex and of the penicillin (binding) type by direct incubation with 8-MOP and by incubation with coated screening cells using barbital buffer.

Results: None of the samples showed reactivity with the direct ddab test as well with coated cells. In addition, there were no autoantibodies detectable.

Conclusion: These results, though limited by number, underline the safety profile of ECP.

**P04-18**

Management of morbus haemolyticus fetalis due to anti-Jsb

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Introduction: A 40 years old pregnant woman of African origin relocated from Tyrol to Upper Austria. It was already known, that she has a rare antibody against Jsb (Matthews) – a highfrequency antigen from the Kell-blood group. At the time of the first visit the patient was 24 weeks pregnant, and there was no sign of fetal distress or anemia. Looking for compatible blood, you would first look for siblings of the mother, but unfortunately they were all unavailable. Anti-Jsb, as all antibodies of the Kell-group are already present on early erythroid precursors at different concentrations. At the concentration of 2 mmol DTT only the antigens of the Kell-group are already present on early erythroid precursors. But it still leads to profound anemia of the fetus.

Methods: The presence of the Jsb-Antibody was confirmed with one of our usual panels, lacking this antigen. Furthermore, you can destroy the individual antigens of the Kell-blood group using DTT (dithiothreitol) at different concentrations. At the concentration of 2 mmol DTT only destroys the Js-Antigens. This treatment abolished all reactions with the whole test cell panel confirming further the specificity of the antibody. Because of the rarity of Jsb-negative units we decided in case that an IUT (intra uterine transfusion) becomes necessary, maternal blood will be used.

Even if the mother and child do not have the same blood group, the baby does not have any agglutinins – so it is save to perform such procedure. At first the obstetrician decided to go for a vaginal delivery. It was already the 6th pregnancy for the patient. She has 3 healthy children, the deliveries were vaginal and without complication. The patient also had one late miscarriage – the child was stillborn. And she has born one live child, which died 1 day later.

Results: Despite the relatively low titer of the antibody (8), the fetus developed severe anemia at 28th week of gestation, which had to be treated by performing an IUT using maternal blood. It had to be done twice without complication. Due to the risk for eclampsia and the baby’s anemia the obstetrician opted for an early cesarean section at 34th week of gestation. The Aggar after delivery was 5/7/7. In addition to the incompatibility to anti-Jsb there was also an ABO-incompatibility. The level of bilirubin required phototherapy. At the time of the discharge the baby’s hemoglobin was 11,9 mg/dl.

Conclusion: The extent of the titer is not predictive how dangerous an antibody is. Based on the highfrequency of the antigen (Caucasian 100%, Blacks 99%) it is very difficult to provide antigen-free blood units. And in such a case it is safe to use maternal blood to perform an IUT even if the blood group of mother and child does not match.

**P04-19**

Evaluation of the new immunohematological fully-automated Eflefixis-System

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Background: Recently, the new fully-automated Eflefixis System for immunohematological routine diagnostic was introduced by Grifols S.A. It was designed for smaller or medium sized blood bank laboratories and reflects a downsized version of the already established Erytra System also using gel agglutination techniques.

Material and Methods: In a comparative study, EDTA blood samples from blood donors and patients were tested on the Eflefixis-System and directly compared to the results of the established methods (Erytra for antibody screening/identification (ABS/ABI), cross-matching and for blood grouping; NEO Iris for blood grouping using direct hemagglutination microplates).

Tests were performed for blood typing including AB0 direct and reverse typing, RhD, C, c, E, e and K on samples of 143 patients and 61 donors; for ABS (indirect anti-human globulin test) on samples of 302 patients and 40 donors – including 20 samples of known antibody specificities, for ABI on 67 samples of patients and donors including 42 samples of known antibody specificities and for cross-matching 269 patient samples tested with 823 blood units – including 25 incompatible blood units (i.a. AB0, Fy(a), M).

Additionally, we recorded the turnaround time for blood grouping for NEO Iris and Erytra, as well as for Eflefixis, and the time for ABS and cross-matching conducted with Erytra and Eflefixis.

Results: AB0/RhD and K typing showed a complete concordance between observed and expected results. The results for C, c, E, e were accurate in the first and the second typing and showed 100% concordance with Erytra and 99.1% concordance with NEO Iris. (discrepancies on 2 samples, which were detected by the Eflefixis as mixed fields). Reaction strength of reverse typing was higher for NEO Iris compared to Eflefixis.

For ABS, we also observed a complete concordance of the results. All known antibodies were positive in both techniques. The comparison of the cross-matching showed a complete concordance. All 25 known incompatible blood units were positive in cross-match.

Comparing turnaround times (TAT) for Eflefixis and NEO Iris for blood grouping (without ABS), we found a weaker performance for the Eflefixis with increasing numbers of samples, although in the case of lower samples (up to 3 patients) the Eflefixis needs less time. For cross-matching (including AB0 and ABS) performed in comparison to Erytra, we recorded nearly the same TAT. The expected disadvantage due to the Eflefixis downsizing
Abstracts

P04-20

Therapy with extracorporeal photopheresis (ECP) – epidemiology and treatment of rare immune disorders in Germany

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Introduction: Extracorporeal photopheresis (ECP) is an autologous immune cell therapy used to treat a variety of auto- and alloimmune mediated diseases. ECP was introduced as treatment for Cutaneous T Cell Lymphoma (CTCL) but is also commonly administered to patients with acute and chronic Graft-versus-Host Disease (aGvHD & cGvHD). Due to the rare occurrence of CTCL and GvHD, data on epidemiology and treatment patterns are scarce. Therefore, our objective with this explorative study was to provide reliable and representative data for the epidemiology and treatment of CTCL and GvHD in Germany.

Methods: Based on the retrospective analysis of a six-year (2009–2014) database from the German Statutory Health Insurance system, we obtained a representative sample of ~3.5 insurants, displaying approximately 5% of the German population. This sample included 48 cases of CTCL and 226 cases of GvHD (acute and chronic). In addition to incidence and prevalence rates, we assessed comorbidities, age and sex distribution of patients as well as their medical care in terms of inpatient procedures as well as medication of CTCL and GvHD patients. Numbers are presented as mean (SD) if not indicated otherwise.

Results: Regarding recent figures, GvHD is the more prevalent disease in Germany (cGvHD: 0.0039%, aGvHD: 0.0017%; 2014), followed by CTCL (0.0012%; 2014). Affecting patients are predominantly of male sex (CTCL 68.5% (4.8%); cGvHD: 62.7% (1.8%); aGvHD: 67.9% (2.5%)). The mean age of patients affected by CTCL, cGvHD and aGvHD is 66.4 (13.2), 51.1 (15.3) and 47.5 (18.6) years, respectively. From 2009 to 2014, patients affected by all 3 conditions received various systemic treatments. For CTCL, common treatments include interferon alpha, bexarotene, and corticosteroids. Unsurprisingly, patients with aGvHD or cGvHD commonly received corticosteroids and calcineurin inhibitors (CNIs). Notably, 21.0% (8.1%), 14.5% (2.1%) and 5.7% (3.9%) of patients with CTCL, cGvHD and aGvHD were treated with ECP, respectively.

Regarding comorbidities diabetes mellitus was a common complication in patients with CTCL, aGvHD and cGvHD. In our sample every 5th patient with GvHD suffered from Candida albicans or Cytomegalovirus (CMV) infections/reactivations. Other commonly observed adverse events such as toxic liver disease and acute kidney failure may be attributed to drug related cytotoxicity as well (Tab.1).

Conclusions: Patients affected by all 3 diseases received various treatments. ECP was administered to less than 20% of the patients but was the most commonly applied second line treatment for GvHD in our sample. Furthermore, this study shows the multitude of comorbidities associated with the three indications, which must be considered for treatment selection (Wolf et al. 2010). Finally, this study demonstrates the usability of claims data to provide insights into demography, associated comorbidities and the actual treatment spectrum of rare diseases.

P04-21

Performance qualification of the pretransfusion antibody screening test of plasma containing Daratumumab (anti-CD 38 antibody) after DTT-treatment of the test cells using gel card and Capture®

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Background: Plasma of patients receiving the therapeutic antibody daratumumab (anti-CD38) induces positive antibody screening results in the indirect antiglobulin test (IAT). Treatment of test cells by dithiothreitol (DTT) disrupts the structure of CD38. No data exist about the performance characteristics of different IAT methods after DTT treatment of the test cells.

Methods: Antibody screening was performed using the microcolumn gel card (BioRad) and solid phase (Capture®) assay pre and after incubation of test red blood cells or microwell strips, respectively, with DTT (0.2M, 30 min, 37°C). Sera with previously identified red blood cell antibodies were tested with and without spiking with daratumumab (10µg/ml).

Results: 61 patient sera containing red blood cell antibodies of one or combined specificities (anti-D, C, -E, -c, -e, -Cw, -Jka, -Fya, -K, -k, -Kpa, -M, -S, -Wra, Vel) were tested. 54 antibodies were specifically detected in the neat sera using gel card and 50 antibodies by Capture®. After spiking with daratumumab binding of anti-CD38 antibodies always caused agglutinates by the gel card but not by the Capture® method (26/50). After DTT treatment anti-K, -k, -Kpa activity became much weaker or disappeared with both methods; also anti-Fya antibodies were not longer detectable by the Capture®. All other antibodies could be detected by both methods using DTT treated test cells. With both methods, unspecified reactions occurred with the DTT treated cells in 40% of tests for the gel card and 7.1% of tests for the Capture® method.

Conclusion: DTT treatment of test cells for the pretransfusion antibody screening in patients with daratumumab treatment can help to identify relevant RBC antibodies anti-D, C, -Cw, -e, -e, -Jka, -S but also causes additional unspecified reactions in 7% (Capture) to 40% (gel card) of tests. The Capture® assay is less sensitive for daratumumab binding compared with the gel card method and can fail to detect anti-Fya after DTT treatment. The known limitation of the DTT method to detect KEL antibodies was confirmed by both methods.

Tab. 1. Comorbidities in CTCL, aGvHD, and cGvHD

<table>
<thead>
<tr>
<th></th>
<th>CTCL (Mean / SD)</th>
<th>aGvHD (Mean / SD)</th>
<th>cGvHD (Mean / SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>32.6%</td>
<td>12.6%</td>
<td>21.7%</td>
</tr>
<tr>
<td>Candidosis</td>
<td>9.5%</td>
<td>12.2%</td>
<td>23.5%</td>
</tr>
<tr>
<td>Thrombocytopenia, unspecified</td>
<td>6.7%</td>
<td>12.2%</td>
<td>15.4%</td>
</tr>
<tr>
<td>Sepsis</td>
<td>2.8%</td>
<td>6.2%</td>
<td>4.9%</td>
</tr>
<tr>
<td>Cytomegaly</td>
<td>0.0%</td>
<td>0.0%</td>
<td>18.0%</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>0.0%</td>
<td>0.0%</td>
<td>16.5%</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>0.0%</td>
<td>0.0%</td>
<td>14.0%</td>
</tr>
<tr>
<td>Toxic liver disease</td>
<td>0.0%</td>
<td>0.0%</td>
<td>10.1%</td>
</tr>
<tr>
<td>Acute kidney failure</td>
<td>0.0%</td>
<td>0.0%</td>
<td>8.4%</td>
</tr>
</tbody>
</table>
P04-22
Prediction of the fetal status in cases of neonatal alloimmune thrombocytopenia
Duong Y.C.; Qui D.; Cooper N.; Giptner A.; Wiencek-Lischka S.; Heidinger K.; Hackstein H.; Santoso S.; Sachs U.J.; Bein G.
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Introduction: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by maternal alloantibodies against fetal platelet antigens (HPA) inherited from the father. The most severe consequence of FNAIT is intracranial hemorrhage (ICH), leading to death or neurologic disability in approximately 10% of clinically symptomatic cases. Conflicting data on etiopathogenetic factors and prediction of the fetal outcome have been published.

Methods: 818 suspected consecutive cases of FNAIT were included in a retrospective cohort study. In 180 confirmed FNAIT cases, the fetal outcome was tested for association with maternal age, gravidity, fetal sex, ABO incompatibility between mother and fetus, presence of maternal HLA class I antibodies against paternal HLA class I antigens, presence of HLA-DRB3*01 in the mother, and HPA-antibody specificity.

Results: 71% of all confirmed FNAIT cases were males (p < 0.001; odds ratio, 2.45; 95% CI, 1.56–3.84). Of the HPA-1a-immunized mothers, 98% carried at least one copy of HLA-DRB3*01 (p = 1.34 × 10–12; odds ratio 92.3; 95% CI, 26.9–317.1). No association between potential pathogenetic factors and neonatal platelet count or the incidence of ICH was detected except for antibody specificity. HPA-1a antibodies were associated with lower neonatal platelet count compared to HPA-3b antibodies (median platelet count 20.0 G/L versus 66.5 G/L; p < 0.0001) and all cases of ICH (n = 17) occurred in mothers that were immunized against HPA-1a (n = 144).

Conclusion: Male sex of the fetus and the presence of HLA-DRB3*01:01 in the mother are strongly associated with the propensity for mounting a humoral immune response against fetal HPA-1a antigen. The manifestation of intracranial hemorrhage in the fetus may be mainly associated with inherent properties of the HPA-1a antibody involved.

P04-23
NAIT due to HPA-1a-incompatibility with initially undetectable alloantibody
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Introduction: Neonatal alloimmune thrombocytopenia is most commonly caused by anti-HPA-1a in Caucasians. The diagnosis is usually based on the detection of the antibody. In rare cases, the antibody is not detectable at birth but it usually appears after a few days in the maternal serum. Here we describe a case of severe NAIT due to anti-HPA-1a with negative findings for platelet antibodies over more than nine weeks.

Case report, laboratory investigation: A male newborn (34+3 weeks of gestation) presented with multiple petechiae and hematoma. The initial platelet count was 14 Gpt/L. Although maternal platelets were HPA-1(a+b) and the platelets of the child were HPA-1(a+b+), the maternal serum contained no detectable concentrations of anti-HPA-1a (indirect MAIPA, indirect platelet immunofluorescence test). In addition, no other platelet alloantibodies were found. Platelet antibody tests remained negative for 68 days. HPA-1a negative and HPA-1a positive platelet transfusions were administered over a period of 36 days. From the 44th day postnatally, platelets remained within the normal range. Sonographic studies revealed no signs of intracranial hemorrhage. Five years later, the mother presented again for genetic counseling in order to learn about the risk of NAIT in a next pregnancy. At this time, a strongly reacting HPA-1a antibody was detected in her serum and the definite diagnosis of NAIT in the first child was made.

Conclusion: It is recommended to test not only for platelet antibodies in newborn babies with severe thrombocytopenia not explained by other possible causes than NAIT, but to determine the genetic HPA-1a-constellation within the family as well. In cases of clinically probable NAIT in HPA-1(a+b+)-children of HPA-1(a-b+) mothers, maternal platelet antibody testing should be repeated over a longer time, before a final decision on the possible diagnosis of NAIT is made.

P04-24
Implementation of blood grouping machine IH500 for patient serology
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Introduction: The implementation of the compact bench-top-system IH500 for patient serology is a measure to increase the safety of blood group reports and to improve the quality of blood group testing. Therefore, in addition to the method validation an extensive device qualification is required.

Methods: As Performance Qualification (PQ) for the laboratory in Zwickau 31 ABO blood groupings with reverse grouping (including 2 D weak), 53 antibody screenings (including 11 antibody identification cases), 18 phenotypings Rhesus/Kell and 61 crossmatchings with ABD identity control were performed by means of IH500. A parallel comparison by manual testing and evaluation on Saxo-ID-Reader was done. The measurements were carried out over a time period of 10 days by different laboratory personnel.

Results: For the comparative measurements no discrepancies between Saxo-ID-Reader and IH500 were observed. A different intensity of agglutination could be detected for 32 of the 122 tested samples (26%). According to the performed test the agglutination was higher either for IH500 or for manual testing.

Conclusion: The device qualification yielded that fully automatic blood group testing by means of IH500 is suitable for automated routine testing in patient serology. With the implementation of IH500 the safety of blood group reports, of error-free result transfer to laboratory IT systems and of dual control during the active standby service can be increased.

P04-25
evaluation of the immunohematological fully-automated ortho-vision system
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Introduction: In our Immunohematological laboratory we are looking for a new fully-automated system, with the ability to automate the full range of immunohematologic tests in pretransfusion testing, including serial dilutions for titration studies and selected cell panels. We have evaluated Ortho-Vision System in order to provide documented evidence of efficacy, specificity and sensitivity.

Methods: The routine testing in our labs is performed in BioRad, ID-Col-umn Agglutination Technique in a column, containing Sephadex gel, using partly manual partly automated technique-Techno Twin Station and Swing Twin Sampler with Saxo ID Reader. The ABO/D typing and Rh/K phenotyping on BioRad system were compared with the Ortho-Vision system, using the BioVue system, consists of a column containing minute glass beads for typing routine pretransfusion patient samples.

Blood grouping ABO/D by two different determinations were determined on 150 samples from patients for routine pretransfusion testing and on 103 samples from blood donors. We included ten samples that had known RhD variant: eight weak RhD and two partial RhD (RhD<sup>+</sup>) poz. Rh/K
CD177-negative. Sequence analysis showed presence of at least one c.829T
MiSeq platform). not pseudogene was subjected to next generation sequencing (Illumina
monoclonal antibody. The whole CD177 gene was amplified by gene-spe
Study design and Methods: In the analysis of 150 samples performed for the AB0/D grouping, the same
results were observed in both systems, except for two sample: on BioRad ID-
cards for RhDVI- it was RhD week (+ +), but on BioVue RhD poz (+++++).
We performed additional testing with ID-Partial RhD Typing set and this samples
were confirm week RhD. Rh/K phenotyping and phenotyping for antigens Fya, Fyb, Jka and Jkb showed complete concordance on both systems. For irregular antibody screening there was nearly complete (96
out of 120 correct negative) concordance, except for one samples, that was negative in BioRad ID cards, but positive in BioVue cassettes (anti-E).
BioVue was able to identify all antibodies present in samples with known antibodies.
Conclusions: The fully automated Ortho-Vision System using the BioVue system, consists of a column containing minute glass beads is reliable and safe for typing routine pretransfusion patient samples and is as specific and sensitive as BioRad ID-Column Agglutination Technique.

PO5-2
KIR-Genotypisierung with NGS
Fürst D.1,2; Neuchel C.3; Tsjamadou C.4; Schrezenmeier H.5,6; Mytilineos J.1,2
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Cross Blood Transfusion Service Baden-Württemberg – Hessen, Transplantation
Immunology, Ulm, Germany, 2Institute for Transfusion Medicine, University Ulm,
Transplantation Immunology, Ulm, Germany

Introduction: The KIR-gene cluster of chromosome 19 is a highly variable
gene region encoding for activatory and inhibitory receptors on
NK-cells, gamma/delta-T-cells and NKT-cells. In hematopoietic stem cell
transplantation they are an important determinant of NK-cell alloreactivity,
influencing relapse control and possibly also GvHd incidence.
There is already evidence that selection of donors with KIR-Bx genotypes,
which contain more activating KIR-genes, may improve outcome for
patients with malignant myeloid disease. We aimed to establish an NGS
based genotyping assay for high-throughput genotyping of registry do-
nors for unrelated hematopoietic stem cell transplantation.
Methods: We established multiplex PCRs amplifying exons 4 and 7 of
all known KIR-Alleles, extended hematopoietic stem cell donor registry typing appears to
be meaningful.

Gruppe 5
Immunogenetik/Molekulare Immunhämatologie

PO5-1
Next generation sequencing analysis of CD177 gene in CD177
negative and positive donors
Bayat B.; Litmeyer A.S.; Wienzek S.; Wolff J.C.; Santoso S.; Sachs U.J.;
Bein G.
Institute for Clinical Immunology and Transfusion Medicine, Justus Liebig
University, Giessen, Germany

Background: Human neutrophil antigen 2 (HNA-2) is located on CD177
which exclusively is expressed on neutrophil surface. CD177 is absent on
neutrophil membrane of about 3–5% of individuals. During pregnan-
cy woman with CD177-null phenotype may form isoantibodies against
CD177 leading to neonatal alloimmune neutropenia. A recent study
reported a nonsense mutation c.829A>T (K263X) that alone or in com-
B: allelic with a single base deletion c.997delG produce premature stop
codons in the CD177 coding region and therefore leads to absence of
CD177 protein on neutrophil surface. Further study however identified
829A>T heterozygous individuals without detectable CD177 protein
expression. Thus, beside the reported premature stop codon, other genetic
variants may lead to CD177-null phenotype. In the current study using
next generation sequencing (NGS) technology we aimed to analyze the
whole genomic sequence of CD177 and further clarify the genetic basis of
the CD177-null phenotype.
Study design and Methods: Expression of CD177 on neutrophils of 250
normal blood donors was analyzed by flow cytometry using specific 7D8
monoclonal antibody. The whole CD177 gene was amplified by gene-spe-
cific long-range PCR. The PCR product contains only CD177 gene but
not pseudogene was subjected to next generation sequencing (Illumina
MiSeq platform).
Results: Out of 250 blood donors, eight individuals were identified as
CD177-negative. Sequence analysis showed presence of at least one c.829T
allele in all CD177-negative individuals; three individuals were TT homo-
yzous and five individuals were heterozygous. The wild type c.829A allele
was detected in all three CD177-positive donors, one homozygous AA
and two AT heterozygous.
Conclusion: Resequencing of the whole CD177 gene by next genera-
tion sequencing confirmed the finding that the CD177-null phenotype
is caused by a homozygous c.829A>T mutation introducing a premature
stop codon at position p.263. However, we identified c.829A/T heterozy-
gous individuals in the CD177-negative cohort, indicating presence of the
additional genetic variations outside the coding sequence of the CD177
gene that may lead to silencing of the second allele in these individuals.

PO5-2
KIR-Genotypisierung with NGS
Fürst D.1,2; Neuchel C.3; Tsjamadou C.4; Schrezenmeier H.5,6; Mytilineos J.1,2
1Institute for Clinical Transfusion Medicine and Immunogenetics, German Red
Cross Blood Transfusion Service Baden-Württemberg – Hessen, Transplantation
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Gruppe 5
Immunogenetik/Molekulare Immunhämatologie

PO5-1
Next generation sequencing analysis of CD177 gene in CD177
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allele in all CD177-negative individuals; three individuals were TT homo-
PO5-3
Step by step – completing the genomic sequence of another rare HLA-DRB1*13 allele
Binder T.M.1; Krammes L.1; Roth T.1; Rabbi S.N.1; Alster I.1; Koehn C.B.1; Kelisch R.1; Schafer M.1; Peter W.1; Eiermann T.H.1
1Universitätsklinikum Hamburg Eppendorf, HLA-Labor, Hamburg, Germany;
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Introduction: Some years ago we found a new HLA-DRB1*13 allele in a family in Germany with roots in Turkey and Armenia. It was found in a male potential stem cell donor, in his own and in his extended family. Sanger sequencing of exon 2 of this allele, later called DRB1*13:54, revealed 3 nucleotide variations at position 157 (T→A), 158 (C→T) and 166 (C→A) in comparison with DRB1*13:24. The remaining DNA sequences beside exon 2 were not determined initially.

Methods: To complete the genomic sequence and to clarify the degree of recombination within this allele, 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 NGSeqine, 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 sequences.

Results: The full-length sequence analysis of the described allele unraveled a quite high similarity to the DRB1*13:04 and *13:24 alleles. The evolutionary history of the DRB1*13:54 could be best explained as recombination product of DRB1*13:04 (acceptor) and DRB1*07 (*07:01-*07:04, *07:07) or DRB1*12 (*12:01, *12:03, *12:05-*12:08) as donor.

Conclusion: LR-PCR and NGS including phased sequence analysis revealed the unambiguous full-length sequence of the HLA-DRB1*13:54 allele. The most likely evolutionary recombination between the DRB1*13 allele and the DRB1*07 or DRB1*12 alleles seems to be restricted to exon 2 only.

PO5-4
Recombinant CR1 protein inhibits HTLA antibodies produced by carriers of a rare and two new CR1 alleles
Flesch B.1; Karnt A.1; Stenzel A.1; Felck U.1; Opitz A.4
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Background: High,titer, low-avidity (HTLA) antibodies in patient sera clearly hamper antibody differentiation in red blood cell (RBC) serology. Recombinant blood group antigen proteins (rBGAP) are an efficient tool to inhibit these antibodies in a specific manner so that the remaining clinically relevant antibodies can be identified.1 Complement Receptor 1 (CR1) carries different blood group epitopes of the Knops blood group system. We describe three cases of HTLA antibodies which could be inhibited by recombinant CR1 protein and resolve the molecular background of the CR1 modifications using PCR-SSP and DNA sequencing.

Methods: Blood samples of one male (1) and two female patients (2 and 3) were submitted to our laboratory within some weeks. Antibody identification was performed by standard gel column indirect agglutination test (IAT, BioRad, Munich, Germany) and an in-house panel. Antibody inhibition by rBGAP followed our in-house algorithm. We first incubated the patients’ sera either with a cocktail of commercially available recombinant blood group proteins (Imusyn, Hannover, Germany) including CR1, Rg(a), Ch(a) and JMH or with single CR1 and JMHRBGAP. Thereafter the pre-treated sera were tested again in IAT. Molecular diagnostics for rare blood group alleles were performed by commercial PCR-SSP (RBC-Ready Gene Rare ID, Inno-Train, Kronberg, Germany) and in-house PCR-SSP (Kna, Knb, McCa, McCh, Yka, Sla, Slb, KCAM, Ch, and Rg). DNA-sequence covering amplified region of CR1 exon 29 and flanking intron sequences and electrophoretic separation in an ABI Prism 310 sequencer using published primer sequences2 and haplotype specific primers.

Results: The patients sera were broadly reactive with nearly all RBC tested so far, including reactivity of sera 1 and 2 with Kna negative RBC. After inhibition with rBGAP cocktail or single CR1 rBGAP, reactivity with the identification panel was completely abrogated. Cross-match of serum 1 was negative with the RBC of patient 2 and positive with patient 3. Patient 1 was typed as Kna+, Yka+, McCa+, McCh+, Sla+, Slb+, Ch+, Rg+. DNA sequencing demonstrated a homozygous CR1*4828T>A mutation (Ser1610Thr) in this patient, inducing the Sl1+, Sl2-, Sl3- genotype.3 In patient 2 compound heterozygosity for a CR1*4828T>A mutation on one allele and a CR1*4838A>T (Glu1613Val) on the 2nd allele induced the missing reactivity with the HTLA antibody in the serum of patient 1 which was supposed to have Sl3 specificity. Patient 3 exhibited a homozygous CR1*4691G>A (Arg1564Gln) mutation. Both new mutations were submitted to Genbank.

Conclusion: Recombinant Knops protein enables inhibition of HTLA antibodies directed against very rare and new CR1 epitopes. The combined application of rBGAP cocktails or single proteins followed by DNA sequencing clearly improves RBC antibody and allele diagnostics.

References:

PO5-5
A patient with trisomy 9 carrying three ABO alleles
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Background: Serological ABO typing in a young female patient resulted in a blood group A phenotype. A very weak expression of the B antigen was assumed which was consistent with missing A and B reactivity in the reverse grouping. Phenotypes were established by standard serological methods using tube and/or gel-column agglutination techniques (Bio-Rad, Munich, Germany). Surprisingly, commercial ABO PCR-SSP by different suppliers (BAG Health Care, Lich, Germany; Innotrain, Krongen, Germany) detected either an O’B or O’B’ genotype. No A genotype was detectable using these commercial kits. Upon further inquiry the clinicians confirmed a trisomy 9 of the patient. Therefore, the existence of three ABO alleles encoded by chromosome 9 was supposed as reason for the inconsistent typing results. ABO DNA sequencing should unambiguously determine the correct alleles.

Methods: ABO sequencing was performed as cycle sequencing of exons 1–7 including flanking intron sequences and the enhancer CBF/NF-Y region of the promoter applying the Big Dye Terminator v3.1 chemistry (ABI, Weiterstadt, Germany) followed by electrophoretic separation in an ABI Prism 310 DNA analyser. Additionally, an A-haplytope specific sequencing of exons 6 and 7 was added in order to separate the A allele from the remaining haplotypes. Determined DNA sequences were aligned to published reference sequences.

Results: Generic DNA sequencing determined an ABO*02 allele, consistent with 106T, 188A, 189T, 220T, 261delG, 464A, 681A, 771T and 829A and an ABO*03 allele. However, additional very small B-specific peaks were visible in the electropherogram, which could not be unequivocally assigned to a special B allele. The enhancer CBF/NF-Y region of the ABO promoter covered one single and a 4fold repetitive 43 bp motive. An A-haplytope specific sequencing of exons 6 and 7 demonstrated an ABO*1A05, including 467T in combination with an *002, including
261delG and a small amount of estimated 7% of *B103, which was determined by additional very low 526G, 703A, 796A, 803C and 930A peaks.

**Conclusions:** We present the case of a patient with trisomy 9 with three ABO alleles. Only an ABO haplotype specific sequencing approach was able to unambiguously separate the three alleles. An ABO*A105 allele (not detectable in PCR-SSP) was compatible with the blood group A phenotype and an ABO*B103 allele in a low quantity was responsible for the assumed weak expression of B in serological typing. These results are consistent with a trisomy 9 mosaic which is characterized by an only partial penetration of the third chromosome within somatic cells.

### P05-7

**Is HLA-B*13:02new a new Null allele?**

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**Introduction:** Some month ago we found a new HLA-B*13:02:01:01-like allele in a new potential stem cell donor using next generation sequencing (NGS) technology. The complete HLA genotype of the potential donor was: A*01:01, *31:01; B*08:01, *13:02new; C*06:02, *07:01; DRB1*03:01, *07:01; DQB1*02:01, *02:02; DPB1*01:01, *04:01. NGS analysis of this new B*13 allele clearly revealed a point mutation in the second position of the last codon at exon 7. This point mutation in the stop codon (TGA → Stop) changes this codon into a translatable codon (TTA → Leucine), leading to an elongation of the HLA-B13 transcript into 3′-UTR. The resulting HLA-B13 protein should have 8 additional amino acids. In HLA C*04:09N the deletion of one nucleotide in the penultimate exon (7), causes a frameshift and an elongation with 32 additional amino acids in the cytoplasmatic tail. For a so far unknown mechanism, this elongation switches C*04:09N1 into the C*04:09N allele and therefore we expected this novel HLA-B*13:02 variant could be a Null allele as well.

**Methods:** To answer this question, we performed a serological typing for HLA-A, B and Cw with the help of CDC typing trays from OneLambda. To verify the mutation in exon 7 of B*13:02new (found with NGS), we used Sanger sequence analysis with specific amplification and sequencing primers.

**Results:** Serological typing revealed the following antigen pattern: A1, A31; B8, B13; Bw4; Bw6; Cw6, Cw7. Sanger sequence analysis confirmed the minor sequence differences between B*13:02:01:01 and B*13:02new. **Conclusion:** The positive recognition of B*13:02new by the anti-B13-antibodies in CDC typing, showed that this allele is expressed on the cell surface and that the HLA-B13 protein is folded correctly. Its functionality in respect of signal transduction needs to be elucidated. Because the stated point mutation is outside from exon 2 and 3, this allele is a part of the B*13:02P and the 13:02:01G groups. With our approach, applying LR-PCR and NGS including phased sequence analysis, we were able to determine the complete gene sequence of HLA-B*13:02new.

### P05-6

**The association of polyglandular autoimmunity with HLA class I is gender related**

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**Background:** The association of glandular autoimmunity, e.g. type 1 diabetes (T1D) or autoimmune thyroid disease (AITD), with HLA class I and class II alleles is well documented. Because of hormonal influences in endocrine autoimmunity a further question is whether HLA association is varying in female and male PGA patients. This study aimed to determine HLA class I allele association with PGA with a special impact on gender-related differences and discrimination for the involvement of AITD.

**Methods:** The study included 143 PGA patients and 350 healthy controls. HLA class I A and B alleles were determined by PCR-SSO (Luminex, Immucor Lifecodes, Nijlen, Belgium) and PCR-SSP (Olerup, Vienna, Austria) on a 2-field level. Evaluation was performed separately for female and male patients and for the contribution of either Graves’ disease (GD) or Hashimoto’s Thyroiditis (HT).

**Results:** No significant differences were observed in HLA-A allele frequencies between healthy controls and PGA patients after correction for the number of comparisons. However, A*02:01 was overrepresented in male PGA patients with a contribution of HT (RR=1.863, p=0.014) while there was no difference in female patients. HLA-B*07:02 was determined as gender-independent protective allele in PGA patients while carriers of B*08:01 had a significantly higher susceptibility for PGA. The B*40:01 allele was associated with a significantly higher risk only in male patients (RR=2.34; p=0.018). Contrary, it proved to be protective for female patients (RR=0.20; p=0.005). These differences would not have been evident without discrimination for gender. This was also shown for PGA patients with a contribution of HT (HLA-HT) with a clearly enhanced risk for men (RR=3.20; p=0.008) and a protective effect for female patients (RR=0.15, p=0.024) with B*40:01 while there was no difference for those patients with a contribution of GD. The B*50:01 allele was only overrepresented in female PGA patients (RR=2.51; p<0.018) and especially in those women with PGA-HT (RR=3.45; p=0.005), while there was no significant difference in female PGA-GD patients.

**Conclusions:** Gender related differentiation opens new insights into HLA class I allele association with PGA. This demonstrates the important influence of e.g. hormones and metabolic parameters on the development of endocrine autoimmunity.

### P05-8

**Assessment of four different protocols for HLA-typing by Next Generation Sequencing**

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**Introduction:** NGS (Next-generation sequencing) based HLA Typing has great potential for generating precise and unambiguous results for multiple samples in a single assay potentially without additional testing to resolve ambiguities. In this study the applicability of four commercially available reagent kits including their corresponding software were evaluated regarding the amplification strategy, target generation, flexibility of the workflow were considered.

**Methods:** 24 samples (21 clinical and 3EPT) were analyzed in parallel using four different kit: A) NGSgo-Ampx (GenDx, Utrecht, The Netherlands); B) TruSight HLA v2 Sequencing Panel (Illumina, San Diego, CA, USA); C) MIA FORA NGS FLEX HLA Typing Kit (Immucor, Inc. Norcross, GA, USA); D) HOLOTYPE HLA (Omixxon, Budapest, Hungary). HLA loci A, B, C, DRB1 and DQB1 were typed. In addition kits B, C and D contain amplification primer for HLA-loci DQA1 and DPB1 and kit C also for DPA1 and DRB3/4/5. Sequences were obtained using the MiSeq Illunima platform and analyzed with the corresponding software NGSequencing, Assign TruSight, MiaFora and Twin, respectively. The final NGS typing results were compared to known SBT and consensus results.
of EPT programs and comprised the verification of homozygous results, rare alleles and typing errors.

**Results:** With all four protocols for MiSeq concordance of the NGS results with the already known typing was experienced. Positive practice in the processing (easy to follow work flow, straightforward analysis) was observed. The minimum number of remaining ambiguities depended partly on the alleles detected.

**Conclusion:** The implementation of the NGS workflow promises to be a highly efficient and reliable method in our HLA-typing routine but its cost effectiveness needs to be followed up.

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**P05-9**

**NGS-technology for HLA-typing in medium or small size HLA laboratories?**

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**Background:** Sequencing based typing (SBT) has become a gold standard for high resolution HLA-typing. However, routine sequencing strategies have mainly focused on exons 2 and 3. Due to the increasing number of new HLA alleles additional typing methods (SSO, SSP) are necessary to resolve ambiguous typing results and null alleles. High-throughput laboratories use NGS (Next Generation Sequencing) based HLA typing routinely for multiple samples and obtain unambiguous typing results in a single run.

**Methods:** 80 samples obtained from EPT, lab exchange, donors or patients with known HLA-genotype (HLA-A, -B, -C, -DRB1 and -DQB1, high resolution) and additional 20 samples with known HLA-DQB1, -DRB1, -DQA1, -DPB1, -DPA1, DRB3/4/5 genotype were analyzed using a modified NGSogo (GenDiX) workflow. Sequences were generated with MiSeq analyzer (Illumina) and assigned with the corresponding software. As quality parameters the number of total reads, the read length, insert size, mappability, read depth, phasing and MiSeq quality were analyzed.

**Results:** Of all samples we obtained accurate HLA-typings concordant with previous high resolution typing results. Sequence data fulfilled our quality criteria. No additional typing methods were needed to resolve ambiguities.

**Conclusion:** High-resolution HLA-typing with the use of NGS is a very promising method to be used in a small or medium sized HLA-laboratory for patient and donor registry typing.

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**P05-10**

**Sealing away PCR volume losses – an experience report**

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**Introduction:** A robust and reliable PCR performance is crucial for the smooth workflow in every molecular genetic laboratory. In case of allelic dropouts or total PCR failures the troubleshooting usually comprises the search for pipetting errors, reagent and cycler functioning, and not to forget DNA quantity and quality issues. Evaporation losses leading to a consecutive increase in salt concentration within the PCR reaction mixtures are usually not considered as a cause of PCR failures. Therefore sealing of PCR reaction vessels might have a major impact on PCR performance.

**Methods:** To measure evaporation or volume losses depending on the sealing method and the cycler type (ABI 7900 and SensoQuest Labcycler) we used compared the total weight of ready to run 96 wells plates (standard semi-skirted 4ti-0760, 4titude)® before and after a long range PCR run. These PCR plates contained a reaction volume of 18 µL/well and the total run time of the amplification lasted approx. 7 hours. We compared a semi-automated thermal sealing approach using the 4s3™ Heat Sealer (4titude®) and the corresponding foil seals (4ti-0536, 4titude®) with the traditional manual sealing using standard adhesive PCR Plate Seals (AB-0558, Thermofisher). The applied 4s3™ sealing parameters (177°C, 2 sec) were chosen according to the manufacturers recommendations. For error compensation the plates were placed on the precision balance (AX105 Delta Range, Mettler Toledo) 2 minutes before taking the measurement.

**Results:** The volume loss in plates sealed with the standard PCR foil was quite obvious and in the range of 200–300 mg/plate. Plates sealed with the 4s3™ Heat Sealer comparatively lost only half the weight on average.

**Conclusion:** This observation clearly demonstrates that semi-automated foil sealing of PCR plates is reducing volume losses during PCR and therefore the method of choice in terms of standardizing PCR performance. Besides the variance elimination due to manual plate sealing the semi-automated procedure could be beneficial during PCR establishment and efforts to minimize input of PCR components.

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**P05-11**

**Full-length description of three novel MICA allelic variants**

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**Introduction:** The MICA (MHC I chain related gene A) gene has a length of 13kb and is located on chromosome 6 within the HLA region. It’s cell stress-inducible, highly polymorphic cell surface proteins function as ligands of the activating NKG2D receptor on natural killer (NK) cells. Recently published papers show benefits of MICA matching in context with hematopoietic stem cell transplants. However, MICA typing is not commonly performed on a routine basis.

**Results:** After development of a full-length MICA next generation sequencing (NGS) assay and implementing it into our NGS HLA typing routine we found so far undescribed MICA coding sequence variants. The first presumably new allele is almost identical to the MICA*009:01 allele except the A>G nucleotide exchange at the position 643 in exon 4 which leads to an amino acid exchange in codon 192 from Glu to Lys. A further sample related to MICA*012:04 revealed an A>T exchange at position 536 causing a Leu to His amino acid exchange in codon 156. In another case we found a MICA*024 with two different nucleotide exchanges. One T>C exchange at position 707 changing Thr to Ile at codon 213 and a further A>G exchange at position 821 replacing Gln by Arg in codon 251.

**Method:** Next generation sequencing was performed on a MiSeq instrument with 500 cycles PE V2 chemistry from Illumina. The initially generated full-length MICA alleles were enzymatically fragmented and adapter ligated basically according to a NEB library preparation protocol. The generated fastq data were analysed with two different sequence analysis tools Twin (Omixon) and NGSeTingle (GenDx). To validate the NGS results we sequenced all 6 exons of these samples again in both directions and reverse using sequence based typing (SBT) method performed on a 3730XL DNA Analyzer (Applied Biosystems) and assigned with the corresponding software. The sequences with Assign SBT v4.7.1 (Conexio Genomics).

**Conclusion:** The sequences generated with the Sanger sequencing method confirmed the nucleotide exchanges at the same positions as shown in the NGS results. The described full-length sequence variants will be submitted to the IMGT database.
Abstracts

P06-1
Traceless Fab-TACS based automated cell isolation method for HLA crossmatch diagnostics in context of kidney transplantation

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Introduction: For a microlymphocytotoxicity test in the context of organ transplantation, isolated lymphocytes of very good purity and viability are required. Current cell isolation methods are based either on magnetic separation or on tetrameric antibodies and ficoll centrifugation. Magnets bound to the cells or stress caused by ficoll centrifugation can influence the quality of diagnostic test outcomes. Automatization of cell isolation procedures would save time for technicians, especially during night shifts and reduce result deviations due to human error. Here we show a fast fully automated magnet-free cell selection system (FABian) based on reversible binding Fab-TACS technology suitable for microlymphocytotoxicity diagnostic tests.

Methods: Whole EDTA blood samples pooled from 4 donors were used for comparison of FABian and the routinely-used standard method based on tetrameric antibodies and ficoll centrifugation. The validation of microlymphocytotoxicity diagnostic tests was performed with blood samples of selected genotyped donors and tested against pre-defined sera containing antibodies against HLA class I or II.

Results: We compared FABian technology to the routinely-used standard cell isolation method for microlymphocytotoxicity tests. We showed higher purity of isolated cells in comparison to the standard method, namely 96% vs. 75% for B-cells, 99% vs. 92% for T-cells and 96 vs. 88% for total lymphocytes, FABian vs. the standard method respectively. While keeping very high purities, we obtained sufficient cell numbers for the microlymphocytotoxicity diagnostic test. Recoveries were 40% for T-cells and 20% for B-cells, and were comparable to the standard method.

Conclusion: The FABian system was successfully validated for usage in this downstream diagnostic test and we have additionally observed lower background signals, most likely due to lower cell damage during isolation.

P06-2
The impact of the counting method of electronic blood counters in quality control of platelet concentrates

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Background: Electronic blood counters are usually used to measure peripheral platelets. The devices are also used in quality control measurements of platelet concentrates with very high platelet counts. To fulfill quality requirements of European guidelines, the accuracy of counting very high platelet numbers has to be validated.

Methods: The following 5 blood counter devices were used to analyse platelet counts: CELL-DYN Ruby [A, optical count] and CELL-DYN Emerald [B, impedance count] (Abbott Diagnostics, Wiesbaden, Germany), Sysmex K-4500 [C, impedance count], Sysmex XN-550 [D, impedance count] and Sysmex XN-550 [E, optical count] (Sysmex, Norderstedt, Germany). Platelets were measured daily from day 1 after collection until day 6 (2 days after expiry). Furthermore, linear regression analyses were performed using 5 defined concentrations of stock solutions which were serially measured 3 times. For precision performances platelet samples were serially measured 5 times. Coefficients of variation were calculated and compared with the manufacturers’ declarations. Furthermore, more than 100 blood samples were compared and the normal hemogram parameters (red blood cells, white blood cells, hemoglobin, hematocrit, platelets) were analyzed.

Results: The comparison shows significant differences between the blood counter devices in measuring high platelet counts as shown in Figure 1. The devices B, C, and E measured significantly higher platelet counts compared to devices A and D (Fig. 1; p < 0.0001) independent of the method of measurement. The manufacturers provide comparable coefficients of variation and linear regressions. We achieved similar results for all counters. All counters showed an expected decrease of platelets at day 6 of storage in a similar manner. All results of the peripheral blood count parameters were comparable.

Conclusion: Our study shows the importance of blood counter validations for platelet concentrates with a high platelet count before implementing devices in routine quality control.

P06-3
Apparently low expression of CD15 on granulocytes detected by flow cytometry: a possibility of misinterpretation

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Introduction: CD15 or Lewis x (Lex) antigen is frequently used as a marker for gating granulocytes in flow cytometric analyses of peripheral blood. This carbohydrate antigen consisting of the trisaccharide Galβ1-4[Fucα1–3]GlcNAc is carried by glycolipids and glycoproteins expressed on all granulocytes. Hence, it is a suitable marker for evaluating granulocyte populations. However, flow cytometric analyses showed an apparently low expression of CD15 on granulocytes.

Aims: The aim of the present study was to validate 5 different blood counter devices focusing on counting high platelet counts.

Methods: The following 5 blood counter devices were used to analyse platelet counts: CELL-DYN Ruby [A, optical count] and CELL-DYN Emerald [B, impedance count] (Abbott Diagnostics, Wiesbaden, Germany), Sysmex K-4500 [C, impedance count], Sysmex XN-550 [D, impedance count] and Sysmex XN-550 [E, optical count] (Sysmex, Norderstedt, Germany). Platelets were measured daily from day 1 after collection until day 6 (2 days after expiry). Furthermore, linear regression analyses were performed using 5 defined concentrations of stock solutions which were serially measured 3 times. For precision performances platelet samples were serially measured 5 times. Coefficients of variation were calculated and compared with the manufacturers’ declarations. Furthermore, more than 100 blood samples were compared and the normal hemogram parameters (red blood cells, white blood cells, hemoglobin, hematocrit, platelets) were analyzed.

Results: The comparison shows significant differences between the blood counter devices in measuring high platelet counts as shown in Figure 1. The devices B, C, and E measured significantly higher platelet counts compared to devices A and D (Fig. 1; p < 0.0001) independent of the method of measurement. The manufacturers provide comparable coefficients of variation and linear regressions. We achieved similar results for all counters. All counters showed an expected decrease of platelets at day 6 of storage in a similar manner. All results of the peripheral blood count parameters were comparable.
Abstracts

on cell membranes of myeloid precursor cells, neutrophils, eosinophils and monocytes. It is not expressed on normal erythrocytes, platelets or lymphocytes. We use CD15 in a routine protocol for flow cytometric diagnosis of paroxysmal nocturnal hemoglobinuria (PNH) and identified a pitfall: rarely misleading results may be obtained when a whole blood staining method is used for the flow cytometric determination of CD15.

Methods: A modified five color protocol was used for the flow cytometric diagnosis of PNH (Sutherland et al., Cytometry B Clin Cytom 2014;86:44–55). We used the following markers: GPI-linked antigens were identified by CD59 and CD58 for erythrocytes and reticulocytes and by FLAER and CD157 for granulocytes and monocytes; granulocytes were identified by CD45 and CD15, monocytes by CD45 and CD64. The monoclonal anti-CD15 antibody 80H5 (Beckmann Coulter) served to detect CD15.

Results: A 62 years old patient presented with unwanted weight loss, weakness, relapsing episodes of thrombophlebitis and pulmonary embolism. The blood count showed significant anemia and reticulocytosis and a sample was referred to us to do a flow cytometric PNH test. After examination of glycosylphosphatidylinositol-linked antigens we found no evidence for the presence of a PNH clone. The only aberrant observation during this test was an abnormal low expression of CD15 on granulocytes (Fig. 1). Since staining had been performed in whole blood, we wanted to exclude an interference of plasma components; therefore, in a further experiment we washed the cells in PBS before staining. Indeed, we now found a normal expression of CD15 on granulocytes. We repeated the experiment using blood of healthy donors. The results of routine whole blood staining for CD15 were normal. However, when the plasma of the donors was replaced by plasma of the patient, again apparently low CD15 expression was observed. In approximately 1500 PNH tests with whole blood staining, we found this phenomenon only with the blood sample of this single patient. Further clinical examinations of the patient revealed extended gastric cancer with multiple metastases of the liver and the peritoneum.

Conclusion: The plasma of a patient with progressive gastric cancer contained a component neutralizing the CD15 antibody 80H5. Studies are in progress to clarify whether this finding is related to the disease of the patient. Such an interference with the flow cytometric CD15 estimation may occur rarely. Nevertheless, our findings suggest that in cases of unexpectedly low expression of CD15 using whole blood staining, the result should be controlled by an additional test using washed cells.

P06-4
Dithiothreitol treatment of test red blood cells to obviate daratumumab interference with compatibility testing for blood transfusion: a retrospective single center study

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Background: Daratumumab (DARA) is a novel therapy for multiple myeloma (MM). This monoclonal antibody recognizing CD38 interferes with routine blood compatibility testing by causing panreactivity during pre-transfusion testing causing positive reactions in indirect antiglobulin tests (IATs) for antibody detection and crossmatches. To overcome this problem, treatment of red cells with dithiothreitol (DTT), a common reagent in blood banks, has been suggested as a simple and practical method to allow alloantibody identification also in presence of DARA during pre-transfusion testing. We retrospectively analyzed data of patients with refractory MM treated with Daratumumab between November 2014 and May 2017.

Method: We retrospectively analysed data of patients with refractory MM treated with Daratumumab between November 2014 and May 2017. Pre-transfusion testing included ABO and Rh typing, antihuman globulin gel testing for antibody screening/crossmatch and direct antiglobulin test (DAT), with and without DTT-treatment.

Results: A total of 34 patients were included in this study. 17 patients have been treated with the drug during clinical trials (group I: median age 73, range 59–88), and 17 patients were treated after drug approval under real-life conditions (Group II, median age 65, range 33–83). Blood samples were referred for serological testing 28 days (range 3–122) and 21 days (range 2–130) after drug administration, respectively. Positive reaction in the antibody screening test was observed by testing initial samples from all patients. Using DTT-treated reagent red blood cells (RBCs) all interferences could be negated. To avoid haemolytic reaction ABO/Rh D phenotypically matched and K-negative RBC units were chosen for crossmatch. In group I, crossmatch testing was performed with 57 RBC units and revealed negative results only after DTT treatment. 6 out of 17 patients in this group received 28 RBC transfusions. In group II, 212 negative crossmatches were obtained after DTT treatment and 13 out of 17 patients received 122 compatible red blood products. While no patient from group I showed a positive DAT, 7 of 17 patients from group II had a slight increase binding of IgG but not C3d. Eluates of patients’ red cells showed no binding to test erythrocytes, excluding an alloimmunization. Most important, no clinically relevant, transfusion-related haemolytic reaction was reported in our study. During the follow-up, 6 out of 17 patients in group II showed negative results in the antibody testing 49 (16–98) days after receiving the last dose of DARA.

Conclusion: DTT treatment of test red cells reagent as well as from RBC units can easily be used in order to eliminate DARA interference with indirect Coombs test of DARA-treated patients. In our cohort, no haemolytic complication was observed indicating that this method can be safely implemented in the pre-transfusion testing of these patients.

P06-5
Daratumumab interference in red cell antibody aetion: specific inhibition with DaraEx

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Introduction: The anti-cancer drug Daratumumab, an anti-CD38 antibody, has recently been approved by the European Commission for treatment of multiple myeloma. CD38 is a surface molecule expressed on a number of tissues and cells, including red cells. The high titers of Daratumumab in patient sera lead to a strong interference in indirect antiglobulin tests (IAT), where most, if not all, of the reactions turn positive suggesting the presence of an alloantibody against a high frequent antigen. This can happen even up to 6 month after the last treatment with Daratumumab. The work-arounds that have been tested so far include:

- the destruction of CD38 on the red cells by treating them with DTT or Trypsin,
- inhibition of Daratumumab with soluble CD38 or an anti-idiotypic antibody, and
- matching by genotyping.

All of these approaches have considerable draw-backs and technical difficulties.

Methods: Imusyn developed a reagent, called «DaraEx», which can specifically inhibit the reaction of Daratumumab with red cells. After pre-incubating the red cells with DaraEx for 30 min at room temperature they can, without washing, be applied in the IAT directly.

Results: In order to evaluate the efficiency and specificity of DaraEx, we tested a number of cells with Daratumumab, sera, or mixtures of Daratumumab and sera. The reaction of Daratumumab with the red cells was completely abolished when the cells were pre-treated with DaraEx. «Hidden» antibodies (here: anti-i, anti-Kell and anti-Fy(a)) could be revealed, indicating that DaraEx is highly specific for Daratumumab. As Daratumumab titers in patients can be different from the concentrations used in the previous experiments, we tried to inhibit the reaction of test cells with sera from a patient treated with Daratumumab. Apart from a mixed-field result, which most likely is not due to Daratumumab, the reaction of the patient serum with red cells was completely inhibited.

Conclusion: DaraEx can specifically inhibit Daratumumab induced agglutination of red cells. The detection of other antibodies such as anti-Kell
is not affected, making DaraEx more reliable than the commonly used DTT-treatment and the method of choice to avoid Daratumumab interference.

**P06-6**

**New irradiation indicator for blood components improves blood safety**

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**Introduction:** To prevent graft versus host disease (GVHD) especially in immunosuppressed patients, blood components are radioactively irradiated with a dose of 30 gray (Gy) thereby inactivating nearly all residual leucocytes. Successful irradiation is controlled by indicators. Most of them switch their color from red into black. Unfortunately, this color change already occurs at lower irradiation doses. Therefore, these indicators cannot guarantee an efficient irradiation with 30 Gy. Moreover, the adhesive strength of the indicator used and the reliability of the color change process are important, since a potential loss of the indicator or its missing legibility after irradiation would prohibit the secureness that the respective blood component is successfully irradiated.

**Methods:** A newly designed irradiation indicator (RAD-Control) and the previously used indicator (RAD-SURE) were tested in parallel at different irradiation doses in 10 Gy steps between 10 and 40 Gy. In a second study part, the stick strength of the new indicator was tested under routine conditions on different blood component (packed red cells and platelets).

**Results:** Both irradiation indicators were tested at an irradiation with 10, 20, 30 and 40 Gy in replicates of n = 50. Figure 1 shows both indicators after the statutory irradiation of 30 Gy. The success of the irradiation procedure with the new RAD-Control indicator is indicated by a scannable barcode («ok») as well as by the notion «bestrahlt». Both become visible during the irradiation after a minimum irradiation dose of 20 Gy. In contrast, the RAD-Sure indicator color changes from red into black which holds already clear at a dose of 10 Gy. Moreover, 500 RAD-Control indicators were tested under routine conditions applying an irradiation of 30 Gy. The barcode was reliable visible and scannable all times without any exceptions.

**Conclusion:** The RAD-Control indicator improves blood safety to monitor the irradiation dose of blood components. A scannable barcode appears after a minimum dose of 20 Gy thereby guaranteeing an efficient irradiation. After the irradiation process, the barcode could be scanned and the results added to the electronic data set stored of the respective blood component. This could be an additional information concerning the blood component independently of the indicator itself. Finally, the indicator can be stored at room temperature, thus facilitating its handling. The new indicator documentation technology described is in line with the requested complete monitoring strategy of all production steps concerning blood components therefore importantly contributing to fulfill the good manufacturing practices.

**P06-7**

**Validation of the BD FACSVia System for the simultaneous determination of residual white blood cells, red blood cells, and platelets in fresh-frozen plasma**

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**Introduction:** According to German guidelines the quality of fresh frozen plasma (FFP) needs to be determined inter alia by counting residual WBCs, rRBCs, and rPLTs prior to freezing. Flow cytometric applications provide a rapid, quantitative, and reproducible method to identify and enumerate residual cell populations. The levels of residual cells in the blood products are regulated in particular by the definition of upper limits. In this study a new flow cytometer using a different detection kit for residual cells was validated in comparison to the established cytometer in order of replacement.

**Materials and Methods:** The BD Plasma Count kit was validated on the BD FACSVia system using the BD FACSvia™ clinical software in comparison to the up to now used BD Leucocount Kit for rWBCs and the detection of rRBCs using anti-glycophorin A on the BD FACScan. rPLTs were calculated by differential blood count on the Sysmex XE-5000. For this purpose 23 plasma samples were tested in parallel on both instruments. The BD Plasma Count assay incorporates BD Trucount tubes to determine absolute cell counts of rWBCs, rRBCs, and rPLTs using a single tube.

**Results:** Both settings showed comparable results regarding residual WBCs and RBCs in plasma. Residual PLTs showed higher results on the FACSvia in comparison to Sysmex XE-5000 due to the higher sensitivity of the FACSvia system.

**Conclusion:** The BD Plasma Count Kit is optimized for the simultaneous detection and quantitation of rWBCs, rRBCs and rPLTs in fresh human plasma, in a convenient single tube assay. The data demonstrate that the test is suitable for routine QC assessment of the cellular contaminants of therapeutic plasma according to the European recommendations.

**Gruppe 7**

**Klinische Hämostherapie und Patient Blood Management**

**P07-1**

**Gender disparities in transfusion of red blood cell concentrates**

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**Introduction:** We analysed the characteristics of patients receiving red blood cell concentrates in a prospective study.

**Material and Methods:** For all transfused red blood cell concentrates (RBCs) in all hospitals of Mecklenburg-Western Pomerania (MV) in the years 2005, 2010, and 2015, age, gender, and patient classification of each patient were obtained. For each respective year data of the population registry of the Federal Statistical Office were used to compute age- and sex-specific transfusion rates.

**Results:** In 2015 more women (50.5%) than men (49.5%) lived in the state MV. However, consistently, more RBCs were transfused to men than to women (2005: 54.2%, 2010: 53.1%, and 2015: 56.8%). This was also the case when single patient categories were analysed (surgical 51.8%, critical 50.5%, emergency 49.5%). However, more women were transfused in the state MV. In patients ≥50 years increasing transfusion rates were calculated for both sexes, in patients ≥50 years increasing transfusion rates were accompanied with more pronounced gender differences (Fig. 1). This gender associated dysbalance in transfusion rates is consistent with other studies [1–4]. Although these similar observations in several countries indicate a generalizable effect, the reasons for this gender disparity are unknown.
Conclusion: Transfusion demand differs depending on patients’ sex. Gender-related differences in transfusion practices should be considered in future studies.

References:

P07-2
Impact of RhD negative blood shortages for RhD negative patients – a survey about current practice in major German hospitals

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Introduction: Transfusion safety also includes prevention of alloimmunization. Data on alloimmunization against red blood cells due to transfusion are not obtained by hemovigilance systems. One reason for anti-D alloimmunization is transfusion of RhD positive (RhD+) RBCs to RhD negative (RhD-) patients. This occurs in clinical practice because the resource of RhD- blood is limited for various reasons: (i) the proportion of RhD- blood donors in the donor population is lower than 15% (7% for 0 RhD- donors), (ii) transfusion of RhD- red blood cell concentrates (RBCs) is mandatory in anti-D alloimmunized patients, recommended for RhD- women at childbearing age, and RhD- patients with chronic transfusion demand, (iii) the (non-recommended) use of 0 RhD- RBC transfusions in emergency bleeding situations until the blood type of the patient has been determined, even though up to 80% of these patients are RhD+. This results in an over-proportional consumption especially of 0 RhD- RBCs, which sometimes necessitates transfusion of RhD+ RBCs to known RhD-patients in urgent transfusion indications. To evaluate the current practice of the use of RhD- RBCs, frequency of RhD- RBC shortages and their impact on RhD incompatible transfusions, a survey is performed among transfusion medicine departments at university hospitals in Germany.

Methods: A questionnaire with 6 questions covering transfusion practice of RhD- RBCs and transfusion numbers of RhD-RBCs over the years 2014–2016 has been sent to 28 university hospitals. Questions include the number of transfused RhD- RBCs, the number of RhD- patients receiving RhD- transfusions, the number of RhD- patients who received RhD+ RBCs and the follow up strategy of these patients to detect possible anti-D alloimmunization. Further questions address the policy for emergency transfusions in patients with unknown blood type.

Results: This survey will provide the first systematic data on the frequency of RhD+ RBC transfusions to RhD- patients in major German hospitals as a marker for RhD- blood shortages. Results will be presented at the DGTI annual meeting in Cologne 2017.

Conclusion: Chronic kidney disease (CKD) has an age-dependent prevalence of ca. 10% in the general population and is associated with anemia, due to diminished production of erythropoietin by the kidney, dysfunctional iron metabolism and shortened erythrocyte survival. A small portion of patients with CKD will progress to end stage renal disease (ESRD) and need kidney replacement therapy or transplantation in their lifetime. The application of blood products is to be used with caution in CKD patients, as it may lead to alloimmunization against potential donors that could complicate kidney transplantation. Most patients are treated by primary care physicians. Presently, no German guideline or recommendations exist on anemia management and transfusion strategy for non-dialysis CKD patients in primary care.

Methods: A systematic review was conducted to identify clinical practice guidelines (CPG) for non-dialysis CKD issued or updated between Jan 2012 and Oct 2016 and the quality of the guidelines was rated with the AGREE-II instrument. Relevant guideline recommendations were adapted for the German healthcare system and the characteristics of the population of CKD patients in primary care via the ADAPTE process. If necessary, additional literature search was conducted.

Results: Recommended therapeutic serum hemoglobin (Hb) targets tended to be lower than normal values in patients without CKD. Most CPG recommended conservative treatment strategies and considering individual patient factors when deciding on anemia treatment. 3 CPG explicitly mentioned restrictive blood transfusion to avoid alloimmunization in cases where transplantation is a treatment option. An additional search for literature on HLA alloimmunization and alloimmunization in CKD patients was conducted. Pragmatic recommendations for German primary care patients with CKD were proposed after integration of evidence of international guideline recommendation and literature search. Recommendations included considering individual patient needs when deciding on diagnosis and treatment of anemia, combining ferritin with transferrin saturation when determining iron status, nephrologist referral if treatment with erythropoietin stimulating agents was deemed necessary and avoiding transfusion of cellular blood components to avoid alloimmunization in patients where transplantation may be a treatment option.

Conclusion: Recommendations for the treatment of patients with non-dialysis CKD in primary care should be pragmatic, as a high percentage of patients with early CKD are elderly and most of these patients will never reach end stage renal disease. These patients may benefit from a more restrictive treatment strategy, focusing on quality of life rather than on rigidly following recommendations. In younger patients or in those with rapidly progressing CKD, alloimmunization by transfusion of cellular blood components should be avoided if possible, to allow for the option of future transplantation.
P07-4
Five years of patient blood management: the effect on transfusion rates at one German University Hospital
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Introduction: Due to the demographic changes with an elderly population at higher percentage of the general population than in prior years, blood donations decline and blood components become a scarce resource. Beyond that, blood transfusions (BTs) are expensive and carry some risks for the patients. Therefore, a Patient Blood Management (PBM) program was set up clinically as an interdisciplinary, multimodal approach to achieve adequate indications for BTs.

Methods: PBM measures were implemented at Muenster University hospital since 2012 as follows: quantitative reporting of administered blood components for all subgroups of all medical services, yearly conversations with the responsible persons for BTs about indications, quantities and risks of transfusions for each clinical department. In 2013, a continuous training of appropriate transfusion indications for all physicians started. In 2014, a consequent prophylactic treatment of iron deficiency anemia prior to elective interventions was added. In the period from 2009 to 2016, all administrations of red blood cells (RBC), fresh frozen plasma (FFP), and platelets (PLT) were documented in all departments of the UKM.

Results: The data presented in this abstract refers to groups of departments with similar clinical tasks such as heart-chest-vascular surgery, general surgery, trauma surgery & orthopedics; hematology, internal medicine and other groups of disciplines. The highest transfusion rate was observed in the heart surgery group, followed by hematology and general surgery. With the implementation of PBM, the percentage of patients transfused decreased for RBCs by -17% and for FFPs by 30% in the period 2009 to 2016. Transfused units decreased for RBCs by -25%, for FFPs by -45%. This effect was more pronounced for surgical disciplines compared to non-surgical disciplines. In contrast, the number of PLTs declined only slightly with the number of transfusional cases being consistent.

Conclusion: To conclude, the PBM program was very successful and effective. There was a reduction of the RBC and FFP transfusion rate. Results were more enhanced in surgical departments. These findings illustrate that the focus on an appropriate indication reduces transfusion rates in the surgical setting.

P07-5
Asking transfusion-experts about their future blood requirements – survey results from 2015 and 2016
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In volatile times of patient blood management, demographic changes, and constant medical innovations it becomes more and more challenging for blood services to plan and provide the appropriate amount of blood products needed to take care of patients in German hospitals and clinics. In addition to internal data and extensive experience the German Red Cross Blood Service Baden-Württemberg Hessen aimed to enrich its knowledge on future developments in the blood consumption by asking transfusion experts in clinics and hospitals about their estimations of future blood product requirements and significant reasons that may cause changes.

Methods: To achieve an assessment of the future blood product requirements from the perspective of transfusion experts a questionnaire was developed in 2015. Beneath general indications such as number of cases or departments it included questions regarding reasons for changes and direction of change in the supply of the most important blood products: concentrated red cells, platelet concentrate, and fresh frozen plasma. Moreover, respondents were invited to predict their requirements of blood products over the coming three years based on their present figures. In 2015 the questionnaire was sent to 317 persons responsible for transfusion in every hospital or clinic that is supplied by the GRC Blood Service Baden-Württemberg-Hessen and its daughter company the Red Cross Blood Service North-East. The same questionnaire was again sent to 340 transfusion physicians in the year 2016.

Results: In 2015 a response of 53.6% and in 2016 of 47.9% was achieved. Seven responses in 2015 and three in 2016 have been excluded as respondents only answered general indications but gave no information on future blood product supply or influence factors of change. In 2015 the respondents predicted a slight decrease in the consumption of red cells and platelet. Only for plasma they assumed an increase of 4% in 2015 and 3% in 2016. With regard to the predicted demand in 2015 for the year 2016 a linear course of y=0.9712x+302.53 with a R² = 0.9644 for the need of red cells revealed a high concordance between the forecast and actual requirements in 2016. The results from the survey of 2016 show a more stable need of the three blood products. For red cells 61.9% (2015: 46.0%), for platelet concentrate 60.4% (2015: 41.4%) and for fresh frozen plasma 63.8% (2015: 51.3%) of the respondents forecast no changes in the consumption for the period ahead.

Conclusion: The annual survey that is also planned for the next years is a valuable additional tool for the German Red Cross Blood Service Baden-Württemberg Hessen. It helps to better assess the prospective needs of blood products and future developments in the market and therewith plan a demand-driven supply of blood products. Each year’s survey results are also shared with the transfusion experts that have participated to return the insights back into the group of experts and to strengthen the dialogue.
Results: APC contained about 40% more platelets than PPC and PPCA (medians, 4.6 x 1011 vs 2.7 x 1011 vs 2.5 x 1011). There were no substantial differences in platelet function between APC and PPC on days 1 and 4 of storage. In contrast to previous studies we observed a more moderate drop in ADP-induced LTA on day 4 (APC, 32%; PPC, 28%; and PPCA 70%, respectively). On day 1 percentages of CD62P and PAC-1 positive platelets were significantly higher and ADP-induced CD62P expression significantly weaker in PPCA compared to APC or PPC. During storage, ADP and collagen-induced TPA dropped more markedly and percentages of activated platelets (CD62P, PAC-1) increased more strongly in PPCA when compared with APC or PPC. Accordingly, after 4-day storage PPCA showed a significantly greater percentage of activated platelets (CD62P, PAC-1) and a significantly weaker ADP and TRAP-induced CD62P expression than APC or PPC. Multiple regression analysis revealed significant linear correlations among LTA induced by ADP, collagen, and TRAP and among flow cytometric assays for ADP and TRAP-induced platelet expression of CD62P and PAC-1, but all correlations were weak (minimum, r=0.34; maximum, r=0.85).

Conclusions: APC contain substantially more platelets than PPC or PPCA. When using platelet function assays in calcium-containing plasma without platelet count adjustment, storage lesion appeared to be more moderate than reported previously. The functionality of PPCA is markedly lower and significantly more affected by storage when compared to APC and PPC. Weak correlations among the results from platelet function assays indicate that the assays applied in this study cannot be used interchangeably.

Gruppe 8
Hämostaseologie

P08-1
Molecular fingerprint of coagulation activation in myeloproliferative neoplasms
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Introduction: Myeloproliferative neoplasms (MPN) are clonal diseases of hematopoietic stem cells, comprising myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET). Patients with MPN are at increased risk for venous thromboembolism (VTE), especially splanchic vein thrombosis (SVT). An association between JAK2V617F, the most common somatic MPN-associated mutation, and an acquired activated protein C (APC) resistance phenotype has been reported. These data suggest an alteration of the thrombin-protein C (PC) axis that may cause increased thrombin formation and subsequent APC generation.

Methods: To further study the contribution of the thrombin-PC axis to the development of a hypercoagulable state in MPN, we quantified plasma levels of thrombin and APC using oligonucleotide-based enzyme capture assays (OECA) in prospectively collected samples from MPN patients (81 samples/45 patients, thereof 23 females), diagnosed with MF (34/19), PV (30/14), and ET (17/12), and in a cohort of healthy probands (n = 30/14), and ET (17/12), and in a cohort of healthy probands (n = 43/22). In addition, activation markers of coagulation and fibrinolysis were measured, including D-dimer, prothrombin activation fragment F1+2, thrombin-antithrombin-complex (TAT), and plasmin-α2-antiplasmin complex (PAP). 38 patients (84%) were positive for the JAK2V617F mutation and three (7%) for the CALR mutation. Significant differences of these biomarkers between patients with MF, PV, and ET were not observed. Compared with healthy controls, samples from MPN patients with (n = 35, 25 thereof SVT) or without (n = 46) a history of VTE, and from patients without anticoagulant treatment (n = 54) or under anticoagulant medication (n = 27) showed significantly higher levels of APC, D-dimer, and PAP while F1+2 was only increased in the first three aforementioned subgroups. Consistent with these findings, F1+2 was the only biomarker, that differed significantly (p = 0.0021) between MPN patients with anticoagulant therapy (0.152, 0.098–0.219 nmol/l) and patients not receiving anticoagulant medication (0.296, 0.185–0.502 nmol/l).

Conclusion: Increased plasma levels of APC are a prominent finding in MPN patients independent of the MPN type. This indicates that MPN-induced thrombin formation as demonstrated by increased levels of F1+2 leads to activation of the anticoagulant PC system. In addition, the increase of PAP indicates activation of fibrinolysis which might contribute to elevated plasma levels of D-dimer. Thus, F1+2 might be better suited than D-dimer to monitor the effect of anticoagulant therapy in MPN.
In patients with increasing D-dimers the intraoperative need for RBC and PC was significantly higher as compared to patients with stable or decreasing D-dimers (RBC: 3.5 vs. 1.9; p < 0.0001; PC: 1.6 vs 0.9; p < 0.0001). Likewise, within transfusion needs 24 hrs postoperatively remained significantly higher (mean RBC: 1.8 vs 1.0; p = 0.001; mean PC 0.7 vs 0.4; p = 0.0006; FFP 2.1 vs 1.1; p < 0.0001) in patients with increasing D-dimers.

Conclusion: Even within the short intra-operative period, sequential D-dimer testing allows the identification of patients with increasing fibrinolytic activity despite tranexamic acid prophylaxis. The probability of 'break-through' fibrinolysis depends on the type of cardiac disease or cardiac surgery. Increasing fibrinolysis is associated with increased bleeding as objectified by higher transfusion needs. According to these data, intra-operative D-dimer monitoring allows for identification of the fibrinolysis-related bleeding risk and potentially, for risk adjusted dosing of antifibrinolytic therapy. Further analyses have to clarify if intra-operative increasing D-dimers are associated also with thromboembolic risks.

A potential role of antibody-mediated glycan modification on platelets and megakaryocytes in autoimmune thrombocytopenia

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Introduction: Immune thrombocytopenia (ITP) is a bleeding disease caused by autoantibodies (AAbs) directed against platelet glycoproteins (GP). A new mechanism of antibody-mediated platelet (PLT) destruction via Ashwell-Morell receptors (AMRs), which recognize glycan changes on cell surface, has been proposed in mice. In the current study, we investigated the effects of AAbs from ITP patients on the glycan pattern of human PLTs and megakaryocytes (MKs) as well as subsequent effects of the these changes on PLT survival in vivo.

Methods: Lectin binding assay (LBA) was used to analyze antibody-mediated change in glycan pattern. CD34+ derived MKs and PLTs were incubated with sera from ITP patients and healthy donors. Modification of glycan pattern was assessed by flow cytometry using different lectins; Ricinus communis agglutinin (RCA), Erythrina cristagalli lectin (ECL) and Peanut agglutinin (PNA) that bind respectively to galactose, N-acetyllactosamine and N-acetylgalactosamine residues. The impact of different glycan patterns on the survival of human PLTs and megakaryocytes (MKs) as well as subsequent effects of the these changes on PLT survival in vivo.

Results: 25 sera from healthy donors and 37 sera from ITP patients were investigated. In the LBA different patterns of glycan modification on PLT surface were observed. 9/37 sera induced high ECL binding, 17/37 sera caused a significant enhancement in PNA binding compared to healthy donors. In contrast, 8/37 sera showed strong decrease in RCA binding. No significant changes were induced by sera from healthy donors. Of note, not only GP-Ib/IX AAbs but also GPIb/IIa AAbs were able to modify glycan pattern. The injection of AAbs induced an accelerated clearance of human PLTs from the circulation in vivo. The destruction of human PLTs by ITP-AAbs was decreased but not completely prevented by a specific neuraminidase inhibitor that blocks glycan changes on PLT surface. Interestingly, we observed that MKs glycan expression can also be changed by exogenous Neuraminidase treatment. In addition, preliminary data showed that some ITP-AAbs were able to modify glycan pattern on human MKs surface.

Conclusion: Our results demonstrate that AAbs from ITP patients are able to induce changes in glycan patterns on both MKs and PLT surfaces. The mechanism of antibody-mediated modification of glycan patterns seems to interfere with PLT production from MKs as well as PLT destruction in the circulation.

Calcium determines the functional capability of large and small platelets

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Background: Platelets play an important role in cellular hemostasis. It is widely anticipated that large platelets are more reactive than small platelets because several studies found a stronger response of large platelets to thrombin or thrombin receptor activating peptide (TRAP) compared to small platelets. However, these studies were performed in Calcium-free media only. As Calcium is important for platelet activation, we sought to determine the influence of the extracellular Calcium concentration and intracellular Calcium mobilization on the response of large and small platelets to TRAP.

Methods: Large and small platelet fractions were separated from healthy blood donors by differential centrifugation. Platelet function was determined by flow cytometry according to CD62P expression after stimulation with TRAP and by light transmission aggregometry. Mobilization of Calcium from intracellular stores was measured using a Calcium release assay based on labelling of free intracellular Calcium with a Fluo-4 AM fluorescent dye. Each experiment was performed with platelets from at least 6 different subjects.

Results: The prepared large and small platelet fractions significantly differed in MPV: 12.03 fl ± 0.88 vs. 7.76 fl ± 0.53, p < 0.0001. In Calcium-free buffer, large platelets showed higher CD62P-expression after stimulation with TRAP compared to small platelets: 45.1 MFI ± 4.06 vs. 40.3 MFI ± 3.6; p = 0.0313. In a Calcium containing buffer, no difference in CD62P-expression was observed between large and small platelets (53.7 MFI ± 7.07 vs. small platelets 53.1 MFI ± 6.81, p = 0.9193). Aggregation of large platelets was stronger (70% ± 9.97 vs. 52% ± 15.91 p = 0.0313) and faster (5s ± 2 vs. 10s ± 2.3, p = 0.0305) after TRAP-stimulation compared to small ones in Calcium free medium. In Calcium containing medium, both platelet fractions showed a similar response (80% ± 8.5 maximum aggregation, p = 0.1563 and 5s ± 3.4 lag time, p = 0.5). Large platelets mobilized more calcium from intracellular stores after stimulation with TRAP (0.276 MFI ± 0.11) compared to small platelets (0.223 MFI ± 0.096; p = 0.0156).

Conclusion: We show for the first time, that large and small platelets have similar functional capabilities after activation of the thrombin receptor, when extracellular Calcium is available. The higher reactivity of large platelets upon TRAP stimulation is only present in calcium free media and explained by better Calcium mobilization in large platelets from intracellular stores. We consider the stronger response of large platelets to TRAP as an in vitro phenomenon caused by Calcium poor conditions.
Abstracts

P08-5
Evaluating the condensed MCMHM-1VWD Bowman Bleeding Questionnaire in the haemostasis clinic
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**Background:** Bleeding Questionnaires can be used to rule out relevant bleeding disorders. Reported negative predictive values (NPV) for von Willebrand disease (VWD) are excellent. We introduced the Condensed MCMDDM-1VWD Bleeding Questionnaire in our Haemostasis Clinic to address the question whether the score could help us to allocate elaborate, time-consuming and expensive diagnostic measures to a defined group of patients.

**Methods:** An open, prospective case-control study was initiated. All initial consultations referred to our Clinic by general practitioner with a suspected bleeding diathesis were enrolled. The condensed MCMDDM-1VWD Bleeding Questionnaire was used in addition to medical history taking from August 2016 onwards. The form was completed by a physician during the patient interview. The laboratory tests were identical for all patients and contained plasmatic and cellular haemostasis tests. After a defined time interval, a total of n = 52 patients with a bleeding score above 4 were matched for gender and age with n = 52 patients with a negative bleeding score for further analysis.

**Results:** In total, 38/52 patients (73%) with a positive score did have at least one abnormal laboratory finding. The final diagnoses were: platelet function defect (n = 15), VWD (n = 11), drug-induced platelet function defect (n = 9), subhaemophilia A (n = 1), dysfibrinogenemias (n = 1) and immune thrombocytopenia (n = 1). 14/52 score-positive patients (27%) did not have any abnormal laboratory finding. In the matched control group with a negative score, 31/52 patients (60%) had no abnormal laboratory finding; including four patients with vasopathies. 21/52 patients (40%) had a negative score, but one of the following diagnoses: VWD (n = 9), drug-induced platelet function defect (n = 8), and heterozygous defects of plasmatic coagulation factors V, VII, or XIII (n = 4). The overall performance characteristics of the questionnaire in our study were: positive predictive value, 73%; NPV, 61.5%; specificity, 70%; and sensitivity, 30%.

When all laboratory data were compared statistically between the two cohorts (score positives versus score negatives), only epinephrine and collagen closure times measured in a PFA 100 device were statistically significant.

**Conclusions:** The questionnaire’s primary intention was the detection of VWD. In the general haemostasis clinic, performance characteristics were disappointing. A NPV of 61.5% appears insufficient to allow for withholding elaborate, time-consuming and expensive diagnostic measures from score-negative patients. A number of false-negative scores were based on the fact that medication (drug-induced platelet dysfunction) and family history (factor deficiencies) are not incorporated. These two items would increase the NPV to 85% in our study. An important limitation of the study is the non-blinded design which is likely to have influenced the interpretation of laboratory data. A blinded follow-up study with an amended questionnaire is underway.

P08-6
The role of platelet proteasome activity for platelet function and viability
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**Introduction:** Although platelets are anucleated cells, they contain a functional system of protein degradation including a poly-ubiquitination apparatus and the main components of the 20S core displaying all three proteasome activities.

However, the role of protein degrading systems in platelet function still remains unclear. Therefore, the intention of this study was to analyze the effects of proteasome inhibition with bortezomib on platelet proteasome activity, poly-ubiquitination, platelet aggregation and *in vitro* viability.

**Methods:** Platelets were prepared from freshly collected venous whole blood. The platelet proteasome activity was determined by changes of fluorescence intensity of the fluorogenic proteasome product AMC, produced in LLVY-AMC-loaded platelets.

The levels of poly-ubiquitinated proteins were measured with an ELISA kit. Light transmission aggregometry was performed with an APACT 4004 aggregometer. Cell viability was measured by means of a fluorometric test kit.

**Results:** Platelet agonists like ADP, collagen and thrombin stimulated both platelet proteasome activity and protein poly-ubiquitination. Proteasome inhibition with bortezomib suppressed basal and collagen-stimulated proteasome activity and increased the accumulation of poly-ubiquitinated platelet proteins in a concentration-dependent manner. Bortezomib concentrations in the nM-range (above 10 nM) completely inhibited both, the basal and the agonist-stimulated proteasome activity, but did not affect *in vitro* platelet viability or agonist-induced platelet aggregation. In contrast, very high bortezomib concentrations (100–200 µM) partially inhibited platelet aggregation and viability.

**Conclusion:** The proteasome system is activated upon platelet stimulation. In contrast, the inhibition of the platelet proteasome activity does not tamper platelet viability and aggregation using bortezomib in the nM-range.

Suprainhibitory concentrations of bortezomib, however, may interfere with platelet responsiveness and *in vitro* viability; presumably due to unspecific and toxic effects. These concentration-dependent interactions with platelets should be considered in the use of proteasome inhibitors like bortezomib for experimental or for therapeutic issues.

P08-7
Tracking binding pathways of magnetic nanoparticles during uptake by platelets
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**Introduction:** To distinguish transfused platelets from patients or probands own cells within the scope of clinical studies, platelets from platelet concentrates (PC) need to be efficiently labeled. Non-radioactive labeling using magnetic nanoparticles (MNP) conjugated with human serum albumin (HSA) has recently shown great potential for platelet labeling. Here, we directly track the binding strengths and pathways of these particles during uptake by platelets using single-molecule force spectroscopy (SMFS). Combining measurement of the binding forces by SMFS and determination of the mean platelet particle content by atomic absorption spectroscopy (AAS), we identified the optimal HSA density for platelet labeling.

**Methods:** We used iron oxide magnetic nanoparticles (Meito Sangyo, Japan) with a mean diameter of 100 nm. MNPs were conjugated with 0.5–2 mg of HSA. Platelets were incubated with HSA-MNP for 60 min at 37 °C. The labeling efficiency was determined as mean iron content per platelet by AAS. Single HSA-MNP conjugated with different HSA-amounts were linked to a tip of a cantilever of the atomic force microscope via PEG linker and allowed to interact with immobilized platelets on a laminin coated glass surface. The binding forces between particles and platelets were determined at the last points in the retraction curves before the tips go back to the rest position. Binding pathways could be tracked by linking single HSA-MNP to the tips via PEG linkers of different length: short –2 nm, medium –30 nm, and long –100 nm to allow the free and flexible single HSA-MNP interacting with platelet compartments.

**Results:** The mean platelet iron content rises with increasing HSA concentration coated on particles and reaches a maximum at 1 mg/mL HSA with 1.3 ± 0.2 pg. With 2 mg/mL HSA, the platelet particle uptake drastically decreases to 0.3 ± 0.06 pg mean iron/platelet. Rupture forces of HSA-
MNP bound on the SMFS tip increase to a maximum at MNP with 1 mg/mL HSA (205.8 ± 2.6 pN) confirming that magnetic labeling efficiency is highest with MNP coated with 1 mg/mL HSA. Depending on PEG length, MNP interact differently with platelets as shown by one-, two- or three rupture force distributions of 36 pN, 172 pN, and 360 pN, which correspond to one-, two- or three binding pathways, respectively. We propose a model that short PEG allows MNP to interact only with the platelet membrane; medium length PEG allows the particle to move further into the open canalicular system (OCS), whereas long PEG promotes the particle transferring from OCS channel to another compartment in platelets.

**Conclusion**: Our study confirms the optimal HSA density for magnetic labeling and provides insights into the pathways of magnetic particles in platelets. The results also suggest three pathways when a single MNP is in contact with a platelet and show that biophysical methods like SMFS have a major potential to characterize processed PC.

**P08-9**

**Nonfatal intravascular hemolysis in a stem cell transplanted pediatric patient after minor incompatitable platelet transfusion**

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**Introduction**: ABO-minor incompatible transfusion of platelet concentrates may rarely result in acute hemolytic reactions that are not predicted by measuring isoaagglutinin in donor's plasma. Therefore, current guidelines recommend that minor incompatible, apheresis platelet concentrates (APCs) should only be given to patients with a body weight over 25 kg.

**Case report**: We describe a 11-year old, 29 kg male patient with myelodysplastic syndrome, who underwent hematopoietic stem cell transplantation from a a blood group identical MLA matched unrelated donor.. The patient showed refractoriness against transfusion of random platelets. Antibodies against HLA-class I were detected in his serum by Luminex technology using single antigen beads. The patient, blood group A, then received apheresis platelet concentrates (APC) form HLA-compatible donors to prevent bleeding by persistent thrombocytopenia. Immediately after administration a minor incompatible APC from a blood group 0 donor, a drop of hemoglobin (from 9.8 g/dl to 6.3 g/dl; normal range: 11.8–15.0) and a significant increase of hemolysis parameters (free Hb: 84.26 mg/dl, normal range: < 10 mg/dl; Haptoglobin < 5 mg/dl, normal range: 17–213 mg/dl; LDH 2107 U/l, normal range: < 299 U/l) were observed. A hemolytic reaction after transfusion was suspected and serological investigations were then initiated. The direct coombs test was positive for IgG but negative for C3d. Anti-A could be confirmed in the eluate from circulating red cells. Additionally, high isoaagglutinin titer against blood group A (titer 2048) was detected in donor’s plasma. In the further course, only blood group identical APCs were administered. A recovery of the hemolysis parameters could be observed within 11 days.

**Conclusion**: Transfusion services and treating physicians should be aware of the potential for adverse consequences of transfusing ABO-non identical APCs. Plasma replacement with additive solutions and screening of blood group O platelet donors should be considered to reduce the risks of hemolytic reaction that may be caused by minor-mismatched APCs.

**P08-10**

**Lupus Anticoagulant-Hypoprophorombinemia Syndrome (LA-HPS): abnormalities in standard coagulation tests paired with concurrent thrombembolic and bleeding complications**

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**Introduction**: The diagnosis of antiphospholipide syndrome (APS) is generally associated with an increased thrombembolic risk, however, this case highlights first that patients may also suffer from an increased bleeding risk and second that taking a standardized patient’s coagulation history (CH) is crucial in identifying this rare but hazardous condition. Recurrent abortion, deep vein thrombosis (DVT) and repeated detection of antiphospholipide antibodies (APA: B2-Glykoproteine-1-Abodies (AB) IgG & -IgM (88 / 100 U/ml), Cardiolipin-AB IgG / IgM (77 / 16 U/ml). A standardized CH revealed no further thrombembolic events (TE) but a history of excessive menstrual bleeding (minimal hemoglobin 6 g/dl) and easy bruising during the last five month. A gynecological cause has been excluded. Current medication: None.

**Results**: Laboratory work-up: Anemia (Hb 9.1 g/dl), PT 33%, INR 2.4 (Ref. 0.8–1.2), PTT 74 sec (Ref. 25–37 sec), PT (LA sensitive) 94 sec (25–37 sec). In order to attenuate APA-induced measuring interferences in factor assays: Measurement of all factor activities with geometrical dilu-
tion, normalization of all factor activities, except prothrombin (F II). Mixing study with normal pooled plasma (NPP) (0, 1 and 2 hours incubation, 37°C): PT and F II activity normalization. The results revealed a genuine F II deficiency. F II activity was 6% (ref. 70–130%), causing an increased bleeding risk for the patient. These findings would not be expected in the first place, since criteria for APS diagnose include TE and recurrent positive APA screening. Phospholipid-binding antibodies in patients blood samples decrease or eliminate the phospholipides in the test tube resulting in faulty standard coagulation parameters (SCP). In our APS case changes of SCP could be interpreted as APS induced, whereas the positive bleeding history should encourage to carry out an extended laboratory work-up. APA are heterogeneous antibodies with an affinity to plasma proteins. In our patient APA binds on F II and accelerates its clearance. Conclusively, the test results revealed a lupus anticoagulant-hypoprothrombinemia syndrome (LA-HPS). A LA-HPS should be excluded if APS patients present with positive bleeding signs and decreased PT (PT is a F II-dependent assay). A F II deficiency can be detected with dilution series and mixing studies with NNP, PPSB or FFP are effective to manage acute bleeding.

Conclusion: A standardized CH is essential for patient safety, especially in the perioperative setting. SLP can be sometimes misleading and therefore the patient’s history contributes highly valuable information in the differential diagnosis of complex conditions.

PO0-11
Alternative anticoagulation with bivalirudin in high-risk cardiac surgery: a case report
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Introduction: Patients with Heparin-induced thrombocytopenia (HIT) are at risk of fatal thromboembolism in case of continued or re-exposure to heparin. After discontinuation of heparin, antibody titres will quickly decline. HIT-patients with antibody titres below the threshold of ELISA testing can be re-exposed to heparin for a short period of time, e.g. during cardiac surgery. Patients with persisting antibodies have to be treated with alternative anticoagulants. We present a case of alternative anticoagulation with bivalirudin (Angiox®) in a dialysis dependent HIT patient undergoing total artificial heart transplantation (TAHT), who had suffered coronary artery thrombosis after heart transplantation (HTX) with heparin re-exposure and boosting of HIT antibodies.

Patient and Methods: A 31 year old female patient with hypertrophic cardiomyopathy had a suspected diagnosis of HIT and was switched to argatroban (Agatran®) preoperatively. Prior to HTX no HIT antibodies were detectable. Thus, re-exposure to unfractionated heparin during HTX. Four hours after heparin infusion was started coronary artery thrombosis was diagnosed. Suspecting HIT type II as likely explanation, ELISA testing was performed showing re-occurrence of HIT antibodies. Due to cardiac deterioration the following days, the transplant had to be exchanged for a total artificial heart device, performed with bivalirudin as anticoagulant. During this surgery standard tranexamic acid therapy was applied. Haemostasis monitoring was performed at narrow intervals using activated clotting time (ACT), conventional haemostatic routine parameters including factor XIII activity and D-dimer testing as well as thromboplastometry (ROTEM®) and impedance aggregometry (Multiplate®). Antithrombogenic therapy was based on real time diagnostic.

Results: With bivalirudin 50mg i.v. at start of extracorporeal circulation followed by continuous infusion with 10mg/h ACT times kept stable. Ultrafiltration was applied during / at the end of operation. Goal-directed haemotherapy was performed with a total of 16 platelet concentrates, 10g fibrinogen, 9,000E Cofact®, 2,750E FXIII-concentrate, 3mg rFVIIa. Thus complete haemostasis was achieved according to clinical judgement.

At entry to the ICU a residual bivalirudin effect with aPTT = 39sec and thrombin time = 87sec was objectified.

Conclusions: No alternative cause besides HIT was detected for the coronary artery thrombosis in our patient. This suggests that boosting of HIT antibodies with manifest thromboembolism may occur even within the very short time-period of intra-operative heparin re-exposure in individual patients. Despite the lack of neutralising agents, alternative anticoagulation with bivalirudin seems to be feasible, even in operations with highest bleeding risk, and even in patients with renal failure, which possibly was achieved by the meticulous real-time monitoring of haemostatic parameters and specific goal-directed haemostatic therapy.

Gruppe 9
Zelltherapie und Tissue Engineering

P00-1
Circadian rhythms regulate release of mature and immature hematopoietic cells into blood
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Background: Small numbers of immature hematopoietic cells (HSPC) circulate in the peripheral blood (PB) during homeostasis. Whether these represent failed bone marrow (BM) retention or active release remains unclear. The number of circulating HSPC markedly rises in situations of (hematopoietic) stress, e.g. via elevation of endogenous G-CSF levels during infection or neutropenia. Given the overlapping signaling pathways of stress and circadian rhythms, we queried whether HSPC release from BM is influenced by circadian regulation.

Methods: Wild-type C57Bl/6 vs. clock gene deficient Bmal1−/− mice, lacking circadian rhythm, were held under conditions of 12/12 light-dark (LD) or continuous darkness (DD). Mice were analyzed for leukocyte (WBC) and HSPC content in hematopoietic organs, using automated hemocytometry, FACS-analysis and CFU-C assay. Cortisol levels were detected by an electrochemiluminescence-immunoassay. Circostosterone and AMD3100 were administered as previously described. Radiation chimeras were generated via cross-transplantation of WT and Bmal1−/− mice. Clock gene expression was analyzed by real-time qPCR.

Results: We observed diurnal rhythmicity of HSPC in PB of WT mice. HSPC peak 2 hours after sunrise and reach trough levels 12 hours later, with WBC 3-fold and CFU-C 4-fold at peak vs. trough. Spleen cellularity was 1/5th reduced at trough; splenectomized mice continued to be rhythmic, ruling out release from splenic reservoirs as causal of the observed oscillation. In contrast, BM HSPC levels were unchanged over time. Experiments were reproducible under DD conditions, confirming the circadian regulation of HSPC oscillation. Cortisol levels peaked 3 hours before HSPC trough. Anti-cyclic substitution of cortisol neutralized rhythmicity and suppressed circulating HSPC counts in WT mice, while cyclic cortisol substitution of Bmal1−/− mice restored HSPC oscillation, suggesting a link between the CRF-ACTH-cortisol axis and HSPC retention factors in BM.

To assess whether peripheral vs. central Bmal1 cycling and alternative CNS mediators regulate the circadian HSPC release, cross-transplantation experiments were performed, using Bmal1−/−. HSPC as donor cells for WT recipients and vice versa. WT mice reconstituted with Bmal1−/− hematopoiesis showed the same circadian HSPC oscillation as WT mice, whereas...
Abstracts

Bma1-/- recipients engrafted with WT cells lacked circadian rhythmicity, indicating non-hematopoietic causes of HSPC release. Enforced mobilization in WT mice at peak and trough using the fast-acting CXCR4 antagonist AMD3100 showed markedly stronger mobilization at peak than at trough. Preliminary data in humans confirm a similar, albeit inverted, rhythmic HSPC egress.

Summary: Hematopoietic-extrinsic signals mediate circadian rhythmicity of HSPC release. These circadian oscillations influence the yield of HSPCs after stem cell mobilization and might be exploited to optimize HSPC mobilization and harvest in humans.

P90-2
The contribution of MICA-129 Val/Met polymorphism on graft rejection and CMV infection in simultaneous pancreas and kidney transplant patients – one-year follow-up

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Introduction: Simultaneous transplantation of pancreas and kidney (SPK) is an established therapy for diabetes type-I patients suffering from chronic renal failure. Emerging evidence suggest that other antigens besides HLA mismatches may play a role in immune cells activation, graft rejection or survival. The polymorphic MHC class I chain-related sequence A (MICA) gene encodes a membrane-bound protein that binds to the NKG2D receptor activating NK and T cells whereas soluble forms of MICA (sMICA) impair the effector functions of these cells. Importantly, a single nucleotide polymorphism (SNP) rs1051792 at position 454A/G of exon 3 causes a valine (val) to methionine (met) exchange at codon 129, which influences MICA expression patterns and binding affinity to NKG2D. Also, met positive molecules contribute to 10- to 50-fold greater capacity to complex NKG2D. Val129Met has been evaluated in hematopoietic stem cell transplantation and autoimmune disorders. However, little is known and the clinical relevance of this dimorphism in solid transplantation is still not clear.

Methods: SPK transplantation recipients were recruited between the year 2012–2016 at Kappaschaftskrankenhaus Bochum, MICA SNP was genotyped in 50 SPK recipients and donors and sMICA levels were determined by ELISA in 21 patients during one-year follow-up. MICA Val129Met typing was performed by NESTED-PCR method.

Results: We evaluate the effect of val/met mismatch situation with regard to CMV infection and graft rejection in the one-year follow up. Distribution of allele (p = 0.261) and genotype (p = 0.411) frequencies of 129 val/ met were similar between donors and recipients. We observed that the val-mismatched patients (n = 7) had a shorter CMV infection-free-survival than the remaining patients (log-rank test, p = 0.004) and higher risk to CMV infection [p = 0.015; hazard ratio (HR) 7.36; 95% CI 1.47–36.9]. Similarly, a shorter kidney rejection-free survival were observed in valmismatched patients (log-rank test, p = 0.007) and higher risk of having acute kidney rejection (padjusted=0.006; HR 6.02, 1.68–21.7). Interestingly, the one-year follow-up sMICA levels could hardly be detected in valmismatched patients (N = 4, range: 0.0–0.02 pg/mL), whereas other patients revealed substantial amount of sMICA (N = 17, range: 0.32–3.70 pg/mL; p < 0.001).

Conclusion: Our study gives for the first time evidence that the functionally relevant mismatch situation of MICA at position 129 has an impact on kidney allograft recognition and CMV infection in the first year post SPK transplantation.

P90-3
Dissecting the origin of dendritic cell and macrophage subsets in human hematopoiesis

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Introduction: According to our recent findings multipotent progenitors (MPPs) do not create common lymphocyte (CLP) and common myeloid progenitors (CMP) as suggested by the classical model of hematopoiesis. Instead, they create lymphoid-primed multipotent (LMP) and erythroid-myeloid progenitors (EMP). Thus, subsets of myeloid cells derive from both branches. In this context, we previously showed that neutrophils are derivatives of LMPs and eosinophils and basophils of EMPs. Monocytes/ Macrophages arise from progenitors of both branches. Without dissecting their concrete origin, dendritic cells (DCs) have been classically discriminated into lymphoid [plasmacytoid DC (pDC)] and myeloid DCs [monocyte-related DC (MoDC), myeloid DC1 (mDC1), myeloid DC2 (mDC2)]. Now, the novel hematopoietic lineage relationships raise the question whether myeloid DCs derive from LMPs and or EMPs. Consequently, in our ongoing work, we aim to unravel the exact origin of both, the different human macrophage subsets as well as of the different DC subtypes at the single cell level. This requires a very robust and effective read out method.

Methods: To establish an effective read out system allowing the analysis of DC and macrophage potential at the single cell level in vitro, we compared different growth conditions and set up flow cytometric protocols to detect the different DC subtypes and macrophages.

Results: So far, we have seeded MPPs into the assay and studied the presence of the different DC subtypes and macrophages in the arising progeny fractions. Upon characterizing obtained cells via multicolor flow cytometry (12 colors), we became able to increase the resolution of the cell surface phenotypes of in vitro generated DCs. Now, we identify pDCs as HLA-DRdim/+CD14-CD1c+CD11clowCD141+CLEC9a+ cells. Accord- ing to the literature MoDCs are defined as HLA-DR+CD14+/CD1c+CD11b+cDC1+cells. Although we obtain numerous cells showing this phenotype, this fraction provides a very heterogeneous cell population, which should be dissected in the future.

Conclusion: By improving the cell surface analysis of in vitro generated DCs, we are able to discriminate different DC subtypes and to study the DC potential of selected progenitor fractions, now.

P90-4
Generation of hematopoietic stem and progenitor cells from human pluripotent stem cells, in vitro

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Introduction: Hematopoietic stem cells (HSCs) are the only cells capable of long-term, multilineage blood-formation after transplantation. Therefore, they are key targets for cell replacement therapy of the hematopoietic system. However, significant in vitro expansion or even maintenance of HSCs has not been accomplished yet. Rather, these cells rapidly differentiate or undergo apoptosis even under the currently best culture conditions. In contrast pluripotent stem cells (PSCs), such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) can be expanded efficiently without losing their «stem-ness». Furthermore, they can differentiate into all cell types of the organism, thus making them an attractive source for generating HSCs, ex vivo. Despite all progress made in the recent years, hPSC-derived HSCs capable of robust engraftment after transplantation into recipient animals have not yet been generated without permanent ectopic expression of a combination of transcription factors. One reason may be that a rate-limiting step of HSC formation, the generation
of the so-called hemogenic endothelium (HE), is not efficiently promoted by the currently existing protocols.

**Methods:** Using human ESCs (H1 line), we tested different protocols with the aim to stepwise develop towards hematopoietic stem and progenitor cells (HSPCs). Embryoid body (EBs) and coculture-differentiation on mouse OP9 stromal cell were compared using published or commercially available (STEMdiffTM Stem Cell Technologies) media formulations.

**Results:** Neither EBs nor OP9 cocultures with or without addition of different cytokine combination led to efficient hematopoietic progenitor formation. In contrast, two-step monolayer-differentiation in STEMdiffTM media (Stem Cell Technologies) promoted efficient and reproducible development of HSPCs. Likewise, mesoderm formation was initiated for the first three days. Within the next 9 days following media exchange, morphological structures reminiscent of HE-colonies appeared which were associated with proliferating suspension cells. Those cells expressed hematopoiesis-associated cell surface molecules such as CD43, CD34 and CD45 (as determined by flow cytometry) and formed myeloid colonies in colony formation (CFC)-assays.

**Conclusion:** We here show that HE and CD43+/CD34+ HSPCs can be robustly generated from PSCs using a novel commercial media formulation. To further increase the efficiency of HSPC development, in-depth immunophenotyping and transcription analysis of genes involved in hematopoietic specification are currently performed to define critical time windows during hematopoietic specification of differentiating hPSCs. Based on these results, selective manipulation of extrinsic and intrinsic cues by small molecules and/or transient expression of fate-determining transcription factors will help to increase HE formation, expansion and its transition to HSPCs.

**P09-5**

**Hemocompatibility of stromal cells depends on tissue factor activity and is influenced by organ origin and culture conditions**

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Mesenchymal stromal cells (MSC) are promising candidates for regenerative medicine and therapy of numerous diseases. Optimal cell source and expansion protocols using fetal bovine serum (FBS) or human platelet-rich plasma (HPRP) are linked to the protective immunity in patients undergoing immunomodulating treatment. In this context, a good laboratory practice (GLP)-compliant protocol for the identification and enumeration of antigen-specific T cells is crucial. Here, we introduce an assay based on no-wash single-platform cell enumeration and HMC Strepnamer staining to determine the WT1-specific T cell immunity in clinical samples.

**Methods:** We analyzed the performance of the WT1-specific MHC class I Strepnamers coupled to PE in direct comparison to CMV- and EBV-specific MHC class I Strepnamer staining by spiking antigen-specific T cells at different densities in non-MHC matched PBMCs. Cells were transferred to TruCountTM Absolute Counting tubes and stained with aCD45 FITC, aCD3 APC, aCD8 V500 and MHC class I-Peptide Strepnamer-PE. To screen for WT1-specific T cells applying a classical wash-protocol, whole blood samples from 88 healthy donors (HD) were obtained by venipuncture. Screening for WT1-specific T cells using the single-platform assay, PBMCs were isolated from 22 Buffy Coats using density gradient centrifugation.

**Results:** The accuracy and linearity of the assay was exceptionally high for all performed experiments with a mean recovery of 94% and a mean linear regression of R2=0.988. Differences were apparent regarding the limit of detection/quantification (LOD/LOQ). While results obtained for WT1 yielded an LOD/LOQ of 0.08 ± 0.04% and 0.11 ± 0.06% (1.33 ± 0.32 cells/µl and 1.9 ± 0.14 cells/µl), the overall LOD/LOQ including virogeneric-specific T cells was 0.08 ± 0.04% and 0.11 ± 0.06% (1.33 ± 0.32 cells/µl). In direct comparison to CMV- and EBV-specific MHC class I Strepnamer staining by spiking antigen-specific T cells at different densities in non-MHC matched PBMCs. Cells were transferred to TruCountTM Absolute Counting tubes and stained with aCD45 FITC, aCD3 APC, aCD8 V500 and MHC class I-Peptide Strepnamer-PE. To screen for WT1-specific T cells applying a classical wash-protocol, whole blood samples from 88 healthy donors (HD) were obtained by venipuncture. Screening for WT1-specific T cells using the single-platform assay, PBMCs were isolated from 22 Buffy Coats using density gradient centrifugation.

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**P09-6**

**Enumeration of WT1-specific CD8+ T cells using an MHC tetramer based no-wash single-platform flow-cytometric assay**

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**Background:** Absolute numbers of antigen-specific T cells in whole blood are linked to the protective immunity in patients undergoing immunomodulating treatment. In this context, a good laboratory practice (GLP)-compliant protocol for the identification and enumeration of antigen-specific T cells is crucial. Here, we introduce an assay based on no-wash single-platform cell enumeration and MHC Strepnamer staining to determine the WT1-specific T cell immunity in clinical samples.

**Methods:** We analyzed the performance of the WT1-specific MHC class I Strepnamers coupled to PE in direct comparison to CMV- and EBV-specific MHC class I Strepnamer staining by spiking antigen-specific T cells at different densities in non-MHC matched PBMCs. Cells were transferred to TruCountTM Absolute Counting tubes and stained with aCD45 FITC, aCD3 APC, aCD8 V500 and MHC class I-Peptide Strepnamer-PE. To screen for WT1-specific T cells applying a classical wash-protocol, whole blood samples from 88 healthy donors (HD) were obtained by venipuncture. Screening for WT1-specific T cells using the single-platform assay, PBMCs were isolated from 22 Buffy Coats using density gradient centrifugation.

**Results:** The accuracy and linearity of the assay was exceptionally high for all performed experiments with a mean recovery of 94% and a mean linear regression of R2=0.988. Differences were apparent regarding the limit of detection/quantification (LOD/LOQ). While results obtained for WT1 yielded an LOD/LOQ of 0.08 ± 0.04% and 0.11 ± 0.06% (1.33 ± 0.32 cells/µl and 1.9 ± 0.14 cells/µl), the overall LOD/LOQ including viro-specific T cells was 0.08 ± 0.04% and 0.11 ± 0.06% (1.33 ± 0.32 cells/µl and 1.9 ± 0.14 cells/µl). Subsequent screening of 22 healthy individuals revealed significantly higher values for
WT1 (0.04 ± 0.02% and 1.5 ± 0.9 cells/μl) than for the irrelevant HIV pol (0.016 ± 0.01% and 0.5 ± 0.4 cells/μl). In contrast, applying the classical wash protocol no increase in detected frequencies of WT1-specific T cells could be identified.

**Conclusion:** Establishing a no-wash-protocol to avoid shear forces potentially leading to the dissolution of weak affine bonds, significantly elevated numbers of WT1-specific T cells compared to its cognate HIV-control were detected in healthy individuals. With an exceptionally high accuracy for all performed experiments, these data imply a high sensitivity of the single-platform no-wash staining. Additionally, considering the high feasibility of this assay, the protocol is of general interest for clinical flow cytometry.

**P09-7**

**Anti-HPA-15b bound to endothelial cells may impair angiogenesis**

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Antibodies against HPA-15b are known to be responsible in different clinical settings of immune mediated thrombocytopenia including platelet transfusion refractoriness (PTR) and fetal neonatal alloimmune thrombocytopenia (FNAIT). However, the serological detection of anti-HPA-15b antibodies is still unreliable based on the low and stability of CD109 expression on platelets. Anti-HPA-15b reacts with CD109, a GPI-anchored protein expressed on activated platelets. Besides on platelets, CD109 was found on the surface of activated T-cells and endothelial cells. Interestingly, truncated CD109 isoform was identified in placenta. Knockdown of CD109 reduced endothelial tube formation and cell migration indicating the role of CD109 in angiogenesis. Recently, we found that anti-HPA-1a antibodies reacted with αvβ3 integrin on endothelial cells could impair angiogenesis, associated with the development of intracranial hemorrhage (ICH) in fetus with severe FNAIT. In rare cases, ICH associated with anti-HPA-15b antibodies has been reported.

In this study, the expression of CD109 on HPA-15 typed human umbilical vein endothelial cells (HUVEC) and the impact of anti-HPA-15b on angiogenesis was investigated. HUVECs from different individuals (n = 39) were typed for HPA-15 by RT-PCR; 8 were HPA-15aa, 9 HPA-15ab and 22 HPA-15bb. Based on these results, the calculated frequencies of HPA-15a and HPA-15b are 0.487, 0.513, respectively which are in accordance to the reported allelic frequencies of HPA-15a and HPA-15b. Flow cytometry analysis using moabs against CD109 (TEA2/16) and CD61 (AP3) revealed comparable amount of CD109 and αvβ3 indicating high CD109 expression on endothelial surface. However, no correlation between CD109 expressions and HPA-15 phenotypes was observed. To evaluate the use of endothelial cells as target cells for the serological detection of anti-HPA-15b antibodies, MAIPA was performed using moab TEA2/16 as capture antibody. In contrast to platelets, significant stronger and reliable reaction of anti-HPA-15b with HPA-15bb and -15ab typed HUVECS was observed. In contrast, no reaction was detected with HPA-15aa typed cells. In contrast to platelets, HUVECS stored in PBS buffer at 4°C for one week did not show any significant decrease of anti-HPA-15 antibody reactivity. To answer the question whether anti-CD109 may impair the angiogenesis of endothelial cells, tube formation assay was performed. Similar to the functional inhibitory moab 23C6 against αvβ3, moab TEA2/16 reacted with endothelial CD109 suppressed tube formation. Similar phenomenon was found with anti-HPA-15b alloantibodies.

Our results demonstrated that in comparison to activated platelets, endothelial cells constitutively and stably express HPA-15 epitopes, and binding of anti-HPA-15 antibodies to endothelial cells can impair angiogenesis which may lead to ICH.

**P09-8**

**Immunologic aspects in tissue transplantation**

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**Introduction:** Whereas in organ transplantation the observance of the immunological compatibility is of crucial importance, these aspects play a subordinate role in the transplantation of tissue. This is one of the main reasons why generally fewer complications occur after the transplantation of a tissue graft. Nevertheless, there are several factors, which must also be considered in tissue medicine.

The present study aims to provide a brief overview of the relevant immunological aspects in tissue transplantation with a particular focus on the corneal transplant.

**Methods:** The most frequently transplanted tissues are ocular corneas, but also human amniotic membranes, musculoskeletal tissues such as bones, soft tissues (fascia and tendons), cartilage tissue, cardiovascular tissues (heart valves and vessels) and skin tissue are transplanted. The individual tissue types are processed differently before application and therefore also show a differently strong immunogenicity, which can lead to complications in the recipient. To compile the knowledge, a literature search with the inclusion of the PubMed database was performed as well as data of the tissue network of the German Society for tissue transplantation (DGFG) were evaluated.

**Results:** Despite the immunological privilege of the cornea and the posterior chamber of the eye, tissue rejections after perforating keratoplasty are still a major postoperative problem. Particularly in high-risk situations, for instance in rekeratoplastics, therefore HLA-matched corneas are transplanted. These were 178 (= 5,9%) in the DGFG network in 2016. In contrast, the transplantation of amniotic membrane with its special properties does not represent a challenge by immunological reactions. This is also the case with the transplantation of musculoskeletal tissue, although the lack of immunogenicity is due to the treatment of the bone graft or the lack of blood circulation of osteochondral allografts. In the same way, the processing of cardiovascular tissue causes rejection reactions to occur extremely rarely since cellular components are altered or completely removed by decellularization. For the use of cryopreserved vascular grafts with vital cells, it is described that HLA matching can reduce the complication rate. The immunosuppression associated with a severe burn injury allows temporary wound care with allogeneic foreign skin.

**Conclusion:** Tissue medication is a part of transplantation medicine for which deviating immunological aspects play a role. The knowledge of the aspects relevant to the individual tissue types is important for the further development of tissue transplantation in the sense of a secure patient care.

**P09-9**

**Towards large-scale isolation of plasmacytoid dendritic cells from healthy donors for use as a next-generation tumor vaccine.**

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Plasmacytoid dendritic cells (pDC) represent a rare type of dendritic cells (DC) with promising features for tumor vaccination. Their precursors circulate in the peripheral blood at a frequency of 0.1–0.5%. When required, they migrate into inflamed areas such as infected or tumor tissue. Within as little as 24 hours, pDC take up antigens and turn into fully functional antigen-presenting cells in the presence of toll-like receptor (TLR) ligands. This is rapid compared to the maturation of other DC types such as monocyte-derived DC (5–7 days), which are currently used in most tumor vaccination trials. A major hurdle in using pDC as an autologous
tumor vaccine is that both their quantity and their function are often compromised due to the underlying disease and its therapy. A promising alternative to autologous monocyte-derived DC may therefore be allogeneic pDC from partly HLA-matched healthy donors. Meanwhile, the isolation of pDC according to good manufacturing practice (GMP) guidelines is possible using magnetically labeled antibodies against blood dendritic cell antigen (BDCA)-4. In the current study, we used a GMP-compliant positive selection kit to directly isolate BDCA-4^+ pDC from leukapheresis products collected from 24 unstimulated healthy donors. Subsequently, we tested the isolated pDC in terms of their response to various TLR agonists and their capacity to activate T cells. We were able to isolate an average of 4.7 × 10^6 pDC (range: 2.3 × 10^6 to 10.5 × 10^6) from 2.3 × 10^7 total leukocytes, which corresponds to 20% of the average leukocyte count in a full leukapheresis product. The average pDC purity was 90.0% (range: 75.1–97.0%); the average pDC viability was 92.4% (range: 81.0–97.0%). Collection of cells directly into a prepared bag with 50ml of donor plasma significantly enhanced the viability of isolated pDC. Another factor associated with higher pDC viability, purity and recovery was a high white blood cell count of the donor and the leukapheresis product. In contrast, a higher hematocrit in the leukapheresis product was associated with lower pDC recovery and viability. Functionally, isolated pDC showed the typical biphasic activation pattern as described earlier. During the first phase, pDC developed a tolerogenic phenotype in response to various cytokines including IL-3, IL-10 and IL-21. This phenotype involved the production of the serine protease granzyme B (GrB). We, and others have shown in recent years, that characteristic of human IL-21-induced B regulatory B cells (Bneg^+). Recently, we and others found that human B cells can differentiate into +induction medium used for the GMP-compliant generation of GrB+ Bneg^+ cells rapidly (within 24 hours) in response to different TLR ligands including IL-3, IL-10 and IL-21. This phenotype involved the production of the serine protease granzyme B (GrB). As little as 0.05%-0.1% of autologous serum were sufficient to significantly enhance the viability of isolated pDC. Another factor as possible and that a standard leukapheresis product may allow the isolation of higher hematocrit in the leukapheresis product was associated with lower pDC recovery and viability. Functionally, isolated pDC showed the typical biphasic activation pattern as described earlier. During the first phase, pDC matured rapidly (within 24 hours) in response to different TLR ligands including IL-3, IL-10 and IL-21. This phenotype involved the production of the serine protease granzyme B (GrB). 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Abstracts

P09-12
Clinical grade manufacturing of genetically modified, CAR-expressing NK-92 cells for the treatment of ErbB2-positive malignancies

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Background: NK-92/5.28.z cell line (also referred to as HER2.taNK) represents a stable, lentiviral-transduced clone of ErbB2 (HER2) specific, 2nd generation CAR-expressing derivative of clinically applicable NK-92 cells. This study addresses manufacturing-related issues and aims to develop a GMP-compliant protocol for the generation of NK-92/5.28.z therapeutic doses. To avoid the delay caused by post-thaw recovery phase our concept encompasses individual patient dose expansion starting from pre-established master cell bank, following maintenance culture with a lifespan of 3 months and on-demand 5-day batch culture ending with the generation and release of IMP. Additionally, we investigated the factors impacting the efficacy and safety of clinical application like γ-irradiation, cytokine re-release and patient premedication.

Materials and Methods: Commercially available GMP-grade culture media and supplements (fresh frozen plasma, platelet lysate) were evaluated for their ability to support expansion of NK-92/5.28.z. Irradiation sensitivity and cytokine release was investigated.

Results: NK-92/5.28.z cells can be grown to clinically applicable cell doses of 5×10^8 cells/L in a 5-day batch culture without loss of viability and potency. Y-Vivo 10 containing recombinant transferrin supplemented with 5% FFP and 500 IU/mL IL-2 in VueLife 750-C1 bags showed the best results. Platelet lysate was less suited to support NK-92/5.28.z proliferation. Irradiation with 10 Gy completely abrogated NK-92/5.28.z proliferation and preserved viability and potency for at least 24 h. NK-92/5.28.z showed higher baseline cytokine release compared to NK-92, which was significantly increased upon encountering ErbB2+ targets (GZMB (2-fold), IFN-γ (4-fold), IL-8 (24-fold) and IL-10 (5-fold)). IL-6 was not released by NK cells, but was observed in some stimulated targets. Irradiation resulted in upregulation of IL-8 and downregulation of SFAI, while other cytokines were not impacted. 24-hour prednisolone treatment did not attenuate the cytotoxicity of NK-92/5.28.z cells.

Conclusion: Our concept suggests NK-92/5.28.z maintenance culture from which therapeutic doses up to 5×10^9 cells can be expanded in 10 L within 5 days. Established process is feasible to analyze NK-92/5.28.z in phase I/II trials.

P09-13
Effect of collection device on product quality in allogeneic and autologous PBSCCT

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Introduction: G-CSF-mobilized peripheral blood stem cells collected by apheresis have been used in hematopoietic transplantation as a common standard. Recently, a new cell separator with an automatic electronic interface management has been introduced refining apheresis technology. Data of allogeneic and autologous HPC products as assessed with the old and new device were analysed for their effect on HPC product quality.

Methods: 125 peripheral blood stem cell collections were performed in 116 individuals. In 44 related donors 46 stem cell harvest were done: 19 applying the manual COBE Spectra cell separator (V5.0; CS), 27 the automatic Spectra Optia (V11.0; SO). 79 apheresis products from 72 patients for autologous stem cell rescue after HDT (36 on CS vs. 43 on SO) were cryopreserved with 10% DMSO in a controlled rate freezer and stored at -140°C. The collections were analysed for differential count, RBCC, PLTs, vital NCs and CD34+ HPCs, in allogeneic donations also for lymphocyte subpopulations and in autologous harvests after cryopreservation for total NC, MNC, viability and CFU-GM. Statistics were calculated by non-parametric tests including binomial logistic regression.

Results: The HPCs collected with CS or SO were equivalent with respect of quantity and viability. This was also true for the doses of CFU-GM and viability measured in autologous products after cryopreservation. But the procedures with healthy donors on SO yielded a significantly better enrichment of MNCs in the apheresis product as compared with CS: monocytes harvested amounted to 2.4 ± 10^9 vs. 1.9 ± 10^9 (p = 0.022) and lymphocytes were 3.2 ± 10^9 vs. 2.5 ± 10^9 (p = 0.035). Lymphocyte subpopulations were similar. In addition, autologous and allogeneic collections with SO contained less contaminating RBCs and PLTs as with CS: RBCs amounted to 0.04 ml/ml vs. 0.05 ml/ml (allogeneic) and 0.03 ml/ml vs. 0.04 ml/ml (autologous), PLT were 43.3 ± 10^9 vs. 72.5 ± 10^9 (allogeneic) and 7.3 ± 10^9 vs. 17.7 ± 10^9 (autologous). All differences except of the RBCs in family donors were significant. Binomial regression analysis of autologous HPCs revealed that the type of cell separator had no significant effect on the yields of CD34+ HPC before or CFU-GM after cryopreservation. As for CD34+ HPC yields the diagnosis and RBCC in the product were best predictive, as were NC viability and concentration after cryopreservation for CFU-GM doses.

Conclusion: These findings demonstrate a higher enrichment of MNCs in the PBSC collections performed with the SO as compared to the CS which paralleled a significantly lower trapping of PLTs in the product. This appears to be advantageous for the safety of donors and patients. The quality of cryopreserved autologous HPC harvests with either device was equivalent. Binomial logistic regression of the autologous setting confirmed that the cell separator was not predictive for stem cell yields in contrast to diagnosis.

P09-14
Optimized timing of chemomobilization and G-CSF administration for a more efficient hematopoietic stem cell collection

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Introduction: Efficient collection of peripheral blood stem cells (PBSCs) depends on the optimal timing of mobilization chemotherapy, application of granulocyte-colony-stimulating factor (G-CSF), and assessment of CD34+ cell number in the peripheral blood (PB). Therefore, implementing a valid timetable depending on the applied chemomobilization regimen is essential.

Methods: For the 10 most commonly used chemomobilization regimens we developed a comprehensive time schedule for PBSC mobilization. A retrospective analysis of the mobilization process of 84 representative selected patients with a variety of diagnoses, including multiple myeloma, malignant lymphoma, and sarcoma was performed (6 to 10 patients per regimen). Clinical and PBSC collection parameters were analyzed including duration of G-CSF application, time point of CD34+ assessment, PB CD34+ cell count, number of leukapheresis (LP) sessions, processed blood volume, and CD34+ collection results.

Results: All patients successfully collected at least one transplant (i.e. >2×10^6 CD34+ cells/kg body weight) and reached their individual collection goal in adherence to the given schedule of chemotherapy, application of G-CSF, measurement of CD34+ cells in the PB and subsequent leu-
kapheresis. Our data underline the validity and efficiency of the applied timetable. In conclusion, we present a comprehensive time schedule which may serve as a blueprint for efficient chemomobilization and stem cell collection, thereby contributing to a more transparent and predictable mobilization process.

P09-15

Cytoskeletal structures and elasticity measures in human MSC cultured in adherence to plastic surfaces or in spheroid bodies

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Relevance: Stress fibers are the most prominent cytoskeletal structures that appear in MSCs when they are cultivated ex vivo in adherence to plastic surfaces or bound to extracellular matrix components. When MSCs are expanded for a potential cell therapeutic approach the occurrence of large bundles of actin filaments that show mechano-sensitive properties and can induce tension forces via focal adhesions to the surrounding peri-cellular matrix might influence their mechanical properties and has an effect on MSC elasticity

Methods: Stress fiber formation, intracellular filament- and mitochondri- al organization as well as focal adhesion formation were investigated in MSCs by immunofluorescence microscopy and by laser scanning micro- scope, cultivated in adherence to plastic surfaces or in hanging droplet cultures forming spheric MSC aggregates. In addition, surface morphology and sub-membrane structure scans were performed by atomic force microscopy in deflection and height mode and elasticity measures were analyzed using the Young's modulus.

Results: The occurrence of stress fibers increased during passaging of MSCs from P1 to P3, while stress fibers running in parallel according to the orientation of the MSCs were substituted during cultivation by robust, thick, crisscrossed pattern of actin cytoskeleton extending most of the length of the cell. MSCs grown in spheric cultures, in contrast, formed very discrete and thin f-actin filaments. The majority of stress fibers observed in adherent and spheric MSC cultures were ventral stress fibers anchored at each end by focal adhesions. Dense mitochondrial networks of P1 MSCs distributed throughout the cytoplasm were replaced by mitochondrial fragmentation in small and larger spheroids located around the nucleus during cultivation. Scanning by AFM confirmed these observations where intense submembrane f-actin filaments were observed in P3 MSCs which decreased elasticity of MSCs.

Conclusion: The occurrence of stress fibers in cultivated MSCs and the influence on elasticity of MSCs has to be taken into consideration when MSCs are expanded for a potential therapeutic use.

P09-16

HOXB4 acts as a hematopoietic fate determinant in pluripotent cell-derived endothelial cells

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Introduction: The embryonic origin of hematopoietic stem cells (HSCs) are rare hematogenically primed arterial endothelial cells. To be able to address the question if and how HOXB4 first acts in early endothelial progenitors we retrovirally expressed HOXB4 or a Tamoxifen-inducible HOXB4-ERT2 fusion protein in Runx1 knock-out ES-cells containing a doxycycline-inducible Runx1 coding sequence (iRunx cells) and differentiated them as embryoid bodies (EBs) for different periods of time, dissociated and sorted them based on expression of the surface markers Flk1 and/or PDGFRα with subsequent co-cultivation on OP9 stroma cells.

Results: Using an in vitro differentiation model of mouse embryonic stem cells (mESCs) we have recently shown that the transcription factor HOXB4 promotes the formation of HSC-forming hemogenic endothelial (HE-) cells. A detailed analysis revealed that HOXB4 did not increase the frequency of blast-colony forming cells (termed hemangioblasts), though. They are thought to be bipotent precursors of the structural and hemogenic endothelium. However, their existence as a defined entity in vivo is controversially discussed. Alternatively, HOXB4 may activate hematopoietic commitment in early endothelial cells. To test this notion, global transcriptome analysis was performed in Flk1+PDGFRα- ESC-derived endothelial cells. HOXB4 significantly upregulated the expression of a number of genes known to be essential for HE specification and hematopoiesis.

Conclusion: Our results strongly suggest that HOXB4 activates a hemogenetic endothelial program in early, Flk1+PDGFRα- endothelial cells by up-regulating a transcriptional program necessary for hematopoiesis. Thus, we conclude that this homeotic selector protein acts as a hematopoietic fate determinant during in vitro development of pluripotent stem cells. Because hematopoietic development is highly conserved throughout all vertebrates, we assume that our results can be directly translated to the development of human iPSC-derived hematopoietic stem- and progenitor cells.

P09-17

Intraoperative radiotherapy for breast cancer treatment efficiently prevents breast adipose tissue-derived mesenchymal stromal cells outgrowth

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Mesenchymal stromal cells (MSC) in the bone marrow have been shown to be radioresistant related to pronounced DNA repair mechanisms. Intraoperative radiotherapy (IORT) during breast conserving surgery is an innovative technique applying low energy x-ray to the tumor bed immediately after removal of the tumour. Aim of this study was to investigate whether IORT affects the outgrowth potential of breast adipose tissue-derived MSC (bASC). After surgical tumour resection, biopsies of the tumour bed before and after IORT with low energy x-rays were taken and processed applying well-established protocols for ASC isolation and characterisation. 100% of tumour bed samples pre-IORT yielded outgrowing bASC with typical ASC characteristics: fibroblastoid morphology, colony formation, proliferation, adipogenic and osteogenic differentiation and ASC surface marker expression. Interestingly, none of the post-IORT samples yielded outgrowth of bASC.

After breast conserving breast cancer surgery relapsing tumours emerge in 90% in close proximity to the initial tumour bed, potentially reflecting a significant contribution of the tumour bed to relapse. Our data show that IORT, besides the proven effect on breast cancer cells, also modifies the tumour environment by having an impact on bASC of the tumour bed. This might help to reduce the risk of tumour relapse.
The peculiarity of stem cell graft sterility release testing

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Background: Antibiotic treatment of autologous stem cell donors may result in false negative results of sterility testing using the recommended standard culture bottles for stem cell grafts.

Methods: We compared two spiked peripheral blood stem cell preparations (PBSC, one patient received ciprofloxacin prophylactically) with the other was treated with ticoplanin for a suspected infection of the central venous line (graft 2) with a fluffy coat (control matrix for PBSC preparations) using the blood culture device BactAlert®3D with the low temperature module (bioMérieux, Nürtinngen, Germany). Samples were spiked twofold > 10 colony forming units (CFU) in standard iAST/INST and in iFA/iFNplus culture bottles, which include resorbing polymers, for 14 days with various microbes with respect to European Pharmacopeia, General Chapter 2.6.1/2.6.27. All aerobic cultures were incubated at 35.0°C and for a direct comparison also at 35.0°C with all anaerobic cultures were incubated at 35.0°C as recommended by the Paul-Ehrlich-Institute.

Results: The BactAlert®3D-System detected all microbes in iAST and iNST culture bottles according to their growth behavior in the fluffy coat matrix. Detection of microbes differed significantly in PBSC products using standard iAST/INST culture bottles and iFA/iFNplus bottles with resorbing polymers: In graft 1 no growth was detected in spiked bottles with Staphylococcus aureus (iAST), Bacillus subtilis (iAST/INST), Clostridium sporogenes (iINST), Propionibacterium acnes (iINST) compared to iFAplus and iFNplus culture bottles wherein growth of spiked microbes was confirmed. Graft 2 showed similar results with no growth but with different microbes depending on the antibiotic treatment: Pseudomonas aeruginosa (iAST/INST), Bacillus subtilis (iAST/INST) and Clostridium sporogenes (iINST/INST). The comparison of incubation temperature showed an expected slower growth at 22.5°C with 1.87 days (d) compared to 1.29 d at 35°C in graft 1 and at 22.5°C with 1.83 d compared to 1.14 d at 35°C in graft 2 using iFAplus and iFNplus bottles.

Conclusions: Our study showed that spiked microbes grow in PBSC using iFA/imFNplus bottles in contrast to iAST/INST bottles depending on the antibiotic treatment of the patient. Only iFA/imFNplus bottles are in line with the results of the spiked fluffy coat, which is recommended as a control matrix by the PEI. Therefore, we recommend iFA/imFNplus bottles for detection of microbes in PBSC using the dual temperature module.

Bioreactor-based expansion of GMP grade adipose-derived mesenchymal stromal cells for clinical applications

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Introduction: Clinical trials showed the promising potency of adipose derived mesenchymal stromal cells (ASC) in the treatment of acute or chronic inflammation and degenerative diseases. High costs for production of clinical doses are still a major limitation for the use of ASC. Bioreactor based systems for production of ASC offer a powerful tool for cost reduction. In addition, monitoring of the production process to obtain a highly standardized product is facilitated.

Methods: For expansion in a customized, Biostat® Bplus (Sartorius) based bioreactor system, cells were grown on Cytoxent (GE Healthcare) microparticles in alphaMEM (Lonza), supplemented with 5% human serum (HS, IKT Ulm) in suspension. Cytoxent carriers are biodegradable polyelectrolyte microparticles. ASC were incubated with Cytoxent1 and pre-expanded in shaker flasks at densities of 1000 or 2500 cells/cm² micro carrier surface to allow cell adhesion under low agitation (≤40 rpm) for 18 hours in alphaMEM containing 0 or 5% HS. Thereafter, agitation rate was either increased or the suspension (ASC on Cytoxent1) was loaded to a Biostat® Bplus system. Cell adhesion in SF and growth in the Biostat® Bplus system was monitored by microscopy of samples taken after 2 to 118 hours. Normoxic (60% pO2) and hypoxic (20% pO2) growth conditions were tested. Parameters analysed included cell count (nuclei extrusion method), doubling time, LDH volume activity, glucose consumption, lactate and NH4+ generation (Konelab, Thermo Scientific) and migration capacity.

Results: The highest degree of ASC-adhesion to Cytoxent1 and lowest degree of aggregation in SF was observed after 18 hours incubation at 40 rpm at a density of 2500 ASC/cm². DT was between 36 and 58 hours. Almost no adhesion was observed when 1000 ASC/cm² were seeded. For the Biostat® Bplus system, shortest doubling time was observed under normoxic conditions, when 50x106 ASC/2000cm² in a volume of 1 liter were seeded (DT: 1:21 h, parallel culture in shaker flask: 33 h) as compared to hypoxic conditions (DT: 801 h, parallel culture in shaker flask: 51 h for ASC/2000cm² in 1 liter medium). Growth of spiked microbes differed significantly in PBSC products using standard iAST/INST culture bottles and iFA/iFNplus bottles with resorbing polymers: In graft 1 no growth was detected in spiked bottles with Staphylococcus aureus (iAST), Bacillus subtilis (iAST/INST), Clostridium sporogenes (iINST), Propionibacterium acnes (iINST) compared to iFAplus and iFNplus culture bottles wherein growth of spiked microbes was confirmed. Graft 2 showed similar results with no growth but with different microbes depending on the antibiotic treatment: Pseudomonas aeruginosa (iAST/INST), Bacillus subtilis (iAST/INST) and Clostridium sporogenes (iINST/INST). The comparison of incubation temperature showed an expected slower growth at 22.5°C with 1.87 days (d) compared to 1.29 d at 35°C in graft 1 and at 22.5°C with 1.83 d compared to 1.14 d at 35°C in graft 2 using iFAplus and iFNplus bottles.

Conclusions: Our study showed that spiked microbes grow in PBSC using iFA/imFNplus bottles in contrast to iAST/INST bottles depending on the antibiotic treatment of the patient. Only iFA/imFNplus bottles are in line with the results of the spiked fluffy coat, which is recommended as a control matrix by the PEI. Therefore, we recommend iFA/imFNplus bottles for detection of microbes in PBSC using the dual temperature module.
Conclusion: Our findings identify ADAR1 as a novel essential regulator of CD103+CD8+ DC subset development adding a new level of complexity in DC homeostasis.

P09-21

Experiencing differences in manufacturing of cell therapy products between Germany and the United States

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Introduction: Tech transfer of two different manufacturing processes, which were developed in the United States (US), was performed to implement these techniques and procedures in Germany. German GMP facility will serve European patients with chimeric antigen receptor (CAR) T-cell products.

Methods: We report our experience, we gained during two tech and analytical transfers for two cell therapy products from two different US companies.

Results: We observed several differences:

1. Starting material of the manufacturing process is an autologous leukapheresis product. Although several patient runs were already performed in the US no specification (e.g. flow cytometric characterization of cells) of the starting material was available. Furthermore, infectious disease marker (IDM) testing of patient peripheral blood at day of collection is not performed in the US.

2. Clean room: Drug products, used in clinical studies, are often manufactured in a laminar air flow bank (class A), which is kept in a clean room class C. So, the cascade according to good manufacturing practices (GMP) pharmaceutical inspection convention (PIC) guidelines is not kept.

3. Qualification of staff: In the US the process engineers are academics. But manufacturing staff is not high qualified. People are often semi skilled and trained on single procedures, but not familiar with the whole process. German technicians are much better qualified concerning problem solving.

4. Batch record: We were very surprised, when we had to translate an 80 pages batch record, in which only selection and cryopreservation procedures were described. Every step is done and verified by two people. That demands higher resources, one technician works as a transcript writer.

5. Shipment on dry ice: Cell therapy products are shipped on dry ice in the US, what is completely unusual in Europe.

Conclusion: Understanding of what is necessary for GMP conform manufacturing is different. A lot of communication is necessary for successful tech and analytical transfer, if US American and European centers are involved.

P09-22

Stem cell donor suitability in the era of molecular diagnostics

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Haematological malignancies are the most frequent indications for stem cell transplantations. With the availability of next generation sequencing a lot of mutations have been described in haematological diseases and even in (so far) healthy persons (CHP, clonal hematopoiesis of indeterminate potential). Donors with a predisposition for a haematological neoplasm should not donate because such disease entities might be transferrable by transplantation and stimulation of peripheral blood stem cells by growth factors like G-CSF might induce progression in such conditions. In this context the broad availability of genetic testing raise the question of a potential role of genetic testing in potential stem cell donors. The interest in genetic testing is also stimulated by the revision of the WHO-classification of myeloproliferative neoplasms, were the upper limit for haemoglobin levels were set to 16 g/dl and 16.5 g/dl for women and men.

Case reports: In 2017 a 63 year old woman was admitted to our collection centre for evaluation of stem cell donation suitability for her 55 year old sister with myelofibrosis with a JAK-2 V617F mutation. The medical history showed a loss of consciousness of unknown aetiology 4 years ago. The laboratory results were normal with the exception of elevated platelets of 507 G/l. During further haematological workup of thrombocytosis a mutation in JAK-2 V617F was diagnosed.

As reported previously a 35 year old male donor was scheduled for collection and cryopreservation of haematopietic stem cells for a 37 year old sister suffering of myelofibrosis with a JAK-2 V617F mutation. Laboratory tests of the donor were normal. The colony assays showed a spontaneous growth of BFU-E. Testing revealed a JAK-2 V617F mutation in the donor. Thus, since 2007 we observed two potential family donors who shared the same JAK-2 mutation than the respective sibling. Many genes have been described recently to be associated with different haematological neoplasms. The relevance of such mutations is not clear in many cases. Some of these mutations increase with age in healthy controls and in elderly patients about 1% per year of these carriers of age-related clonal haematopoiesis evolve to overt haematological malignancy. Currently it is not clear which implications such mutations would have in healthy donors. Laboratory tests would therefore be difficult to interpret. Genetic testing should therefore be limited to cases were a specific disease is suspected based on medical history and routine laboratory testing. It should be limited to genes with strong association with a particular neoplasm. Further guidelines on donor selection should address the rapidly evolving field of clonal hematopoieses of indeterminate potential and a consensus on the implication if such findings in a potential stem donor should be reached.

P09-23

Heparin affects gene expression of mesenchymal stromal cells in a source-dependent manner

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Introduction: Pooled human platelet lysate (pHPL) has been proven to be a valid alternative replacing animal sera in protocols for expansion of cell-based medicinal products. To prevent pHPL-media from clotting, addition of porcine heparin is required. We have recently developed a heparin-free protocol for the culture of human mesenchymal stromal cells (MSCs) by mechanical depletion of fibrinogen (J Transl Med. 2015; 13: 354). Even though porcine heparin is safely used in patients for decades by now, its impact on MSC biology remains elusive. We investigated whether heparin effects on gene expression of MSCs (from umbilical cord (UC), white adipose tissue (WAT) and bone marrow (BM)) are globally or tissue-specific.

Methods: UC-, WAT- and BM-MSCs (each n = 3) were cultured using three different alpha-MEM medium formulations: (1) 10% pHPL + heparin, (2) 10% fibrinogen-depleted pHPL and (3) 10% fibrinogen-depleted pHPL + heparin. Immunophenotyping and differentiation assays were performed for all MSCs and proliferation and colony forming units (CFU) assays were done over four passages. All MSCs were subjected to whole genome expression analysis (affymetrix Human Gene 2.1 ST array). Data
were analyzed using R/Bioconductor and Panther, KEGG, Biocarta and Reactome analysis tools and confirmative qRT-PCR was conducted.

**Results:** Independent of MSC origin and medium composition, flow cytometry revealed a characteristic MSC phenotype and a comparable in vitro osteogenic, adipogenic and chondrogenic differentiation potential. WAT-MSCs displayed unvaried cell proliferation and clonogenicity in different pHPL-media. Proliferative and clonogenic capacity of UC- and BM-derived MSCs however, was significantly altered in the absence of fibrinogen and enhanced by heparin in early passages. Whole genome expression profiling revealed distinct and source-dependent genes being differentially regulated by heparin. Functional enrichment analysis identified source-specific heparin-induced regulation of signaling cascades such as the Notch pathway in WAT-MSCs only. Depending on the source of MSCs, further signaling pathways were found to be regulated by heparin: integrin signaling, cadherin signaling, Notch-, Wnt-, TGFbeta-, p53-, EGF-, FGF- and PDGF-pathways. qRT-PCR of selected target genes confirmed our array results.

**Conclusion:** Here we demonstrate that the influence of heparin strictly depends on the source of MSCs: WAT-MSCs’ proliferation and clonogenicity are not altered by different pHPL-medium conditions, whereas BM- and UC-derived MSCs respond to the mitogenic stimulus of heparin. Immunophenotype and in vitro trilineage differentiation potential are shown to be independent of MSC origin and medium type used. Our gene expression data show that heparin regulates distinct sets of genes and signaling pathways depending on the source of MSCs. Whether the use of heparin-free media is a meaningful approach for clinical MSC propagation has to be shown in further studies.

**P09-24**

**Transfusion requirement after hematopoietic stem cell transplantation**

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**Targeted genome editing mediated by CRISPR-Cas nucleases holds great promise for translation into the clinic. We have established GMP-compliant protocols to edit the genome of hematopoietic stem and progenitor cells (HSPCs) with high efficiency. To this end, we compared (a) multiple delivery platforms, including RNA or ribonucleoprotein (RNP)-based delivery of the CRISPR-Cas system, (b) various modified gRNA structures (dual versus single), (c) several electroporation conditions, and (d) different clinical-grade media and cytokine cocktails to conserve long-term repopulating HSPCs (LT-HSC) in the CD34+ cell population. Remarkably, the remaining fraction of LT-HSC at the end of the manufacturing process was not only dependent on the cytokines but also on the GMP-grade medium used during culturing of the CD34+ cells. When applying optimized electroporation conditions, we observed up to 90% gene disruption at the endogenous CCR5 locus by combining chemically end-protected single gRNA with either Cas9 mRNA or RNP. These high gene editing frequencies were observed in all CD34+ cell subpopulations, although the frequency was slightly lower in LT-HSC. Unexpectedly, Cas9 was detected as long as six days post-nucleofection when employing RNPs, raising concerns of immunogenicity. To introduce targeted chromosomal deletions, we simultaneously employed two CRISPR-Cas nucleases that cleave at two adjacent sites within the CCR5 locus. Almost 50% of all CCR5 alleles revealed the expected deletion, and three out of four tested CRISPR-Cas9 nucleases demonstrated high specificity, as determined by targeted next-generation amplicon sequencing. In summary, we have developed a GMP-compatible manufacturing process to generate gene edited HSPCs with high efficiency.

**P09-25**

**Efficient GMP-compatible CRISPR-Cas mediated genome editing in hematopoietic stem and progenitor cells**

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**A highly efficient and GMP-compliant protocol to manufacture CCR5-edited cells to treat HIV infection**

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**Targeted genome editing in blood and immune cells enable new therapeutic applications, especially for infectious diseases. We present a GMP-compliant protocol to manufacture CCR5-edited CD34+ hematopoietic stem and precursor cells (HSPCs) with the goal to cure patients suffering from chronic infection with human immunodeficiency virus type 1 (HIV1). We hypothesize that genetic disruption of the CCR5 gene, which encodes the major HIV1 co-receptor, in HSPCs will give rise to an HIV-resistant immune system after transplantation. We have developed engineered nucleases based on transcription activator-like effector nucleases (TALENs) targeting CCR5. Electroporation of CD4+ T-cells and CD34+ HSPCs with mRNAs encoding TALENs revealed disruption of up to 80% of CCR5 alleles in CD4+ T-cells and over 90% of alleles in HSPCs. The high gene editing frequencies in T-cells and HSPCs were confirmed by deep sequencing, and no cleavage activity above background levels were detected at the top 20 predicted off-target sites. CCR5-edited CD4+ cells preserved their proliferation capacity and their biological function. Importantly, these cells showed significantly reduced CCR5 expression**
and became resistant to infection with the RS-tropic HIV-1<sub>RS</sub> virus. The CCR5-edited HSPCs maintained their proliferation potential and 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 cells. In summary, our developed protocol enables highly efficient and GMP-compliant knockout of the CCR5 locus in clinically relevant cells, so forming the foundation for a planned phase I/II clinical study.

**P09-27**

**Bone marrow aspirate concentrates enrich stem cell populations and trophic factors**

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**Background:** The number of Mesenchymal Stem/Stromal Cells (MSCs) in the human bone marrow (BM) is small compared to other cell types. BM aspirate concentration (BMAC) may be used to increase numbers of MSCs, but its efficacy, as well as the composition of MSC subpopulations and trophic factors after processing, is currently unknown. The purpose of this study is to assess the enrichment of stem/progenitor cells including MSC subpopulations in BM aspirate by two different commercial concentration devices versus standard BM aspiration from single donors.

**Methods:** 120 mL of BM was aspirated from the iliac crest of 9 human male donors. Each sample was processed simultaneously by either Emcyte Genesis<sup>®</sup> (Emcyte) or Harvest SmartPReP<sup>®</sup>2 BMAC (Harvest) devices and compared to untreated BM aspirate. Samples were analyzed with multicolor flow cytometry for cellular viability and expression of hematopoietic and MSC subpopulations markers. Stem cell content was verified by quantification of colony forming unit-fibroblasts (CFU-F). Platelet, red blood cell and mononuclear cell content was determined using an automated hematology analyzer. Trophic factors were analyzed with enzyme-linked immunosorbent assays.

**Results:** Cell viability after processing was over 90% in all groups. Compared to control, Harvest concentrated CD45-CD73+CD90+ (11.44 fold), CD45-CD10+ (3.68 fold), CD45-CD29+ (1.54 fold), and CD45-CD119+ (5.52 fold) cells, whereas Emcyte concentrated CD45-CD73+ cells (13.90 fold). Both devices mainly enriched the CD45-CD73+CD90+ (11.44 fold), CD45-CD73+CD10+ (3.68 fold), CD45-CD29+ (1.54 fold), and CD45-CD119+ (5.52 fold) cells, whereas Emcyte concentrated CD45-CD73+ cells (13.90 fold). Both devices mainly enriched the CD45-CD73+CD90+ (11.44 fold), CD45-CD73+CD10+ (3.68 fold), CD45-CD29+ (1.54 fold), and CD45-CD119+ (5.52 fold) cells, whereas Emcyte concentrated CD45-CD73+ cells (13.90 fold). Both devices mainly enriched the CD45-CD73+CD90+ (11.44 fold), CD45-CD73+CD10+ (3.68 fold), CD45-CD29+ (1.54 fold), and CD45-CD119+ (5.52 fold) cells, whereas Emcyte concentrated CD45-CD73+ cells (13.90 fold). Both devices mainly enriched the CD45-CD73+CD90+ (11.44 fold), CD45-CD73+CD10+ (3.68 fold), CD45-CD29+ (1.54 fold), and CD45-CD119+ (5.52 fold) cells.
Pre-coded sample tubes improve blood safety

Streis W.1; Adler M.2; Wersch K.; Grawunder M.; Stenzel Z.; Kriegelsteiner I.; Rüster B.; Pfeiffer H.-U.; Seifried E.; Schmidt M.3

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Background: Usual blood donor samples were labeled at the donation side before venipuncture. In case of unsuccessful venipuncture re-labeling of samples tubes might be necessary. In the blood donor service Baden-Württemberg – Hesse two donors were donating blood side by side separated by a table in the middle of two beds. Mix up failures between both donation occurred approximately 2–3 times per year and will be identified in the laboratory. The user has to refer to the control measurements of the manufacturers to detect a potential failure to avoid post-donation mix up failures.

Conclusion: The introduction of pre-coded sample tubes with alphanumeric bar-codes is feasible and able to improve blood safety in order to prevent any sample mix ups at the blood donation side. The use of pre-coded sample tubes reduce the electronic devices at the blood donation sides and optimize scanning of sample tubes in the laboratory because all barcodes were labeled exactly at the same place. Finally all samples tubes were linked to the blood donation number on the sample bags. In the laboratory all numbers (donor number, donation number and pre-coded sample tube numbers) were handled by the laboratory information system. After testing all test results were transferred into the blood donation programme (Inlog Edge blood).

Methods: For comparison measurements, the three non-contact infrared thermometers Geratherm Non Contact, Bosotherm diagnostic and Medisana Infrared thermometer FTN were used. Each of these devices can perform a measurement in the body or object mode. The thermometers were calibrated with a device qualification for uncritical and calibrated devices. This included a adapted Installation Qualification (IQ), an Operational Qualification (OQ) and a Performance Qualification (PQ). As PQ a comparative measurement with the aid of a temperature-controlled oil bath and the calibrated measuring system Almeno was carried out on the one hand and a comparative measurement with a calibrated ear thermometer and a test person on the other hand. Both measurements were performed in the body mode setting. The measurements in the oil bath were carried out at 37°C, 38°C, 39°C, 40°C and 41°C.

Results: The PQ showed that all measurements in the body mode were comparable with the temperatures of the oil bath with the measured values of the ear thermometer. The non-contact thermometers determined the acceptance limit +/- 1 K for all measuring points. For future usability tests a comprehensive calibration as in this study made by the German Red Cross Blood Donation Service North/East is not provided. The user has to refer to the control measurements of the manufacturers according to the German Medical Products Law.

Conclusion: The measurements in the body mode gave valid results, but an adequate calibration according to EU-GMP Guide is only possible with a dummy comparable to the human body. Because the Guide requires a praxis-based qualification of devices and a praxis-based validation of methods, the operator depends on the device approval by the manufacturer according to the German Medical Products Law.

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Validation of microbiological testing in cardiovascular tissue preservation

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Introduction: The aim of this study was to validate the microbiological testing of different samples (cell culture medium with and without antibiotics, tissue samples) in the preservation process of cardiovascular tissues according to the method suitability test defined by the European Pharmacopoeia.

Methods: 10–100 colony forming units of Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Bacillus subtilis, Aspergillus niger, Clostridium sporogenes, Propionibacterium acnes and Enterococcus faecium were inoculated in the different samples: cell culture medium with and without antibiotics, tissue samples with and without antibiotics; (MEM-199; Merck, Biochrom, Berlin, Germany); antibiotic content: Amikacin 12 µg/ml, Metronidazole 12 µg/ml, Flucytosin 29 µg/ml, Vancomycin 12 µg/ml, Ciprofloxacin 3 µg/ml.
The spiked samples were given to blood culture bottles, to Thioglycolate and Tryptic Soy Broth respectively. The samples were than incubated at 36 ± 1°C or room temperature until a positive reading, or for at least 14 days. The same vials were also inoculated directly with all tested microorganisms as growth control.

Results: All tested microorganisms were detected and showed a similar time to detection compared to the growth controls.

Conclusions: The microbiological testing in cardiovascular tissue preservation could be successfully validated and showed a sufficient sensitivity.

Physician made cell-based medicinal products: regulatory pitfalls due to ATMP-regulation in cartilage repair and in the use of the stromal vascular fraction

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Introduction: The project evaluates the legal handling of physician made cell-based medicinal products in point-of-care (PoC) settings for cartilage repair and for the use of (cells out of) the stromal vascular fraction. These two fields have been chosen since they represent an emerging and growing market in the field of trauma and reconstructive surgery. Beside numerous case series conducted as non-randomized controlled trials (non-RCT) with stem cell preparations of different origins and specifications actually only few RCTs processed allogeneic bone marrow derived mesenchymal stem cells for intraarticular cell injection for the treatment of osteoarthritis. Cell preparations of the stromal vascular fraction were also generated in bed-side models for the treatment of a multitude of diseases. Such individualized physician made PoC medicinal products are considered to have benefits, e.g. being rapid and/or immune-friendly. However, the regulatory framework for such products, mostly known as «Advanced Therapy Medicinal Products» (ATMP), or – if in combination with a medical device, such as scaffolds for 3D-structure – bears numerous legal pitfalls for physicians. Also, the current ATMP legislation leaves loopholes which seem to open doors for unsafe, non-efficient or unproven therapies.

Methods: First, the project evaluates the state of the art in physician made cell-based PoC therapy models for cartilage repair and for the use of fat tissue. Second, the project analyses the current regulatory handling of such therapy concepts in an interdisciplinary approach. Additionally, the project extrapolates the current technical development to examine technical advancements of such therapies.

Results: The regulatory handling of cell-based therapies in PoC settings is incomplete. Such physician made cell-based medicinal products do not need market approval since in regulatory terms they are not brought to the market. Only medicinal products need to be tested in clinical trials which are brought to the market. Depending on the processing of the biopsied cells and/or tissue only in some cases a manufacturing license is mandatory. This lack of regulation causes that certain physician made cell based medicinal products in PoC therapies do not have to be manufactured under Good Manufacturing Practice (GMP). Therefore, in certain settings such products are either unregulated or only partly regulated although these cells have the character of cell-based pharmaceuticals comparable to other regulated cell-based ATMP therapies.

Conclusion: The assurance of safety, efficient, and proven cell therapies in PoC settings needs a legal ATMP and/or medical device framework in all settings. The major obstacle is currently the separation of medical device law and medicinal product (ATMP) law. The project proposes changes in legislation where the current legislation seems to be insufficient to ensure safe and efficient treatments for physician made cell-based medicinal products.

Immortalization of erythroid precursor cells by c-myc and BCL-XL using antibiotic inducible lentiviral vector system

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Background: Blood pharming using embryonic, bone marrow or induced stem cells presents a new and fascinating option to warrant the blood supply in the future. However, little is known about the mechanisms and factors that would allow the most efficient in vitro generation of enucleated blood cells. Since the differentiation from stem cells and/or IPS cells into the erythroid lineage would involve considerable amount of cytokines and purification steps, we aimed to immortalized cells already determined to the erythroid lineage.

Materials and Methods: Using an antibiotic inducible lentiviral vector system we transduced erythroid precursor cells derived from CD34⁺ stem cells from mobilized peripheral blood (blood group O RhD) with five different oncogenes (c-myc, BCL-XL, SV40 LargeT, Bmi-1, Lhx2) and combinations of each two. 48h after transduction gene expression was induced by the addition of doxycycline. In the absence of doxycycline oncogene expression is turned-off. Using single cell printing technology single cell colonies were obtained, expanded and analysed for CD235a expression and morphology.

Results: The transduction of each oncogene alone does not lead to immortalization of the cells. Only combined transduction of c-myc and BCL-XL leads to immortalization of erythroid precursor cells and continuously proliferation of them for more than 2 years. Furthermore we obtained more than 270 single cell colonies from these cells with different morphology and expression pattern for different surface marker (CD71, CD45, CD34, CD235a). After removal of doxycycline a part of these cells were differentiated into normoblasts and reticulocytes within 7 days with an enucleation rate of 0.5–1%.

Conclusions: Immortalization of erythroid precursor cells is feasible using stable inducible lentiviral gene transfer for c-myc and BCL-XL. Withdrawal of dox allows differentiation in to further differentiated red blood cells. The conditions to obtain high yields of enucleated red blood cells, which then would also not harbour the risk attributed to the proto-oncogenes, has to be further optimized.
P11-2

Analysis of the functional impact of mutant RUNX1 on the expansion of hematopoietic stem and progenitor cells

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Introduction: The transcription factor RUNX1 is essential for definitive hematopoiesis and regulates critical processes in adult hematopoiesis. C-terminally truncated mutants of RUNX1 encode proteins that lack transactivation activity while strongly binding DNA. Their dominant-negative effect on wildtype RUNX1 induces hematopoietic stem (HSC) expansion. However, the functional consequences, notably the downstream pathway and the key target genes remain unclear.

Methods: Hematopoietic stem and progenitor cells (HSPCs) were transduced with lentiviral vectors conferring the expression of mutant RUNX1. Transduced HSPCs were cultured for in vitro studies or analyzed in vivo six months after transplantation into lethally irradiated mice. The regulation of mutant RUNX1-expression was employed under the doxycycline-inducible gene expression system Tet-ON. Gene expression analysis was performed in sorted lineage-negative, Kit+ cells (LK) and lineage-negative, Kit+, Sca1+ cells (LSK) before and after mutant RUNX1-expression was turned off. Mutant RUNX1-binding sites were identified by chromatin immunoprecipitation followed by sequencing (ChIP-Seq).

Results: Mouse HSPCs were immortalized in vitro by the expression of the dominant-negative, C-terminally truncated isoform RUNX1a. Survival of these cells continuously depended on RUNX1a activity. Immunophenotypical analysis revealed that the majority of these cells (86%) were lineage-negative cells that contained 46% of LSK cells. Importantly, removal of doxycycline changed this phenotype and led to differentiation and cell death. Of note, in this setting LSK cells rapidly lost Sca1+ positivity and became LK cells. We investigated differential gene expression in LK and LSK cells of mutant RUNX1-expressing immortalized cells compared to those where RUNX1a expression was turned off. To define primary target genes of RUNX1a, we identified 7262 RUNX1a genomic DNA binding sites in vitro in the immortalized murine HSPCs. Furthermore, RUNX1a-expressing cells showed a strong competitive advantage in an in vivo reconstitution setting that was associated with a differentiation block.

Conclusion: Expression of RUNX1a led to an expansion of mouse HSPCs in vitro and in vivo, confirming the presence of an immature subpopulation with self-renewal activity. Combining the data set mapped of genomic binding sites with the gene expression profiles, we aim to identify genes affected by mutant RUNX1. Our model will contribute to define molecular targets of mutant RUNX1 and to understand their function. Thus, ultimately, molecular targets that promote HSC expansion for potential therapeutic applications will be identified.

P11-3

Change in growth factor content of human serum for use as eye drops during frozen storage for 1 year

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Introduction: Growth factors are thought to be among the active components in serum used for treatment of dry-eye syndrome. Stability of growth factors during frozen storage in mini containers (140 µL) is unknown. If these products can be stored at 18°C, it will be feasible to store this product in 3-star household freezers, making the product available for patients in need of serum eye drops. The aim of this study was to demonstrate stability of growth factor content in human serum during long-term storage at 18°C or < 25°C. During storage at 3 months intervals, samples were tested for several growth factors, using Magpix LumineX Multiplex assays and compared to control samples stored at < 80°C. Growth factors tested were PDGF-AA&AB/BB, TGF-ß1/2/3, VEGF, EGF, FGF2. The study was a fact-finding study, without pre set acceptance criteria.

Results: PDGF-AB/BB and TGF-ß1 were the most abundant growth factors, on average 35, resp. 40 ng/mL. Also, PDGF-AA was detected at relatively high concentration in human serum, on average 11 ng/mL. TGF-ß2, EGF and VEGF were detected at relatively low values, resp. 3 ng/mL, 0,5 ng/mL and 0,3 ng/mL. Average levels of FGF2 and TGF-ß3 were close to detection limit (< 0.2 ng/mL). The controls stored at < -80°C showed for all growth factors close to 100% of the initial values in samples at T=0 (moment of filling mini containers). For serum stored at < 25°C for up to 12 months, most factors showed less than 2% decrease, except for PDGF-AA and TGF-ß2, showing 6% resp. 3% lower values. For serum stored at 18°C, the values for TGF-ß1, EGF and VEGF were stable, whereas PDGF-AB/BB, PDGF-AA and TGF-ß2 showed a decrease of 9, 17 and 3%, respectively.

Conclusion: Human serum eye drops can be stored in the new micro dose device at 18°C (3-star household freezers) or < 25°C (professional freezers) for at least one year after preparation without large decreases in growth factor content. The maximum decrease was found for PDGF-AA in serum stored at 18°C. It is yet unknown if the tested components add to the in vivo effectiveness of serum eye drops and what the minimal concentration is to ensure in vivo effectiveness. Further stability testing in combination with in vitro and in vivo application is required to extend the shelf-life beyond 1 year.

P11-4

Autologous serum eye drops: five years’ experience of manufacturing

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Introduction: The therapy with autologous serum eye drops (ASED) containing epitheliothrophic factors (growth factor, fibropectin, and vitamins) may lead by patients suffering especially from ocular surface disorders (dry eye, persistent epithelial defects, ocular GvHD) to a rapid and essential improvement of clinical symptoms. Here the experiences of a 5 year period are presented.

Methods: Since 2012 ASEDs are manufactured in our institute in a closed system (Meise, Schalksmühle, Germany) with a manufacturing license of the local authority. The number of patients, the respective disease / indication, the amount of donations per patient, side effects of donation, problems in logistics, the clinical benefit, and potential side effects were evaluated.

Results: Between 2012 and 2016 in total 284 patients referred by the treating ophthalmologist were accepted for autologous blood donation; 602 products were manufactured. In 2016 83 patients donated blood for the manufacturing of ASED (3 pt donated 4x, 20 pt 3x, 37 2x and 23 one time). Quality control was performed on each donation with no bacterial contamination detected. According to the German pharmaceutical law ASEDs have to be delivered via a pharmacy. Therefore some products were lost because of logistic problems. Moreover a loss of ASEDs occurred in very few cases due to leakage of the plastic bags during centrifugation or due to thawing (breaking of the cold chain due to prolonged shipment on dry ice or dysfunction of freezers in private pharmacies). Most of the patients reported a substantial relief of the symptoms. No serious side effects were observed or reported neither for donation nor for application. In 2 patients a decreased therapeutic effect was discussed in one subsequent donation.

Abstracts

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Conclusion: In long term evaluation autologous serum eye drops are a save therapeutic option for severe cases of dry eye, which cannot be treat- ed by other therapies. Due to benefits of ASEDE application, an increasing demand of this therapy is expected. Unfortunately the delivery process is still uncomfortable and a change of German regulations allowing a direct delivery to the patients is urgently necessary.

P11-5
Knockout of adenylate kinase 2 in mice leads to early embryonic lethality and to profound anemia in a conditional setting

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Introduction: Defects in the human adenylate kinase 2 (AK2) cause reticu- lar dysgenesis (RD), the most severe form of inborn severe combined immunodeficiencies (SCID). RD is characterized by almost complete leu- kopenia and bilateral sensorineural deafness. The aim of this study was the development and characterisation of an Ak2 knockout mouse model to provide a model system for further studies on the pathophysiology of reticular dysgenesis.

Methods: The effects of a constitutive and a conditional haematopoie- sis-specific AK2 deficiency (knockout of exon 3 and 4) have been analysed in mice by FACS analyses, digital PCR, colony formation assays, histo- pathological and immunohistochemical analyses. Sorted murine hematopoietic cells were analysed by Western Blot.

Results: In contrast to humans, constitutive AK2 deficient mice are non- viable and die before embryonic day 8.5 most likely due to a non-haematopoietic cause. Embryonic stem cells from blastocysts with a homozygous Ak2 knockout are viable and were obtained in normal Mendelian ratios. The consequences of AK2 deficiency on hematopoiesis/lymphopoiesis were investigated using conditional Ak2 knockout mice (Vav-iCre mice) which survive only until embryonic day 18.5. Fetal mice present with growth retardation, they show delayed organ development and die due to a lack of mature erythrocytes caused by an early block in the development of erythroid progenitors. In addition, the B-cell development was impaired and at embryonic day 18.5 the thymus hardly contained any T cells. Protein analyses of sorted hematopoietic cells revealed differences in the expression of the adenylate kinases 1 and 2 between mice and humans especially in erythrocytes. Murine erythrocytes have only AK2 protein in contrast to human erythrocytes which contain both adenylate kinases.

Conclusion: The consequences of an adenylate kinase 2 knockout in mice differ from the knockout in humans in that mice die before embryonic day 8.5 due to a non- hematopoietic cause.

P11-6
A modified Mixed Lymphocyte Reaction as a functional assay for extracellular vesicles

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Introduction: Extracellular vesicles (EVs), such as exosomes and microvesicles, are shed by all cell types and found in all body fluids. EVs transmit specific information from their cells of origin to specific target cells and are key factors in a novel form of intercellular communication. Depending on their origin, EVs can modulate immune responses and act either pro-inflammatory (e.g. mature DC-EVs) or anti-inflammatory (e.g. mesenchymal stem cell- and many tumor cell-derived EVs). Aiming to analyze the immune-modulating properties of EVs from different sources in vitro, we established a novel form of a standardized mixed lymphocyte reaction (MLR) assay.

Methods: Peripheral blood-derived mononuclear cells were pooled from 10–12 healthy donors warranting high cross-reactivity even being main- tained after introducing an optimized freezing and thawing cycle. EVs are administered to the MLR followed by a 5 days incubation time. By ana- lyzing the expression of defined lineage and activation markers via flow cytometry, the immunomodulatory effects of EV samples on different immu- ncell types can now specifically be studied.

Results: The administration of EVs changed the expression level of activation and defined lineage marker on immune cells in the MLR compared to the untreated control. The amount of CD14+ (monocytes) and CD56+ (natural killer cells; NK) cells was increased after incubation with MSC- and platelets- (PL) derived EVs, whereas CD4+ and CD8+ T cells showed a slight decrease. MSC-EVs lead to higher expression levels of CD19 (B cells) compared to PL-EVs and the untreated control. All activation markers (CD54, CD97, CD25 and CD71) were lower expressed on T cells as in the control without EVs. In addition the expression of activa- tion marker was increased for T cells or not different to the control, when treated with PL-EVs. Different patterns for the expression of CD97 and CD25 on NK, B cells and CD16+ cells was observed, where higher levels of these activation markers were seen.

Conclusions: It could be observed that EVs of different origins have distinct effects on the immune cell types in the MLR. The lower activation of T cells and the higher expression of CD97 and CD25 on NK and B cells could lead to the assumption that MSC-EVs are influencing the immune response to a more tolerant state. To give a clear statement, how MSC-EVs are modulating the immune response and do identify their function, we are currently investigating the lineage subsets in more detail using flow cytometry.

P11-7
Mesenchymal stem/stromal cells release different subtypes of extracellular vesicles

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Introduction: Due to their reported regenerative and immunomodu- latory properties, mesenchymal stem/stromal cells (MSCs) represent a promising therapeutic agent for multiple diseases. Initially, the MSCs were thought to act in a cellular manner. However, recent investigations suggest that they rather act in a paracrine manner. Moreover, within the MSC conditioned medium (CM), the small extracellular vesicles (sEVs), such as exosomes and microvesicles, seem to be the key players for such therapeutic effects. On the other hand, the results from previous MSC studies are highly diverse and sometimes even controversial, suggesting that MSCs are actually a heterogeneous population, with only a sub-frac- tion providing therapeutic activity. Previous experiments performed by our group revealed significant molecular and immunomodulatory differences between the sEV fractions isolated from 20 different MSC samples. Consequently, it is of interest to identify sEV markers whose presence correlates with their functionality. However, none of the available methods allowed yet a detailed study of the molecular content of sEVs at a single vesicle level.

Methods: We established a new antibody-based approach for the analysis of single sEVs by imaging flow cytometry using the ImageStream® X Mark II imaging flow cytometer.

Results: This technique allowed, through the detection of CD81 and CD9 surface markers, the discrimination of MSC-sEVs and human platelet lysate (used as a culture supplement)-sEVs within the MSC-CM. Further- more, with a three parameter antibody staining analysis, this method also allowed the identification of further MSC-sEV subpopulations.
Conclusion: This technique rises as a powerful tool for the characterization of sEVs at a single vesicle level, including identification of novel sEV markers and subpopulations. Moreover, functional studies with the detected sEV subfractions will help to unravel their potency and to better understand the MSCs mode of action.

Allogeneic eye drops for therapy of the dry eye

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Background: Various disorders are combined with the symptoms of dry eyes. A common entity for dry eyes is the chronic graft vs host disease (cGvHD) after allogeneic stem cell TX. However, serum eye drops (SEDs) are usually prescribed when conventional treatments fail. Commonly they are derived from autologous whole blood which implicates a good venous status of the patients, a good health condition and no prevalence of any viral infection as HCV, HBV or HIV. While most of the commercial types of eye drops are free of any healing potential serum derived drops contain a cocktail of tissue repairing cytokines.

Material: In the year 2010 we started with the production of autologous serum eye drops in an open system. In 2013 we switched to a closed system which covers all production steps. Whole is resting overnight at 4°C to coagulation, after centrifugation the serum is collected and dispensed without dilution into the connected applicators. Quarantine storage till delivery is done < minus 18°C till sterility is proven. The donors were recruited from our routine donor pool and fulfilled the requirements for blood donation. All donors were tested for infectious diseases by antibody- and NAT screening. CMV IgM positive individuals were rejected from donation.

Patients: In the last 2 years 18 patients (8males, 10females) received allogeneic serum eye drops (median age 53yrs, range14–90). The leading diagnose was cGvHD and Sjögren Syndrome, followed by therapy resistant corneal ulcers. ABO blood group identical donors were selected for therapy. Cytoablation, the matching of blood type and ABO Isotherapy were not a problem. While transplants for corneal epithelia even seemed to be closed. No adverse reactions were reported; blood types were not matching (father A, child 0). As a consequence of this success the amount of SED’s could be reduced from 5 to 4 drops daily for each eye and follow-up examinations will be performed quarterly.

Conclusion: The treatment with allogeneic SEDs seems to be an effective, if not even equal, alternative to the autologous variety if donation is impossible. As already shown in the literature, an «individual healing attempt» with allogeneic SEDs with AB serum of male donors without blood borne diseases, that never received blood products and not taking any kind of medication, seems to be a promising option.

Serum eye drops: first experience of manufacturing and treatment in allogeneic setting

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Introduction: The therapy with autologous serum eye drops (ASED) is a highly effective treatment for patients suffering from ocular surface disorders. Nevertheless in some cases the therapy with ASEDs cannot be applied due to patient’s lack of suitability for blood donation including underlying disease, poor venous access, low haemoglobin, older or younger age. For these reasons, the use of allogeneic serum eye drops (SED) might be an option. This case report presents our first experience in the production and use of allogeneic SEDs.

Methods: Allogeneic SEDs were manufactured in a closed system (Meise, Schalksmühle, Germany) according to the process for ASEDs. A consent of the local authority for an «individual healing attempt» was necessary in advance because this procedure, in contrast to other countries (Norway, Denmark, Australia, New Zealand), is not performed regularly in Germany yet.

Results: A deep anterior lamellar keratoplasty and an amniotic membrane transplantation had been executed on a 2 year old child suffering from neurotrophic keratopathy in both eyes with major lesions in the left eye. However the healing process remained difficult. For that reason the indication for a treatment with serum eye drops was given. Because a donation of 500ml blood was impossible for a 2 year old child it was decided to make an allogeneic attempt. Finally the child’s father was selected and after consent of the local authority, donation and manufacturing was realized. One month later the first follow-up examination was carried out: a distinct shrinking of lesions in both eyes was observed. On the right eye the corneal epithelia even seemed to be closed. No adverse reactions were reported; blood types were not matching (father A, child 0). As a consequence of this success the amount of SED’s could be reduced from 5 to 4 drops daily for each eye and follow-up examinations will be performed quarterly.

Conclusion: The treatment with allogeneic SEDs seems to be an effective, if not even equal, alternative to the autologous variety if donation is impossible. As already shown in the literature, an «individual healing attempt» with allogeneic SEDs with AB serum of male donors without blood borne diseases, that never received blood products and not taking any kind of medication, seems to be a promising option.
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