Transfusion Medicine and Hemotherapy

Abstracts

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V1 Immunohematology, Maternofetal Incompatibility, Autoimmune Reactions against Blood Cells

V 1.1

D Category IV Comprises Phylogenetically Heterogeneous Alleles of Clinical Importance

von Zabern I.[1], Wagner F.F.[2], Hanna D.[3], Moulds J.M.[3], Flegel W.A.[4]

[1]Institut für Klinische Transfusionsmedizin und Immungenetik Ulm, Immunhämatologie, Ulm, D; [2]DRK Blutspendedienst NSTOB, Institut Springe, D; [3]LifeShare Blood Centers, Shreveport, Shreveport, LA, USA; [4]Dept Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, USA

Purpose: Carriers of partial D may produce anti-D following exposure to D positive blood. Current D typing strategies applied in several European countries protect recipients carrying the partial D DVI from immunization by normal D. However, carriers of other partial D can still get immunized, which is particularly relevant in pregnancies. While most of the clinically important partial D were comprehensively characterized, a full record was missing for DIV. We describe the molecular basis and clinical importance of DIV variants.
Methods: RHD nucleotide sequences were determined from genomic DNA. D epitope patterns were established with commercial monoclonal anti-D panels.
Results: Cases of D positive patients with anti-D were collated in an internet-based survey. 123 patients with anti-D were reported to our institution between 1998 and 2008, 73 of whom carried partial D. Allo-anti-D was observed in 12 cases with DVI, 12 with DIV, 11 with R0Har, 9 with DNB, 6 with DVII, 5 with DIII, 4 with weak D type 4.2 (DAR) and 2 with DV. Among 12 carriers of DIV with anti-D were 10 females, 7 of whom were likely immunized by pregnancy. DIV and its 2 subgroups DIVa and DIVb differ not only by serology, but also by molecular structures, evolutionary origins and ethnic prevalences. The DIVa phenotype is expressed by the allele DIV type 1.0 which in addition to 350H harbors the 3 dispersed amino acids 62F, 137V and 152T. The DIVb phenotype is expressed by the alleles DIV type 2 to type 5 which represent RHD-CE-D hybrids. 350H is common to all DIV types. We detected new D variants which provided information on the properties of DIV. DWN differs from DIV type 4 by 350D and lacks the epitope pattern characteristic of DIV. DNT carries the primordial substitution N152T of the DIVa cluster which causes a high D antigen density on RBC.
Conclusions: D category IV comprises alleles of different evolutionary origin. While DIV type 1 with DIVa phenotype belongs to the oldest extant human RHD alleles, DIV type 2 to type 5 with DIVb phenotype arose from more recent gene conversions. 350H is the only variant amino acid residue shared by all DIV and determines the DIV phenotype. The current serological D typing strategy for recipients, using two monoclonal antibodies not recognizing DVI, could be modified. A set of 2 monoclonal anti-D could be used with one not recognizing DVI, and another not recognizing further clinically important D variants, including DIV. Discrepant results should prompt transfusion of D negative RBC units and anti-D prophylaxis in pregnancies. Panels of monoclonal anti-D and genotyping methods are available to identify the recognized aberrant D.

V 1.2

Molecular Basis of Samples Negative with Discrepant LU or CO Results in Molecular and Serologic Testing

Wagner F.[1], Döscher A.[2], Bittner R.[1]

[1]DRK Blutspendedienst NSTOB, Institut Springe, D,
[2]DRK Blutspendedienst NSTOB, Oldenburg, D

Purpose: Molecular prediction of antigens may fail in carriers of rare alleles. To identify such alleles, we checked donors predicted to be Co(a+b+) or Lu(a+b+) for their true serologic phenotype. Here we report on the results of the molecular analysis of discrepant samples.
Methods: Samples predicted by pooled capillary electrophoresis to be Co(a+b+) and Lu(a+b+) but found to be Co(a-b+), Co(a+b-), Lu(a-b+) or Lu(a+b-) were analyzed by sequencing of the exons of the CO and LU genes from genomic DNA, respectively. Adsorption/elution was used to check for faint antigen expression.
Results: Of three Lu(a+b-) samples, two showed previously unknown missense mutations, one an in frame two amino acid deletion (table 1). In one of two Co(a-b+) and one of two Co(a+b-) samples, a missense mutation was identified. Of three Lu(b-), one Co(a-) and two Co(b-) tested by adsorption/elution, all but one Lu(b-) sample were positive.
Conclusion: The molecular background of Lu(b-), Co(a-), and Co(b-) samples missed by single SNP testing is diverse. Most of these seemingly antigen negative samples are positive on adsorption/elution. Missing such samples does not relevantly reduce the efficacy of molecular donor screening.

Sample	Phenotype	Adsorption/ elution	Assumed causative variation	Additional variations
A	Lu(a+b–)	Positive	6 bp deletion 98 to 103 (34_35 del RL)	230 G/A (R77H, Lu^a/Lu^b), 586 G/A (V196I, Lu^a-associated) 1615 T/A (T539A, Au^a/Au^b)
B	Lu(a+b–)	Positive	997 G/A (G333R)	230 G/A (R77H, Lu^a/Lu^b), 586 G/A (V196I, Lu^a-associated) 1671 C/T (silent) 1742 A/T (Q581L, Lu13-associated)
C	Lu(a+b–)	Negative	529 G/A (G177E)	230 G/A (R77H, Lu^a/Lu^b), 586 G/A (V196I, Lu^a-associated) 1615 T/A (T539A, Au^a/Au^b)
D	Co(a-b+)	Positive	None identified	134 C/T (A45V, Co^a/Co^b)
E	Co(a-b+)	Pending	494 G/A (G165D)	134 C/T (A45V, Co^a/Co^b)
F	Co(a+b–)	Positive	502 G/A (A168T)	134 C/T (A45V, Co^a/Co^b)
G	Co(a+b–)	Positive	None identified	134 C/T (A45V, Co^a/Co^b)

V 1.3

Molecular Blood Group Typing Improves Transfusion Support

Wagner F.[1], Döscher A.[2], Bittner R.[1], Müller T.[1]

[1]DRK Blutspendedienst NSTOB, Institut Springe, D,
[2]DRK Blutspendedienst NSTOB, Oldenburg, D

Purpose: Recently, high throughput methods have been developed to predict minor blood group antigens. Information on the impact of systematic donor typing for minor transfusion relevant antigens on routine transfusion care is still emerging. Here we report on our experiences with a typed database of more than 25,000 donors.
Methods: An IT solution was introduced guiding typed units with useful typing results (molecular or serologic) to a specific laboratory inventory and to locate typed units in the blood service. As baseline, typed units delivered in a 6 month period in 2008 by one of our laboratories were compiled from the laboratory documentation. Units guided to the laboratory inventory, blood service stock and laboratory stock were determined.
Results: In the baseline period of 6 month, 480 Jk(a-), 472 M-, 447 Fy(a-), 137 S-, 95 Jk(b-), 44 N-, 21 Fy(b-) and 2 s- units were released. Currently, typing information of about 199 donations is available per day. In 2 month, 751 units were guided to this laboratory inventory, including 434 M neg, 399 S neg, 377 Fy(a-) and 348 Jk(a-) units. Although not used as guidance criterium, 110 Jk(b-), 96 N neg, 67 Fy(b-) and 34 s neg units were scheduled, too. At May 3rd, the laboratory inventory consisted of 250 units, of which the following negative phenotypes were known: 44 Fy(a-), 30 Jk(a-), 63 M-, 55

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S-, 19 Fy(b-), 26 Jk(b-), 16 N-, 6 s-, 2 Lu(b-), 1 Co(a-), 1 Kp(b-). Multiple negative units included 23 Fy(a-)/ Jk(a-) and 10 Jk(a-)/M-/S-. In the whole blood service, there were 4425 units, including 156 Fy(a-), 155 Jk(a-), 157 M- and 255 S- units. Multiple antigen negative units were 55 Fy(a-) / Jk(a-); 15 Jk(a-) / M- / S- and 11 Fy(b-) / Jk(b-) / S-. Rare units in the inventory included 1 Kpb-, 2 Lu(b-), 4 Co(a-) units.
Conclusion: Routine typing need may be satisfied using selective guidance rules focusing on Fy(a-)/ Jk(a-) and M neg units, selected for specific Rh phenotypes. Units negative for other antigens are available in sufficient number due to the full typing of units devised for any antigen. Transfusion support is improved by the rapid availability of screened donations, including those negative for antigens of high frequency like Co(a) and Lu(b).

V 1.4

Easy Identification of Antibodies to High-Prevalence Scianna Antigens and Detection of Admixed Allo-Antibodies Using Soluble Recombinant Scianna Protein

Seltsam A.[1], Grueger D.[2], Blasczyk R.[2], Flegel W.A.[3]

[1]German Red Cross Blood Service NSTOB, Institute Springe, D,
[2]Institute for Transfusion Medicine, Hannover Medical School, Hannover, D,
[3]German Red Cross Blood Donor Service Baden-Württemberg-Hessen, Institute Ulm, D

Purpose: Identification of antibodies against high-prevalence Scianna (Sc; ERMAP) antigens, like Sc1 and Sc5, is difficult and may incur delays in blood procurement and costs. The detection of additional clinically significant allo-antibodies is hampered in the presence of anti-Scianna antibodies. Soluble recombinant Scianna protein is demonstrated to facilitate antibody diagnostics in both cases.
Methods: Soluble recombinant Scianna protein (Sc:1,-2,3,-4,5,6,7) was produced comprising the antigenic extracellular domain fused to a V5-His tag. The protein was isolated from eukaryotic cell culture supernatants of stably transfected HEK293 cells. Seven serum samples with anti-Sc1, anti-Sc2 and anti-Sc5 antibodies and 30 serum samples with antibodies to other blood group antigens were evaluated in hemagglutination inhibition assays. Antisera with mixed antibody specificities and auto-antibodies were also tested.
Results: Soluble Scianna protein inhibited specifically antibodies to the high-prevalence Scianna antigens Sc1 and Sc5. No antibodies were neutralized that were directed to the low-prevalence Sc2 antigen or to a large representative set of antigens from other blood group systems. Clinically relevant antibodies could be identified despite being masked by anti-Sc1 and anti-Sc5 antibodies. A mixture of Scianna and JMH proteins allowed detecting a common antibody despite the presence of antibodies to high-prevalence antigens of the Scianna or JMH blood group systems.
Conclusions: Antibody detection systems comprising soluble recombinant Scianna protein provide an easy single-step method for detection and identification of antibodies to high-prevalence Scianna antigens. Reagents with Scianna and other recombinant blood group proteins and mixtures of such proteins would be useful routine reagents in immunohematology.

V 1.5

New Differential Diagnosis of a Positive Direct Antiglobulin Test: Shift to Extravascular Hemolysis Due to Efficient Eculizumab Therapy in Paroxysmal Nocturnal Hemoglobinuria (PNH)

Höchsmann B., Flegel W.A., Schrezenmeier H.

IKT Institut für klinische Transfusionsmedizin und Immunogenetik Ulm, D,
DRK Blutspendedienst Baden-Württemberg/Hessen, Institut Ulm, D

Purpose: Eculizumab is a monoclonal C5 antibody preventing terminal complement activation with a highly significant reduction of intravascular hemolysis in PNH. We observed a shift from intravascular to extravascular hemolysis in PNH- pts with complete blockade of the terminal complement cascade during eculicumab therapy and evaluated low dose steroid therapy as additional treatment option.
Methods: In 17 pts receiving eculizumab GPI-AP flow cytometry, LDH, total/direct bilirubin, AST, Hb, ferritin and a direct antiglobulin test (DAT) was performed before and during eculizumab therapy. DAT was also performed in 28 PNH pts without eculizumab. In 2 patients with clinical significant extravascular hemolysis low dose steroid (5 mg Prednison/d) was started and therapy effects were monitored.
Results: Most important results in the table below. Only 3/39 pts without eculizumab showed a positive DAT with polyspecific antiglobulin serum, two of them with monospecific anti-IgG and one of them with monospecific anti-C3d. 4 PNH pts had anti-erythrocytic allo-antibodies (anti-D, -C, -E, -Lua, and -Kpa) and two of them had additional warm auto-antibodies. In contrast, all but one of the 13 pts during eculizumab therapy reacted positive with polyspecific antiglobulin serum and monospecific anti-C3d. The only pt with a negative DAT during eculizumab was treated for PNH-related thrombo-embolic events without having transfusion dependent hemolytic anemia. DAT-positivity due to eculizumab seems to be reversible as DAT gets negative again after therapy stopp. The two patients with clinical significant extravascular hemolysis showed a reduction of RBC transfusions and a decrease of C3d positivity during the ongoing low dose steroid therapy.
Conclusion: 1) A positive direct antiglobulin test seems to be a consistent finding during Eculizumab therapy. As Eculizumab does not block the early stages of complement activation C3 cleavage products may occur and bind to CD55/CD59-deficient RBC 2) We recommend the monitoring of signs of extravascular hemolysis and ferritin levels during eculizumab therapy. 3) A low dose steroid therapy seems to be efficient in cases of clinical significant extravascular hemolysis but need to be confirmed.

mean value +/– standard deviation	DAT C3d positive pat (%)	CD59 neg ery. (%)	CD24/66b neg granulo. (%)	Hb (g/dl)	LDH (U/l)	total bilirubin (µmol/l)	direct bilirubin (µmol/l)	Ferritin (ng/ml)
before eculizumab	2.6	32.1+/-19.4	90.2+/–9.3	9.2+/–2.0	2609+/–1039	47.6 +/–31.3	8.6+/–15.8	223+/–535
latest visit during eculizumab therapy	91.7	58.9+/-26.8	86.9+/–9.1	10.7+/–1.4	253+/–54	43.0 +/–20.3	11.2+/–5.4	666+/–622

V 1.6

Reaction Strength of 2+ in Serologic RhD Typing Is a Reasonable Threshold to Initiate RHD Genotyping

Jorks S.[1], Döscher A.[2], Kroll H.[1]

[1]Red Cross Blood Transfusion Service NSTOB, Institute for Transfusion Medicine Dessau, D,
[2]Red Cross Blood Transfusion Service NSTOB, Institute for Transfusion Medicine Oldenburg, D

Purpose: The Rh system is the most polymorphic and clinically relevant human blood group system. Amino acid variations in the RhD protein induced by single point mutations in the RHD gene and hybrid genes between RHD and RHCE or other mutations are responsible for weak and partial Ds. Patients with partial or weak D show mostly weak agglutinations in phenotyping of the RhD antigen. However, partial and weak D alleles must be distinguished to avoid the risk of transfusion-related antibodies in recipients. In this study, we systematically analysed the RHD genes in patients with weak agglutinations in serologic RhD typing to define a threshold for initiation RHD genotyping.
Methods: RhD phenotyping of patients of the immunohematologic laboratory in Dessau was performed by tube agglutination technique with two different monoclonal anti-D antibodies that did not react with D category VI. All samples that showed weak agglutinations or different reaction strengths between the two sera were further analysed by genotyping. RHD genotyping was performed by polymerase chain reaction with sequence-specific primers (CDE SSP, weak D SSP) and in selected cases by direct gene sequencing using dye terminators.
Results: A total of 147 patients were analysed. RHD variants were detected in 145 (98.6%) patients. In detail, the following RHD genes were identified: weak D type 1: 79 (53.7%), weak D type 2: 38 (25.9%), weak D type 3: 14 (9.5%), weak D type 4: 1 (0.7%), weak D type 18: 1 (0.7%), weak D type 41: 1 (0.7%), weak D type 42: 1 (0.7%), weak D type 61: 1 (0.7%), weak D type 63: 1 (0.7%), weak D type 64: 1 (0.7%), D cat VII: 3 (2.0%), DHMi: 1 (0.7%), DFR2: 1 (0.7%), RHD-CE(3,4)-D: 1 (0.7%), wild type D sequence: 2 (1.7%). Interestingly, four new weak D alleles (weak D type 61, weak D type 63, weak D type 64, weak D type 33 like) and one new hybrid allele (RHD-CE-D) were found. In all patients the strength of agglutination with at least one monoclonal anti-D antibody was 2+ or less.
Conclusions: The reaction strength of 2+ in the tube agglutination technique is a reasonable threshold to initiate RHD genotyping. However, we did not found a clear association between strength of agglutination with a certain weak or partial RHD allele. In 98.6% of the patients the weak reactions in

RhD phenotyping were caused by weak or partial RHD alleles. Eleven different weak D types and four different partial RHD alleles were found and, in addition, five new RHD alleles were identified.

V 1.7

Non-Invasive Prenatal Diagnosis of the Fetal RHC-, RHc-, and RHE-Status from Maternal Plasma: Characteristics of Two Different Real-Time PCR Approaches

Gutensohn K.[1], Müller S.P.[2], Thomann K.[2], Suren A.[3], Körtge-Jung S.[4], Schlüter G.[5], Legler T.J.[6]

[1]University Hospital Eppendorf and AescuLabor, Dept. of Transfusion Medicine, Hamburg, D, [2]Universitätsmedizin Göttingen - Abt. Transfusionsmedizin, Göttingen, D, [3]Göttingen, D, [4]Pränatal-Medizin und Genetik, Düsseldorf, D, [5]Pränatalmedizin und Genetik, MVZ Nürnberg, D [6]Universitätsmedizin Göttingen, D

Purpose: Beside anti-D antibodies, anti-C (or anti-G), anti-c and anti-E are frequently involved as a cause of haemolytic disease of the fetus and newborn. Recently, we have published a large scale evaluation of an automated extraction method for fetal DNA from maternal plasma. In order to establish a clinical service for the determination of the fetal RhC-, Rhc and RhE-status the published DNA extraction method was evaluated in combination with two different PCR protocols.
Methods: Fetal DNA from maternal plasma was extracted using the Chemagic viral DNA/RNA kit in conjunction with the Magnetic Separation Module I (Chemagen AG, Baesweiler, Germany). Thereafter, real-time PCR was applied for the detection of the C, c, E alleles with two different published PCR protocols. A total of 233 specimen (46 for C, 87 for c, 100 for E) were included from volunteer, not immunized women at weeks 10 to 22 of gestation. The mother was always negative for the respective Rh antigen. Fetal genotyping results from amnion fluid were used as a reference.
Results: The sensitivity obtained from the first PCR protocol was 100% for RHC, 38% for RHc, 59% for RHE, respectively. With the second PCR protocol using a smaller size of amplicons sensitivity could be increased: The sensitivity for RHC was 100%, for RHc 100%, and for RHE 100%. The specificity for all assays was found between 99% and 100%.
Conclusions: Automated routine analysis of fetal RHCE alleles from maternal plasma with current protocols is feasible. Furthermore, results demonstrate that sensitivity is dependent on the PCR protocol and amplicon size used for amplification and detection.

V 1.8

Development of a New Platelet-Phagocytosis Assay and Its Applications for Better Understanding of Immune Thrombocytopenia

Gallin A.[1], Giptner A.[2], Sachs U.[2], Santoso S.[2], Bein G.[2], Bakchoul T.[2]

[1]Justus-Liebig Universität Giessen, Klinische Immunologie und Transfusionsmedizin, Giessen, D,
[2]Justus-Liebig Universität Giessen, D

Purpose: Although it is generally accepted that the central immunopathological disturbance in immune thrombocytopenia is the destruction of antibody-coated platelets by phagocytic cells in the reticuloendothelial system, no in vitro mean is available to determine the phagocytosis. The aim of this study is to develop a new phagocytosis assay to investigate phagocytosis induced by platelet-reactive antibodies via Fc receptors on leukocytes and to test new therapeutic approaches.
Methods: Human platelets (PLTs) were labeled with the fluorescent dye CMFDA, opsonized with sera from patients with autoimmune thrombocytopenia (ITP) and neonatal alloimmune thrombocytopenia (NAIT). Opsonized PLts were then incubated with monocytes isolated from whole blood of healthy donors using the automated magnetic cell sorter (autoMACS). Intracellular fluorescence of monocytes was subsequently analyzed by flow cytometry.
Results: Compared with NAIT-antibodies opsonized platelets, phagocytosis of ITP-antibodies opsonized platelets was significantly slower. Additionally, while all sera from NAIT-patients induced efficiently phagocytosis of opsonized platelets, not all ITP-sera were capable of this, suggesting that another mechanism of antibody-mediated platelet clearance is implicated in ITP than phagocytosis.
Conclusion: The results of our study suggest that the kinetics how monocytes engulf opsonized platelets and induce phagocytosis differ between NAIT- and ITP-sera. Further mechanisms than phagocytosis could be involved in autoantibody-mediated platelet clearance in ITP-Patients. The use of an in vitro phagocytosis assay will potentially enhance the understanding of the mechanism of in vivo destruction of platelets and help in developing and testing new therapeutic approaches.

V 1.9

Bacterial Infections Confer Susceptibility to Heparin-Induced Thrombocytopenia by Priming the Immune Response Against PF4/Polyanion Complexes

Krauel K.[1], Pötschke C.[2], Fürll B.[2], Keßler W.[3], Bagemühl J.[2], Weber C.[4], Hammerschmidt S.[4], Bröker B.M.[2], Greinacher A.[2]

[1]Institut für Immunologie und Tranfusionsmedizin, Transfusionsmedizin, Greifswald, D, [2]Institut für Immunologie und Transfusionsmedizin, Greifswald, D, [3]Klinik und Poliklinik für Chirurgie, Greifswald, D, [4]Interfakultäres Institut für Genetik und Funktionelle Genomforschung, Greifswald, D

Purpose: Heparin-induced thrombocytopenia (HIT) is the most frequent immune mediated adverse drug reaction with a high risk for new thrombotic complications. HIT is caused by antibodies against complexes of platelet factor 4 (PF4) and the polyanion heparin. Even patients without any previous heparin exposure develop anti-PF4/heparin IgG from on day 4 of heparin treatment. This early onset of IgG after first antigen contact is atypical for a classical primary immune response and suggests preimmunization with an antigen that mimics the PF4/heparin complex. The bacterial cell wall consists of polyanions. Therefore we analysed whether PF4 interacts with bacteria thereby exposing the epitope(s) recognized by anti-PF4/heparin antibodies.
Methods: First, binding of increasing concentrations of biotinylated PF4 to Gram-positive (S. aureus, S. pneumoniae, L. monocytogenes) and Gram-negative bacteria (E. coli, N. meningitidis) was quantified in the presence of heparin, dextran sulphate, or buffer by using streptavidin-PerCP-Cy5.5 and flow cytometry. Second, HIT sera were adsorbed by a Protein A deficient S. aureus strain (SA113spa) and a non-pathogenic E. coli strain (JM109), preincubated with PF4 or buffer; thereafter, bound antibodies were eluted (pH3), neutralised, and tested by PF4/heparin enzyme immunoassay (EIA). Third, C57BL/6 mice underwent colon ascendens stent peritonitis (CASP) surgery (model for polymicrobial abdominal sepsis) or sham surgery (control for surgical intervention), or were left untreated. At day 1, 3, 7, 14, and 28 after surgery serum was analysed by PF4/heparin EIA.
Results: PF4 bound specifically to both Gram-positive and Gram-negative bacteria. PF4 binding was inhibited by heparin and dextran sulphate suggesting a charge dependent mechanism. PF4-coated bacteria adsorbed anti-PF4/heparin IgG of HIT-patient sera and the respective eluates of these bacteria reacted in the PF4/heparin EIA. C57BL/6 mice (not previously exposed to heparin) developed anti-PF4/heparin IgM already at day 3 after induction of bacterial infection and low IgG levels from on day 14.
Conclusions: PF4/polyanion complexes on the bacterial surface prime the immune response. This might be an explanation for the rapid anti-PF4/heparin IgG production after heparin medication in HIT. HIT seems to be a misguided secondary immune response, during which heparin coated platelets mimic previously encountered bacteria.

V2 Hemostasis I

V 2.1

A New Strategy to Bypass Factor VIII for the Treatment of Patients with Hemophilia A and Inhibitory Antibodies

Schüttrumpf J.[1], Milanov P.[2], Abriss D.[2], Lyssy P.[2], Tonn T.[2], Seifried E.[2]

[1]DRK-Blutspendedienst Baden-Württemberg - Hessen, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt am Main, D,
[2]DRK-Blutspendedienst Baden-Württemberg - Hessen, Frankfurt am Main, D

The treatment of patients with inhibitory antibodies remains a major challenge in hemophilia therapy. Currently, infusion of activated proteases is the therapy of choice. Unfortunately, available substances, like activated factor VII or a mixture of pre-activated proteases, only have short half lives and long term administration of activated proteases raises safety concerns. Here, we generated FIX variants which do not require FVIII and confirmed their therapeutic potential in patient samples and in the hemophilia A mouse model. In the initial screening one single mutation (K265T) was identified which exhibits 6.6% clotting activity at physiological levels (FIX antigen 100%) in FVIII deficient plasma and 191% clotting activity in presence of

FVIII. This mutation was further combined with other candidate mutations which led to the generation of FIX variants with 17% clotting activity (V181I/K265T/I383V) in absence of FVIII (~100,000-fold increase compared to wild type FIX). FVIII inhibitor bypassing activity was confirmed in plasma of patients with high titers of inhibitory antibodies. Further, three different variants were expressed in FVIII knockout mice using non-viral gene transfer. At FIX expression levels ranging from 7500 to 19000ng/ml partial normalization of the aPTT (from 70 to <50 sec.) and of blood loss following tail clip assay (1.5 and 3 mm) were observed in all three variant groups (n=5-9 mice/group, p<0.05-0.005), while wild type FIX expressing mice did not differ from untreated animals. Similar results were obtained in FVIII knockout mice with high titers of anti-FVIII antibodies. Further, the efficacy of the FIX variants with FVIII bypassing activity was confirmed following laser induced injury of a cremaster arteriole by in vivo imaging technology. While no formation of a stable clot could be observed in FVIII knockout mice treated with wild-type FIX, a stable clot formed at 8 out of 8 injury sites using the V181I/K265T/I383V variant. The described FIX variants therefore offer a new FVIII inhibitor bypassing strategy. The use of these not previously activated proteases could presumably allow prophylactic treatment or even gene therapy in inhibitor patients.

V 2.2

Non Viral Coagulation Factor VIII Gene Transfer for the Treatment of Hemophilia A and for Immune Tolerance Induction

Abriss D.[1], Milanov P.[2], Seifried E.[2], Tonn T.[2], Schüttrumpf J.[2]

[1]DRK-Blutspendedienst Baden-Württemberg - Hessen, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt am Main, D,
[2]DRK-Blutspendedienst Baden-Württemberg - Hessen, Frankfurt am Main, D

Hemophilia A is a bleeding disorder caused by a deficiency of factor VIII (FVIII). A major problem in both, factor replacement therapy and development of gene transfer strategies, is the development of inhibitory antibodies. We therefore explore a non-viral gene transfer approach based on the generation of minicircle vectors for gene therapy and gene-based immune tolerance induction. Minicircles differ from standard plasmid vectors by the lack of bacterial sequences and therefore minimize the risk of gene silencing and immune response induced by inflammatory unmethylated CpG motifs. We constructed a minicircle for a B-domain-deleted version of FVIII driven by a strong liver specific promoter. Initially, we applied 50 µg (high dose) or 10µg (low dose) of minicircle vector by hydrodynamic injection technique into the tail veins of C57Bl/6 mice and compared FVIII expression with levels in mice that received an additional dose of 50µg bacterial pUC19 plasmid DNA as contaminant (n=10 per group). Mice treated with 50µg of minicircle vector showed higher expression levels (up to 0.5 IU at day 3 after injection) compared to the low dose (10µg) treatment group (~ 0.2 IU; day 3). No effect of the pUC19 co-injection was observed on expression levels. Starting 14 days following injection, anti-FIX antibodies (IgG1) were observed in around half of the mice. Occurrence of antibodies coincided with the disappearance of FVIII expression. In the groups with a higher vector dose, the onset of the immune response was faster and more pronounced. However, 12 weeks post gene transfer FVIII expression returned in 4 out of 4 mice receiving 50µg of minicircle, in 2 out of 4 mice receiving 50µg of both, minicircle and pUC19, and in none of the mice treated with a lower minicircle dose (0/4 for 10µg of minicircle and 0/2 for 10µg of minicircle + 50µg pUC19). In a different experiment, FVIII knockout mice were treated with high and low dose of either minicircle or pSL1180 plasmid vector (n=10 mice per group). Although the minicircle vector led to slightly higher expression levels (2.8 IU vs 1 IU or 0.8 vs 0.8, for high and low dose at day 3, respectively), a drop in FVIII expression and the occurrence of anti-FVIII antibodies was observed in all mice. 20 weeks post treatment, an immunotolerance characterized by a drop in antibodies and a rise in FVIII levels was observed in 50% or 14% of mice treated with high or low dose minicircle and in 40% or 0% treated with high or low dose plasmid, respectively. In conclusion, a non-viral minicircle vector, free of bacterial DNA, can improve efficacy and the immunological consequences of FVIII gene transfer.

V 2.3

The Prognosis of Acquired Hemophilia Can Be Significantly Improved: Results of the Düsseldorf Study

Scharf R.E.[1], Hoffmann T.[2], Bomke B.[2], Gheisari R.[2], Dücker C.[2], Stockschläder M.[3]

[1]Heinrich Heine University Medical Center and Biological Medical Research Center, Heinrich Heine University, Department of Experimental and Clinical Hemostasis, Hemotherapy and Transfusion Medicine, Hemophilia Comprehensive Care Center, Düsseldorf, D,
[2]Heinrich Heine University Medical Center, Düsseldorf, D, [3]Marburg, D

Background: Acquired hemophilia A (AHA) is a rare but significant hemostatic disorder caused by autoantibody inhibitors against factor VIII coagulant protein (FVIII:C). The annual incidence of AHA is low with 1.3 to 1.5 cases per one million individuals; however, the lethality due to severe hemorrhages (and comorbidity) was high, reaching 22% in some series related to age, inhibitor levels, and response to treatment. Patients in whom the inhibitor cannot be eliminated may have a mortality rate as high as 42%.
Design, Patient Characteristics and Clinical Endpoints: We have performed a monocenter study on 24 consecutive patients with AHA who were referred to the Düsseldorf Hemophilia Center between March 2001 and April 2009. The cohort included 15 males (age ranging from 59 to 87 yrs.) and 9 females (age 28 to 76 yrs.). For laboratory evaluation, a standardized staged protocol of APTT, FVIII:C activity and concentration, mixing studies with patient and normal plasma, characterization and quantitation of inhibitor titers (Bethesda assay) was used. Diagnostic work-up included elaborate examinations for any underlying disorder. Clinical endpoints were control of bleeding, eradication of the inhibitor, remission, relapse or death.
Results: Only in 5 of the 24 patients (21%), an underlying disorder (breast cancer; lymphoma; multiple sclerosis; rheumatoid arthritis; postpartum state) was identified, while in 79% AHA was "idiopathic". Upon admission, 11 of the 24 patients (46%) presented with life-threatening hemorrhages. Control of bleeding was achieved in 10 of these 11 patients by high doses of rFVIIa (NovoSeven), one patient required combined FVIII bypassing agents (rFVIIa plus FEIBA). In the other 13 patients, bleeding also subsided in response to rFVIIa. Concurrent immunosuppression consisted of prednisone (2 mg/kg/day), cyclophosphamide (2 mg/kg/day), and 4 weekly doses of rituximab (375 mg/m^2). Fourteen of 24 patients (58%) had high inhibitor titers above 30 Bethesda units (ranging up to 168 BU). Of these 14 patients, 10 individuals required daily large-volume immunoadsorption (Ig-Therasorb) for up to 4 weeks to accelerate inhibitor elimination. Induction of immunotolerance was achieved without administration of exogenous rFVIII:C. In total, 21 patients experienced complete remission, one had a relapse, 2 patients died (one of acute myocardial infarction, one of sepsis).
Conclusion: These monocenter data demonstrate that control of life-threatening bleeding by rFVIIa, eradication of the inhibitor by combined immunosuppression and immunoadsorption and induction of tolerance to endogenous factor VIII have significantly improved the clinical outcome of acquired hemophilia.

V 2.4

Peripheral Blood Smear: Immunostaining for Assessment of Patients with Macrothrombocytopenia

Althaus K., Blumentritt C., Stein K., Greinacher A.

Ernst-Moritz-Arndt-Universität, Institut für Immunologie und Transfusionsmedizin, Abteilung Transfusionsmedizin, Greifswald, D

Purpose: Persisting thrombocytopenia is a diagnostic challenge. In a subgroup of these patients, hereditary thrombocytopenia is the underlying cause. Congenital macrothrombocytopenias are a heterogeneous group consisting of MYH9-related diseases, glycoprotein Ib/IX receptor defects, lack of α-granules in the gray platelet syndrome, and abnormalities in the transcription factors like the Paris-Trousseau thrombocytopenia, or the X-linked macrothrombocytopenia. Patients are often misdiagnosed as having ITP. We developed a method to diagnose the most frequent causes of hereditary macrothrombocytopenia using blood smears.
Methods: Air dried blood smears from peripheral blood were fixed, permeabilized, stained by monoclonal antibodies, and visualized by immunofluorescence. Mutation analysis in the MYH-9 gene was performed by standard methods using PCR-RFLP/SSP and sequencing.
Results: Blood smears are stable at room temperature for at least one week and can be shipped by regular mail. Expression of glycoprotein Ib/IX and IIb/IIIa as well as the presence of alpha granule proteins, and dense granule expression can be visualized by immunofluorescence semiquantitatively.

Structure and distribution of non-muscular myosin clusters can be analysed in platelets and granulocytes. Using this method we now established the correct diagnosis in 50 unrelated families with congenital macrothrombocytopenias, most of them with MYH9-related diseases, five families with Bernard-Soulier syndromes, and one patient with gray platelet syndrome. In all patients the initial diagnosis by blood smear immunofluorescence was later confirmed by standard methods, e.g. flow cytometry, platelet aggregometry or mutation analysis. In MYH9-related disorders we also established a close correlation between the microscopical structure of the clusters of non-muscular myosin and the mutated exon. This enables relatively rapid identification of the genetic cause within this 40 exon gene.
Conclusions: Congenital macrothrombocytopenia are underestimated in their frequency and are often misdiagnosed as ITP. We now offer a tool to diagnose these patients on the basis of peripheral blood smears, which are easy to obtain even in young children.

V 2.5

Chemical Chaperones Improve Secretion of Coagulation Factors VIII or IX in Gene-Corrected Hemophilia Mice

Roth S., Abriss D., Milanov P., Schüttrumpf J., Seifried E., Tonn T.

Red Cross Blood Donor Service Baden-Württemberg - Hessen, Institute for Transfusion Medicine and Immunohematology, Frankfurt, D

Purpose: Hemophilia A and B are x-chromosome linked recessive bleeding disorders caused by coagulation factor VIII or IX deficiency. The current substitution therapy is based on regular intravenous administrations of factor concentrates. A gene therapy approach would be a more desirable alternative due to a continuous reconstitution of blood coagulation. To date only low human Factor VIII (hFVIII) or Factor IX (hFIX) plasma levels could be reached by different gene therapy trials in mouse models. As an increasing number of studies showed that low-molecular-weight compounds called "chemical chaperones" (CC) can support intracellular folding of proteins, we wanted to proof if the CC Betaine can improve FVIII or FIX plasma levels in gene-corrected Hemophilia A or B mice.
Methods: In the Hemophilia B mouse model (C57Bl/6/129sv, n=5) a liver-specific expression plasmid carrying the hFIX mini-gene was hydrodynamically injected in the tail veins. Plasma samples of mice were collected retro-orbitally in 2-week intervals and analyzed for hFIX by indirect ELISA. When the levels remained relatively constant, mice were divided in two groups. Betaine-dissolved tap water (2% w/v) replaced regular tap water in one group. After 3 and 17 days of Betaine treatment blood samples were tested for hFIX levels. C57Bl/6 Hemophilia A mice were hydrodynamically injected with Minicircle-DNA expressing a B-domain-deleted version of hFVIII (BDD-FVIII) or BDD-FVIII with a missense mutation (BDDaa305Q>P). After 24 hours mice were divided in a control and Betaine-treated group for 3 days, then treatment was interchanged for a further 3-day-intervall. At both time points hFVIII levels were determined by indirect ELISA.
Results: Five days post injection Hemophilia B mice showed mean hFIX plasma levels of 3412+/–369 ng/ml. After a 5-week-decline levels remained relatively constant implicating the start of Betaine treatment on day 55 post injection. Human FIX levels in control mice altered insignificantly (n=2), whereas Betaine supplementation (n=3) yielded in an mean increase of 40% hFIX in mouse plasma after 3 days (from 268+/–60 to 375+/–68 ng/ml) with an further increase after 2 additional weeks. In five out of six Hemophilia A mice BDD-FVIII plasma levels also rose 1.6 to 3fold in the period of drug administration. Mutant BDD-FVIII levels increased 3fold on average in 9 out of 10 injected mice.
Conclusions: Oral Betaine administration increases hFVIII and hFIX plasma levels in Hemophilia mice after a non-viral gene therapy approach significantly. As even BDD-FVIIIaa305Q>P levels rose upon treatment, Betaine could be promising in patients suffering from Hemophilia due to a trafficking-defect in FVIII or FIX.

V 2.6

Classification of Platelet Aggregation Defects by Characterization of Dysfunctional Integrin AlphaIIb Beta3

Scharf R.E.[1], Kirchhoff E.[2], Hoffmann T.[2]

[1]Heinrich Heine University Medical Center and Biological Medical Research Center, Department of Experimental and Clinical Hemostasis, Hemotherapy, and Transfusion Medicine, Düsseldorf, D,
[2]Heinrich Heine University Medical Center, Düsseldorf, D

Background and Objectives: Normal platelet aggregation requires (i) agonist-induced activation of αIIbβ3, (ii) binding of fibrinogen (Fg), and (iii) postoccupancy events following ligand binding. To assess aggregation defects in these terms, we used flow cytometry and specific antibodies that can distinguish between resting, activated, and ligand-occupied forms of αIIbβ3.
Design and Methods: We studied 30 MPD patients. PAC1 (directed to activated αIIbβ3) and anti-LIBS-1 (recognizing a ligand-induced binding site on β3) were used. LIBS is either expressed upon receptor occupancy by Fg, a process which requires activation, or by Fg-mimetic RGD peptides, which bind to αIIbβ3 by an activation-independent manner. This approach offers a strategy to distinguish defects in activation, ligand binding, or postoccupancy events.
Results: Among the patients, 25 had aggregation defects in response to epinephrine (EPI) and/or ADP despite normal expression of αIIbβ3. Of these 25 patients, 15 failed to bind PAC1 upon stimulation with EPI or ADP, but 11 of them bound PAC1 in response to PMA, a signal mimetic which circumvents agonist-induced platelet activation by directly activating protein kinase C. In 11 of the 15 patients, binding of anti-LIBS-1 was concomitantly absent in response to EPI or ADP, but intact upon incubation with RGDS. In the other 4 patients, RGDS failed to induce binding of anti-LIBS1. The remaining 10 patients showed intact binding of PAC1 and anti-LIBS-1 in response to EPI, ADP, or PMA, indicative of postoccupancy dysfunction.
Conclusion: Platelet aggregation abnormalities can be classified with regard to defects of activation, ligand binding, or postoccupancy dysfunction of αIIbβ3.

V3 Immunogenetics, Immunohematology

V 3.1

Semaphorin 7A: A New Modulator of NK Cell Function

Figueiredo C.[1], Zenk J.[1], Grüger D.[1], Eiz-Vesper B.[1], Blasczyk R.[1], Seltsam A.[2]

[1]Hannover Medical School, Institute for Transfusion Medicine, Hannover, D,
[2]Hannover Medical School, Institute for Transfusion Medicine; German Red Cross Blood Service NSTOB, Institute Springe, Hannover, Springe, D

Purpose: Semaphorins are a wide family of phylogenetically conserved signalling molecules. Semaphorin 7A (Sema7A) is expressed on a variety of myeloid and lymphoid cells as well as on red blood cells. It has shown the ability to promote axon outgrowth and modulate T cell-mediated immune responses through integrins. In this study, we investigated the influence of Sema7A on NK cell phenotype and function.
Methods: Recombinant soluble Sema7A was expressed in HEK cells and used to stimulate primary NK cells. The NK cells were measured for activation of the mitogen-activated protein kinase (MAPK) pathway and changes in the surface expression of KIR2DL1, KIR2DL2, NKp30, NKp44, NKp46, NKG2A and NKG2D receptors. NK cell proliferation assays were performed in the presence or absence of Sema7A. Cytotoxic assays using K562 cells were performed with non-stimulated or Sema7A pre-stimulated NK cells. To study whether the effect of Sema7A on NK cell function was mediated by integrins, a mutated Sema7A protein with an altered integrin-binding motif was used as negativecontrol.
Results: Soluble Sema7A showed the ability to bind NK cells and to induce phosphorylation of the non-receptor protein kinase focal adhesion kinase (FAK) and extracellular regulated kinases (ERK) 1 and 2. Sema7A caused a downregulation of NKG2D, NKp30 and NKp46 expression by up to 30%, 47% and 43%, respectively. The proliferation rate of NK cells decreased by 62% in presence of Sema7A, in comparison with non-stimulated NK cells. Pre-incubation of NK cells with Sema7A reduced the cytotoxic activity of NK cells against K562 cells by up to 60%. The mutated Sema7A protein did not show any effect on NK cell proliferation or cytotoxicity suggesting that integrin receptors are involved in Sema7A-mediated NK cell activation.

Conclusion: This study demonstrates for the first time that Sema7A is a potent inhibitor of NK cell function. This observation further highlights Sema7A as an important effector molecule in cell-based immunity.

V 3.2

Junctional Adhesion Molecule-C Induction via the Calcium-NFAT Pathway in Activated T Cells

Immenschuh S.[1], Naidu S.[2], Chavakis T.[3], Beschmann H.[4], Ludwig R.[5], Santoso S.[2]

[1]Hannover Medical School, Institute for Transfusion Medicine, Hannover, D, [2]Institute for Clinical Immunology and Transfusion Medicine, Justus-Liebig-University Giessen, D, [3]Experimental Immunology Branch, NCI, National Institutes of Health, Bethesda, USA,[4]Department of Dermatology, Johann-Wolfgang-Goethe-University, Frankfurt am Main, D,[5]Department of Dermatology, University of Lübeck, D

Purpose: T cell activation is an important process of the adaptive immune system, which requires recognition of MHC-associated antigens by antigen presenting cells (APCs) via the T cell receptor (TCR). To induce a productive T cell response the interaction of T cells with APCs needs to be stabilized by adhesion molecules. Junction adhesion molecules (JAMs) are a recently discovered group of immunoglobulin (Ig) superfamily proteins, which are involved in the regulation of various inflammatory and vascular events. The third member of the JAM protein family, JAM-C, is highly expressed in platelets and endothelial cells, whereas expression in T cells is largely unknown.
Results: To investigate the regulation of JAM-C in T lymphocytes, we determined JAM-C gene expression in quiescent and activated human T cells. Treatment with the polyclonal T cell activator phytohemagglutinin (PHA) increased surface and total JAM-C expression in T cells time- and dose-dependently, as determined by flow cytometry and immunoblot analysis. By contrast, no up-regulation of JAM-A in activated T cells was detectable. The highest level of JAM-C up-regulation by PHA was observed in CD3+FoxP3+ and CD4+CD25high T cells. Moreover, T cell receptor activation with combined anti-CD3 and anti-CD28 stimulation induced JAM-C expression in T cells. JAM-C induction occurred at the mRNA level suggesting a transcriptional regulatory mechanism of JAM-C expression. Accordingly, we studied the regulation of the human JAM-C gene promoter in transiently transfected T cells. Luciferase activity of a JAM-C promoter gene construct with three potential consensus sites for the transcription factor NFAT was markedly induced in activated T cells. Finally, pretreatment with two pharmacological inhibitors of calcineurin, cyclosporin A and FK-506, but not with MAPK inhibitors, blocked JAM-C induction in activated T cells.
Conclusion: In summary, the present data indicate that JAM-C is induced in activated human T lymphocytes via a transcriptional mechanism and suggests a major regulatory function of JAM-C for the T cell response.

V 3.3

Inhibition and Genetic Deficiency of p38 MAPK Upregulates the Anti-Inflammatory Heme Oxygenase-1 Gene via Transcription Factor Nrf2 in Monocytes

Naidu S.[1], Santoso S.[1], Kietzmann T.[2], Immenschuh S.[3]

[1]Justus-Liebig-University Giessen, Institute for Clinical Immunology and Transfusion Medicine, Giessen, D,
[2]Department of Biochemistry, University of Kaiserslautern,
[3]Hannover Medical School, Institute for Transfusion Medicine, Hannover, D

Purpose: Heme oxygenase (HO)-1 is the inducible isoform of the first and rate-limiting enzyme of heme degradation. It has been shown in HO-1 knock out mice and in human genetic HO-1 deficiency that this enzyme has potent anti-inflammatory functions and may be a major target in transplantation medicine. Although HO-1 has previously been shown to be induced by various stimuli via activation of the p38 MAPK signaling pathway, the role of this protein kinase for HO-1 gene regulation is largely unknown.
Results: In the present study it is demonstrated that pharmacological inhibitors of p38 and siRNA-dependent knock down of p38α induced HO-1 expression in monocytic cells. Moreover, basal HO-1 gene expression levels were markedly higher in untreated murine embryonic fibroblasts (MEF) from p38α-/- mice as compared to that from wild type mice. Transfection studies with luciferase reporter gene constructs indicate that increased HO-1 gene expression via inhibition of p38 was mediated by the transcription factor NF-E2-related factor-2 (Nrf2), which is a central regulator of the cellular oxidative stress response. Accordingly, inhibitors of p38 induced binding of nuclear proteins to a Nrf2 target sequence of the HO-1 promoter, but did not affect HO-1 protein expression and promoter activity in Nrf2-/- MEF. Genetic deficiency of p38 led to enhanced phosphorylation of ERK and increased cellular accumulation of reactive oxygen species (ROS). In addition, pharmacological blockage of ERK and scavenging of ROS with N-acetylcysteine reduced HO-1 gene expression in p38-/- MEF, respectively. Taken together, it is demonstrated that pharmacological inhibition and genetic deficiency of p38 induce HO-1 gene expression via a Nrf2-dependent mechanism in monocytic cells and MEF.
Conclusion: These findings not only give new insights into the complex signaling events of HO-1 regulation during the inflammatory response, but may also help to develop novel therapeutic approaches for inflammatory diseases and in transplantation medicine.

V 3.4

Cloning and Expression of Recombinant Viral Proteins and Generation of Adenoviral-Specific T Cells

Tischer S.[1], Maecker-Kolhoff B.[2], Heim A.[3], Bajor A.[1], Blasczyk R.[1], Eiz-Vesper B.[1]

[1]Hannover Medical School, Institute for Transfusion Medicine, Hannover, D, [2]Hannover Medical School, Department of Pediatric Hematology/Oncology, Hannover, D, [3]Hannover Medical School, Institute for Virology, Hannover, D

Purpose: The infection with human adenovirus (ADV) is a prevalent complication after allogeneic hematopoietic stem cell transplantation (HSCT) and is associated with a high morbidity and mortality. The incidence of ADV infection ranges between 5% and 30%, with pediatric recipients showing the highest rates of infection. The cumulative incidence of ADV-associated mortality ranges between 8% and 15%. Therapy for ADV infection includes the use of antiviral agents (Cidofovir or Ribavirin), which can be associated with severe side effects. It is evident that the elimination of ADV is only achieved by recovery of cellular immunity. Studies showed that children with ADV-associated mortality had no ADV-specific T cells, whereas patients who cleared ADV infection had normal frequencies of antiviral T cells. Therefore, it is appreciated that T-cell reconstitution is required for the control of ADV infection and that drug therapy may limit, but not clear the infection. Across the ADV subgenera the capsid protein hexon is highly conserved and serves as an immunodominant T-cell target antigen. Up to now only some hexon-derived immunodominant epitopes are known, which are restricted to HLA-A*01, 02, 24, B*07, and B13.
Methods: The aim of this study was the identification of adenoviral-specific T-cell targets for the ADV type C and the generation of anti-viral T cells for adoptive immunotherapy. We analyzed the specificities of T-cell response to the immunogenic capsid protein pentonbase and the non-structural protein E1B of adenovirus strain 5. In order to facilitate the eukaryotic expression and isolation procedures we constructed HLA-A*0201/ADV fusion proteins (sADV5_pentonbase, sADV5_E1B), which are secreted into the supernatant of HEK293 cells.
Results: ADV-specific CD4+ as well as CD8+ T cells were isolated based on the secretion of interferon-γ after short in vitro stimulation with sADV5_pentonbase. Only a limited amount of ADV-specific T cells could be isolated after stimulation with the sADV5_E1B protein.
Conclusion: From these data we conclude that the adenoviral capsid proteins hexon and pentonbase contain immunodominant epitopes, which can be used to generate antiviral T cells for adoptive immunotherapy.

V 3.5

Genome Sequencer Based HLA Typing for High-Resolution Allele Identification

Gabriel C., Danzer M., Hackl C., Hofer K., Polin H., Stabentheiner S., Pröll J.

Blood Center Linz, A

Purpose: We describe the development of a novel, human leukocyte antigen (HLA) sequence-based typing (SBT) method by exploring the next-generation sequencing technology as provided by the Genome Sequencer FLX (GS-FLX) system (Roche/454 Life Sciences, Branford, CT, USA).
Methods: The established system allows for mostly ambiguity-free, high-throughput, high-resolution HLA typing with the potential for high-grade automation. The on average 259 bp long sequence reads generated by the Genome Sequencer FLX system in this study, span most of the HLA exons of interest enabling an amplicon sequencing strategy. We developed a list of 11 HLA-A and -B locus-specific intronic primers for exon 1, 2, 3 and 4.

Primers and GS-FLX specific adaptors were lengthened with patient-identifying barcode sequences encompassing 4 bp´s to identify each of 8 patients within one single multiplex sequencing run gaining 185.000 high-quality sequence reads. GS-FLX sequencing protocols were performed exactly according to suppliers instructions.
Results: For data analysis the latest version of the GS-FLX Amplicon Variant Analysis (AVA) software supporting multiplex identifier sequence tags was used. The software enables the download of all read-sequences, qualified for absolute occurrence and read length. This AVA software package maps the GS-FLX reads against the HLA reference sequences and reports the type and frequency of the found variants. It clusters reads with the same variant pattern into consensus reads. After renaming, files containing these consensus reads can be imported to the Assign SBT 3.5 software (Conexio Genomics, Fremantle, Australia) for final HLA allele identification.
Conclusion: Our results using the 454 GS-FLX, compared to normal SBT HLA typing, show that with an average of 1000 reads per amplicon every patient was identified correctly.

V 3.6

Sanglifehrin A Is a Novel Dendritic Cell Chemokine and Migration Inhibitor

Immecke S.[1], Bein G.[2], Wilhelm J.[3], Hackstein H.[3]

[1]University of Giessen, Clinical Immunology and Transfusion Medicine, Giessen, D, [2]University of Giessen, [3]Giessen, D

Objective: Sanglifehrin A (SFA) is an immunosuppressive drug. It is a representative of a class of macrolides produced by the actinomycetes strain Streptomyces A92-308110 that bind to cyclophilin A (CypA), the binding protein of Cyclosporine A (CsA). Although SFA has a higher affinity than CsA for cyclophilin, SFA does not inhibit the activity of calcineurin phosphatases. Different groups have reported that SFA exerts unique suppressive effects on human and mouse dendritic cells (DC). SFA effectively suppresses antigen uptake and bioactive IL-12 production of DC in vitro and in vivo but it does not inhibit DC differentiation and surface costimulatory molecule expression. The mechanism of action of SFA is still unknown. Our goal was to investigate the gene expression profile of SFA exposed human DCs with subsequent confirmation on the protein level and functional in vitro and in vivo assays.
Material and Methods: Human monocytes were isolated from buffy coats of healthy blood donors. CD14+ monocytes were cultured with IL-4 and GM-CSF to generate DCs. On day 5, SFA or vehicle was added to the culture for one hour. After 12 hours stimulation with Lipopolysaccharid, RNA was isolated and microarrays were performed. Expressions of proteins are studied by ELISA and FACS. In vitro migration was estimated with chemotaxis assays. FITC-skin-painting method was used to study the in vivo migration of DC towards the inguinal lymph node by C57BL/6NCrl mice.
Results: Global gene expression analysis and subsequent protein level confirmation revealed that SFA suppressed CCL5, CCL17, CCL19, CXCL9 and CXCL10 expression in human monocyte-derived DC. Direct comparison with the related agent cyclosporine A (CsA) and the classical corticosteroid dexamethasone indicated that SFA uniquely suppresses DC chemokine expression. Given the emerging critical role of chemokines production and the important role for migration of DC to initiate innate and adaptive immune responses, we investigated the effect of SFA on DC migration in vitro and in vivo. In vitro, functional assays demonstrated impaired migratory activity of maturing, SFA-exposed DC against CCL19. Moreover, SFA suppressed expression of the ectoenzyme CD38 that was reported to regulate DC migration and cytokine production. In vivo analyses confirmed SFA's inhibitory effect on DC migration.
Conclusion: This first systematic genome-wide study on SFA and dendritic cells revealed a novel and unique anti-inflammatory mode of action of SFA. Our results identify SFA as a novel DC chemokine and migration inhibitor.

V 3.7

In Stem Cell Transplantation for Treatment of Myeloic Leukemia the Genotypic Difference of Patients and Donors for the Inhibitory KIR-Receptors 2DL1 and 2DL3 is Correlated with Reduced Survival

Richter R.[1], Skrablin P.[2], Bug G.[3], Serve H.[4], Seifried E.[2], Martin H.[3], Seidl C.[2]

[1]DRK Blutspendedienst Baden-Württemberg Hessen, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt am Main, D, [2]DRK Blutspendedienst Baden-Württemberg Hessen, Frankfurt, D, [3]Internal Medicine II, Hematology and Oncology, Stem Cell Transplant Unit, Goethe University Hospital, Frankfurt, D, [4]Internal Medicine II - Hematology and Oncology, Goethe University Hospital, Frankfurt, D

Purpose: Natural killer (NK) cells are critical for the innate immunity and are relevant for the immune control of tumors. It was recently suggested that the success of stem cell transplantation in myeloic leukemia and MDS patients is dependent on the NK-cell activation status regulated by inhibitory killer immunoglobulin receptors (KIRs). On the other hand, in functional analyses it was shown that the activity of NK cells lacking inhibitory KIR receptors is predominantly regulated by the lectin-like receptor NKG2A, which is associated with a constant inhibition of the NK cell activity and a hypo-responsiveness to HLA class I-deficient target cells. Therefore, we aimed to evaluate the significance of the deficiency for inhibitory KIR receptor genes in the donors for the outcome of the stem cell transplantation.
Methods: The study included 109 patients (94 AML, 10 CML, 4 MDS). Patients were transplanted with HLA matched unrelated donors or family related donors. KIR typing was performed as previously described. Clinical analysis included acute and chronic GvHD, relapse, TRM and overall survival (OS) with a follow-up of 4-8 years.
Results: A lack of inhibitory receptor genes in donors or patients was found for KIR2DL1 in 3.7% and 4.6%, for KIR2DL2 45% and 45%, for KIR2DL3 in 10% and 11%, and for KIR3DL1 in 10% and 14%, respectively. A lack of KIR2DL1 or KIR2DL3 in the donors in conjunction with the presence KIR2DL1 or KIR2DL3 (P+/D- situation) in the recipient was correlated with a significantly reduced survival of the patients (log rank, $p = 0.016$). Out of 6 AML patients with this mismatch 5 patients died within 4 years after transplantation, whereas 52% of the patients without the mismatch were still alive ($p = 0.05$). Interestingly, a lack of KIR2DL1 or KIR2DL3 in graft-vs.-host/host-vs.-graft direction (GvH/HvG; (P+D-/P-D+ situation) was associated with a significantly reduced overall survival of the patients (log rank, $p = 0.02$). Concerning the AML patients, a lack of KIR2DL1 or KIR2DL3 in GvH/HvG direction was associated with a significantly reduced overall survival of the patients (log rank, $p = 0.03$).
Conclusion: Our results support the significance of KIR2DL1 and KIR2DL3 for the immune control of tumors. A lack of these receptors in the transplant or in the patients may be correlated with a reduced survival after stem cell transplantation. KIR2DL1 or KIR2DL3 deficiency is not considered by the missing-self hypothesis; therefore, the KIR2DL1 or KIR2DL3 deficiency may be an additional criterion for identification of the best fitting stem cell transplant.

V4 Demographic Change and Blood Supply, Quality Management in Hemotherapy

V 4.1

Who Are the Blood Recipients Today and in 2020?

Greinacher A.[1], Fendrich K.[2], Brzenska R.[1], Hoffmann W.[2]

[1]Institut für Immunologie und Transfusionsmedizin, Greifswald, D
[2]Institut für Community Medicine, Greifswald, D

Background: The population structure is currently shifting from younger to older age groups. This demographic change will lead to an increased transfusion demand and at the same time a reduction of the number of blood donors. Little is known about the characteristics of patients who are the blood recipients.
Methods: We identified for all recipients of allogeneic transfusions of red blood cell concentrates (RBCs) in the 34 hospitals in the Federal State Mecklenburg-Vorpommern (MV) in the year 2005 the following characteristics: age, sex, medical specialty (medicine, surgery, intensive care/emergency room, pediatrics). Each transfused RBC was counted as one event. Population data in 1-year age groups for males and females were retrieved from the population registry for MV for 2005 and 2020.

Results: In 2005 95,477 RBCs (45.8% to females) were transfused in MV. Median patient age was 68.9 years with a peak at 65-79 years (47% of all transfusions). 37.4% (35,737 RBCs) of all RBCs were transfused to medical patients; 24.6% (23,446 RBCs) to patients in the emergency room and in intensive care units (combined medical and surgical patients); 35.1% (33,530 RBCs) to surgical patients; 0.7% (695 RBCs) to pediatric patients (2.2% (2,069 RBCs) not classified). Projections of the overall blood demand and blood donations predicted a shortfall of 56,083 RBCs for in-hospital patients in 2020 with a demand of ~46,000 RBCs for medical patients; 28,000 RBCs for patients in the emergency room / intensive care units; 42,000 RBCs for surgical patients; 600 RBCs to pediatric patients.
Discussion: In 2005 already more than half of the RBCs were transfused to medical patients and to intensive care/emergency patients. These patients will be treated with higher priority than elective surgery patients for medical and ethical reasons. Hence, the shortfall in RBCs will likely lead to a substantial reduction of elective interventions, which could severely affect hospital budget planning and challenge the provision of medical care.

V 4.2

The Greifswald DONOR-SHIP (Study of Health in Pomerania)-Study: Sociodemography of Blood Donors

Greinacher A[1], Konerding U.[2], Alpen U.[3], Lubenow N.[3], Hoffmann W.[2], Kohlmann T.[2]

[1]Greifswald, D, [2]Institut für Community Medicine, Greifswald, D, [3]Institut für Immunologie und Transfusionsmedizin, Greifswald, D

Purpose: Information on socioeconomic status of blood donors are scarce. We report results of a systematic assessment of blood donors, the Greifswald DONOR-SHIP Study.
Methods: In the DONOR-SHIP study over a 1 year period every 6th person enrolling for blood donation was asked to fill in a structured questionnaire on characteristics of the socio-economic situation, the subjective health status, and the health related behaviour. All data were adjusted for oversampling due to multiple donations.
Results: 2503 blood donors participated; females 54.6%; mean age 29.7 years, 23.5% were married; most blood donors had a higher grade of school education: 30% primary (Hauptschule), 30% intermediate (mittlere Reife), 40% secondary education ([Fach-]Abitur). Only 33% were full time working; 32% were in training (total students 24%), unemployed were 16%. 68% came to blood donation from home, 19% from work. Median single way to the donation center were 4km (quartiles 1.9–24.75 km) (~median time 15 min, quartiles 9.5–27.5 min). Information about blood donation were obtained in 90% of donors by family members, 30% by newspaper, and 20% in school, while radio broadcast and internet had a minor role (5% and 7%). Motivation for blood donation were (multiple answers possible): I want to help 98%; wanted my blood assessed 80%; I feel more satisfied 67%; renumeration (Aufwandsentschädigung) 64%; feel physically better 43%; to know my blood group 40%; to receive a donor passport 40%; wanted to be seen by physician 38%; other blood donors took me with them 31%; family member required blood transfusion 11%; wanted HIV test 6.6%.
Conclusion: Most blood donors are well educated. To help others but also personal benefits (blood assessment and renumeration) are strong motivators for blood donation. Full time employees are underrepresented. These data may be used to design blood donor campaigns.

V 4.3

Trends in the Supply of Blood Products in the Period 2000 to 2007 – Data Pursuant to Section 21 Transfusion Act (TFG)

Henseler O.[1], Heiden M.[2], Seitz R.[2], Haschberger B.[2], Hesse J.[2]

[1]Paul-Ehrlich-Institut, Transfusion Medicine, Langen, D,
[2]Paul-Ehrlich-Institut, Langen, D

Purpose: Which amounts of blood components and plasma derivatives are annually available in Germany and how many of these blood products are used to treat patients? The data collected and published as annual reviews by the Paul-Ehrlich-Institut provide an overview of blood collection and manufacture, import and export, as well as consumption of blood products over the past 8 years. Moreover, data are compared with data from other European countries.
Results: In the period from 2000 to 2007, whole blood donations were fairly stable with about 4.7 millions annually, while the number of aphaeresis donations fluctuated between 0.7 and 2.4 million. The proportion of autologous donations decreased from 4.7 to 1.1%. The annually distributed number of red cell concentrates remained nearly constant at 4.5 million, platelet concentrates increased by 30% to 480,000, and single plasma units reached 1.2 million in 2007.
Germany is the European country where the highest amount of plasma for fractionation was collected. Over the years, constantly about 1 million litres came from whole blood donations, 0.6 to 1.5 million litres from aphaeresis. In 2007, a total amount of 2.2 million litres were collected, 1.4 million litres thereof fractionated in Germany.
Since 2004, the number of autologous peripheral blood stem cells remained balanced between 6,996 and 7,648 preparations per year, while the number of allogeneic preparations increased from 3,616 to 4,682. In 2007, 2,027 allogeneic preparations were transplanted, 1,810 exported, and only 179 imported from other countries. Remarkable is the 55% increase of autologous cord blood stem cell preparations within one year to 10,313 in 2007 while no autologous transplantation was reported; 2,657 allogeneic cord blood preparations were deposited, 15 had been transplanted.
Conclusions: There is an increasing supply with several blood components in Germany over the last years, with a fluctuating share of aphaeresis. The stably reported number of 58 whole blood donations per 1,000 inhabitants should guarantee a sufficient blood supply, however shortages of red cell concentrates are discussed as a serious problem. The collection of plasma by far exceeds the fractionation capacity. Due to significant amounts of imports and exports of plasma, intermediates and products, the assessment of self sufficiency with plasma derivatives is only limited. Nevertheless, products are apparently available in sufficient quantities to meet patient's need. Stem cell donations are obviously thus well organised in Germany, so that a large portion can be even exported.

V 4.4

Socio-Demographic Data Ascertainment of Blood Donors at the Institute of Transfusion Medicine at the University Clinic of Magdeburg

Lüdke J.[1], Schulze S.[2], Heim M.U.[2]

[1]Medizinische Fakultät der Otto-von-Guericke-Universität Magdeburg, Institut für Transfusionsmedizin u. Immunhämatologie mit Blutbank, Magdeburg, D,
[2]Medizinische Fakultät der Otto-von-Guericke-Universität Magdeburg, D

Purpose: The steady increase for demand of blood products requires continuous motivation of permanent donors towards regular blood donation in addition to acquisition of new donors to ensure the availability of blood. Effective motivational campaigns and advertising efforts both depend on knowledge of the socio-demography of blood donors. In this study the socio-demographic data of the blood donors at our Institute ascertained by a survey is analyzed.
Methods: Over a period of ten weeks the donors of the Institute of Transfusion Medicine were interviewed about their socio-demographic data via questionnaire. The participation in these interviews was anonymous and voluntary. The questions inter alia covered the topics religion, family, education, job, spare time activities, voluntary involvement, and satisfaction with health as well as financial means.
Results: A total of 1,568 questionnaires were returned and evaluated. At the time of the interviews 22.1% of the participants were attending an education (school, professional training, study). The majority of the interviewed donors (i.e. 59.6%) were gainfully employed, and only 4.6% were unemployed. In contrast, in this time the general unemployment rate in Magdeburg was 13.5%. Among the gainfully employed donors 26.8% were satisfied/very satisfied with their financial means. Among the students and trainees declared 28% to be very satisfied with their financial status. Totally 432 donors (27.6%) consider their financial positions satisfactory. In Saxony-Anhalt 30% of the inhabitants are having a membership or involved voluntarily in clubs. In the interview 33.7% of the donors confirmed participations in clubs. 18.9% of the interviewed donors are working voluntarily in their spare time. Only 12.5% of the unemployed, trainees and pupils are participating clubs, but 54.7% of the students. 49.2% of the interviewed donors are married or cohabit. The majority of the interviewed donors (i.e. 81.3%) do not belong to any religion.
Conclusions: The Institute of Transfusion Medicine Magdeburg pays remunerations for expenses. In spite of this payment the portion of the unemployed donors are very below the unemployment rate in Magdeburg. The majority of donors (i.e. 62.2%) are motivated by family and friends and not by the remunerations for expenses. The ascertainment of socio-demographic data of the blood donors is required for implementing successful advertising tactics in blood donation establishments. The gathered data should be evaluated according to the regional and cultural environment, as well as in the context of motivational research.

V 4.5

The EuBIS Project: European Quality Management Standards for Blood and Blood Components Related to the Public Health Programme of the European Commission

Seidl C.[1], Bregeon T.[2], Cermakova Z.[3], Delaney F.[4], van Galen J.P.[5], Huber H.-M.[6], Nightingale M.[7], MacMillan Douglas A.[8], Siegel W.[9], de Wit J.[10], Seifried E.[11]

[1]DRK Blutspendedienst Baden-Württemberg Hessen, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt am Main, D, [2]European Commission, DG Sanco, Brüssel, B, [3]Fakultni nomocnici Ostrava, CZ, [4]FMD, Luxembourg, L,[5]Sanquin, Groningen, NL, [6]Paul Ehrlich Institut, Langen, D, [7]NHS-BT, Southampton, GB, [8]GMP, Forfar,GB, [9]Regierungspräsidium, Darmstadt, D, [10]Sanquin, Amsterdam, NL, [11]DRK Blutspendedienst Baden-Württemberg Hessen, Frankfurt, D

Purpose: In recent years, significant progress has been made with the entry into force of EU legislation on blood, based on Directive 2002/98/EC and its technical annexes. The Public Health Programme (Directorate General Sanco) gave impetus to the need for equivalent recognition of inspections of blood establishments among Member States (MS) through the development and implementation of commonly accepted criteria and standards by funding the European Blood Inspection Project (EuBIS) (www.eubis-europe.eu). Without these criteria and standards, the levels of risk from having a blood transfusion in the MS could continue to differ.
Methods: EuBIS aims to provide assistance to EU MS in their implementation of regulatory requirements set out in Directives 2002/98/EC, 2004/33/EC, 2005/61/EC and 2005/62/EC.
These include inter alia:
• designation, authorisation, accreditation or licensing of blood establishments (BEs)
• authorisation of the activities which can be undertaken and the applicable conditions for blood collection
• provisions for ensuring the quality and safety of blood and blood components, and
• requirements for imported blood and blood components.
Results and Conclusion: EuBIS comprises 25 collaborating partners from 19 MS, cooperative working partnerships with five organisations and three projects, affiliations with six partners involved in conducting its survey, and is supported by the European Blood Alliance (EBA). The initial work phase of the project has been completed by performing a survey on the currently used standards and criteria for inspection (Tab). Based on currently used standards as Eudralex-GMP, CoE-EDQM, PIC/S and ISO, the EuBIS project consortium and its working groups have developed guidelines including inspection checklists and criteria for the evaluation of inspection results. With respect to the different standards and guidelines that are currently in place, the inspection guide summarizes the most critical aspects to be addressed in order to achieve good practice. For each of these critical points, the Guide provides references to commonly used standards for the inspection of BEs. In addition, defined principles for the structure of quality systems will assist in implementing or expanding the currently used quality management systems in BEs.
Acknowledgements: The EuBIS Project is cofunded by the EC, DG Sanco, grant agreement No. 800053. The Project would like to acknowledge the support of the WHO-Europe (V. Hafner) JACIE (E. McGrath), the Eustite Project (D Fehily), the Council of Europe, EDQM (M. Spieser) and the continuous support of the EBA.

V 4.6

Pandemic Influenza-Planning for Austrian Blood Donor Services

Schennach H.

University Hospital Innsbruck, Central Institute for Blood Transfusion and Immunology, Innsbruck, A

Purpose: In the case of a pandemic, caused by H5N1 or any other similarly virulent virus, blood establishments will face great problems concerning donor recruitment, management of donor sessions, processing, testing and distribution of blood components. Main objective of the Austrian Pandemic Plan is to minimize the effects of a pandemic on the blood supply.
Methods: This plan is based on assumptions of the European Blood Alliance-Emergency Planning and Action Group (EBA-EPAG) and was harmonized with the generic Austrian Pandemic Plan. In a first step important stakeholders were identified, including blood donors, staff of the blood donor services, media, health authorities, the "Emergency planning group of the Austrian Red cross blood donor services", hospital blood banks, Austrian blood establishments, main suppliers.
Results: Major risk factors were identified. In a decentralized system like in Austria rules for the communication between the different blood establishments had to be implemented. A web based "blood pool" showing the reserves of each blood bank was implemented during the European football championships 2008 and is kept up to date. Health authorities were not aware of the specific problems a blood donor service will face: Closing of schools, assembly ban, restricted access to resources as fuel. Agreements had to be made. Lists of reserve donor sessions were created, a list of so called emergency donors is on the way. Special security measures for donors and staff, including triage check points, the use of face masks, aprons were specified in SOPs. Acceptance criteria for donors such as minimum haemoglobin levels were reviewed. Convalescent persons would be accepted as donors. Messages for donors (distributed via media) were prepared. Special considerations were made for platelet donors. Staff has to be informed in advance about personal inconveniences: Taking care for their children will be difficult, because schools and kindergartens will be closed. Many of the employees won't go to work because of personal illness or taking care for sick relatives. Work load for the remaining staff will be high. Hospital blood banks have to be informed, that stocks will decrease, and that the have to implement triage plans for their patients. There should be queries of the business continuity plans of the main suppliers (bags, reagents).
Conclusions: Each blood organisation should plan, prepare and remain prepared for a severe human influenza pandemic. An influenza pandemic response plan should be constructed and adapted in accordance with national and local public health guidance.

V 4.7

Antibody Screening of Female PLT-Aphereses Donors According to the TRALI Directive in Germany

Petershofen E.K.[1], Baume H.[2], Look P.[2], Borowsky A.[3], Grabowski C.[3], Kroll H.[3]

[1]Institut Bremen-Oldenburg, DRK Blutspendedienst NSTOB, Cytapherese-Abteilung Oldenburg, D, [2]Institut Bremen-Oldenburg, DRK Blutspendedienst NSTOB, Oldenburg, D, [3]Institut Dessau, D, DRK Blutspendedienst NSTOB, Dessau, D

Purpose: In 2008 a new directive for pre-screening of female platelet donors was introduced in order to detect antibodies that might be a cause for the development of TRALI syndrome.
Methods: All female donors of the Oldenburg donor centre have been reviewed for pregnancies and birth and were afterwards screened for the occurrence of HLA-, HPA- or HNA-antibodies using the following tests: tests included a) LCT / CDC technique, b) anti-HLA class I &II with Luminex technology, c) granulocyte aggregation testing (GAT), d) granulocyte immune fluorescent testing (GIFT), e) in selected cases MAIGA or MAIPA. For consistency all tests have been performed in the PLT-laboratory at Institute Dessau.
Results: The Oldenburg donor pool comprises 47% male and 53% female donors. In total 116 female donors had to be tested. 95 samples were stated "non-relevant", while 21 samples showed relevant positive test results and women had to be excluded from the donor pool.
Test results were as follows: anti-HLA class-I in 10 cases; anti-HLA class-II in 14 cases; 5x positive GAT; 7x positive GIFT; 7x positive MAIPA; 0x positive MAIGA. One donor exhibited an Anti-HNA3; 7 donors were positive for HLA-class I & II, MAIPA and GAT or GIFT. Others were only positive in a single test. The median age of these 21 women was 44 years (range 38–67years), having on average 1.5 births / 1.8 pregnancies.
Conclusion and
Discussion: After checking our female donors we had to take 21 women from the PLT donor pool, comprising 10,1% of all women and 5,3% of total donors, respectively. The acute loss of 5% of our donors has to be compensated during the next months. For a few donors it was rather difficult to accept that they were excluded from the donor pool and are not allowed to donate in future, while they have been donating for years before. In addition we checked 32 PLT-transfusion reaction cases in our region from 2006 to 2008: 11 cases with male and 21 cases with female donors were found. One case had a suspect for TRALI syndrome and included a female donor without any antibodies. One donor was excluded due to HLA-class I & II antibodies, positive GAT and MAIPA; she was involved 3 times in cases with "weak" reaktions.

V5 Infectious Diseases Transmitted by Transfusion, Immunologically Mediated Transfusion Reactions

V 5.1

Stability of Transfusion Transmitted Viruses in Whole Blood

Schmidt M.[1], Hourfar M.K.[2], Klose T.[3], Putzker M.[3], Sireis W.[2], Gubbe K.[4], Schrezenmeier H.[5], Hellmann I.[6], Geusendam G.[6], Seifried E.[2]

[1]German Red Cross Institute Frankfurt, Blood donor screening, Frankfurt, D, [2]German Red Cross Institute Frankfurt, D, [3]Central Institute of the Federal Armed Forces Medical Services Koblenz, D, [4]German Red Cross Institute Frankfurt, D, Plauen, D, [5]German Red Cross Institute Frankfurt, D, Ulm, D, [6]German Red Cross, Lütjensee, D

Purpose: In order to reduce the diagnostic window period, real time mini-pool nucleic acid amplification technology (mini-pool NAT) was implemented into blood donor screening at the German Red Cross in 1997. Currently, approximately 10,000 donations from Austria, Luxemburg, Baden-Württemberg, Hessia, Saxonia, Berlin, Brandenburg, Schleswig-Holstein and from the German Federal Armed Forces (Bundeswehr) are tested daily by NAT for HAV, HBV, HCV, HIV-1 and B19 at our blood donation service by three testing laboratories in Frankfurt/Main, Plauen, and Ulm. Blood samples arriving by midnight, are pooled over night, extracted and amplified the next morning. Especially during the Christmas and Easter holidays the time period before separation of the diagnostic sample into plasma and red cells should be possible to extend to 72h. The current study evaluated the influence of different pre-analytic conditions on the analytical sensitivity of the Baden-Württemberg – Hessen mini-pool NAT system.
Methods: Blood donor samples (576 in total) were pooled into 144 pools (pools of 4) with a total volume of 15 ml each. Haematocrit was examined from all pools by Sysmex XT 1800i. In the next step the whole blood pools were spiked with six different concentrations of HBV, HCV and HIV-1 (each concentration was tested in 24 replicates) and adjusted to the plasma volume in each mini-pool. After spiking, the plasma from one ml of whole blood from each pool was frozen at –40°C. The residual volume of the mini-pools (14 ml) was divided into two aliquots and stored at room temperature and 4 °C–8 °C for additional time points. Plasma was taken from each mini-pool after 24h, 48h, 72h and 168h, respectively. All plasma samples were extracted by the automated Chemagen extraction system and investigated by the German Red Cross NAT system for HBV, HCV and HIV-1.
Results: The analytical sensitivity remained stable from day 0 to day 7 independent of storage conditions (room temperature or 4 °C–8 °C) for HBV, HCV, and HIV-1, respectively.
Conclusions: Whole blood storage up to seven days in EDTA sample tubes did not influence the analytical sensitivity of transfusion-transmitted relevant viruses listed in annex II list A 79/98/EU in combination with the extraction and amplification method of the German Red Cross Baden-Württemberg – Hessen. These data enable an extension of the pre-analytic time period before centrifugation up to 72h at room temperature without increasing the residual transfusion- transmitting infectious risk for blood components, and gives blood banks more flexibility to organize screening processes especially on public holidays.

V 5.2

Evaluation of the Chemagen Viral DNA and RNA Extraction Combined with GFE Blut HIV/HCV/HBV Real-Time NAT for Rapid Automated Screening of Blood Donations

Legler T.J.[1], Holzhausen S.[1], Weis C.[2], Schuller A.[2], Roth W.K.[2]

[1]Universitätsmedizin Göttingen, Abt. Transfusionsmedizin, Göttingen, D, [2]GFE Blut mbH, Frankfurt a.Main, D

Purpose: We were interested if we can replace the current HIV/HCV/HBV NAT (COBAS AmpliScreen, Roche Diagnostics, Mannheim, Germany) by the combination of a fully automated nucleic acid extraction system and real-time NAT. Since the fully automated systems from Roche and Chiron are not cost-effective in most blood-centres of university hospitals, manual nucleic acid extraction is still frequently applied and cost-effective automated alternatives have to be developed.
Methods: We evaluated the automated Chemagic viral DNA/RNA kit in conjunction with the Magnetic Separation Module I (Chemagen AG, Baesweiler, Germany) and used novel HIV/HCV/HBV testkits (GFE Blut mbH, Frankfurt a.m., Germany) for subsequent real-time NAT. Plasma-Pools from 24 donations were tested using the 4.8 ml protocol (200 microliter per single donation). We spiked 1,029 pools with defined concentrations of reference material and tested 100 pools negative for the viruses. Results were compared with detection limits published for the AmpliScreen assay.
Results: The assays were highly specific. A 95 percent detection limit of 35 IU per mL of pooled single donation was determined for HCV, 22 IU per mL for HBV and 1,725 IU per mL for HIV-1, respectively. The detection limits are comparable (HIV-1) or even better (HCV and HBV) than the published detection limits for AmpliScreen (504 IU per ml for HCV, 120 IU per mL for HBV, 1,470 for HIV-1). HCV genotypes 1-6, HBV genotypes A-G and HIV-1 genotypes A-H and O were detected. Most notably we can screen up to 240 donations (10 pools) and two controls within 3.5 hours with the new automated procedure, whereas the current process takes 6.5 hours for the same number of specimen.
Conclusions: First data demonstrate a good performance of this novel automated workflow for virus-screening in smaller donation centers. In the next phase we will determine the robustness of the system in the daily routine.

V 5.3

Natural Course of Primary Cytomegalovirus Infections in Blood Donors

Ziemann M.[1], Unmack A.[2], Steppat D.[3], Juhl D.[2], Görg S.[2], Hennig H.[2]

[1]Universitätsklinikum Schleswig-Holstein, Institut für Transfusionsmedizin, Lübeck, D, [2]Universitätsklinikum Schleswig-Holstein, Lübeck, [3]Universitätsklinikum Schleswig-Holstein, Kiel, D

Purpose: The course of primary human cytomegalovirus (CMV) infections in immunocompetent individuals is important for the identification of possibly infectious blood donors.
Methods: Monitoring of healthy blood donors primarily seropositive for CMV antibodies for CMV DNA in plasma identified 13 subjects with ongoing primary CMV infection. They were asked for CMV-related symptoms and the possible source of infection. Urine and blood samples were monitored regularely for CMV antigens, CMV DNA, parameters of humoral immune response against CMV and unspecific markers of organ function and infectious disease.
Results: After presentation of some preliminary data at the annual congress of the DGTI in 2007, this abstract summarizes the final results after termination of the study. CMV antigens and CMV DNA were detectable in peripheral blood for up to 54 and 269 days, respectively, and in urine for more than 420 days. Clearance of CMV DNA from peripheral blood correlated with clearance of CMV IgM antibodies, development of IgG antibodies against the CMV membrane glycoprotein gB, and development of high avidity CMV IgG antibodies. 85% of subjects with primary CMV infection, but even 69% of matched controls reported possibly CMV-related symptoms during the last 6 months prior to seroconversion. One donor developed a febrile illness accompanied by increased levels of CMV DNA in peripheral blood 2 to 3 weeks after seroconversion.
Conclusions: The mostly subclinical but prolonged course of primary CMV infections could explain residual cases of transfusion-transmitted CMV infections. Therefore, blood from newly seroconverted donors should not be used for severely immunocompromised patients.

V 5.4

Mechanism of Transfusion-Related Acute Lung Injury Induced by HLA Class II Antibodies

Sachs U.[1], Wasel W.[2], Berghöfer H.[2], Reil A.[3], Bux J.[3], Bein G.[2], Santoso S.[2], Weissmann N.[2]

[1]Justus Liebig Universität, Institut für Klinische Immunologie und Transfusionsmedizin, Giessen, D, [2]Justus Liebig Universität, Giessen, D, [3]DRK-Blutspendedienst West, Hagen, D

Purpose: Transfusion-related acute lung injury (TRALI) is a serious, frequently fatal side effect of transfusion. In the majority of cases, antibodies against HLA class II antigens are present in the implied donor plasma. Since HLA class II antigens are not expressed on the surface of neutrophils or lung endothelium, the mechanism by which these antibodies precipitate TRALI in the recipient is currently unclear.
Methods: The ability of sera containing HLA class II antibodies to stimulate HLA-matched or non-matched monocytes was investigated. Supernatants from stimulated monocytes were used (1) in a functional endothelial barrier

assay studying the transfer of FITC-labelled albumin in the absence and presence of neutrophils and (2) in an ex vivo rat lung model studying their ability to induce TRALI. In addition, HLA class II-induced TRALI was mimicked in the rat lung model by the use of monocytes, neutrophils, and plasma before and after IgG depletion.
Results: Donor sera were capable of activating HLA class II-matched monocytes as demonstrated by the release of IL-8, GRO-alpha, and TNF-alpha. Supernatant from matched, but not from non-matched monocytes, induced a sharp increase in endothelial permeability in the presence, but not in the absence of neutrophils. Suppression of ROS production prevented this effect. In the rat lung model, only matched supernatants induced a TRALI reaction if the rats had been pre-stimulated with LPS. A combination of monocytes, neutrophils, and plasma induced TRALI even in the absence of LPS. Depletion of IgG from plasma prevented TRALI.
Conclusion: HLA class II antibodies are capable of inducing TRALI by three step-mechanism involving monocytes, neutrophils, and the endothelium. Activated monocytes release cytokines that induce a neutrophil response, which includes release of ROS. These mediators initiate a breakdown of the endothelial barrier, resulting in acute lung edema as the hallmark of TRALI.

V 5.5

High-Throughput Screening for Granulocyte-Specific Antibodies in Blood Donors

Nguyen X.D.[1], Dostmann N.[2], Flesch B.[3], Dengler T.[4], Klüter H.[5]

[1]Medical Faculty Mannheim, Heidelberg University, German Red-Cross Blood Service of Baden-Württemberg - Hessen, Institute of Transfusion Medicine and Immunology, Mannheim, D, [2]Medical Faculty Mannheim, Heidelberg University, German Red-Cross Blood Service of Baden-Württemberg - Hessen, Mannheim, D, [3]Institute of Transfusion Medicine, University Hospital Schleswig-Holstein, Campus Kiel, D, [4]Institute of Transfusion Medicine, German Red-Cross Blood Service of Baden-Württemberg - Hessen, Baden-Baden, D, [5]Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, University Heidelberg, Mannheim, D

Background: Granulocyte associated antibodies can cause allo- or autoimmune granulocytopenia. Since granulocyte allo-antibodies can lead to severe pulmonary transfusion reactions (TRALI) they attracted attention recently. However, investigation of a large number of blood donor samples using the standard granulocyte immunofluorescence (GIFT) and granulocyte agglutination test (GAT) proved to be difficult to perform due to the time consuming process and the large number of test cells required. Here we describe the novel Flow-GIFT method, (flow cytometric granulocyte immunofluorescence), which allows rapid detection of granulocyte antibodies using automation in pipetting of samples and flow cytometric analysis. We investigated the prevalence of granulocyte antibodies in female blood donors with this novel method.
Methods: Out of 1140 sera collected from female blood donors, 697 (61.1%) were from females with a history of pregnancy. For testing, MNC from two HNA-typed donors were isolated using cell sedimentation in a ficoll density gradient. Subsequent pipetting steps into 96-deep well plates were automated using the machine Biomek NXp Workstation. Antibody binding to test cells was detected using FITC-conjugated antibodies and analysed on the flow cytometer FC 500 MPL. 7-AAD was used to exclude dead cells. Standard GIFT and GAT were also performed as reference methods. For the detection of HLA class I and II IgG antibodies, AB screen ELISA assay was used.
Results: In 169 (24.2%) of 697 females with a history of pregnancy, specific antibodies against granulocyte-antigens (n= 10; 1.43%), HLA class I (n= 105; 15.1%), HLA class II (n= 25; 3.6%) and HLA class I as well as class II (n= 29; 4.2%) could be detected. However, only in 39 of 443 (8.8%) females without history of pregnancy antibodies against granulocyte-antigens (n=3; 0.68%) and HLA class I (n= 38; 8.5%) were detectable. The granulocyte-antibodies in females with history of pregnancy were determined as anti-HNA-1b (n=1), anti-HNA-2a (n=4), anti-HNA-3a (n= 2); anti-CD16 (n=2), one antibody with unclear specificity. Interestingly, in 3 females without history of pregnancy, granulocyte antibodies with the specificities anti-HNA-1b (n=1) and anti-CD16 (n=2) could also be detected.
Conclusion: The automated Flow-GIFT permits rapid and simple detection of granulocyte antibodies while requiring less donor test cells in comparison to current methods. Thus this high throughput method potentially opens the way for screening of granulocyte antibodies in large donor populations in order to prevent TRALI due to transfusion of FFP and platelet apheresis products effectively.

V 5.6

Screening of Whole Blood and Platelet Apheresis Donors on Presence of HLA- and HNA Antibodies in View of TRALI under Consideration of Gender, Pregnancy and Transfusion Anamnesis

Carbol A., Marandiuc D., Runkel S., Hitzler W.E.

Universitätsmedizin Mainz, Transfusionszentrale, Mainz, D

Purpose: The availability from HLA- and HNA antibodies preferentially in therapeutic plasma or plasma-containing products like platelet concentrate can lead in rare cases to the serious complication of a transfusion-related acute lung insufficiency (TRALI). Pregnancies, transfusions and transplants are known as sensitising events for formation of HLA- and HNA antibodies. Against the background of the discussed TRALI the fraction of HLA- and HNA antibody positive donors from Transfusionszentrale Mainz was researched. Correlation with pregnancy and transfusion had to be examined. Finally implementation of a high throughput method in routine testing should be proved being able to move the recommendation of the National Advisory Comittee “Blood” and the upcoming graduated plan (Stufenplan) from the Paul-Ehrlich-Institut (PEI) to test women with positive pregnancy anamnesis for HLA Class I + II Ab and HNA-1a, -1b, -2a, -3a Ab.
Methods: 891 test samples of donations (224 platelet apheresis and 667 whole blood donors) were collected and a questionnaire was filled to the pregnancy and transfusion anamnesis. The samples were examined with the LABScreen Mixed HLA Class I + II + HNA (1a,1b,1c,2a) by the Luminex 200 Flow-Analyzer using the xMAP technology with a Normalized Background Value of 1.5 as cutoff.
Results: The median age of the donors was 33 years. In 38,6% (344/891) of the donors we found HLA and/or HNA-antibodies (43,4% female and 36,3% male). HLA antibodies were detected in 21,1% (188/891) and HNA antibodies in 22,3% (199/891). From the sensitised group 53% (43/81) female donors with pregnancy anamnesis and 58,8% (10/17) donors with transfusion anamnesis were positive for antibodies. Also 31,0% (246/793) unsensitised donors within 17,2% (103/600) of the male donors without immunogenes events showed a presence of antibodies against HLA and 21,8% (131/600) against HNA.
Conclusions: In 53% (43/81) of the female donors with pregnancy anamnesis and in 59% (10/17) anticipated Ab (HLA and/or HNA) could be proved. After the new recommendation for risk minimisation in view of TRALI their plasma should furthermore not used as therapeutic plasma. Unexpected was the high percentage of 31,0% (246/793) unsensitised donors with positive antibody detection especially by male donors. Where they have there antibodies from, which role does they play or if they are an artefact of high sensitivity has to be further investigated. According to our data the scientific basis of the graduated plan of the PEI excluding plasma from woman with a positive pregnancy anamnesis is doubtful and should be reevaluated.

V 5.7

Detection of Leukocyte Antibodies by a Rapid Whole Blood Flow Cytometric Assay for the Prevention of TRALI

Hoyer P.[1], Büttner A.[2], Borowsky A.[2], Grabowski C.[2], Kroll H.[2]

[1]Red Cross Blood Transfusion Service NSTOB, Dessau, D, Institute for Transfusion Medicine Dessau, Dessau-Roßlau, D, [2]Dessau-Roßlau, D

Purpose: Screening of blood donors with a history of pregnancy or transfusion for antibodies (abs) against human leucocyte antigens (HLA) and neutrophil antigens (HNA) may help to prevent immune transfusion related acute lung injury (TRALI). Here, we describe a whole blood flow cytometric assay which allows rapid detection of relevant antibodies.
Methods: Thirty microlitres EDTA-anticoagulated whole blood from selected donors were lysed with 200 µL NH4Cl lysing solution for 7 min at 4 °C in a microtiter plate. After one wash step with PBS the white cells were incubated with 10 µL donor plasma containing well defined HNA or HLA abs for 30 min at 37 °C. Following two washing steps, bound abs were detected with 20 µL of FITC-conjugated rabbit antihuman IgG or IgM for 20 min at 20 °C. At least 10.000 white blood cells were analysed by flow cytometry using a FACSCanto™II (BD Biosciences). A mean fluorescence intensity ratio (donor plasma/control plasma) above 2 was defined as positive. Microscopic granulocyte immunofluorescence and aggregation tests were used as reference methods.
Results: Abs against HNA-1a, -1b, and -2a reacted clearly with granulocytes but not with other leukocytes. In contrast, anti-HNA-3a showed the strongest reactions with lymphocytes and weaker reactions with granulocytes. Anti-HNA-4a reacted positive with granulocytes and stronger with a lymphocyte

subpopulation. HLA-class I abs reacted strongly with lymphocytes and weakly with granulocytes whereas HLA-class II abs showed reactivity only with B-cells. To compare the sensitivity of flow cytometric and microscopic assays titrations with selected abs were performed. The titers of anti-HNA-3a were 64 vs. 16 and of anti-HNA-1b were 128 vs. 8, indicating higher sensitivity of the flow cytometry assay. An anti-HNA-1a of IgM class could be detected using an anti-IgM conjugate. Exclusion of avital 7-AAD-stained cells in some experiments did not improve the sensitivity.
Conclusions: First experiences with our whole blood flow cytometry assay showed sensitive detection of HLA- and HNA-specific abs. Compared to standard tests this technique requires only low amounts of donor cells and has the potential of fast and large scale analysis.

V6 Preparation of Blood Components, Pathogen Inactivation

V 6.1

Procedures to Reduce the Risk of Immune TRALI by Systematic HNA and HLA Antibody Screening in Platelet and Plasma Apheresis Donors

Schmidt J.-P.[1], Jorks S.[2], Grabowski C.[2], Borowsky A.[2], Kroll H.[2]

[1]Institute for Transfusion Medicine Dessau, Red Cross Blood Transfusion Service NSTOB, Blood donation, Dessau, D, [2]Institute for Transfusion Medicine Dessau, Red Cross Blood Transfusion Service NSTOB, Dessau, D

Purpose: Immune transfusion related acute lung injury (TRALI) is caused by HNA- or HLA-antibodies present in donor plasma. Exclusion of donors only on the basis of an anamnestic immunizing event (pregnancy or transfusion) would lead to a considerable loss of donors without risk factors. Selection on the basis of antibody status seems rational. However, experience with or consensus about suitable techniques and a "safe exclusion threshold" in prospective screening is limited. We report on our strategy to reduce TRALI risk by different screening assays and the resulting drop out rate of apheresis donors.
Methods: A total of 537 apheresis donors were interviewed for previous pregnancies and transfusions. Donors with an immunizing event were analyzed by lymphocytoxic test (LCT), beta2 microglobulin-specific enzyme immunoassay (MAIPA), bead array technique (HLA class I and II, Tepnel), enzyme immunoassay (EIA, Biotest AbScreen HLA class II), granulocyte immunofluorescence test and granulocyte agglutination test. MAIPA (HLA class I) and EIA (HLA class II) were compared to the bead array. The results from identical samples were compared to define the cutoff for donor exclusion.
Results: From 537 donors (252 male, 285 female) 209 (38.9%) reported a history of immunization: male: 11 (4.4%) transfusion; female: 165 (57.9%) pregnancy, 5 (1.8%) transfusion, 28 (9.8%) pregnancy and transfusion. Antibody screening was negative in all tests in 126 (60.3%) donors. Antibodies were detected in two men after transfusion and in 81 women (68 pregnancy, 13 pregnancy and transfusion): Anti-HLA class I: 34, class II: 22, class I and II: 26. Most antibodies were detected in the bead array either alone or in combination with MAIPA (14) or EIA (19). Two donors showed positive reactions only in LCT (Anti-HLA-B17 and B61). One granulocyte-specific antibody (anti-HNA-1b) was found in a female donor. For HLA class I antibodies an adjusted fluorescence ratio cutoff of 15 in bead array or optical density cutoff of 0.4 (MAIPA) were used for exclusion. HLA class II and HNA antibodies detectable in either method led to donor exclusion. By using these criteria 58 (27.8%) of our apheresis donors with immunizing event and 10.8% of our total donor population had to be excluded. With completely negative test results 83 (15.5%) would drop out from donation.
Conclusions: HNA and HLA antibody screening allows a rational exclusion of apheresis donors who carry a higher risk of immune TRALI. Since the quantity of antibodies which is necessary to induce TRALI is completely unclear, we propose to use a threshold which accepts very weak HLA class I antibodies. Using this strategy the number of dropouts of donors is acceptable.

V 6.2

Efficacy and Side Effects of Granulocyte Mobilization and Collection in Healthy Donors

Sütterlin F.[1], Kramer M.[2], Blechschmidt M.[2], Zimmer K.[2], Ehninger G.[2], Bornhäuser M.[2], Hölig K.[2]

[1]Universitätsklinikum Carl Gustav Carus, Medizinische Klinik und Poliklinik I, Dresden, D, [2]Dresden, D

Purpose: Collection of peripheral blood stem cells (PBSC) has become a routine procedure, but mobilization and collection of granulocyte concentrates (GC`s) are less commonly performed. In a retrospective analysis a database of granulocyte donors was established and analysed regarding factors predicting the efficacy of GC collection and the side effects of this procedure.
Methods: 378 related donors (221 males, 157 females) underwent 841 GC collections between 4/1999 and 12/2007. The donors received rhG-CSF (lenograstim) at a median dose of 5.6 µg/kg with or without 4mg dexamethasone. Demografic data and laboratory values of the donors were correlated with the granulocyte yield in the products. Side effects were recorded by donor monitoring and interview (questionnaire).
Results: The median granulocyte yield in the apheresis products was 7.78×1010 (3.07×1010–16.8×1010). We observed significant correlations between donor sex, weight, height, regular smoking status and baseline leukocyte count and the granulocyte content of GC`s. Donor age had no influence. Mobilization regimen with dexamethasone had the most significant impact on granulocyte yield (median yield with dexamethasone: 8.47×1010, without dexamethasone 6.65×1010; p=0.000). Multivariate regression analysis included weight, height, baseline neutrophile and platelet count, dexamethasone-application and smoking. Side effects of granulocyte mobilization were generally mild (bone pain in 31.4%, headache in 19.6%, fatigue in 15.7% of the donors). The most common side effects occurred during the apheresis: paresthesia (32.3%), circulatory disturbance (2.3%) and difficult venous access (4.3%) were observed. Central venous access was never required in granulocyte donations. During the follow up period pruritus and/or exanthema were reported by 17.6% of the donors. Administration of dexamethasone had no impact on the incidence of side effects.
Conclusions: Granulocyte mobilization with a dose of 5.6 µg/kg lenograstim proved to be effective to collect adequate cell doses for the treatment of adult patients. Administration of dexamethasone in addition to rh-G-CSF results in an improvement of granulocyte yield. Side effects of G-GCF administration and leukapheresis were tolerable and less pronounced than in PBSC donors. Reversible HES-associated pruritus is a typical side effect of GC donation and has to be mentioned in informed consent. No serious adverse events occurred during G-CSF-administration or leukapheresis. Further monitoring of healthy GC donors remains useful to establish optimal standards for granulocyte mobilization and collection.

V 6.3

Venous Access Problems (VAP): Incidence and Characteteristics of this Most Common Untoward Event during Preparatory Plasmapheresis

Diekamp U.

Haema AG, Leitende Ärztliche Person, PMS-Zentren, Bremen, D

Plasmapheresis donors may donate twice weekly. Blood flow requirements during the phases of separation (100 ml/min.) and return (130 ml/min.) mandate a large bore intravenous cannula (16 G) for adequate access to the donor's antecubital vein. Use of such needles can result in a set of difficulties encountered in every plasmapheresis centre. For a better understanding of this problem we analyze the VAP observed in 2008.
Methods: In 2003, we establish an untoward events-documentation system in six of our donor centres. It is updated January 1, 2008 to document all mishaps during and after donation, distinguishing local from systemic and from technical problems and grading the former two by severity. We review all events periodically.
Results: The table shows our results.

parameter	n	%	.	VAP-category	n	% of donat.
plasma donations	286,081		.	repeat venous puncture	4.371	1.528
VAP (% donations)	5,288	1.85	.	mild hematoma	631	0.221
broken off donat. (% donat.)	2,701	0.94	.	moderate hematoma	96	0.034
plasma donors (PD)	18,679		.	severe hematoma	23	0.008
PD with VAP (% PD)	3,679	19.70	.	mild pain/spasm	149	0.052
PD with >1 VAP (% PD)	967	5.18	.	moderate pain/spasm	16	0.006
PD with broken off donat. (% PD)	1,987	10.64	.	severe pain/spasm	3	0.001
mean donations / PD	15.32		.	arterial puncture	1	<0.001

Discussion: VAP occur at the start of plasmapheresis as outright unsuccessful veni-punctures or later on during the procedure, when - while the donor contracts his fore-arm musculature in an attempt to improve blood flow – the cannula slides out of the vein or when the venous wall no longer withstands the changing pressures. 82.6% of VAP lead to repeat venipuncture. Due to VAP 0.94% of collections are ended early with substantial economic impact: the plasma centre incurs the donation cost without obtaining a marketable product. Hematomas and pain at the venipuncture site are much less common and infrequently lead to significant donor discomfort or morbidity.
Conclusions: 1.85% of preparatory plasmapheresis procedures are associated with VAP. Severe donor morbidity occurs at a rate of 0.009%.

V 6.4

Routine Quality Control of Platelet Apheresis Concentrates Determined by Multiple Electrode Aggregometry MEA – Experience of 18 Months

Wittmann G.[1], Berghammer B.[2], Romann A.[2], Giebl A.[2], Jambor C.[2], Wagner B.[2], Grützner S.[2], Spannagl M.[2]

[1]Klinik f.Anästhesiologie Klinikum der Universität München, Abteilung f. Transfusionsmedizin und Hämostaseologie, München, D, [2]München, D

Purpose: Currently the only mandatory tests for apheresis concentrates (APHC) by the German or European guidelines are volume, plt count and content, residual leukocytes, swirling and pH measurement. A test for the functional capacity of platelets (PLT) which is applicable for routine analysis is desirable. Currently there is no golden standard procedure for this application. This study describes the launch and follow-up of multiple electrode aggregometry (MEA, Multiplate analyzer) as a routine quality control (RQC) of APHC in our institution.
Methods: Standard MEA analysis was adapted for the analysis of platelet concentrates: 150 µl of the undiluted concentrate were added to 450 µl of PBS buffer. After 3 minutes incubation 3.2 or 6.4 or 9.6 µg collagen / ml were added as the trigger. Aggregation is recorded for 8 min and quantified by the area under the curve (AUC). In a first series from 01-04/2007 81 APHC were included. From 0 (d0) until day 7 (d7) concentrates were tested: volume d0, plt count and content d0+d7, residual leukocytes d0, swirling, ph measurement and MEA with 6.4 µg/ml collagen on d0,d1,d2,d3,d4,d6+d7. In a second series from 05/2007–11/2008 221 APHC were tested on d0 and d7.
Results: The MEA measurements showed a continuous decline from an AUC of 1.059+/–246 (mean+/–sd) at d0 to 116+/–54 on day 4. Findings of the first 81 APHC and the second series of 221 APHC showed a good agreement.
Conclusions: We present a simple measurement technique for the functional capacity of PLT during the shelf-life of APHC in addition to the standard mandatory tests. Unlike other proposed techniques the MEA based assay is neither laborious nor time-consuming and can be done in parallel to the routine work.

V 6.5

Influence of Prestorage Leukoreduction and Subsequent Day +14 Irradiation on In Vitro Red Blood Cell (RBC) Storage Variables of RBCs in the Additive Solution SAG-M

Zimmermann R.[1], Wintzheimer S.[2], Weisbach V.[2], Strobel J.[2], Zingsem J.[2], Eckstein R.[2]

[1]University Hospital Erlangen, Transfusion Medicine And Hemostaseology, Erlangen, D, [2]University Hospital Erlangen, D

Purpose: There exists only very few data on in vitro and in vivo effects of gamma irradiation of red blood cells (RBCs) that have been leukoreduced by filtration before a subsequent irradiation on day +14. Reported studies reflect neither the current Food and Drug Administration (FDA) nor the European recommendations on timing of irradiation and subsequent storage.
Methods: We studied 40 RBC units that were prepared from inline filtered whole blood and 40 RBC units that were filtered after component separation. All RBCs were leukoreduced on the collection day and stored subsequently in the additive solution saline-adenine-glucose-mannitol (SAG-M). In both groups, 20 components were irradiated on Day +14 with 30 Gy, and 20 served as nonirradiated controls. In vitro evaluation of both irradiated and nonirradiated RBC units was performed before and after irradiation on Days +1, +7, +14, +21, +28, +35, and +42 from the collection day.
Results: Gamma irradiation induced enhanced leakage of potassium ions and lactate dehydrogenase and an enhanced in vitro hemolysis rate in the irradiated components. However, in vitro hemolysis rate of both nonirradiated and irradiated components was remarkably lower than 0.8 percent, and the preservation of adenosine triphosphate over 42 days was satisfying.
Conclusions: This study was the first presenting data on the influence of gamma irradiation on day +14 on RBCs that had been leukoreduced by filtration on the collection day. Our findings together with recent results of other investigations on the effect of gamma irradiation on leukoreduced RBCs allow the proposal that a storage time up to 28 days after irradiation is allowable.

V 6.6

Double-Dose Platelet Concentrates Prepared from Pools of 7 or 6 Buffy Coats for Pathogen-Inactivation with the Intercept System

Henschler R.[1], Wiesneth M.[2], Pfeiffer H.-U.[3], Müller M.[4], Andresen S.[5], Krämer A.[5], Irsch J.[5], Lin L.[5], Corash L.[5], Schrezenmeier H.[2], Seifried E.[6]

[1]Institute for Transfusion Medicine and Immune Hematology, DRK Blutspendedienst Baden-Württemberg - Hessen gGmbH, Frankfurt / Main, D, [2]Institute for Transfusion Medicine, DRK BLutspendedienst Baden-Württemberg - Hessen gGmbH, Ulm, D, [3]Institute for Transfusion Medicine and Immune Hematology, DRK Blutspendedienst Baden-Württemberg - Hessen gGmbH, Frankfurt, D, [4]Institute for Transfusion Medicine, DRK BLutspendedienst Baden-Württemberg - Hessen gGmbH, Frankfurt, D, [5]Cerus Inc., Concord, USA, [6]Institute for Transfusion Medicine and Immune Hematology, German Red Cross Blood Donor Service, Frankfurt, D

Purpose: The Intercept Blood SystemTM is a technology for the pathogen inactivation of platelet components (PC) that has been implemented in more than 50 European blood centers. Implementation of the Intercept technology is feasible in component processing laboratories and the procedure requires limited additional handling steps to produce a pathogen-inactivated blood component. Processing results in loss of 8 to 12% of platelets. A novel dual-bag disposable set for the production of two pathogen-inactivated therapeutic platelet doses from a single Intercept treatment recently received CE Mark registration. This approach will reduce labor, cost, and platelet loss per therapeutic dose. To increase the efficiency for production of platelet components treated with PI procedures in which 6 or 7 buffy coats (BC) can be pooled to produce a double dose PC treated in a single inactivation step to provide two therapeutic PC.
Methods: The volume of the BC was optimized to comply with the guard-bands for the Intercept procedure. The volume settings for the Compomat (Fresenius) were adjusted to 49±2mL (mean±SD) per BC for pools of 7, and to 46±2mL (mean±SD) for pools of 6BC. The volume guard bands for the Intercept procedure are 300–420mL for 2.5–7.0x10E11 platelets with a 32–47% plasma concentration and < 4×10E6/mL for residual erythrocytes.
Results: The volume of the pools achieved with this approach was 600±6 mL for 7BC and 542±4.5 mL for 6BC respectively after the addition of additive solution (InterSolTM). The hematocrit was 21±0.9% and 16.6±1.7%

and the total plt dose (mean±SD) was 7.3±0.7×10E11 and 5.7±0.3×10E11 for 7BC (n=23) and n=8 (n=6), respectively. After centrifugation and leukofiltration the platelet concentrates had volumes of 389±16mL with a plasma content of 42±1% (pool of 7BC) and 374±8.9mL with 38.9±1.4% plasma (pool of 6BC). The mean total platelet number was 5.6±0.5x10E11 and 4.8±0.4x10E11 respectively. The RBC contents also passed the requirement for Intercept guardbands.
Conclusions: Here we demonstrate the possibility to produce double dose PC from pools of Buffy Coats generated from 7 or 6BC. While we were successful when pooling 7 buffy coats, which on average contained 5.6×10E11 platelets and could thus result in 2 therapeutic units after splitting, we need to further optimize the procedure for the generation of a pool of 6 buffy coats. These results demonstrate that the pooling of up to 7BC is a feasible approach to generate a double dose PC product ready to be used with the Intercept dual bag set and thus will help to reduce cost, cell loss and labor per therapeutic unit.

V7 Therapeutic Hemapheresis, Cellular Therapeutics

V 7.1

A Novel Photopheresis Technique for Children and Critically ill Patients with Extracorporeal Photopheresis Contraindications

Hackstein H.[1], Misterek J.[2], Nockher A.[2], Reiter A.[2], Bein G.[2], Woessmann W.[2]

[1]University of Giessen, Clinical Immunology and Transfusion Medicine, Giessen, D, [2]University of Giessen, D

Background: Conventional extracorporeal photopheresis (ECP) has proven efficacy for the treatment of graft-versus host diesease (GVHD), cutaneous t cell lymphomas and acute heart allograft rejection but is limited to patients with sufficient body weight. We have developed a novel simplified photopheresis technique called mini buffy coat ECP that allows treatment of small children and patients with apheresis contraindications.
Study
Design and Methods: Leukocyte-rich buffy coat fractions were prepared from 5–8 ml/kg whole-blood donations in a closed-system, diluted and ultraviolet A (UVA)-irradiated after addition of 8-methoxypsoralen (8-MOP). Apoptosis and cell death were analysed by AnnexinV and 7-aminoactinomycin staining (7-AAD). Lymphocyte proliferation was measured after CD3/CD28 and phytohemagglutinin (PHA) stimulation. Autologous residual blood and UVA-irradiated buffy coat were returned to the patients. 56 mini buffy coat ECP procedures were applied to three children with acute steroid-refractory skin GVHD and apheresis contraindications.
Results: Average whole blood and buffy coat volumes were 166ml (±61.8) and 8 ml (±1.6), respectively and resulted in a hematocrit of 2.2% (±0.4) after saline dilution (median±SD). UVA irradiation of 8-MOP buffy coat preparations resulted in significant induction of leukocyte apoptosis at 48h and 72h ($p \leq 0.001$). Leukocyte proliferation was significantly inhibited both after CD3/CD28 stimulation and after PHA stimulation when compared to controls ($p \leq 0.001$). No clinical or laboratory side effects were observed during mini buffy coat ECP procedures and the three patients responded to the therapy.
Conclusion: Mini buffy coat ECP induces comparable rates of apoptosis and lymphocyte proliferation inhibition as conventional ECP. We propose mini buffy coat ECP as a simple and inexpensive alternative to classical ECP in children and adult patients with apheresis contraindications.

V 7.2

Immunoadsorption – Optimal Anticoagulation?

Rummler S.

Universitätsklinikum Jena, Institut für Transfusionsmedizin, Jena, D

Purpose: One of the main problems of extracorporeal treatment regimens is the high thrombogenicity of all of these systems. Our aim is to provide patients with optimal, effective and individual anticoagulation treatment that is low in side effects.
Methods: We assessed 613 immunoadsorptions (IA) in 58 patients aged from 6 to 72 years (33 female and 25 male patients). 45% of all IA were conducted on patients before and/or after a solid organ transplant, and 46% on patients with autoaggressive disorders. The whole blood was separated mainly through peripheral venous access, using a Cobe spectra (CaridianBCT) separator. Anticoagulation was basically administered with ACD-A in a concentration ranging from 1:13 to 1:16 or a combination of ACD-A and Heparin (0.5 IU/ml whole blood as bolus and 0.125 IU/ml hourly to the end of treatment and citrate [1:25]), without exceeding the recommended maximum ACD-A infusion rate of 1.2 ml/min/l of total blood volume (TBV). IA were performed on three consecutive days or on alternate days; they lowered the immunoglobulin level by more than 90% compared with the baseline value.
Results: 0.71% of our patients presented with clinical signs of hypocalcaemia. At no time were there any incidences of bleeding complications or respectively clotting in the extracorporeal circulation.

Parameter	Therapy	Mean	Min - Max
Serum citrate concentration in mmol/l (n- 50)	before	0.29	0.11 - 0.90
	after	0.86	0.3 - 1.87
ACD-A use in ml		835	563 - 1196
Duration of procedure in min		318	204 - 440
Total Ca in mmol/l [standard range 2.20–2.65]	before	2.24	1.34 - 2.56
Ionized Ca (iCa) in mmol/l [standard range 1.15–1.29]	before	1.11	0.74 - 1.35
	after	0.99	0.58 - 1.23
Substitution iCa in mmol/l per h	during	0.93	0.56 - 1.12
Intact parathormone in ng/l [standard range 17.3–72.9]	before	94.86	28.50 – 357.00
	after	140.13	32.70 – 457.00

Conclusion: An effective and individually controlled anticoagulation treatment is possible.

V 7.3

Homing Receptor Repertoire of ex vivo-Expanded Mesenchymal Stromal Cells (MSCs) Shows Mimicry of Pathways Used by B- and T-Lymphocytes, Dendritic Cells and Phagocytes

Deak E.[1], Ciuculescu F.[2], Giesen M.[2], Seifried E.[3], Hartmann T.[4], Henschler R.[3]

[1]German Red Cross Blood Donor Service, Institute for Transfusion Medicine and Immune Hematology,, Frankfurt am Main, D, [2]Institute for Transfusion Medicine and Immune Hematology, Frankfurt am Main, D, [3]Institute for Transfusion Medicine and Immune Hematology, German Red Cross Blood Donor Service, Frankfurt, D, [4]Laboratory for Immunological and Molecular Cancer Research, University of Salzburg, A

Background: Intravenously applied Mesenchymal Stromal Cells (MSCs) are being increasingly used in preclinical and clinical studies. Examples for clinical uses of i.v. adminstered MSCs include (1) suppression of immune reactions such as Graft versus Host Disease, (2) integration into tumor microvasculature to modulate tumor angiogenesis, and (3) improvement of clinical outcome in patients with severe sepsis. Here we wished to investigate adhesion receptors normally used by immune cells subsets to mediate their tissue specific egress, which i.v. administered MSCs might employ during their homing to tissues.
Methods: Human MSCs were isolated from normal healthy donors and characterized using differentiation assays, RT-PCR and flow cytometry. Rolling, adhesion and transmigration were determined under shear stress in parallel plate flow chambers that were precoated with endothelial cells or recombinant adhesion receptor ligands.
Results: MSCs showed functional P- and E-selectin ligands, whereas L-selectin binding was hardly detected, indicating a T helper type repertoire of rolling receptors. Of integrin receptors, beta1 integrins were highly expressed, whereas the beta2 integrins LFA-1 and Mac1 were present only in low amounts. Unexpectedly, MSCs expressed a plethora of chemokine receptors of both the CCR and the CXCR families. In shear stress assays using laminar flow chambers, MSCs strongly reacted with rolling, arrest and transendothelial migration upon contact with chemokine ligands for CCR7, the lymph node homing receptor for naive T- and B lymphocytes and dendritic cells. Also, the lymph node entry receptor for B cells, BLR/CXCR5, was expressed and activated on MSCs upon stimulation with its cognate ligand CXCL13, to arrest MSCs. Moreover, MSCs arrested effectively through interleukin-8 receptors and the phygocyte activating chemokine,

CCL15. Arrest and subsequent transendothelial migration were blocked after pretreatment of MSCs with the G alpha I inhibitor pertussis toxin and the Rac GTPase specific inhibitor NSC23766, confirming active involvement of chemokine induced G protein coupled receptor signalling.
Summary/Conclusions: MSCs show an unexpected profile of adhesion receptor usage when binding endothelial cells under shear stress, employing a multitude of chemokine receptors which are normally specific for either B- or T-lymphocytes, or monocytes or granulocytes. MSCs bind endothelial cells also through E- and P-selectin and beta1 integrins, but show little or no response to L-selectin and beta2 integrins. Our data provide an understanding for previously unexplained homing events of MSCs and show new possibilities for receptor-based graft engineering in MSCs.

V 7.4

A Four-Cytokine Combination Allows Sustained Expansion of Adult Bone Marrow-Derived Erythroid Progenitor Cells

Hintze C.[1], Nilmaer O.[2], Goettig S.[2], Seifried E.[2], Henschler R.[2]

[1]Blood Transfusion Centre of the German Red Cross, Institute of Transfusion Medicine and Immunohaematology, Frankfurt, D,
[2]Blood Transfusion Centre of the German Red Cross, Frankfurt, D

Purpose: Substitution therapy using in vitro generated erythrocytes from stem cell cultures has been discussed as a novel approach to overcome limitations in availability of donor-derived red cell concentrates. We here investigated a recently published protocol (Lodish et al, Nature Medicine 12, 240, 2006) that allows in vitro expansion of adult bone marrow derived hematopoietic stem cells, and asked for its ability to support expansion of primitive progenitors of the erythrocytic lineage and the ability of these progenitors to mature to hemaglobinized and enucleated erythrocytic cells.
Methods: Unseparated murine bone marrow cells were inoculated into IMDM culture medium supplemented with Stem Cell Factor, Thrombopoietin, Insulin-like Growth Factor-1 and Fibroblast Growth Factor-2. Cells were seeded at high density (10E6/ml) and supplemented three times per week with new medium for a total of 21 days. Ensuing cells were analyzed in semisolid colony forming assays for CFU-E and BFU-E formation, via flow cytometry and through morphological analysis on cytospin stains.
Results: After a 10-14 day lag phase, we observed an exponential growth phase of the cultures in two waves, peaking at days 14 and 21 and leading to a mean 30-fold expansion in total cell numbers (n=5). The clonogenicity of the seeded cells remained preserved unchanged during this period, or in the case of day 9-BFU-E colony forming cells increased. This indicated an approximately 25–50 fold amplification of CFU-E and BFU-E erythroid colony forming cells during culture period. Flow cytometric evaluation showed that proportion of CD71+ the cells increased from 32 ± 4% to about 71 ± 13% during weeks 1-3 of the culture. Percentages of Glycophorin A-associated antigen (Ter119) positive cells were found between 31 ± 5 and 48 ± 6% (means ± SD) at start and day 21, respectively, indicating constant production of erythrocytic cells. Erythrocytic colony-forming units showed a high degree of hemoglobinization.
Conclusions: A four-cytokine substituted ex vivo expansion protocol showed a high preservation of cells with a primitive and undifferentiated hematopoietic phenotype, starting from undifferentiated cells and leading to a massive (30 fold) amplification of erythopoietic colony forming cells with a high capacity to hemoglobinize in EPO-containing media. Therefore, the generation of erythrocytic cells from adult progenitors might using this protocol might provide an attractive alternative to genetically manipulated or embryonic cell-derived cultures when generating red blood cells at high numbers in vitro.

V 7.5

Specific Donor Lymphocyte Infusions by Streptamer-Based Selection of Leukemia Antigen-Specific CD8+ T Cells

Schmitt A.[1], Wang X.[2], Chen B.[3], Xu X.[2], Freund M.[2], Schmitt M.[2]

[1]University of Rostock, Internal Medicine III, Rostock, D, [2]University of Rostock, D, [3]Southeast University Clinical Medical School Nanjing, CN

Background: Donor lymphocyte infusions (DLIs) after allogeneic hematopoietic stem cell transplantation have the potential to generate a desirable graft-versus-leukemia (GVL) effect, but bear the risk of eliciting a noxious graft-versus-host disease (GVHD). To improve the GVL effect and to minimize the risk of a GVHD, a positive selection of leukemia (antigen)-specific T cells would be highly desirable. In this study we focused on the leukemia-antigen Wilms Tumor gene 1 (WT1). In patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), a good correlation of WT1 expression and the number of leukemia blasts could be demonstrated. Several groups described immunogenic T cell epitopes derived from the WT1 protein.
Material and Methods: Here, we used the technology of streptamers available on a GMP level to detect the frequency of HLA-A2 restricted CD8+ T cells in the naïve peripheral blood (PB) from both healthy donors (HDs) and AML patients. Such WT1-specific CD8+ T cells were further characterized for the expression of CD27, CD28, CD45RA, CCR7 and CD107a. In the next step, WT1-specific cells were positive selected by MACS columns after labeling with streptamers and thereafter immunophenotyped. Moreover, mixed lymphocyte peptide cultures (MLPCs) were preformed to enrich WT1 specific T cells derived from the PB of HDs. At last, WT1 specific T cells were evaluated in a cytotoxicity assay.
Results: 21 of 40 HDs showed naïve WT1 specific T cell frequencies of 0.5 to 2.0% of all CD8+ T cells. In two of ten AML patients, also 0.4 to 3.6% of WT1-specific T cells could be detected. These cells revealed to be CD8+WT1 _Streptamer+CD28+ CD45RA+CD69-CD107a+CCR7-CD137- effector T cells in flow cytometry. After a maximum of three rounds of MLPC, only a frequency of 2–5% could be achieved, thus demonstrating the power of the streptamer technology. In cytotoxicity assays, WT1-specific CD8+ T cells were able to lyse HLA-A2+WT1+ cells at an effector/target ratio of 20:1.
Conclusion: In summary, the streptamer technology allows to select a pure fraction of WT1-specific effector T cells with cytotoxic properties. In analogy to DLIs specific for viral antigens, production of leukemia specific DLIs is feasible on a GMP level. Further leukemia antigens are currently evaluated by our group.

V 7.6

Collection Efficiency of CD 14+ 16+ Monocyte Subpopulations Collected by the Cobe Spectra and the COM.TEC Cell Separator

Strasser E.[1], Brügel J.[2], Achenbach S.[2], Weiss D.[2], Eckstein R.[2]

[1]Universitätsklinikum Erlangen, Transfusionsmedizinische und Hämostaseologische Abteilung, Erlangen, D, [2]Universitätsklinikum Erlangen, D

Purpose: The CD14+ CD16+ monocyte subpopulation play an increasing role in infection and inflammation (1) and reduction of this monocyte population may be useful in therapeutic apheresis of chronic inflammatory bowel disease (2). The subject of this study was an evaluation of the collection efficiency of two different cell separators to collect CD14+ CD16+ monocytes due to therapeutic apheresis.
Methods: Two studies were performed in non-cytokine stimulated blood donors to compare the monocyte subpopulations of leukapheresis products. The first paired study compared two leukapheresis procedures with the COM.TEC cell separator by use of different instrument settings (n=5, MNC program:1000 rpm vs. 1500 rpm). The second paired study compared the COM.TEC with the Cobe Spectra cell separator (n=6). The instrument settings of the COMTEC separator: MNC program with a centrifuge speed of 1500 rpm. The settings of the Cobe Spectra: WBC program, separation factor 250 (centrifuge speed: 646 rpm). Both cell separators were performed with an inlet blood flow rate of 50 ml per minute. The collection efficiency of monocyte subpopulations of all leukapheresis procedures was calculated. The blood count was performed on the ADVIA® 120 Hematology System (Siemens Diagnostics, Germany). The differentiation of the monocyte subpopulations (CD14 + and CD 14+ CD 16+) were performed on a flow cytometer (FACS Calibur, BD, USA).
Results: Study 1: By use of a lower centrifuge speed (MNC program), we found a significantly lower leukocyte content by use of 1000 rpm vs. 1500 rpm (23x10e9 WBC per L vs. 52,3x10e9 WBC per L, p = 0,02). CE of monocyte populations (1000 rpm vs. 1500 rpm): CD14+CD16- monocytes: 45,0% vs. 75,3%, p=0,17; CD14+CD16+ monocytes: 57,8% vs. 96,8%, p=0,21; CD14-CD16+ leukocytes: 51,1% vs. 68,4%, p=0,45. Study 2: By use of the Comtec (MNC program) and the Cobe Spectra (WBC program) cell separators, we found a nearly equal WBC concentration (39,7x10e9 WBC per L vs. 39,0x10e9 WBC per L, p = 0,95) by use of different donation time (COM.TEC 98min. vs. Cobe Spectra: 127 min., p = 0,046). The CE of different monocyte populations (Cobe Spectra vs. COM.TEC): CD14+CD16- monocytes: 36,2% vs. 66,6%, p=0,30; CD14+CD16+ monocytes: 49,7% vs. 95,6%, p=0,21; CD14-CD16+ leukocytes: 48,7% vs. 72,4%, p=0,21.

Conclusions: All monocyte subpopulations showed a trend to higher collection efficiencies (CE) by use of of the COM.TEC cell separator (centrifuge speed: 1500 rpm) compared to the standard WBC program of the Cobe Spectra cell separator. Thus, we recommend the MNC program of the COM.TEC device with the standard instrument setting to collect monocyte subpopulations.

V8 Hemostasis II

V 8.1

Conformational Signaling of Activated Platelet Integrin αIIbβ3 Variants Expressed in HEK293 Cells

El Khattouti A.[1], Stoldt V.R.[2], Scharf R.E.[2]

[1]Heinrich Heine University Medical Center, Department of Experimental and Clinical Hemostasis, Hemotherapy, and Transfusion Medicine, Düsseldorf, D, [2]Heinrich Heine University Medical Center, Düsseldorf, D

Background and Objectives: The HPA-1 polymorphism of αIIbβ3 arises from a Leu→Pro exchange at residue 33 of the β3 subunit resulting in HPA-1a (Leu33) or HPA-1b (Pro33). We have documented that patients with coronary artery disease who are carriers of HPA-1b experience their myocardial infarction 5.2 years earlier than HPA-1a/1a patients (JTH 2005; 3: 1522–1529). Based on these observations, it has been postulated that HPA-1b is a prothrombotic variant of αIIbβ3. We have now generated a model overexpressing fluorescent proteins fused with αIIbβ3 in transfected HEK293 cells. To explore the molecular nature, specifically the possibility that the HPA-1 polymorphism modulates the allostery of integrin αIIbβ3, dynamic measurements were performed using fluorescence resonance energy transfer (FRET).

Design and Methods: A yellow (YFP) and a cyan fluorescent protein (CFP) were cloned to the C-termini of the β3 and αIIb subunits prior to transfection of HEK293 cells, subsequently expressing the fusion proteins of both HPA-1 isoforms. Using flow cytometry, Western blotting and specific antibodies directed against αIIb or β3, we identified 12 HPA-1a and 11 HPA-1b positive clones expressing equal amounts of fluorescent fusion proteins, i.e. a 140 kD αIIb-CFP and a 113 kD β3-YFP.

Results: Functional integrity of both integrin variants and proper membrane insertion were documented by intact activation through G protein-coupled receptors with organic acid (OA; 1-stearyl-2-arachidonyl-sn-glycerol), phorbol-12-myristate-13-acetate (PMA)-induced activation of protein kinase C and by specific binding of Alexa647 fibrinogen to αIIbβ3. In the presencence of pertussis toxin or abciximab, activation or ligand binding of αIIbβ3 were completely (>98%) inhibited in both isoforms. Analysis of Src, a tyrosine kinase associated with αIIbβ3, revealed that activation of the phosphotyrosine motif at residue 418 was higher in adherent HPA-1b than HPA-1a cells ($p<0.01$). Upon activation of αIIbβ3 by OA or PMA in the presence of Mn2+, analysis by FRET showed a similar signal disappearance of 40+5% in both HPA-1 variants, indicative of a lateral spreading of the cytoplasmic tails due to receptor activation.

Conclusion: We therefore conclude that the prothrombotic phenotype of HPA-1b (Pro33) is related to increased outside-in signaling rather than to different allosteric changes of the C-terminal cytoplasmic tails of αIIbβ3.

V 8.2

Dysfibrinogenemia and Thrombophilia

Sittinger K.[1], Bidlingmaier C.[2], Harbrecht U.[3], Heller C.[4], Krause M.[5], Miesbach W.[4], Mondorf W.[4], Weiss D.[6], Oldenburg J.[3], Geisen C.[4], Seifried E.[4]

[1]Institute of Transfusion Medicine and Immunohaematology, DRK Bood Donor Service, Frankfurt, D, [2]München, D, [3]Bonn, D, [4]Frankfurt, D, [5]Wiesbaden, D, [6]Erlangen, D

Purpose: Congenital dysfibrinogenemia is highly heterogeneous in respect to mutational spectrum as well as clinical presentation. Apart from bleeding and asymptomatic manifestation thrombosis occur in approximately 20%. Here we focus on "thrombophilic" dysfibrinogen mutations and dysfibrinogenemia families with a background of thrombosis at a very young age.

Methods: FGA, FGB and FGG genes as well as other thrombophilic genes were analysed by direct sequencing on an automated sequencing system (ABI Prism 3100).

Results: Venous thrombotic events were more common than arterial and patients suffered from them at a mean age of 32 years (range 2 to 46 years). In approximately 50% genetic testing revealed the thrombin cleavage site mutations AaArg16Cys and AaArg16His but the overall incidence of thrombosis in patients with these mutations was very low (21%). By contrast 88% of known families with the BbArg14Cys mutation showed severe thrombotic episodes as did our two year old dysfibrinogenemia child with heterozygous protein S deficiency and prothrombin mutation G20210A as well. Its mother with the same defects but without dysfibrinogenemia had no clinical symptoms. Mutations creating new cystein residues are suspected to provoke thrombotic events by formation of disulfide linked protein complexes. In favour of that thromboembolism was present in a family with the new gTrp253Cys mutation. Two other patients with amino acid changes to cystein in the FGB gene (BbArg44Cys and BbArg169Cys) have not shown thrombotic events, so far, maybe due to their young age (4 and 35 years). In the FGG gene a deletion of aminoacid residues Asn319_Lys320 was present in two families with an increased tendency towards severe thrombotic events. In a patient with multiple ischemic attacks variation AaArg19Gly was detected, previously reported in patients with the same clinical picture.

Conclusions: Our data suggest that there is apparent correlation between certain dysfibrinogenemia mutations and a thrombotic clinical phenotype. Good characterization of genetic and clinical data may help to identify additional modifier genes and risk factors for thrombosis. Thus, molecular diagnosis may influence therapy and improve clinical outcome of dysfibrinogenemia patients.

V 8.3

CD40 Ligand (CD40L) Increases Platelet Thrombus Stability and Outside-in Signaling through Integrin αIIbβ3

Hasse M.[1], Stoldt V.R.[2], Scharf R.E.[2]

[1]Heinrich Heine University Medical Center, Department of Experimental and Clinical Hemostasis, Hemotherapy, and Transfusion Medicine, Düsseldorf, D, [2]Heinrich Heine University Medical Center, Düsseldorf, D

Background and Objectives: CD40L, a transmembrane protein of the TNF family, stabilizes arterial thrombi by an αIIbβ3-dependent mechanism. We explored the effect of soluble (s) CD40L on thrombus formation under flow conditions in vitro. The HPA-1 polymorphism of αIIbβ3 was examined in relation to a possible sCD40L-modulated outside-in signaling. Moreover, putative effects of sCD40L with regard to mechanotransduction upon platelet adhesion onto fibrinogen were studied.

Design and Methods: Citrated blood from healthy volunteers (n=8) genotyped for HPA-1 was perfused at a shear rate of 1000 sec-1 through a collagen type I-coated flow chamber, following incubation of platelets with mepacrine and sCD40L. Quantitation of fluorescently stained thrombi was performed by confocal microscopy and a voxel-based software. For signaling experiments, washed platelets were treated with 1 µg/ml sCD40L for 5 min prior to adhesion onto fibrinogen at 37 °C. Phosphorylation of Src was assessed by immunoblotting using antibodies specific for the active Src (pSrc418) or total Src. The signals were quantified by densitometry. P-selectin and sCD40L colocalized on the surfaces of thrombi formed upon perfusion. In the absence of CD40L, the mean thrombus volume (±SD) was 34,922±5,278 μm^3 after 10 min. Addition of sCD40L (5 ng/ml) caused an increase in thrombus volume 70,333±19,745µm3 ($p<0.01$, n=10). Interestingly, homozygous HPA-1b platelets showed elevated thrombus heights and volumes, as compared to the HPA-1a/1a genotype ($p<0.05$). This difference further increased upon addition of 5 ng/ml sCD40L ($p<0.01$). Concomitantly, adhesion of HPA-1b/1b platelets onto fibrinogen enhanced the αIIbβ3-mediated phosphorylation of Src (pSrc418) by >100% upon sCD40L pretreatment, whereas HPA-1a/1a did not show such an effect. In thrombin-activated platelets, pSrc418 was suppressed by >85% by the Src inhibitor PP1.

Conclusion: Our findings demonstrate that the HPA-1b variant of αIIbβ3 increases both, sCD40L-modulated thrombus formation under arterial flow and pSrc418 kinase activation upon platelet adhesion onto fibrinogen. This outside-in signaling provides a link to the cytoskeleton, may modulate mechanotransduction, and can foster thrombus stability upon addition of sCD40L.

V 8.4

The Pro33 (HPA-1b) Variant of Platelet Integrin αIIbβ3 is Prothrombotic due to Enhanced Outside-In Signaling

Gyenes M.[1], Hasse M.[2], Stoldt V.R.[2], Scharf R.E.[2]

[1]Heinrich Heine University Medical Center, Department of Experimental and Clinical Hemostasis, Hemotherapy, and Transfusion Medicine, Düsseldorf, D, [2]Heinrich Heine University Medical Center, Düsseldorf, D

Background and Objectives: αIIbβ3 is polymorphic at residue 33 (Leu or Pro) of the β subunit. Pro33 platelets can display a prothrombotic character. Thus, we have documented that patients with coronary artery disease (CAD)who are carriers of the HPA-1b allele experience their myocardial infarction 5.2 years (median) earlier than CAD patients who are HPA-1b negative (p=0.006) (JTH 2005; 3: 1522–29). To explore the biochemical nature of the prothrombotic phenotype of HPA-1b (Pro33), we now examined Leu33 (HPA-1a/1a) or Pro33 (HPA-1b/1b) platelets and transfected αIIbβ3-CHO cells expressing either HPA-1a or HPA-1b. Activation of αIIbβ3 stimulates the tyrosine kinase Src and the extracellular signal-regulated kinase (ERK2).
Design and Methods: To determine whether αIIbβ3-dependent signaling is responsible for a polymorphism-related modulation, we performed adhesion experiments with fibrinogen in the absence or presence of Mn2+. Specific activation of the phosphotyrosine motif pSrc-418 and phosphorylation of ERK2 were determined by Western blotting and quantified by densitometry. Platelet adhesion onto fibrinogen (100 µg/ml) or incubation with Mn2+ (0.5 mM) resulted in the activation of pSrc-418 to the same extent. Combination of both stimuli caused a 3-fold increase in pScr-418 activity of Pro33 platelets at 2.5, 5 and 10 min of adhesion time, as compared with Leu33 platelets ($p<0.01$). This difference further increased at 20 (4-fold) or 40 min (6-fold). Incubation of transfected CHO cells expressing αIIbβ3 (either in the HPA-1a or HPA-1b isoform) with Mn2+ resulted in a lower ERK2 phosphorylation than their adhesion onto fibrinogen ($p<0.05$). Adherent CHO cells exhibited only a 1.5-fold higher ERK2 activity in the presence of Mn2+. By contrast, 3-fold higher levels of phosphorylated ERK2 were detected in adherent Pro33 than in Leu33 CHO cells ($p<0.05$). Incubation with PP1 (Src kinase) and Y27632 (Rho kinase) completely blocked ERK2 activation indicating that αIIbβ3-mediated ERK2 signaling is regulated by Src and Rho kinases.
Conclusion: Our results demonstrate that integrin-mediated outside-in signaling via Src and ERK2 can be modulated by the Leu33 to Pro exchange in the β3 subunit. The Pro33 isoform appears to be more sensitive to Mn2+ with regard to the activation of Src rather than to ERK2. Pro33 platelets are characterized by increased signal transduction corresponding to their prothrombotic phenotype.

V 8.5

Efficacy of Antibody-Deglycosylation as a New Approach to Prevent Platelet Clearance in an in vivo Model for Immune Thrombocytopenia

Bakchoul T.[1], Sachs U.[2], Dehl J.[2], Bein G.[2], Santoso S.[2]

[1]Justus-Liebig Universität Giessen, Klinische Immunologie und Transfusionsmedizin, Giessen, D, [2]Justus-Liebig Universität, Giessen, D

Purpose: Immune thrombocytopenia (ITP) is a relatively common autoimmune disorder caused by IgG-autoantibody directed against antigens on platelets (PLTs). IgG-coated platelets will be cleared from the circulation causing the characteristic low platelet count and mucocutaneous bleeding. Currently, It is firmly established that the IgG effector functions are crucially dependent on the interaction between a branched sugar moiety attached to asparagine (Asn) 297 residue on the heavy chain of the constant region (Fc) and the Fc gamma receptors (Fcγ Rs) on leukocytes. Endo F, an endoglycosidase, has been shown to efficiently hydrolyze asparagine-linked glycans (N-glycans) from glycoproteins under native conditions. The purpose of this work is to study the ability of Endo F to hydrolyze Asn279-linked N-glycan from platelet-reactive IgG, and to investigate whether Endo F can inhibit antibody-mediated clearance of platelet from the circulation in an in vivo mouse model for ITP.
Methods: PLT-reactive monoclonal antibodies (mAb) were purified and treated with recombinant Endo F. The Glycan modification was analyzed using lectin-blotting and MALDI-TOF mass spectrometric analysis. In order to study the impact of the glycosylation status on the effector function of platelet-reactive antibodies, we employed a recently-developed NOD/SCID mouse model of human platelets circulating in mice. Resting human PLTs from Blood donors were injected into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. Circulating PLTs were exposed to Endo F-treated or non-treated platelet-reactive antibodies. Mouse blood samplings were taken over time and analyzed by flow cytometry.
Results: LCA-blotting of Endo F-treated mAbs revealed no signal indicating a complete digestion of the N-glycan of the heavy chain. MOLDI-TOF analyses showed that Endo F specifically cleaves the N-glycan attached to the Asn-297 residue. In vivo, while non-treated antibodies sufficiently cleared human PLTs from mouse circulation, pre-treatment of with Endo F reduced significantly antibody-mediated platelet clearance.
Conclusions: The major finding of this study ist that Endo F treatment of platelet reactive IgG-antibody prevents platelet clearance in vivo. Hence, Asparagine-linked glycan on PLT-reactive autoantibodies plays a crucial role in ITP. Our results indicate that Endo F might be a potential therapeutic agent especially for patients with refractory ITP. In the future, Endo F may be combined with new thrombopoiesis- stimulating agents to take advantage of the different mechanisms of therapeutic effect. Additional studies are required to further evaluate the safety and confirm the optimal dosing in human.

V 8.6

Massive Platelet Transfusion in Patients with Refractory Autoimmune Thrombocytopenia

Meyer O.[1], Salama A.[1]

[1]Charité - Institut für Transfusionsmedizin Berlin, D

Purpose: Patients with refractory autoimmune thrombocytopenia (ITP) may develop life-threatening bleeding that cannot be immediately controlled by drug administration. To date, there have been no studies conducted to evaluate the efficacy of platelet transfusion alone in such cases.
Design and Methods: Ten patients with refractory ITP and bleeding or a high bleeding risk were consecutively transfused (one unit/30 min) apheresis platelet concentrates (APC) without the administration of new drugs. The used APCs (average 3–7 units) contained 2.7×1011 (medium) platelets and were leukodepleted (≤1×106 leukocytes/unit). Platelet serology was performed using standard techniques.
Results: Platelet transfusion resulted in an increase in the platelet count to 84 –157×103/µl, and the cessation of bleeding in all patients without any serious adverse effects. Although platelet counts gradually decreased within a few days post-transfusion, bleeding was sustained in all cases.
Conclusion: These findings indicate that consecutive platelet transfusion using APCs is the most effective means of emergency treatment in patients with refractory ITP.

V9 Preparation and Therapy with Hematopoietic Stem Cells, Related Subjects

V 9.1

Intracoronary Stem Cell Therapy in Patients with Acute Myocardial Infarction – a Randomized, Double-Blind, Placebo-Controlled Trial (SCAMI)

Mailänder V.[1], Wöhrle J.[2], Schauwecker P.[3], Merkle N.[2], Nusser T.[2], Bommer M.[4], Schwarz K.[5], Maccari B.[5], Wiesneth M.[5], Hombach V.[2], Schrezenmeier H.[5]

[1]Institute of Clinical Transfusion Medicine and Immunogenetics, DRK Blood Donor Service Baden-Württemberg - Hessen, Institute of Transfusion Medicine, University of Ulm, D, [2]Clinic for Internal Medicine II, University of Ulm,D, [3]Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, [4]Clinic for Internal Medicine III, University of Ulm, D, [5]Institute of Clinical Transfusion Medicine and Immunogenetics Ulm, Institute of Transfusion Medicine, University of Ulm, D

Conflicting results of intracoronary bone-marrow cell (BMC) therapy for improvement of left ventricular ejection fraction (LVEF) in patients with acute myocardial infarction (AMI) have been reported. In this study we analyzed the effect of BMC on left ventricular ejection fraction (LVEF) at 6 months (primary endpoint) in a randomized, double-blind setting with particular attention to complete assurance of double blinding by use of autologous erythrocytes within the placebo preparation.
Methods: We performed a double-blind, placebo-controlled, randomized trial in AMI patients with successful stenting >6 hours after symptom onset. Patients were 2 : 1 randomized to BMC vs. placebo therapy, and stratified according to age, AMI localization and LV function. BMC were prepared from bone marrow aspirate by a Ficoll density gradient. Patients in the BMC group received a median of 324×106 bone marrow mononuclear cells with a viability of 97%. The absolute number of CD34+/CD45+ cells was 2.7×106 and the number of CD133+/CD45+ cells was 1.5×106. The frequency of colony-forming units in these cell preparations was as follows: 1750 BFU-E/106 cells, 319 CFU-GEMM/106 cells, and 16 CFU-F/106 cells (median). Placebo syringes contained 0.9% sodium chloride with 2% human albumin and autologous erythrocytes with a hematocrit of 0.1% without bone marrow cells. Placebo and verum could not be visually distinguished assuring a rigorous double blinding.

Results: 42 patients were enrolled (29 BMC and 13 placebo). Baseline clinical data and cardiac magnetic resonance imaging (CMRI) parameters did not differ. As compared to baseline, the difference in LVEF for placebo vs. BMC treatment at 3 months was 3.1 +/– 6.0% vs. 1.9 +/– 3.4%, and at 6 months (primary endpoint) 5.7 +/– 8.4% vs. 1.8 +/– 5.3%, respectively. CMRI studies did also not demonstrate significant treatment-related differences in LV muscle mass, LV enddiastolic and LV endsystolic volume indices at 1, 3, and 6 months follow-up. There was no difference between groups in the amount of infarct size as it was for the reduction during follow-up. The study was terminated since stopping criteria were met.
Conclusion: We did not observe an improvement of LVEF for intracoronary administration of autologous BMC as compared to placebo therapy. The importance of careful preparation of placebo to avoid accidental unblinding in double-blind, placebo-controlled cell therapy studies is emphasized.

V 9.2

Peripheral Blood Stem Cell Apheresis in Low Weight Infants

Wagner B.[1], Albert M.H.[2], Adam M.[3], Gruetzner S.[4], Wittmann G.[4], Schramm W.[4], Schmid I.[2]

[1]Klinikum der Universität München, Transfusionsmedizin und Haemostaseologie, München, D, [2]Haematologie & Onkologie Dr. von Haunersches Kinderspital, München, [3]Institute of Clinical Chemistry, München, D, [4]Transfusionsmedizin & Haemostaseologie Klinikum der Universität München, D

Purpose: High dose chemotherapy with stem cell rescue has been generally accepted as first line therapy for a variety of malignancies. Especially in low weight infants it is crucial to establish safe and consistent collection procedures. We examined the effect of additional heparin to ACD-A anticoagulation during apheresis on stem cell yield and safety.
Methods: 21 infants <25 kg body weight with different malignancies performed 30 collection procedures (11 with ACD-A=standard, 19 with ACD-A + Heparin) using a continuous-flow cell separator (COBE Spectra, Caridian) after mobilisation with chemotherapy + rHu-G-CSF yielded CD34+ blood counts >10/µl. 2.5–3fold TBV was processed using MNC programme V6.1. Heparin was infused continously at 20 I.U./kg BW*h starting 2h before the begin of apheresis or following a bolus injection of 50 I.U./kg BW. ACD-A/blood ratio was 1:12–1:18 ("standard") and 1:16–1:28 (ACD-A+Heparin). The stem cell product was analysed on a blood counter (KX-21, Sysmex) and a flow cytometer (Calibur, BD). Blood counts before and after stem cell harvest were done to determine needs for transfusion.
Results: The patients were in the median 3.5 years (range 0.5–12.4) old with 13.5 (7.5–24) kg body weight. All procedures were run after priming the device with irradiated RBCs adjusted to the patient´s haematocrit. At in the mean 141 (CI 93–189) CD34+ cells/µl in peripheral blood 12.6 (CI 7.6–17.6)*10E6 CD34+ cells/kg were harvested per procedure. Like in adults no other blood count but CD34+ cells correlated with stem cell yields: R^2 was 0.953 and 0.872 for the standard anticoagulation and with additional heparin, respectively. Despite similar platelet counts at baseline, platelet counts after the procedure were significantly lower in the ACD-A only group (mean 50.4/nl) as compared to patients receiving heparin+ACD-A (93.5/nl). The same was true for the procedure related platelet loss (1- the ratio of post/prae platelet counts): 0.39 (ACD-A only) vs.0.21 (ACD-A+Heparin). On dual anticoagulation platelet counts before stem cell harvest correlated with those afterwards (R^2=0.756) in contrast to the standard regimen. Haemoglobin concentration was only marginally affected by the procedure: before vs. after apheresis 11.34g/dl and 11.27g/dl. No clinical signs of bleeding and other serious procedure related adverse events were recorded.
Conclusion: The additional application of heparin during stem cell apheresis did not affect stem cell yield. The procedure related platelet loss was significantly lower as compared to the standard procedure. Thus, the safety of the procedure with these high-risk patients has been improved significantly.

V 9.3

Mobilization of Hematopoietic Stem/Progenitor Cells without G-CSF

Bonig H.[1], Papayannopoulou T.[2]

[1]German Red Cross Blood Center and Institute for Transfusion Medicine and Immunohematology of the JWGU Frankfurt, Cellular Therapeutics, Frankfurt, D, [2]University of Washington, Seattle, WA, USA

G-CSF mobilized hematopoietic stem/progenitor cells (HSPC) have become the prime source of transplantable cells; reasons include donor/collection facility preference, and favorable properties as grafts. Insight into the molecular mechanisms of G-CSF mediated HSPC mobilization has identified alpha4-integrin and CXCR4 as rational targets of enforced mobilization. The potential of such mobilizing regimes as alternatives for G-CSF refractory or intolerant donors remained incompletely explored, most importantly with respect to the advantageous properties of G-CSF mobilized grafts. Here we provide data generated in mice, non-human primates and humans, about efficiency of alternative mobilizing agents and properties of HSPC mobilized with these. The efficacy of AMD3100, a short-acting small-molecule antagonist of CXCR4, is modest if given as suggested by the manufacturer (single dose). Its efficacy can be improved 10-fold if administered by continuous infusion. Alternatively, a derivative of AMD3100, AMD3465, provides greater potency and efficacy. Transplant-related properties of AMD3100 mobilized HSPC were analyzed in detail; AMD3100 mobilized HSPC showed equally good homing as G-CSF mobilized cells, markedly superior to bone-marrow derived HSPC. Engraftment kinetics of equal volumes of G-CSF or AMD3100 mobilized peripheral blood (optimized mobilization schemes) were similar, despite at least 2-fold differences in circulating progenitor cells. This suggests that the frequency of engrafting stem cells in AMD3100 mobilized grafts may exceed that in G-CSF mobilized grafts. Alpha4-integrin blockade was similarly considered as a rational pathway for HSPC mobilization. Interference with alpha4-integrin function (anti-functional antibodies or genetic ablation) led to increased circulating HSPC in mice, monkeys and humans. Although such cells home relatively poorly, they provide engraftment of lethally irradiated recipients. Short-acting alpha4-integrin blocking reagents (similar to AMD3100 on CXCR4) will overcome this effect, which currently limits its clinical applicability. Further, the combined mobilizing effect of alpha4-integrin blockade and CXCR4 blockade was greater than additive in mice and monkeys. These data indicate that a) alpha4-blockade and CXCR4-blockade target independent, but interacting pathways of HSPC retention, and b) combination of the two modalities, once small-molecule alpha4-blockers become available, will provide a potent, G-CSF-free mobilizing scheme satisfying all requirements of transplantable cells. Our data provide novel insights into properties of mobilized HSPC, and demonstrate potent alternatives to G-CSF for HSPC mobilization.

V 9.4

Successful Mobilization and Harvest of HPC Independent of Leukocyte Counts by G-CSF, Plerixafor (MozobilTM) and Large Volume Aphereses in 4 Formerly Poor Mobilizing Patients

Humpe A.[1], Günther A.[2], Valerius T.[2], Schmidt A.[2], Buwitt-Beckmann U.[2], Gramatzki M.[2]

[1]Section of Stemcell and Immunotherapy, Second Department of Medicine, University Clinic Schleswig-Holstein Campus Kiel, D, [2]Section of Stemcell and Immunotherapy, Second Department of Medicine, Kiel, D

Purpose: In the majority of patients successful mobilization of hematopoietic progenitor cells (HPC) can be achieved by chemotherapy and G-CSF. But in some patients due to extensive previous chemotherapy or radiotherapy mobilization fails and alternatives are needed. A novel cytokine receptor blocker plerixafor (MozobilTM), an antagonist of the CXCR4 receptor, seems to be such an alternative. We report a series of 4 patients that initially failed to mobilize sufficient numbers of HPC by standard mobilization regimens who were successfully mobilized and harvested by plerixafor together with large volume leukaphereses (LVL).
Methods: In a series of 4 patients, 2 male and 2 female, with a median age of 48.5 (range: 44–65) years and diagnosed with multiple myeloma (n=3) or NHL (n=1) plerixafor mobilization was performed after failure of mobilization with standard mobilization regimens. Mobilization consisted of G-CSF (5 µg/kg body weight (bw) subcutaneously twice daily) and addition of plerixafor (240 µg/kg bw subcutaneously 10 hours before apheresis) starting in the evening of the fourth day of G-CSF administration.
Results: In the respective previous failing mobilization cycles a median maximum number of CD34+ cells/µL in PB of 6 (range: 2.5–14) was reached in the 4 patients. During the mobilization cycle with plerixafor, in the morning of the fourth day of G-CSF administration the median number of leukocytes and of CD34+ cells/µL in PB were 24,900 and 4 (range: 5,600–62,800 and 0–6) respectively, with an increase on the first morning after plerixafor addition to 34,800 and 23 (range: 8,500–72,600 and 18–37) respectively. A median of 2.5 (range: 2–3) aphereses processing a median of 4.5 (range: 2.9–6.4) times the total peripheral blood volume were performed with a median yield of 6.24 (range: 2.87–7.56)x10E+06 CD34+ cells/kg bw.

So far 3 of the 4 patients were transplanted after high dose chemotherapy exhibiting successful engraftment.
Conclusions: Administration of plerixafor was well tolerated and its addition to G-CSF mobilization together with LVL enabled, independent of leukocyte counts, successful harvest of HPC numbers sufficient for high dose therapy and subsequent transplantation. Addition of plerixafor already during a poor mobilization cycle consisting of chemotherapy and G-CSF could be a further desirable improvement in the future.

V 9.5

Long-Term Culture of Human MSCs Induces Variations in Genomic Stability

Schallmoser K.[1], Rohde E.[2], Reinisch A.[2], Obenauf A.C.[2], Bartmann C.[2], Lanzer G.[2], Linkesch W.[2], Strunk D.[2]

[1]Medical University of Graz, Stem Cell Research Unit of Graz, University Clinic of Blood Group Serology and Transfusion Medicine, Graz, A,
[2]Medical University of Graz, A

Human multipotent mesenchymal stromal cells (MSCs) are currently tested in clinical trials for immunomodulatory and regenerative therapy. We and others have recently established MSC propagation with pooled human platelet lysate (pHPL) substituting fetal bovine serum (FBS). As serious doubts regarding the use of MSCs cultured with FBS and their possible genomic instability have arisen, we investigated safety aspects of short- and long-term cultures with FBS in comparison to pHPL.
Unmanipulated bone marrow aspirates were seeded in Alpha-MEM with pHPL. Clinical-scale expanded MSCs were harvested directly after primary culture (short-term). Representative cultures were continued for long-term expansions each with directly comparing pHPL and FBS stimulation for a maximum of 46 to 51 population doublings until MSC proliferation ceased. Comparative genome hybridization (array-CGH) was carried out with short- as well as long-term expanded MSCs using a whole genome microarray platform and CGH analysis software.
pHPL is highly efficient in stimulating MSC expansion resulting in 780±150 million MSCs after one passage. Flow cytometry revealed more than 95% viability, more than 95% CD73/90/105 reactivity and less than 2% hematopoietic contamination. We could show adipo/osteo/chondrogenic differentiation potential, endotoxin levels below 0.025 EU/mL and negative bacterial/fungal/mycoplasma testing. In all short-term MSC products, array-CGH revealed balanced genomic profiles. We detected several small copy number variations previously found in healthy individuals not associated with phenotype changes. In contrast, after long-term culture with FBS as well as pHPL, MSCs showed de novo copy number amplifications not reported in the database of genomic variants, hitherto.
Despite a high proliferation rate in the short-term pHPL-driven culture, MSCs showed genomic stability according to array-CGH results. These data support our earlier findings that MSCs expanded under humanized conditions did not form tumors in vivo in animal experiments. It is not clear whether in vitro genomic variations in long-term propagated MSCs under humanized as well as xenogeneic culture conditions may be associated with a risk of malignant transformation rather than representing replicative senescence. Therefore safety concerns have to be vigilantly addressed parallel to the clinical use of MSCs.

V 9.6

Iron Labeling of Mesenchymal Stem/Stromal Cells for Magnetic Resonance Imaging: Studies on Innovative Poly-L-Lactic Acid - Iron Nanoparticles

Schmidtke-Schrezenmeier G., Urban M.**, Sharma S.+, Dausend J.*, Rojewski M.*, Mailänder V.**, Rasche V.+, Landfester K.**, Schrezenmeier H.**

*Institute for Transfusion Medicine, University Hospital of Ulm and Clinical Transfusion Medicine and Immunogenetics Ulm, DRK Blood Donor Service Baden-Württemberg-Hessen, Ulm, Germany
**Max-Planck-Institute for Polymer Research, Mainz, Germany
+Department of Internal Medicine II, Cardio-MR, University Hospital of Ulm, Germany

Background: Cell therapy with MSC is of high interest. Little is so far known on the trafficking and homing of MSCs after ex-vivo expansion and transplantation into the host. Currently it is not known which application mode (i.v., local injection, local application of MSC on scaffold) is optimal. Thus a method enabling in vivo observation of the MSCs is desirable. Magnetic resonance imaging (MRI) is a method technically suitable for this (no ionizing radiation; high spatial resolution at near cellular level). A prerequisite for this is labeling of the MSCs with a MR contrast agent fulfilling following criteria: sufficient uptake to alter a MR signal, intracellular persistence for a sufficient in-vivo observation period, lack of toxicity, lack of influence on function and phenotype of the MSC, and no interference with ex-vivo MSC expansion process. Biodegradable iron-loaded poly-L-lactic (PLLA) nanoparticles (particles) synthesized via the miniemulsion process and equipped with the "dual-reporter" property, which is the inclusion of a fluorescent dye (for FACS detection) and iron oxides (for MRI detection) in the PLLA-polymer, were studied.
Methods: Ex-vivo expanded MSCs were labeled with iron-PLLA nanoparticles. Using transmission electron microscopy, flowcytometry, confocal laser scanning microscopy, and Prussian blue staining we studied intracellular uptake, persistence, viability, proliferation, surface marker expression, trifunctional differentiation. MR phantom studies with iron-PLLA particle labeled cells were performed in a 3 Tesla clinical grade MR scanner.
Results: Particles are taken up rapidly by MSCs and persist up to 144 h after particle removal (mainly in endosomal / lysosomal compartment). In MRI 96 h after particle removal 2x10^5 labeled MSCs could be clearly discriminated from 5x10^4 MSC and from unlabeled MSC in an agarose phantom. Particle-labeled MSCs maintained their differentiation potential and showed an excellent viability. Standard surface markers were not influenced by the labeling with exception of CD71. In particle labeled MSCs a down regulation of CD71 on day 2 and 6 after particle removal could be observed in comparison to unlabeled cells. Iron content of individual cells (detected by Prussion blue) was very heterogeneous.
Conclusion: These innovative iron-PLLA particles are suitable for MSC labeling for MR imaging in an agarose phantom. In a next step MR imaging of labeled cells in vivo has to be tested. The mechanism and the impact of CD71 down regulation and heterogeneity of intracellular iron persistence needs further studies.

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P1 Immunohematology (Red Cells, Platelets, Granulocytes)

P 1.01

A Novel ELISA Method for the Detection of HNA-2a Neutrophil Antibodies

Bayat B., Werth S., Sachs U., Santoso S.

Justus Liebig University, Institute for Clinical Immunology and Transfusion Medicine, Gießen, D

Purpose: Antibodies to human neutrophil antigen (HNA)-2a is responsible for a number of immune mediated neutropenia disorders. Although several methods exist for the identification of anti-HNA-2a, all these methods have several limitations. In this study,we developed a solid phase ELISA using recombinant HNA-2a antigen (rHNA-2a) allowing rapid detection of HNA-2a antibodies.
Methods: Soluble rHNA-2 was generated by transfection of insect cells with CD177 vector. Purified rHNA-2a was immobilized on microtiterwells coated with anti-CD177 and was applied to analyse 10 sera containing HNA-2a antibodies. For the evaluation of the ELISA method results were compared with the standard assay, MAIGA (Monoclonal Antibody Antigen Capture Assay) for detection of neutrophil antibodies.
Results: The specificity of HNA-2a antibodies in all sera were confirmed by immunoblotting. Sera were then tested simultaneously in ELISA and MAIGA assays. 9/10 sera showed positive reactions in ELISA, whereas only in 7/10 sera reacted in the standard MAIGA assay. No reaction was observed with different sera containing neutrophil reactive antibodies (6 anti HNA-1a, 4 anti HNA-1b, and 20 anti-HLA class I and II). Notably, sera containing anti-proteinase 3 (PR3) from patients with Wegener's granulomatosis reacted in MAIGA. In contrast, this antibody showed no reaction in ELISA with purified rHNA-2a.
Conclusions: These results demonstrated that ELISA with rHNA-2a provides a good method for detecting HNA-2a antibodies in human serum. This assay enables to exclude the presence of autoantibody against PR3 in patient's sera which can not be differentiating from anti HNA-2a with current serological methods.

P 1.02

Do Donors with Non-Deletional Blood Group O Alleles Boost Anti-A and Anti-B Titers in Blood Group O Recipients?

Scharberg E.A.[1], Seyboth S.[2], Panter K.[2], Ernst A.[2], Hagel J.[2], Richter E.[2], Rink G.[3], Janetzko K.[3], Bugert P.[3]

[1]Institut Baden-Baden, DRK-Blutspendedienst Baden-Württemberg-Hessen, Immunohematology, Baden-Baden, D, [2]Institut Baden-Baden, DRK-Blutspendedienst Baden-Württemberg-Hessen, Baden-Baden, [3]Institut Mannheim, DRK-Blutspendedienst Baden-Württemberg-Hessen, Mannheim, D

Purpose: The ABO blood group gene is located on the human chromosome 9. It encodes glycosyltransferases that mediate the expression of the A and B antigens on red cells. DNA sequence variations in the coding region of the gene determine the quality (A or B) and the quantity (A2, A3, Ax, Ael, Bel etc.) of the antigens. There are more than 200 known ABO alleles. Some non-deletional O alleles have no serologically detectable A/B antigens though the missing or weak isoagglutinins might suggest a low A or B antigen expression. In this retrospective study we investigated, if red cell concentrates of blood group O donors with ABO alleles O03, Aw08, Bw20 and Ax03 transfused to blood group O patients boosted their anti-A and anti-B titers.

Methods: Serological ABO typing was performed on Olympus PK7200 using monoclonal anti-A and anti-B reagents. Donors with missing isoagglutinins were additionally tested in the tube technique with reference monoclonal anti-A and anti-B. Red cell units with clear serological ABO antigen typing were used for transfusions but if the isoagglutinins were still weak in the tube test (reverse A1, A2 or B less than 2+) the samples were analyzed for the ABO gene by PCR-SSP and exon re-sequencing. When ABO gene variants were found and the units were cross-matched in our laboratory in 2007, a look back procedure in patients was started, as blood samples of all 6,387 patients who had been tested in our reference laboratory in 2007 were frozen and stored at -30°C. The procedure included titration of anti-A and anti-B in patients' serum (gel test, neutral and antiglobulin cards) at the time of the cross-match of the ABO variant and in follow up samples.

Results: In 2007 red cell units (n=35) with variant ABO alleles were randomly cross-matched in our laboratory. In 21 cases a look back and a follow up procedure were possible because patients' samples were available. Following ABO alleles were transfused as group O: O03 (n=17), Bw20 (n=2), Aw08 (n=1) and Ax03 (n=1). At the time of the cross-match of the ABO variants the titers of anti-A in patients varied between 1 and 64 (IgM) and between negative and 512 (IgG). Anti-B titers varied between negative and 32 (IgM) and between negative and 128 (IgG). The follow up samples were drawn 6 to 283 days after transfusion of the ABO variants. In all 21 recipients anti-A and anti-B titers remained unchanged.

Conclusions: The transfusion of red cell units with the ABO alleles O03, Aw08, Bw20 and Ax03 typed and transfused as group O did not boost anti-A and anti-B titers in blood group O recipients. For these variants no donor referral because of missing or weak isoagglutinins seems to be necessary.

P 1.03

Is there a New Therapy Option in Autoimmune Hemolysis? – Case Report of Successful Eculizumab Treatment in a Patient with Combined Cold and Warm Autoantibodies

Höchsmann B.[1], Bommer M.[2], Flegl W.[3], Schrezenmeier H.[3]

[1]IKT Institut für klinische Transfusionstherapie und Immunogenetik Ulm, DRK Blutspendezentrale Ba-Wü/Hessen, Institut Ulm, D, [2]Universitätsklinik Ulm, [3]DRK Blutspendedienst Baden-Württemberg/Hessen, Institut Ulm, D

Purpose: Cytolytic activity due to cold autoantibodies is mediated by complement activation forming the membrane attack complex C5b-9. C5 antibody eculizumab, which is approved for the treatment of Paroxysmal Nocturnal Hemoglobinuria (PNH), is a potent inhibitor of terminal complement activation by prevention of C5 cleavage. Rare data are published concerning treatment of cold agglutinine disease by eculizumab. We describe the first case of eculizumab response of a hemolytic patient with cold and warm autoantibodies.

Case Report: A 50 year old caucasian male with Chronic Lymphatic Leukemia and chronic hepatitis B as well as history of Hodgkin disease presented with severe autoimmune mediated hemolytic anemia (8 packed red blood cell (PRBC) transfusions/week). Serologic testing revealed cold and warm autoantibodies as well as erythocyte allo-antibodies type anti-Cw and anti-Lua. Hemolysis was refractory to prednisolon, rituximab, cyclophosphamide, immunoglobulins and combination therapy of alemtuzumab/rituximab. Therefore we started eculizumab therapy off label with the aim to reduce at least the part of cold autoantibody mediated hemolysis. Meningitis vaccination and eculizumab dosage was done like recommended for PNH. Before 1st /15th eculizumab administration the following values were collected: Hb 7.2/ 12,9 g/dl, reticulocytes 342/ 106 K/µl, LDH 372/181 U/l, haptoglobin < 0.13/ < 0.13 g/l, total bilirubine 101/ 19 µmol/l. Direct antiglobulin test (DAT) was positive with polyspecific antiglobulin serum and monospecific anti-IgG, anti-IgA, anti-C3c and anti-C3d before eculizumab start and got negative for monospecific anti-IgA and anti-C3c during treatment. Indirect antiglobulin test showed a decrease of free autoantibodies titre from 32 to 4 during therapy. As anticipated no change in the known erythocyte allo-antibodies type anti-Cw and anti-Lua was observed. Number of transfused PRBCs dropped from 131 units during the 7 mo before start of eculizumab-therapy to 10 units in the first 7 mo after start of eculizumab-therapy. The last PRBC transfusion was necessary 70 d (7. application) after start of eculizumab therapy. Although we tapered and finally stopped eculizumab dosage Hb values are still stable and up to now transfusion independency is lasting for 11 months.

Conclusion: We report a case of severe pretreatment-refractory autoimmune mediated hemolytic anemia due to cold and warm autoantibodies successfully treated by the C5 antibody eculizumab. Therefore we conclude that eculizumab could be a therapy option for immune mediated hemolysis also in the presence of warm autoantibodies. For more extensive recommendations data had to be confirmed in a larger cohort.

P 1.04

PCR-SSP Genotyping of ABO, RH, KEL, JK, FY and MNS Alleles: A Population Study with Black American Blood Donor Samples

Prager M.[1], Boje P.[2], Legler T.J.[3], Nitschke C.[3], Seltsam A.[4], Moulds J.J.[5], Billingsley K.[5], Moulds J.M.[5]

[1]BAG Health Care GmbH, Molecular Genetics, Lich, D, [2]BAG Health Care, Lich, D, [3]Dept. of Transfusion Medicine, University Medical Center Goettingen, Georg-August-University, Goettingen, D, [4]German Red Cross Blood Service NSTOB, Institute Springe, D, [5]LifeShare Blood Centers, Shreveport LA, USA

Background: Genotyping assays based on the Polymerase Chain Reaction with Sequence Specific Primers (PCR-SSP) are helpful to define unusual immunohematology findings. The aim of this study was to examine the suitability and reliability of ready-to-use PCR-SSP assays to detect common and rare ABO, RH, KEL, JK, FY and MNS alleles in black American population.

Methods: We analyzed 161 DNA samples, derived from random USA Black donors, previously phenotyped for ABO and Rhesus antigens and additionally genotyped for KEL, JK, FY and MNS alleles with BeadChip™ Technology (BioArray Solutions, Warren, New Jersey, USA). Common and rare blood group alleles were determined by sequence-specific priming (BAG Health Care, Lich, Germany) and, if necessary, further investigated by sequencing. The SSP results have been interpreted with the novel evaluation software BAGenotype (BAG Health Care) and crosschecked manually.

Results: Unequivocal results were obtained with the majority of the samples in accordance with the BeadChipTM Technology and serologic typing. Eight (5%) of the 161 DNA samples had unusual ABO genotypes, caused by variant ABO*O alleles and identified by sequence analysis only. Three ABO SSP typings (1.9%) were not concordant with serology but have been confirmed by sequence analysis. These cases have to be further investigated. Five inconclusive D typings (3.1%) came from variant RHD alleles and were defined by sequencing. Among 149 D positive samples we detected 43 (29%) DAU, 13 (9%) RHD/RHD psi or RHD/Cdes and 1 (0.7%) weak D type 4.0/4.1 genotype. Two of the seven D negative samples were homozygous for RHD psi. Deletion of the RHD gene was detected with the other five samples. Two (1.2%) variant MNS alleles were indicated by negative reactions with big S and little s.

Conclusion: The use of a PCR-SSP technique which is mainly designed according to White population-specific allele frequencies proved to be a reliable and suitable tool to determine most of the blood group genotypes in Blacks. Further blood group genotype screening studies are needed to systematically elucidate the allele repertoire and frequencies in the Black populations and, if necessary, to adapt PCR-SSP assays accordingly.

P 1.05

ABO Blood Group Genotyping of South-African Blood Donors Reveals Unknown ABO Alleles

Strate A.[1], Wetzel E.[2], Hellberg A.[3], Smart E.[4], Prager M.[5], Legler T.[2], Olsson M.L.[6]

[1]Universitätsmedizin Göttingen, Abteilung Transfusionsmedizin, Göttingen, [2]Universitätsmedizin Göttingen, D, [3]Univeristetsjukhuset i Lund, S, [4]Blood Donation Centre Pinetown, ZA, [5]BAG Health Care GmbH, Lich, D. [6]Lund University, Lund, S

Background: Blood group genotyping has become a remarkable alternative to traditional blood group serology. Considering the increasingly multiethnic population in Western countries and the variety of blood group alleles reported from non-Caucasian individuals, evaluation of blood group genotyping tests should not be restricted to Caucasian individuals only. We therefore evaluated a commercially available ABO blood group genotyping test with unselected South-African blood donors of African origin.
Methods: A total of 99 samples were drawn from apparently healthy random donors at the blood donation center in Pinetown, South-Africa. Genomic DNA was prepared by magnetic separation technology. ABO serology was performed according to standard laboratory methods. All samples were genotyped with the BAGene ABO-TYPE PCR test kit based on sequence specific primers (SSP). ABO genotyping results were compared with ABO serology, and samples with inconsistent results were further analyzed by sequence analysis of the ABO gene.
Results: According to serology, the most common ABO type was blood group O (49 donors) followed by blood group A (30 donors), B (17 donors) and AB (3 donors). Genotyping results were consistent with serology in all but four cases (4%) that included three cases with inconclusive amplification results. One group O donor had a BO1 amplification pattern due to an O1v-B hybrid allele which is known to be common in Africans. Sequencing of another group O donor with a BO1 amplification pattern revealed a rare and difficult-to-define ABO genotype involving two hybrid alleles with both O1v- and B- specific nucleotide sequences. A group B donor with an inconclusive A1A1 amplification pattern revealed heterozygosity for A1 and B sequences with suspicion of a mutation outside exons 1 to 7 of the A1 sequence, possibly leading to a novel Aweak allele or a non-deletional O allele. Sequencing of the fourth sample, phenotyped AB and conclusively genotyped O1O1, revealed heterozygosity for O1 and an O1v-like allele with 595C>T (O12 according to dbRBC nomenclature); a mix-up of samples is the most likely explanation of this discrepancy.
Conclusion: ABO blood group genotyping performed with a commercially available SSP-based PCR test kit can lead to inconclusive results in blood donors of African origin. In our study, four out of 99 donors could not be accurately genotyped with SSPs designed for Caucasian individuals due to ABO alleles either common among individuals of African ancestry or previously not encountered. Considering the genetic variation in different ethnic groups, genotyping in general should be handled with care if applied in non-Caucasian individuals.

P 1.06

Spontaneous Rh Phenotype Splitting: Loss of Heterozygosity on Chromosome 1 in Different Blood and Bone Marrow Cell Subsets

Körmöczi G.F.[1], Dauber E.-M.[2], Swoboda S.[2], Mansouri Taleghani B.[3], Fontana S.[4], Hustinx H.[4], Sikyr S.[3], Colucci G.[3], Schwarzinger I.[2], Fritsch G.[5], Mayr W.R.[2]

[1]Medical University of Vienna, Blood Group Serology and Transfusion Medicine, Vienna, A, [2]Medical University of Vienna, A, [3]University Hospital, Bern, CH, [4]Regional Blood Transfusion Service of the Swiss Red Cross, Bern, CH, [5]Children's Cancer Research Institute, Vienna, A

Purpose: In a patient with progressive Rh antigen loss, the molecular changes next to the RHD and RHCE loci (1p36.11) in different cell subsets from blood and bone marrow, in cultivated red cell precursors and hair roots were studied.
Methods: Red cell phenotyping was carried out by extended serology and flow cytometry. Cell subsets from peripheral blood and bone marrow aspirate were isolated using fluorescence-activated cell sorting. Erythropoietic burst-forming units (BFU-E) were cultivated from peripheral blood mononuclear cells. Chimerism was ruled out by analysis of 15 short tandem repeat (STR) markers located outside chromosome 1. DNA samples from different cell subsets sorted from peripheral blood and bone marrow cells as well as red cell precursor colonies were analyzed using chromosome 1 STR markers.
Results: Rh phenotyping of a 62-year-old male patient revealed mixed-field agglutination in D and E testing independent from transfusion, without further blood group abnormalities. About 48% of his red cells were D+. Six years later, routine serology indicated total D and E antigen loss, and further diagnostic work-up resulted in findings being highly suspicious for primary myelofibrosis. At this time, analysis of DNA derived from blood using PCR-SSP demonstrated the presence of all RHD exons and a cDdEe genotype. Only with adsorption/elution of anti-D and anti-E, a minimal expression of these antigens was detected. Now, less than 1% of his red cells were D+, as evidenced by flow cytometry. Chimerism was ruled out by standard STR analysis of DNA from whole blood and hair samples. Loss of heterozygosity (LOH) was detected testing ten informative (heterozygous) STR markers of the short arm of chromosome 1. In contrast, no LOH on 1q was found. Further STR analysis showed complete LOH of 1p in DNA samples from granulocytes (peripheral blood and bone marrow) and monocytes (peripheral blood). Total LOH on 1p was also found in bone marrow normoblasts and CD34+ cells. Moreover, all tested BFU-E colonies showed such LOH. Conversely, no LOH on chromosome 1 was seen in DNA samples from hair roots and blood or bone marrow lymphoid cells.
Conclusions: A largely parallel pattern of LOH of 1p encompassing the RH locus in different cell subsets from blood and bone marrow was found. The consequent elimination of one RH haplotype is the major mechanism of spontaneous Rh antigen loss often associated with hemato-oncologic disease.

P 1.07

Targeting the Immunological Synapse by Silencing PECAM-1 Expression for Organ Transplantation

Jaimes Y., Figueiredo C., Zenk J., Aufderbeck S., Eiz-Vesper B., Blasczyk R.

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

Purpose: Immune recognition and rejection of allogeneic transplants is the main obstacle for long term allograft survival. Platelet endothelial cell adhesion molecule (PECAM-1) is an immunoglobulin-like glycoprotein, involved in leukocyte migration, cellular adhesion, immunological synapse stabilization, and signal transduction. We specifically knocked down PECAM-1 expression by RNA interference (RNAi) to circumvent allorecognition.
Methods: We designed lentiviral vectors that express short hairpin RNA sequences (shRNA) to target PECAM-1. Silencing of PECAM-1 on monocytes and endothelial cells was measured at protein level by flow cytometry and at RNA level by Real Time RT-PCR. The surface expression of PECAM-1 molecules was quantified in an antigen binding capacity assay. The cytotoxic potential of T cells directed against PECAM-1 silenced cells was evaluated by measuring granzyme B mRNA levels using Real Time RT-PCR.
Results: The expression of PECAM-1 was decreased by up to 80% at the protein as well as the mRNA level on monocytes and endothelial cells. The antigen capacity assay showed that PECAM- 1 silencing induced a reduction in cell surface expression by up to 80%. Granzyme B production decreased by up to 85% on the T cells stimulated with the PECAM-1 silenced monocytes in comparison with the non-specific shRNA expressing monocytes.
Conclusions: In conclusion, we show that silencing PECAM-1 expression is feasible in a permanent way. Silencing of PECAM- 1 expression demonstrated an efficient destabilization of the immunological synapse, and may substantially decline the allogeneic inflammatory process by limiting leukocyte extravasation and graft allorecognition. Targeting the immunological synapse and lymphocyte migration may provide a new approach to overcome rejection in allogeneic transplantation.

P 1.08

Prozone Phenomenon of Anti HLA-A2 in Lifecodes Luminex Single Antigen Class I, Tepnel (LSA Class I)

Stenzel A.[1], Bringmann G.[2], Koch K.[2], Strathmann K.[2], Zeiler T.[2]

[1]DRK-Blutspendedienst West, Zentrum für Transfusionsmedizin Breitscheid, Ratingen, D, [2]DRK-Blutspendedienst West, Ratingen, D

Purpose: The prozone or (high-dose) hook effect, which was first described over 60 years ago, can cause false negative results in serological tests of multiple purpose due to massive excess of the analyte to be tested. Here we describe a high-dose hook effect in the LSA Class I-test.
Case Report: We received blood specimens of a 68-year-old female patient with a hematological disease who had developed refractoriness to platelet transfusion.

HLA class I alleles (HLA-A *24, *26; B *40, *44) were determined by SSP-PCR.
HPA and HLA antibody screening (GTI PakPlus, GTI-Diagnostics) revealed the presence of HLA antibodies, HPA antibodies were not detectable. For the differentiation of the HLA antibodies, LCT (HLA-ABC 60, Biotest) and LSA Class I-test were used.
In LCT (with DTT), anti-A2 was doubtlessly detectable. In LSA Class I, 46 of 90 beads responded positively, but surprisingly none of the existing three A2 beads.
Without referring to a high dose hook effect the producer only recommends dilutions of the patient's serum when beads produce MFIs of more than 15.000. However, we rather assumed that the missing of anti-A2 was potentially due to a high dose hook effect and thus performed dilution studies. Anti-HLA-A2 could be detected in a serum dilution of 1:10.
Conclusion: In LSA Class I a high-dose hook effect is possible. Following the here described first case we could observe this phenomenon in several additional cases. This should be taken into account when implausible test results occur and is highly recommended to be examined by dilution studies.

Results of the dilution studies: The 3 highest raw MFI values of undiluted serum vs. A02			
Bead	MFI undiluted	MFI dilution 1:2	MFI dilution 1:10
B1801	10.542	7.468	2.637
A6901	9.980	11.716	10.127
B5401	9.969	9.625	3.905
A0201	2.394	2.417	13.950
A0206	1.395	1.687	13.665
A0203	1.471	1.709	12.736
(Cutoff: 3000 (adjusted value 1))			

P 1.09

HLA-Antikörpernachweis mittels CDC-und Luminex-Technologie bei refraktärer Thrombozytentransfusion

Thierbach J.[1], Braisch M.[2], Sunic K.[2]

[1]Rotkreuzstiftung, Regionales Blutspendezentrum SRK, St. Gallen, CH, [2]RBSZ, St. Gallen, CH

Im vorliegenden Fall wurden bei einer 60-jährigen Patientin mit einer AML bei Z. n. Chemotherapie mit Thrombozytopenie regelmässige Thrombozytensubstitutionen durchgeführt. Mit zunehmender Thrombozytenkonzentratgabe kam es zu einem immer schlechteren Anstieg der 1 Std.- und 24 Std.-Thrombozytenwerte. Die Suche nach HLA-Antikörpern mit dem herkömmlichen CDC-Test war primär bei Aufnahme negativ, mit zunehmender Anzahl transfundierter HLA-nicht identischer Präparate konnten jedoch einige zu den Mismatchen korrespondierende HLA-Antikörper im CDC-Test gefunden werden. Eine Kontrolle mit der hochsensitiven Luminex-Methode brachte den Nachweis, dass bei einem MFI von 500 nahezu jeder Mismatch der transfundierten Thrombozytenpräparate als HLA-Antikörper bei der Patientin nachgewiesen werden konnte. Eine retrospektive Kontrolle der Rückstellproben mittels der Luminex-Methode zeigte, dass schon zum Zeitpunkt der Aufnahme bei der Patientin HLA-Antikörper nachweisbar waren. Diese könnten unter Umständen auf die Gabe von 8 Erythrozytenkonzentraten im Jahr 2001 und zwei vorausgegangene Schwangerschaften zurückgeführt werden (wird z.Zt. abgeklärt). Diese Fallstudie zeigt, dass refraktäre Thrombozytensubstitutionen auf HLA-Antikörper zurückgeführt werden können, die mit der herkömmlichen CDC-Methode bisher nicht nachweisbar waren, mittels der Luminex-Methode jedoch nachweisbar sind und klinisch auch sicher berücksichtigt werden müssen. Weitere grössere klinische Studien könnten zeigen, ob diese Situation auch bei anderen therapierefraktären Thrombozytensubstitutionen vorliegt.

P 1.10

Antibody against NS1 Antigen in Dengue Infected Patients Induces Upregulation of Anti-Apoptotic Heme Oxygenase-1 Gene in Endothelial Cells

Rahayu P.[1], Naidu S.[1], Bayat B.[1], Rachman A.[2], Immenschuh S.[3], Santoso S.[1]

[1]Justus-Liebig University, Institute for Clinical Immunology and Transfusion Medicine, Gießen,D, [2]University Indonesia,Department of Oncology and Hematology, Jakarta, RI, [3]Hannover Medical School,Institute for Transfusion Medicine, Hannover, D

Purpose: Dengue virus (DV) infection is one of the most serious mosquito-borne flaviviruses of human that cause morbidity and mortality. There are two life-threatening forms of the disease; dengue hemorrhagic fever (DHF) and dengue shock syndromes (DSS) characterized by thrombocytopenia, increased vascular permeability and bleeding. The pathomechanism of this severe dengue forms is incompletely understood. Several evidences suggests that antibodies against DV nonstructural glycoprotein NS1 play a role in the pathogenesis of severe dengue due to cross-reaction of these antibodies with platelet and endothelial cells of patients. Recently, it has been demonstrated that antibodies against NS1 induced endothelial cells to undergo apoptosis. The exact mechanism underlying this antibody mediated cell apotosis, however, is not known yet. Heme oxygenase-1 (HO-1) is an enzyme originally classified as the metabolic determinant of heme degradation, which oxidizes heme to biliverdin, carbon monoxide and free iron. In addition to its metabolic functions, HO-1 has been defined as a cytoprotective and acute phase gene involved in numerous physiological and pathophysiological processes.
In this study, we investigated the influence of NS-1 antibodies from DHF patients on the regulation of HO-1 gene in HUVEC cells.
Methods and Results: Sera derived from patients with DHF (n=57) were screened for anti-NS1 NS1 by solid phase ELISA. In 47/57 sera antibodies against NS-1 were found. Incubation of HUVEC cells with purified IgG containing anti-NS1 antibodies (n=12) caused HO-1 gene expression in time and dose manner. This upregulation was not observed with IgG fraction from patients without detectable anti-NS1 antibody (n=6), Similary, treatment with monoclonal antibody against NS-1 markedly induced HO-1 expression in HUVEC cells (n=5), which was attenuated by the presence of NS1 antigen. Furthermore, reduction of HO-1 gene expression was observed, when HUVECs were pretreated with PI3K pathway inhibitors (LY 294002 and wortmannin) prior to stimulation with anti-NS1. No influence was detectable with p38 MAPK inhibitors (SB 203580). Finally, treatment with NS1 antibody led to I HUVEC, which was sensitive to PI3K inhibitors.
Conclusion: These finding suggest that binding of anti-NS1 on endothelial cells cause upregulation of the apoptotic HO-1 gene expression via activation of PI3K and NF- Understanding the role of HO-1 expression during dengue infection may have potential therapeutic benefits.

P 1.11

ABO Titer Assessment in ABO-Incompatible Living Kidney Transplantation-Titer Course of 10 Successfully Transplanted Patients and Influence of ABO Antibodies in IVIG Preparations

Wienzek S.[1], Bakchoul T.[1], Weimer R.[2], Bein G.[1]

[1]Institute for Clinical Immunology and Transfusion Medicine, Gießen, D, [2]Department of Internal Medicine, Gießen, D

Background: The increased incidence of end-stage renal disease and scarcity of cadaveric organs lead to an increase in living kidney donation. It has been suggested that a further 20% increase in living kidney transplantation (Tx) may be achieved when ABO-incompatible donors may successfully donate their kidney. However, in 36% of all intended living donor kidney Tx the potential donor has an incompatible ABO blood group. Over the past few years, new successful protocols were developed using Rituximab, intravenous immunoglobulin (IVIG) and ABO-antigen specific immunoadsorption (IA). These new protocols are able to decrease successfully the titer of ABO antibodies. The assessment of ABO antibody titers against donor erythrocytes is necessary to guide pretransplant IA treatments and possibly necessary IA treatments in the first 2 weeks posttransplant. However, ABO antibody assessment and interpretation of the results has some perfidies. Two years ago we reported that IVIG application affects the ABO antibody titer thus we usually assess the ABO titer in different IVIG preparations to choose the preparation with the lowest content of ABO antibodies.

Material and Methods: ABO antibody titer in serum samples from a recipient of a kidney from a living related donor were measured during pretransplant treatment period. The titer of anti-donor IgG and IgM antibodies was determined using a standardized tube technique. The impact of IVIG preparations on ABO titer assessment was evaluated by the same technique.
Results: In the early phase of IA treatment the anti-donor erythrocyte titer decreased as expected. However, an increase in antibody titer was observed following IVIG administration despite ongoing IA treatments. To verify our suspicion that IVIG might affect ABO antibody titers we analysed the applied and other IVIG preparations by tube techniques and direct Coombs test. The different tested IVIG preparations revealed titers between 8 to 256, 2 to 32 and 8 to 64 against A1, A2 and B red blood test cells, respectively. Therefore, the clinicians usually apply the IVIG preparation with the lowest content of ABO antibodies. Moreover in the presented single center experience we show you the course of antibody titer assessment and clinical outcome of 10 successfully transplanted patients in a 30 months follow up.
Conclusion: ABO titer assessment in IVIG preparation is a helpful tool for choosing the suitable IVIG preparation and to save costs for additional immunoabsorptions treatments.

P 1.12

Apparent Non-Expression of RHD Associated with a Splice Site Mutation

Daubere E.-M.[1], Jungbauer C.[2], Renz R.[2], Hobel C.[2], Neumüller J.[3], Mayr W.[4]

[1]Medical University of Vienna, Blood Group Serology, Wien, A, [2]Red Cross Blood Donation Centre, Vienna, [3]Medical University of Vienna, Department for Cell Biology and Ultrastructure Research, Vienna, A, [4]Medical University of Vienna, Division of Blood Group Serology, Vienna, A

Purpose: A 65-year old, male Caucasian individual showed discrepant phenotyping and genotyping results at the RHD locus. In-depth analysis including flow cytometry and RHD sequencing was carried out to clarify the situation.
Methods: A series of different monoclonal and polyclonal anti-D reagents were used in antiglobulin testing, absorption/elution experiments and flow cytometry. Genotyping of the RH genes was carried out with the Ready Gene CDE kit for all RHD exons and the weakD SSP kit (Innotrain), which is able to detect weak D types 1 to 5, 11, 14, 15 and 17. Additionally, an in-house method was performed. Sequencing of the exons 1-10 of the RHD gene was carried out including the promoter region and the intron/exon boundaries, for exon 3 with two different sets of PCR and sequencing primers.
Results: The phenotype D-C+c+E-e+ obtained in routine blood group serology was confirmed applying a series of additional anti-D reagents in antiglobulin testing, absorption/elution experiments and flow cytometry. SSP-Genotyping showed positive reactions for the RHD exons and negative reactions in the weak D SSP kit. Thus, many partial and weak D types were ruled out. Sequencing of all RHD exons showed a point mutation in the 5' splice site of intron 3, which was confirmed applying alternative primers.
Conclusion: This splice site mutation is highly suggestive to be the reason for the non-expression of this RHD allele. In a further step, close relatives will be tested to elucidate the genetics of this silent RHD allele.

P 1.13

Detection and Differentiation of Platelet-Binding Immunoglobulins Using PIFT vs. SASPA

Göbel M.[1], Baade M.[2], Klüter H.[3], Nguyen X.D.[3]

[1]Medical Faculty Mannheim, Heidelberg University, Red-Cross Blood Service of Baden-Württemberg - Hessen, Germany, Institute of Transfusion Medicine and Immunology, Mannheim, D, [2]Institute of Transfusion Medicine and Immunology, Mannheim, D, [3]Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Heidelberg University, Mannheim, D

Objectives: Glycoprotein (GP)-specific platelet-binding antibodies can cause allo- or autoimmune thrombocytopenia. The detection and differentiation of relevant antibodies is a prerequisite for an adequate clinical treatment. Two principle methods are currently being used. One is the platelet immunofluorescence test (PIFT), mainly used as a screening assay to detect platelet-binding immunoglobulins. For further analysis of platelet antibodies a glycoprotein-specific assay is necessary. We use the novel SASPA assay (Simultaneous Analysis of Specific Platelet Antibodies). This assay allows rapid and simultaneous detection of platelet specific IgG and IgM antibodies by flow cytometry. Here, we performed a serial investigation on platelet-binding immunoglobulins and platelet-specific antibodies using PIFT vs. SASPA.
Materials and Methods: Sera from 220 patients with or without thrombocytopenia with various underlying diseases were investigated for platelet-binding immunoglobulins and platelet-specific antibodies against glycoprotein GPIIb/IIIa, GPIX, GPIa/IIa, HLA class I and CD32 (FcγRII), CD36 using PIFT and SASPA, respectively. For PIFT and SASPA, the same test platelets from 4 different HPA-typed donors were used. AB-sera and sera containing antibodies with known specificities were used as controls.
Results: Out of 220 sera tested, 32 sera (14.5%) were detected positive in both assays. This included all sera containing single or several specific anti-GPIIb/IIIa (n=9), anti-HPA-1a (n=1) and anti-CD36 (n=3). Also sera containing anti-HPA-5b (n=6) and anti–GPIa/IIa were positive in both assays. 17 sera (7.7%) were positive only in PIFT but negative in SASPA. Notably, 16 sera (7.3%) were positive only in SASPA. Glycoprotein-specific analysis of these sera revealed single or multiple antibodies against HPA-5a (n=2), HPA-5b (n=6); GPIa/IIa (n=4), CD32 (n=6) and HLA-class I (n=10). Finally, 155 sera (70.5%) were negative in both assays.
Conclusion: The platelet immunofluorescence test (PIFT) shows limited sensitivity regarding anti-GPIa/IIa, particularly anti-HPA-5, and HLA-class I specific antibodies. The SASPA assay is a suitable method for detecting and differentiation of platelet-specific antibodies. A combination of PIFT and SASPA proved to be reasonable for the detection of clinically relevant platelet antibodies.

P 1.14

Successful Management of Platelet Transfusion Refractoriness during Haematopoietic Stem Cell Transplantation

Wolf Z.[1], Saure C.[2], Hoffmann T.[3]

[1]Düsseldorf University Hospital, Institute for Hemostasis and Transfusion Medicine, Düsseldorf, D, [2]Düsseldorf University Hospital, Department of Haematology, Oncology and Clinical Immunology, D, [3]Düsseldorf University Hospital, Institute of Hemostasis and Transfusion Medicine, D

Platelet transfusions are essential to prevent fatal haemorrhage with myeloablative therapy and haematopoietic stem cell transplantation (HSCT). While leukodepletion of cellular blood products has substantially reduced the incidence of HLA antibodies in transfusion recipients, alloimmunization in woman with former pregnancies still is a major problem with respect to the transfusion support in haematologic malignancy.
Here we present a case of a 41-year-old woman with a secondary acute myeloid leukaemia and refractoriness to platelet transfusion occurring during induction chemotherapy. Multispecific anti-HLA class I-antibodies as well as an anti-HPA-5b alloantibody were shown causative. Clinical relevance of the anti-HPA-5b antibody was objectified, as transfusion efficacy of HLA compatible platelet concentrates depended on HPA-5-matching. Four donors with HLA-A/-B-full match and one donor with a 3/4 match were identified from the donor pool of our blood donation centre, all of them with HPA-5b-negative genotype.
During myeloablative HSCT a total of 19 platelet units from these 5 donors were administered. Platelet count increments were effective and major bleeding was prevented during the whole period of haematopoietic aplasia. Stable engraftment with respect to platelet counts was achieved at day +25.
Forceful diagnostic approaches with selection of donors from extended pools of typed donors allow offering myeloablative treatment and HSCT even to polyimmunized patient.

P 1.15

Severe Ceftriaxone-Induced Immune Thrombocytopenia with Antibodies against Platelet Glycoprotein IIbIIIa

Winterstein K.[1], Otto C.[2], Büttner A.[2], Kroll H.[2]

[1]Red Cross Blood Transfusion Service NSTOB, Institute for Transfusion Medicine Dessau, D, [2]Dessau, D

Purpose: Drug-induced immune hemocytopenias are rare but potentially severe side effects of several drugs. Detection of the responsible antibodies may prevent the patient from reexposition to the drug.
Case Report: An 81-year-old female patient suffering from biliary obstruction and post-ERCP-pancreatitis with multiple abscess formations received a daily dose of ceftriaxone intravenously because of repeated fever. Further medications were metronidazole, paracetamol and low molecular weight heparin (enoxaparin) for about 8 weeks. The platelet count was normal

(243,000/μL) before treatment. On Day 3 of ceftriaxone therapy epistaxis developed and the platelet count dropped to 4,000/μL. On the next day, extensive petechiae appeared at a platelets count of 3,000/μL. Serologic examination was initiated because immune thrombocytopenia was suspected. After discontinuation of ceftriaxone the platelet count rose immediately to 154,000/μL and petechiae as well as epistaxis resolved spontaneously during the following days.
Methods: The serum was analysed by heparin /PF4 enzyme immunoassay, heparin-induced platelet activation assay, platelet suspension immunofluorescence test (PSIFT) and glycoprotein-specific immunoassay (MAIPA).
Results: On initial analysis the serum gave negative results in all assays. Upon flow cytometric PSIFT the serum was strongly positive in the presence of 1 mg/mL ceftriaxone but negative in the absence of the drug (MFI ratio: 40) indicating ceftriaxone-dependent platelet-reactive antibodies. No reactivity was detectable in the presence of metronidazole and paracetamole. The target of ceftriaxone-dependent antibodies was further localized on glycoprotein IIbIIIa by MAIPA assay. In contrast, no binding was detected on platelet glycoproteins IbIX or IaIIa as well as on red blood cells indicating clear epitope specificity.
Conclusions: We describe a further case with severe ceftriaxone-induced thrombocytopenia. This drug seems to bear a special risk for the induction of drug-dependent antibodies against different blood cells. Interestingly, multiple distinct targets have been observed. Physicians who use ceftriaxone should be aware of this side effect.

P 1.16

Association of X-Linked Chronic Granulomatous Disease with the Rare McLeod Phenotype – a Case Report

Siderow A.[1], Ries J.[2], Grossrieder B.[2], Reichenbach J.[3], Seger R.[3], Hergersberg M.[4], Röthlisberger B.[4], Frey B.M.[2]

[1]Zürcher Blutspende, Immunhämatologie, Schlieren ZH, CH,
[2]Zürcher Blutspende, Schlieren, [3]Kinderspital, Zürich,
[4]Zentrum für Labormedizin, Aarau, CH

Background: McLeod syndrome (MLS) is characterized by the absence of the high frequency red blood cell (RBC) antigen Kx (>99.9%), weakened expression of KEL antigens, acanthocytosis and hemolytic anemia. MLS results from mutations in the XK-Gen at Xp21.1. Mutations of the X-chromosomal gene encoding gp91-phox (CYBB), a subunit of cytochrome b (-245) cause the X-linked granulomatous disease (X-CGD). X-CGD caused by deletion of the CYBB locus might be associated with the rare MLS. In these cases, the XK gene locus at Xp21.1 adjacent to CYBB locus is affected by an extend deletion of CYBB including Xp21.1 leading to disturbed expression of XK-KEL protein complex on RBC membrane and Kx neg RBC phenotype. To date, 8 different mutations translating into X-CGD associated MLS are known. All affected carriers are males as is expected in X-linked hereditary diseases.
Case: We report a 10-year old boy with X-CGD and therapy refractory pulmonary aspergillosis. Because of lack of HLA-identical stem cell donor, he was treated by genetically (gp91phox) corrected autologous blood stem cell transplantation (ABSCT). Following mild conditioning for ABSCT, he became transfusion dependent and he received 19 RBC-units. On pretransfusion compatibility testing he presented with a high-titer anti-Kx antibody (>1:8'000) preventing allocation of compatible allogeneic RBC from blood storage repository. Search of rare donor data files provided one healthy Swiss blood donor carrying McLeod RBC phenotype who was recruited for directed blood donation. Unfortunately, following transfusion of Kx neg RBCs of this donor, the patient developed additional RBC alloantibodies (anti-E, anti-K20) which required worldwide donor search. One compatible donor was identified and provided several units of RBC that were successfully transfused. After sufficient erythropoietic engraftment the patient became transfusion independent again. Later on, following an infection, the patient was again successfully transfused with Kx neg, K20 neg and E neg RBCs. The patient continued to produce RBC that are expected to be of McLeod phenotype but without sign of hemolysis. By Array Comparative Genomic Hybridization (CGH) the deletion of Xp21.1 encompassed the complete XK-gene and the first three exons of the CYBB gene causing the clinical phenotype of X-CGD associated MLS.
Conclusions: X-CGD is rarely associated with hereditary MLS harbouring the risk of allosensitisation against the high-frequency RBC antigen Kx which may severely compromise transfusion support. Therefore, these patients should require immunohematological work-up and extensive organisational preparation for transfusion support prior to invasive regimens.

P 1.17

HIT II Diagnostics Including HLA- and Platelet Antibody Testing – Experiences in Jena

Oberle V., Barz D.

University Hospital Jena, Institute for Transfusion Medicine, Jena, D

Introduction: In difference to most laboratories performing HIT II diagnostics in Jena there is always a full program of additional tests running, which should prevent wrong HIT II test results, clarify different ones of different test methods and exhibit alternative reasons for a thrombocytopenia.
Material and Methods: We use two commercial available heparin antibody detection tests the PAIFA-Test from Akers Bioscience and the heparin/PF4-ELISA from GTI diagnostics.
HLA- and platelet antibodies are tested with the Pak2-LE from GTI and in case of positive ELISA results with the Luminex-assay and /or LcT-Test SeraScreen FCT60 from BAG.
Results: In ca 70% of all cases the results of the two Heparin antibody testing methods are identical. In most cases 50% of all, or 66% tests, the HLA- and platelet antibody inclusive, are negative.
The rest of the results show HLA antibodies in14%, and platelet antibodies in 7% of these cases.
If there is a different testing result related to the two heparin antibody tests (30% of all) there are no additional antibodies detectable in 65%, whereas HLA antibodies in 2.7% and platelet antibodies in 1.5% were present studying these cases. If patients are several times tested the result will be in 75% reproducible, whereas in only 25% there is a discrepancy in the results. This discrepancy might be caused by an increase in HLA-antibody titre and is related to the heparin-ELISA-method. But the numbers of these cases are too low to be statistically proven and need further investigations.
Discussion: Our experiences with a complete testing program in Hit II Diagnostic exhibit an entire view on the thrombocytopenic event. In case of differences between test results using different testing methods, it is possible to give more necessary information to the clinician for therapy. There might be a connection between a positive heparin-ELISA result and a high HLA-antibody titre in the patients serum.

P 1.18

Anti-Jr(a) in Pregnancy – a Case Report

Thomas E.[1], Förstemann E.[2], Scharberg E.A.[3]

[1]DRK-BSD Ost gGmbH, ITM Dresden, Immunhämatologisches Labor, Dresden, D, [2]DRK-BSD Ost gGmbH, ITM Dresden, D,
[3]DRK-BSD Ba-Wü-Hessen gGmbH, ITM Baden-Baden, D

We investigated a blood sample of a 20 years old Caucasian woman, who developed an antibody against a high frequency antigen during her first pregnancy. Her serum showed positive reactions (2+) with all test cells in indirect antiglobulin test in the gel technique and in the tube test. Only three different Jr(a) negative cells of a special red cells panel negative for different high frequency antigens reacted negatively. The direct antiglobulin test of the patient was negative.
The patient was typed Jr(a) negative using two single donor source of anti-Jr(a).
The patient's serum was also tested against red cells from her mother, father and brother, who were all found to be reactive with her serum and also to be Jr(a) positive. However, the reactions with red blood cells from her mother and father were considerably weaker than those of her brother or random panel cells. This suggests that red cells from her father and mother express a single dose of Jr(a) antigen.
After an uneventful pregnancy and delivery the newborn showed a positive direct antiglobulin test (IgG 1+, C3d 2+) but did not develop a hemolytic disease of the newborn. The patient did not need a red cell transfusion.
Conclusion: In few cases anti-Jr(a) has been reported to cause hemolytic transfusion reactions and a hemolytic disease of the newborn. If possible Jr(a) negative red cells are usually recommended be used for transfusion. In our case a newborn of a mother with anti-Jr(a) did not develop a hemolytic disease of the newborn. The mechanism of immunization against the Jr(a) antigen in the first pregnancy remains unclear.

P 1.19

Seroepidemiologic Evaluation of Rh System Major Antigens(D,C,E,c,e) and their Phenotypes among the Blood Donors in Khorramabad, Iran

Abdi J.[1,3], Kiani A.A.[2], Engels F.[3]

[1]Iranian Blood Transfusion Organization Research Center, Tehran, Iran,
[2]Lorestan University of Medical Sciences, Khorramabad, Iran,
[3]Division of Pharmacology & Pathophysiology; Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands

Background: Rh blood group system is the most clinically important one after the ABO system. This system contains at least 50 blood group antigens, but the most important of them are the five antigens(D,C,E,c,e) with D being the most immunogen. Getting knowledge of Frequency of these antigens is necessary for population studies, managing the inventories in blood centers and also in paternity tests.
Materials and Methods: The study was carried out as census on 1240 blood donors referring to Khorramabad blood center. The 2-5% cell suspention in saline was incubated with Rh antiserums(anti-D, anti-C, anti-E, anti-c, anti-e, and anti-CDE as control) with all the steps being done just according to available SOPs and manufacturers instructions. Depending upon the type of reactions, and also on the basis of most frequent genotypes by referring to the tables of Rh antigens frequency in black and white populations, Rh phenotype for main above antigens were determined. **Findings:** Out of all of the observed reactions, the most common one was associated with the e antigen(94.4%) and the least with the E antigen(43.5%). As to D antigens, 92.6% of individuals were positive and 7.3% were negative. The results were 73.5% and 71.2% for C and c antigens, respectively. **Conclusion:** A 92.6% frequency for D antigen indicates a high difference with other home regions and in the world. As a result , considering the Rh negative blood units in the region deserves much notice. In the meantime, the relatively significant frequency of e negative donors in the region addresses the necessity of setting up the e negative blood units bank much more than before.

Frequency(%)	number	antigens
94.4	1166	e+
5.6	70	e-
71.2	883	c+
28.5	353	c-
43.5	540	E+
56.1	696	E-
73.5	911	C+
26.2	325	C-
92.6	1148	D+
7.10	88	D-

P 1.20

Antibody Screening with Enzyme-Treated Test Cells Detects Potentially Transfusion-Relevant Allo-antibodies

Geisen C.[1], Klarmann D.[2], Sireis W.[2], Seifried E.[2], Bonig H.[2]

[1]DRK Blutspendedienst BW-Hessen, Institut Frankfurt am Main, Immunhämatologie, Frankfurt am Main, D, [2]DRK Blutspendedienst BW-Hessen, Institut Frankfurt am Main, D

Purpose: Allo-antibody screening (ABS) with defined test cells is a critical component of pre-transfusion diagnostics. However, the titers of some "historical" allo-antibodies may fall below the detection threshold of even very sensitive ABS techniques, such as the gel centrifugation assay, but can be rapidly boostered by transfusion of incompatible erythrocytes and then cause delayed hemolytic transfusion reactions. It is known that some such antibodies can be detected by ABS with enzyme-treated test cells, but this test is not routinely performed.
Methods: Using papain-treated ABS test cells, we prospectively studied the frequency of "enzyme-only" allo-antibodies in 4133 indirect antiglobulin test (IAT) ABS-negative routine patient plasma samples (LISS-suspended test cells on IAT gel centrifugation cards).
Results: 189/4133 IAT-negative samples were reactive in the enzyme milieu. Of these, 32 (0.77% of total) were positive for a total of 34 specific allo-antibodies, the remainder contained auto-antibodies or pan-agglutinating antibodies. Allo-antibodies were mostly directed against Rh antigens (n=27), in addition, 1 anti-K1 and 3 typical cold-reactive antibodies (anti-Le(a)/(b), anti-P1) were detected. Transfusion history, in as far as it was available, was not informative.
Conclusions: Given the significant cost associated with enzyme ABS and antibody differentiation, where 120 enzyme ABS and 6 differentiations had to be performed to detect one antibody-positive patient, some immunohematologists may consider this approach not cost-effective for routine pre-transfusion diagnostics. Alternatively, we propose to extend Rh subgroup and K1 typing to all patients, and to select compatible blood products whenever possible.

P 1.21

Evaluation of an Immunohematological Methods Using the Cellbind Technology on the Magister Sanquin System

Köcher K., Korb M., Pretzel K.J., Pruß A., Schönfeld H.

Institute of Transfusion Medicine, Charité - Universitätsmedizin Berlin, D

Introduction: Sanquin has developed a new technology (micro column affinity test) for blood grouping (AB0, Rh factor), Rh phenotyping (C, c, E, e), Kell and the detection or identification of red cell antibodies (ABS), suitable for automation.
The method is based on a micro column affinity test system in which sensitized red cells from a suspension will bind to a gel matrix containing immobilized protein G, anti-IgM and anti-C3d in an enhancing Medium. The principle of the test is the affinity technique, which is based on antigen-antibody reaction. The cell suspension is added to the incubation compartment, together with the plasma/ blood grouping reagent. After a three phases centrifugation sensitized red cells are attached to the affinity matrix and will adhere on top of the gel matrix, while the non- or very weakly sensitized red cells will move toward the bottom of the micro column, as discrete button of red cells.
Material and Methods: In a comparative study, EDTA blood samples from blood donors and patients were tested on Cellbind-Technology and the observed data were directly compared with the results of the Charité blood bank method:
• Olympus PK 7200 for AB0/ Rh blood grouping and Rh phenotyping
• Galileo-Immucor System for blood grouping and ABS
• DiaMed gel card centrifugation technique for antibody screening
Tests were performed for blood grouping (AB0 with direct and reverse typing, Rh factor) and Rh phenotyping (C, c, E, e), Kell by two determinations on 579 samples of donors and patients and as for ABS (indirect anti-human globulin test) on 517 samples of donors and patients.
Results: AB0/ Rh grouping showed a 93.6% concordance on common erythrocyte groups between observed results and expected ones. 37 doubtful or false positive reactions were found in reverse typing due to an artefact issue (fibrin residues and lipemic samples) (6.4%). After some adaptations of the programming the results of the C, c, E, e, K tests were accurate in the first and second determination and showed 99.0% concordance between observed and expected results. Furthermore, we observed discrepancies on 3 of 6 weak D samples, which were also discrepant by the reference methods as well.
450 of 461 samples for antibody screening test Cellbind technique were correct negative (97,6%). 51 of 56 known antibodies were confirmed by the Cellbind technique (91.1%). 5 of 56 Samples were false negative (8.9%). 4 of 446 Samples revealed unknown antibodies in comparison to the reference technique and were later confirmed in complementary tests (DiaMed, Immucor, Ortho, Biotest). All of these revealed antibodies were confirmed as Anti-Lea (IgM) antibodies. 7 of 461 Samples showed false positive results related to fibrin residues and lipemic samples (1.5%).
Conclusion: The Magister equipment is very easy to use and could be introduced in a regular lab working schedule. Still we recommend a necessarily improvement of the evaluation software. The manually reading of the Cellbind-Cards needs some experience/ training.
Magister provides a full automation system (equipment and reagents) and is reliable and safe for AB0/ Rh grouping and Rh phenotyping.

P2 Immunogenetics, Transplantation Immunology

P 2.01

Mechanism of Immunosuppression by Mesenchymal Stem Cells or Fibroblasts

Mailänder V.[1], Hennel E.[2], Eicher L.[2], Flören M.[3], Rojewski M.[2], Reichel H.[3], Schrezenmeier H.[2], Mailaender V.[4]

[1]University Medicine of the Johann-Gutenberg University, Mainz, III. Medical Clinic, Mainz, D, [2]University of Ulm, D, [3]University of Ulm, Department of Orthopedics, Rehabilitation Clinic Ulm, D, [4]III. Medical Clinic, University of Mainz, D

Purpose: While Mesenchymal stem cells (MSC) are increasingly used for therapy of GvHD or in treatment of autoimmune diseases the mechanism of immunosuppression is still unknown. We investigated if this depends on close cell-cell contact or is mediated by a soluable factor and if this is specific for MSC. Therefore different cell populations and their supernatant were studied if they can suppress a mixed lymphocyte reaction (MLR): untouched MSC, conditioned MSC, i.e. MSC preincubated with two populations of lymphocytes, fibroblasts and the supernatant of these cell populations. Furthermore the role of indoleamine 2,3-dioxygenase (IDO) as IDO metabolizes tryptophan was studied.
Methods: MSC from bone marrow were characterized by FACS and differentiation assays. MSC, conditioned MSC or fibroblasts were either added in a 1:1 ratio to responder lymphocytes to the standard MLR or used in transwell inserts in the same ratio. Supernatant was 0.22 µm filtered. We also added tryptophan (add-back) or 1-methyl-tryptophan (1-MT) (inhibitor of IDO) to our MLRs.
Results: Untouched MSC in a ratio of 1:1 suppressed the proliferation of lymphocytes in the MLR to <50% repeatedly. Transwell experiments and preincubated MSC showed the same amount of reduction (to around 50% and <40%).In experiments with MSC supernatant we did not find any suppressive effect, independent if conditioned or not. Fibroblasts show a suppression to <50%, while supernatant from fibroblasts was also ineffective.
Add-back of Tryptophan has no effect on the suppression of MLR, neither does the inhitibion of IDO with 1-methyl-tryptophan.
Conclusions: Immunosuppression by MSC can be detected in direct cell-cell contact and in transwell experiments pointing towards a soluable factor. Judging from our results this soluable factor cannot be transferred effectively by supernatant. This could be explained if the soluable factor is rather instable but is needed for a prolonged time during the MLR.
Fibroblasts interestingly showed a considerable amount of suppression of proliferation.
1-MT did not show an effect on immunosuppression nor add-back of tryptophan, suggesting that this is not the cruciable factor.
The mechanism of immunosuppression is still not fully understood and further investigation is needed.

P 2.02

Human Antigen Presenting Cells Generated under the Influence of Heat Shock Proteins Can Improve the Adoptive Cellular Immunotherapy

Bajor A.[1], Lukis S.[1], Rokitta D.[1], Struß J.[1], Figueiredo C.[1], Wittmann M.[2], Blasczyk R.[1], Eiz-Vesper B.[1]

[1]Hannover Medical School, Institute for Transfusion Medicine, Hannover, D, [2]Department of Dermatology and Allergology, Hannover Medical School, Faculty of Biological Science, University of Leeds, Hannover, D, Leeds, GB

Purpose: Calreticulin (CRT), an endoplasmic reticulum (ER) resident protein, is involved in critical cellular functions, such as protein folding and antigenic peptide cross presentation. Furthermore, this chaperone has been proposed to act as an adjuvant during the activation of dendritic cells (DCs) in vivo. We assessed human eukaryotically expressed CRT for its potential to induce NF-κB regulated maturation of monocyte-derived DCs.
Methods: In order to facilitate eukaryotic expression procedures, we established and compared three different methods to express recombinant endotoxin-free CRT to be secreted in the supernatant of HEK 293 cells: (1.) the complete, unmodified CRT coding sequence was cloned into the pcDNA3.1V5/His vector (euCRT), (2.) the C-terminal ER-retrieval KDEL amino acid sequence was mutated into KDQL in order to disturb the endoplasmic retention and support the protein secretion (euCRT_KDQL) and (3.) a shRNA was designed to knock down the expression of aminoacyl-tRNA synthetase-interacting multifunctional protein-1 (AIMP-1), which is known to regulate protein retention in the ER. An efficient shRNA sequence specific to AIMP-1 transcripts was delivered to HEK 293 cells which were afterwards transfected with the CRT-expressing vector (euCRT).
Results: No relevant differences between these different approaches were observed in regard to mRNA levels determined by Real Time PCR as well as protein expression levels of CRT determined by ELISA. Thus, for large scale expression of CRT the first strategy with the unmodified CRT sequence (euCRT) was chosen. The functional capability of CRT to induce maturation of DCs was tested. By flow cytometry the translocation of NF-κB into the nuclei of the monocytes after stimulation with CRT could be demonstrated. Using low-dose CRT (10 µg/ml) the phenoptype of the immature DCs changed to a more matured one, as indicated by an increased surface expression of CD40, CD86, CD83.
Conclusion: In summary, our data indicate that CRT can act as an adjuvant for in vitro maturation of DCs and therefore has the potential to assist in T-cell stimulation and expansion protocols.

P 2.03

Clinical Significance of HLA-E*0103 Homozygosity on Survival after Allogeneic Hematopoietic Stem Cell Transplantation

Gabriel C.[1], Danzer M.[1], Polin H.[1], Pröll J.[1], Haunschmid R.[2], Hofer K.[1], Stabentheiner S.[1], Hackl C.[1], Kasparu H.[3], Hauser H.[4], Krieger O.[3]

[1]Blood Center Linz, A, [2]Federal Agency for Water Management, Mondsee, [3]Elisabethinen Hospital, Linz, A, [4]3Elisabethinen Hospital, Linz, A

Purpose: Hematopoietic stem cell transplantation (HSCT) is a well-established treatment in various hematologic malignancies, but the outcome depends on disease relapse, infections and the development and severity of acute and chronic graft-versus-host disease (GvHD). Some evidence has revealed an important role for the non-classical major histocompatibility complex (MHC) class I molecules in transplantation, most notably human leukocyte antigen (HLA)-E. The present study evaluates the impact of HLA-E alleles on transplantation outcome after HLA matched allogeneic HSCT.
Methods: We genotyped DNA for HLA-E polymorphism from 83 recipients and their respective donors by real-time polymerase chain reaction (PCR) following melting curve analysis and compared the results with clinical outcome.
Results: HLA-E*0103 homozygous patients showed a higher probability of overall survival (P=0.003) and disease-free survival (P=0.001) in a univariate model. Cox regression analysis confirmed HLA-E*0103, 0103 (P=0.006; relative risk [RR]: 1.12; 95% confidence interval [CI]: 0.31-1.94) and early stage of disease (P=0.005; RR: 1.16; 95% CI: 0.45-1.86) as independent factors improving overall survival. Moreover, homozygosity for HLA-E*0103 was associated with a significant decreased incidence of transplant-related mortality (P=0.01).
Conclusions: We found an association between HLA-E*0103 homozygosity and the significant reduction of transplant-related mortality in related and unrelated HSCT. The risk of post transplant complications was significantly reduced when the donor possesses the HLA-E*0103, 0103 genotype and this was translated in a better overall survival.

P 2.04

A Novel HLA-DQB1*06 Allele with an Exon 3 Amino Acid Modification Found in a Voluntary Blood Stem Cell Donor of Caucasian Origin

Skrablin P.[1], Brixner V.[2], Richter R.[2], Seifried E.[2], Seidl C.[2]

[1]German Red Cross Blood Donation Service Baden-Wuerttemberg - Hessen, Institute of Transfusion Medicine and Immunohematology, Frankfurt, D, [2]German Red Cross Blood Donation Service Baden-Wuerttemberg - Hessen, Frankfurt, D

Purpose: HLA-molecules are characterised by a high polymorphism. To optimize transplant outcome, complex HLA typing techniques are therefore required to achieve a high level of histocompatibility between donor and recipient. Currently, there are 3,635 HLA alleles described by the HLA nomenclature, including 96 different HLA-DQB1 alleles with 45 of them belonging to HLA-DQB1*06. We describe a novel HLA-class II, HLA-DQB1*06 that has been observed in a voluntary blood stem cell donor of Caucasian origin from our local blood stem cell donor registry (donor ID FFM 58536) and his son.
Methods: DNA was isolated from peripheral blood cells (Qiagen). Low resolution typing of HLA class I (HLA-A, HLA-B and HLA-Cw) and class

II (HLA-DQB1 and HLA-DRB1) was performed by PCR-SSO (Invitrogen). High resolution HLA-DQB1 typing was conducted by DNA sequence based typing (PCR-SBT, Invitrogen). Sequences were analysed using the SEQUENCE PILOT 3.1.0.4 Software (JSI medical systems GmbH, Kippenheim, Germany).

Results and Conclusions: High resolution HLA-DQB1 typing gave the following ambiguous results, each with one nucleotide mismatch: HLA-DQB1*0603,*0604/*0607,*0608/*0621,*0632. A second typing from an independently blood sample of the donor revealed the same result. Comparison with donor´s direct relatives (mother and son) revealed a novel DQB1*0604 with a sequence variation of HLA-DQB1*0604new in contrast to HLA-B*060401 at nucleotide position 557 (274 of exon 3) where T was exchanged for C. This nucleotide change results in a codon change at position 186 (GTG to GCG) and amino acid exchange from Valin to Alanin. Sequence analysis of the donor´s son revealed the same HLA-DQB1*0604new. HLA-phenotype of the donor was determined to be HLA-A*03,*02; HLA-B*15,*40; Cw*03; DRB1*13; DQB1*0603,*0604new. Family based genotype analysis defined that the novel HLA-DQB1*0604 allele belongs to the paternal haplotype HLA-A*02, HLA-B*40; HLA-Cw*03; HLA-DRB1*13 and donor´s son inherited this haplotyp.

The sequence of HLA-DQB1*0604new (exon 3) has been reported to the EMBL database (FN256435) and to the IMGL nomenclature commission (HWS10006272) to be named.

P 2.05

A Novel HLA-B Allele: HLA-B*9553 – Influence on the Polypeptide Structure?

Wienzek S.[1], Ernst-Schlegel M.[2], Lang C.[2], Scharf M.[2], Müller K.[2], Bein G.[1]

[1]Institute for Clinical Immunology and Transfusion Medicine, Gießen, D, [2]Gießen, D

The extensive polymorphism of the human leukocyte antigen (HLA) class I and class II loci in the human genome comprises more than 1100 HLA-B alleles that have been reported until January 2009 (IMGT/HLA Database).

Here, we describe a novel HLA-B allele, HLA-B*9553, which has been detected by an unexpected SSP pattern at the occasion of donor recruiting for the volunteer haematopoietic stem cell donor registry. The ethnic origin of the 25 year-old male is Caucasian, Central Europe. Further investigation using haplospecific sequencing-based typing (SBT) showed that the new allele is most closely related to a HLA-B*1518 allele. HLA-B*9553 differs from the sequence of HLA-B*1518 by one coding substitution at nucleotide position 488 (C to G) resulting in an amino acid substitution (Ala to Gly) at position 139 of the mature polypeptide.

The amino acid substitution at residue 139 of the HLA-B polypeptide is located in a highly conserved position of the α2 domain in the transition of a beta-sheet into the alpha-helix structure. Therefore, it can be expected that this novel HLA-B allele might have an influence on the mature polypeptide structure.

The antigen genotype of the volunteer donor is HLA-A*29,*68; HLA-B*9553,*44; HLA-Cw*07,*16; HLA-DRB1*07,*16; HLA-DQB1*02,*05.

Finally, the family members were genotyped for HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 to identify the parental haplotype and to confirm the sequence of the new allele in a second individual. Informed consent was obtained from all family members.

P 2.06

Phenotypic and Functional Characterization of the Alloreactive Donor-Derived NK Cell Repertoire to Improve Clinical Outcome of Stem Cell Transplantation

Skrablin P.[1], Richter R.[2], Martin H.[3], Bader P.[4], Seifried E.[2], Seidl C.[2]

[1]German Red Cross Blood Donation Service Baden-Wuerttemberg - Hessen, Institute of Transfusion Medicine and Immunohematology, Frankfurt, D, [2]German Red Cross Blood Donation Service Baden-Wuerttemberg - Hessen, Frankfurt, D, [3]Department of Internal Medicine II, Hematology and Oncology, Stem Cell Transplant Unit, Johann Wolfgang Goethe University, Frankfurt, D, [4]Department of Paediatric Stem Cell Transplantation, Johann Wolfgang Goethe University Hospital, Frankfurt, D

Purpose: Allogeneic stem cell transplantation (alloSCT) may trigger NK cell-mediated graft versus leukemia (GvL) effects leading to improved outcome for patients with malignant haematologic diseases. GvL effects of donor-derived NK cells are caused by a repertoire of alloreactive NK cells expressing inhibitory killer cell immunoglobulin-like receptors (iKIR) for self HLA-ligands missing in the recipient (KIR-MM). Currently, no general conclusion can be drawn from the genetically predicted iKIR repertoire for the beneficial effect of the NK cell alloreactivity. In addition, since iKIR are clonally distributed in the NK cell population with expression frequencies highly variable between individuals, prediction of alloreactivity by HLA and KIR genotyping alone does not consider the actual frequency of functionally mature alloreactive NK cells. Thus, an extensive phenotypic and functional characterization of donor NK cells is needed.

Methods: HLA class I genotyping was performed by PCR-SSO or PCR-SBT and KIR genotyping by PCR-SSP. Peripheral blood NK cells of healthy individuals were isolated by negative selection and used for the establishment of phenotypic and functional analyses. To analyze the NK cell mediated cytotoxicity, K-562 or B-LCL-stimulator target cells were stained with the fluorescence membrane dye DiO or PKH67. After coculture with NK cells target cell viability was determined by propidium iodide or 7 aminoactinomycin D. To determine the frequency of alloreactive NK cells for potential donor-recipient pairs, iKIR with specificities for the polymorphic HLA class I motifs (C1, C2 and Bw4) and NKG2A are analyzed by flow cytometry using different antibody combinations.

Results and Conclusions: We established a cell-mediated cytotoxicity assay to analyze the functional potential of donor-derived NK cells. Isolated peripheral blood NK cells killed K 562 target cells with similar efficiency as the NK cell line NK92. KIR phenotyping of NK cells confirmed KIR genotyping results, but showed inter-individually variations in the presence of NK cell populations expressing certain receptors. Previously, we have shown that AML patients with a genetically determined KIR-MM have an increased event-free and overall survival compared to patients without a KIR-MM. To further characterize the beneficial impact of the KIR-MM, the NK cell reactivity of patients that underwent alloSCT will be analyzed to confirm the correlation between NK reactivity and patient survival. Phenotypic and functional characterization of NK cell subsets in addition to HLA and KIR genotyping will help to identify donors with an optimal NK cell repertoire to improve alloSCT outcome.

P 2.07

Acute Antibody Mediated Rejection after Living Kidney Transplantation despite CDC Negative Crossmatch – a Case Report

Wienzek S.[1], Weimer R.[2], Bein G.[1]

[1]Institute for Clinical Immunology and Transfusion Medicine, Gießen, D, [2]Department of Internal Medicine, Gießen, D

Background: Living kidney transplantation which is increasingly performed due to the shortage of cadaver kidneys provides the advantages of short waiting time, well planned preparation of the transplantation and short cold ischemia time. One disadvantage is the intervention in the physical integrity of a healthy individual.

Case: Here, we describe the case of a 46-year-old female with end stage renal disease. First, the patient, multipara, should receive a kidney donation of her husband. However, this plan was refused by reason of presence of a HLA antibody received during the pregnancies against the HLA-B27 antigen of her husband resulting in a positive CDC crossmatch. The Anti-HLA B27 antibody was detected both by CDC and ELISA testing.

After excluding her husband as living donor the brother of the patient was willing to donate his kidney. The CDC crossmatch between donor and recipient was two times negative. The pretransplant PRA (Panel reactive antibodies) values were between 22-30%. After living kidney transplantation the kidney was lost due to antibody-mediated rejection. Immediately after Tx, antibody screening by CDC was negative and after explantation of the kidney the PRA value increased up to 78%. HLA-class I ELISA was positive (90%), HLA class II ELISA negative. The two weeks after explantation performed antibody screening by CDC showed a broad reactivity against Anti-Bw4 positive epitopes including donor-specific antigens. Retrospective analysis of pre- and posttransplant serum samples using Luminex single antigen bead techniques revealed the Anti-Bw4 reactivity of non-complement activating antibodies.

Conclusion: There is no consensus of clinical relevance of non complement activating HLA antibodies. Detection and characterization of donor specific antibodies using Luminex technique could potentially prevent antibody-mediated rejection or lead to specific strategies before transplantation like plasmapheresis or treatment change in immunosuppressive regimens. Further evaluation of data and studies are necessary to determine the role of non complement activating HLA-antibodies.

P3 Preparation of Blood Components

P 3.01

Cryopreservation and Thawing of Red Blood Cells Using Sterile Tubing Connections in a Closed System

Schäfer U., Wiesneth M., Koerner K., Schauwecker P., von Zabern I., Schrezenmeier H.

Institute Ulm, German Red Cross Blood Transfusion Service Baden-Württemberg - Hessen, Ulm, D

Purpose: Cryopreservation of rare red blood cells (RBC) allows to supply compatible blood products to patients with antibodies directed against high-frequency blood group antigens. However it is associated with high costs and labor. Here we describe a functionally closed bag system using sterile tubing connections (TSCD, Terumo, Germany) for glycerolization as well as for deglycerolization of RBC to avoid the necessity of clean room conditions. The method is licensed by the Paul-Ehrlich-Institute for storage of autologous and allogeneic RBC up to 10 years.
Methods: RBC were cryopreserved in a 1000 ml bag (CryocyteTM, Baxter, Germany) with a final concentration of 18.5% glycerol and 1.5% sorbitol after 1:1 adding of 250 ml freezing solution (1-SB-250-F Erythrocyte Freezing Solution, Fresenius Austria) and stored at –140 °C in the vapor phase of liquid nitrogen. The frozen units were thawed at +41 °C in a heat device (Plasmatherm, Barkey, Germany) and sterilely connected to the first washing solution bag (250 ml NaCl 3%, Na2HPO4 0.35%; 1-SB-250 W Erythrocyte Washing Solution, Fresenius, Austria). For deglycerolization the cells were washed three times at room temperature with 250 ml SAG-M (Fresenius) and finally resuspended in 50 ml of a solution containing 0.9% NaCl, 0.2% glucose and 1.5% mannitol (1-SB-50-RS Erythrocyte Resuspension Solution, Fresenius, Austria) for transfusion within 24 hours after resuspension.
Results: Since 2006 overall 59 autologous and allogeneic cryopreserved RBC units were thawed and transfused to patients. Sterility tests of all products were negative. Parameters of the final RBC units are given as mean ± standard deviation: volume 195±19 ml, Hct 0.62±0.07 L/L, Hb 41±6 g/unit, hemolysis 0.2±0.1%, supernatant osmolarity 394±15 mOsmol/kg H2O, storage time 3.5±2.1 (range 0.5–9.0) years.
Conclusions: The described method for cryopreservation and thawing of RBC is easy and cost efficient. The in vitro quality of the thawed products meets the requirements of the German and European guidelines. Post thaw storage up to 24 hours resulted in an adequate post transfusion increment of RBC. Prolongation of the post thaw period would be helpful in daily practice and will be further investigated.

P 3.02

Changing the Age Criteria for Blood Donors: Influence on Plasma Quality and Parameters of Coagulation

Madla W.[1], Alt T.[2], Jungk H.[2], Heil U.[2], Markovic A.[2], Schottstedt V.[3]

[1]DRK Blutspendedienst West Zentrum für Transfusionsmedizin Bad Kreuznach gemeinnützige Gesellschft mit beschränkter Haftung, Herstellung/Präparation, Bad Kreuznach, D, [2]DRK Blutspendedienst West Zentrum für Transfusionsmedizin Bad Kreuznach gemeinnützige Gesellschaft mit beschränkter Haftung, Bad Kreuznach,D, [3]DRK Blutspendedienst West Zentrum für Transfusionsmedizin Hagen gemeinnützige Gesellschaft mit beschränkter Haftung, Hagen, D

Purpose: A several years process of shifting towards older blood donors forces to raise upper age limits for donation in order to maintain an adequate blood supply. For locking on this option donor safety and blood component quality has to be proven.
Methods: In order to investigate the influence on blood product quality three cohorts at different ranges of age have been defined:
1 = 69–71 years, 2 = 66–68 years, 3 = 50–52 years.
Whole blood of 50 donors each of a cohort has been separated after filtration into the components red cell concentrate and plasma by standard techniques. Immediately after separation plasma units have been devided into aliquots for freezing in liquid nitrogen. After thawing plasma quality was tested for different parameters as follows: global tests (PT, PTT), coagulation factor activity (fibrinogen, V, VIII), coagulation inhibitor activity (antithrombin, protein S) and plasminogen. The effect on the quality of red cell concentrates has been presented elsewhere.
Results: Regarding the global tests for PTT no statistical differences ($p > 0,05$, calculated by T-test) could be seen but for PT with lower values for cohort 1 compared to cohort 2 and 3. The latter phenomenon is reflected by lower but not significant levels of factor V (e. g. cohort 1 vs. cohort 2: $p=0,09$) and is focussed to male donors of cohort 1. In addition PTT-values of male donors of all cohorts have been higher ($p< 0,05$) that that of female donors. With respect to the data of fibrinogen, antithrombin and plasminogen the cohorts showed conformity. In contrast factor VIII-values of cohort 3 male donors, but not female, demonstrated striking decrease ($p < 0,005$) versus those of the older donors.
The female part of cohort 3 impressed with slightly lower mean values of protein S in opposite to cohort 1 and 2 women. Donation frequencies did not differ between the cohorts.
Conclusions: In accordance to published data for some but not all parameters of plasma quality tested, age and sex dependent differences could be stated. Only PT data indicate a decline for older, male donors, nevertheless all but one of the values have been in normal reference range. The remaining factors of this study, representative for activation or inhibition of coagulation and fibrinolysis, give evidence that raising up of blood donors age up to 71 does not affect quality of blood plasma products adversely.

P 3.03

Utilizing the COM.TEC Cell Separator for Dry Platelet Production

Martens J., Goudeva L., Tell-Lüersen M., Schmitt-Thomssen A., Heuft H.-G.

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

Purpose: To investigate whether the Fresenius COM.TEC (Software Version 04.02.00, Plt-5d Program, Set C5L) is suitable to collect plasma reduced platelet (PLT) concentrates (PCs) for storage in additive solution. The study was carried out as a comparison to our standard procedure for double PC production: 5.6×10E11 PLT/apheresis in 450 mL plasma (corresponding 2.8×10E11 PLT in 225 mL plasma per transfusion unit [TU]).
Methods: A total of 20 runs from 13 healthy donors were performed to collect 5.6x10E11 PLT, initially in 200 mL plasma (10 runs), in a second step in 180 mL (5 runs) and 160 mL plasma (5 runs), respectively. The automatically calculated best-flow presettings were accepted. Donors height, weight, blood volume and pre-apheresis PLT counts were consistent (± 10%) with the average values of our apheresis donor group. The PCs were suspended in Composol® additive solution immediately after apheresis targeting a total volume of 450 mL, followed by both a resting and an agitation time of 60 min at 20–24°C. PLT concentration and yield were measured, and PLT collection efficiency (CE) and collection rate (CR) were calculated for each donation. Residual WBC and RBC counts were determined using microscopical and FACS measurements. Storage parameters include pH, glucose consumption, lactate, CD62p, and hypotonic shock reaction.
Results: Compared to our standard procedure, reduced (8% for apheresis yield), but stable target cell parameters were observed in the dry PLT groups. WBC and RBC contamination were below 1×10E6 and 3×10E9 per TU. Mild PLT aggregates occurred during the procedure in the collection line, but completely dissolved after dilution with Composol® (18/20 aphereses, 90%). The storage parameter evaluation is currently under way.
Conclusions: Despite the reduced target cell counts, the COM.TEC cell separator seems to be suited for collection of dry PCs with a plasma content of 35%. Our preliminary results with the 160 mL group are going to be supported by a larger donor group including storage parameters.

Plasma (mL),%	PLT/apheresis ($x\ 10^{11}$)	PLT concentration ($x10^6/\mu L$)	CE (%)	CR ($\times10^9$/min)
450 mL, 100	5.5 ± 0,6	1.24 ± 0.13	60.9 ± 7.0	10.2 ± 2,8
200 mL, 44	4.9 ± 0,3	2.46 ± 0.17	51,6 ± 2,1	6,7 ± 1,2
180 mL, 40	5.1 ± 0,3	2.85 ± 0.14	54,3 ± 3,3	7,2 ± 1,7
160 mL, 35	5.1 ± 0,5	3.21 ± 0.27	53,1 ± 2,8	6,8 ± 2,0

P 3.04

Pro-Inflammatory Mediators Accumulate in Stored Whole Blood While Awaiting Component Separation / Pre-Storage Leukocyte Depletion

Chudziak D.[1], Sireis W.[2], Pfeiffer H.-U.[2], Henschler R.[2], Seifried E.[2], Bonig H.[2]

[1]German Red Cross Blood Center and Institute for Transfusion Medicine and Immunohematology of the JWGU Frankfurt, Hematopoietic Cell Research Group, Frankfurt, D, [2]German Red Cross Blood Center and Institute for Transfusion Medicine and Immunohematology of the JWGU Frankfurt, D

Background and Objectives: Pre-storage leukocyte depletion has reduced frequency and severity of febrile non-hemolytic transfusion reactions (FNHTR), likely due to reduced concentrations of leukocyte-derived inflammatory/pyrogenic cytokines contained in such blood products compared to non- or post-storage-leukocyte-depleted products. However, pre-storage leukocyte-depletion has not eradicated FNHTR. Prior to component separation, donor whole blood is stored at room temperature for 4–24 hours, to allow for phagocytosis of residual bacteria, while ensuring integrity and function of platelets and plasma proteins. To remain within this time window was assumed to limit the quantity of soluble mediators, although this did not influence decision-making when specifications were devised. We hypothesized that during this period of time, inflammatory cytokines might accumulate in plasma.
Materials and Methods: Serial cell-free plasma samples were collected from donor whole blood or leukocyte-depleted platelet-rich plasma (to distinguish between leukocyte- and platelet-derived factors) 4, 10 and 20h after donation. Concentrations of 1β (IL-1β), IL-6, IL-8, tumor necrosis factor α (TNF-α), soluble CD40 ligand (sCD40L) and high mobility group box 1 (HMGB1) were measured by commercially available ELISA.
Results: Serial cell-free plasma samples were analyzed. Baseline concentrations (i.e., 4h our values) of all mediators were low but measurable, with remarkably small intra-individual variation. IL-6 concentrations decreased over time; all other pro-inflammatory parameters were increased at the later time-points, although not in all cases statistically significantly. Dramatic increases were observed for IL-8 (> 20-fold @ 20h) and sCD40L (>6-fold @ 20h). However, final concentrations remained well below those documented previously after 5 days of storage (IL-1β 1%, TNF-α 5%, and IL-8 2.5%, Aye et al. Transfusion 1996). Platelets could be ruled out as a significant source of these mediators.
Conclusion: Although inflammatory cytokines increase over time during preprocessing, concentrations may not be sufficient to cause febrile reactions except possibly in certain sensitive individuals. Thus, while on their own these data do not provide a rationale to generally advocate leukocyte depletion within 10 h of donation, we propose to consider these findings (i.e. the potential benefit of early leukocyte depletion), in as far as these are compatible with achievement of an optimal functional quality of the components.

P 3.05

Iron Balance in Repeat Blood Donors – the Role of Hepcidin

Schorer G.[1], Brittenham G.[2], Darnuzer R.[3], Westerman M.[4], Frey B.M.[3]

[1]Blutspende Zürich, Medizinischer Dienst, Schlieren, CH, [2]Columbia University, New York, USA, [3]Blutspende Zürich, Schlieren, CH, [4]Intrnsic Life Science LLC, La Jolla, USA

Purpose: Blood donation (D) results in iron loss (ca 250 mg/D) by the donor (BD). With regular D, stimulated erythropoiesis requires an increased supply of iron from mobilisation of body iron stores (BIS) and increased iron absorption, adaptations thought to be mediated by hepcidin, the chief controller of body iron supply and storage. To examine the effects of sequential D on body iron stores and serum hepcidin, we have prospectively studied iron and hematologic parameters in newly recruited BD. Here we present a preliminary evaluation of the ongoing study.
Methods: Since August 1st, 2007, newly recruited BDs (male(m):female(f) = 1:1), entered into the study after written consent, have agreed to make at least four sequential Ds (450 ml each) with a minimal interdonation interval (≥10 weeks). In a venous sample collected before each donation, measurements are made of hemoglobin (Hb, g/L), reticulocyte hemoglobin content (CHr, pg), serum ferritin (SF, µg/L), soluble transferrin receptor (mg/L), C-reactive protein (CRP, mg/L), alaninaminotransferase (ALAT, U/L) and, in a subset of donors, of serum hepcidin (ng/ml; Blood 2008;112:4292) and serum erythropoietin (EPO, mIU/ml).
Results: We present results from the first 67 BDs (36 m, 31 f) who have completed 3 consecutive Ds; accrual of 200 BDs is planned. With sequential D, body iron stores, as assessed by SF, decreased progressively from the initial to the third donation, from a geometric mean of 112 (range: 34–296) to 48 (13–199) µg/L in m and from a geometric mean of 39 (13–151) to 11 (1–55) µg/L in f. Compared to m, the geometric mean SF in f was initially lower and decreased proportionately more. In these 67 BD, Hb remained above the level required for D (m>135 g/l, f>125 g/l) and the mean CHr remained in the normal range. No BD had an elevated CRP or ALAT. In a subset (n=10), mean serum hepcidin decreased, from 58.8±9.6 (SD) to 43.0±17.2 ng/ml in m, and from 54.4±15.1 to 26.5±14.2 ng/ml in f in parallel with body iron stores; EPO did not change significantly.
Conclusions: 1. Consecutive D leads to depletion of body iron stores and a decrease in serum hepcidin that should lead to a compensatory increase in iron absorption. 2. Due to lower iron stores in f compared to m, females may be affected earlier and more severely by iron loss with regular D. 3. Measurement of serum hepcidin may help identify BD who can maintain BIS despite regular sequential D. 4. Firm conclusions on the value of hepcidin measurement in blood donors may be taken, once the study is completed and data of deferred donors are co-evaluated.

P 3.06

Transmission Electron Microscopy of Platelets after Pathogen Reduction

Gathof B.[1], Barham M.[2], Picker S.[3], Oustianskaja L.[1], Krafcyk T.[2], Neiss W.[2]

[1]University Hospital, Transfusion Medicine, Köln, D, [2]Anatomie II, University, Köln, D, [3]University Hospital, Köln, D

Purpose: Evaluation of the influence of different pathogen reduction technologies (PRT) on platelet (PLT) metabolism and ultrastructure during storage.
Methods: 27 split triple-dose apheresis PLT´s were PRT treated using UV light with either psoralen (I) or riboflavin (M) or remained untreated (C). Samples taken on days 0, 1, 5, 7, and 8 were analysed for mitochondrial metabolic activity. The volume of mitochondria in percent of PLT cell volume was estimated in electron micrographs at days 0, 1 and 7. This volume fraction is related to the maximal metabolic capacity of mitochondria in PLT.
Results: In qualitative examination the PLT of all groups C, I and M showed no signs of ultrastructural pathology up to 7 days of storage. PLT fresh from apheresis (day 0) contained 2.4 vol% mitochondria. After PRT respectively standard treatment the PLT of M contained 1.6% mitochondria at day 1 and still 1.5% at day 7, those of group C 1.6% at day 1 and 1.3% at day 7, those of group I 1.3% at days 1 and 7. Though there was a tendency in favour of group M, this was not statistically significant with n=8 PLT donors.
Discussion: The tendency for differences in mitochondrial volume fraction between the groups coincides with the metabolic differences observed (Picker et al. Transfusion 2009, Picker et al. Vox Sang 2009).
Conclusion: Different pathogen reduction technologies may influence platelet metabolism differently. Further studies, larger study groups, and particularly clinical studies will be recommended to judge the clinical significance of the findings reported in this study.

P 3.07

Performance of the Trima© Accel Version 5.2 in the Collection of Triple Platelet Products plus Plasma

Daskalakis M.[1], Schulz-Huotari C.[2], Meike B.[2], Klink I.[2], Umhau M.[2]

[1]Universitätsklinikum Freiburg, Transfusionsmedizin, Freiburg, D, [2]Freiburg, D

Purpose: A current limitation to the collection of multiple platelet products plus plasma from individual donors is the total volume removal. The Trima© Accel Version 5.2 was designed to allow accurate collection of high yield platelet products as hyperconcentrates (dry platelets), which are then automatically diluted by the system with platelet additive solution for storage. This study was undertaken to test the ability of the Trima 5.2 to collect triple platelet products and double doses of plasma from suitable donors and to determine the residual leukocytes in the platelet units.
Methods: Triple platelet products (target 7.5–9.0×1011 platelets) and 430 mL plasma products were collected from randomly selected donors using Trima Accel 5.2, configured to collect at a concentration of 2800×103 plts/µL and were diluted in SSP+ storage solution with a plasma carryover of

43%. Yields were determined and compared with Trima© Accel target yields. The residual leukocyte content of the product was measured by flow cytometry. Apheresis times and volumes removed were also determined.
Results: One hundred fourteen (114) collections (81m, 33f) were done on donors with ave. wt = 82kg, ave. TBV = 6130mL, ave. Plt precount = 306,000/µL. In 107 collections (94%) a triple Plt product was collected. In 86 (75%) of these the inclusion criteria of at least 7.5x1011 plts was met. In 87 cases (76%) a double plasma product (>430mL), in 7 cases (6%) only a single plasma product (>220mL) was collected. In 71 (62%) cases both a triple platelet product and a double plasma product were collected. The average procedure time was 87 minutes (range: 54–120 min.).
The average number of platelets (±sd) collected per procedure was 8.5±1.4×1011, which was 1.07±0.14 times the final Trima target. This resulted in average doses of 2.88±0.46×1011 platelets in 225±33 mL giving an average concentration of 1280±150×103 platelets/µL. An average of 375 ml SSP+ storage solution was used to dilute and store the platelets for each procedure.
The residual leukocyte content per transfusion dose averaged 0.17×106 with a range of 0.01–0.91×106.
Conclusion: The Trima© Accel, SW version 5.2 collects high yield platelet products as hyperconcentrates and automatically adds metered platelet additiv solution (PAS) following the collection. The collection efficiency is high and the yields are reliably obtained. The residual leukocyte content of all platelet transfusion doses was less than one million. Collection as hyperconcentrates followed by dilution saved an average of 375 mL plasma per collection, allowing concurrent double dose plasma collection in the majority of the procedures.

P 3.08

Influence of the Blood-Drawing Location for Hemoglobin Measurement and Resulting Effects on Admission of Blood Donors

Brandenburg F.[1], Faustmann J.[2], Adolf D.[2], Dumschat C.[3], Heim M.[2]

[1]Universitätsklinik Magdeburg, Institut für Transfusionsmedizin, Magdeburg, D, [2]Universitätsklinik Magdeburg, D, [3]EKF-Diagnostic GmbH, Barleben, D

Purpose: Hb determination before admission to blood donation is mandatory. Capillary Hb is measured from either earlobe or fingertip samples. Discrepancies to the venous values are described. In regard to these deviations and to evaluate effects on donor qualification, the capillary Hb values from earlobe and fingertip were compared to the venous Hb levels in a blood donation center under routine conditions.
Methods: A total of 108 paired capillary and venous blood samples were obtained from randomly selected blood donors. Capillary blood samples were analysed using 2 photometers (acid-metHb-method): Hb201+, HemoCue and Hemo_Control, EKF-Diagnostic. Standardized HemoCue Safty Lancets (incision: 2.25 mm) delivered capillary samples by earstick and fingerstick in sitting position of all prospective donors. The 1st drop of blood was discarded, the 2nd and 3rd drop were analysed immediately with Hb201+ device and Hemo_Control device in alternating order. Venous EDTA-blood from the predonation sample bag was measured twice with CellDyn 1700 (Abbott).
Results: Venous Hb levels obtained using the reference method (CellDyn 1700) ranged from 7.30 mmol/l to 10.35mmol/l (11.8 g/dl to 16.7g/dl). Females showed average Hb values of 8.25 mmol/l (13.3 g/dl), males of 9.27 mmol/l (14.9 g/dl). The mean deviation between earstick and venous Hb (CellDyn) was 0.75 mmol/l [CI: 0.64; 0.85] (1.2 g/dl [CI: 1.03; 1.37]) higher, while fingerstick Hb values were 0.33 mmol/l [CI: 0.25; 0.41] (0.5 g/dl [CI: 0.40; 0.66]) lower than the reference values. The correlation between capillary and venous Hb ranged from r = 0.75 to r = 0.84 (all p<0.001). Venous Hb levels below the donation cut-off were found at 11.1% of the participants (females: 17.1%; males: 7.5%). Based on fingerstick Hb values 21.3% (Hb201+) and 23.1% (Hemo_Control) of participants would have been rejected, although venous Hb was above the threshold value. Whereas earstick samples permitted donation below venous measured cut-off in 9.3% (Hb201+) and 10.2% (Hemo_Control).
Conclusions: In comparison to the venous reference method, fingerstick and earstick Hb values showed significant deviations in Hb values and permitted blood donations (McNemar: all p < 0.006). Based on fingerstick Hb values eligible donors will be rather rejected, while earstick samples permit more blood donors in comparison to the venous reference. The influence of personnel has to be taken in consideration, when comparing capillary and venous blood samples, since especially capillary samples depend on skilled laboratory assistants.

P 3.09

Comparison of Different Additive Solutions and their Influence to the In Vitro Quality of Platelets in Double Platelet Concentrates Collected with the MCS+ Collection System

Baumann-Baretti B.[1], Jaster G.[2], Brys B.[3], Krause K.[3]

[1]Haema AG, Qualitätskontrolle, Berlin, D, [2]Haema AG, Berlin, D, [3]Haema AG, Leipzig, D

Purpose: The advantages of platelet concentrates produced in additive solution (PAS) are well known. The production of double unit platelet concentrates (DPC) in additive solution using the MCS+ collection system (Haemonetics) has not been described yet; the different additive solutions P.A.S. III (I), Fenwal and SSP+ (S), Macopharma and their influence to the in vitro quality of platelets in DPC were compared.
Methods: DPC were obtained from regular donations performed with the MCS+ device; P.A.S. III (500 ml formulation) and SSP+ (250 ml formulation) were used as additive solutions with a Plasma : PAS ratio of 40 : 60. 24 DPC were obtained with a total of 12 apheresis procedures. Samples were taken for the analysis of in vitro metabolic and activation quality parameters during 7 days of storage.
Results: Mean product volume (195-212 ml), mean platelet yield per unit (2,7×10E11/TU) and residual white blood cells per unit (0,02×10E6/TU) showed similar values in I- DPC and S-DPC. Regarding pH and base access the pH increased in S-DPC whereas in I- DPC a slight decrease of pH and a higher descent of base access and bicarbonate was observed. The baseline CD 62 expression [%] showed no significant differences but lower values in S-DPC (d0: 23,84±7,74; d5: 33,33±9,07; d7: 39,10±8,3) compared to I- DPC (d0: 22,74±4,6; d5: 40,75±11,79; d7: 49,09±12,06) during the whole storage time. After stimulation with ADP a similar increase of CD 62 expression [%] was mentioned in both DPC (S-DPC: d0: 21,27±5,45; d5: 11,03±5,28; d7: 7,38±6,27 versus I- DPC: d0: 26,45±9,13; d5: 11,29±5,25; d7: 7,27±3,7). Hypotonic shock reaction (HSR) values decreased during storage in all units; all HSR scores throughout storage in I- DPC were significant below S-DPC. The metabolic parameters as lactate dehydrogenase (LDH), lactate and glucose showed different results, less consumption of glucose and minor increase in LDH and lactate in S-DPC.
Conclusions: Apheresis was performed safely and efficiently in all procedures. Satisfactory platelet content according to current standards was found in all products and residual white blood cells met the current quality control criteria. No significant differences in cellular and flowcytometric variables between DPC treated with SSP+ or Intersol were observed though the platelet metabolism seemed to be perpetuated in a more efficient way in DPC treated with SSP+. Overall the collected data of in vitro quality parameters of DPC in SSP+ and Intersol produced by MCS+ were consistent with recently published data of platelet concentrates obtained with other apheresis devices.

P 3.10

Proteomic Profiling of Platelet Concentrates during Storage after Gamma Irradiation

Thiele T.[1], Steil L.[2], Hammer E.[2], Mohr H.[3], Müller T.[3], Voelker U.[2], Greinacher A.[4]

[1]Universität Greifswald, Abteilung Transfusionsmedizin, Greifswald, D, [2]Abteilung für funktionelle Genomforschung, Greifswald,D, [3]Blutspendedienst NSTOB, Springe, D, [4]Abteilung Transfusionsmedizin, Greifswald, D

Purpose: Gamma irradiation of platelet concentrates (PCs) is fundamental in clinical medicine for the transfusion of neutropenic or severly immunocompromised patients. However Gamma irradiation leads to a reduced recovery and survival of transfused platelets. This raises the issue how Gamma irradiation alters platelet integrity and produces storage lesions. Using sensitive 2D-DIGE-proteomics and mass spectrometry we assessed the impact of Gamma irradiation on the proteome of platelets stored in PCs.
Methods: Plasma-reduced buffy coat derived PCs (biologically identical, n=2) were irradiated with Gamma (25Gray) or left untreated, respectively. PCs were stored under blood bank conditions and aliquots were taken one, five and nine days after production. Samples were prepared for the Differential In-Gel-Electrophoresis (2D-DIGE; pH-range 4–7), applying three different fluorescent dyes with one dye serving as an internal standard. Quantitative assessment of the 2D-pattern was performed with the Delta-2D and GeneSpring software packages. Protein spots of interest were identified using mass spectrometry.
Results: 1264 protein spots were quantitatively monitored in both study arms on the three time points investigated. Gamma irradiation induced

proteome changes in a subset of 165 spots observed on day one after treatment. After five days of storage 15 protein spots differed compared to control samples on the same day and after nine days only 9 protein spots showed different spot intensities, respectively.
Conclusion: 2D-DIGE is a tool for comparing cytosolic protein alterations in PCs induced by Gamma irradiation. Changes provoked by Gamma-irradiation appear to be reversible with ongoing platelet storage suggesting effective repair mechanisms in platelets stored in vitro.

P 3.11

Increasing CD40L Levels in Prestorage Pooled Platelet Concentrates May Be Caused by Matrix Metalloprotease-2 (MMP-2)-Dependent Shedding

Wenzel F.[1], Weichert S.[2], Lasshofer R.[3], Giers G.[3], Stahl D.[3], Weber A.A.[2]

[1]Salzburger Landesklinik, Institut für Blutgruppenserologie und Transfusionsmedizin, Salzburg, A, [2]Institut für Pharmakologie, Essen, D, [3]1Universitätsklinik für Blutgruppenserologie und Transfusionsmedizin, Salzburg, A

Introduction: Platelets are the major source of soluble CD40 ligand (sCD40L) (1) in the blood. It has been demonstrated that CD40L is cleaved from the surface of activated platelets to release sCD40L. CD40L is involved in the stabilization of arterial thrombi (2), and is supposed to trigger adverse transfusion reactions like TRALI (3). Therefore we examined CD40L concentrations in prestorage pooled platelet concentrates.
Material and Methods: CD40L concentrations were determined in prestorage pooled platelet concentrates (n=6) before and after platelet stimulation (recalcification and clot formation) at day 1, 3, 5 and 7 under routine storage conditions. CD40L concentrations were determined by a commercially available ELISA Kit (R&D Systems). Additionally, platelet-rich plasma (PRP) from healthy volunteers (n=6) was incubated with different pharmacological inhibitors (MMP-2/MMP-9 Inhibitor I, MMP-9 Inhibitor I, MMP-2 Inhibitor I, recombinant ADAM 10, and recombinant ADAM-17) during platelet activation.
Results: In platelet concentrates, recalcification and clot formation caused an increase in CD40L concentrations. Expression of CD40L by stimulated platelets decreased from 29,019 pg/mL ±3,599 pg/mL at day 1 to 19,247 pg/mL ±2,093 pg/mL at day 7. In contrast, CD40L concentrations in storage medium increased over time from 1,185 pg/mL ±214 pg/mL at day 1 to 4,579 pg/mL ±667 pg/mL at day 7 in a hyperbolic manner. Using the MMP-9 inhibitor (100 nM) and the MMP-2/9 inhibitor (3 µM) sCD40L release could be inhibited by >60%. Interestingly, the MMP-2 inhibitor (17 µM) completely prevented the shedding of sCD40L from activated platelets.
Conclusions: CD40L levels in prestorage pooled platelet concentrates were dependent on storage duration. Additionally, these data support the hypothesis that MMP-2 might be the protease, primarily responsible for CD40L cleavage from platelet surface.

References:
1. Wenzel et al.: Clin Hemorheol Microcirc 2008;39: 17–22.
2. Andre et al.: Nat Med 2002;8:247–52.
3. Khan et al.: Blood 2006;108:2455–62.

P 3.12

Epitheliotrophic Capacity and Biochemical Quality of Autologous Serum Eyedrops after 6 Month Storage at -20 °C

Opitz A.[1], Fischer K.[2], Kasper K.[3], Geerling G.[3], Böck M.[2]

[1]Institute of Clinical Transfusion Medicine and Hemotherapy, University Hospital Wuerzburg, D, [2]Institute of Clinical Transfusion Medicine and Hemotherapy, Wuerzburg, D, [3]Department of Ophthalmology, Wuerzburg, D

Purpose: Autologous serum eyedrops (ASE) have been successfully used in the treatment of severe dry eye, persistent corneal epithelial defects (CED) and other severe ocular surface disorders. For improving patient care and increasing the efficiency, extended storage of ASE is desirable. Up to date, there are no data of ASE quality and efficacy after 6 months of preservation in the freezer (–20 °C). Study
Design and Methods: After written informed consent 10 healthy volunteers donated 300 ml whole blood in sterile tubes. The preparation was performed according to the protocol of Geerling at al.. Samples of ASE (undiluted, diluted to 50% or 20%) were examined at day of production, at day 7 (stored at +4 °C), after 3 respectively 6 months (stored at –20 °C). The concentrations of epidermal growth factor (EGF), fibronectin, vitamin A and E, igA and albumin were evaluated with ELISA, HPLC, turbidimetry or photometry. The epitheliotrophic capacity was tested in a cell culture model of human SV-40 immortalised corneal epithelial cells (HCE-T). Cell proliferation and migration were evaluated by means of a luminescence-based ATP assay, a colony-dispersion assay and a scratch wound assay.
Results: During the 6 month preservation period no significant decrease of the mentioned parameters were measured. Concentration in undiluted ASE before and at the end of storage: EGF: 465 ± 110 / 472 ± 133 pg/ml; fibronectin: 141±17 / 133±22 ng/ml; vitamin A: 2,8±0,6 / 3,1±0,8 µmol/l; vitamin E: 27,9±4,4 / 29,6±4,8 µmol/l; albumin: 4,7±0,2 / 4,8±0,3 g/dl; IgA: 248±50 / 252±48 mg/dl. The storage period had no statistically significant impact on the relative cell growth as determined with the ATP assay. Cells incubated with undiluted ASE after 6 month storage did migrate to the same extend as with ASE at day of production.
Conclusion: The epitheliotrophic capacity and the biochemical quality of autologous serum eyedrops did not change during 6 months of storage at –20 °C. ASE storage life extension could optimize production by processing larger quantities of whole blood.

Geerling et al. An optimised protocol for the production of autologous serum eyedrops. Graefe`s Arch Clin Exp Ophthalmol 2005; 243:706–714.

P 3.13

INTERCEPT Platelets Treated and Stored in SSP+ Meet Council of Europe Standards for In Vitro Function after 7 Days of Storage

Deane M., Rivera P., Sawyer L.

Cerus Corp, Concord, USA

Background: The INTERCEPT Blood System™ for platelets was developed to prevent transfusion-transmitted infections by inactivating pathogens in donor blood. This system utilizes amotosalen HCl and UVA illumination and has been demonstrated to inactivate a broad spectrum of blood-borne pathogens and leukocytes in platelets and plasma. The INTERCEPT Blood System is CE Marked for the treatment of platelets concentrates suspended in approximately 35% plasma and 65% InterSol™ platelet additive solution. SSP+™ is a recently introduced platelet additive solution that differs from InterSol primarily in the addition of magnesium and potassium. This study evaluated in vitro platelet storage parameters of platelets treated and stored in two different platelet additive solutions, InterSol and SSP+.
Method: For each replicate experiment (N=6) a double-dose platelet unit, with or without the addition of an ABO-matched single-dose unit, was split to create two identical units. Each unit was processed to remove excess plasma and resuspended in approximately 285 mL of either 35% plasma/65% InterSol or 35% plasma/65% SSP+, resulting in units with a mean platelet dose of 3.5 $x10^{11}$ and 3.4 $x10^{11}$, respectively. Platelets were then treated with the Small Volume INTERCEPT™ Processing Set for Platelet Units and stored for 7 days. In vitro parameters were analyzed on Day 7.
Results: See table. Small but statistically significant differences between platelets treated and stored in InterSol and those treated and stored in SSP+ were seen in several function parameters, however all units met EU guidelines for transfusable platelet units.
Conclusion: In vitro function of apheresis platelet units INTERCEPT-treated and stored in SSP+ is as good as or better than that of platelets treated and stored with InterSol.

Mean Platelet Function on Day 7 Following INTERCEPT Treatment and Storage in Indicated Additive Solution (N=6)

Function Parameter	InterSol	SSP+	Function Parameter	InterSol	SSP+
Platelet Dose ($\times 10^{11}$ /unit)	3.27	3.15	ESC (%)	4.2	5.9
pH (22 °C)	6.70	6.79	HSR (%)	19.7	28.5*
ATP (nmol/10^8 platelets)	1.49	2.33*	LDH release (% lysis)	7.1	4.9*
P-selectin (% expression)	51.11	47.19	Lactate (mM)	15.1	14.3
Blood Gases: pCO_2/pO_2 (mmHg)/ HCO_3^- (mM)	15.7/ 131.5/ 1.4	16.3/ 132.7/ 1.8	Glucose (mM)	0.1	0.8

*Significant differences, paired Student's t-test, $p \leq 0.05$.

P 3.14

Unplanned Incomplete Return of Donor Red Cells (IRRC) during Preparatory Plasmapheresis (PPP): Incidence, Causes and Consequences

Diekamp U.

Haema AG, Leitende Ärztliche Person, PMS-Zentren, Bremen, D

The expected loss of donor red cells (RC) per PPP performed with the A200 (Fenwal) is around 4 ml; the saline rinse-protocol reduces this loss to 0.7 ml. A donor may thereby lose 27–152 ml of RC with the maximum of 38 donations Germany allows annually. RC-loss may also be a result of IRRC due to several untoward events during PPP. We want to know more about IRCC and analyze those observed in 2008.

Methods: In 2003, we establish an untoward events-documentation system in six donor centres. It is updated January 1, 2008 to document all mishaps during and after donation, distinguishing and grading by severity local and systemic events, and listing technical problems such as IRRC. We review all events periodically.

Results: The commonest causes of IRRC relate to venous access problems during PPP; others are machine malfunction, plugging of the filter in the separation chamber, leakage of the harness, clots within in the system, and/or occlusion of the membrane for air exchange within the RC-reservoir. The table shows our results:

parameter	n	%		donation frequencies in 2008		%
plasma donors (PD)	18,679			mean donat./PD	15.32	
PD with IRRC (% PD)	1,28	6.85%		mean donat./PD, no IRRC nor deferral	14.88	
PD with >1 IRRC (%PD)	140	10.94%		donat. from PD with IRRC	21.58	
male : female	541 : 838			donat. from PD deferred after IRRC	20.92	
plasma donations	286,081			donat. from PD with IRRC then deferred	21.79	
IRRC (% donat.)	1,485	0.52%		PD with donat. after IRRC & deferral	1,104	86.25%
>1 IRRC/PD (% IRRC)	312	21.01%		PD with no donat. after IRRC & deferral	172	13.44%

Hemoglobin levels prior to IRRC and prior to the donation following the RC-loss differ only by 0.14 and 0.10 mmol/l among male and female donors, respectively.

Discussion: IRRC affects the most dedicated donors, as they donate substantially more often than donors not experiencing IRRC. The loss of RC does not seem to impair their ability to donate again. Unfortunately, the amount of RC-loss is not quantified in our documentation with sufficient accuracy to permit detailed analysis.

Conclusions: IRRC is an occasional untoward event affecting 0.52% of PPP. It has limited impact on donor hemoglobin levels and does not impair the motivation of donors nor their ability to further actively participate in PPP.

P 3.15

Evaluation of the EKF Hemo_Control Device for Hb Screening of Blood Donors

Faustmann J.U.[1], Brandenburg F.[2], Dumschat C.[3], Adolf D.[2], Heim M.[2]

[1]Universitätsklinik Magdeburg, Institut für Transfusionsmedizin, Magdeburg, D, [2]Universitätsklinik Magdeburg,D, [3]EKF-Diagnostic GmbH, Barleben, D

Purpose: Aim of the study was to evaluate the use of the EKF Hemo_Control device as hemoglobin measuring system in a blood donation center under routine conditions. EKF-diagnostic GmbH designed a new hemoglobin microcuvette with improved filling behavior which is easier to handle. Furthermore the probability of forming air bubbles is reduced.

Methods: A total of 108 paired capillary and venous blood samples were obtained from randomly selected blood donors and measured with Hemo_Control device, HemoCue Hb 201+ analyzer and CellDyn 1700 (Abbott). Standardized Safety Lancets (incision 2.25 mm) were used to obtain capillary samples by earstick (ES) and fingerstick (FS) of all donors. The 1st drop of blood was discarded, the 2nd and 3rd drop were analysed with Hb201+ and Hemo_Control in alternating order. Venous sample was measured twice with CellDyn, Hemo_Control and Hb201+.

Results: The best agreement for both photometers and nearly no difference were found for venous sample. Compared to the CellDyn we found mean differences for venous samples from 0.15 mmol/l [CI 0.13;0.18] for Hb201+ and 0.14 mmol/l [CI 0.10; 0.17] for Hemo_Control whereas the correlations between photometers and CellDyn were excellent (r=0.99 resp. r=0.98, p<0.001 each). The datas for capillary samples are summarized in table 1. The mean values for men and women were significantly different. There are no significant differences between the photometers for male and femal donors.

Conclusion: The results show an excellent correlation between the Hemo_Control and the Hb201+ for venous and capillary samples. The higher experimental error obtained with capillary samples compared to venous ones reflects the problem of capillary sampling, which is partially influenced by staff-dependent training skills. The Hemo_Control system with the new microcuvette is well suited for hemoglobin screening of blood donors.

Table 1: Comparison of Hemo_Control and Hb201+

	Men n=67				Women n=41			
	MD	CI	p	r	MD	CI	p	r
FS	0.02	-0.12; 0.15	0.749	0.78	0.13	-0.02; 0.27	0.080	0.73
ES	-0.11	-0.21; 0.00	0.043	0.87	-0.19	-0.32; -0.05	0.008	0.86
Venous	-0.02	-0.05; 0.00	0.057	0.99	0.00	-0.04; 0.03	0.848	0.97

MD-mean difference; CI-95% confidence interval; p-unadjusted p-value; r-Pearsons correlation coefficient (significant with p<0.001 each).

P 3.16

Influence of NaCl 0.9% during Apheresis on IgG Content in Plasma

Evers J.[1], Betz J.[1], Blankenburg T.[2], Engelen T.[1], Galle G.[2], Laitinen T.[1], Taborski U.[1]

[1]DGH Plasmaspende, Aachen, D, [2]Institut für Klinische Chemie, DRK Ost, Chemnitz, D

Purpose: The aim of this validation was to evaluate the influence of an infusion of NaCl 0.9% 500 ml during preparative plasmapheresis on the IgG concentration of separated plasma.

Methods: 32 donors of plasma (16m/16f) with an average weight of 70 kg were studied in a cross-over design and after informed consent during automated apheresis on a day without infusion of NaCl (A), and on another day with infusion of 500 ml NaCl 0.9%(B). Aphereses were performed with Haemonetics plasma collecting system 2 (PCS2). Volume of separated plasma was 760 ml. Sodium citrate 4% anticoagulant solution was added to venous blood during the draw phase in a ratio of 1 part to 16 parts of whole blood. On day (B) an infusion of NaCl 0.9% 500 ml was gradually given in divided doses through the PCS2 after each cycle. After venipuncture blood samples were taken from the tube of the fistula needle 1st before and 2nd after aphereses for IgG concentrations in serum. Samples of plasma were taken after aphereses from the separated plasma for IgG levels in plasma. IgG concentrations in serum and in plasma were measured by an immunturbimetric assay with Olympus apparatus AU 640. The percentages of IgG concentrations in plasma were calculated by dividing the mean serum IgG concentrations according to the formula:
% IgG in plasma = IgG in plasma x 100 / mean IgG serum concentration,
mean IgG serum concentration = (1st IgG in serum + 2nd IgG in serum) / 2.
Mann-Whitney-U-test was used for statistical analysis.

Results: Without giving an infusion of NaCl 0.9% during aphereses (A) the average percentage of IgG in plasma in relation to the mean IgG serum concentrations was 85.5 (±2.3)%, and it was 80.5 (±3.4)% when NaCl 0.9% was given (B). The difference was highly statistically significant (p<0.001).

Conclusions: In agreement with earlier studies we reasoned that the difference between the mean serum concentrations and the plasma concentrations of IgG during standard aphereses without NaCl was due to the infusion of sodium citrate 4% anticoagulation solution. We concluded that the further highly significant difference of 5.0 (±3.8)% (or 5.8% in relation to 85.5%) in aphereses with NaCl 0.9% was due to the concomitant infusion of NaCl. This implies that the diluting effect should be considered in the addition of NaCl 0.9% solution during preparative plasmapheresis.

P 3.17

A Modified AutoPBSC Procedure with Enhanced Values for CD3+ Cell Collection

Schenck P.[1], Baume H.[2], Petershofen E.[2]

[1]DRK Blutspendedienst NSTOB Institut Bremen-Oldenburg, Zytapherese, Oldenburg, D, [2]DRK BSD NSTOB Inst. Bremen-Oldenburg, D

Introduction: The AutoPBSC program is an automatic discontinued stem cell/ mononucleated cell collecting system for Cobe Spectra cell separators. The lymphocytes/ monocytes are concentrated in the buffy coat layer until a certain blood volume has been processed and then transferred into the collecting bag. The intervals between these single collections (by the meaning of processed Volume) are calculated by the AutoPBSC software using values for total leucocyte and monocyte count. We modified the collecting procedure to set the intervals between the collection phases manually in order to achieve at least eight collections per apheresis, which means one collection every 800-1000 ml of processed volume.
To evaluate our modified AutoPBSC procedure we compared 9 unstimulated lymphocyte collections of healthy donors with modified settings and 24 collections with the original settings.
Results:

AutoPBSC setting	"original" median (min. /max.)	"modified" median (min. /max)
processed blood volume	13.339 (7348/ 21.684) ml	14.047 (9024/ 18.114) ml
CD3+ in product	61,45 (27/ 102) x10^8	98,2 (52,4/ 134) x10^8
extraction factor	0,54 (0,22/ 0,83)	0,61(0,45/ 0,83)
Hb in product	0,8 (0,2/ 4,6) g	3,1 (1,6/ 7,9) g
platelets in product	1,72 (1,11/ 3,44) x10^{11}	3,3 (2,17/ 4,47) x10^{11}

Note: CD3+ extraction factor: total CD3+ in product/CD3+ in processed volume
Discussion and Conclusion: One reason to use a modified approach was that collection intervals were sometimes too long, resulting in fewer collections thereby producing apheresates with low volume and poor cell counts.
Using the original AutoPBSC setting the requested CD3+ cell amount could in several cases not be obtained. We found, that our modified AutoPBSC procedure yielded higher amounts of mononucleated cells/ CD3+ cells than the original Auto-PBSC procedure. On the counterside the amounts of thrombocytes and hemoglobin were higher compared to standard settings. For further evidence additional apheresis data will be collected.

P 3.18

A New Closure for Blood Bag Systems

Agildere A.[1], Richter E.[2]

[1]DRK Blutspendedienst Baden-Württemberg - Hessen, Institut Baden-Baden, Produktion, Baden-Baden, D, [2]DRK Blutspendedienst Baden-Württemberg - Hessen, Baden-Baden, D

Purpose: Restricted blood flow can be a reason for hemolysis. Current closures have to be opened manually and with a certain force often straining fingers and joints. Incompletely opened they can cause restricted blood flow and damage the red cell layer during separation. Fresenius-Kabi designed a novel closure called Compoflow which is opened either by an individual device or a device integrated into a separator: the Compomat G5. After breaking Compoflow's aperture is supposed to guarantee an undisturbed blood flow. Tests were done to verify that the new closure does not cause hemolysis and is suitable for routine use.
Methods: For separation a new separator by Fresenius-Kabi was used: the Compomat G5 . Before using the Compoflow blood bag system in routine procedure 32 donations were separated with the buffy coat method, stored for 42 days and tested for residual leukocytes, Hb content and hemolysis. For routine use close to 4800 donations with Compoflow were separated by G5. For quality control (QC) 163 (3.4%) red cell concentrates (RCC) processed with the G5 were stored for 35 days and assessed at day 1 for hematocrit, hemoglobin, and leukocyte count and at day 35 for hemolysis. Additionally the Compoflow system was used at the current separator Compomat G4. Here the closures were opened manually.
The performance and suitability of Compoflow were recorded by experienced staff.
Results: The RCC of the preliminary test with 32 WB donations had a volume of 290 ml ±14 ml, an Hb content of 54.3 ±4.3 g/unit, and a hemolysis rate of 0.29% after 42 days of storage. The RCC quality data with up to 4800 WB had a mean volume of 287ml ±15ml, an Hb content of 55.9 ±5.0 g/unit, a leukocyte count of 0.02±0.06x106/unit, and a hemolysis rate of 0.25 ±0.16%.
Tests opening Compoflow manually were all within the specification.
The staff appreciated breaking the CompoFlow closures by G5. They reported less problems of their fingers and joints. There were no major complaints and incidents seen working with Compoflow.
Conclusion: All results were within the specifications of the European Guidelines. No hemolysis was seen with the new closure. Further tests evaluating the performance of CompoFlow in routine procedures e. g. on different separators should be performed. The Compoflow was suitable for routine use with the Compomat G5.

P 3.19

Production of Platelet Concentrates iIn Additive Solution Using the COM.TEC Cell Separator

Janetzky S.[1], Thiele T.[2], Hartenstein G.[3], Fürll B.[2], Strobel U.[2], Greinacher A.[2]

[1]Ernst-Moritz-Arndt-Universität Greifswald, Institut für Immunologie und Transfusionsmedizin, Greifswald, D, [2]Institut für Immunologie und Transfusionsmedizin, Greifswald, D, [3]Fresenius Kabi Deutschland GmbH, Bad Homburg, D

Purpose: Platelet concentrates (PC) using additive solution (PAS) as storage medium build the basis for the use of new photochemical treatments (PCT) for pathogen inactivation. Moreover, the reduction of plasma in PCs minimizes the risk of plasma-associated transfusion reactions like TRALI or AB0-minor incompatibility. We present a protocol to obtain PCs with PAS as storage medium using the COM.TEC cell separator (Fresenius) without the need of an additional centrifugation step.
Methods: Single-donor PCs were collected by apheresis (COM.TEC blood cell separator, Fresenius) in reduced plasma volume (125ml). Subsequently the same amount of PAS (SSP+, MacoPharma, 125ml) was added via sterile-docking. For quality comparison, PCs of the same donors were collected again in plasma after an interval of two weeks. In vitro quality parameters (platelet count, MPV, total protein concentration, pH, pO2, pCO2, glucose, lactate and LDH) were determined on day 0 (day of donation) and days 1, 6, and 9 of storage. Functional assays were performed using hypotonic shock response (HSR) and maximum aggregation induced by TRAP, ADP and collagen. Expression of CD62P and PAC-1 was analyzed by flow cytometry on resting PLTs and thrombin receptor agonist peptide (TRAP) stimulated PLTs.
Results: PCs (n=4) contained 2.82E11±7.12E09 platelets, target yield was 3.00E11. Total protein content after PAS-addition was 34.52±0.9% of donor plasma protein concentration. Glucose level and LDH activity in plasma-PCs were higher than in PAS-PCs throughout day 0 to 6 (glucose, $p<0.0001$; LDH, $p=0.0004$), lactate and pH levels in both groups did not differ at day 1 (lactate, $p=0.1130$; pH, $p=0.6835$), but lactate increased more in plasma-PCs (day 6, $p=0.0156$) accompanied by a decrease in pH (day 6, $p=0.0095$) during storage. HSR and aggregometry revealed similar results for both groups, but ADP-induced aggregation which was reduced in PAS-PCs (day 1, $p=0.0003$). In resting platelets PAC-1 and CD62P expression levels of both platelet preparations were comparable (day 6, PAC-1, $p=0.8818$; CD62P, $p=0.6020$).
Conclusion: This pilot study shows that the COM.TEC cell separator can be used to prepare plasma reduced PCs of reasonable quality.

P 3.20

Suboptimal Storage of Platelet Concentrates (PC): Effects of Agitation and Oxygen Access?

Krause M.[1], Zimmermann B.[1], Müller T.[2], Doescher A.[3]

[1]German red cross blood transfusion service, Bremen, D, [2] German red cross blood transfusion service, Springe, D, [3]German red cross blood transfusion service, Oldenburg, D

Background: The transportation from blood banks to the recipients is difficult to standardize and potentially affects the quality of PC. We developed a model to examine the effects of transportation to BC-PC's by standardized reduction of the surface of the storage bag available for gas exchange combined with agitation or non-agitation. The quality of the PC was evaluated by measurement of pH and the levels of glucose, lactate, beta-thromboglobulin and solube p-selectin.

Methods: PC were prepared from 4 buffy coats of whole blood donations. PC(n=64) were stored either continuously agitated (group I) or without agitation (group II). The surface of the bag was covered with a gas impermeable foil by 0% (control), 25%, 50% or 100%. PC were stored for 8 days and samples were taken on day 1, 5, 7 and 8. Biochemical and activation parameters were measured by commercial assays (Roche, Germany, Greiner, Germany, R&D Systems, USA).

Results: Mean values of the results for 8 PC per treatment are summarized in table 1

Conclusions: Changes in pH during storage are significantly higher in non-agitated platelets compared to agitated. While the 25% reduction of gas exchange can be tolerated in agitated BC-PC the same treatment combined with non-agitation of the container results in a pH at the lowest level of specification.

		day 1				day 5				day 7				day 8			
		control	25%	50%	100%	control	25%	50%	100%	control	25%	50%	100%	control	25%	50%	100%
agitated	ph (22 °C)	7.27	7.17	7.11	6.84	7.35	7.25	7.15	6.61	7.31	7.22	7.11	6.63	7.27	7.20	7.09	6.64
	glucose consumption *	7.96	5.24	5.83	5.10	5.75	3.28	3.57	1.16	4.57	2.50	2.60	0.55	3.52	1.94	1.97	0.30
	lactate production *	4.80	4.19	4.51	6.75	8.18	6.29	7.92	14.09	10.81	8.48	9.91	14.76	11.98	9.35	11.17	15.76
	β-TG**	8.25	–	–	5.75	12.58	–	–	14.52	14.32	–	–	16.92	15.22	–	–	17.28
	soluble P-Selectin***	37.40	–	–	28.50	57.07	–	–	61.15	72.66	–	–	99.53	77.35	–	–	120.90
non-agitated	ph (22 °C)	7.14	7.10	6.98	6.86	6.99	6.88	6.70	6.57	6.93	6.84	6.72	6.57	6.98	6.83	6.73	6.61
	glucose consumption *	6.57	7.33	4.99	5.30	2.90	2.00	0.42	0.43	1.90	1.31	0.32	0.32	1.25	0.95	0.31	0.32
	lactate production *	5.71	6.25	6.26	7.37	13.89	15.91	19.67	21.80	16.26	18.94	22.93	22.67	15.14	18.94	21.01	19.30
	β-TG**	7.50	–	–	6.72	11.80	–	–	19.61	14.51	–	–	22.98	13.64	–	–	21.60
	soluble P-Selectin***	30.40	–	–	35.29	59.67	–	–	93.69	82.33	–	–	136.60	79.39	–	–	136.40

* (mmol/10E + 9 Plt)
** (U/10E + 6 Plt)
*** (µg/10E + 6 Plt)

P 3.21

Development of A New Closure Device Concept to Optimize Workflow, Workload and Product Quality in Blood Component Processing

Boecker W.[1], Reidel A.[2]

[1]Fresenius Kabi Deutschland GmbH, Transfusion Technology, Oberursel, D,
[2]Fresenius Kabi Deutschland GmbH, Oberursel, D

Purpose: Since the introduction of flexible blood bag systems the closure devices (bag breakers) remained nearly unchanged. Staff members often complain about mechanical damage of fingers, joints and tendons as well as skin deteriorations that represent symptoms of Repetitive Strain Injuries (RSI). This finally may lead to haemolysed RCCs caused by insufficiently broken closure devices and additional sick leave of staff members which causes an enormous money drain from blood services.

Methods: The redesign of the closure device alone will not solve all above mentioned problems. A complete concept including an automated opening of the closures was developed consisting of blood bag systems containing a CompoFlow® closure and two different opening devices (blood component separator CompoMat G5® and battery driven handheld opener CompoSure®).

Results: During feasibility studies at Blood Research Center of CBS Canada with CompoFlow blood bag systems the device showed an excellent performance with no impact on haemolysis or activation of platelets com-pared to standard breakers. The flow characteristics of CompoFlow were much better (free flow space nearly double). Preliminary data of more than 5,000 processed CompoFlow bag systems in combination with CompoMat G5 at German Red Cross BDS Baden-Baden and smaller studies in Modena/Italy and Mainz in combination with CompoSure clearly demon-strated the superiority of the concept.

Conclusions: Haemolysis is the most common form of noxa that beside leakages lead to an unnecessary discard of blood products. It is possible to reduce the occurrence of haemolysis by technical measures like the implementation of the CompoFlow Concept. Further calculations will show not only the financial benefit but also a better supply situation for urgently needed blood products. On the other hand the CompoFlow system being an integrated solution to avoid RSI at the most critical production step of opening bag breakers adds additional benefit in respect of haemolysis avoidance. The concept is designed to keep the process of component preparation very simple with the highest staff satisfaction by avoiding painful pinch grip opening movements. In combination with the CompoMat G5 even staff in contact with blood bag systems will get fewer skin lesions, tendinitis and carpal tunnel syndrome. All this leads to lower staff replacement cost and a higher job satisfaction.

P4 Hemotherapy

P 4.01

Platelet Function Testing Using the Impact-R in Monitoring Platelet Substitution

Meyer O., Aboud N., Radtke H., Hemmati P., Theis T., Kiesewetter H., Arnold R., Salama A.

Charité - Institut für Transfusionsmedizin Berlin, D

Purpose: To date, platelet transfusions are usually monitored by measuring the post-transfusion platelet count and/or the corrected count increment (CCI). However, an increase in the platelet count does not necessarily correspond to an improvement in primary haemostasis. Although the response to platelet transfusions can be somewhat assessed by the physician treating the affected patient, the real effect remains frequently questionable. The application of platelet function assays might therefore be helpful in the assessment of platelet transfusions.

Design and Methods: We investigated 66 single-donor platelet transfusions (Median 2.5×1011 platelets/unit) to patients who underwent allogenic blood stem cell transplantation. Blood samples were obtained before and after transfusion. EDTA-blood samples were used to measure the platelet count and corrected count increment (CCI). Citrated blood samples were used to investigate platelet adhesion and platelet aggregation using the Impact-R device (DiaMed, Ottobrunn, Germany).

Results: All patients were thrombocytopenic before transfusion (Median 6/nl; Range 0–21/nl), and platelet transfusion resulted in an increase of platelet count after 1h (Median 24/nl; Range 1–53/nl). The Median 1 h CCI was 12 (Range 0–33). Median platelet adhesion as expressed by the surface coverage (SC) measured by the Impact-R increased from 0.3 (Range 0.1–2.6) to 0.7 (Range 0.1–8) 1 h after transfusion. The median platelet aggregation (AS by the Impact-R) was 45 (Range 10–114) before and 43 (Range 11–183) 1h after transfusion. Multi-Regression analysis showed a strong correlation of SC as measured by the Impact-R (Coef 5.46; p>0.001; 95% Conf. Interval 2.407458–8.521375) to 1 h CCI. No correlation was found for AS.

Conclusion: The Impact-R is very simple to handle and requires a very small volume of blood (0.2 ml). In our experience, the test demonstrated reproducible results, even in patients with platelet counts <30/nl. Furthermore, the results of the Impact-R are independent of other variables (e.g. haematocrit and coagulation factors). However, the Impact-R does not provide any additional information in patients that require platelet transfusions, since we found a strong correlation of platelet adhesion (SC) and CCI after 1 h of transfusion.

P 4.02

Effects of the Reduction of the Platelet Concentrate Storage Period to Four Days on Platelet Logistics and Transfusion Success

Heuft H.-G., Goudeva L., Tell-Lüersen M., Schmitt-Thomssen A., Martens J., Pastucha L., Blasczyk R.

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

Purpose: To investigate the effects of an apheresis platelet concentrate (PC) storage period reduction to four days, we evaluated the logistics (PC production schedule, acquisition from external providers, waste, storage time from apheresis to transfusion, degree of AB0 identical PC transfusions) and the success of PC transfusions (corrected count increment [CCI], parallel administration of other blood components, major bleeding events) in adult haematological transplant patients.
Methods: To assess logistical parameters, the electronic data base of our institution was evaluated for the time periods July, 2007 to January, 2008 (5.992 PC, 5-days-storage) versus July, 2008 to January, 2009 (5706 PC, 4-days-storage). Clinical parameters were determined by a comparison of patient records from September to November, 2007 (5-days-storage, 85 patients, 654 PC transfusions) versus September to November 2008 (4-days-storage; 78 patients, 580 PC transfusions).
Results: The total number of PC aphereses per week (n=105) remained stable, but the apheresis schedule changed from an uniform scheme with 5×21 aphereses (Monday–Friday) per week to enlarged numbers on Monday and Friday (2×26 aphereses) and reduced numbers of 18/day on Tuesday to Thursday. There was no increase in the PC numbers that had to purchased from external providers (2007, n=17; 0.29% vs 2008, n=14; 0.25%) or in the PC waste (2007, n=148; 2.5% vs 2008, n=153; 2.7%). The time from termination of apheresis to transfusion was reduced from 79 ±30 hours (h) in 2007 to 52 ±25 h in 2008; p≤0.0001. The number of AB0 identical PC transfusions decreased from 4356 (2007, 72.7%) to 4029 (2008, 70.6%; p=0.08). For transfusion data, see table.
Conclusions: The reduction of the PC storage period to four days did not cause severe logistical difficulties with respect to PC delivery, waste or the degree of AB0 identical transfusions. The improved PC transfusion results are likely to be due to the significant decrease of the storage time prior to transfusion to approximately 2 days.

Year, PC storage (days)	PC transfusion needs /patient, n	RCC/FFP transfusion episodes in parallel, n (%)	CCI ($\times 10^3/\mu L$)	Bleeding events, n
2007, 5 days	6 (1–33)	101 (15.5%)	11 ±5	8
2008, 4 days	5 (1–40)	80 (13.8%)	16 ±6	4
p-values	0.14	0.03	0.01	0.001

P 4.03

Is the Low Platelet Transfusion Trigger in the New German "Leitlinien" of Risk in Outpatient Setting? Prophylactic Platelet Transfusions in Aplastic Anemia

Höchsmann B.[1], Seidel N.[2], Marx-Hoffmann A.[2], Wiesneth M.[2], Schrezenmeier H.[3]

[1]IKT Institut für klinische Transfusionsmedizin und Immunogenetik Ulm, DRK Blutspendedienst Baden-Württemberg/Hessen, Institut Ulm, D, [2]IKT Ulm Institut für klinische Transfusionsmedizin und Immunogenetik, Ulm, [3]DRK Blutspendedienst Baden-Württemberg/Hessen, Institut Ulm, D

Purpose: Recent publications recommend a strong restriction of prophylactic platelet concentrat (PC) transfusions with a transfusion trigger of platelets <10 G/l without major bleeding signs. Also the new German "Querschnitts-Leitlinien 2008" recommand a trigger <5 G/L for prophylactic transfusions in hematologic patients with chronic thrombocytopenia . We agree to the necessity of a restrictive transfusion policy due to potential adverse events, availability and costs of PC. Here we report our data on chronic PC transfusion support in adult aplastic anemia (AA) patients treated in an outpatient setting to show that PC transfusion guidelines have to be evaluated for different diseases and situations.
Methods: Analysis of patient (pt) characteristics and transfused PC units. Therapy efficacy and adverse events were evaluated by blood count, vital parameters, clinical symptoms, CMV-/HBV-/HCV-/HIV –status and antibody testing.
Results: In total we examined 247 patients (pts) with a median duration of outpatient transfusion therapy of 256 d (1–4954 d). 12 pts (7 male/5 female) of this cohort were diagnosed with AA without stem cell transplantation. Pt characteristics were: median age: 63 (20–76) years; no SCT; median duration of PC transfusion therapy: 13 (6–171) mo; median number of transfused PC units: 101 (16–328) with a median of 52 (2–328) pooled PC (P-PC) and 4 (1–175) apheresis PC (A-PC) units. Median increase after the first PC was 51 (17–68) G/l. There was no hint for a decrease of clinical efficacy during PC transfusion therapy. In none of the pts a seroconversion in CMV-/HBV-/HCV-/HIV –status or the development of new anti-HLA-antibodies were observed. After PCs 15 mild (none severe) transfusion reactions occured. One pt is still transfusion dependent, 6 pts are independent for PC now and 4 pts died due to infections caused by AA related neutropenia. One pt died due to a cerebral hemorrhage after reduction of the transfusion trigger to <10 G/l. This pt was without any major bleeding complications during 11 mo and 328 P-PCs upon a transfusion trigger of <20 G/l for prophylactic PC-transfusions.
Conclusions: 1) Our data show that outpatient transfusion therapy is safe and efficient even in long term therapy. 2) We strongly recommend individualized transfusion guidelines based on the underlying disease and the patient situation. 3) Especially in outpatient care without fulltime observation and with more physical strain than in hospital a higher transfusions trigger may be necessary than in hospitalized pts.

P 4.04

A Simple Guideline Reduces the Need for Red Blood Cell (RBC) Transfusions: A Prospective, Multicenter, Before-and-after Study in Elective Hip and Knee Replacement

Fontana S.[1], Müller U.[2], Perler M.[2], Lädrach A.[3], Krummen L.[3], de la Cuadra C.[3], Luginbühl M.[4], Mansouri Taleghani B.[3]

[1]Regional Blood Transfusion Service of the Swiss Red Cross, Bern, CH, [2]Institute for Evaluative Research in Orthopaedic Surgery, University of Bern,CH, [3]Department of Hematology and Central Hematological Laboratory, Inselspital, University Hospital, Bern, CH, [4]Department of Intensive Care Medicine, Emergency Medicine and Anaesthesiology, Inselspital, University of Bern, CH

Introduction: Limited data and no general guidelines on transfusion practice in elective orthopedic surgery are available in Switzerland. Therefore we launched a Swiss study group and initiated a study, analyzing our pre-intervention transfusion practice in elective hip and knee replacement and the effects following the introduction of a straightforward guideline on RBC transfusion.
Methods: Prospective, multicenter before-and-after study comparing the use of RBC in adult elective hip or knee replacement before and after the implementation of a transfusion guideline in 10 Swiss hospitals. During the first 6 months we monitored RBC use and patient outcomes (mortality and cumulative complication rate – cardiovascular, bleedings, infections). In a following transition period a RBC transfusion guideline, jointly developed by the participating hospitals, was introduced and implemented. We continued monitoring of data until completion of a subsequent post-interventional period of 6 months.
Results: Preliminary evaluation of 2134 patients (1238 before and 896 after, 42% knee and 58% hip replacements, median ASA 2 (range 1-4)) is available. The two groups are comparable for patient- and intervention-related variables. The proportion of transfused patients (PTP) decreased from 20.9% before to 16.9% after intervention (4%, 95% C.I. 0.7 to 7.4, p=0.02), the number of transfused RBC units (NTU) from 0.5 to 0.4 per patient (0.1, 95% C.I. 0.08–0.2, p=0.014), and the haemoglobin value before the first transfusion from 82.6 (inter-hospital range 76.9–88.9) to 78.2 g/L (68.5–81.2, p=0.0001). The variability between hospitals decreased in the second phase (PTP 4.6–43.7% to 6.9–28.3%, NTU 0.11–1.16 to 0.14–0.69). We didn't observe changes in in-hospital mortality (0.4% vs. 0%, p=n.s.) and morbidity (4.1% vs. 4.0%, p=n.s., median hospital length of stay 9 days before and after), follow up mortality (0.4% vs. 0.2%, p=n.s.) and morbidity (6.9% vs. 6.0%, p=n.s., median follow up 52 days before and 49 days after).
Conclusions: The preliminary evaluation of this first Swiss multicenter study shows that the introduction of a simple transfusion guideline reduces and standardizes the use of RBC by decreasing the haemoglobin transfusion trigger, without negative effects on the patient outcome.

P 4.05

Red Blood Cell (RBC) Use in Elective Cardiac and Vascular Surgery: A Prospective Assessment of the Effect of a Transfusion Guideline in a Swiss University Hospital

Fontana S.[1], Müller U.[2], Perler M.[2], Eberle B.[3], Regli B.[3], Schmidli J.[4], Carrel T.[4], Krummen L.[5], Lädrach A.[5], Mansouri Taleghani B.[5]

[1]Regional Blood Transfusion Service of the Swiss Red Cross, Bern, CH, [2]Institute for Evaluative Research in Orthopaedic Surgery, University of Bern,CH, [3]Department of Intensive Care Medicine, Emergency Medicine and Anaesthesiology, Inselspital, University of Bern, CH, [4]Department of Cardiovascular Surgery, Inselspital, University Hospital and University of Bern, CH, [5]Department of Hematology and Central Hematological Laboratory, Inselspital, University Hospital, Bern, CH

Introduction: Limited data and no general guidelines on transfusion practice in elective cardiac and vascular surgery are available in Switzerland. We started a single center study, analyzing our pre-intervention transfusion practice in elective cardiac and vascular surgery and the effects following the introduction of a straightforward guideline on RBC transfusion.
Methods: Prospective, before-and-after study comparing the use of RBC in major adult cardiac and vascular surgery (valve replacement, coronary bypass, open abdominal aortic aneurysm procedures) before and after the implementation of a transfusion guideline. During the first 6 months we monitored RBC use and patient outcomes. During this phase, aprotinin use was withdrawn from the market and substituted by tranexamic acid. Thereafter, an institutional RBC transfusion guideline, jointly developed by the participating services, was introduced and implemented. We continued monitoring of data until completion of a subsequent period of 6 months
Results: Preliminary evaluation of 797 patients (414 before and 383 after, 91.3% cardiac and 8.7% vascular surgery, median ASA physical status 3, median Euroscore 5) is available. The proportion of transfused patients is 67.9% before and 75.7% after intervention (Diff –7.8%, 95% C.I. –1.6 to 14.1%, p=0.015), the number of transfused RBC units per patient 2.5 and 3.0 (+20%, p=n.s.), and the haemoglobin value before the first transfusion 80.1 (+/–10.9) g/L prior to guideline implementation and 75.2 (+/–7.5) g/L thereafter (p<0.0001). We did not observe changes in in-hospital mortality (1.2% vs. 2.1%, p=n.s.), morbidity (15.2 vs. 12.5%, p=n.s., median hospital length of stay 8 days before and after), and follow up mortality (1.2 vs. 2.6%, p=n.s.) and morbidity (19.1 vs. 17.5%, p=n.s., median follow up 39 days before and 42 days after). Additional data related to the change of the antifibrinolytic prophylaxis will be presented.
Conclusions: Our preliminary evaluation shows that the introduction of a simple guideline modified the transfusion behaviour by lowering the haemoglobin transfusion trigger, but did not prevent an actual increase over time in the proportion of transfused patients nor the number of transfused RBC transfused. Apparently, more influential factors overrode the effect of lowering the transfusion trigger by the guideline. E. g., the change in antifibrinolytic prophylaxis alone may explain most of the increased proportion of transfused patients.

P 4.06

Coombs Cross Match after a Negative Antibody Screen

Selleng K.[1], Lange J.[2], Selleng K.[2], Heddle N.[3], Traore A.[3], Greinacher A.[2]

[1]Institut für Immunologie und Transfusionsmedizin, Abt. Transfusionsmedizin, Greifswald, D, [2]Institut für Immunologie and Transfusionsmedizin, Ernst-Moritz-Arndt Universität, Greifswald, D, [3]Department of Medicine (Hematology), McMaster University, Hamilton, CDN

Purpose: Full antiglobulin cross matching (AHG XM) is mandatory for patients scheduled for red blood cell (RBC) transfusion in Germany and Austria. We assessed how many biologically relevant RBC alloantibodies are identified by the AHG XM in patients with a negative antibody screen (ABS), depending on the conventional tube test (CTT) or the gel card test (GT).
Methods: All AHG XMs at the Department of Transfusion Medicine at the Ernst-Moritz-Arndt University Hospital Greifswald were retrospectively analyzed for an 8 year period. Data source were the protocols for ABS, AHG XMs and antibody differentiation. One patient population was screened by 2 test cells in the ABS and another population by 3 test cells using the GT.
Results: 312,275 RBC XMs were assessed. Using the GT for AHG XM five times more XMs were reactive after a negative ABS than by the CTT (CTT 0.05% vs. GT 0.25%; p<0.001). Excluding anti-A1, RBC alloantibodies were found with a very low frequency regardless to the method used (CTT 5/105,647 [0.005%], GT 5/206,628 [0.002%]; p=0.3) (table). Only in 1% of the reactive GT-XMs RBC alloantibodies were identified. After changing from 2 to 3 test cells for ABS, not a single biologically relevant alloantibody was identified in 126.333 XMs.
Conclusions: The probability to detect biologically relevant RBC antibodies by AHG XM after a negative ABS is similar for CTT or GT. If a 3-cell-ABS by GT is negative and anti-A1 antibodies are excluded, the AHG XM contributes minimally to transfusion safety.

	CTT-XM (n=105,647)			GT-XM (n= 206,628)			p-value
Alloantibodies identified after reactive XMs	n	% of reactive XMs	95% CI	n	% of reactive XMs	95% CI	
RBC alloantibodies	5*	10.2	[1.7;18.8]	5**	1.0	[0.1;1.9]	0.001
anti-A1 antibodies	5	10.2	[1.7;18.8]	80	15.5	[12.4;18.6]	0.526
Other causes of reactive XMs							
Autoantibodies of recipient	6	12.2	[3.0;21.4]	33	6.4	[4.3;8.5]	0.153
Reactive DAT of RBC concentrates	1	2.1	[0;5.9]	137	26.5	[22.7;30.3]	0.003
No antibody identified	22	44.9	[31.0;58.8]	191	36.9	[32.7;41.1]	0.486
Not further investigated	10	20.4	[9.1;31.7]	71	13.7	[10.8;16.7]	0.301
TOTAL	49	100.0		517	100.0		
* anti-P1 (4XMs), anti-Wr^a ** anti-Jk^a, anti-Kell (2XMs), anti-P1, anti-M							

P 4.07

Platelet Transfusion Support in a Polyimmunized Patient

Duecker C.[1], Strupp C.[2], Holder A.[3], Homey B.[2], Korfmacher S.[2], Hoffmann T.[2], Scharf R.E.[2]

[1]University Clinic of Duesseldorf, Institute for Haemostasis and Transfusion Medicine, Duesseldorf, D, [2]University Clinic of Duesseldorf, D, [3]DRK-Blutspendedienst West, Duesseldorf, D

Transfusion support may be substantially hindered in polyimmunized patients.
We report a 57 yrs old patient with myelodysplastic syndrome scheduled for allogeneic stem cell transplantation. Due to two pregnancies and / or prolonged transfusion therapy she had developed blood-group antibodies, HLA antibodies and HPA antibodies, respectively. Furthermore, the latest transfusion of platelet concentrates was repeatedly complicated by anaphylactoid reactions with drop of systolic blood pressure to <60mmHg. Neither intensive premedication nor selection of HLA compatible concentrates allowed to prevent the adverse reactions. IgA deficiency was excluded. An IgE-mediated etiology was not to be suspected. Dermal testing provided evidence for allergenic plasma-components, rather than serum-components to be responsible; alternatively, stabilizing agents of platelet concentrates could be causative.
Accordingly, test-transfusions were performed. A washed pool-platelet concentrate was administered without adverse reaction, but didn´t achieve an increment in platelet counts as expected. In contrast, transfusion of a washed single donor apheresis concentrate, chosen according to compatibility with the patient´s HLA-type as well as compatibility in the platelet crossmatch, was effective (CCI >7,5) as well as adverse event-free.
Consequently, allogeneic stem cell transplantation with provision of HLA compatible washed platelet concentrates is now under way. Washing of the stem cell transplant will be considered as well as lowering the transfusion needs by using a reduced conditioning regimen.

P 4.08

Hemostasis-Navigated Hemotherapy: Conception and Realization

Scharf R.E.

Heinrich Heine University Medical Center and Biological Medical Research Center, Heinrich Heine University, Department of Experimental and Clinical Hemostasis, Hemotherapy, and Transfusion Medicine, Düsseldorf, D

Appropriate treatment of bleeding patients with complex hemostatic defects requires both, adequate transfusion of hemotherapeutics and close monitoring of their effects. While improvement or restoration of oxygen transport upon replacement therapy with packed red blood cells is easy to examine in anemic patients, administration of fresh frozen plasma, prothrombin complex and/or coagulation factor concentrates, and platelet units requires close control. This is best achieved by longitudinal laboratory assessment within short-term intervals. Screening tests of coagulation, i.e. prothrombin time (PT), activated partial thromboplastine time (APTT), fibrinogen, and as measure of fibrin cross-linking such as D-dimer/fibrin degradation product should be performed together with platelet counting. In addition, evaluation of platelet function using PFA-100 or performing global hemostasis testing by the use of thrombelastography will provide useful information to guide hemotherapy and document its effects. Thus, instead of "empirical" substitution, simply controlled by clinical observation, such as cessation of bleeding, a rational, efficient, and also economic hemotherapy can be achieved, replacing specifically those components that are depleted in the individual patient. This procedural approach has several advantages: (1) It strictly follows the guidelines of modern hemotherapy by administrating cellular or plasma products targeted and adjusted to the individual needs of a given patient ("as much as required, as less as needed"), (2) prevents from overtreatment, (3) indicates therapeutic failure, and (4) allows early detection of imminent side-effects or complications, e.g. disseminated intravascular coagulation (DIC) in due time. To illustrate how these principles of hemostasis-navigated hemotherapy are best realized in daily clinical practice, typical cases will be discussed with special emphasis on the hemotherapeutic management and laboratory monitoring.

P 4.09

Supportive Homotherapy in a Polyimmunized Patient with Myelodysplastic Syndrome

Dücker C.[1], Strupp C.[2], Holder A.[2], Homey B.[2], Korfmacher S.[2], Hoffmann T.[2], Scharf R.E.[2]

[1]Heinrich Heine University Medical Center, Department of Experimental and Clinical Hemostasis, Hemotherapy, and Transfusion Medicine, Düsseldorf, D, [2]Heinrich Heine University Medical Center, Düsseldorf, D

Supportive hemotherapy may be substantially hindered in polyimmunized patients. We report on a 57-yr-old female patient with myelodysplastic syndrome of the chronic myelomonocytic leukemia (CMML) subtype who was referred for allogeneic hematopoietic cell transplantation. Along with two previous pregnancies and/or prolonged hemotherapy with red blood cells units and platelet concentrates, the patient had been alloimmunized and now presented with multiple antibodies against blood group antigens, human leukocyte (HLA) and human platelet (HPA) antigens. Upon admission, hemotherapy with platelet concentrates was repeatedly complicated by severe anaphylactoid reactions, including hypotension, tachycardia, dyspnea, and generalized urticaria. Neither selection of HLA and HPA compatible platelet donors nor intense premedication prevented the patient from transfusion-associated adverse reactions. Selective IgA deficiency and an IgE-mediated mechanism were excluded. Subsequent pretransfusion dermal testing of the patient with plasma or serum specimen from selected individual donors was indicative of allergenic plasma rather than serum components being responsible for the adverse reactions. However, it could not be excluded that stabilizing agents of platelet concentrates were also involved. Consequently, test transfusions were carefully performed using washed platelet preparations under intensive care conditions. Fortunately, hemotherapy with washed single-donor apheresis platelet concentrates from carefully selected compatible donors tested by platelet crossmatch was tolerated by the patient without any adverse side effects. Moreover, subsequent transfusions of washed plateletpheresis concentrates were effective, as documented by the 1-hr corrected count increment (CCI >20 x 109/L). Consequently, allogeneic hematopoietic cell transplantation with supportive hemotherapy of HLA and HPA compatible washed single-donor plateletpheresis concentrates is now under way. To decrease the posttransplantation transfusion needs, a less intense conditioning regimen will be used. Moreover, to prevent any further adverse reactions and to improve the outcome, washing of the hematopoietic cell transplant may be also required in this specific clinical setting.

P5 Preparation and Therapy with Hematopoietic Stem Cells

P 5.01

Conversion of Pluripotent Stem Cells towards Hematopoietic Stem Cells, In Vitro, Mediated by HOXB4

Klump H.[1], Lesinski D.[2], Heinz N.[2], Luikenga S.[1], Schiedlmeier B.[2], Baum C.[2], Horn P.[1]

[1]University Hospital Essen, Institute for Transfusion Medicine, Essen, D, [2]Hannover Medical School, Dept. of Experimental Hematology, Hannover, D

Purpose: Somatic cells which have been reprogrammed back to an induced pluripotent state (iPS) will likely become a key source for the in vitro generation of patient-tailored hematopoietic stem cells (HSCs) in future gene and cell therapy. This opens avenues for efficient selection of molecularly characterized, "safe", gene-corrected clones at the pluripotent, undifferentiated level. However, protocols for directed differentiation of pluripotent cells towards hematopoietic stem cells (HSCs) have to be established and subsequent expansion of the artificially generated stem cells must be achieved up to levels useful for transplantation. We and others have previously shown that ectopic expression of the human homeobox transcription factor HOXB4 mediates HSC expansion both of mice and humans, in vitro and in vivo, and also enhances the in vivo repopulation ability of in vitro differentiated mouse ES-cells in a dosage dependent manner (1,2). Thus, HOXB4 is considered one the most attractive candidates for therapeutic use. Nevertheless, an understanding how HOXB4 promotes the conversion of pluripotent cell derivatives to HSCs is still in its infancy (3).

Methods & Results: Using a novel, tightly regulable expression system, we here demonstrate that induced HOXB4 expression enforces the development of the earliest known (CD41hi) hematopoietic cell during ES-cell differentiation and promotes its subsequent expansion in stroma-cell free suspension culture. These cultures lead to long-term engraftment after transplantation into immunodeficient, Rag2(-/-)gammaC(-/-) mice.

In conclusion, the ability of HOXB4 expressing cultures to engraft may correlate with the presence of CD41hi cells in these cultures. Thus, tight and fine-tuned regulation of HOXB4 or the use of "HOXB4-mimetic" drugs (3) may also support the de novo generation and expansion of safe (gene-corrected) human HSCs derived from patient-specific induced pluripotent stem cells, entirely in vitro.

References:

1 Kyba,M., Perlingeiro,RC, and Daley,GQ. (2002) Cell, 109, 29–37.

2 Pilat,S., Carotta,S., Schiedlmeier,B., Kamino,K., Mairhofer,A., Will,E., Modlich,U., Steinlein,P., Ostertag,W., Baum,C., Beug,H and Klump,H. (2005) Proc. Natl. Acad. Sci. USA, 102, 12101–12106.

3 Schiedlmeier B, Santos AC, Ribeiro A, Moncaut N, Lesinski D, Auer H, Kornacker K, Ostertag W, Baum C, Mallo M, Klump H (2007) Proc. Natl. Acad. Sci. USA 104, 16952–16957.

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P 5.02

Functional Characterization of the Impact of the Par/aPKC-Complex and Cdc42 on Cell Fate Decisions, Polarity and Migration in Human Hematopoietic Stem and Progenitor Cells

Andre Goergens A., Horn P.A., Giebel B.

University Hospital Essen, Institute for Transfusion Medicine, Essen, D

Freshly isolated hematopoietic stem and progenitor cells (HSC/HPCs) are small round cells which acquire a polarized cell shape upon cultivation. They form a leading edge at the front and a uropod at the rear pole. We and others have shown that this polarization process depends on the activity of the phosphoinositol-3-kinase; evidence was provided that the GTPase Cdc42 is also involved in this process.

As it has been shown in model organisms, polarity is an important prerequisite for asymmetric cell divisions and it is known that the Par/aPKC-Complex and Cdc42 are required for the establishment of this polarity. Since we showed that HSC/HPC cells can divide asymmetrically, we wondered whether these evolutionary conserved proteins are also involved in cell fate specification processes within the human hematopoietic compartment.

Preliminary results of our group show that the main components of the Par/aPKC-Complex are expressed in cultivated HSC/HPCs. To analyse the impact of these proteins on primitive hematopoietic cells by means of gain or loss of function experiments, we have set up efficient methods to genetically manipulate primary human HSCs/HPCs first. Furthermore, we have established assays to analyse effects on the cell polarity, on the migration behaviour and the cell fate of the manipulated cells.

P 5.03

The Mannheim Cord Blood Bank: Evaluation of Transplant and Patient Data

Lauber S.[1], Latta M.[2], Klüter H.[2], Müller-Steinhardt M.[2]

[1]Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Heidelberg University, Red Cross Blood Donor Service Baden-Württemberg-Hessen, Mannheim, D, [2]Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Heidelberg University, Mannheim, D

Purpose: Cord blood (CB) as a source of hematopoietic stem cells is an alternative to bone marrow or peripheral stem cells in clinical transplantation. Currently the Mannheim cord blood bank (established in 1996) collects CB donations from 7 different hospitals in South-West Germany. At present storage criteria are: whole blood volume >68 ml, nucleated cell count (NC) >8×108 and time between collection and cryopreservation <48 hours. To date about 1750 allogeneic CB units were stored and recorded to the German National Bone Marrow Donor Registry (ZKRD) for distribution. In order to optimize our preparation procedure we evaluated stored CB units with regard to transplant and patient characteristics. All units were analyzed for volume, NC, mononucleated cell (MNC) and CD34+ cell count, colony-forming units, HLA class I/II, ABO, Rhesus and ethnic background of the donor.
Results: Only 25% of all collected CB met the storage criteria. Main reasons for exclusion were: 1. little volume (58%) and/or 2. cell count (7%), and 3. exceeding time between collection and cryopreservation (19%). Up to now 34 CB units have been released for transplantation, mainly to transplant centers in North-America (41%) and Europe (53%). 62% of the recipients were children under 15 years of age, but also adults (mean 19 yrs, median 9 yrs). Transplant indications were haematological diseases (76%) and less often immune deficiencies and metabolic diseases (each 6%). Transplanted CB units showed significantly higher cell counts for NC, MNC and CD34+ versus stored units: 12.3±5.8 vs. 7.0±4.0×108 NC (mean ± SD), 4.8±2.4 vs. 2.8±1.9×108 MNC and 3.2±2.0 vs. 1.6±2.1×106 CD34+. Related to patient body weight (BW), a mean NC cell dose of 4.7×107 NC/kg BW was determined. Among transplanted CB units we found donors from ethnic minorities in 34% of cases versus 20% in stored units.
Conclusions: CB is a valuable source of hematopoietic stem cells that is successfully applied for allogeneic transplantation. Even though cell count and volume are key parameters for the eligibility of CB units, our data also indicate the ethnic background of the donor to play a major role. Although recipients are mainly children, CB transplantation also offers an alternative treatment for adults.

P 5.04

Hepatitis B Reactivation during Autologous Stem Cell Collection – Case Report

Schauwecker P.[1], Reinhardt P.[2], Schilling U.[3], Else H.[3], Mayr-Wohlfahrt U.[4], Schmidt M.[5], Wiesneth M.[2], Schrezenmeier H.[2]

[1]Institute for Clinical Transfusion Medicine and Immunogenetics Ulm; German Red Cross Blood Transfusion Service Ba-Wü - Hessen, Spenderabteilung, Ulm, D, [2]Institute for Clinical Transfusion Medicine and Immunogenetics Ulm; German Red Cross Blood Transfusion Service Ba-Wü - Hessen, Ulm,D, [3]Diakonie-Klinikum Stuttgart Medizinische Klinik II, Stuttgart, D, [4]Institute for Clinical Transfusion Medicine and Immunogenetics Ulm; DRK Blood Donor Service Baden-Württemberg - Hessen, Ulm, D, [5]Institute for Clinical Transfusion Medicine and Immunohematology Franfurt; DRK Blood Donor Service Baden-Württemberg - Hessen, Frankfurt, D

Purpose: The „Bundes-Gesundheitssurvey“ from 1998 reports the seroprevalence for Anti-HBc-Antibodies of 7.7% in the population of the former West-German states. The frequency of Hepatitis B virus persistence (occult Hepatitis B) in patients who seemingly have eliminated the virus is not clear. Recently growing incidence of Hepatitis B reactivation during immunosuppression has come up.
Methods: We report about a 44 year old male patient with B-NHL (B-CLL and Richter-Syndrome) with Hepatitis B reactivation during stem cell mobilization and collection of autologous stem cells. GPT (ALT), HBs-Ag, Anti-HBs, Anti-HBc IgG, Anti-HBc IgM, Anti-HBe (all CE-certified). Standard resolution HBV-PCR-Nr (detection limit <100 IU/ml) and high resolution HBV-PCR-Hr (detection limit <10 IU/ml).
Results: Before initiation of the first chemotherapy (high-dose-Methotrexate, high-dose-Ifosfamid as well as Cytarabin i.th.) the patient's Hepatitis B infection appeared to be cured (HBV-PCR-Hr, HBs-Ag, Anti-HBs, Anti-HBc IgM negative; Anti-HBc IgG positive). Autologous stem cell collections were performed on three consecutive days following the second chemotherapy cycle and mobilization treatment with G-CSF. On these days we observed rising GPT (126–190 U/l) and Anti-HBs (50–190 IU/l). Two out of three HBV-PCR-Hr and one of three HBV-PCR-Nr had turned positive. Anti-HBe was also positive.
Conclusions: Taken together we interpret these results as a reactivation of a Hepatitis B infection of the patient during stem cell mobilization and collection. Although a past HBV-infection is not a rare event, little to no data are available concerning HBV-reactivation during stem cell mobilization and collection, thus we recommend a close monitoring of these patients.

P 5.05

Analysis of Transcription Factors Differentially Expressed in the Primitive Human Hematopoietic Compartment

Zeidler S., Goergens A., Horn P.A., Giebel B.

University Hospital Essen, Institute for Transfusion Medicine, Essen, D

Somatic stem cells are required to maintain homeostasis in different tissues. In this context stem cells give rise to differentiating cells which replace cells getting lost in the lifetime of a multi-cellular organism. To fulfil this function over a long period of time, it is essential that the pool of stem cells remains a constant size. Since both the abnormal loss as well as the uncontrolled expansion of stem cells is fatal for organisms, the decision of self-renewal versus differentiation needs to be tightly regulated. The understanding of such mechanisms will not only be essential for the clinical use of these cells in regenerative medicine but will also increase our understanding of certain aspects of tumor formation and degenerative diseases.
At the example of the hematopoietic system, a few transcription factors, e.g. HoxB4, AML1/Runx1, SCL/Tal1, Meis1, have been identified, taking part in the decision process self-renewal versus differentiation of primitive hematopoietic stem cells. While loss of function of these transcription factors is generally associated with defects in the development of the hematopoietic system, the aberrant expression often results in an expansion of primitive hematopoietic cells and seems to be connected to different forms of leukemia.
With the aim to identify additional transcription factors required for the self-renewal process of primitive human hematopoietic cells, we have performed genome wide GeneChipTM analyses of different cell fractions, containing either primitive or more mature hematopoietic cells. We identified a number of transcription factors encoding genes which are specifically expressed in the most primitive hematopoietic cell fractions, whose function has not yet been associated with hematopoiesis. In order to characterize the early hematopoietic function of some of these candidate genes we decided to perform over expression as well as RNAi mediated knock down experiments. We are using a lentiviral strategy to genetically manipulate primary human umbilical cord blood derived CD34+ cells and analyze effects on the cell fates of transduced cells in different functional read out systems.

P 5.06

Mobilisation of Cd133 Positive Cells in Healthy Volunteers

Rehfeld I.[1], Kreissig C.[2], Bux J.[3]

[1]DRK BSD-West, Zentralbereich Stammzelle, Ratingen, D, [2]DRK BSD-West, Ratingen,D, [3]DRK BSD-West, Hagen, D

Introduction: CD133 is a marker of early hematopoietic cells. Different cell selection strategies are developed for different therapeutic approaches. If CD133 antigen is used for positive selection, it is important to know mobilisation efficiency under G-CSF application.
Methods: We examined CD34 and CD133 expression in 12 peripheral blood samples (PB) collected from unrelated donors after four days of G-CSF mobilisation and in apheresis products (AP) from these donors. We used four color flow cytometry on FACSCalibur device: In forward and side scatter threshold was depleted, then CD45 positive cells were stained with 7AAD to

exclude dead cells. CD45-positive cells were examined for CD34 expression. CD34 positive cells have to coexpress CD133 antigen.
Results: While CD34 cell counts in PB range from 26 to 179/µl (median 80/µl) CD133 cell counts reached 17 to 160/µl (median 55/µl). That means 0,15% (0,06–0,32%) of leucocytes were stained positive for CD34 antigen and 0,10% (0,04–0,28%) for CD133 antigen.
In leukapheresis products stem cells were enriched effectively. In AP 0,91% of leucocytes showed CD34 antigen (0,30–1,74%) and 0,68% (0,20–1,40%) CD133 antigen. That means 1605 (650–10680) CD34 positive and 1526 (433–9009) CD 133 positive cells were detectable per µl blood.
Discussion: We compared mobilisation of CD133 expressing cells under G-CSF injection to CD34 mobilisation. In peripheral blood fewer CD133 positive cells were detectable, but there is a correlation of mobilisation efficiency of both cell types: 78% of CD34 positive cells coexpress CD133 antigen.
In apheresis products CD34 and CD133 expressing cells are enriched in the same relation. The enrichment factor for CD34 positive cells was 30 fold, for CD133 cells 28 fold.
There is a lot of experience concerning mobilisation of CD34 positive cells. Regarding to our results we can pre-estimate CD133 kinetic during G-CSF administration and collection efficiency in peripheral blood stem apheresis procedures.

P 5.07

Response of Human Hematopoietic Stem and Progenitor Cells to Energetic Carbon Ions

Becker D.[1], Fournier C.[2], Seifried E.[3], Tonn T.[3]

[1]Institute for Transfusion Medicine & Immunohaematology, German Red Cross Blood Donation Service, JWG University Clinics Frankfurt, Gesellschaft für Schwerionenforschung (GSI), Darmstadt, D, [2]Gesellschaft für Schwerionenforschung (GSI), Darmstadt,D, [3]Institute for Transfusion Medicine and Immunohaematology, German Red Cross Blood Donation Service, Frankfurt, D

Background: The increasing application of heavy ions in radiotherapy is a strong motivation to expand the fundamental research with respect to long term side effects of heavy ions in different cell systems. The estimation of radiation induced chromosomal aberrations by cytogenetic analyses is usually conducted with differentiated lymphocyte populations only. Here many chromosomal aberrations may not be detected, due to apoptosis and cell death of damaged cells. In addition, the impact of heavy ion irradiation to hematopoietic stem cells is not well analysed and may be of relevance in the malignant formation of cancer stem cells. The purpose of this study was to characterize the radiation response of human hematopoietic stem and progenitor cells (HPCs) with respect to X and carbon ion irradiation.
Methods: CD34+ HPCs were immunomagnetically enriched (CD34 Micro Bead Kit, Miltenyi Biotech) from the peripheral blood of G-CSF mobilized healthy donors and subsequently submitted to different doses of X ray [16mA, 250 kV] and Carbon ion irradiation (29 keV/µm monoenergetic beam and 60-85 keV/µm spread-out Bragg peak) at the SIS facility at GSI, mimicking radiotherapy conditions. Apoptotic cell death, cell cycle progression and the frequency of chromosomal aberrations were determined.
Results: After radiation exposure no inhibition in the progression of the cell cycle was detected. However, an enhanced frequency of apoptotic cells and an increase in aberrant cells were observed, both effects being more pronounced for carbon ions than for X rays (relative biological effectiveness, RBE, 1.4–1.7). The fraction of complex-type aberrations was higher following carbon ion exposure.
Conclusions: RBE values of carbon ions are low, as expected for radiosensitive cells. The observed frequencies of apoptotic cells and chromosome aberrations in HPCs are similar to those reported for human peripheral blood lymphocytes suggesting that the effects observed in mature lymphocytes reflect the radiation response of their proliferating progenitors.

P 5.08

CD34 Quantitation Depending on Method of Determination

Gebauer W., Barklage I.

DRK BSD NSTOB, Oldenburg, D

National and international cooperation gives today many patients the opportunity to receive blood progenitor cell (bpc) transplants from allogeneic donors. Flow cytometric determination of CD34 positive cells among bpc in the collecting institution not necessarily is performed according to procedures used in the transplantation centre.
We determined CD34 positive bpc in apheresis products according to three different established procedures. Method A determines total nucleated cells in a blood cell counter and via flow cytometry percentage of CD34 positive cells after immunological staining, lysis of erythrocytes followed by fixation. Method B and C allowed direct enumeration of CD34 positive cells in the flow cytometer only by using counting beads in the assay. For Method B and C reagent sets were available from different commercial suppliers both without fixation of analyzed cells.
In thirteen bpc products determination of CD34 positive cells by method A showed good correlation to method B (r2 0.97) and method C (r2 0.97) although different amounts of CD34 positive cells were detected by method A compared to method B and method C.
The authors of this paper do not want to vote in favour of one of the methods described above but together with CD34 amounts in interlaboratory exchange some declaration on methods employed would be desirable.

P 5.09

Shifts within Nucleated Cell Subgroups of Stored Stem Cell Transplants

Schauwecker P.[1], Fischer C.[2], Brink A.[2], Maccari B.[2], Reinhardt P.[2], Wiesneth M.[2], Schrezenmeier H.[2]

[1]Institute for Clinical Transfusion Medicine and Immunogenetics Ulm; German Red Cross Blood Transfusion Service Ba-Wü - Hessen, Spenderabteilung, Ulm, D, [2]Institute for Clinical Transfusion Medicine and Immunogenetics Ulm; German Red Cross Blood Transfusion Service Ba-Wü - Hessen, Ulm, D

Purpose: For matched unrelated donor transplantations, collection centre and transplant centre may be located on different continents. If two aphereses are required, the 72-hour shelf-life of non-cryopreserved transplants may almost be reached by the time of transplantation. On the other hand, immunophenotyping of weekend collections of autologous stem cell grafts may be delayed and not take place immediately after collection but after cryopreservation. Thus we analyzed allogeneic and autologous stem cell collections of our institute for shifts in the subpopulations of nucleated cells (NC) over time.
Methods: 15 allogeneic and 15 autologous stem cell collections were assessed for differential NC-count (Abbott CellDyn 3200) and CD34+-cells (BD FACSort, dual platform) immediately after collection. A similar second measurement was performed of an 1 ml aliquot (in a 1.5 ml polyprophylene tube) after a 72-hour storage at +4±2 °C without agitation. The t-test for paired samples was used for statistical evaluation.
Results: A significant change of CD34+-cells between the two time points analyzed was not observed, neither for allogeneic (445×106 vs 428×106 , p=0.72) nor for autologous collections (556×106 vs 412×106 , p=0.17). As expected prolonged storage did lead to a significant reduction of the total NC-count (allogeneic: 624×108 vs 462×108 , p<0.01; autologous: 316×108 vs 240×108, p<0.02). Because the autologous patients had different underlying diseases and therapies prior to stem cell collection we only addressed the question in the allogeneic group which leukocyte subtype is affected by the approximately 30% decrease of NC. The reduction of the neutrophils is about 28% (p<0.01), but the largest reduction is classified by the cell counter as "lymphocyte" population (–40%, p<0.001). The monocytes (–22%, p<0.05) and basophils (–10%, p=0.08 ns) are only slightly reduced.
Conclusions: Storage of stem cell transplants over 72 hours at +4±2 °C does not influence the amount of stem cells but leads to a significant reduction of total nucleated cells. The distinct decrease of the lymphocytes was not expected and is subject to further evaluation.

P 5.10

Bone Marrow, Collected from Unrelated Donors – a Source of Mesenchymal Cells for Therapeutic Approch

Kreissig C.[1], Rehfeld I.[2], Bux J.[3]

[1]DRK BSD-West, Zentralbereich Stammzelle, Ratingen, D, [2]DRK BSD-West, Ratingen, [3]DRK BSD-West, Hagen, D

Introduction: Mesenchymal stromal cells are an exciting tool for research and therapeutic application regarding different diseases. They can be isolated from different sources such as bone marrow, umbilical cord blood or adipose tissue. Under specific culture conditions they can differentiate into mesodermal and even non-mesodermal cells. For development of treatment strate-

gies the question, which cell source can give sufficient amount of cells in a save and simple way, has to be answered.
Methods: We examined CD34/CD45 and CD90/105 expression in 20 bone marrow samples collected from unrelated donors for the purpose of hematopoietic transplantation. We used three color flow cytometry on FACSCalibur device. The gating strategy was as follows: In forward and side scatter mononuclear cells were selected, then gated for CD34 expression. CD34- and CD45-negative cells were examined for CD90 and CD105 expression.

	Volume (ml)	Leucocyte count/µl	CD34 cells/µl	HPC in product	CD90/105 cells/µl	MSC in product
median	997	17.890	184	184	133	162
range	569–1569	12.320–24.550	122–316	89–301×10E6	69–412	61–442×10E6

Discussion: Bone marrow from allogeneic related and unrelated donors is collected for hematopoietic transplantation for more then 30 years. It is a safe and simple method for preparation of a cell therapy product.
We could show, that bone marrow collection in healthy volunteers give high numbers of mesenchymal stromal cells. The cell counts are comparable to amounts of hematopoietic cells collected. Unlike to cord blood the volume, which has to be collected, can be calculated depending on cell count necessary for successful therapy.
We consider bone marrow to be a good source for mesenchymal stem cells for different cell therapy strategies.

P 5.11

Collaboration of a GMP-Facility with a Central Apheresis Unit for the Production of Stem Cell-Transplants

Kleine H.-D.[1], Edelberg A.[1], Freund M.[2], Gloger D.[3]

[1]Seracell Stammzelltechnologie GmbH, Rostock, D, [2]Dept. of Hematology and Oncology, University of Rostock,D, [3]HAEMA AG, BSZ Rostock, D

Backround: In 2007 Seracell Stammzelltechnologie GmbH rolled out with a newly developed GMP facility for the production of stemcell- and tissue transplants in Rostock with four clean rooms of class B EU-GMP equipped with laminar-air-flow hoods providing a class A environment for the manipulation of cellular products. To provide the complete service for hospitals including stem cell collection and preparation, a collaboration with the Haema AG, Rostock was settled up in 2007. After stem cell mobilization treatment carried out by the hospital itself apheresis has been performed by the Haema AG based on §14 part 4 German Drug Law (AMG) for the Seracell Stammzelltechnologie GmbH.
Methods: 94 apheresises in 68 patients or related donors have been carried out since November 2007. 78 apheresis products were collected for autologous and 16 products were collected for allogenic treatment. All procedures were performed using the Cobe spectra™ (Protocol MNC).The procedure time was in mean 190 minutes,the AC-ratio 1.12 to 1:13 ,only ACD-A was used for anticoagulation.
Results: The autologous apheresis products collected with a volume of 197 ml in mean leading to 7.17×10E6 viable CD34+ stem cells/kg BW in mean after cryoconservation. In 84.5% of all patients one apheresis was suitable to reach at least one transplantation dose. In 15.5% of all patients a second apheresis was necessary to reach at least a single transplantation dose.
Conclusion: A central apheresis facility combined with a GMP laboratory working for different hospitals has the advantage of a more cost effective stem cell production with less trained personal necessary.

P6 Hemostasis

P 6.01

Prothrombotic Effects of CD40L on Platelet Thrombus Formation under Arterial Flow Conditions

Reiff E.[1], Stoldt V.R.[2], Scharf R.E.[2]

[1]Heinrich Heine University Medical Center, Department of Experimental and Clinical Hemostasis, Hemotherapy, and Transfusion Medicine, Düsseldorf, D, [2]Heinrich Heine University Medical Center, Düsseldorf, D

Background and Purpose: CD40 ligand (CD40L), a transmembrane protein of TNF family, stabilizes arterial thrombi by an integrin αIIbβ3-dependent mechanism. Moreover, it has been shown that activated platelets express CD40L on their surface. We now explored the effects of soluble (s) CD40L on thrombus formation under arterial flow conditions in vitro.
Design and Methods: Citrated blood obtained from healthy volunteers was perfused in a rectangular flow chamber (flow path 80 µm height) coated with collagen type I at a shear rate of 1,000 sec-1. Prior to perfusion, blood samples were incubated with mepacrin (10 µM) and sCD40L at concentrations of 1, 5, 10, 20, and 100 ng/ml. Thrombus formation was detected 3- dimensionally by confocal laser scanning microscopy over time and quantified by ECCET imaging software (www.eccet.de).
Results: Under control conditions, the mean thrombus volume (±SD) was 8,412±1,472 µm^3 after 5min and 34,922±4,776 µm^3 after 10min. Addition of 1 or 5 ng/ml sCD40L caused a significant increase in the thrombus volume, i.e. 20,641±6,604 µm^3 (5min) and 64,131±13,840 µm^3 (10min) at 1 ng/ml or 31,091±15,740 µm^3 (5min) and 69,707±35,373 µm^3 (10min) at 5 ng/ml, respectively. At sCD40L concentrations of 10 to 20 ng/ml, thrombus volumes were only marginally increased, i.e. 12,920±1,808 µm^3 (5min) and 43,785±5,401 µm^3 (10min), and 100 ng/ml of sCD40L did not show any significant effect on thrombus formation compared to the control.
Conclusions: Under arterial flow conditions, platelet thrombus formation shows a sCD40L concentration-dependent growth. Clinically relevant concentrations of sCD40L (1 to 5 ng/ml) increase platelet thrombus volumes, while higher concentrations of sCD40L do not affect thrombus growth. These in vitro data confirm the prothrombotic effects of CD40L and indicate that CD40L may modulate arterial thrombus formation in vivo.

P 6.02

Ectopic Expression of the Brain Type Creatine Kinase in Human Platelets

Bugert P.[1], Arnold H.[2], Hoffmann M.M.[3], Hinz K.[4], Scheuerbrandt G.[5], Janetzko K.[4], Klüter H.[4]

[1]Institute of Transfusin Medicine and Immunology, Medical Faculty of Mannheim, University of Heidelberg, Mannheim, D, [2]University of Freiburg, D, [3]Division of Clinical Chemistry, Department of Medicine, University Medical Center, Freiburg, [4]Institute of Transfusion Medicine and Immunology, Mannheim, D, [5]CK-Test-Laboratorium, Breitnau, D

Objective: Creatine kinase is an enzyme for ATP regeneration usually located in nervous (brain type, CK-B) and muscle (muscle type, CK-M) tissues. The active enzyme is a homo- (CK-BB or CK-MM) or a hetero- (CK-MB) dimer and CK-MB activity can be detected in serum of healthy individuals at low levels. During a newborn screening program for Duchenne muscular dystrophy in the late 70's one family with unusual high levels of CK-BB activity in erythrocytes and some families with unusual high levels of this enzyme within platelets were identified. This ectopic expression of CK-B (CKBE; OMIM: 123270) is an autosomal dominant inherited anomaly with an estimated frequency of about 1 in 5,000 persons. The causal CKBE mutation has been mapped to chromosome 14q32 where the CKB gene is located, however, the molecular basis remains unknown. In this study we investigated the expression of CK-B leukocytes and platelets of CKBE probands and normal controls.
Methods: Citrated whole blood was used to isolate platelets and leukocytes of 22 CKBE probands (from 10 unrelated families) and 11 unrelated controls. Total CK activity was measured in platelets, leukocytes and serum using standard tests (Roche Diagnostics). A monoclonal antibody specifically inhibiting CK-M was used to determine the proportion of CK-MB activity of the total CK activity. The levels of CKB and CKM gene expression in platelets and leukocytes were investigated using quantitative real-time PCR. DNA sequencing of the coding region of the CKB gene was performed at the genomic level.
Results: All CKBE probands and controls showed normal blood cell values. Serum CK levels were comparable between probands and controls (72.3±23.7 versus 61.8±51.4 U/L: p=0.459). CKBE probands revealed significantly higher CK activity compared to controls in platelets (188.4±50.7 versus 13.4±5.9 U/10ex12 PLT; p<0.0001) and leukocytes (4.1±1.9 versus 0.6±0.3 U/10ex9 leukocytes; p<0.0001). The CK acitivity is generated exclusively by the CK-BB isoenzyme because the anti-CK-M antibody did not affect CK activity in platelets and leukocytes. First results of gene expression analysis indicated that platelets harbour CKB but not CKM gene transcripts. Whether the mRNA level differes between CKBE probands and controls is under current investigation. The CKB coding DNA sequence analyzed in 8 CKBE probands did not reveal a mutation so far.
Conclusions: We found unusual CK activity in platelets and leukocytes of CKBE probands. The assumed gene mutation alters the pattern of tissue specificity leading to CKB gene expression in bone marrow cell lineages. Probably, a mutation or methylation of the CKB promoter is the molecular

basis of CKBE. The probands do not show signs of platelet dysfunction, thus, it is unlikely that the high CK activity significantly affects platelet function. However, platelets may benefit from an enhanced ATP regeneration and a higher granular adenine nucleotide pool.

P 6.03

Discriminative Inhibition of Platelet Aggregation Response to Different Agonists by Nitric Oxide (NO)

Zimmermann R.[1], Simeunovic V.[2], Filipovic M.[3], Ivanovic-Burmazovic I.[3], Eckstein R.[2]

[1]University Hospital Erlangen, Transfusion Medicine And Hemostaseology, Erlangen, D, [2]University Hospital Erlangen, D, [3]Department of Chemistry and Pharmacy, Erlangen, D

Purpose: Nitric oxide (NO) is a diffusible, short-lived, diatomic free radical ubiquitously produced by mammalian cells. The NO signaling pathway involves mainly the activation of soluble guanylyl cyclase to produce cyclic GMP (cGMP) as a second messenger and downstream mediator. Nitric oxide (NO) is known to inhibit platelet function by this induction of cGMP production. However, there exists only very few data on the discriminative effect of nitric oxide on the in-vitro aggregation response of human platelets to different agonists as measurable by the classical Born aggregometry.
Methods: We studied the aggregation response to arachidonic acid, collagen, ristocetin, and thrombin receptor-activating peptide (TRAP) using Born Aggregometry and a PAP-4 aggregometer. The effect of nitic oxide (NO) on the different aggregation responses was measured using diethylamine NONOate sodium salt hydrate (DEA-NONOate) as a NO donor.
Results: DEA-NONate inhibits completely the aggregation response to arachidonic acid. The response to collagen is decelerated and decreased in the presence of NO. Using TRAP as agonist, platelet aggregates can be induced even in the presence of NO but these aggregates disaggregate. Only the aggregation response to ristocetin is not inhibited by NO aside from very high NO cencentrations.
Conclusions: This study is the first presenting data on the discriminative influence of NO on the aggregation response to different platelet agonists. Nitric oxide does not inhibit all aggregation responses at the same NO concentration. The aggregation response to ristocetin is much less sensitive to the inhibition by NO than the aggregation responses to arachidonic acid, collagen, and TRAP.

P 6.04

Comparison between Flow Cytometry and ELISA Methods to Detect Microparticles in Blood of Healthy Donors and in Plateletpheresis Products

Strasser E.[1], Happ S.[2], Zierk J.[2], Weiss D.[2], Eckstein R.[2]

[1]Universitätsklinikum Erlangen, Transfusionsmedizinische und Hämostaseologische Abteilung, Erlangen, D, [2]Universitätsklinikum Erlangen, D

Purpose: Evaluation of two new methods to detect biological microparticles (MP) in 30 healthy persons (blood donors) and plateletpheresis products (paired comparison). The microparticle content of blood of healthy donors was compared with plateletpheresis products. Because of the variable size of microparticles, standardized detection by flow cytometry is difficult. Therefore, a new ELISA test was evaluated and compared with the results of flow cytometry (FACS).
Methods: The FACS analysis of MP was performed on a flow cytometer (FACS Canto II, BD, USA) by use of microbeads (0.5–1.0 µm) to optimize the cytometer settings (Megamix, Biocytex, Marseille, France). The ELISA assay (Hyphen BioMEd, Neuville/Oise, France) measures the procoagulant activity of microparticles by use of Factor Xa, thrombin inhibitors and biotinylated Annexin V. Citrated blood of 30 healthy blood donors was collected before and after plateletpheresis. In addition, a sample of each platelet product was analysed. All samples were prepared by the same procedure: 15 min. centrifugation at 2000 g followed by a second centrifugation step at 13,000 g, all steps at RT (25 °C). Thereafter, all plasma samples were stored at –40 °C for twelve weeks until analysis.
Results: By use of FACS analysis, the results were as follows: pre-donation, 4463 MP per µl, post-donation, 4918 MP per µl, $p>0.05$; plateletpheresis products, 25325 MP per µl, $p<0.001$. Analysis with the ELISA showed the following results: pre-donation, 3,63 nmol per ml, post-donation, 5,53 nmol per ml, $p=0.002$; plateletpheresis products, 27,2 nmol per ml, $p<0.001$. We found a significant correlation (Pearson) between the results of the ELISA and the flow cytometry: pre-donation, $r=0.38$ ($p=0.048$); post-donation, $r=0.51$ ($p=0.005$) and plateletpheresis product, $r=0.46$ ($p=0.017$).
Conclusions: By use of the ELISA we found a significantly higher activity of MP in citrated plasma after plateletpheresis compared to pre-donation blood samples. In plateletpheresis products, the activity of MP (ELISA), as well as the content of MP (FACS) was significantly higher compared to per- and postdonation blood samples. The ELISA and the FACS analysis showed a weak but significant correlation of the results.

P 6.05

APC Resistance and Patent Foramen Ovale in Stroke Patients

Schleef M.[1], Feurer R.[2], Sadikovic S.[2], Bockelbrink A.[3], Poppert H.[2]

[1]Klinikum rechts der Isar der TU München, Institut für Klinische Chemie, München, D, [2]Klinikum rechts der Isar der TU München, D, [3]Institut für Sozialmedizin, Epidemiologie und Gesundheitsökonomie, Hamburg, D

Purpose: As patent foramen ovale (PFO) is a frequent finding in cryptogenic cerebral ischemia, paradoxical embolism has been discussed to be a possible pathogenic mechanism. APC resistance as a well known risk factor for venous thromboembolism may cause paradoxical emboli and contribute to the risk of ischemic stroke in patients with PFO. We aimed to investigate the role of APC resistance and PFO in stroke patients.
Methods: The records of 567 patients with definite diagnosis of cerebral ischemia at discharge were analyzed retrospectively. All patients had undergone PFO detection based on transcranial Doppler ultrasonography. All patients were screened for APC resistance; Factor V Leiden was demonstrated by polymerase chain reaction. Stroke origin had been subtyped using the TOAST classification criteria. All patients were contacted for follow up by e-mail or telephone.
Results: Patients with Factor V Leiden were as frequent in the PFO positive group as in the PFO negative group (6,8 vs. 9,0%, p=0,4). According to the TOAST criteria 215 of 567 cases were classified as cryptogenic stroke. As expected, positive PFO detection was more frequent among patients with cryptogenic stroke than among those with stroke classified other than cryptogenic (43 vs. 28%, p<0,001). The prevalence of Factor V Leiden did not differ significantly between these two groups (6,5 vs. 9,4%, p=0,3).
Among patients with cryptogenic stroke, Factor V Leiden was not significantly more prevalent in patients with PFO than in patients without (7,6 vs. 5,7%, p=0,4).
Complete follow up was obtained in 490 patients (86,4%). Among the responding patients with PFO (n=165), recurrence of cerebral ischemia was observed in 1 patient (11,1%) positive for Factor V Leiden and in 7 patients (4,5%) with no evidence of APC resistance (p=0,4).
Conclusion: Our data suggest that APC resistance is not a strong risk factor for ischemic stroke among patients with patent foramen ovale.

P 6.06

Coagulation Activation, Fluid Retention, and Transient Autoimmune Hepatitis Associated with the Use of Black Cohosh: A Case Study

Zimmermann R.[1], Frieser M.[2], Weiss D.[2], Strobel J.[2], Eckstein R.[2]

[1]University Hospital Erlangen, Transfusion Medicine And Hemostaseology, Erlangen, D, [2]University Hospital Erlangen, D

Purpose: Black cohosh (Actaea racemosa L.; Cimicifuga racemosa [L.] Nutt.) is one of the most popular herbal therapies for premenstrual discomfort, hot flushes, and other climacteric and menopausal symptoms. Most often, it is tolerated well. However, there are some recent reports on serious adverse events probably associated with this complementary and alternative herbal medicine.
Methods: We report a case of coagulation activation, fluid retention, weight gain, and transient autoimmune hepatitis most likely triggered by the use of black cohosh over four weeks.
Results: Laboratory evaluation revealed the following pathological results: D-dimers of 1304 mg per mL (ref <500), an alanine transaminase (ALT, SGPT) of 104 U/L (ref <34), an aspartate transaminase (AST, SGOT) of 66 U/L (ref <31); and a positive antinuclear antibody (ANA) screening. The ANA titer was 320 (ref <100). Further tests revealed a weak reaction in a test for antibodies against SSA/Ro-52. An abdominal sonographic evaluation showed a liver of normal size and structure . A duplex sonography of the veins of both legs revealed no signs of thrombosis. However, there was a

remarkable fluid accumulation in the subcutaneous tissue. An echocardiographic examination showed normal atrial and ventricle chambers. Virologic evaluations excluded an infection with hepatitis viruses A, B, C, and G, cytomegalovirus, adenovirus, picorna virus, and parvovirus B19. All pathologic values returned to normal within two weeks, when the black cohosh ingestion was stopped.

Conclusions: Diagnostic procedures aimed to explain lower leg edema are not uncommon in the age group of women suffering from climacteric and menopausal symptoms. Therefore, herbal-induced fluid retention and coagulation activation should be considered in differential diagnosis of coagulation activation that is indicated by elevated D-dimers, especially if thrombosis has been excluded. This is of special importance because herbal therapies for climacteric and menopausal symptoms are extremely popular.

P 6.07

Acquired von Willebrand Syndrome and Acquired Platelet Dysfunction Caused by Amyloidosis

Buender R.[1], Hoffmann T.[2], Schieren G.[3], Braunstein S.[4], Gabbert H.E.[4], Rump C.L.[3], Scharf R.E.[2]

[1]University of Duesseldorf, Institute of Hemostaseology and Transfusion Medicine, Duesseldorf, D, [2]Institute of Hemostaseology and Transfusion Medicine, Duesseldorf, D, [3]Clinic of Nephrology, Duesseldorf, D, [4]Institute of Pathology, Duesseldorf, D

A 54-years old patient suffering from amyloidosis of the kidney and intestine was due to monoclonal gammopathy in 2001 followed by steam cell transplantation in 2004. In 2008 the patient was clinical stable on chronic dialysis, but in the beginning of 2009, bleeding symptoms occurred.

The hemostaseologic workup showed an acquired von Willebrand Syndrom (vWS) with an decreased ratio (ristocetin cofactor activity/ vWF-antigen) less than 0.3, an impaired platelet dysfunction demonstrated by thrombocyte aggregometry and a prolonged in-vitro-bleeding time with the platelet function analyzer.

Despite substitution of the von Willebrand factor (vWF) the bleeding complications, as spontaneous hematomas and prolonged bleeding after shunt punction, remained. Because of a retroperitoneal haemorrhage of one kidney, nephrectomy was performed. The histology of the kidney showed amyloid deposits in the blood vessels consistent with a severe vasculopathy. By subsequent substitution of vWF-concentrate, the ratio of the vWF-activity to the vWF-antigen was postoperative normalized, but returned shortly after nephrectomy to the initial baseline levels of the acquired vWS. In contrast, the platelet function remained normal up to 6 weeks after the nephrectomie as shown by thrombocyte aggregometry. No bleeding complications appeared during this time. Afterwards the platelet function deteriorated, followed by recurring bleeding complications.

Summerizing our observations, severe amyloidosis can cause an acquired vWS as well as an acquired platelet function defect.

Amyloidosis-induced bleeding in our patient seems to be based on the acquired platelet function defect rather than the acquired vWS.

P7 Preparation of Tissues

P 7.01

Increased Foam Cell Potential of Human Blood and Marrow-Derived Cells after Pro-Angiogenic Induction as an Apparent Risk Factor of Therapeutic Angiogenesis

Rohde E.[1], Schallmoser K.[2], Reinisch A.[2], Hofmann N.A.[2], Pfeifer T.[2], Froehlich E.[2], Rechberger G.[3], Lanzer G.[2], Kratky D.[2], Linkesch W.[2], Strunk D.[2]

[1]Medical University of Graz, Stem cell Research Unit Graz, University Clinic of Blood Group Serology and Transfusion Medicine, Graz, A, [2]Medical University of Graz, A, [3]Karl-Franzens University Graz, A

Current clinical trials for therapeutic angiogenesis use blood- or marrow-derived transplants containing monocytes, mesenchymal stromal cells (MSCs) and endothelial progenitor cells (EPCs) to support vascular regeneration. Safety concerns regarding atherosclerosis aggravation have emerged since all three cell types can also contribute to atheroma formation. We therefore examined foam cell formation of these cells after lipid exposure in vitro as a surrogate marker for potential pro-atherogenic side effects.

Foam cell development was tested with human monocytes, MSCs and EPCs either immediately exposed to acetylated lipoproteins (acLDL) or subjected to a three day pro-angiogenic induction prior to lipid loading. Intracellular lipid accumulation was detected by Nile Red staining with fluorescence laser-scanning microscopy. After 3D mapping, the number of lipid droplets (LDs) per cell was determined. Morphometric observations were confirmed by flow cytometry and cholesterol measurement using gas chromatography–mass spectrometry.

Foam cells developed from monocytes with and without pro-angiogenic induction. However, 12h of lipid exposure after a 3-day pro-angiogenic induction resulted in increased numbers of LDs/cell, as compared to 12 hour lipid-exposed fresh monocytes (42; [29/54] versus 2; [1/9], n=6, data presented as median; [q1 / q3]). A prolonged lipid exposure for 84h after a 3-day pro-angiogenic induction enhanced lipid accumulation in monocytes as compared to 84h exposure without pro-angiogenic conditioning (102; [69/130] versus 19; [13/27] LDs/cell). MSCs and EPCs did not accumulate lipids during culture mimicking conditions applied in clinical trial protocols. The quantification of total cholesterol revealed an increase within lipid-stressed monocytes, whereas highest total cholesterol concentrations were found after 84h with acLDL following pro-angiogenic pre-culture.

The foam cell potential of monocytes in vitro was strikingly evident, especially after prior pro-angiogenic culture, while MSCs or EPCs were devoid of lipid accumulation. These data raise serious concerns that cellular therapy with monocyte-containing hematopoietic cell preparations may be counterproductive or even aggravate atheroma formation in patients with cardiovascular disease if underlying pathologic conditions are not appropriately reverted. We therefore support previous arguments that the role of transplanted cells in the various aspects of vascular homeostasis, regeneration and therapeutic angiogenesis must be re-examined prior to further clinical trials.

P8 Blood, Tissues, Stem Cells – Regulatory Aspects

P 8.01

Tiam-1 Mediates Rac Activation in the Murine Hematopoietic Progenitor Cell Line, FDCPmix

Jochheim-Richter[1], Koczan D.[2], Nilmaer O.[3], Duthey B.[3], Goettig S.[3], Seifried E.[3], Henschler R.[3]

[1]German Red Cross Blood Donor Service, Frankfurt am Main, Institute for Transfusion Medicine and Immune Hematology, Frankfurt, D, [2]*Institute of Immunology, Department of Medicine, University of Rostock, D, [3]Institute for Transfusion Medicine and Immune Hematology, German Red Cross Blood Donor Service, Frankfurt, D

Background: Tiam-1 (T cell lymphoma invasion and metastasis 1) is a guanine nucleotide exchange factor (GEF) that is known to activate the Rac GTPases. Cells that ectopically overexpress Tiam-1 showed increased metastatic potential. To analyze to which extent Tiam-1 regulates additional functions associated with metastasis, we silenced the Tiam-1 gene in a murine hematopoietic progenitor cell line (FDCPmix) with short interfering RNAs, and asked for regulation in components in signalling pathways.

Methods: Tiam-1 gene expression was silenced by 2 Tiam-1-specific siRNAs in FDCPmix hematopoietic progenitor cells. Changes in Rac1 activation induced by Tiam-1 siRNA were determined in a PAK binding pulldown assay (Benard & Bokoch, 2002). Gene regulation was studied using array technology. Cells were additionally transfected with scrambled siRNA that served as negative control. RNA was isolated and 5µg each were used for array hybridizing (Mouse genome 230.2 Arrays, Affymetrix). Hybridization and scanning procedure was carried out as described recently (Jochheim-Richter A, 2006). Inhibition of Tiam-1 was observed in all samples analyzed (6-fold/4-fold down-regulation). Anova (Analysis of variance) was carried out followed by filtering the probe sets by criteria derived from the MAS5.0 (MicroArray Suite 5.0, Affymetrix) to yield more stringency. The remaining 77 probe sets were grouped by the annotation in Gene Ontology and visualized by TreeView 1.60.

Results and Conclusions: To evaluate the effects of Tiam-1 gene silencing, a pulldown assay was carried out and a reduced Rac activation was observed compared to scrambled siRNA treated cells, indicating a regulatory function of Tiam-1 in the Rac dependent pathway. Furthermore, array analysis showed regulation of several genes including Never in mitosis, G protein-coupled receptor 34 (Gpr 34) and Receptor activity modifying protein 1 and 2 (RAMP1/2). Moreover, the gene for Nek3 which is known to contribute to Prolactin-mediated breast cancer cell motility through mechanisms involving Rac1 activation (Miller et al., Oncogene, 2007) was highly up-regulated. A highly down-regulated gene is RAMP2. Disruption of RAMP2 leads to embryos that die at midgestation due to vascular fragility (Ichikawa-Shindo et al., J Clin. Invest, 2008). The analyzed changes of gene regulation in

hematopoietic progenitors point to the participation of Tiam-1 in more than one Rac dependent signalling network, and point out potential new ways to interfere with hematopoietic progenitor cell migration and mobilization.

P 8.02

Mycoplasma Detection by Realtime PCR: A Method for Quality Control of Cell Cultures

Janetzko K.[1], Rink G.[2], Bieback K.[2], Klüter H.[2], Bugert P.[2]

[1]DRK-Blutspendedienst Baden-Württemberg - Hessen gGmbH, Institute of Transfusion Medicine and Immunology, University of Heidelberg, Medical Faculty of Mannheim, D, [2]DRK-Blutspendedienst Baden-Württemberg - Hessen gGmbH, Mannheim, D

Purpurse: The European Pharmacopoeia (EP) 5.8, Section 2.6.7, testing for Mycoplasma is recommended for cell banks and control cell cultures. Testing can be performed using culture methods as well as nucleic acid amplification techniques (NAT). The list of bacterial strains to be detected includes M. gallisepticum, M. fermentans, M. hyorhinis, M. orale, M. pneumoniae, M. synoviae and Acholeplasma lailawii. Here, we describe a NAT based on the Mycoplasma-specific realtime amplification of part of the 16s rDNA gene. The test validation included the determination of detection limits for 12 different strains, spiking experiments and evaluation of specificity with regard to other bacteria.
Methods: For optimization of primer/probe design we used commercially available DNA-samples (Minerva Biolabs) of 12 different Mycoplasma strains. Detection limits were determined using dilution series of the DNA samples and were calculated by PROBIT analysis using SPSS statistical software. Lyophilized M. orale bacteria were used to spike cell culture supernatants and to compare the sensitivity with the detection limit based on DNA dilution series. The specificity of the assay was proven with the use of DNA samples of common bacteria such as Clostridium acetobutylicum, Lactobacillus acidophilus and Streptococcus pneumoniae.
Results: We designed a single tube assay with a mixture of 7 forward primers, a single reverse primer and a single probe for the detection of 12 different Mycoplasma strains (including all strains demanded by EP 5.8). We also included a synthetic DNA molecule detected by a different probe as internal control. Detection limits differed between the strains and revealed a sensitivity of less than 2,000 genomes/ml (95% probability) for most of the strains. Spiking experiments of cell culture supernatants confirmed this sensitivity. Specificity of the assay could be demonstrated by negative results for bacterial strains not belonging to the Mycolasma family.
Conclusions: Our realtime PCR method is suitable to detect all Mycoplasma strains demanded by the EP 5.8. Testing can directly be performed on 5 µl of cell culture supernatant without need of DNA isolation.

P 8.03

GCSF Mobilization in Healthy Stem Cell Donors Increases Both Plasminogen Activator Inhibitor-1 Activity and Tissue Factor Expression on Monocytes

Strate A.[1], Pechmann C.[2], Salge-Bartels U.[3], Heiden M.[3], Seitz R.[3], Legler T.[2]

[1]Universitätsmedizin Göttingen, Abteilung Transfusionsmedizin, Göttingen, D,[2]Universitätsmedizin Göttingen, D, [3]Paul-Ehrlich-Institut, Langen, D

Background: Tissue factor (TF) is the physiologic initiator protein of the coagulation cascade enhancing the catalytic activity of factor VII. It can be found both at extravascular sites and intravascular in the circulating blood. Circulating TF has been demonstrated to play a crucial role in the systemic inflammatory response syndrome potentially leading to life-threatening disseminated intravascular coagulation with considerably elevated plasma and monocyte TF levels. In light of the thrombogenic potential attributed to TF in inflammatory diseases, increased TF levels might be a significant risk of coagulation disorders in pathological conditions only mimicking a severe systemic inflammation, too, such as stem cell mobilization with granulocyte-stimulating factor (GCSF). We therefore investigated plasma TF antigen and TF antigen expressed by monocytes before and after administration of filgrastim with voluntary healthy donors of allogeneic peripheral blood stem cells (PBSC).
Methods: TF antigen expressed by monocytes was analyzed by flow cytometry using PE-conjugated anti-CD14 and FITC-conjugated anti-TF antibodies. TF antigen in plasma was determined by ELISA technology. Plasma activity of TF pathway inhibitor (TFPI) and plasminogen activator inhibitor-1 (PAI-1) were analyzed by functional chromogenic assays. All parameters including white blood cell count (WBC) were performed before and after daily administration of 10 µg/kg body weight filgrastim for 3 days before PBSC apheresis.
Results: A total of 47 healthy PBSC donors were analyzed (flow cytometry only with 44 donors). After filgrastim, both median WBC and median monocyte count significantly increased from 7,300 to 41,050/µL and from 315 to 1965/µL, respectively. Median fluorescence intensity of TF on each monocyte slightly decreased (27 to 24 U), but total TF expression on monocytes (TF fluorescence intensity x monocyte count) increased by the 4.8 fold due to the increased monocyte count after filgrastim ($p<0,001$). Both plasma TF antigen and TFPI activity slightly declined from 136 to 124 pg/mL and from 1.40 to 1.28 U/mL, respectively, whereas median PAI-1 activity increased from 0.24 to 0.93 ng/mL ($p<0,001$).
Conclusion: According to the parameters investigated in this study, increased PAI-1 activity and total TF antigen expression on moncytes can be observed in healthy PBSC donors after administration of GCSF. Since none of the PBSC donors suffered from thrombembolism during or after stem cell mobilization, it should be further investigated whether these in vitro changes result in an increased risk of thrombembolism in vivo.

P 8.04

Differential Numbers of Endothelial Progenitor Cells in Peripheral Blood of Patients with Various Degrees of Heart Failure

Fabris M.[1], Thude H.[2], Fiedler N.[3], Barz D.[4]

[1]Institut für Transfusionsmedizin Uniklinikum Jena, Bad Langensalza, D, [2]Hamburg, D, [3]Weimar, D, [4]Jena, D

Purpose: Peripheral circulating endothelial progenitor cells (EPCs) are bone marrow derived and characterised by the expression of CD34, CD133 and vascular endothelial growth factor receptor 2 (VEGFR-2). EPCs are capable to contribute to neovascularization, to improve the myocardial function after injury and are involved in the pathophysiology of heart failure. We determinded the number of EPCs and correlated them with New York Heart Association (NYHA) functional class.
Methods: Surface expression of CD34, CD133 and VEGFR-2 was quantified by flow cytometry in venous blood of 111 male patients with various degrees of heart failure (NYHA I-IV) and 41 male healthy adults without any cardiovascular risk.
Results: The number of EPCs inversely correlated with NYHA functional class. EPCs decreased in NYHA class III and IV compared with NYHA class I and II. In the same way levels of EPCs are lower in patients with heart failure compared with healthy adults.
Conclusion: The severity of heart failure influences the number of peripheral circulating EPCs. This fact may be used to differentiate the several functional classes of heart failure more exactly. In the same way the determination of EPC levels could show the development and possibly the progression of heart failure, which could relate with clinical consequences.

P 8.05

Transfusion Medicine in Sweden and in Germany – Comparison of Two European Countries

Lubenow N.[1], Norda R.[2], Åkerblom O.[2], Säfwenberg J.[2], Knutson F.[2]

[1]Uppsala University Hospital, Clinical Immunology and Transfusion Medicine, Uppsala, S, [2]Uppsala University Hospital, Uppsala, S

Purpose: Sweden and Germany are countries with a long history of active participation in the development of transfusion medicine. Preparation and use of blood components is in both countries affected by European Community (EC) legislation and Council of Europe (CoE) guidelines and also regulated on a national level. Differences between the countries can exemplify difficulties in European harmonisation as well as indicate areas of interest for cooperation and research.
Methods: Brief descriptions of the transfusion medicine services are provided, and the regulatory standards for production and use of blood components are reviewed. Differences are pointed out and interpreted in view of potential interest for cooperation.
Results: In Sweden (9.2m inhabitants) 474,617 units of whole blood were collected 2007 (51.7/1,000 inhabitants); in Germany (82.1m inhabitants) 4,708,259 (58.0/1,000) units. Whereas in Sweden 24 major blood establishments (including 7 university based) function as hospital based, county-council led units, in Germany the Red Cross, as well as 55 state-communal

(e.g. university based) and 20 private blood establishments secure the blood supply. In Sweden the national authority responsible for the authorisation of blood component production is the national board of health and welfare. Notably, unlike in Germany, the pharmaceutical law does not apply to blood components for use in patients. Differences in the production process compared to Germany include: an individually unique national registration number for all citizens, delegation of routine donor approval and release of blood components to nursing and technical staff, no statutory demand for screening by NAT-technique, only two different bloodbank softwaresystems. Safe use of red cell concentrates is achieved without routine crossmatching or "bedside-test" bloodgroup control by use of blood group check, antibody screening and electronic matching. Education of medical doctors comprises a 5 year curriculum to become a transfusion medicine and clinical immunology specialist. The corresponding qualification (also 5 years) in Germany is transfusion medicine.
Conclusions: Both in Sweden and Germany EC directives and CoE guidelines apply. Nevertheless, varying national legal frameworks and differing strategies in practice lead to remarkably different organisations and procedures. Possible differences in cost (personnel, laboratory testing) as well as safety (rate of DHTR, transfusion to wrong patient) should be studied in detail. As blood component therapy is perceived to be safe and effective in both countries, a dialogue could lead to mutual improvements.

P 8.06

Retrospective Analysis of Confidential Self-Exclusion from HIV-, HCV- and HBV-Positive Donors for the Period of 2004–2008

Michel P.[1], Lebhard E.[2], Ruf E.[2], Schuller A.[2], Weinauer F.[2]

[1]Blutspendedienst des BRK, Institut München, D, [2]Blutspendedienst des BRK, München, D

Purpose: We retrospectively analysed the benefit of confidential self exclusion from donors, who were found positive for either HIV, HBV or HCV for the period of 2004 to 2008.
Methods: During 2004–2008 the BSD/BRK collected about 2.8 million blood donations. 373 donors were found to be positive for one of the three parameters HIV, HBV or HCV in accordance with the RKI criteria on infectious diseases and had to be reported unter the terms of § 22 TFG.
We analysed retrospectively if the donors had chosen the confidential self-exclusion
- at time of the positive donation
- at time of the last donation before there were found positive
- at any previous donation

Results: From the total of 373 positive donors (313 first time donors, 60 multiple time donors) only five donors (1.3%) had chosen confidential self-exclusion at time of the positive donation. Four of these donors were positive for HBV, one for HCV.
None of the 60 positive donors with previous donations had chosen confidential self-exclusion at time of the last donation before there were found positive.
Only one regular donor had chosen confidential self-exclusion at one of his previous donations. This donor, who had given blood 56 times, was found positive for HIV in August 2007. The only positive self-exclusion hin his history was for a blood donation in September 1992. The donor gave another 40 blood donations afterwards without using the confidential self-exclusion.
Conclusions: These data show no evidence that donors at high risk of being infectes exclude their donations by using the confidential self-exclusion. On the other hand a lot of donations are destroyed every year because of incorrect use of self-exclusion. Therefore, we suggest a reevaluation ot this system.

P9 Pathogen Inactivation of Blood Components

P 9.01

Theraflex UV-Platelets: No Evidence for Intolerance and Antibody Formation in Dogs During a 10-Week Safety Pharmacology Study

Pohler P.[1], Lehmann J.[2], Veneruso V.[2], Lambrecht B.[3], Mohr H.[3], Müller T.H.[3], Axel S.[3]

[1]DRK-Blutspendedienst NSTOB, Forschung und Entwicklung, Springe, D, [2]Fraunhofer Institut für Zelltherapie und Immunologie, Leipzig, D, [3]DRK-Blutspendedienst NSTOB, Springe, D

Backround: The Theraflex UV-System uses UVC light for pathogen inactivation in platelet concentrates (PCs). Previous studies have shown that UVC can potentially damage proteins. This may impact the drug tolerance in humans and influence the immunogenicity of the blood-derived therapeutics. The aim of this study was to assess the safety pharmacology of UVC-treated PCs in dogs following intravenous administration and to analyze whether UVC-irradiated PCs are capable of eliciting a humoral response against platelets and plasma proteins.
Study Design and Methods: In a GLP study, 12 male Beagle dogs were infused every second week over 10 weeks with approximately 100 mL autologous canine platelet concentrates (cPCs). One group of 6 animals received UVC-treated cPCs, while the control group received untreated cPC. During the application period, safety pharmacology parameters including the haematological, biochemical, respiratory and cardiovascular parameters were assessed in the dogs. In order to test for the presence of newly formed antibodies against platelet or plasma neoantigens, dog sera were taken prior to cPC transfusion and 14 days after the 5th administration and tested against UVC- and untreated platelet and plasma proteins by western blot. In addition, the presence of platelet-directed autoantibodies were analyzed in the dogs by flow cytometry.
Results: Safety pharmacology study for Theraflex UV-platelets did not show any signs of local or systemic intolerance against UVC-irradiated cPCs. Even after serial administrations of UVC-irradiated cPCs, antibodies against plasma or platelet proteins could not be detected by immunoblotting. In addition, flow cytometric results did not reveal antibodies against platelet surface antigens after UVC treatment.
Conclusion: Repeated transfusions of UVC-treated autologous cPCs were well tolerated in dogs. UVC irradiation did not cause significant plasma and platelet protein modification capable of inducing a humoral immune response in dogs.

P 9.02

Theraflex UV-Platelets: In Vitro Assessment of Platelet Quality and Storage Stability

Castrillo A.[1], Arcas C.[1], Martinez-Reboredo N.[1], Adelantado M.[1], Tolksdorf F.[2], Cabrera J.[1]

[1]Centro de transfusión de Galicia, Santiago de Compostela, E, [2]MacoPharma International GmbH, Langen, D

Introduction: The Theraflex UV pathogen reduction system for platelet concentrates (PCs) uses short-wave UV light (UVC, 254 nm) in combination with strong agitation. UVC is absorbed by DNA/RNA in pathogens and the induced damage prevents nucleic acid replication. We investigated the influence of Theraflex UV treatment on buffy coat platelets prepared by the OrbiSac® method. In vitro quality and storage stability of plasma-reduced PCs was evaluated throughout storage.
Design and Methods: In vitro quality of UVC treated (test, n=12) platelets was evaluated as compared to untreated (control, n=12) platelets in a pool and split trial. 24 plasma-reduced PCs were prepared from pools of 5 buffy coats each using the OrbiSac® system (Caridian BCT) and suspended in additive solution SSP+ (MacoPharma) in a ratio of 65:35 (SSP+:Plasma). Two of which underwent a pool and split step yielding two identical platelet units. One unit was UVC treated using the Macotronic UV illumination device, one remained untreated and both were stored at 22 °C until day 7 after donation. In vitro parameters tested on day 2, 5 and 7 were: pH, glucose, lactate, MPV, CD62, Annexin V, LDH and swirling.
Results: Theraflex UV treated PCs showed slightly lower pH on day 7 (7.27±0.08 vs. 7.36±0.04). At the same time the glucose level was decreased (3.35±0.64 vs. 4.37±0.38 mM/L) and the lactate level (12.23±2.52 vs. 9.83±1.41 mM/L) was increased. LDH levels on day 7 were 87.0±14.7 vs. 81.8±16.4 U/L. Platelet activation was assessed by CD62p expression, which remained almost unchanged after treatment (29.7±3.5 vs. 24.1±4.5%). Detec-

tion of apoptosis was performed using an Annexin V assay. Annexin V binding percentage in UVC treated PCs was marginally higher (5.47±1.86 vs. 4.40±1.64%). In addition, mean platelet volume (MPV) was slightly increased compared to control platelets (9.61±0.27 vs. 9.20±0.23 fL). Swirling scores were similar in both groups and did not change during storage.
Conclusion: In Theraflex UV treated PCs there was a good maintenance of in vitro platelet quality and storage stability throughout storage for 7 days. Study results indicate that OrbiSac® platelets are compatible with the Theraflex UV process.

P 9.03

Quantitation of Bacteria: A Comparison of Conventional Culture Methods with Rapid Flow Cytometry-Based Detection Methods

Lambrecht B.[1], Spengler H.-P.[2], Störmer M.[3], Montag T.[3], Müller T.[1], Seltsam A.[1]

[1]DRK-Blutspendedienst NSTOB, Springe, D,
[2]BD Biosciences, Erembodegem, B, [3]Paul-Ehrlich-Institut, Langen, D

Background: The transfusion of blood components containing high counts of bacteria can cause immediate septic shock, multiple organ failure and frequently death of the recipient. Rapid detection methods for quantitation of bacterial titers are useful for both bacteria screening in standard blood products and the development of pathogen inactivation methods. We compared two standard culture methods with three rapid flow cytometric procedures allowing determination of bacteria titers within 30 min.
Methods: Four different blood bacteria standards (BBS) proven to grow in platelet concentrates (PCs) even at low titers were used. Frozen bacteria stocks were prepared by the Paul-Ehrlich-Institute from growing cultures by a standardized proven protocol containing live bacteria after rethawing. Two standard culture methods (manual spread or by spiral plater) were compared to three flow cytometry-based assays (FACSCalibur, BD Biosciences) using thiazole orange (TO) to label the nucleic acids of living and dead bacteria. In one method bacteria were counterstained by propidium iodide to label (dead) bacteria with a permeable membrane (Cell Viability Kit (BD). The flow cytometric methods allowed quantitative determinations when Trucount beads (BD) were used in the sample tube.
Results: Both culture methods gave comparable results at fivefold determination. Differences of parallel determinations of a single sample diverged in the range of factor 0.5 to 1.6 within the culture methods. The precision of the spiral plater was similar or superior compared to the manual spread of bacteria. Differences of flow cytometric methods in comparison to the standard cell culture method were in the range of factor 0.6 to 2.8. Bacteria could be detected in PCs by flow cytometry within 30 minutes whereas 1 to 2 days incubation were necessary for the culture methods.
Conclusions: Flow cytometric methods can be used to determine bacterial numbers in growth media and PCs rapidly. Only the BD Cell Viability Kit cannot be used for bacteria enumeration in PCs. Following the assumption that bacteria are alive in a preparation, the flow cytometry-based methods give comparable results and have the potential to replace the time consuming culture methods. However, because of the higher detection limit of the flow cytometry-based assays, lower bacterial concentrations (e.g. directly after spiking of blood products) have still to be determined by the conventional culture methods.

P 9.04

Storage Stability of Methylene Blue-Treated Plasma

Gravemann U.[1], Reichenberg S.[2], Struff W.[2], Seltsam A.[3], Mueller T.H.[3]

[1]Blood Center of the German Red Cross, Chapters of NSTOB, Research and Development, Springe, D, [2]MacoPharma International, Langen, D, [3]Blood Center of the German Red Cross, Chapters of NSTOB, Springe, D

Background: Treatment with methylene blue (MB) and light is a well-known procedure for the inactivation of blood-borne viruses in fresh frozen plasma (FFP), which recently received marketing authorisation from the German authorities.
Aims: The purpose of this study was to assess the stability of MB/light-treated plasma (MB plasma) processed by the MacoPharma Theraflex MB-Plasma® system. Preparation was done under worst-case conditions for routine processing to evaluate the worst plasma quality to be expected during production.
Methods: Plasma from four different plasma pools was photodynamically treated and stored for up to 39 months at < –30 °C. Treatment was done under worst-case conditions with regard to the preservation of product quality: minimum volume of 235 ml (specification for the Theraflex system: 235–315 ml), maximum MB concentration (1.15 µmol/l) during illumination (specification: 0.85–1.15 µmol/l), maximum storage time of whole blood before separation (4 °C, 17h), maximum storage time of MB plasma before freezing (1h). Sampling was done after 1 week and 3, 9, 18, 27, 33 and 39 months. Several plasma parameters were determined.
Results: No significant changes were observed for factors V, VIII, XI and fibrinogen (Clauss) (table). Also factors II, IX, TT, AT III, vWF:RCo, Plasmininhibitor, α1-Antitrypsin, Protein S and Protein C were not significantly altered during storage for 39 months at < -30°C.
Conclusion: Quality of MB plasma was well maintained for up to 39 months even after preparation under worst-case conditions. A shelf life of 36 months for MB plasma was accepted by the German authorities.

Parameter	1st week	9th month	18th month	27 th month	39 th month
		mean +/– SD			
Factor V (U/ml)	1.06 +/– 0.9	1.05 +/– 0.09	0.99 +/– 0.06	1.08 +/– 0.04	1.07 +/– 0.4
Factor VIII (U/ml)	0.73 +/– 0.16	0.73 +/– 0.14	0.74 +/– 0.15	0.82 +/– 0.13	0.71 +/– 0.13
Factor XI (U/ml)	0.78 +/– 0.07	0.79 +/– 0.06	0.72 +/– 0.04	0.88 +/– 0.03	0.83 +/– 0.07
Fibrinogen (Clauss) (mg/dl)	190.5 +/– 12.6	190.8 +/– 11.3	254.5 +/– 13.4	227 +/– 11.7	213.0 +/– 16.3

P 9.05

The Introduction of a Novel Prion Protein (PrPSc) Removal Technology for the Pharmaceutically Licensed Plasma OctaplasLG®

Heger, A.[1], Neisser-Svae A.[2], Bailey A.[3], Gregori L.[4], Behizad M.[5], Jordan S.[5], Römisch J.[2], Svae T.-E.[2]

[1]Octapharma PPGmbH, Research & Development, Vienna, A, [2]Octapharma PPGmbH, Vienna, A, [3]ViruSure GmbH, Vienna, A, [4]VAMC & UM, Baltimore, USA, [5]ProMetic BioSciences Ltd., Cambridge, GB

Purpose: The aim of our studies was to evaluate the biochemical quality and prion safety of the pharmaceutically licensed plasma OctaplasLG® after implementation of an affinity ligand chromatography for selective binding of pathogenic prion proteins (PrPSc) and reduction of the solvent/detergent (S/D) process time from 4–4.5 to 1–1.5 hours.
Methods: Pilot batches of OctaplasLG® (Octapharma PPGmbH, Vienna, Austria) were tested on global coagulation parameters, fibrinogen levels, activities of coagulation factors and protease inhibitors, markers of activated coagulation and fibrinolysis, as well as von Willebrand factor multimers. In parallel studies, plasma pool was spiked with exogenous spike material, derived from brains of hamsters infected with hamster-adapted scrapie 263K, and a down-scale of the OctaplasLG® manufacturing process was performed. Validated Western blot assays were used to investigate the PrPSc binding capacity of the resin.
Results: Our studies demonstrated that PrPSc binds rapidly and with a very high affinity to the ligand gel. Based on the amount of PrPSc captured, as determined by Western blotting of both the product fractions and resin, a very high and robust binding capacity in the order of 6 log10 ID50 bound per ml resin was demonstrated. OctaplasLG® and Octaplas® produced with and without the ligand chromatography for selective PrPSc capture demonstrated an identical quality. In OctaplasLG® a parallel reduction of the S/D virus inactivation step led to significantly higher activities of plasmin inhibitor.
Conclusions: This biochemical characterisation and prion removal studies confirmed that the affinity ligand chromatography under the developed conditions can be introduced into the Octaplas® manufacturing process, as a mean to reduce potentially present PrPSc. OctaplasLG® has the same clinical safety and efficacy profile compared to that demonstrated by Octaplas® over the last 17 years, except for the increased safety margin in terms of prion disease transmission and the possible effect of a significantly increased plasmin inhibitor activity.

P 9.06

A New Cell Free, Pathogen Inactivated Single Donor Plasma

Lehmann C.[1], Rummler S.[2], Kummer C.[2], Barz D.[2]

[1]Institut für Transfusionsmedizin, Herstellungsbereich, Jena, D, [2]Jena, D

Background: Plasma (FFP) derived from apheresis as well as whole blood is not completely cell free and may contain pathogens such as bacteria, viruses and protozoa. The remaining leukocytes, platelets and pathogens pose an immunological risk to the recipient. The residual cells can be removed by filtration and pathogens can be inactivated with the INTERCEPT Blood System by adding amotosalen followed by UVA-illumination.
Objectives: The aim of our study was to improve the safety of FFP by filtration and subsequent pathogen inactivation (PI).
Material and Methods: Plasmapheresis was performed using the Baxter A200 and Haemonetics MCS+ machine resulting in a volume of 600 ml. We reduced the residual cells of the apheresis plasma with the Plasmaflex PLAS4 filter (Macopharma) to achieve a cell free product. Afterwards the plasma was pathogen inactivated with the INTERCEPT Blood System. After pathogen inactivation amotosalen was depleted with a compound adsorption device (CAD) and the plasma was split into 3 units of about 200 ml to be frozen. By using a specific validation protocol, several in-vitro parameters were tested.
Results:

Parameter	FFP before filtration n=19	FFP after filtration and PI n=19
WBC (10^6/U)	0,060	0,0
RBC (10^9/U)	0,000	0,0
PLT (10^9/U)	1,81	0,0
Volume (ml)	596	572
Factor VIII (%)	111	84
Factor V (%)	128	130
Factor XI (%)	115	99

The plasma products were cell free after filtration. Plasma coagulation recovery in PI-FFP was 76% for Factor VIII with an average content of 84%, 96% for Factor XI with an average content of 99%, Factor V remained unchanged. The averaged FFP loss was 24 ml.
Conclusion: We were able to produce cell free pathogen inactivated single donor plasma which meets all criteria of the national guidelines with enhanced safety. Residual cells and pathogens cannot affect the product safety any more. No quarantine storage is needed for PI-FFP before releasing the products to the clinic. Standardised FFP-therapy can be accomplished because the plasma was split into 200 ml units.

P10 Infectious Diseases Transmitted by Transfusion

P 10.01

Fatal Outcome of a Hepatitis B Virus Transfusion-Transmitted Infection

Niederhauser C.[1], Weingand T.[2], Wuillemin W.[3], Maier A.[2], Tinguely C.[4], Gowland P.[4], Fontana S.[4], Stolz M.[4]

[1]Blood Transfusion Service SRC Berne, Laboratory diagnostics, Berne, CH, [2]Blood Transfusion Service SRC Central Switzerland, Lucerne, CH, [3]Division of Hematology and Central Hematology Laboratory, Kantonsspital Lucerne and University of Berne, Lucerne, CH, [4]Blood Transfusion Service SRC Berne, CH

Purpose: In Switzerland today 75% of the Swiss blood donor population is tested with HBV nucleic acid test in either individual donation (ID-NAT) format or in minipools of 6 donations. HBV NAT will be declared as mandatory in Switzerland from September 2009. The present case illustrates the importance of the introduction of very sensitive NAT screening methods for HBV.
Methods: Multiple samples from the implicated donor and infected recipient were tested for HBV serological and molecular markers.
Results: The implicated donor was a 51 years old male repeat donor. At that time of the donation the donor was healthy with a negative HBsAg test and an ALAT of 22 IU/ml.
Ten weeks later he was suffering from a stomach ache and nausea. 3 days later he consulted his doctor with dark urine and a strong jaundice. A further 5 days later the donor informed the BTS that a fresh Hepatitis B infection was clinically diagnosed. This diagnosis was confirmed with the following positive tests: HBsAg, HBeAg, anti-HBc IgG, anti-HBc IgM and anti-HBe. Only the anti-HBs test was not yet positive. Five months later (eight month after donation) the donor had seroconverted with an anti-HBs concentration of 162 IU/ml. Retrospectively the archive sample from the donation was tested negative for anti-HBc total, but the viral load was 17 IU/ml. The recipient was an 85 year old male patient with a myodysplastic syndrome (refractory cytopenia with multi-linear dysplasia), a coronary artery three-vessels disease and monoclonal gammopathy of undetermined significance,. He was transfused with the HBV-contaminated RBC. Three months after the transfusion the recipient was still negative for the following HBV markers: HBsAg, HBeAg, anti-HBc total and anti-HBe. A retrospective investigation done on the corresponding archive samples revealed a viral load of 3'111 IU/ml. Two months later the recipient had following positive HBV markers: HBsAg, HBeAg, anti-HBc total and HBV DNA positive (8.4 x 107 IU/ml). Anti-HBc IgM, anti-HBe and anti-HBs were negative. Two weeks later the recipient died from complications associated with a Hepatitis B infection in the context of his underlying diseases. The genotypes of the donor and recipient isolates belonged to genotype A.
Conclusions: The transmission of HBV by a donor with an early HBV infection was shown by the seroconversion of both the donor and recipient. The present case shows the importance of the introduction of highly sensitive HBV NAT screening strategies to prevent possible HBV transfusion-transmitted infections from donors with low viral loads.

P 10.02

Novel Flow Cytometry-Based Screening for Bacterial Contamination of Platelet Concentrates in Comparison to Other Rapid Bacterial Detection Methods

Vollmer T., Kleesiek K., Dreier J.

Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, D

Background: Bacterial contamination is currently the major infectious hazard of platelet transfusion. The detection of bacterial contamination in platelet concentrates (PCs) has been implemented in several blood services as a routine quality control testing, but to date transfusion-transmitted bacterial sepsis has not been completely prevented. In this context we developed a novel flow cytometry-based method for point-of-issue screening of PCs.
Methods: The BactiFlow flow cytometer was used to detect and count bacteria based on esterase activity in viable cells. The assay was compared to incubation (BacT/Alert culture system) and rapid nucleic acid-based or immunoassay detection methods (RT-PCR, Pan Genera Detection Technology).
Results: A protocol for bacterial screening of PCs was established by enzymatic digestion and centrifiltration for the elimination of viable platelets and selective labeling of bacteria with a fluorescent esterase substrate. Results from the BactiFlow showed an excellent correlation to traditional plate count results. The lower detection limit of the assay was determined to 150 CFU/mL, whereas the time-to result was less than 1 hour.
Conclusion: Our study demonstrates that the BactiFlow flow cytometry is most suitable for rapid bacterial screening of PCs and fulfils the requirements for a point-of-issue-testing of PCs with acceptable time-to-result, specificity, sensitivity and costs.

P 10.03

Possible Transfusion Transmitted Hepatitis A

Gubbe K.[1], Loehr M.[2], Pawlowski-Nähring U.[3], Galle G.[4], Rothe R.[2], Blankenburg T.[4], Karl A.[5], Elke G.[2]

[1]DRK Blutspendedienst Ost gemeinnützige GmbH, Institut für Transfusionsmedizin Plauen, D, [2]DRK Blutspendedienst Ost gemeinnützige GmbH, Cottbus, D, [3]DRK Blutspendedienst Ost gemeinnützige GmbH, Berlin, D, [4]DRK Blutspendedienst Ost gemeinnützige GmbH, Chemnitz, D, [5]DRK Blutspendedienst Ost gemeinnützige GmbH, Plauen, D

Purpose: The most common mode of transmission of hepatitis A virus (HAV) is person-to-person, resulting from fecal contamination and oral ingestion. However, a few HAV transfusion-transmissions have been reported. This case report describes the first possible transmission of HAV via the transfusion of a thrombocyte concentrate in our blood donation service.
Methods: All donations were routinely screened by in-house RT-PCR for HAV in pools of 96 with a sensitivity (95% cut off) of 155IU/ml in the

individual donation. Quantitative ID-PCR (95% cut off 16IU/ml) was used for retesting donor material from the archive samples and samples from the recipient. Anti-HAV-IgM/IgG and ALAT values of all samples were examined.

Results: A 26-year-old asymptomatic male donor made plasma and thrombocyte donations since October 2008. At the end of March 2009 a plasma donation was tested HAV-RT-PCR positive by pool-PCR whereas no IgM and IgG were detectable at this time. A low viral load of 193IU/ml was detected by ID-PCR. The alanine aminotransferase level was not elevated. The donor developed clinical symptoms three weeks after his first HAV-PCR-positive donation and became antibody positive. The alanine aminotransferase levels increased to 740 IU/l.

A thrombocyte concentrate was transfused just three days before the first HAV-pool-PCR-positive donation was detected. The recipient was a female oncological patient. She gave a sample two weeks after transfusion. The sample was tested HAV-positive with RT-PCR (48 IU/ml) but HAV-seronegative for anti-IgM/IgG. Investigations in order to detect a seroconversion and to confirm a transmission of HAV by blood products are in progress.

Conclusions: For the first time a HAV-positive blood donor and a possible transfusion-transmitted HAV infection was evidenced by PCR in our blood donation service. The risk of transfusion transmitted infection with HAV is extremely small but still exists.

P 10.04

Five Parameter NAT Screening by TaqScreen MPX with Cobas S201 in Switzerland: Validation, Implementation and First Experiences

Glauser A.[1], Hardegger K.[2], Gottschalk J.[2], Frey B.M.[2]

[1]Blutspende Zürich, Screeninglabor, Schlieren, CH,
[2]Blutspende Zürich, Schlieren, CH

Purpose: The system cobas s201/TaqScreen MPX test (Roche Diagnostics) is a fully automated multiplex nucleic acid test for blood screening for hepatitis B virus (HBV) DNA, hepatitis C virus (HCV) RNA, HIV-1 RNA (groups M and O) and HIV-2 RNA. We implemented this test system at the ZURICH Blood Transfusion Service SRC for routine analysis in April 2008 based on a head to head comparison of alternative system. Limit of detection validated by probit analysis and first results of routine testing are presented and we show the overall performance and suitability of the system for high throughput blood donor screening aiming to improve transfusion safety.

Methods: The cobas s201 platform (Roche Diagnostics) consists of automated pooling of blood donations using Hamilton Star pipettor, automated sample preparation using cobas Ampliprep instrument and automated amplification (real time PCR) and detection using cobas TaqMan analyzer. The test cobas TaqScreen MPX for use on this platform is a CE labeled in vitro diagnostics (IVD) for detection of HBV DNA, HCV, HIV-1 and HIV-2 RNA in a multiplex assay. In reactive samples, the individual reactive parameters have to be identified using alternative testing. Routine samples were tested in pools of six. Resolution of positive pools is performed by single donation re-testing.

Results: The 95% limit of detection (LOD) for HBV, HCV and HIV were 2.3 IU/ml, 7.4 IU/ml and 31.1 IU/ml (HIV-1), respectively. These results are comparable with those of other European testing sites using the same platform and are even better than indicated in the test manual by Roche Diagnostics.

From April 2008 to April 2009 we screened more than 93'000 donations. From 17 reactive donations, 9 were confirmed positive for HBV (HBsAg positive by ELISA), 5 were positive for HCV (HCV Ab positive by ELISA) and 2 were confirmed positive for HIV-1(HIV Ab and p24 Ag positive by ELISA). 1 donation was “isolated PCR reactive” (ELISA screening negative for all parameters tested). This sample was shown to be HBV PCR positive and could later be identified as HBV windows case. Actual data including nature and frequency of problems will be presented.

Conclusions: LOD of the cobas s201 TaqScreen MPX fulfills well the national and international requirements for NAT screening and proves to be most suitable for high throughput blood donor analysis. Operational consistency is excellent. The first results indicate already a net safety benefit on provision of transfusion products by application of this screening approach for Swiss blood donors.

P 10.05

Evaluation of Anti-HBc Testing in Blood Donor Screening

Juhl D.[1], Glessing P.[2], Görg S.[2], Hennig H.[2]

[1]Universitätsklinikum Schleswig-Holstein, Institut für Transfusionsmedizin, Lübeck, D, [2]Universitätsklinikum Schleswig-Holstein, Lübeck, D

Purpose: Screening of blood donations for antibodies against Hepatitis B core antigen (anti-HBc) is mandatory in Germany since 2006. To investigate the efficiency of anti-HBc screening concerning transfusion safety and to assess the loss of donations due to false positive anti-HBc testing, the serologic and NAT results of Hepatitis B screening were evaluated retrospectively.

Methods: Data from samples taken in our institute either by blood donations or by preliminary screening of intended blood donors were investigated. All samples were screened for anti-HBc and Hepatitis-B surface antigen (HBsAg) by chemiluminescent enzymeimmunoassay (ChLIA, Abbot Architect Anti-HBc and HBsAg) and by minipool NAT containing up to 96 samples (COBAS AmpliPrep/COBAS TaqMan HBV Test, Roche Diagnostics). Plasma samples of intended blood donors were not screened by minipool NAT routinely, but at their first donation. Positive results of anti-HBc testing were confirmed by microparticle enzymeimmunoassay (MEIA, Abbott Axsym Core), by testing for antibodies against Hepatitis B surface antigen (ChLIA, Abbott Architect Anti-HBs) and by single sample NAT testing (COBAS AmpliPrep/COBAS TaqMan HBV Test, Roche Diagnostics). By database query, donations and intended blood donors tested positive for anti-HBc by ChLIA within a two and a half years period (01.07.2006 – 31.12.2008) were identified and evaluated. As precautionary measure, all donations tested repeatedly positive for anti-HBc by ChLIA were abolished and donors were rejected from donation irrespective of the confirmatory test results.

Results: Overall 124,424 samples were screened for anti-HBc. Of those, 508 (0.4%) were tested repeatedly positive for anti-HBc by ChLIA. 182 ChLIA positive results were confirmed by MEIA. Of the ChLIA and MEIA positive samples, 6 were tested positive for HBsAg and for HBV-DNA, all of them were tested negative for anti-HBs, indicating an acute or chronic HBV infection. In non of the samples tested positive for anti-HBc, isolated HBV-DNA without HBsAg was detectable. Of 326 samples tested positive for anti-HBc by ChLIA only, 209 were tested negative for anti-HBs, indicating an unspecific test result. 28/182 ChLIA and MEIA positive samples were negative for anti-HBs, indicating a remote HBV infection, or more probably, unspecific reaction in both tests. 271/508 ChLIA positive samples were anti-HBs positive, either due to former infection or due to unspecific reaction in the anti-HBc test and former vaccination.

Conclusions: No HBsAg escape mutants or HBsAg low-level carriers were identified by anti-HBc-testing, probably indicating a better sensitivity of the currently used HBsAg assays compared with those used in previous serosurveys. However, anti-HBc testing results in a donation loss of up to 0.4%, but then, it might yields an additional benefit for blood safety by identifying donors with serologiocal criteria of subsided HBV infection, which are even so in individual cases at risk for transmitting HBV.

P 10.06

Application of the Pan Genera Detection Immunoassay for the Rapid Detection of Bacterial Contamination in Platelet Concentrates

Vollmer T.[1], Hinse D.[2], Kleesiek K.[1], Dreier J.[1]

[1]Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, D, [2]Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, D

Background: Bacterial contamination of platelet concentrates (PCs) still represents an ongoing risk in transfusion transmitted sepsis with a significant morbidity and mortality. Recently the Pan Genera Detection (PGD) system has been developed and FDA licensed for screening for bacterial contamination directly prior to transfusion. The test principle is based on the immunological detection of lipopolysaccharide (gram-negative bacteria) or lipoteichoic acid (gram-positive bacteria). In the present study we analyzed the applicability of this method with regards to detection limit, implementation and performance.

Methods: PCs were spiked with different strains of Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli. Bacteria were grown for 24h at 22 °C with agitation. The presence of bacteria was assessed by plating culture and the PGD immunoassay.

Results: The PGD assay is very simple to perform with a time-to-result less than 1.5 hours. Gram-positive bacteria where detected in the given range of 8.2 E03 – 5.5 E04 CFU/mL. Nevertheless, some gram-negative strains of Klebsiella pneumoniae and Escherichia coli where only detectable with titers higher 1 E06 CFU/mL or results were difficult to interpret due to faint bands.
Conclusions: Our study demonstrates that the PGD immunoassay is a rapid and easy-to-perform bed-side-like test for the detection of bacterial contamination in PCs. However to date there are some shortcomings in the detection and result interpretation of some strains of gram-negative bacteria.

P 10.07

Preparation of a HCV-Seroconversion Panel

Gubbe K.[1], Frank K.[2], Elke G.[3], Karl A.[2]

[1]DRK Blutspendedienst Ost gemeinnützige GmbH, ITM Plauen, D, [2]DRK Blutspendedienst Ost gemeinnützige GmbH, Plauen, [3]DRK Blutspendedienst Ost gemeinnützige GmbH, Cottbus, D

Purpose: A serial collection of samples was taken from a single donor while markers of an infection are emerging. The performance of several serological screening and confirmatory tests was measured to characterize the seroconversion samples.
Methods: Routine screening was performed with ABBOTT PRISM HCV and in-house pool-PCR (95% cut off 950 IU/ml in the individual donation). Viral load was quantified by in-house Individual donation PCR (95% cut off 29 IU/ml). The ABBOTT PRISM HCV assay was compared with ABBOTT AxSYM HCV and Ortho Anti-HCV-Elisa. The Inno-LIA HCV Score Blot is routinely used for confirmation of repeated anti-HCV reactive samples. This blot was tested by contrast with the recomBlot HCV 2.0 IgG from Microgen and the Chiron-HCV-RIBA 3.0 from Ortho. The determination of the HCV genotype was performed with Innogenetics Versant HCV genotype 2.0 assay.
Results: A male 20 year old repeat donor was tested HCV-pool-PCR positive but Abbott PRISM HCV negative. The time-point of infection could be ascertained. Ten follow-up samples were obtained from the donor and tested with various HCV antibody assays. HCV titers were measured by quantitative ID-PCR. All anti-HCV-screening assays became reactive 42 days after infection whereas HCV-pool-PCR could detect HCV on day 17 after infection. Inno-LIA HCV Score blot was the first confirmatory test that became positive (core1 and core 2 band). Chiron HCV-RIBA 3.0 and the Microgen recomBlot HCV 2.0 showed weak reactions for only one band (c22p resp. core) at this time. The viral titers fluctuated between 10E5 and 10E7 IU/ml in the individual donation. The HCV genotype was identified as 3a.
Conclusions: A HCV-converting donor gave us the opportunity to establish a well characterized HCV seroconversion panel in order to monitor and compare the performance among screening and confirmatory tests and to evaluate current and future HCV-assays.

P 10.08

Donor Loss Incurred by Newly Developed Anti-HBc

Wagner F.

DRK Blutspendedienst NSTOB, Institut Springe, D

Purpose: In Germany, if a donor developes a reactive infectious disease test result, a look back procedure and donor referral is advocated only if the specificity of the result is supported by additional tests. Anti-HBc is an exception, because a look back procedure and donor deferral is advocated, if the positive result is reproduced in the same test system, irrespective of the results of different anti-HBc tests or other HBV-related tests. This policy might lead to the exclusion of donors who could remain in the donor pool without posing a risk to patients.
Methods: The results of routine donor testing and seroconversion work-up were collated. Enzygnost Anti-HBc by Siemens (formerly: Behring) was used as primary anti-HBc test.
Results: From February 2006 to December 2008, 455 donors were excluded who did not test positive in the first anti-HBc tested donation (~0.02% of donations). One of these donors was positive for HBV PCR in the first anti-HBc positive donation, in two other donors the last Anti-HBc negative donation was HBV DNA positive in sensitive testing. All three donors were positive for Anti-HBc IgM (Abbott) and Anti-HBc (Abbott, AxSym system) and had boostered or newly developed anti-HBs. Two of these donors were positive for anti-HBe. Thirty-five donors had a negative HBV PCR and an anti-HBs >100 IU/l and were removed from the donor pool because of the seroconversion. In 12 of these donors, anti-HBs was constant, in further 10, anti-HBs was >1000 IU/l already prior to the first anti-HBc reactive donation. In 43 donors, the only sign of possible HBV infection was a reactive initial anti-HBc test.
Conclusion: The observed seroconversions involving viremia were concordant in different Anti-HBc tests and accompanied by the development of anti-HBs. The currently advocated strategy leads to the loss of donors whose newly developed reactive anti-HBc test may be due to other reasons than HBV infection. Rules for the re-entry of such donors might be considered.

P 10.09

Analysis of 277 Donor Self Deferrals

Kiessig S.[1], Krause K.-P.[2]

[1]Haema AG, NRW, Dortmund, D, [2]Haema AG, Leipzig, D

Aim of the Study: The donor self deferrals from 11 blood service centres of the Haema AG in the last 36 month should be evaluated for the reason and the impact on the blood supply.
Methods: After a self deferral the donor was interviewed if possible. The self deferral data were evaluated after categorization. Six categories were used:
1. unclear, if no further from the donor could be gained
2. accidental, if the donor war not aware of the consequences of his action
3. wrong, if there was a complete misunderstanding the sense on the self deferral
4. conscious, if the donor has deferred himself with full intent.
5. missing, if the self deferral form was not filled
6. forgotten, if the self deferral form was not filled at the time of the donation, but filled latest 24 h after the respective donation

In every case the last serological test and NAT result was evaluated too.
Results: In this time 604,306 donations with 277 self deferrals were observed in the respective centres. 48.01%, the majority of the self deferrals were accidental. Typical events were lipaemic or haemolytic plasma or a heedless donor. Another 23.10% were consciously. Two main reasons were identified: a) real risk behaviour, or b) the volition to harm the blood service for several reasons like deferrals. The donors in these groups were well informed about the self deferrals. Three of them were found to be reactive in the serological testing. 22.74% were categorized as unclear. No further information was available. The respective donors did not return into the centre. In 3.25% the self deferral from was missed. 2.17% of the donors deferred themselves due a misunderstanding of the reasons for self deferrals. Typical answers in the interview were “alcohol in the last 24h” or use of antibiotics. Only 0.72% of the donors forgot the self deferral.
Conclusion: The average loss of donation was at 0,046% and varies between the different centres (min: 0,006%; max: 0,175%). The majority of all self deferrals were either accidental, wrong or due to misunderstanding. This shows clearly, that more efforts form all staff members in explaining especially the procedure to the first time donors. The test seekers were found in the second strongest group. Here most of the donors were informed about the self deferral and test procedures in a blood service centre. The influence of the stop of the gratuitous HIV-testing in some big German cities was very obvious. All the reactive donors found belong to this group. The blood services and the patients necessitate blood or blood products taking a huge risk. The governmental and local health authorities have to be aware on this despite of the financial crisis.

P11 Immunologically Mediated Transfusion Reactions Including TRALI

P 11.01

Severe TRALI with Death Caused by Antibodies against HNA-3a, HNA-2a And HLA-Antigens after Transfusion of Two Platelet Concentrates

Barz D.[1], Oberle V.[1], Jütte H.[1], Hofmann M.[2], Arnold R.[3]

[1]Universitätsklinikum Jena, Institut für Transfusionsmedizin, Jena, D, [2]Klinik für Anästhesiologie und Intensivmedizin, Jena, D, [3]Institut für Gerichtsmedizin, Jena, D

Transfusion associated acute lung injury is a severe complication after transfusion of blood products (plasma, thrombocytes, erythrocytes). We reported of a 65-old woman with carcinoma in the colon. After surgery the patient were transfused with two platelet concentrates (80 GPt/l was the patient thrombocytes). Within 30 minutes the patient became an acute lung injury und died in the next day. The result of the forensic and histological

investigations showed results of shock symptoms, lung edema, injury to the pulmonary architecture.
All results in the lab of pretransfusion diagnostic and sterile culture was negative. The antibody screening against HNA-, HLA-antigens was negative. In the blood of the two platelet donors we found antibodies against HNA- and HLA-antigens. First donor (men): Auto-Antibodies against HNA-2aa Second donor(woman): Anti-HNA-3aa (titre 1:32), Auto-Anti-HNA-2aa, weak Anti-HLA
In the patient we could typed the corresponding antigens HNA-2a and HNA-3a. The crossmatch was strong positive (titre 1:32)
Conclusion: TRALI is a severe complication after transfusion. We conclused, that all donors from platelet concentrates should be negative for HNA-antibodies.

P 11.02

Transfusion-Related Acute Lung Injury (TRALI) in a 3-Year Old Patient after High-Dose Chemotherapy

Marx M.[1], Stephan B.[2], Pindur G.[2], Eichler H.[2], Schenk J.F.[2]

[1]University Hospital of Saarland, Institute of Clinical Hemostaseology and Transfusion Medicine, Homburg/Saar, D, [2]University Hospital of Saarland, Homburg/Saar, D

Introduction: Transfusion-related acute lung injury (TRALI) is a serious, life-threatening complication of hemotherapy. It typically presents within 6 hours after transfusion as a clinical syndrome characterized by acute respiratory distress, hypoxemia and a bilateral pulmonary edema on x-ray with normal cardiac function. The pathogenesis of TRALI has been related to the infusion of antileukocyte antibodies, resulting in activation of complement cascade, pulmonary leukostasis and neutrophil mediated lung injury. Look-back studies of donors with specific antibodies directed against HLA or granulocyte antigens demonstrated that the infusion of donor antibodies into recipient expressing the cognate antigen resulted in TRALI in only a minority of patients. This implies that also the current clinical condition of the recipient may be important for the development of TRALI.
Methods: A 3-year-old boy suffered from a neuroblastoma without infestation of the bone marrow. In the clinical course of surgical intervention and high-dose chemotherapy with autologous peripheral blood stem cell support, allogeneic RBC and apheresis platelet units were infused and well tolerated. Treatment with corticosteroids and antihistamines has been carried out to avoid anaphylactic reactions.
Results: In a follow-up cycle of chemotherapy, the patient suffered from a severe reaction during an ABO identical RBC transfusion, showing rapid onset of tachypnea, cyanosis, dyspnea, and fever. A radiographic examination revealed diffuse, fluffy infiltrates consistent with pulmonary edema. Treatment consisted of aggressive respiratory support, including supplemental oxygen and mechanical ventilation. A volume overload, heart failure, and IgA deficiency syndrome could be excluded. Examination of bacterial contamination of the blood component was negative, and no infection of the patient could be detected. The donor of the respective RBC product prior donated for 24 times without any recipient's side effect.
Conclusion: The 2-event model of TRALI postulates that the first event is the clinical status of the patient leading to pulmonary sequestration of polymorphonuclear leukocytes, and that the second event is the infusion of biologic response mediators like antibodies directed against HLA, neutrophil antigens or lipids, or cytotoxic substances leading to the activation of granulocytes.

P12 Therapeutic Hemapheresis

P 12.01

Therapeutic Erythrocyte-Apheresis for Hemochromatosis Using a Single-Needle Procedure

Reinhardt P.[1], Kulaksiz H.[2], Flor K.[3], Wiesneth M.[3], Schrezenmeier H.[3]

[1]IKT-Ulm, Apheresis, Ulm, D, [2]IM 1, Universitätsklinikum Ulm, D, [3]IKT-Ulm, D

Purpose: Hereditary hemochromatosis leads to impaired function of heart, liver and various endocrinous organs due to progressive iron overload. Patients' first line therapy consists of frequent phlebotomies drawing some 300 to 600 ml of whole blood every other week. Some patients do not tolerate the repeated punctures, the volume shifts or the frequent loss of plasma involved in this treatment, for others the iron depletion remains insufficient. We have adapted an established preparative single-needle red blood cell (RBC) apheresis procedure to the needs of a therapeutic (not preparative) erythrocyte-apheresis.
Methods: Hemochromatosis patients' whose previous therapy had proven insufficient or no longer tolerable, were referred to us for therapeutic erythrocyte-apheresis. Depending on the patient's clinical status, body blood volume (gender, height, weight) and hemoglobin concentration, a TRIMA accel cell separator (version 5.21, Caridian BCT) was programmed to collect 219 ml, 350 ml or 438 ml of concentrated RBC's (Hct 80%). Limits were the relative reduction of the initial hemoglobin of up to 20%, however not below 100 g/l absolute final hemoglobin concentration.
Results: Five patients (2 female, 3 male), average age 61 years (range 54–70), average weight 79 kg (range 66–107) were treated with a total of 25 RBC-procedures (range 2–7 aphereses/pat.). All procedures were very well tolerated and every patient preferred the apheresis to the previous, conventional blood drawing regimen. The only side effect was an expected, temporary fatigue due to the hemoglobin reduction. Within 3 to 7 aphereses the therapeutic aim of a ferritin reduction to 60 ng/ml was reached in all but one patient, who was later diagnosed to also suffer of a rheumatoid disease.
Conclusions: This single-needle procedure replacing 100% of the collected volume with 0,9% NaCl-solution, avoids clinically relevant blood volume shifts. The easily adjustable blood draw warrants gentle collection- and return-cycles, thus even challenging peripheral veins allow repeated aphereses. In our hands collection intervals of 2 to 4 weeks were feasible and sufficient to achieve the required ferritin reduction. This therapeutic approach has little impact on the usually high quality of life of the common hemochromatosis patient, leads to faster iron-store depletion and is clinically less demanding for those patients with advanced organ impairment.

P 12.02

U.K.-Scandinavian Consensus on the Use of Extracorporeal Photopheresis (ECP) for Treatment of CTCL and Chronic GvHD

Berlin G.

Linköping University, Department of Transfusion Medicine, Linköping, S

Purpose: To provide guidelines on the use of ECP for the treatment of cutaneous T-cell lymphoma (CTCL) and chronic graft-versus-host diease (cGvHD).
Background: ECP is a cell-based immunomodulatory therapy using psoralen (8-MOP) and UVA-irradiation of leukocytes. The mechanism of action is not fully understood but involves cross-binding of DNA and apoptosis of irradiated cells. The effect is also believed to be generated through T-cells with specificity for target cells and immunosuppression via T-regulatory cells. ECP has a favourable safety profile with very few immediate adverse events and no long-term adverse effects.
ECP has been used for the treatment of CTCL for more than 20 years and is also widely used in GvHD and in rejection after solid organ transplantation. However, guidelines using evidence-based medicine and best medical practice have been lacking.
Methods: Existing reports and guidelines on the use of ECP in CTCL and cGvHD were identified through literature search of medical databases. A U.K.-Sacndinavian photopheresis expert group met several times during a one year period with the aim of producing a consensus statement using evidence-based medicine and best medical practice of the group members.
Results and Conclusions: A consensus document providing standardized eligibility, assesment and treatment strategies for the use of ECP in CTCL and cGvHD will be presented and discussed.

On behalf of the U.K.-Scandinavian Photopheresis Expert Group.

P 12.03

Case Study: 40 Years Therapy in a Patient with Familiar Hypertriglyceridemia

Otto J.

Deutsches Hämapheresezentrum gGmbH, Köln, D

Familiar Hypertriglyceridemia is a hereditary metabolism disorder, which can cause in particular a life-threathenung pancreatitis. The therapy of the first choice is a change of the lifestyle, mainly an adaptation of the diet.
Now we refer for a patient, who suffered recurrent pancreatitises since 1969 with triglycerid-levels more then 170 mmol/l (15000 mg/dl). As a result of the lipometabolism disorder developped an insulin-dependent diabetes as well as a coronary heart disease with repeated myocardial infarction.

Because of a lacking of compliance, there were multiple dietetic excesses with exacerbations.
Since 1969 the patient was treated with plasma exchange therapy, initially if required, afterwards in constant intervals.
Thus a progress of the complications could be avoided successfully.
By efforts of the physicians for decades, at last the the manner of alimentation could be influenced positively, so that the intensity of plasma exchange therapy could be reduced.

P 12.04

Therapeutic Leukocyte Depletion in CLL at Hb-Level of 37g/l

Braisch M.[1], Thierbach J.[2], Hitz F.[3]

[1]Rotkreuzstiftung, Regionales Blutspendezentrum SRK, St. Gallen, CH, [2]RBSZ, St. Gallen, CH, [3]KSSG, St. Gallen, CH

Purpose: chronic lymphocytic leukemia (CLL) is a neoplastic disease characterized by the accumulation of small, mature-appearing lymphocytes in blood, marrow and lymphoid tissues. The causes of this disease are unknown, although genetic factors likely contribute to its development. Leukapheresis has been used successfully to ameliorate clinical symptoms associated with leukostasis and lower the risk for incurring adverse reactions to subsequent antileukemia therapy. We report a case of a 56 years old female patient with CLL with leukocyte counts of about 676 G/l, Hb 37 g/l, PLT 56 G/l and signs of leukostasis (Dyspnoe NYHA 3). The initial diagnosis was in 08/1998, in 2006 the patient got 5 cycles of chemotherapy (Leukeran, Prednison d1-5) afterwards she refused any further therapy.
Methods: we performed leukapheresis on 3 consecutive days (cell separator COBE Spectra, MNC programme) using a central venous access with a blood flow rate of 50 ml/min and 1 unit of red cells, leukocyte-depleted was pre-filled into the apheresis set.
Results: the mean processed volume was 7919 ml corresponding to >2-fold the patient blood volume (body weight 64 kg). The removed volume ranged from 507 ml to 581 ml. The result was a reduction of the leukocyte count of peripheral blood to 327 G/l without a reverse effect on the Hb-level (Range: 37–33 g/l). The leukapheresis was well tolerated by the patient and no complications occurred.
Conclusions: the clinical situation of the patient forced us to do a leukapheresis even though the Hb level was so low. On grounds of hyperviscosity syndrome the transfusion of red cells unit was contraindicated. Therapeutic leukocyte depletion with pre-filled apheresis set was an efficient and save procedure. The reported case implies the possibility in special constellations (Hb 37 g/l) to reduce the tumor load of leukemic patients, to improve the clinical situation of the patient and to induce the chemotherapy.

P13 Maternofetal Incompatibility (HDN, NAIT)

P 13.01

Health Economic Evaluation of RHD NIPD in Germany

Haverkamp A.[1], Legler T.[2]

[1]Hanover Medical School, Dep. of Epidemiology, Social Medicine and Health System Research, Hannover, D, [2]Department of Transfusion Medicine, University Medical Center Göttingen, D

Purpose: The routine antenatal anti-D prophylaxis (RAADP) is unnecessarily given to about 40% of women carrying an RhD-negative fetus. Noninvasive prenatal diagnosis (NIPD) tests of fetal Rhesus D (RhD) in combination with an indication-related antenatal RhD prophylaxis could be used to avoid unnecessary expense, wasting time or adverse effects of prophylaxis. The cost of RhD NIPD are unknown so far and suitable cost-utility analyses are missing. We identified the test cost of RhD NIPD for the analysis by the Chemagic Magnetic Separation Module and compared conceivable alternatives for a new RHD prophylaxis in the view of the German health care setting as diagnostic procedures, treatment options and health care cost.
Methods: We estimated the direct medical costs of RhD NIPD in form of stated resource consumption for personnel expenditures, the use of medical facilities and equipment as well as necessary supplies and overheads. An economic decision model was developed to differentiate the benefits, risks and costs of alternative strategies of RhD prophylaxis.
Results: The cost für RhD NIPD testing ranges from € 42.6 to € 135.2 per test whereas the cost variation mainly depended on the patent payments, number of replications and retests or the grade of automation. The expected health care cost ranged between € 124.5 for conventional RAADP, € 140.6 for a prophylaxis with integrated RhD NIPD and € 167.6 for an only postnatal prophylaxis. The break-even price of € 26.5 for NIPD test is achievable by utilising economies of scale as rationalised workflows or higher throughputs in centralised diagnostic centres.
Conclusions: RhD NIPD requires a high throughput to become a cost-efficient alternative to RAADP. However, RhD NIPD reduces unnecessary administration of anti-RHD prophylaxis and eliminates the subliminal risks of blood products.

P 13.02

Identification of a Fetal Specific Histone Protein for the Enrichment of Cell-Free Fetal DNA for Improved Non-Invasive Prenatal Diagnosis

Müller S.P.[1], Legler T.J.[2], Avent N.D.[3]

[1]Universitätsmedizin Göttingen, Abt. Transfusionsmedizin, Göttingen, D, [2]Universitätsmedizin Göttingen, D, [3]Centre for Research in Biomedicine, University of the West of England, Bristol, GB

Purpose: One major obstacle of current approaches for non-invasive prenatal diagnosis targeting cell-free fetal (cff) DNA in maternal plasma is that overwhelming maternal DNA is hampering the detection of fetal sequences. This fact is complicating the detection of single nucleotide polymorphisms (SNPs) which may be involved in haemolytic disease of the newborn (e.g. HDN due to Anti-K) or neonatal alloimmune thrombocytopenia (e.g NAIT due to anti-HPA-1a). The purpose of this study was to identify a fetal specific histone protein which should allow the enrichment of cff-DNA for improved detection of fetal-specific SNPs.
Methods: Procedures for the isolation of histone protein fractions from placental chorionic villi and adult mononuclear cells were optimized and evaluated. Differential in Gel Electrophoresis (DIGE) technology was applied in order to compare core histone protein expression in fetus and adult. Differences in fluorescence intensity between samples were evaluated using DeCyder software (GE Healthcare, Buckinghamshire, UK).
Results: A novel histone purification kit (Actif Motif, Rixensart, Belgium) was the method which proved to be most suitable for histone enrichment, as purest fractions were obtained whilst post-translational modifications were preserved. Compared with histones from adult, a shift of fetal histones to the basic end was observed on DIGE gels, indicating potential post-translational modifications changing respective pI's of fetal histones.
Conclusions: This proteomic approach indicated fetal specific DIGE spots. Identification of the protein spots via LC-ESI-MS/MS is required for final conclusions. In addition, this DIGE workflow has to be used in a larger sample cohort, in order to verify differences between fetal and adult histone expression. Subsequently, fetal-specific anti-histone antibodies have to be developed before this new knowledge can be applied for improved detection of fetal-specific SNPs from maternal plasma in cases of HDN or NAIT.

P 13.03

A Case of Massive Fetomaternal Haemorrhage and High Dose Rho (D) Immune Globulin Administration

Grabmer C.[1], Achleitner R.[2], Mitterschiffthaler G.[2], Brezinka C.[2], Mühlbacher A.[2], Utz I.[2], Pircher E.[2], Schennach H.[2], Kilga-Nogler S.[2]

[1]General Hospital and University Clinics Innsbruck, Central Institute for Blood Transfusion and Immunology, Innsbruck, A, [2]University Hospital Innsbruck, A

Purpose: Massive fetomaternal haemorrhage (MFH) involving volumes of blood greater than 30 mL can cause substantial fetal morbidity and mortality. MFH occurs in approximately one per 1000 pregnancies and in case of a Rh D positive fetus, it can lead to alloimmunization of a Rh D-negative mother. We report the clinical management of a Rh D negative mother with MFH from a Rh D positive fetus.
Methods and Results: A 26-year-old woman, gravida III para II, blood group A Rh D negative, was admitted to the hospital at 38 weeks of gestation due to absence of fetal movement. Because of pathologic cardiotocogramm the patient underwent emergency delivery by cesarean section of a 3150-g infant with Apgar score 3/3/3, NapH 7.02 and Hb 4.0 g/dL. The infant's blood group was 0 Rh D positive. In spite of reanimation and transfusion of red blood cells the infant died shortly after delivery. Kleihauer-Betke testing resulted in a FMH volume of approximately 270 mL blood. At an Intermediate Care Unit 2700 µg Rho (D) Immune Globulin (Rophylac®, 50 mL NaCl 0,9%/ 300 µg) was administered to the mother intravenously over a time period of 9 hours. The patient showed no serious adverse effects despite

slight signs of hemolysis (LDH 510 U/L, Bilirubin 2.49 mg/dL) and an elevated D-dimer level of 25232 µg/L on day 2 (40 hours after initiation of therapy). Maternal fetal haemoglobin was measured at regular intervals and declined to 0‰ on day 3. The patient was discharged on day 7 with a declining D-dimer level of 1447µg/L and an Anti-D titer of 1:4.
Conclusion: MFH in case of a Rh D negative mother and a Rh D positive fetus must be treated with high dose Rho (D) Immune Globulin in order to prevent alloimmunization of the mother. Intravenous administration of Rho (D) Immune Globulin should be performed slowly (300 µg/h) and requires strict monitoring of the patient.

P 13.04

Multiple Maternal Antibodies: A Challenge in the Treatment of Haemolytic Disease of the Newborn

Schulte U.[1], Meyer-Wittkopf M.[2], Hoffmann H.-G.[2], Walther-Wenke G.[3]

[1]DRK-Blutspendedienst West, Zentrum für Transfusionsmedizin Münster, D,
[2]Gesundheitszentrum Rheine Mathias-Spital, Rheine, D,
[3]DRK-Blutspendedienst West, Münster, D

Background: Rhesus prophylaxis has reduced the sensitisation rate of Rh(D)-negative pregnant women to 0.2–0.5% (1). Nevertheless, in a few cases anti-D plays an important role as a cause of haemolytic disease of the newborn. The care of foetuses and neonates of mothers with multiple antibodies poses a particular challenge.
Case Report: A 39-year-old pregnant woman (3 healthy children, 2 miscarriages) with the blood group B Rh-negative (ccddee) presented in the 29th week of pregnancy with the alloantibodies anti-D, anti-C and anti-Jk(b). The father was Rh(D)-positive. Significant intrauterine anaemia of the foetus necessitating transfusion had occurred in the woman's previous pregnancy.
Methods and Results: After the previous finding of anti-D, anti-C and anti-Jk(b) antibodies had been confirmed using the gel card system, red cell concentrate that had been cross-matched with maternal blood and irradiated with 30 Gy was obtained for intrauterine transfusion. The foetus was given successful intrauterine transfusions in the 29th and 30th weeks of pregnancy. In the 32nd week of pregnancy testing with the gel card system revealed an anti-Fy(b) antibody in addition to the already known alloantibodies. As a result, the proportion of compatible red cell concentrates (blood group 0 Rh neg, Fy(b) neg, Jk(b) neg) fell from 26 to 5% (2). Additional intrauterine transfusions were required in the 32nd and 34th weeks of pregnancy. In the 36th week of pregnancy the foetus was delivered by primary cesarean section. (Apgar score 9/10/10, Hb 18.3g/dl). Five topup transfusions were required in the first nine weeks postpartum.
Conclusion: Close and timely cooperation between gynaecologists, paediatricians and transfusion specialists can prevent the development of severe haemolytic disease of the newborn even when multiple irregular alloantibodies are present in the mother.

Literature:
1 The National Institute for Clinical Excellence: Technology Appraisal Guidance 41. "A review of the clinical effectiveness and cost effectiveness of routine anti-D prophylaxis for pregnant women who are rhesus (RhD) negative", London NICE 2002.
2 M. E. Reid, C. Lomas-Francis, The Blood Group Antigen Facts Book, London 2007.

P 13.05

Sucessful Treatment of an Alloimmunthrombcytopenia Caused by HLA-B7 Using Immunadsorption(IA) onto Protein A

Barz D.[1], Rummler S.[1], Oberle V.[1], Thude H.[1], Schleussner E.[2]

[1]Universitätsklinikum Jena, Institut für Transfusionsmedizin, Jena, D,
[2]Universitätsklinikum, Klinik für Geburtshilfe, Jena, D

Fetal alloimmune thrombocytopenia (FAIT) due to transplacentally acquired maternal platelet alloantibodies is a rare event. The majority (>75 percent) of cases are due to fetomaternal incompatibility for the platelet specific alloantigen, HPA-1a, whether human leucocyte antigen (HLA) antibodies can cause FAIT is still controversial.
In this case a woman lost after the first uncomplicated pregnancy two children due to severe thrombocytopenia and coagulopathia after preterm birth. Maternal sera were tested for HPA and HLA antibodies by monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay, solid phase-linked immunosorbent assay (ELISA), lymphocytotoxicity assay (LCT) and flow cytometric analysis find no HPA, but HLA B7 antibodies (AB) (titer 1:512). Typing result show the HLA-B7 antigen in the father and first child. During the 4th pregnancy 44 immunadsorbtion were performed up to three times weekly starting in early pregnancy. An amniocentesis detected maternal HLA-B7 antibodies in the amnion fluid (titer 1:32) and HLA-B7 antigen in the fetal cells. The maternal sera were tested for HLA- and HPA-AB by ELISA after each IA. The antibody titre was 1:512 to 1:8.
Under this therapy the pregnancy was ongoing without complication up to 34 weeks. By primary caesarean section a healthy eutrophic neonate was born with only mild thrombocytopenia which normalized immediately after immunoglobulin therapy.
This is the first report about fetal alloimmunthrombocytopenia caused by HLA-B7 antibodies demonstrating a successful therapeutic approach.

P 13.06

Neonatal Alloimmune Thrombocytopenia Caused by Maternal Low-Affinity Anti-HPA-1a Antibody

Marandiuc D.[1], Carbol A.[1], Runkel S.[1], Kunz-Kostomanolakis M.[1], Böckelmann S.[2], Mildenberger E.[2], Hitzler W.[1]

[1]Transfusionszentrale, Universitätsmedizin der Johannes Gutenberg-Universität Mainz, [2]Zentrum für Kinder- und Jugendmedizin, Universitätsmedizin der Johannes Gutenberg-Universität Mainz, D

Purpose: We report two cases of NAIT in the presence of anti-HPA-1a and anti-HLA-Bw4 antibodies (Abs). Case 1: Full-term male baby born with thrombocytopenia and petechial spots on chest area, to a 27-year-old woman (gravida1/ para1). Case 2: Female baby born with intracranial hemorrhage, hydrocephaly and thrombocytopenia to a 23-year-old woman (gravida4/ para2).
Methods: Anti-platelet-Abs were assayed with Capture-P®-Ready Screen®, Platelet Immunofluorescence Test, MAIPA and ELISA. Anti-HLA-Abs were investigated using CDC-NIH. HPA and HLA genotyping were determined using PCR-SSP and PCR-SSO.
Results: Case 1: At delivery day, a strong reactive anti-HLA-Bw4-Ab was found in the mother´s serum which made the anti-platelet-Abs test-results inconclusive. Analyses of HPA genotypes revealed a HPA-1 mismatch: mother HPA-1b1b, newborn HPA-1a1b. Thus, the possibility of an undetectable anti-HPA-1a-Ab existed. Because of this constellation and based on the frequency with which this Ab causes thrombocytopenia in newborns, the first two transfused single-donor aphaeresis platelets (SDAP) were HPA-1a-negative (neg), but HLA-Bw4-positive (pos). The platelet count increased from 8/nL to 138/nL on day 0 and from 17/nL to 146/nL on day 8. In the absence of a detectable anti-HPA-1a-Ab, on day 13 and 14, HPA-1a-pos, HLA-Bw4-neg and pos SDAPs were inefficiently transfused. Considering the risks of persistent thrombocytopenia, on days 15 and 24 two washed SDAPs from the mother increased the platelet count from 20/nL and 23/nL to 481/nL and 273/nL, respectively. The anti-HPA-1a-Ab was detected by retesting the mother´s serum on day 15, 24 and 34. Retrospectively, by ELISA testing this Ab had been detectable from day 0. Case 2: Two random SDAPs were transfused inefficiently. The analyses revealed anti-HPA-1a and anti-HLA-Bw4-Abs. The transfusion of a HPA-1a-neg, HLA-Bw4-pos SDAP increased the platelet count from 23/nL to 118/nL. No further SDAPs were needed.
Conclusion: We observed an inconsistency between the efficiency of HPA-1a-neg, HLA-Bw4-pos SDAP transfusions and the inefficiency of HPA-pos, HLA-neg/pos SDAP transfusions, in the single presence of an anti-HLA-Bw4-Ab. The detection of the anti-HPA-1a-Ab from day 15 on (with ELISA from day 0) leads us to the conclusions that the Ab´s affinity increases after first pregnancy´s delivery day. The involvement of anti-HLA-Bw4-Ab is unlikely in both cases. If possible, we always recommend in cases of suspected NAIT (HPA-1 mismatch) the transfusion of compatible (antigen-neg) SDAPs. Our experience showed the inefficiency of random SDAP transfusions in NAIT.

P14 Autoimmune Reactions against Blood Cells: AIHA, AITP, Autoimmune Neutropenia

P 14.01

Elevated PF4 Binding to Activated Platelets of Women as a Possible Explanation for the Gender Imbalance in Heparin-Induced Thrombocytopenia

Krauel K., Saafan H., Fürll B., Wessel A., Greinacher A.

Institut für Immunologie und Transfusionsmedizin, Transfusionsmedizin, Greifswald, D

Purpose: Heparin-induced thrombocytopenia (HIT) is an immunological-based drug complication with several features remaining to be elucidated. One of these is why women have a higher incidence for developing HIT. The pathogenesis of HIT is mediated by anti-platelet factor 4 (PF4)/heparin antibodies which bind to and activate platelets. We assessed whether PF4 level in plasma, PF4 platelet content, platelet activation sensitivity, PF4 secretion and PF4 binding to rested and activated platelets is gender-dependent.
Methods: In a blinded study hirudinized whole blood was collected from 100 healthy blood donors stratified for gender and age. Plasma, platelet rich plasma (PRP) and gel-filtered platelets were prepared by standard methods. Platelets in PRP were lysed by alternating cycles of freezing and thawing and supernatants were obtained. Gel-filtered platelets preincubated with abciximab were incubated with increasing concentrations of PF4, or buffer, or activated with rising concentrations of convulxin, fixed with paraformaldehyde and surface-bound PF4 and P-selectin expression were determined by flow cytometry using a FITC-labelled polyclonal rabbit anti-PF4 antibody or a PE-Cy5 conjugated mouse anti-human CD62P antibody, respectively. PF4 levels in plasma, platelets and supernatants were quantified by a commercial PF4 enzyme immunoassay. Differences between genders were calculated by Wilcoxon rank sum test.
Results: PF4 plasma levels (P=0.27), platelet PF4 contents (P=0.27), secreted PF4 amounts (P=0.75) as well as PF4 binding to rested platelets (P=0.95) did not differ between genders. However, more platelets of female donors rebound platelet-derived PF4 after convulxin activation compared to platelets from male donors (P=0.04). This was not due to a major difference in platelet activation as P-selectin expression of platelets from female and male donors was comparable at any of the convulxin concentrations tested (P=0.90).
Conclusions: Increased rebinding of platelet-derived PF4 after platelet activation might be one reason for the elevated HIT incidence in women compared to men. This can be explained neither by a higher PF4 storage in platelets of female nor by a higher sensitivity of platelet activation and therefore augmented PF4 release. As PF4 binding to rested platelets did not vary between sex either, activation-dependent mechanisms are most likely to be responsible.

P 14.02

Severe Idiopathic Auto-Immune Haemolytic Anaemia Predominantly Caused by an IgA-Autoantibody of Unknown Origin in a 16-Year-Old Girl: A Case Study

Strobel J.[1], Stachel D.[2], Holter W.[2], Zimmermann R.[2], Eckstein R.[2]

[1]Universitätsklinikum Erlangen, Transfusionsmedizinische und Hämostaseologische Abteilung, Erlangen, D, [2]Universitätsklinikum Erlangen, D

Purpose: In auto immune haemolytic anaemia (AIHA), immunoglobulins and/or complement components are found bound to red cells by positive direct antiglobulin test (DAT). In most cases, IgG or IgM and C3d or C3c can be characterized with monoclonal antisera. Only in very rare cases IgA is the only identified immunoglobulin.
Methods: We report the case of an adolescent presenting with jaundice and severe auto immune haemolytic anaemia predominantly caused by an IgA autoantibody, the induction of which could not be specified.
Results: A 16-year-old Caucasian girl presented with jaundice and fatigue. There was no history of infection, malignancy, or an autoimmune disease. Laboratory evaluation gave the following results: Haemoglobin 5.3 g/dL, haematocrit 17.6%, MCV 138 fL, MCH 41 pg, reticulocytes 34.5%, leukocytes 19,750/µL, bilirubin 15.1 mg/dL, LDH 2,711 U/L, haptoglobin 2 mg/L, and AST (SGOT) 153 U/L. Platelets, CRP, ALT (SGPT), gamma-GT, potassium, Vit.-B12, and folic acid were within normal range. The blood type was A Rh pos., CCD.ee, Kell neg. The indirect antiglobuline test was negative. However, the DAT with polyclonal and monoclonal antisera showed only IgA, and no complement fixation. An eluate was made and tested with monoclonal Coombssera. This procedure identified a very weak additional IgG-component beside the IgA. We found no cold agglutinins, and a Donath Landsteiner test excluded paroxysmal cold haemoglobinuria. A bone marrow biopsy, serum electrophoresis and screening for auto immune diseases (p-ANCA, c-ANCA, antistreptolysin titer, ANA directed at Hep2-cells, DNA antibodies) revealed no pathologies. No haematological causes or infectious agents responsible for the haemolytic anaemia could be detected. Therefore, an idiopathic autoimmune haemolytic anaemia was diagnosed. The patient was treated with corticosteroids. One month later, all pathological laboratory values had returned to normal.
Conclusions: IgA-mediated autoimmune haemolytic anemia is a rare event. In the majority of pediatric cases, idiopathic autoimmune haemolytic anaemia is caused either by warm reactive auto antibodies of IgG class or cold reactive auto antibodies of IgM class. Still, the direct antiglobulin test must include monoclonal antiserum specific for IgA antibodies because severe haemolytic anaemia caused by IgA antibodies is possible and polyclonal antisera fail to detect IgA bound to red cells. In the presented case, the haemolysis was primarily caused by IgA and no underlying cause could be identified. It may be worthwhile to screen the patient repeatedly for auto immune diseases and haematological malignancies.

P15 Demographic Change and Blood Supply

P 15.01

Adverse Reactions in Elderly Blood Donors beyond the Age of 68 Years

Zeiler T.[1], Lander-Kox J.[2], Kühnel D.[3], Weischer P.[4], Spier B.[3], Möller U.[5], Hettinger J.[6], Bux J.[7]

[1]DRK Blutspendedienst West, Zentrum für Transfusionsmedizin Breitscheid, Ratingen, D, [2]DRK Blutspendedienst West, ZTM Breitscheid, D, [3]DRK Blutspendedienst West, ZTM Münster, D, [4]DRK Blutspendedienst West, ZTM Hagen, D, [5]DRK Blutspendedienst West, ZTM Bad Salzuflen, D, [6]DRK Blutspendedienst West, ZTM Bad Kreuznach, D, [7]DRK Blutspendedienst West, Hagen, D

Background: Starting in 2006 we initiated a prospective multicenter study, to assess the risk of adverse donor reactions in elderly donors. Preliminary data published in 2008 showed no increased rate of adverse donor reactions in donors beyond the age of 68. The study was continued to confirm these data.
Study Design and Methods: Repeat donors aged from 69 to 71 were invited to continue blood donation on mobile collection sites of the German Red Cross Blood Service West. Two control groups (50–52 years and 66–68 years) were established. Admission of donors by discretion of a physician followed the national German guidelines for blood donation. Donor deferrals and all kinds of adverse reactions during donation were monitored by staff members. Adverse reactions in the 48 hrs following donation were recorded with a standardized questionnaire. Data were collected and evaluated using statistical software.
Results: 51.085 valid cases (blood donations) were entered in the study. The rate of adverse reactions during donation in all three groups was lower than the average rate of adverse reactions in the blood donation service (0,2%). Reported adverse reactions following within 48 hrs after blood donation were only partially (66%) explained by the previous blood donation and decreased with age (see table). Adverse reactions were more frequent in female donors; however this difference decreased with age.
Conclusions: Our data confirm, that elderly regular blood donors may safely continue blood donation beyond the age of 68 at least to the age of 71.

Donors		Adverse reactions			
		During donation		Following within 48 hrs	
Age (years)	n	male	female	male	female
50–52	16.470	0,04%	0,13%	0,49%	2,49%
66–68	17.936	0,03%	0,05%	0,34%	2,08%
69–71	16.679	0,04%	0,19%	0,21%	0,77%

P 15.02

Analysis of the Donor Behaviour Using the Epidemiological Data according to § 22 TfG

Kießig S.T.[1], Gloger D.[2], Rivero J.[3], Krause K.-P.[4]

[1]Haema AG, NRW, Dortmund, D, [2]Haema AG, Rostock, D, [3]Haema AG, Dresden, D, [4]Haema AG, Leipzig, D

Last year Haema performed more than 870.000 whole blood, red cell, thrombocyte and plasma donations. Depending on demand and their characteristics the donors are directed to the different donation procedures.
Purpose: Epidemiological data has to be reported according to §22 TfG in regular time frames. This data include also information's about the product structure, the age and gender distribution of the donors, and the donation frequencies. Correlations between these data should allow conclusions for directed donor recruitment.
Methods: The epidemiological data were evaluated according to the predetermined structure (age groups, first time donors, repeated donors by time distance, number of donation by product) by Excel sheets. The donation frequencies by age, gender and product were analysed.
Results: The highest proportion of donors for both genders was found in the youngest donors. Especially those had the lowest donation frequency. Donors with higher donation frequencies (average 11 per year) presenting only 5% of the active donors.
Conclusion: Donor recruitments in the elder population are limited due to several medical and demographical reasons. Activities in the donor recruitment for the younger population have to be focused on the donor engagement. Only by focusing on the younger population the increasing requirement for blood and blood products can be fulfilled. The donor recruiting strategies has to be adapted with regard to the age of the potential donors. Younger donors need to get more attraction to the blood service than the older one. Cooperation is required for all governmental and private organisations.

P 15.03

A Two-Year Analysis of the Donor Population Demography in the Blood Donation Centre of the Haema AG in Rostock

Gloger D.[1], Bermaoui B.[1], Schmitt C.[2], Löwe G.[1], Proske J.[1], Krause K.[3]

[1]HAEMA AG, BSZ Rostock, D, [2]HAEMA AG BSZ Berlin, D, [3]HAEMA AG BSZ Leipzig, D

Background: The purpose of this study was to characterize the donor population of a new, independent blood and plasma donation centre (PDC) in a town with approximately 200.000 inhabitants, in which three blood and plasma donation services already were established.
Against the background of an aging population and birth rate, which has been decreasing since 1990, we were interested in the demographic distribution of the first-time donor population and especially in the resulting number of donors who never donated blood or plasma before. We evaluated the impact of excluding female plasma donors with a positive pregnancy history from the donation of therapeutic plasma as demanded in the Votum 39 of the National Advisory Committee for Blood.
Methods: A two-year analysis (5/2007 to 5/2009) was performed using the blood bank software Progesa/MAK. We analyzed the information on our 5,711 first-time donors, which were registered during this time. We conducted an analysis of their age, gender, return rate after first donation, qualification for plasma donation, and donation frequency for blood and plasma. Furthermore we collected information about the pregnancy history of our female donors.
Results: 51.4% (n=2,935) of our first-time donors had never donated blood or plasma before, hence can be regarded as newly recruited donors. Of all donors 56.1% (n=3,203) were younger than 25 years.
In the group of our true first-time donors 71.7% (n= 2,105) were aged 18–25 (rate of 18–25 year olds males compared to the whole population 58.1% (n=1,224), for females 41.9% (n=881)).
76% of the male and 68% of the female donors returned to our centre to donate at least at one subsequent occasion. We qualified 60% of our first-time donors for plasma- or erythrocyteapheresis. A first analysis of positive pregnancy history revealed that 46.2% of female donors had been pregnant at least once.
Conclusion: Our results indicate a high participation of young donors in the provision of blood and plasma products in Germany. Only with the ongoing support of this age group can the demand for blood and plasma products be met. The most important task for the immediate future is to implement effective measures to bind first-time donors and to motivate a higher percentage of the population to donate blood and blood products.
With pregnancy rates of 46.2% the exclusion of women with a positive preganancy history from the donor pool could severely decrease the availability of therapeutic plasma.

P 15.04

Regaining Inactive Donors

Hettinger J.[1], Weimann W.[2], Alt[2], Markovic A.[2]

[1]DRK-BSD West Bad Kreuznach gemeinnützige Gesellschaft mit beschränkter Haftung, Bad Kreuznach, D, [2]Bad Kreuznach, D

Introduction: The growing demand for blood products is not only to be covered by first-time donors, but especially through a reliable number of regular, continuous long-time donors. It is important to maintain this group of loyal donors, but also to find out why -after several donations - the one or the other donor is not available any longer. We have taken contact with such donors by telephone.
Method and Realization: Persons minimally employed were trained for these telephone calls. Donors were selected from the database, who – after donating on regular basis in the past - had not donated blood for a period of two years. The donors were also asked about their reasons for not coming to donation. If the reasons were not compelling, especially not of medical nature, they were invited to come to the next appointment.A suitable donation appointment within a couple of days of the phone call was suggested to these people. Afterwards, the databank was upgraded according to the information given by the donors.
Results: In 2008, 9146 donors were reached by telephone.
2778 donors refused.
3232 donors were not sure.
2620 donors could be motivated donate again.
Conclusions: Telephone contact is a developable and effective measure to regain inactive donors.

P 15.05

Donor Motivation and Donor Satisfaction

Sönnichsen I.[1], Bohnen H.[2]

[1]Zentrales Institut des Sanitätsdienstes d. Bundeswehr, Blutspendedienst, Koblenz, D, [2]Zentrales Institut d. Bundeswehr, Koblenz, D

Purpose: In Germany, 4.8 million units of stored blood are needed per year, i.e. 13,000 per day. Of these, approximately 75% are provided by the German Red Cross, approximately 25% by municipal and private blood transfusion services and approximately 0.8% by the Bundeswehr Blood Bank Service. While the demand for blood products is increasing as a result of demographic development (increasing number of successful treatments in oncology, cardiac and vascular surgery etc. and a growing life expectancy) the number of first-time donors, the population and the willingness to donate blood are decreasing.
Methods: The motives for deciding in favour of or against the donation of blood, the satisfaction with the blood transfusion service before, during and after the donation and the age distribution of the donors were analyzed.
A questionnaire intended to determine both the motivation and the satisfaction of Bundeswehr blood donors was prepared and evaluated.
Results: The donors' answers with regard to their motivation very often mentioned self and buddy aid, the day off and the health check included; but the fact that some were directly affected (through deployments abroad, family members, friends, acquaintances) also played an important role, as well as group dynamics, appeals and posters or simultaneous blood typing and "rewards" such as medals/certificates or the recognition by superiors and comrades.The donors were satisfied without exception; negative impressions concerned "waiting times" and "procedural information". The donors felt well looked after following the donation; the snack provided was considered satisfactory despite its frugality.
Conclusions: The willingness to donate blood could be increased by a combination of several non-monetary incentives (granting of compensatory time off, social recognition in the form of symbolic awards in public).
The efforts should concentrate on "customer care" with a focus on education/advertising (dispelling fears and creating trust and transparency), optimization of donor care (waiting times, friendliness, atmosphere, snack) and rewards/recognition.

P16 Immunogenetic Basis of Transfusion and Transplantation

P 16.01

Released Factors from Damaged Tissue Influence Immunoregulatory Function of MSCs by Inhibiting IDO Expression

Lotfi R.[1], Solgi G.[2], Fuchs K.[3], Becker T.[3], Rojewski M.[3], Meister A.[3], Baur G.[3], Schrezenmeier H.[3]

[1]University of Ulm and German Red Cross Blood Services Baden-Württemberg-Hessen, Institute of Transfusion Medicine and Institute of Clinical Transfusion Medicine and Immunogenetics Ulm, D, [2]Molecular Immunology Research Centre, Tehran University of Medical Sciences, Tehran, IR, [3]University of Ulm and German Red Cross Blood Services Baden-Württemberg-Hessen, Institute of Transfusion Medicine, Ulm, D

Purpose: Rejected allografts and necrotic tumor cells share the characteristic feature of releasing damage associated molecular patterns (DAMPs) which may, on the one hand, act as alarmins inducing an immune response, or on the other hand, initiate wound healing processes associated with activation of mesenchymal stem cells and immune suppression. Thus DAMPs play an important role in modifying allograft and tumor microenvironment. Mesenchymal stem cells have been reported to influence immune response due to their immunosuppressive capacities which have lead to their use in clinical studies aiming to control autoreactivity or graft rejection. We aimed to investigate a possible mechanism underlying the immune regulatory function of DAMPs to find out possible strategies to modify DAMPs-induced immune response.
Methods: MSCs were generated from human femoral bone marrow and were characterized by FACS analysis of specific surface markers. DAMPs were obtained by repeated freeze-thaw-cycles of a colorectal cell line (HCT-116) or alternatively of human platelets. Expression of indoleamine dioxygenase (IDO) was assessed by measuring its product kynurenine in supernatants of DAMPs-challenged MSCs after 7 days. MSC proliferation was measured using CyQuant-Kit according to manufacturer's instructions.
Results: We demonstrate that not only lysates from human platelets but also DAMPs from lysed HCT-116 cells are capable of enhancing MSC proliferation in a dose dependent manner, MSC proliferation in presence of DAMPs was about five fold higher compared to control. Surprisingly, IFNγ-induced expression of the immunoregulatory enzyme IDO by MSCs was suppressed in presence of DAMPs down to 50%. The described effects of DAMPs on MSCs could be abolished once these DAMPs have been trypsinized or oxidized. Further studies on DAMP treated MSCs in other functional assays (e.g. mixed lymphocyte reaction) are warranted.
Conclusions: All together immunomodulatory properties of MSC seem to be altered by the effects of DAMPs on MSCs. Our results have implications for attempts to modify to optimize graft tolerance, modify tumor microenviroment, and for understanding further details of wound healing.

P 16.02

Long-Term Follow Up of Non-Cytokine Stimulated Blood Donors after Repeated Leukocyte Donation

Strasser E.[1], Hofer M.[2], Weiss D.[2], Strobel J.[2], Zimmermann R.[2], Eckstein R.[2]

[1]Universitätsklinikum Erlangen, Transfusionsmedizinische und Hämostaseologische Abteilung, Erlangen, D, 2Universitätsklinikum Erlangen, D

Purpose: Leukocyte collection in healthy donors is a frequently used new apheresis procedure in our hospital in Erlangen to obtain monocytes for the generation of Mo-DCs and lymphocytes for tumor patients. This study presents first data on leukocyte subpopulation counts of non cytokine stimulated leukocyte donors, who repeatedly donated leukocytes between 2005 and 2009. The donation frequency was restricted to five leukocyte collections per year.
Methods: The regular leukocyte collection procedure is performed on the Cobe Spectra cell separator or the COM.TEC device (former AS.TEC 204). The donation time of each leukapheresis was 120 minutes. The predonation count of leukocytes was between 3.000 leukocytes per microliter and 10.000 leukocytes per microliter. We calculated the total yield of leukocytes and monocytes of all leukapheresis procedures of each blood donor. We compared the collection results of the leukocyte products with the donor leukocyte predonation counts. In addition every other six month a blood count and FACS analysis was performed on lymphocyte populations (CD3+4+, CD3+8+ lymphocytes). The blood count was performed on the ADVIA® 120 Hematology System (Siemens Diagnostics, Germany). The analysis of the CD14+ monocytes and the CD3+ lymphocytes were performed on a flow cytometer (FACS Calibur, BD, USA).
Results: The mean leukocyte yield collected with both apheresis devices was 6,02 x10e9 leukocytes, the lymphocyte yield was 4,28 x10e9 and the CD14+ monocyte yield was 1,22 x10e9. In this study we compared data of the first leukapheresis with the following leukaphereses of each donor. We did not find a significant difference of the yields of leukocytes, lymphocytes and CD14+ monocytes. In addition, the predonation counts of leukocytes and the percentage of monocytes did not differ significantly between the first and the subsequent leukoapheresis procedures. Regarding the lymphocyte counts performed every six months, we did not find a significant difference of leukocytes, CD45+ lymphocytes, CD3+CD8+- and CD3+CD4+ T-cells.
Conclusions: The frequency of additionally five leukocyte donations per year did not show a significant reduction of the leukocytes, lymphocytes and T-cell populations as well as the percentage of CD14+ monocytes in healthy non-cytokine stimulated blood donors. The yield of CD14+ monocytes and CD45+ lymphocytes did not decline due to that leukocyte donation frequency.

P17 Automation, Informatics in Blood Banking and Hemotherapy

P 17.01

Evaluation of a Fully Automated Analyser (Techno TwinStation, Diamed AG) for Gelcards

Strobel E.[1], Betten S.[2]

[1]Städtisches Klinikum München GmbH, Department Mikrobiologie, Immunologie und Krankenhaushygiene, München, D, [2]Städtisches Klinikum München GmbH, München, D

Purpose: The gel-centrifugation system (DiaMed-ID Micro Typing System, DiaMed AG, Cressier s/Morat, Switzerland) is used for blood grouping and antibody detection in many laboratories. For a long time, we worked with this system manually. Now, a fully automated analyser for gelcards is offered. We evaluated this analyser for its use in a clinical routine laboratory.
Methods: Sensitivity was tested by detection of complete and incomplete antibodies using a dilution series of the antibodies. Furthermore we examined several samples with weak antigens, with mixtures of different red blood cells (RBCs) and with hyperbilirubinemia, hyperlipidemia or partial hemolysis, and compared the results of the automated reading with our visual reading of the gelcards taken out from the machine.
Results: Automated reading of the titers of complete (anti-A) and incomplete antibodies (anti-D, anti-Fyb) showed the same results as visual reading. In one case (anti-B) the analyser recognized the highest positive dilution of the antibody more clearly (1+) than our visual reading ("?"). Two weak D samples were correctly recognized by the analyser as well as one of the two A2B samples. In the other sample the negativ control tube of the gelcard was assessed as doubtful ("?") by the analyser because of a small clot, making necessary manual interpretation of the complete results, but the A and B antigen had been correctly identified by automated reading. Mixtures containing 10% D+ (in D-), 10% B (in O) or 90% B (in O) RBCs were recognized as a doubtful ("?") result by the analyser, while 90% D+ (in D-) gave positive results for the most part, not indicating the mixed field agglutination. Mixtures containing 95% B (in O) or D+ (in D-) or 5% B (in O) RBCs gave positive rsp. negative results without indicating a mixed field agglutination, 5% D+ (in D-) RBCs gave inconstant (positive and negative) results. Blood group antigen testing was not influenced by hyperbilirubinemia, hyperlipidemia and hemolysis. Antibody screening and reverse typing was not influenced in the icteric samples, whereas in the lipemic and in the hemolytic samples the analyser reported doubtful results ("?") making visual reading necessary.
Conclusions: Techno TwinStation is an automated analyser that performs pipetting, identification and documentation of samples and reagents, centrifugation of the gelcards and reading the results. Interpretation of the results may be disturbed in some difficult samples, but in most of these cases the analyser has not reported a false result, but has shown a doubtful ("?") result. Therefore, validation of all results by the operator with the help of the colour pictures on the screen cannot be abolished.

P 17.02

Bloodbank-Software

Goebel S., Hering J.

Martin-Luther-University, Institute for Transfusion Medicine, Halle (Saale), D

Purpose: A good bloodbank-software is important, so it is to the satisfactory in the daily routine. A good bloodbank-software covers all demands. The users have an easier task – without any limitation. Each concentrates on their own job. The users play together, everyone in their own rhythm, but in the tune of the bloodbank.
Methods: Everybody is introduced in an overview of the section "Automation and Data processing" listed suppliers by bloodbank- software, 9 passive and 11 active suppliers. The web page of every supplier serves the mediation of the first impression of the application. Addresses can be used with an establishment of contact helpfully.
Results: The following bidders have remained in 2009 in the market in Germany (see below).
Important overviews are given :
- Requirements for the software
- Legal bases for bloodbank-software
- Questions to a software manufacturer
- Documents who are to be supported by the software
- Training demands by the introduction
- Expenses to be expected
- Licensing
- Tools for development

The V-model and the Phase-model as well as the life cycle of a software development attract attention.
Conclusion: The overview supports potential prospective customer in new bloodbank-software with the decision-making.

Name	Legal form	Setting up	Install-ation	Server	Client	Data bank
Becom Blutdepot	AG	1995	10	Win 2003	XP, Vista	Sybase
Blood Banking Center	GmbH	2004	3	Unix	XP, Vista	Oracle
Blues	AG	1984	10	Unix	XP, Vista	Informix
BluWin	GmbH	1978	27	Win 2003	XP, Vista	Oracle
BTS	GmbH	1963	9	Win 2003	XP, Vista	Oracle
Edgecare	GmbH	1996	35	any	XP, Vista	any
eProgesa	GmbH	1984	35	any	XP, Vista	Oracle
Eurolab	AG	1989	35	Linux	XP, Vista	Oracle
PC-Blut	GmbH	2004	35	any	XP, Vista	Pervasive
Swisslab	GmbH	1977	35	any	XP, Vista	Sybase
WinTransmed	GbR	1986	4	Win 2003	XP, Vista	SQL

Connection of an Immunohaematological Laboratory Automat to a Pre-Existing Laboratory Computer System by a Bidirectional Interface

Lüdicke C.[1], Schwarz M.[2], Hölig K.[3]

[1]University Hospital Dresden, Medical Clinic I, Transfusion Medicine, Dresden, D, [2]Martin Schmidt Software GmbH, Schwäbisch Hall, [3]Dresden, D

Purpose: The requirements of an immunohaematological laboratory in a hospital of maximum care are steadily growing. Under routine conditions, an increasing number of different tasks have to be addressed without further increasing the consumption of resources and in front of economic restrictions. These challenges can only be met using sophisticated automation.
Methods: In this contribution, an automated solution is presented that combines the flexibility of the manual processing of emergency samples with the advantages of an automated sample handling, such as continuity, low hands-on time, constant quality, flawless documentation, permanent quality control regarding reagents and test results. For this purpose, an immunohaematological-automated system (Galileo by Immucor Company, Rödermark, Germany) was connected to a pre-existing, previously validated laboratory computer system (PC-Blut by Martin Schmidt Software Development Company Ltd., Schwäbisch Hall, Germany). This connection was facilitated by a newly developed bi-directional interface allowing full integration into in the domain of the existing hospital computer network.

Results: This solution permits sample processing without an explicit assignment of work orders by the laboratory software to the automat. Thus, a large part of the validated protocols of the electronic job allocation can be used without additional changes. Within the further work process, an option exists for the combination of manual and automated handling of partial orders. This allows the flexibility necessary for the work process, taking into account the actual situation in a given facility. It does not cause additional labor related to the adaptation of electronic job allocation into the automated system.
Conclusion: This solution saves work force, and it enables us to combine the benefits of manual processing of emergency samples with those of automated sample processing.

P18 Quality Management in Hemotherapy

P 18.01

Certification of a Blood Donation Service according to DIN EN ISO 9001: Procedure and Avail in the Case of the BRK Blood Donation Service

Nagl D.[1], Wohlfart U.[2]

[1]Blutspendedienst des BRK, Qualitätsmanagement, Augsburg, D,
[2]Blutspendedienst des BRK, Augsburg, D

Purpose: In Germany the legal regulations (AMG, AMWHV, TFG) are substantial concerning quality assurance and management. Therefore, the most important yet indispensable quality certificate is the manufacturing license according to the AMG by the competent supervisory authority. Thus the certification of a blood donation service according to the industrial standard DIN EN ISO 9001 is optional.
Methods: The management of the BRK Blood Donation Service decided in 2007 to get the entire company certified according to DIN EN ISO 9001:2000, including the company' s pharmaceutical and administrative depart-ments and the "Biobank" of blood donors. The main steps on the way to a successful certification were to establish a project team, to get involved with a consulting firm, to choose an appropriate certification body ("TÜV Süd"), to create a quality manual, to implement and train quality representatives at all sites and departments, to spread the ISO 9001 standard matters and intentions in terms of a vertical and horizontal transmission into all sectors, and to prepare all sites and departments for the certification audit with the help of internal audits executed by QA and consultant.
Results: From 21 to 24 July 2008 the certification audits were executed by the "TÜV Süd". On 25 August 2008 the successful certification of all sites and departments of the BSD/BRK was documented. In retrospect a great number of positive effects through this voluntary non-governmental certification process can be stated:
- implementation of QA structures in the non-GMP or administrative sector
- transfer of ISO 9001 standard structures and thinking into the GMP sector
- fixing of responsibilities by identification of interfaces and blank spots
- provision of a basis for the reorganization, specification and improvement of running processes and the establishing of new processes by mapping of the entire process landscape in the quality manual
- competitive edge, customer loyalty and corporate image benefits
- introduction of yet unpaired topics like mission statement, management organization, and balanced scorecard into an overall concept (quality manual)
- dissemination of QA thinking in the company as a whole
- enhancement of the acceptance and importance of the QA unit

Conclusions: Due to the undeniably positive effects it is recommended for every blood donation service to subject to the not officially required certification process according to DIN EN ISO 9001.

P 18.02

Influence of Production Process and Interruption of Cold Chain on RBC Quality

Weiss D.R.[1], Fortenbacher S.[2], Strasser E.[2], Zimmermann R.[2], Eckstein R.[2]

[1]Universitätsklinik Erlangen, Transfusionsmedizinische und Haemostaseologische Abteilung, Erlangen, D, [2]Universitätsklinik Erlangen, D

Purpose: The prescribed storage conditions for red blood cell concentrates (RBC) include a storage temperature of +4°C +/- 2°C. However, in the blood bank routine, the cold chain can be interrupted up to some hours (during crossmatching and between release of blood bank and refundment from ward). Conventional quality controls regarding the shelf life of RBC do not regard such interruptions. Besides quality loss of RBC by storage conditions, some differences of RBC quality could exist because of different production processes by different manufacturers.
Methods: One day after the expiration date of RBC, we performed a haemogram, a blood gas analysis, the analysis of K+, phosphate, lactate dehydrogenase (LDH), glucose, lactate, total and free haemoglobin (Hb). Furthermore, we analyzed the total content of ATP and 2,3-diphosphoglycerate in the RBC suspension. The volume of the units was determined by weighing. The frequency, but not duration, of storage interruptions was visible in our blood bank administration software.
Results: The frequency of interruptions accounted from 0 to 11. In no assembly of data we could find any correlation between the frequency of interruptions and the rate of haemolysis or other parameters which could point to an impairment of the blood product. However, we found some significant differences of some abovementioned parameters comparing our two main RBC suppliers. The content of Hb in RBC of manufacturer A ("A") was 20% higher than in units of manufacturer B (65.8 vs. 51.9 g/unit). The higher haematocrit of A (66.7 vs. 63.9%) may explain the lower level of glucose (261 vs. 349 mg/dl) and a higher level of lactate (283 vs. 232 mg/dl) in the storage solution. Due to the enhanced lactate accumulation, the energy status of A was impaired (lower ATP content, lower 2,3-diphosphoglycerate, resulting in the higher O2 concentration in the storage solution of A). The rate of haemolysis in A on average was higher than in B (0.54 vs. 0.19%); 16% of A, but none of B, exceeded a haemolysis rate of 0,8%. LDH was enhanced in storage solution of A (379 vs.105 U/l). The differences persisted even after 42 day storage of RBC of both suppliers (declared shelf life A: 42 days; B: 35 days)
Conclusions: (1) Transitory interruptions of cold chain of RBC with rise of temperature up to room temperature does not impair standard quality parameters of RBC concentrates.
(2) There are remarkable quality differences between RBC of different manufacturers.
In summary, the impact of the production processes of the manufacturers on the RBC quality even at the end of shelf life is higher than the impact of manipulations within the hospital.

P 18.03

Consistent Error Reporting Reduces Blood Sample Mislabelling

Henneberg-Quester K.-B.[1], Fuchs N.[2], Holtmann M.[2], Knop D.[2], Mueller N.[2], Horn P.A.[2], Peine S.O.[2]

[1]University Hospital Essen, Institute for Transfusion Medicine, Essen, D,
[2]University Hospital Essen, D

Purpose: Any wrong blood group determination can lead to serious adverse events ranging from antibody formation to delayed transfusion reaction up to ABO incompatibility-related transfusion fatalities. In order to improve our internal error-risk-management we established a continuous reporting system to document any case of blood group discrepancies detected in our laboratory, which has been in effect since 1991.
Methods: Between 1991 and 2009 we discovered 150 cases of blood group discrepancies. In this period of time approximately 22.000 blood samples per year were sent in to perform pre-transfusion cross match testing and approximately 40.000 units of packed red cell units were transfused per year. All deviations occurred between a current sample sent in for cross matching in comparison to a preceding blood group determination which had been performed days, months or years earlier. In each case the blood group determination was repeated with the same material and checked twice with new blood samples from the patient.
Results: Over the 18 years reviewed approximately 400.000 blood samples were examined in the context of pre-transfusional cross-matching. Due to these examinations discrepancy in blood group typing showed up in 150 patients. In the majority of cases the ABO-blood group determination revealed the mistake. However, in 13% the Rhesus system typing or a different result in antibody screening or DAT gave the decisive hint. In 70% the blood sample for pre-transfusional cross-matching was identified as a mislabelled sample from a different person. The remaining 30% were likely due to a mislabelled sample during initial blood group determination; however, for these a laboratory error or sample mix-up cannot be excluded. Importantly, reporting each blood group discrepancy to the clinician reduced mislabelling by almost 50%.
Conclusions: In a university hospital setting we uncovered approximately 8 blood group discrepancies per year, most of which could have resulted in

serious adverse events due to ABO major incompatibility. The majority of discrepancies could be attributed to sample mislabelling by the clinician. The fact that the frequency of mislabelled samples was reduced by 50% by implementing a stringent reporting system underscores the importance of implementing a critical incident reporting system. Since our laboratory was obviously unable to uncover discrepancies in cases for which no previous blood group result was available, the importance of taking two different samples (from two separate withdrawals) for blood group typing and cross matching is emphazised.

P 18.04

Safe System for Transport of Blood Samples at Recommended Temperature (2–8 °C)

Klose T.[1], Prokoph B.[1], Seidel K.[1], Schmidt M.[2], Hourfar K.[2], Putzker M.[1]

[1]Central Institute of the Federal Armed Forces Medical Servives Koblenz, Blooddonation Center, Koblenz, D, [2]Institute of Transfusion Medicine and Immunohematology; German Red Cross, Johann Wolfgang Goethe University, Frankfurt, D

Purpose: The blood donation service of the German Federal Armed Forces (Bundeswehr) in Koblenz collects about 40,000 whole-blood donations from military and civilian volunteers annually in garrisons and other facilities all over Germany via mobile donation teams. The filled blood bags and the corresponding sample tubes are carried to the institute overnight. European and German authorities have not yet prescribed temperature limits for this pre-analysis transport of laboratory blood specimens. Nevertheless, all relevant textbooks and publications on microbiology and laboratory medicine recommend 2–8°C as the storage temperature. We evaluated a conception based upon this temperature interval for qualified transportation of sample tubes from the location of the blood donation to the examining laboratory. **Methods:** Six commercially available transport box systems of varying capacity characterized by either efficient isolation technique were investigated in a climatic chamber for simulation of temperature (Weiss Umwelttechnik, Reiskirchen, Germany). Sets of novel 4 °C latent heat/cold storage elements (4C Akku 3L/1L/0.2L; delta T, Giessen, Germany) were positioned into each box that had been previously chilled at 4 °C for 24 hours in a cold storage room. Up to 450 sample tubes at room temperature were added following a standardized packaging protocol. The systems were incubated at defined temperature conditions of –10 °C, +20 °C, or +40 °C, respectively. Using calibrated data loggers (Junior; Escort, Hohentengen, Germany), the temperature profile was measured over seven days at three critical control points (bottom, middle, top) in the respective box. **Results:** The optimal isolation boxes, MT 12 and RCB 25 (Dometic, Hosingen, Luxembourg), and PharmaCase Blueline 30 L (deltaT, Giessen, Germany) containing 100, 200, or 350 sample tubes permitted handling by a single person. The packaging regimen was composed of latent heat/cold accumulators (+4 °C) forming a cube with the sample tubes placed inside, entirely enclosed in bubble wrap. Conventional inter-product data loggers measuring every 10 minutes facilitated sufficient monitoring in comparison with the external temperature supervision of the climatic chamber. The data shown represent each the worst critical control point in the box. **Conclusions:** When the preparation process is followed as described, temperature stability can be ensured for up to 21 hours (PharmaCase Blueline 30 L) or up to 36 hours (each MT 12 and RCB 25) provided that the environmental temperature does not exceed –10 °C or +40 °C. The evaluated systems are rapid and easy to handle and contribute to a potential increase in blood safety.

P 18.05

Temperature-Stable Transport of Blood Products across Europe on Varying Environmental Demand: Finally Working with Fresh Frozen Plasma (FFP) as Well

Putzker M.[1], Seidel K.[1], Schmidt M.[2], Hourfar K.[2], Friedrich I.[1], Bohnen H.[1], Klose T.[1]

[1]Central Institute of the Federal Armed Forces Medical Services Koblenz, Blooddonation Center, Koblenz, D, [2]Institute of Transfusion Medicine and Immunohematology; German Red Cross, Johann Wolfgang Goethe University, Frankfurt, D

Purpose: Besides supply of German or Allied Forces' field hospitals worldwide with red blood cell concentrates (RBCs), one additional task of the German Armed Forces' blood donation center in Koblenz is to provide military and civilian hospitals in the native country with fresh frozen plasma (FFP). European and German authorities have prescribed different temperature limits for RBC transport (2–6 °C vs. 1–10 °C) but there is lack of any definite regulation for the transport of FFP ("...in a deep-frozen status...", as is worded by the German hemotherapy guidelines). According to the standard procedure concerning frozen plasma for fractionation, we evaluated a transport conception based upon a temperature maximum of –20 °C. **Methods:** A set of novel –30 °C latent heat/cold storage elements (–30C Akku 3L/1L/0.2L; delta T, Giessen, Germany) were positioned into the validated passively cooling transport box (RCB 25 E; Dometic, Luxembourg) that was previously chilled at < –30 °C for 24 hours in a cold storage room. Thirty-two FFP units (< –30 °C) were added following a standardized packaging protocol. In an experimental climatic chamber for simulation of temperature (Weiss Umwelttechnik, Reiskirchen, Germany) the system was incubated at de-fined temperature conditions in terms of –10 °C, +20 °C, or +40 °C, respective-ly. Utilizing three inter-product data loggers (Junior, Escort, Hohentengen, Germany) per box, the temperature profiles were measured over 72 hours at three critical control points located in the bottom and the top corners as well as exactly in the middle of the box. **Results:** The insulation box RCB 25 containing up to 32 FFP units permitted loading and handling by a single person according to military requirement. The packaging protocol composed of latent heat/cold storage elements (< –30 °C) forming a cube with the products placed inside after they had been in total enclosed in a double layer of bubble wrap was capable to maintain the inter-product temperature below –20 °C for at least 27 hours. This result represented the worst critical control point at the top corners of the box. **Conclusions:** When the proce-dure is precisely performed as described, the assumed temperature limit of –20 °C among the products according to the usual habit for fractionation plasma can be ensured for at least 27 hours, as long as the environmental temperature does not exceed -10°C or +40°C, respectively. The system is rapid and easy to handle and standardized. Since an actual scenario rarely meets such extreme temperature influence during the complete course, significantly increased transportation time synonymous with distance to the addressee may be achieved facilitating within receivers all over Europe.

P 18.06

Change Control at the Bavarian Red Cross Blood Donation Service

Willemsen T.[1], Piana M.[2]

[1]Blutspendedienst des BRK, Qualitätsmanagement, Augsburg, D, [2]Blutspendedienst des BRK, Augsburg, D

Purpose: An essential component of a functional quality management system in pharmaceutical industries is to keep a tight grip on changes of components or procedures that ensure the quality of products. Every uncontrolled change endangers the valid state of affected processes. Maintenance of high product quality therefore requires a formal change control procedure according to annex 15 of the EC GMP guidelines.

Methods/Results: We developed and established a semi-automatic workflow in Microsoft SharePoint Services (R) to ensure the execution of a change process in accordance with the GMP guidelines. An implemented audit trail allows to trace every step of the process. The workflow starts with a formal "request for change" (rfc), where additional data are included to guide the following steps of the workflow. The rfc requires an approval by a responsible person before it is sent automatically to the QA unit. There the rfc is reviewed regarding legal regulations. The next steps are to obtain the approval of affected other departments and to assign a project leader who is responsible for the execution of the change procedure (detail planning, validation/qualification, implementation etc.). He also has to add all the required documents like project plan, risk analysis, validation report etc. After the documents are reviewed and approved by the QA the change is authorized by a responsible person.

Conclusions: Although the workflow in Microsoft SharePoint (R) had to be adapted to our specific requirements it turned out to be a suitable tool to handle the change management process in all its aspects. The main benefits are a well-controlled transaction, an automated information of concerned partners, a proper documentation, an auditability of every step of the process, enabling of a user-friendly statistical evaluation, and the overall network-compatibility. Moreover, the standardization of the process helps to avoid unnecessary operations and offers a good overview of the actual state of change processes at any time.

P 18.07

The First 27 Months of a Mobile Electronic Patient Diary in Hemophilia – Deductions from Adoption Rates and Usage Data

Westfeld M.[1], Mondorf W.[2], Klamroth R.[3], Siegmund B.[4], Kosch M.[5], Tuischer J.[5], Pollmann H.[6]

[1]Wyeth Pharma GmbH, Medical, Münster, D, [2]In Thera, Hämostas Frankfurt, D, [3]Vivantes Netzwerk für Gesundheit GmbH, Berlin, D, [4]ITH Institut f. Thrombophilie u. Hämostaseologie, Münster, D, [5]Wyeth Pharma GmbH, Münster,D, [6]ITH – Institut für Thrombophilie und Hämostaseologie, Münster, D

Background and Objectives: Electronic Health Records are believed to improve transparency, empower patients, and enhance overall quality of care. We introduced a PDA-based mobile electronic patient diary (Haemoassist®) for haemophilia home care documentation (on-demand treatment and prophylaxis), which has been described previously. The system is adaptive and constantly adjusted to meet patients' needs. For the first time we report findings from initial adoption rates and usage data.
Methods: Data collection and storage of Haemoassist® is carried out by an independent research organization, therefore aggregated data was available for this evaluation. We descriptively analyzed the chronological sequence of participating patients, the total number of PDAs given to patients, and the number of electronically submitted documentations (ESD) per month.
Results: During the first 27 months of operation (Jan 2007 to Apr 2009), the number of participating patients increased from 11 to 98, who conducted a total of 9480 ESDs. The mean percentage of active (>1ESD per month) patients per month increased from 57% (1st half 2007) to 67% (2nd half 2008). The average percentage of prophylaxis patients increased from 73% (1st half 2007) to 87% (2nd half 2008). Average monthly documentation frequency increased from 5.6 (Q1/2007) to 7.9 (Q1/2009) ESDs/patient, with high month-to-month variation (average fluctuation 33.8% compared to the previous month).
Conclusions: High month-to-month variation in Haemoassist® usage could be the result of varying numbers of bleedings and subsequent ESDs in participating on-demand patients, whereas irregular patient compliance needs to be taken into account as well. Despite an increase of prophylaxis patients in the Haemoassist® cohort, we believe that the higher frequency of ESDs per patient per month -together with an overall rising number of participants and a larger share of active patients- indicates a growing acceptance of the Haemoassist® system among Hemophiliacs.

P 18.08

Statistical Methods for Process Monitoring in Quality Control in Blood Transfusion Service

Alt T.[1], Schottstedt V.[2], Madla W.[1], Markovic A.[1]

[1]DRK-Blutspendedienst West Zentrum für Transfusionsmedizin Bad Kreuznach gGmbH, Bio/Medizinstatistik, Bad Kreuznach, D, [2]DRK-Blutspendedienst West Zentrum für Transfusionsmedizin Hagen gGmbH, Hagen,D

Purpose: In blood donation facilities a partly big number of product-destroying random checks are necessary according to production amount for the quality control of blood components. Every product not necessarily to be tested is therefore a profit for the patient's care. On the basis of the immense data continuance of the qualitiy controls carried out up to now in the DRK BSD West a statistical procedure was developed with which the random check number of erythrocyte concentrates could be drastically lowered with constant security of the supervision of the process stability and product quality. This was put in connection to recognise the true value of the run-away's rate in the basic set with a high probability.
Methods: Basis for the production of the statistical model were the quality control results of leukocyte reduced erythrocyte concentrates of the DRK BSD West from the year 2008. The suggested formula to the regulation of the random check size is derived from the theory of Laplace probabilities. With a confidence of 95% and a permissible runaway's rate of 5% a random check size of 59 pieces is calculated regardless of the basic amount, in contrast to present approach (1% of the produced products).
Results: A simulation of the reduction of the data collected up to now at basis of 1% on monthly 60 tests with the help of a chance generator showed that the examined attributes of the reduced random check size correspond to the original data and show a reduced, but realistic image of the basic data. As a controlling mechanism serve the so-called operating characteristic curves which show the topical confidence interval with given runaway's number. As a trend supervision and runaway's identification dynamic Shewhart-control maps were introduced for every examined parameter. With this model it is possible after definition of the probability with which x% of OOS results should be recognised in the basic data to determine the required random check size and to supervise afterwards also statistically. Arrangements with appearance of trends and Patterns were defined.
Conclusion: The investigations and comparisons with the reference database of all results from 2008 show that the parameter averages of the reduced random check size correspond to those with 1% rule determined ones. This assumes constantly low variations (SD). An increase of the variations implies a rise of the random check amount. With overstepping of the random check size of 60 a saving of the examining preparations can be reached with stable production process by introduction of the new model.

P 18.09

Venous Access Problems (VAP), the Most Common Untoward Event during Preparatory Plasmapheresis; A Parameter for Staff Performance?

Diekamp U.

Haema AG, Leitende Ärztliche Person, PMS-Zentren, Bremen, D

Plasmapheresis donors may donate twice weekly. Blood flow requirements during the phases of blood separation (100 ml/min.) and red cell return (130 ml/min.) mandate a large bore intravenous cannula (16 G) for adequate access to the donor's antecubital vein. Use of such needles can result in a set of difficulties encountered in every plasmapheresis centre. To better understand them we analyze the VAP observed in 2008.
Methods: In 2003, we establish an untoward events-documentation system in six of our donor centres. It is updated January 1, 2008 to document all mishaps during and after donation, distinguishing local from systemic and from technical problems and grading the former two by severity. We review all events periodically.
Results: In 2008, we accept 286,081 plasma donations by apheresis from 18,679 individuals in these donor centres. We record 5,290 untoward local events due to VAP (1.85% of donations) and additional 4,186 technical problems (TP) associated with VAP (1.46% of donations). The table lists the number of events, their severity, and their frequency in percent of donations.

VAP-category	n	% of don.	VAP-category	n	% of don.	TP-category	n	% of don.
needle change	4.371	1,528	pain/spasm, mild	146	0,051	collection		
hematoma, mild	631	0,221	pain/spasm, mod.	16	0,006	discontiued	2.701	0.944
hematoma, mod.	96	0,034	pain/spasm, sev.	3	0,001	RBC-return		
hematoma, sev.	23	0,008	artery punct., mild	1	0,0003	incomplete	1.485	0,519

Needle consumption exceeds the number of donations plus repeat plus unsuccessful venipunctures by 0.52%. The centre with the highest excess use of cannulas (3.20%) reports the lowest rate of unsuccessful (0.12%) and repeat venipunctures slightly below average (1.48 vs. 1.53%). We notice transient higher frequencies of venous access problems in centres with personal turnover. The centre with the greatest proportion of donors rejected for reasons of poor venous status (3.37% of applicant donors) has the highest rates of unsuccessful (0.31%) and repeat venipunctures (2.13%), while the centre with the lowest proportion of donors deferred for reasons of poor veins (0.38% of donor applicants) has the lowest rates of unsuccessful (0.12%) and below average repeat venipunctures (1.48%).
Conclusions: These findings suggest a need to train physicians (to assess suitability of veins for donation) and phlebotomists (to proficiently establish venous access).

P 18.10

Implementation of an Automated Hematology Analyzer in a Mobile Blood Donation Environment

Hartmann L.[1], Kawaters A.[2], Zeiler T.[2]

[1]DRK Blutspendedienst West, Zentrum für Transfusionsmedizin Breitscheid, Ratingen, D, [2]DRK Blutspendedienst West, Zentrum Breitscheid, Ratingen, D

Objectives: National German guidelines for blood donation request the determination of red cell-, platelet- and leukocyte counts before a blood

Methods: The study was a non-interventional trial. Patients with haemophilia A of any severity, treated with Moroctocog alfa could be included in the study. Safety was assessed by documentation of all (serious) adverse events during treatment with ReFacto. Special focus is on the development of inhibitors in PTPs and PUPs. Efficacy assessment is performed e.g. by evaluating the number of exposure days per bleeding episode.
Results: Until April 2009, 287 patients were recruited in 60 centers in Germany and Austria. 24 (8.4%) were previously untreated (PUPs) and 263 (91.6%) previously treated patients (PTPs). 231 patients (80.5%) suffered from severe haemophilia A (FVIII:C <1%). 27 PTPs had a positive inhibitor history at baseline. De novo inhibitors developed in 4/263 (1.8%) PTPs and 3/24 (12.5%) PUPs. Treatment was effective with a median number of 1.33 exposure days per bleeding episode.
Conclusions: The PE of ReFacto® is the first long-term analysis of a currently marketed FVIII product in Germany and Austria under routine clinical conditions. Data from ten years duration and 287 patients underline the safety and efficacy of ReFacto® in treatment of haemophilia A in daily clinical practice.

P 18.12

Assessment of Platelet Quality and Function – New Aspects in the Analysis of Quality of Platelet Concentrates Prepared by Thrombocytapheresis

Brys B.[1], Baumann-Baretti B.[2], Jaster G.[2], Krause K.-P.[3]
[1]Haema AG, QK- Labor, Leipzig, D, [2]Haema AG, Berlin, D,
[3]Haema AG, Leipzig, D

Purpose: The analysis of in vitro platelet (plt) quality parameters of platelet concentrates (PC) as the basic of studies in the context of validations or changes in existing accreditations is a well known procedure. The demonstration of different methods to analyse in vitro platelet quality and gain information about platelet function when produced as PC in plasma by apheresis is purpose of the work.
Methods: PCs were obtained from regular donations performed with the MCS plus (Haemonetics) and Trima 5.1 (Caridian) device. During 7 days of storage samples were analysed at day 0, 1, 5 and 7. P-Selectin expression (CD62) was measured by flow cytometry (FACS Calibur, Becton Dickinson); platelet stimulation was performed with ADP and TRAP 6. To evaluate the aggregation Apact 4s Plus (Rolf Greiner BioChemica) was used; aggregation was initiated by using TRAP 6, arachidonic acid, collagen and ADP as agonists. Soluble p-selectin- and serotonin were analysed using ELISA-test kits (IBL).
Results: The baseline CD 62 expression increases during the whole storage time without significant differences between PC obtained by MCS or Trima. The capacity of activation after stimulation with ADP (MCS:d0 48,8%, d7 19,8%; Trima:d0 49,9%, d714,3%) is lower than after stimulation with TRAP 6 (MCS: d0 76,8%, d7 55,4%; Trima:d0 70,9%, d748,2%) also the decrease of activation is higher when stimulated with ADP. The capacity of aggregation with collagen and ADP (MCS:d0 82,2%, d7 8,1%; Trima: d0 94,8%, d7 13,5%) as agonists significantly drops whereas the decreases of aggregation induced by TRAP 6 (MCS:d0 91,2%, d7 91,7%; Trima: d0 95,8%, d7 93,7%.) and arachidonic acid are low. Soluble p-selectin increases during storage time in all PC; the concentration of serotonin does not change significantly and demonstrates therefore the integrity of cell membrane.
Conclusion: The testing of different plt quality and plt function parameters in vitro allows a more detailed description of the platelet status during storage over 7 days; better knowledge of in vitro plt quality parameters might enable the performance of test systems with higher predictive value of the in vivo plt quality in the routine quality control.

P 18.13

Introduction of Computer Based Document Management

Gathof B.[1], Picker S.[2], Grunenberg R.[2], Radojska S.[2]
[1]University Hospital, Transfusion Medicine, Köln, D,
[2]University Hospital, Köln, D

Purpose: All documents relevant to blood donation and transfusion should be available at the time needed at each work place in the actual version.
We evaluated the implementation of a computer based document management system in blood donation and transfusion.
Methods: We observed the implementation of a new soft ware system (Pergamon, Ainea, Ludwigshafen, Germany), which replaces document management by staff (and electronic word processing) only.
Results: All documents in use were stored in the new system. Revision of Standard operating procedures (SOP), is now performed in the new system. The "old" versions are stored in the respective archive. Each step in revision is documented. Pergamon supports education of staff by a system connected to the email system. Specific tests for the success of education are possible online. Validation of the system is still in process and will be reported.
Conclusion: Document management is essential in blood donation and transfusion to ensure optimal quality. Document management systems which are computer based may add to safety and productivity. Further validation and experience will be required to determine whether the system described above fulfils all requirements.

P 18.14

Positive Experiences with a Coequal Direction of the Blood Ccollection Department

Markovic A., Stroh K., Hettinger J., Halfer-Wirkus H., Weinstock C.
DRK-Blutspendedienst West ZTM Bad Kreuznach gGmbH, Bad Kreuznach, D

Purpose: The necessity to enlarge blood collection departments (BCD) in supraregional blood donation services in order to satisfy the growing needs of hospitals for blood products has brought problems in supervision. Mostly a head nurse is appointed to support the head of the BCD, but as an assistance, not in a coequal position. The lack of autonomy often shows to be an encumbrance to realize needed action.
Methods: To improve adherence to given standards we deployed an operational head of the BCD, who has to assure customer orientation and disciplinary behavior in the unit. This items are of great importance in our ISO-certified institution. The operational head is absolutely coequal to the medical head of the BCD, who attends to all medical and pharmaceutical aspects.
Results: After initial problems in restructuring the leadership we have made very positive experiences. Both heads are working together on a confidential basis with respect to the different main focus. The formerly subordinated operational head now is not longer blocked with delegated minor tasks.
Conclusions: The strengthening of the position responsible for customer-related and behavioural aspects has reduced insufficient performance and corresponding complaints. It has also enabled the medical head to concentrate its effort more on medical and pharmaceutical topics.

P 18.15

Qualification of Laboratory Automats for Hematological Investigations Considering The Characteristics Correctness, Precision, Linearity

Reinhardt F.
DRK-Blutspendedienst Ost gemeinnützige GmbH, Institut für Transfusionsmedizin Plauen, D

Background: Proof of correct hematological investigations as well as qualification of a complex analysis system require the use of predefined guidelines for performing a design, installation, functional and performance qualification of the device in the framework of a method validation.
Material and Methodology: At the automat Sysmex K4500 are to be furnished for the parameters of the small blood cell count (WBC - leukocyte content, RBC - erythrocyte content, PLT - Thrombozytengehalt, HGB - hemoglobin) the proof of the correct analysis. A requirement specification was created by the users in the course of the design qualification on the basis of which key functionalities for automated hematological investigations were defined. Practically all requirements were already considered by the device manufacturer in the enhanced development of the device and were also already included in the specification. Using the procedure defined in the quality management system, the qualification focused on technical features rather than validation of the methodology.
Result: In the framework of the installation, function and performance qualification, important system modules were assessed in accordance with the requirements of the Common Technical Specification. Following the

successful device qualification, the methodology for automated hematological investigations was validated on the basis of considering the characteristics correctness, precision, linearity. The documented proof that the equipment in its enterprise functions exactly the same, as it is intended. This is ensured by an examination of the equipment under material conditions regarding the surrounding field and setting of tasks. The Performance Qualification examines critical parameters of the equipment on the basis suitable testing methods. The correctness of the WBC, RBC, PLT and HGB regulation is to be with 2%. The examination of the correctness takes place via measurement with a second independent testing method (with well-known correctness). For the determination of the precision at least the repetition precision of the entire analysis procedure is by multiple regulation for WBC, RBC, PLT and HGB on the basis of the homogeneous sample (n > 6) to occupy. The linearity of the analysis to the determination of the WBC, RBC, PLT and HGB is determined by measurement of a row of standards with at least 5 different concentrations.
Conclusion: The use of the algorithm for device qualification, which is prescribed in the quality management system of the DRK Blood Donation Service allows the successful qualification of laboratory automats for hematological investigations.

P19 Miscellanea

P 19.01

Specimen Anticoagulation for the Measurement of So-Called "Circulating" Platelet-Derived Growth Factors and Cytokines

Zimmermann R.[1], Hauck B.[2], Ringwald J.[2], Strasser E.[2], Eckstein R.[2]

[1]University Hospital Erlangen, Transfusion Medicine And Hemostaseology, Erlangen, D, [2]University Hospital Erlangen, D

Purpose: Elevated levels of circulating platelet (PLT)-derived growth factors and cytokines as platelet-derived growth factor AB (PDGF-AB) or vascular endothelial growth factor (VEGF) have attracted much attention. They were found in a wide spectrum of malignancies and in very different non-malignant diseases and clinical conditions. However, the question which is the best specimen for the measurement of circulating PDGFs or VEGF, serum or plasma, has not been answered, and the question which kind of plasma should be used – if plasma is used at all - has attracted even less attention.
Methods: From thirty blood donors, plasma from blood anticoagulated with citrate-theophylline-adenosine-dipyridamole buffer (CTAD), sodium citrate, or EDTA, and serum was examined. In all four samples, the growth factors VEGF, PDGF-AB, and transforming growth factor ß1 (TGF-ß1) were measured. In addition, we examined the spontaneous CD62-expression in the three anticoagulated specimens.
Results: In CTAD blood the spontaneous CD 62 expression was very low, and CTAD plasma contained the lowest mean levels of all measured growth factors. The in-vitro activation of PLTs in citrate blood was slightly higher, and the mean levels of PDGF-AB and TGF-ß1 in citrate plasma were higher than in CTAD plasma ($p<0.05$). EDTA lead to pronounced in-vitro platelet activation in some samples and to minor activation in others. Concurrently, there was a strong correlation between the absolute number of activated, CD62 positive PLTs per mL and the levels of PDGF-AB, TGF-ß1, and VEGF. Serum contained much higher levels of all four growth factors than anticoagulated specimens. There was a weak correlation of growth factor levels in serum with different fractions of white blood cells.
Conclusions: Measurements of circulating growth factors which originate from PLTs should be performed from CTAD plasma if the aim is to detect only molecules that have been released in vivo. EDTA plasma must not be used for any measurement of circulating plated-derived growth factors because of the extreme interindividual variation of PLT activation and concurrent in-vitro growth factor release by EDTA.

P 19.02

Trypsinizing Tumor Cells Which are Supposed to Be Used for Pulsing Dendritic Cells (DCs) Affects their Ability to Induce Maturation of DCs

Herzog G.[1], Solgi G.[2], Schrezenmeier H.[3], Lotfi R.[3]

[1]University of Ulm, Institute of Transfusion Medicine, Ulm, D, [2]Molecular Immunology Research Centre, Tehran University of Medical Sciences, Tehran, IR, [3]University of Ulm and German Red Cross Blood Services Baden-Württemberg-Hessen, Institute of Transfusion Medicine, Ulm, D

Purpose: Lysates from tumor cells are reported to induce maturation of DCs and are used in clinical settings for DC-based vaccination against solid tumors. Using three individual adherent colorectal tumor cells we could not confirm the DC maturating effect of tumor lysate. Therefore we analyzed possible differences in preparing tumor cell lysate in order to find a way to optimize vaccination strategies.
Methods: Colorectal tumor cell lines (HCT-116, CACO-2, COLO-678) were purchased from DSMZ and cultured in DMEM containing 10% human AB serum. Tumor lysates were obtained by repeated freeze-thaw-cycles. Human monocytes were separated from whole blood using CD14+ magnetic beads (Miltenyi). DCs were generated from monocytes cultured in presence of IL-4 and GM-CSF according to standard protocols. DC maturation was assessed by flow cytometric analysis of surface markers CD 40, CD80 and CD 86.
Results: Lysated from tumor cells which have been detached from culture flasks by trypsinizing did not induce DC maturation while non-trypsinized tumor cells induced upregulation of CD 40 and CD86 on DCs. This effect was abolished once the lysated was exposed to minimal amounts of trypsin.
Conclusions: Trypsinizing is a very common way of harvesting adherent cells from culture flasks. Our results shall call investigators' attention to the enzymatic activity of trypsin degrading some possibly important proteins on the surface of cultured cells, specifically for DC-based vaccination against tumor antigens investigators should avoid trypsinizing.

P 19.03

Donor Questioning on the Occasion of One Year Follow Up after Allogenic Stem Cell Donation

Völker G., Kreissig C., Fischer I.

DRK-Blutspendedienst West, Zentralbereich Stammzelle und Transplantationsimmunologie, Ratingen, D

Question: In our institution we perform round about 120 allogenic stem cell collections from unrelated donors per year. 85 till 90% of them are mobilised peripheral blood stem cell donations and10 till 15% bone marrow donations. All donors are contacted one year after donation for follow up interview and check up.
According to ZKRD standard a contact between unrelated donor and recipient is possible for the first two years after transplantation as a controlled anonymous correspondence. Afterwards a direct contact is allowed, if both want to establish it. We wanted to know how close the relationship between donor and unknown patient is and how many donors want to get to know their recipient.
Donors and Method: On the occasion of one year follow up check up and interview we asked unrelated donors if they want to get information about the state of health of the recipient and if they plan to allow the contact with the patient, whose life they saved. In 24 month 300 questionnaires were mailed to our donors.
Results: We have got back 128 questionnaires. Despite of the fact that in donors education before donation all information about anonymity and donor-recipient-contact is included, 28 donors said they were not aware of the fact, when and how a contact is possible. 34 of 128 donors wanted a direct contact with patients after expiration of the 2 year time limit. Additional 23 donors wanted to get information concerning patient health status and well being. Only 10 of 128 donors denied any information or contact at any time.
Conclusions: It is conspicuous, that despite of intensive oral and written education concerning anonymity and approach many donors do not know, if, when and how a donor-recipient-contact could be established. We assume that during donor interview, education and physical examination too much informations are communicated. So some facts are not acquired. Concerning patients health status a lot of donors expect information by registries or collection facilities self acting.

P 19.04

Retrospective Aanalysis of Transfusion Reactions to Cellular Blood Components in 2004–2008

Kühnel D.[1], Spier B.[2], Schulte U.[2], Walther-Wenke G.[2]

[1]DRK Blutspendedienst West, Zentrum für Transfusionsmedizin Münster, D, [2]DRK Blutspendedienst West, Münster, D

In accordance with §63b Arzneimittelgesetz (German Medicines Act) and §16 Gesetz zur Regelung des Transfusionswesens (German Transfusion Law), adverse reactions to blood products are reported to the pharmaceutical company concerned and serious adverse reactions are additionally reported to the federal regulatory authority.

Purpose: Investigation of donor and production-specific characteristics on transfusion reactions occurring in the period 2004-2008.

Methods: A retrospective analysis of transfusion reactions in terms of cause, age of product, amount transfused, patient data, clinical outcome and donor gender.

Results: In the period 2004-2008 a total of 1,197,785 units of RBCs and PCs were supplied to medical care establishments and 712 transfusion reactions were reported. The reporting physicians classified 11.38% (n=81) of these reactions as serious and 88.62% (n=631) as non-serious.

Table 1: Cause of transfusion reactions (TR) (N = 712)

	%	N
Acute hemolytic TR	0.14	1
Delayed hemolytic TR	0.99	7
Febrile nonhemolytic TR	56.74	404
Allergic TR	26.69	190
Anaphylactic TR	1.97	14
Septic TR	0.28	2
No relationship	2.25	16
Not detectable	10.96	78

Table 2: Number of units transfused per TR (1) and age of product [d] (2)

Product	Mean/Median/Range(1)			Mean/Median/Range (2)		
RBCs (N=827)	1.38	1	(0–9)	14.6	13	(2–42)
PCs(apheresis/pooled) (N=164)	1.50	1	(0–5)	3.3	4	(2–6)

Table 3: Patient data

Age	N	%	Mean, y	Median/range, y
Female	429	60.25	67.91	73.44 (0.04–97.13)
Male	283	39.75	68.16	71.82 (1.61–95.49)

Table 4: Clinical outcome

Recovery without deficit	57.16**%**	Death due to other cause	0.28%
Death due to basic disease	1.83%	Unknown	40.73%

Table 5: Gender distribution of donors

	M	F	Chi-squared test:
Units of RBCs produced	768,203	492,253	$X^2 = 0.5056$
Number involved in TR	514	313	p = 0.47 (not significant)

Conclusions: Transfusion reactions were reported in association with one per 1209 products supplied. The rate of reactions was not associated with donor gender and age of product.

P 19.05

Follow-Up of G-CSF Stimulated Granulocyte Donors by Questionnaire

Macher S.[1], Posch U.[2], Drexler C.[2], Lanzer G.[2]

[1]Medical University Graz, Univ. Clinic of Blood Group Serology and Transfusion Medicine, Graz, A, [2]Medical University Graz, A

Purpose: Despite the development of modern broad-spectrum antibiotics, antimycotics, and haematopoetic growth factors infections remain the main cause of morbidity and mortality in neutropenic patients. Studies in the 1970ies showed a benefit of granulocyte transfusions. Although at that time the yield of transfused cells was far below the yield we are able to collect since granulocyte-colony stimulating factor (G-CSF) administration to the donors. To evaluate long term side effects as well as donor`s mental and physical experiences with G-CSF stimulation and granulocytapheresis (GA) we sent a questionnaire to 73 former granulocyte donors.

Methods and Results: 57/73 (78%) donors replied to the questionnaire (median age 36 years [range 22–68]; 48 male, 9 female). 52/57 (91%) donors had previous experiences as blood donors. The time from GA to follow up ranged from 6 months to 5 years. All donors had received G-CSF (5µg/kg per day) for 1 up to 8 days. The median number of concentrates collected from each donor was 4 (±1,63 SD). 7/57(12%) donors had donated more than 4 times (5–8) for 5 children with less than 35 kg body weight. In those cases only 3500 ml was processed during each apheresis (otherwise 7000 ml). Collections were performed by standard leukapheresis (COBE, Caridian BCT) via peripheral venous access. At the time of recruiting the greatest concerns were pain or complications during the apheresis procedure (23/57; 40%), long-term adverse effects (15/57; 26%) and medication side effects (8/57; 14%). Complaints during G-CSF stimulation were bone pain (25/57; 44%), fatigue (20/57; 35%), vertigo (5/57; 9%), fever (2/57; 3.5%), headache (2/57; 3.5%), elevated blood pressure (1/57; 1.8%) and edema (1/57; 1.8%). 6/57 (11%) donors reported no complaints. Bone pains were rated by 21/57 (36%) as moderate to severe and were located mostly at the back and/or pelvic/hips. 14/57(25%) donors received oral pain medication and achieved pain reduction to analgesia. The 57 evaluated donors underwent 215 apheresis procedures. During 24/215 (11%) procedures a citrate reaction was observed that could be managed by i.v. or oral Ca++ administration. During the follow-up 55/57 (96,5%) donors confirmed excellent health and would recommend GA to other persons, 44/57 (77%) would agree to a granulocyte donation again.

Conclusions: Among the former 57 granulocyte donors neither a haematological nor any other severe malignancy was observed. The administration of G-CSF to healthy donors to collect granulocytes does induce short-term side effects such as bone pain, which can be managed by medication, but may not induce negative long term consequences.

P 19.06

Storage Duration Influences TPO Clearance in Prestorage Pooled Platelet Concentrates

Wenzel F.[1], Gruber W.[2], Lasshofer R.[2], Wiedemann H.[2], Stahl D.[2], Giers G.[2]

[1]Salzburger Landesklinik, Institut für Blutgruppenserologie und Transfusionsmedizin, Salzburg, A, [2]1Universitätsklinik für Blutgruppenserologie und Transfusionsmedizin, Salzburg, A

Introduction: Thrombopoiesis is regulated by the hormone thrombopoietin (TPO) dependent on a feed back mechanism. TPO levels are influenced by clearing via TPO receptors of platelets followed by internalization. The TPO clearance addiotionally reflects functionality of platelets and may be a marker for alterations in platelet concentrates.

Methods: In prestorage pooled platelet concentrates (n = 4) TPO clearance was determined at day 1, 3, 5, 7 during routine storage conditions. TPO was adjusted to 500 pg/mL at the start of an incubation period for 5h at 37 °C. TPO levels were determined by a commercially available ELISA-Kit (R&D Systems).

Results: Incubation of prestorage pooled platelet concentrates for 5h at 37 °C reduces TPO levels significantly compared to the respective control values before incubation. As example, the average TPO level was 289 +/– 27 pg/mL before incubation and 132 +/- 19 pg/mL after incubation at day 1. During the observed 7 day period of routine storage, TPO clearance decreased from 30 +/– 5 zg TPO/platelet/h at day 1 to 19 +/- 8 zg TPO/platelet/h at day 7. Control samples did not show any difference.

Conclusion: TPO clearance by platelets is dependent on storage duration. In prestorage pooled platelet concentrates, TPO clearance was reduced to about 70% of the respective control values after a storage period for 7 days. The observed reduction of TPO clearance by the platelets is in line with literature data indicating that a loss of platelet functionality of about 20% will occur after 5d of storage.

P 19.07

Experience with a GPS-Assisted Automatic Temperture Monitoring System in Transporters of Blood Products

Brys B.[1], Gerbatsch K.[2], Krause K.-P.[2]

[1]Haema AG, Logistik, Leipzig, D, [2]Haema AG, Leipzig, D

Purpose: In blood donation services the monitoring blood product temperature during transportation is one of important parameters of quality control. Our objective was to introduce an efficient system for uninterrupted acquisition of temperature data at real time during the blood transport. Alternatively, this system should allow us to analyse several data retrospectively.
Methods: For transportation blood products and test samples of Haema AG a GPS-assisted temperature monitoring system from the company Euroscan has been used. VW Caddy and VW T5 have been used as vehicles. The data collected by two sensors in each transport chamber were transferred to a central computer via satellite at defined interval time from registration equipment. This data are accessible via internet for the user. The driver has always the possibility to have a look at the current temperature of the registration equipment Euroscan X2. Rregistered data can be printed and used for further analysis.
Results: The transport vehicles of Haema AG were completely retooled on Euroscan system. All vehicles were hereby equipped with registration equipment X2 including two temperature sensors per each transport chamber. Euroscan was responsible for the accessibility of vehicles, system control and maintenance of the system. The efficiency of the system was validated using qualified digital temperature logger and therefore its applicability for temperature monitoring during transportation has been approved.
A tour logbook was used to collect time-depended data of transport activities including all specialities during the transportation of products and this has been compared with GPS-assisted recordings.
All temperature variations are detected by the system and can be analyzed by controller and evaluation will be made by the tour leader. After conducting risk assessment the final decision will be rendered to transported products.
Conclusion: An automatic GPS-assisted temperature controlling system is routinely applied for transports by Haema AG. This system is currently unavoidable for uninterrupted transport and evaluation of blood products as well as during their processing according to the GMP guideline. Therefore, this system is of particular importance for daily practice in our company.