P 2.23
Automated Whole Blood Processing with the ATREUS 2C+ System Exceeds Conventional Preparation Method
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Objectives: The aim of the study was to evaluate the in-vitro quality of blood components processed by the ATREUS 2C+ System (AS) in comparison to results achieved using a conventional centrifugation method (CM). Design and Methods: The AS is an automated device for blood component filtration to separate a single unit of whole blood (WB) into a unit of red blood cells (RBCs), a plasma unit and a Buffy Coat (BC). The RBCs are leukoreduced (LR) offline through an integrated filter. Single BC units can be pooled together for further processing into a leukoreduced pooled plasma concentrate (PPC). Using the CM whole blood is LR in-line and the components are separated by centrifugation followed by a blood press process. By using a specific validation protocol, several in-vitro parameters of the blood components were tested. (1) BC-derived LR-PCs made out of 5 BCs: Volume, Factor VIII and residual cell contamination. (2) Fresh blood concentrates (PC). Using the CM whole blood is LR inline and the components are separated by centrifugation followed by a blood press process. By using a specific validation protocol, several in-vitro parameters of the blood components were tested.

Results: The AS enables us to improve the quality of LR-RBCs, LR-FFP and LR-BCPs. The AS is an automated device for blood component filtration to separate a single unit of whole blood (WB) into a unit of red blood cells (RBCs), a plasma unit and a Buffy Coat (BC). The RBCs are leukoreduced (LR) offline through an integrated filter. Single BC units can be pooled together for further processing into a leukoreduced pooled plasma concentrate (PC). Using the CM whole blood is LR in-line and the components are separated by centrifugation followed by a blood press process. By using a specific validation protocol, several in-vitro parameters of the blood components were tested.

Plasma: Volume, Factor VIII and residual cell contamination. Results:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Platelet pool (AS) n=15</th>
<th>EK (AS) n=16</th>
<th>EK (CM) n=50</th>
<th>FFP (AS) n=17</th>
<th>FFP (CM) n=50</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^6/U)</td>
<td>0.02</td>
<td>0.02</td>
<td>0.08</td>
<td>0.0</td>
<td>0.06</td>
</tr>
<tr>
<td>RBC (10^6/U)</td>
<td>1.0</td>
<td>1560</td>
<td>1602</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>PLT (10^9/U)</td>
<td>322</td>
<td>0.7</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>251</td>
<td>254</td>
<td>255</td>
<td>248</td>
<td>246</td>
</tr>
<tr>
<td>pH - day 2 (day 6)</td>
<td>6.7</td>
<td>7.1</td>
<td>24.4</td>
<td>24.9</td>
<td>24.4</td>
</tr>
<tr>
<td>Hb (mmol/l)</td>
<td>0.0</td>
<td>0.04</td>
<td>0.01</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>0.57</td>
<td>0.57</td>
<td>248</td>
<td>246</td>
<td>248</td>
</tr>
<tr>
<td>Hemolysis (%) - 1 day/1 day 42</td>
<td>0.08/0.36</td>
<td>0.04/0.2</td>
<td>0.04/0.2</td>
<td>0.04/0.2</td>
<td></td>
</tr>
<tr>
<td>n=18</td>
<td>4.4</td>
<td>3.2</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All blood products processed with the AS and CM compare with the guide for the preparation, use and quality assurance of blood components. Conclusion: The AS enables us to improve the quality of LR-RBCs, LR-FFP and LR-BCPs.

P 2.24
Reduction of the Plasma Content in Red Cell Units Using a New 5-Port-Technology in a Top/Top Blood Bag System
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Objectives: The major advantage of the Top/Top systems for blood component fractionation is the optimized yield of red blood cells (RBCs) and plasma. However, the preparation process in the standard blood bag systems causes an additional contamination of the packed RBCs with plasma resulting in a loss of plasma for therapeutic and fractionation purposes. A novel Top-Top blood bag design with a 5-port-technology was developed to further improve the purity of the blood components. Design and Methods: Whole blood donations (500 ml) from 37 healthy, randomized donors were paired according to the ABO blood groups, pooled and transferred in equal volumes to the standard and the new blood bag systems. Both blood bag systems were produced from Maco Pharma. Hematocrit, hemoglobin content and cell counts were measured using the Sysmex 2000; protein content was determined by the Bradford method. Results: Blood component preparation using the newly developed bag system led to a significantly lower plasma content in the RBC unit (mean 7.9 ±0.9 g/L) than the preparation in the standard Top/Top system (mean 10.5 ±1.1 g/L). Accordingly, a higher yield of plasma (mean 3.3 ml) in the plasma unit was obtained with the new 5-port-technology. Conclusion: The new Top/Top blood bag system with the 5-port-outlet design provides an optimal yield and separation of RBCs and plasma, fulfilling the requirements of a modern blood bag system. In particular, minimizing the plasma content in RBC units may help to reduce the incidence of transfusion-related acute lung injury (TRALI).

P 2.25
Filtration Times of Leukocyte depleted Red Cell Concentrates
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According to GMP regulations, all steps during processing of blood products should be defined and closely monitored. The objective of this study was to establish a method to record filtration times of leukocyte depleted red cell concentrates (RCC). A newly developed filtration device will be presented and the data obtained during routine processing of RCC. Design and Methods: After implementation of the device, filtration times (FT) were recorded during routine processing of RCC. Whole blood donations were obtained using two different blood collection systems. One was equipped with a hard-shell filter (T3987, Fresenius Hemocare, EK-Inline Filter), the other with a soft shell filter (LQ7241, Maco-Pharma, LRC-5 Filter). In both systems PAGGS-M was used as additive solution. Filtration data spanning one year were analyzed retrospectively. Results: FT using the hard-shell filter were recorded correctly. Readings from the soft shell filter had to be corrected due to the void volume of the filter and the detection principle of the device. FT were 41±11 min. (Mean=SD) for the T3987 system (n=28,635) and 24±4.9 min for the LCR-5 system (n=9,523), respectively. Using the T3987 system, there was a substantial fraction (3.9%) of RCC with FT of over 60 minutes. With the LCR-5 system, the respective fraction was only 0.27%. No evidence of filter damage was found by leukocyte counts of RCC with very short FT. Time dependent analysis of FT of the T3987 system revealed a high variation from day to day, superposed by substantial intermediate term variations. The latter could partially be correlated to the use of different lots of the blood collection systems. FT of the LCR-5 system showed only little variation from day to day as well as between different lots. Conclusion: The filtration device offers a method for studying the kinetics of leukocyte filtration, thus providing a means for a better understanding of the filtration process. Monitoring of filtration times according to GMP regulations is made feasible by this device. Further adaptations in the detection principle will facilitate monitoring of soft-shell filters.

P 2.26
Sterility Testing of Blood Components
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Objectives: Bacterial contamination of blood components is one of the most common and dreaded causes of transfusion-transmitted disease. Here in particular the platelets are affected due to their storage conditions. In Germany the mortality rate related to bacterial contaminated platelets is about 1 per 400,000 platelet units disposed. We report on the data of sterility testing of homologous blood components within routine quality control. Moreover we report on our experiences with testing blood products after adverse transfusion reactions. Methods: 0.4 x the square root of produced blood components per month are tested for bacterial contamination using original blood products. Testing of cellular blood components takes place at the expiry date + maximal 3 days; plasma is tested after production. Bacterial contamination of blood products is tested with BacT/ALERT automated microbial detection system. A 10 ml sample is taken under the laminar flow hood and incubated at 37 °C for 7 days in aerobic and anaerobic media, respectively. Positive samples are tested twice again in the same media. If the positive result can be confirmed the sample is classified as positive. When the differentiated bacteria are identical in both approaches the sample is confirmed positive, otherwise the sample is classified as potentially positive. Results: In the years 2006 and 2007 10,552 homologous blood components were tested for bacterial contamination using original blood products. Testing of cellular blood components takes place at the expiry date + maximal 3 days; plasma is tested after production. Bacterial contamination of blood products is tested with BacT/ALERT automated microbial detection system. A 10 ml sample is taken under the laminar flow hood and incubated at 37 °C for 7 days in aerobic and anaerobic media, respectively. Positive samples are tested twice again in the same media. If the positive result can be confirmed the sample is classified as positive. When the differentiated bacteria are identical in both approaches the sample is confirmed positive, otherwise the sample is classified as potentially positive. Results: In the years 2006 and 2007 10,552 homologous blood components were tested for bacterial contamination. In 2 (0.02%) of 5515 erythrocyte concentrates, in 2 (0.04%) of 2159 platelets and none of 2878 FFPs a bacterial contamination could be confirmed. The identified bacteria were skin commensals. However, in the same period a bacterial contamination in 1.11% of inquiries carried out after adverse transfusion reactions was determined. Conclusion: Our data show that bacterial contaminations are a very rare event. The contamination rate of platelets is 2-fold higher than the contamination rate of erythrocyte concentrates. In agreement with our data, the contamination rate of platelet concentrates is cited to be between 0.02 and 1.2%, depending on the production and method of bacterial testing. The
Concentrates in Plasma (Plasma-PC) were compared. Solution (PAS) and plasma as well. Productivity, donor safety and in vitro separator enables the delivery of standardized platelet products in additive Fenwal (AM) has been previously described. An integrated procedure of the Objective:

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3. Labor 28, Berlin, D, 3Institut für Transfusionsmedizin der Charité – Universitätsmedizin Berlin, D

Objective: Kmod describes an inherited rare phenotype, in which expression of Kell antigens is markedly reduced. So far several different point mutations of the KEL alleles contributing to this phenotype have been described, either existing in homozygosity or in compound heterozygosity with a KEL*2null allele. We report the case of a 70 year old male patient with a novel mutation in the KEL*2 allele. The patient became apparent because of a seemingly K0 phenotype, later corrected to Kmod phenotype. Design and Methods: Serological investigations were carried out in standard gelcard technique. Commercial reference antisera and donor samples containing Anti-Ku and Anti-KL were used for direct antigen investigation. Genetic analysis was performed after DNA-extraction using primers as previously described for PCR and sequencing. Results: The patient’s red blood cells were found to be negative with commercial anti-K, and there were trace agglutinations with anti-k and with anti-Ku. The serum of the patient did not exhibit anti-K, anti-k, or other Kell-antibodies. The results of KEL genotyping showed one of the two parental KEL-alleles to be a KEL*2 (IVS3+1G>A) allele, which is the most frequent Knall allele observed worldwide so far. In the second parental KEL allelic, a single-nucleotide mutation was detected by generic DNA sequencing in exon 19 of the KEL-gene: 2111C>A, predicting an amino-acid change from Proline 704 to Histidine resulting in KEL*2(P704H). Conclusions: Mutations involving Proline are known to cause drastic changes in steric characteristics of primary structures of peptides. It is therefore well conceivable to explain the appearance of a drastically diminished Cellano-expression resulting in a Kmod phenotype by the described mutation in the presented case. The molecular basis of Kmod in this patient is a novel mutation, which has not been described previously.

P 3.02 Anti-S and Rh-Antibodies Are Associated with Red Blood Cell Alloantibodies

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Objective: Alloantibodies (ALLO) to red blood cells (RBC) are frequently associated with autoantibodies (AAB). The association between ALLO specificity and AAB has not yet been described. Design and Methods: All patients and healthy blood donors screened for RBC antibodies at the Charité between 2000 and 2006 were included. The odds ratio (OR) for ALLO in patients with AAB compared to those without AAB was correlated with the OR of general ALLO prevalence in patients with AAB (normalized OR). Results: ALLO were found in 4626 out of 204,330 patients and healthy blood donors (2.3%). The ALLO were associated with AAB in 413 cases (8.9%). Among the specificities, anti-S with a normalized OR of 2.9 was overrepresented. This was most evident in pregnant women who showed a normalized OR of 15.1 for anti-S and AAB. The normalized OR revealed an additional association between Rh antibodies and AAB. No association was found between ALLO to the Kell glycoprotein, Duffy protein, Lewis, or Glycophorin A (MUN) and AAB. Conclusion: The majority of associated ALLO and AAB are directed against neighboring antigens of the Rh complex and Glycophorin B.
**Abstracts**

**P 3.03**

**Shortage of Rhesus-D-Negative RBC: Frequency of Rhesus-D-Immunization**

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**Background:** Demographic change of age distribution in German population causes an increase of patients who need blood transfusions and a decrease of potential blood donors. Therefore the restricted transfusion policy switching the supply of D- patients to D+ RBC in case of emergency, which is based on historical data from D-immunization of healthy volunteers, should be revised. **Methods:** 32 D-negative patients (period 2002–2007, age 17 to 91 years) from surgical wards and intensive care units were retrospectively evaluated for the development of erythrocyte antibodies after transfusion of D+ RBC by a follow-up of antibody screening tests (enzyme-test, IAT, DAT; DiaMed-ID System, DiaMed Germany). **Results:** In total 688 D+ RBC units (mean: 21 [1–170] D+ RBC units) were transfused to the patients after receiving a total of 279 D– RBC units (mean: 12 [0–30] D– RBC units). 11 (34%) patients died in the clinical course due to the underlying disease. In the eligible 21 patients follow up antibody screening tests were performed (mean: 206 days [34–1060] days). Anti-D was detected in 3/21 (14%) patients on day 42/62/132 after initial D+ RBC transfusion (1/4/8 RBC units). All D-immunized patients acquired additionally antibodies such as anti-C/anti-C,-Jk(b)/anti-E,-Wr(a),-Lu(a). In further 2/21 patients IAT revealed reactions without specificity particularly no anti-D. 1/21 patient developed temporarily HTLA-antibodies. 2/21 patients exhibited positive DAT with anti-IgG, without detectable (IAT) antibody in plasma or eluate. Patient records revealed no signs of significant hemolysis. **Conclusions:** In accordance to latest published studies with surgical patients the frequency of anti-D-formation was found to be 14%. Anti-D-formation seems not to depend on the number of D+ RBC units transfused, and there might be an individual disposition for responder and nonresponder of alloimmunization, although the underlying pathomechanism is not yet understood. With consideration of the data, it is justified to change transfusion policy in a less restricted way in times of shortage. To optimize the early selection of D- patients for supply with D+ RBC a scoring system was developed, in respect to age, sex, risk profile and amount of requested RBC. This approach should save RBC resources for the provision of D-patients who have mandatory need of D- RBC.

**P 3.04**

**Mixed Type Autoimmune Hemolytic Anemia: Differential Diagnosis and a Critical Review of Reported Cases**

Mayer B.1, Yürek S.2, Genth R.2, Herziger A.2, Kiesewetter H.2, Salama A.2

1Charité - Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Berlin, D, 2Institute for Transfusion Medicine, Charité - University Medicine, Berlin, D

**Objectives:** Autoimmune hemolytic anemia (AIHA) is usually classified as either warm or cold type. During the last few decades, mixed types have also been described in a number of cases (6–8% of AIHA). However, serological data to support the diagnosis have often been incomplete. We demonstrate that the incidence of mixed type (Mx) AIHA is extremely rare. **Design and Methods:** Between August 1998 and August 2007, all in- and outpatients with detectable warm autoantibodies (WABs) were included in this study. Serologic testing was performed using standard techniques for the detection of antibodies to red blood cells (RBCs). **Results:** From a total of 2194 patients with detectable WABs, only two patients (< 0.1%) developed both, significant WABs and cold agglutinins (CAs) satisfying the criteria for Mx AIHA. However, only one of these patients showed cold and warm hemolysis. Insignificant CAs at temperatures ≤ 24 °C were found in 242 patients. **Conclusions:** There is evidence that the presence of CAs with high thermal amplitude, and WABs may lead to confusion and misdiagnosis in some patients with AIHA. This study demonstrates that Mx AIHA is less common than previously reported.
P 3.05 Evaluation of Immunohematological Methods Using the Capture-Technology on the Galileo Immucor System Pruß A., Pretzel K., Kempe K., Köcher K., Korb M.
Charité-Universitätsmedizin Berlin, Institute of Transfusion Medicine, Berlin, D, Berlin, D

Objectives: Immucor has developed a technology (Capture-R-Technology) for the detection of unexpected IgG antibodies (ABS) by solid phase red cell adherence methods, suitable for high throughput automation. The method is based on a modified solid phase antibody detection system. Blood grouping is performed on microtiter plates (MTP) with regular antisera. Design and Methods: In a comparative study, EDTA blood samples from 403 blood donors and 574 patients were tested on Capture-R-Technology for antibody screening and from 523 blood donors and 574 patients for blood grouping (ABo with direct and reverse typing, Rh factor) and Rh phenotyping (C, c, E, e), Kell, by two determinations. The observed data were directly compared with the results of the Charité blood bank methods: gel centrifugation technique on DiaMed gel cards for antibody screening and Olympus PK 7200 for ABo/Rh blood grouping and Rh phenotyping. Results: 891 of 977 samples for antibody screening test Capture-R-Technology were correct negative. 75 of 84 known antibodies were correct positive in the two techniques. Capture-R-Technology showed 9 false negative results (0.9%). These were confirmed as IgM antibodies, which are not detectable by this anti-IgG technology. 2 of 977 samples (Anti-Jk(a) and Anti-E) were found only by Galileo. These antibodies were later confirmed in complementary tests (DiaMed, Ortho, Biorad). Anti-Jk(a) (Anti-E) was found only by the gel centrifugation method. ABo/Rh grouping showed a complete concordance on common erythrocyte groups between observed results and expected ones. We observed discrepancies in 3 weak D samples, which were detected by the reference method but not by Galileo system. All the results of the C, E, c, E, K tests were accurate in the first and the second determination and showed 100% of concordance between observed results and expected ones. Conclusions: The performed ABS comparative study reveals that Capture-R-Technology test is as sensitive and reliable as the existing gel centrifugation techniques for the detection of clinically relevant IgG antibodies. Galileo provides a full automation system (equipment and reagents) and is reliable and safe for ABo/Rh blood grouping and Rh phenotyping, excluding the tests of weak D on MTP.

P 3.06 The Performance of Three Different Tube IAT Methods and the Microplate Test System Solidscreen II in the Detection of Red Cell Alloantibodies Weisbach V., Teufel M., Bredehoff A., Strobel J., Eckstein R.
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Objectives: To evaluate whether there is a difference in sensitivity of the detection in red cell alloantibodies (ab) between three different tube IAT methods and the Solidscreen II. Previously, we and others had shown a lower sensitivity of the tube LISS-additive-IAT compared to microcolumn and microplate tests. Some critics argued that this difference in sensitivity would not have been observed if polyethylene glycol (PEG) or LISS-suspension would have been used in the tube IAT. Design and Methods: In a comparative study, we tested 379 sera which were previously demonstrated to contain red cell ab by means of the Scangel (Bio-Rad Laboratories GmbH, München, Germany) and the Immucor Capture-R Ready-Screen (4) (Immucor medizinsche Diagnostik GmbH, Roedermark, Germany) in our routine laboratory. In the present study, the following tests were performed strictly in parallel: tube LISS-additive IAT, tube LISS-suspension IAT, tube PEG IAT (all three manually) and the Solidscreen II (automated using the Tango Optim) (Bistest Medical Diagnostics GmbH, Dreieich, Germany). Tests were performed simultaneously by a single person on freshly thawed sera which had been cryopreserved at −20°C. Results: The detection rate of ab which are assumed to be clinically significant (cs-ab, for example Rhesus, Kell, Duffy or Kidd ab) (n=339) was: tube LISS-additive IAT: 230 (67.8%), tube LISS-suspension IAT: 222 (65.5%), tube PEG IAT: 241 (71.1%), Solidscreen II: 290 (85.5%).The detection rates of 40 ab which are assumed to be of minor clinical significance (ms-ab, M, N, P1 or Lewis ab) was: tube LISS-additive IAT: 15 (37.5%), tube LISS-suspension IAT: 16 (40.0%), tube PEG IAT: 20 (50.0%), Solidscreen II: 22 (55.0%). Conclusions: The sensitivity of all three methods in the detection of cs-ab was markedly inferior to the sensitivity of the Solidscreen II. The sensitivity of the tube IAT is highest in the PEG IAT and lowest in the LISS-suspension IAT. Specificity can not be tested with the design of the present study. No single test system of the systems used in this study can detect all ab which can be detected by the combination of two different other test systems.

P 3.07 Typing of Neutrophil Antigens (HNA) to Establish a Screening Panel to Prevent Immune Transfusion-Related Acute Lung Injury (TRALI) Grabowski C., Jorks S., Borowy A., Reil A., Krohl H.
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Purpose: Granulocyte-reactive antibodies are involved in several diseases. Transfusion-related acute lung injury (TRALI) is one of the most dangerous hazards of transfusion and can be induced by granulocyte-reactive alloantibodies. Prospective screening of plasma-containing blood products (platelet concentrates, fresh frozen plasma) for granulocyte antibodies is one of the effective measures to reduce the incidence of TRALI. We, therefore, established a screening panel of well characterized granulocyte donors. Methods: A total of 138 unrelated healthy blood donors were included into the study. 110 donors were typed for the HNA-1, HNA-4, and HNA-5 polymorphisms by molecular methods. In 105 donors serologic HNA-2 and HNA-3 typing was performed. DNA was extracted from EDTA-anticoagulated blood using chemagic Magnetic Separation Module I (Chemagen AG, Baesweiler, Germany) or Genom6 workstation (GenoVision Vienna, Austria). Genotyping was performed by PCR-SSP (Bux et al. 1995/1997, Steffensen et al., 1999, Sachs et al., 2004/2005) with modified PCR conditions. Serologic typing was performed by granulocyte adherence and immunofluorescence tests with well defined antisera. Results: The following antigen frequencies were found in 110 genotyped donors: HNA-1a: 65 (59.1%), HNA-1b: 93 (84.6%), HNA-1c: 7 (6.4%), HNA-4a: 109 (99.1%), HNA-4b: 26 (23.6%), HNA-5a: 101 (91.8%), and HNA-5b: 55 (50.0%). The genotypes were: 1a+b-c-: 13 (11.8%), 1a+b+c-: 45 (40.9%), 1a+b+c+: 45 (40.9%), 1a+b-c+: 4 (3.6%), 1a+b+c+: 3 (2.7%), 4a+b+c+: 84 (76.4%), 4a+b+c+: 25 (22.7%), 4a+b+: 1 (0.9%), 5a+b-: 55 (50.0%), 5a+b+: 46 (41.8%), 5a+b-: 9 (8.2%). The gene frequencies were calculated: 1a: 0.373, 1b: 0.627, 1c: 0.032, 4a: 0.877, 4b: 0.123, 5a: 0.709, 5b: 0.291. It was assumed that the HNA-1c allele was always located on the same chromosome as the HNA-1a. From 105 genotyped donors 105 (100%) carried the HNA-2a antigen and 102 (97.14%) were HNA-3a positive. Conclusions: We typed our blood donors for HNA alleles as more accurate selection of panel cells is possible. Typed panel cells can be employed for the screening of plasma-containing blood products for granulocyte antibodies to reduce the incidence of TRALI. The HNA gene frequencies of the investigated blood donors from Sachsen-Anhalt corresponded well to the gene frequencies described earlier in the German population.

P 3.08 Cellular Sources for Elevated Tumor Necrosis Factor alpha Cytokine Levels in Carriers of TNAlpha Risk Allele Wiezens K., Kissel K., Breitkamp K., Lang C., Nockher A., Hackstein H., Bein G.
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Objectives: Carriage of the TNF rs1800629 A allele (-308 A/G) has been recently identified to be associated with the development of sepsis syndrome, fatal outcome, and incremented TNF alpha plasma concentration in severely traumatized patients (Menges et al. 2008). Based on these results, the aim of our present study was to elucidate the cell type responsible for increased TNFalpha release in carriers of TNF -308 A allele. Design and Methods: Healthy blood donors between 18 and 45 years of age were randomly selected. Following isolation of distinct peripheral blood cells, we examined different ligands and costimuli to determine their potential to stimulate a TNFalpha release in vitro. In the next step, TNFalpha expression of distinct blood cells and monocyte-derived macrophages triggered with LPS and IFNalpha was measured in ELISA assay. Genotyping of TNFalpha promoter polymorphism rs1800629 of the individuals was performed using a TaqMan technique in an independent analysis. The possible association of TNFalpha promoter polymorphism rs1800629 (-308 A/G) and TNFalpha phenotype of the individuals was calculated in statistical analysis (two-tailed Mann-Whitney-U-test). Results: Using ELISA assay, TNFalpha cytokine production was detected in supernatants of different stimulated peripheral blood cells and monocyte-derived macrophages in variable amounts. CD14+CD16+ monocytes were identified as the major source of TNFalpha release in peripheral blood cells, while neutrophils revealed very little amounts of TNFalpha. An association between carriage of the risk allele...
TNFalpha rs1800629 A and the phenotype high TNFalpha expression could not be verified neither in distinct human peripheral blood cells nor in monocyte-derived macrophages as representatives of tissue resident cells. **Conclusions:** Our findings indicate that high TNFalpha levels detected in blood shortly after multiple traumas are not the result of a release from circulating blood leukocytes and macrophages. In contrast, tissue resident cells like mast cells are good candidates for elevated TNFalpha cytokine shortly after trauma. Since TNFalpha rs1800629 A has been associated with high TNF levels, it is of high clinical importance to identify the cellular source of TNFalpha to improve anti-inflammatory therapy and to develop new intervention strategies for carriers of the risk allele.

**P 3.09** Tango Optimo versus Galileo – a Large-Scale Comparison of the Routine Performance of Two Fully Automated Systems in Blood Typing and the Detection of Red Cell Alloantibodies

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**Objectives:** To compare the performance of two fully automated immuno-hematology systems under routine blood bank conditions. **Design and Methods:** We tested in parallel 3104 blood types (A1B0,D,C,e,E, and K) and 5206 red cell alloantibody (ab) screening tests using the Tango Optimo (Biomet Medical Diagnostics GmbH, Dreieich, Germany) and the Galileo (Immucor medizinische Diagnostik GmbH, Roedermark, Germany). The ab detection system in the Tango was the Solidiscore II (Galileo: Capture-R Ready-Screen (4)). All tests were performed automatically. When necessary, the automated reading was corrected by the investigator. Discrepant results in ab screening were controlled in the Diamed Gel System (Diamed Deutschland GmbH, Ottobrunn, Germany). Blinded replicate tests were performed using 60 aliquots of a negative and 60 samples of a weak positive plasma. **Results:** Both systems performed very good in the 3102 blood types, we observed no false results. In the Tango, in 73 (2.4%) of the blood types single reactions had to be repeated manually in the tube test (Galileo:103 (3.3%)). Reasons for repeated tests were inconclusive results in serum testing (Tango 46 (1.5%), Galileo 56 (1.8%), pipetting errors (no serum or too much red cells) (23 (0.7%) versus 25 (0.8%) and discrepant results in double testing of Rhesus or Kell antigens (4 (0.1%) versus 22 (0.7%). In red cell ab screening, both systems together detected 54 ab which are assumed to be clinically significant (a-aab, for example Rhesus, Kell or Kidd ab), (Tango 40, Galileo 51). The Tango Optimo showed 9 ‘false’ or unwanted positive results (inconclusive positive results, only HLA alloantibodies could be detected, IgG autoantibodies without hemolysis in the patient) in ab screening, the Galileo showed 35 of these ‘false’ or unwanted positive results. The Tango showed 42 (0.81%), the Galileo 65 (1.25%) unwanted positive autoantibodies (no sign of auto- or alloimmune hemolysis in the patient). Blinded replicate tests showed 3 false positive and 1 false negative result in the Galileo, in the Tango Optimo all replicates were diagnosed correctly. **Conclusions:** The performance of both systems blood typing was excellent with a small advantage for the Tango Optimo. In screening for red cell ab, the Galileo showed a higher sensitivity, while the specificity was considerably better in the Tango Optimo.

**P 3.10** A Two-Steps-Strategy for Testing Samples Weakly Reacting with Anti-D: Review of Results

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**Objectives:** In our centre all new samples of patients and blood donors that are tested for the Rhesus D antigen and show weak reactions in the serology are immediately tested with a serological identification kit for partial Rhesus D to exclude some of the most frequent partial D phenotypes and on the following working day undergo a molecular testing. Aim of this study was to compare the findings of the serological and the molecular testing and to evaluate if the results obtained with the serological identification kit could reliably guide transfusion decisions. **Design and Methods:** From April 2000 to December 2007 361 patients and 206 blood donors (total 567) were tested for the Rhesus D antigen according to German guidelines. The serological identification screening for partial Rhesus D was performed with the D-Screen, Ds gauge, Loos, France and the molecular testing was carried out with the BAGene, BAG, Lic, Germany or with the Ready Gene, Inno-Train, Kronberg, Germany. According to the results of the serological identification kit two groups of samples were defined: the one in which a partial D was detected (group A) and the one in which no specific result was shown (group B). The latter was assumed to identify weak D. The findings of each group were compared with the results of the molecular testing. **Results:** Of the 223 samples in group A 59 (26.5%) were confirmed to be partial D, 125 (56.1%) were shown to be weak D and 39 (17.5%) were neither partial nor weak D. Of the 344 samples in group B 319 (92.7%) were confirmed to be weak D, 5 (1.5%) were shown to be partial D and 20 (5.8%) were neither partial nor weak D. With the molecular testing clear results were obtained in 508 samples (444 weak D and 64 partial D), while no weak and no partial D could be identified in the remaining 59 samples. **Conclusion:** Only a quarter of the partial D found with the serological identification screening are confirmed with the molecular testing. Moreover 92% of samples in which no partial D was defined appear to be weak D. These findings can guide transfusion policy in case there is no time to wait until the results of the molecular testing are available. With the molecular testing 90% of the samples reacting weakly with anti-D could be safely defined in their specificity. The remaining 10% identify probably a normal D, although a few samples could also be a rare variant, which is undetectable with the test in use.

**P 3.11** Severe Autoimmune Hemolytic Anemia of an 18-Month-Old Boy with Auto-Anti-E

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4Institute of Immunohematology, University of Düsseldorf, D,
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**Purpose:** The occurrence of autoimmune hemolytic anemia (AIHA) in children and adolescents is rare. The exact incidence is unknown, but the rate is estimated to be less than 1/100,000 individuals below the age of 20 years. We report the rare case of a severe AIHA in an 18-month-old boy caused by auto-anti-E. **Case report:** An 18-month-old boy was admitted to the hospital with clinical signs of vomiting, fever, abdominal pain and icteric sclera. The initial blood tests showed severe anemia (Hb 21 g/l, Hct 7.6 l/l, reticulocytes 270.000), signs of hemolysis (LDH 528 U/l), haptoglobin <0.07 g/l and leukocytosis (21000/μl). Pretransfusional antibody screening and the cross-matching with A Rh positive (ccD.Ee) RBC (red blood cells) in the hospital laboratory were negative, the direct antiglobulin test (DAT with anti-IgG) was positive. The positive DAT in the initial test was not further investigated and the boy was transfused with A Rh positive (ccD.Ee) RBC. Four days after the first transfusion, antibody screening in the hospital laboratory became positive and specimens were sent to our regional immunohematologic reference laboratory. Child: A Rh positive (ccD.Ee) serology test, DAT positive (monospecific anti-IgG), auto-Anti-E (reactive in indirect antiglobulin test) in plasma and platelets. Autoimmune hemolytic anemia due to auto-anti-E was diagnosed. A variant of the E antigen was excluded by molecular analysis. Two compatible RBC concentrates (A Rh positive, ccD.ee) were transfused and the child was released from the ward in stable condition after additional treatment with immunoglobulin. **Materials:** Labo-
ryatory tests were performed with blood specimens (EDTA). ABO and Rhesus blood grouping were tested by tube technique. Antibody screening, identification tests, and DAT were performed by using the gel card method (DiaMed). RBC-bound antibodies were eluted by an acid elution kit (Dia-
Cidel, DiaMed). A variant of the E antigen was excluded (NSTOB, Olden-
burg) by multiplex PCR and sequencing (AB-Prism 310, Applied biosys-
tems). **Conclusions:** Due to the fact that positive DAT with anti-IgG in the initial test of the hospital laboratory was not further investigated, auto-anti-E initially was not detected and ccD.ee red cells were transfused. This case shows that the further investigation of a positive DAT has to be done at any signs of hemolysis and anemia of unknown origin. The timely investigation before the first transfusion can avoid the transfusion of incompatible red cells, the boost of the antibody and the rapid decrease of the Hb after the first transfusion.

**P 3.12** Haplotype-Specific Extraction: A Simple and Efficient Method for Comprehensive Polymorphism Analysis

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1QIAGEN GmbH, R&D, Hilden, D,
2QIAGEN GmbH, Hilden, D

**Determination of allelic polymorphism combinations is required for many different genotyping applications. Several methods including sequence-
specific priming (SSP) or sequencing-based typing (SBT) can be employed.**
Abstracts

P 3.14 Application of the Particle Gel Agglutination Assay in the Typing of Single Human Leukocyte Antigens

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Purpose: The majority of laboratories utilize flow cytometry for typing of single human leukocyte antigens, for example HLA-B27. The microlymphocytotoxicity test (LCT) and polymerase chain reaction (PCR)-based techniques may also be used, but these techniques are laborious and cannot be implemented in most laboratories. Recently, we described a particle agglutination assay (PaGIA) for HLA-B27 typing. This test has been modified and extended to include the detection of HLA-A2 and HLA-B7. Design and Methods: Superparamagnetic streptavidin particles were coated with biotinylated monoclonal antibodies to HLA-A2, HLA-B7 and HLA-B27. Anticoagulated whole blood samples from healthy blood donors (n = 118) with known HLA patterns were incubated with monoclonal antibody coated particles, transferred into a standard ID-gel card, and subsequently centrifuged. Samples were evaluated macroscopically, with antigen-positive samples resulting in a visible agglutination reaction. Results: The results obtained were found to be in complete agreement with the known HLA pattern of the donors. A clear distinction could be made between all positive and negative samples tested. Fifty-seven samples were found to be positive for HLA-A2 (48%), 26 samples for HLA-B7 (22%), and five samples for HLA-B27 (4%). Conclusion: The described PaGIA is suitable for rapid typing of single antigens. The advantages of this assay include its simplicity and potential to be implemented in any routine laboratory.

P 3.15 Prospective RHD Genotyping in Patients with Weak Agglutinogens in Serologic RhD Typing

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Purpose: The Rh system is the most immunogenic and clinically important protein-based blood group system. Single point mutations in the transmembrane region lead to altered RhD protein expression (weak D). Other mutations or hybrid genes between RH and RHCE are responsible for partial Ds. Since some patients with partial or weak D can form anti-D they should be well characterized. Here, we systematically analyzed the RHD genes in all patients with atypical reactions upon phenotyping. Methods: RhD phenotyping of patients of the immunohaematologic laboratory in Dessau was performed by tube agglutination technique with two different monoclonal anti-D antibodies that did not react with D category VI. Over a period of 18 months, all samples that showed agglutinations of ≥1/2 were further analyzed by genotyping. Results: A total of 117 patients with weak agglutinogens in serologic RhD typing were analyzed. In 115 patients (98.3%) a weak D (n = 111, 94.9%) or partial D (n = 4, 3.4%) was detected. In detail, the following RHD genes were identified: weak D type 1: 65 (55.4%), weak D type 2: 29 (24.7%), weak D type 3: 12 (10.2%), weak D type 4: 1, weak D type 42: 1, cat VII: 2 (1.7%), DHHM-D, wild type D sequence: 2 (1.7%). Interestingly, three new weak D alleles: weak D type 61: 1, weak D type 63: 1, weak D type 64: 1 and one new hybrid allele (RHD-CE-D) were found. Conclusion: Weak reactions in RhD phenotyping were caused by weak or partial RHD alleles in 98.3% of the patients. Eight different weak D types and three different partial RHD alleles were found. However, no clear association between strength of agglutination and with a certain weak or partial RHD allele was detected. We think that a ≥1 agglutination in the tube technique is a reasonable threshold to initiate RhD genotyping. In addition, our strategy allowed the identification of four new RHD alleles.

P 3.13 Comparison of Iso-Haemolysin and -Agglutinin Testing of Platelet Donors in the Context of the Prevention of Haemolytic Transfusion Reactions

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Background: Testing for iso-haemolysins (IH) in platelet donors is mandatory in Switzerland. However, the few case reports of haemolytic reactions after minor incompatible platelet transfusion are related to high iso-agglutinin (IA) titres (>1:50 or >1:100). Data about a correlation between IH and IA titres are lacking. Methods: Testing for IA and IH in parallel in 2 different methods (IH1, IH2) by tube technique were performed in consecutive platelet donors. IH1: serum dilution 1:4 with addition of AB plasma as complement source (single titre analysis); IH2: classical titration without complement source. Specificity (Spec), sensitivity (Sens), positive (PPV) and negative (NPV) predictive values of IH1 (cut off >1:4) and IH2 (cut off >1:12) in detecting or excluding IA titres >64 and >128 are calculated. Results: Our results show 71/188 donations (37.8%) with IH1/>64 and 34/188 (18.1%) with IA titres >128. The correlations with the specified cut offs of IH1 and IH2 are summarized in the table.

<table>
<thead>
<tr>
<th>(%)</th>
<th>IH1/&gt;64</th>
<th>IH1/&gt;128</th>
<th>IH2/&gt;64</th>
<th>IH2/&gt;128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sens</td>
<td>45.1</td>
<td>55.9</td>
<td>29.6</td>
<td>41.2</td>
</tr>
<tr>
<td>Spec</td>
<td>89.7</td>
<td>83.8</td>
<td>90.6</td>
<td>88.5</td>
</tr>
<tr>
<td>PPV</td>
<td>72.7</td>
<td>43.2</td>
<td>65.6</td>
<td>43.7</td>
</tr>
<tr>
<td>NPV</td>
<td>72.9</td>
<td>89.6</td>
<td>67.9</td>
<td>87.2</td>
</tr>
</tbody>
</table>

Conclusions: Despite 1/3 false positive results (low PPV), IH1 and IH2 detect only 1/2 of the donations, using the accordingly defined cut off IA titres (low Sens). Our preliminary data suggest that IH testing may be of limited significance in the discrimination of high titre IA donations. But its value in platelet donation screening will be further assessed after completion of the data collection, including analyses for additional IH cut offs, different donor ABO blood groups and donor follow up.
Discrimination of Null and Low Expression of HLA by Secretion Assays Using Soluble HLA-A*3014L

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The disruption of disulfide bridges can abolish or decrease expression of HLA class I molecules on the cell surface. Such disulfide bridges are formed by cysteine residues between aa positions 101/164 (α2 domain) and 203/259 (α3 domain). Ten HLA-A molecules have been described to be affected by sequence alterations in codon 101, 164, 203 or 259. All of these variants result in null alleles except for A*3014L and A*3211Q. HLA-A*3014L shows a transversion at nucleotide position 563, resulting in the substitution for cysteine to serine at position 164 of the mature polypeptide. By standard microlymphocytotoxicity assay HLA-A30 was not detectable. To verify low or non expression of the allele, we cloned soluble A*3014L and A*3001 into a eukaryotic expression vector and transfected K562, C1R and HEK293 cells. In aneandand TNF-αaddition we investigated the effect of the cytokines IFN-γ in combination on the expression pattern of the recombinant HLA. Soluble HLA was measured in the cell culture supernatant using a w6/32 and anti-β2-microglobulin sandwich ELISA. The mRNA transcripts of both alleles were determined by real-time RT-PCR. We were unable to detect A*3014L in the supernatant of transfected cells. Addition of IFN-γ and TNF-α slightly increased A*3014L secretion to a detectable amount, whereas the expression of A*3001 was increased up to 1000fold. In contrast, no difference was found in the increase of he mRNA levels of both the A*3014L and A*3001 as assessed by real-time RT-PCR. Because the increase in the mRNA level of A*3014L was comparable with A*3001, the protein expression defect is likely caused by an incorrect disulfide bond formation in the α2 domain. In conclusion, exposing the cells to cytokine stress showed to be helpful for discrimination of low- and non-expressed alleles. This discrimination may be important in allotransplant settings, where a cytokine storm follows pretransplant myeloablative as well as posttransplant immunosuppressive treatment.
P 4.04

Long-Term Storage of Peripheral Blood Progenitor Cells (PBPC) at –80 °C Leads to a Decrease of the Haptenomimetic Potential Compared to Storage over Liquid Nitrogen

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Objective: Usually, autologous PBPC for therapeutic use are stored below -170 °C directly in or over liquid nitrogen or at -80 °C in mechanical refrigerators. The aim of this study was to investigate the above-mentioned storage temperatures with regard to leukocyte (WBC) recovery, membrane integrity (trypan blue exclusion) and colony forming potential (CFU) in semisolid media. Clonogenic capacity (%)

Fifty-two frozen reference samples (1.5 ml) of PBPC concentrates obtained from 13 patients. The samples contained 10% (v/v) DMSO and had been cooled down to -90 °C at 1.5 °C/min in a controlled rate liquid nitrogen operated freezer. After storage for 3 and 6 months at -80 °C and below -170 °C, respectively, they were thawed within 3 min using a shaking water bath. Terminology: group 1: <–170 °C/3 months, group 2: –80 °C/3 months, group 3: –80 °C/6 months, group 4: –80 °C/6 months. Paired samples with identical storage times (but different storage temperatures!) were thawed together and analyzed in parallel by the same person on the same day. This may be the major reason why others, to date, have failed to detect differences in WBC recovery, membrane integrity and colony forming potential of PBPC stored at –80 °C versus below –170 °C.

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45+/7-AAD- (%)</td>
<td>CD45+/7-AAD- (%)</td>
</tr>
<tr>
<td>81 (25–100)</td>
<td>92 (80–97) *</td>
</tr>
<tr>
<td>CD45+/- (AAD) (%)</td>
<td>CD45+/- (AAD) (%)</td>
</tr>
<tr>
<td>97 (0–100)</td>
<td>98 (74–100)</td>
</tr>
<tr>
<td>NC trypan blue negative (%)</td>
<td>NC trypan blue negative (%)</td>
</tr>
<tr>
<td>72 (12–96)</td>
<td>90 (74–95) *</td>
</tr>
<tr>
<td>Functionality:</td>
<td></td>
</tr>
<tr>
<td>n=16</td>
<td>n=12</td>
</tr>
<tr>
<td>Clonogenic capacity (%)</td>
<td>Clonogenic capacity (%)</td>
</tr>
<tr>
<td>6 (0–52)</td>
<td>9 (5–64)</td>
</tr>
</tbody>
</table>

* p<0.05

P 5 Experimental Cellular Therapy

P 5.01

Dendritic Cell Vaccination Therapy of Glioblastoma

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Objectives: Dendritic cell (DC)-based immunotherapy is a promising approach in glioblastoma. Patients are vaccinated with tumor-antigen loaded...
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DC. After vaccination, the DC have to migrate to lymph nodes and there to induce specific anti-tumoral T-cell responses. We have established such a protocol and report here on initial results. Methods: Autologous tumor material is obtained from surgery. After minimal mechanical dissection, necrosis is induced and lysates are prepared. Monocyes enriched from leukapheresis products are used as starting population for DC culture. Successive generation of immature and mature DC for tumor lysis-loading and vaccination, respectively, are generated in a two-step culture system. Five vaccines are prepared and applied weekly by intradermal injection. Results: 24/24 tumor samples processed yielded a preparation of sterile, avital tumor cell lysates. For 14/24 of these preparations, diagnosis of GBM and a tumor cell content of at least 70% was confirmed neuropathologically. For 6 patients, vaccines were produced. From leukapheresis products containing 1.61×10^10 leukocytes with a monocyte frequency of 24.2±1.5%, monocyes were enriched to 97.6±0.7% CD14+ purity. Immature and mature DC obtained after 6 and 9 days of culture showed typical morphotypic and functional properties of bona fide DC, including induction of anti-tumoral responses in vitro. Overall, for each patient 5 vaccines containing 3.8×10^7 DCs were prepared and administered as final purity of DC83+ mature DC of 87.6±2.4%. Vaccination was well tolerated and severe adverse events have not been observed. In two patients there was clear evidence of anti-tumoral immunity induced by the vaccines. Two of the patients progressed and died early. One patient vaccinated after 3rd recurrence died 260 after the last resection (668d after 1st resection). One patient vaccinated between the last two resections (668d after 1st resection). Five vaccines are prepared and applied weekly by intradermal injection.

Objectives: To evaluate the immunostimulatory capacity of DC vaccines generated in a two-step culture system. Results: For 6 patients, vaccines were produced. From leukapheresis products containing 1.61×10^10 leukocytes with a monocyte frequency of 24.2±1.5%, monocyes were enriched to 97.6±0.7% CD14+ purity. Immature and mature DC obtained after 6 and 9 days of culture showed typical morphotypic and functional properties of bona fide DC, including induction of anti-tumoral responses in vitro. Overall, for each patient 5 vaccines containing 3.8×10^7 DCs were prepared and administered as final purity of DC83+ mature DC of 87.6±2.4%. Vaccination was well tolerated and severe adverse events have not been observed. In two patients there was clear evidence of anti-tumoral immunity induced by the vaccines. Two of the patients progressed and died early. One patient vaccinated after 3rd recurrence died 260 after the last resection (668d after 1st resection). One patient vaccinated between the last two resections (668d after 1st resection). Five vaccines are prepared and applied weekly by intradermal injection.

Conclusion: Vaccine production in glioblastoma patients is feasible and early results of vaccination, including induction of anti-tumoral immunity, are promising.

P 5.02 Evaluation of a Tubing System with an Incorporated DMSO-Resistant Sterile Filter for Cryopreservation of Cellular Products outside of Cleanroom Facilities

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Objectives: Processing of cellular therapeutics in an open system according to GMP guidelines requires a cleanroom grade A with surrounding grade B conditions. In a former study with a different system but the same sterile filter a MIS of incubation. In the NaCl series, 35 to 50% of the spiked bacteria could be detected after spiking and before filtration. After passing the sterile filter, bacterial growth was no longer detectable. Bacteria spiked in DMSO showed a time dependent decline of growth with a complete growth inhibition after 5 minutes of incubation. In the NaCl series, 35 to 50% of the spiked bacteria could be detected after spiking and before filtration. After passing the sterile filter, bacterial growth was no longer detectable. Conclusions: Although certainly most of the potential bacterial contaminants of hematopoietic progenitor cell (HPC) grafts are not viable after treatment in 99% DMSO (similar results were obtained for S. epidermidis as published recently) the sterile filter offers a security measure to eliminate potential contaminants. Additional validation experiments of the systems with media fill runs simulating all processing steps of HPC grafts are the next task.

P 5.03 Apoptosis of Lymphocytes and Monocytes during Short-Term Storage of MNCs Obtained from LRS Chambers of the Trima Accel Cell Separator

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Objectives: There is great interest to obtain small amounts of mononuclear cells (MNCs) for experimental purpose, to test T-cell or dendritic cell (DC) function, to optimize DC culture. Recently, this new source of peripheral mononuclear cells (PBMCs) obtained from the leukocyte reduction system chambers (LRSs) of the Trima Accel apheresis machine was described. Methods: The LRSs of Trima Accel of nine (n=9) plateletspheresis procedures were analysed for the apoptosis rate of lymphocytes and monocytes. The blood volume of each LRS was transferred into a plastic bag (150 mL, PVC). The bags were stored for 72 hours (RT, in the dark), no agitation) and the apoptosis of lymphocytes and monocytes was analysed one hour after production, after 6, 24 and 72 hours. A blood count was performed with the ADVIA TM 120 analyser (Siemens, Germany). The lymphocytes and CD14-positive monocytes were analysed by flow cytometry (FACS Calibur, BD, USA). Apoptosis was analysed by Annexin-V-staining (FACS Calibur). Irreversible cell death of cells was shown by the 7-AAD uptake of cells. Results: The mean leukocyte (WBC) concentration of LRS chambers was 93.0±32.2 WBC per microliter. We found 64.2±9.6% lymphocytes (LYM) and 11.2±5.7% monocytes (MON) in the LRSs. The Annexin-V-positive cells were found as follows: one hour after production (LYM: 24.7±12.8; MON: 48.5±8.8), after 6h (LYM: 22.0±9.5; MON: 34.0±10.5), after 24h (LYM: 23.1±10.1; MON: 29.9±5.9), after 48h (LYM: 23.4±5.7; MON: 50.8±8.4) and after 72h (LYM: 22.3±12.8; MON: 48.5±8.8). Only Annexin-V-positive monocytes differed significantly between the different periods of storage. Conclusions: Lymphocytes obtained from the LRSs of the Trima Accel machine and stored in PVC-bags are stable within a period of at least 72 hours. The apoptosis, measured by Annexin-V-staining, did not differ significantly between day of production and Day 3 of storage. However, CD14-positive monocytes showed significant differences during the storage period. The percentage of Annexin-V-positive cells was high after production, decreased until 24h of storage and increased between Day 2 and Day 3 of storage. If monocytes obtained from LRSs are used for research application, the cells should be stored for 6 to 12 hours at RT before usage.

P 5.04 Bacterial Contamination of Hematopoietic Progenitor Cell Grafts Leading to Detection of a Contaminated Port System in a Patient with NHL

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Purpose: Essential basis for harvest, production and release of hematopoietic progenitor cell (HPC) products of sufficient quality is a well established quality management system with a timely exchange of information between the laboratory and clinic unit. We report a case of a patient with a Non-Hodgkin lymphomas (NHL) of the central nervous system with bacterial contaminated HPC products caused by a contaminated port system. Case Report: HPCs were collected by apheresis from a 46-year-old female patient with NHL and subsequently three products were cryopreserved. Sterility testing results showed that all products were contaminated with Staphylococcus epidermidis. Retesting reference tubes from all products confirmed the results. The products were not released and an investigation for errors during processing was initiated. The positive microbial results were reported to the clinician of the patient leading to further examinations of the patient. Swabs and speciments for blood culture were taken from several sites of the patient. All samples taken from the thoracic port system were contaminated with Staphylococcus epidermidis at a low level. As consequence the patient was treated with Vancomycin and the port system was removed. After a second cycle of chemotherapy and G-CSF administration, a new apheresis was performed and a sufficient amount of HPCs for autologous transplantation was harvested and cryopreserved. Microbial testing of the products revealed that all products from this apheresis series were sterile and the products were released. Conclusions: The reported case demonstrates that trouble-shooting of contaminated HPC products should include all process steps ranging from the patient to the final processing of the product. In the reported case the
Abstracts

P 5.05
Transfusion Lin-CD117+ Bone Marrow Cells in Lethally Irradiated Mice
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2Charles University in Prague, Medical Faculty and Teaching Hospital in Hradec Králové, CZ, University of Defance, Medical Faculty, Hradec Králové, CZ

Experiments presented here were aimed at the description of hematopoiesis repair and in vivo homing of transplanted separated lin-CD117+ bone marrow cells after whole-body lethal irradiation at LD 90. ROSA 26 mice were used as donors of marrow cells for transplantation [B6; 129S/Gt (ROSA)26Sor] were tagged with lacZ gene and F2 hybrid mice [B6129SF2/J] were used as recipients of bone marrow transplanted cells. Hematopoietic repair was provided by transplantation both suspension of whole bone marrow cells and isolated lin-CD117+ cells. Mice survived up to thirty day after irradiation. We demonstrated that transplantation of suspension of whole bone marrow cells led to faster recovery of CFU-GM in a bone or spleen compared with whole bone marrow transplantation. The difference in CFU-GM numbers in the bone marrow and spleen found on day 30 posttransplantation has no influence on survival of lethally irradiated mice (30 days follow-up).

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P 6.8/01
Chemical Chaperones as a New Concept to Improve Factor VIII Secretion
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Current substitution therapy for patients with hemophilia A is primarily based on the administration of recombinant FVIII (FVIII:Fc) concentrate, conventionally produced in mammalian cells. In such cell factories, secretion of recombinant FVIII is still inefficient due to failure in intracellular FVIII-trafficking. An increasing number of studies showed that low-molecular-weight compounds called ‘chemical chaperones’ (CC) can restore trafficking of misfolded proteins in storage diseases. CC may be an attractive tool to help to reduce the risk for TRALI by exclusion of potentially sensitized donors.

We have previously described a dose prediction formula for the MNC collection setting on Cobe Spectra, which does predict harvest dose more accurately than using peripheral CD34+ count alone (Journal of Clinical Apheresis 23(1):21–22). We had hoped that it would be possible to perform a similar exercise for the Auto-PBSC collection setting on Spectra. However, it appears that collection efficiency is significantly more variable for the Auto-PBSC than for the MNC setting. This is somewhat counter-intuitive, as the Auto-PBSC is a highly automated procedure, while MNC requires continuous interface monitoring by the operator. However, collection efficiency during the Auto-PBSC procedure appears significantly dependent on initial data entry for a large number of variables (including patient mononuclear cell count, hematocrit, chase volume, harvest volume and number of collection cycles), while the MNC procedure is much less dependent on initial data entry and therefore has more predictable efficiency.

P 6.8/03
Comprehensive Antibody-Screening in Apheresis Donors with Suspected Risk for Induction of TRALI
Fürst D.1, Pabst K.2, Reinhardt P.2, Mayr-Wohlfart U.1, Kress R.1, Koemer K.1, Wiesnet M.3, Schrenzeimer H.3, Mytilineos J.1
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TRALI is a serious complication in transfusion therapy with a high mortality rate. Alloreactive HLA and HNA antibodies are implicated in an immune-mediated pathway triggering non-cardiac pulmonary edema. Rapid, reliable and cost-effective antibody-screening for HLA and HNA-antibodies could help to reduce the risk for TRALI by exclusion of potentially sensitized donors.

Female apheresis donors with history of pregnancy or transfusion (n=104) and male donors with transfusion history (n=22) were screened for HLA Class I and Class II as well as HNA 1a, 1b, 1c, 2a and 4 antibodies with a commercial, Luminex based method. Consistency of HLA antibody screening was assessed by concurrent ELISA Class I and Class II testing with a commercial kit.
Platelet glycoprotein specific autoantibodies to platelets were detected in both patients, and remained detectable in patient no. 1 following treatment with low dose prednisolone. An increase in the platelet count from 30 × 10^11/ml to roughly 90 × 10^11/ml was observed. Patient No. 2 was previously treated with prednisolone and an increase in the platelet count from 13 × 10^11/ml to 200 × 10^11/ml was observed. Autologous platelets to platelets became detectable, whereas the platelet count dropped to 50 × 10^11/ml. Both patients were found to suffer from progressive Sneddon’s Syndrome.

Conclusion: Sneddon’s Syndrome remains often unrecognized. Autoantibodies appear to be responsible for the thrombocytopenia observed in affected patients.
level HBV-DNA or confirmed seroconversion could be identified. Most of the HBsAg positive test results could be explained by vaccination. Up to now, HBsAg testing in addition to screening for HBV-DNA and anti-HBc, didn’t show any evidence of increased safety of our blood products.

P 6/8.9 Evaluation of Safety and Efficacy of Recombinant Factor IX in Daily Clinical Practice: A Pharmacovigilance Evaluation of BeneFIX

Westfeld M.1, Kosch M.2, Pollmann H.1, Huth-Kühne A.1, Eisert R.1, Laws H.J.1, Niekrens C.1, Girisch M.2, Sevier K.1, Ries M.1, Oldenburg J.1

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Objectives: Haemophilia B is a rare coagulation disorder with an incidence of about 1:25,000 in male newborns. Treatment of choice is prophylactic or on-demand replacement of coagulation factor IX (FIX) in a home-treatment setting. Today, two different types of factor concentrates are marketed in Germany: plasma-derived products and a recombinant product (nonacog alfa, BeneFIX). Nonacog alfa has proven its safety and efficacy in clinical studies including PTPs, PUPs and during surgery. A new formulation of nonacog alfa was introduced to the market in October 2007. Clinical studies have confirmed bioequivalence between the former and new formulation as well as safety and efficacy for the new formulation. Since haemophilia B is a very heterogeneous disease only a limited number of patients can be included in clinical trials, a non-interventional post-authorisation study with a special focus on safety parameters appears to be adequate. We here report on the study design and first results of a non-interventional pharmacovigilance evaluation (PE) of BeneFIX. Methods: To prospectively evaluate BeneFIX in the usual health care setting we started a non-interventional study including haemophilia B patients of any severity that are treated with reformulated BeneFIX. Primary objective of the study is the long-term evaluation of safety and efficacy of BeneFIX. Inclusion criteria for patients are: diagnosis of haemophilia B, treatment with reformulated BeneFIX and written informed consent prior to data collection. The aim is to include approx. 80–100 patients within the first three years. Results: With regulatory and ethic committee’s notification the PE started in Germany in February 2008. The study is set up and managed by the medical department of Wyeth in collaboration with a scientific advisory board. Data collection and data-management as well as monitoring is supported by a clinical research organization. The data collection period will last for at least 3 years and is very likely to be extended beyond this point. Until the end of April 2008 9 centers have been initiated, and 8 patients have been included.

Conclusions: Non-interventional trials in the usual health care setting are adequate means to assess the safety and efficacy of a treatment in the post-authorization phase. Especially in very rare diseases such as haemophilia B the conduct of this kind of study appears to be reasonable in order to monitor a larger cohort of patients over a longer period of time. First results will be presented in Sept. 2008.

P 6/8.10 Hemovigilance Goes Active – Local Initiative to Improve Adverse Event Reporting in Transfusion Medicine

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Background: Hemovigilance in Germany is a rather passive system, where manufacturers have to rely on full reporting of adverse events (AE) by hospitals, private practice and clinical colleagues. Compared to other European hemovigilance systems, which took a more proactive approach, the rate of adverse reporting after transfusion of blood products is rather low in Germany. Objectives: An investigator initiated trial (IIT) reporting acute transfusion reaction rate (ATR) and serious adverse transfusion events (SATE) was jointly established in three university hospital settings by the departments of hematology and the institutes of transfusion medicine. Methods: A novel ATR and SATE reporting form was established and introduced on the hemovigilance wards. Furthermore, all platelet concentrates (PC) distributed to the hematology departments are equipped with a transfusion reaction form attached to the blood bag. Following each PC transfusion, it has to be documented on these forms, whether or not a transfusion reaction was observed. These barcoded forms covering patient data, date and time of transfusion and name of the transfusing physician, will then be entered into an electronic database. All transfusion reactions observed have to be described in detail on the novel ATR and SATE reporting form. Results: In 2005, 2006 and 2007, 7.5, 6.1 and 7.5 AE per 10,000 transfused packed red cell concentrates (PRCC), 8.3, 11.9 and 6.8 AE per 10,000 transfused platelet concentrates (PC) as well as 4.6, 8.1 and 5.8 AE per 10,000 fresh frozen plasma (FFP) were reported from one university hospital. This serves as a basis for comparison for our novel approach in order to establish an evidence-based decision model in our clinics. Conclusions: Combining modern tools for hemovigilance in a jointly established system helps to improve reporting accuracy of adverse transfusion reactions.

P 6/8.11 Implementation of Transfusion Guidelines for Red Blood Cells (RBC) in Elective Hip and Knee Replacement: A Prospective, Multicenter, Before-and-after Study in 10 Swiss Hospitals

Fontana S.1, Müller U.1, Staub L.1, Lugibühel M.1, Kwassma M.1, Schmid P.1, Krummen L.1, Läderach A.1, de la Cuadra C.4, Mansouri Taleghani B.4

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Background: 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-transfusion transfusion practices in elective hip and knee replacement and the effects following the introduction of a straightforward guideline on RBC transfusion. We report the data of the first, observational phase. Methods: Prospective, multicenter before-and-after study comparing the use of RBC in elective hip or knee replacement before and after the implementation of a transfusion guideline in 10 Swiss hospitals. During the first 6 months...
(08.2007–02.2008) we monitored RBC use and patient outcomes. In a following transition period a RBC transfusion guideline, jointly developed was introduced and implemented. Currently we continue monitoring the data of the pre-interventional phase. Methods: Before-and-after study comparing the use of RBC in major adult cardiac and vascular surgery (valve replacement, coronary bypass [CABG], open abdominal aortic aneurysm procedures) before and after the implementation of a transfusion guideline. During the first 6 months (08.2007–02.2008) we monitored RBC use and patient outcomes. In a following transition period an institutional RBC transfusion guideline, jointly developed by the participating services, was introduced and implemented. Currently we continue monitoring the data of the post-interventional period (03.–10.2008). Results: Preliminary data of 351 patients are available (median age 68 y, 74.6% male, median ASA physical status 3, 409 cardiac procedures [176 valvular, 234 CABG] and 23 abdominal aortic procedures including several combined interventions). 214 of 351 patients (61.0%) received a total of 683 RBC units. This corresponds to a mean of 1.95 RBC units per patient and 3.19 units per transfused patient. Median length of hospital stay was 8 days. In-hospital mortality and cumulative complication rate (cardiovascular, bleeding, infections) during hospitalization were 0.8% and 21.6%, respectively.

Conclusions: We report the first data on perioperative transfusion practice for cardiac and vascular surgery in a Swiss university hospital. The preliminary observed pre-interventional transfusion practice seems to be more liberal than reported in the literature. It is expected to be positively influenced by the introduced guidelines.

P 6/8.13 Red Blood Cell (RBC) Use in Elective Cardiac and Vascular Surgery: A Prospective Assessment Prior to the Implementation of Transfusion Guidelines in a Swiss University Hospital Fontana S.1, Müller U.1, Staub L.1, Eberle B.1, Regli B.1, Carrell T.-P.1, Schmidt J.1, Kwasa M.1, Schmid P.1, Mansouri Taleghani B.1
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Background: 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. We report the data of the first, observational phase. Methods: Prospective, before-and-after study comparing the use of RBC in major adult cardiac and vascular surgery (valve replacement, coronary bypass [CABG], open abdominal aortic aneurysm procedures) before and after the implementation of a transfusion guideline. During the first 6 months (08.2007–02.2008) we monitored RBC use and patient outcomes. In a following transition period an institutional RBC transfusion guideline, jointly developed by the participating services, was introduced and implemented. Currently we continue monitoring the data of the post-interventional period (03.–10.2008). Results: Preliminary data of 351 patients are available (median age 68 y, 74.6% male, median ASA physical status 3, 409 cardiac procedures [176 valvular, 234 CABG] and 23 abdominal aortic procedures including several combined interventions). 214 of 351 patients (61.0%) received a total of 683 RBC units. This corresponds to a mean of 1.95 RBC units per patient and 3.19 units per transfused patient. Median length of hospital stay was 8 days. In-hospital mortality and cumulative complication rate (cardiovascular, bleeding, infections) during hospitalization were 0.8% and 21.6%, respectively.

Conclusions: We report the first data on perioperative transfusion practice for cardiac and vascular surgery in a Swiss university hospital. The preliminary observed pre-interventional transfusion practice seems to be more liberal than reported in the literature. It is expected to be positively influenced by the introduced guidelines.
pathologic value in a patient with known thromboembolism may have impact on further decisions concerning anticoagulation therapy.

P 6/8.15 Temporal Changes in Antibody Reactivities in 64 Patients with Anti-PF4/Heparin- IgG, -A, and -M Antibodies

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Objectives: IgG-antibodies against complexes of heparin and platelet factor 4 (PF4) can cause heparin-induced thrombocytopenia (HIT) by platelet activation via FcγRIIA-receptor. Anti-PF4/heparin IgM and -A antibodies, and non-platelet activating PF4/heparin-IgG-antibodies are of minor clinical relevance, justifying the continuation of heparin. However, the frequency of a seroconversion toward a more pathological serological profile is unknown.

Methods: From patients tested positive in a monovalent (IgG, IgA, IgM) PF4/heparin enzyme-immunoassay (EIA) a second blood sample was obtained about 10 days later to assess changes in the immunoglobulin class distribution and the platelet activating capacity. All samples were investigated by heparin induced platelet activation (HIPA) test. Results: 6/64 initial samples contained anti-PF4/heparin IgG antibodies with or without IgM and/or IgA antibodies. In 2/64 initial samples only IgM and IgA antibodies were detectable. 12/64 samples became EIA-positive in the second sample. Of 30 sera being HIPA positive in the first sample, 11 became HIPA negative in the second sample, however, also 7/32 initially HIPA negative sera became HIPA positive about 10 days later. None of the isolated anti-PF4/heparin IgM and -A positive sera became anti-PF4/heparin IgG positive subsequently.

Conclusion: The rapid disappearance of HIT antibodies indicates their adsorption from the circulation even after cessation of heparin, compatible with a transient autoimmune disorder. Therefore, patients with acute isolated HIT may benefit from prolonged anticoagulation. Continuation of heparin in patients with non-platelet activating IgG antibodies needs a carefully follow-up, as about 20% of HIT-antibodies change their reactivity pattern and cause a positive HIPA test.

P 6/8.16 Transfusion Reactions 2002 to 2007 at the University Hospitals in Jena: The Savety Experience with the Solvent Detergent Plasma Octaplas

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Objectives: A haemovigilance system is a prerequisite for the safety of transfusion and is required by the German transfusion law (TFG). Each transfusion reaction is to be reported to Transfusion Medicine. We looked at data collected in Jena with special regard to the transfusion of fresh frozen plasma. Jena uses the solvent detergent plasma OCTAPLAS only, excluding the risk of viral infection hereby. Furthermore, Barz et al. showed already in 1994, that no antigen bearing cells or cell fragments can be found in Octaplas and U.J. Sachs et al. found no HLA class I or II and no granulocyte specific antibodies 2005. This reduces the risk of immunological caused adverse effects like TRALI.

Methods: 310 Reports of transfusion reactions at the University Hospital Jena over a period of six years were analyzed with special regard to the side effects of OCTAPLAS. Results:

<table>
<thead>
<tr>
<th>Year</th>
<th>Erythrocyte concentrates (EC) transfused</th>
<th>Thrombapheresis concentrates (TAC) transfused</th>
<th>Octaplas transfused</th>
<th>Transfusion reactions reported</th>
<th>Severe reactions reported</th>
<th>Transfusion reactions with Octaplas involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>20 408</td>
<td>6 287</td>
<td>1 0167</td>
<td>32</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>2003</td>
<td>22 136</td>
<td>7 650</td>
<td>9 966</td>
<td>32</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>2004</td>
<td>23 054</td>
<td>8 323</td>
<td>11 577</td>
<td>54</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2005</td>
<td>21 270</td>
<td>8 789</td>
<td>11 018</td>
<td>46</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2006</td>
<td>22 843</td>
<td>9 137</td>
<td>10 489</td>
<td>43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2007</td>
<td>26 379</td>
<td>7 864</td>
<td>13 408</td>
<td>52</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>136 090</td>
<td>48 050</td>
<td>66 625</td>
<td>279</td>
<td>18</td>
<td>7</td>
</tr>
</tbody>
</table>

Compared to cellular blood components, the transfusion of OCTAPLAS is fairly safe. The reports involving OCTAPLAS shows 4 cases of allergic reactions and 2 of fever. All but one report included other cellular blood components. Therefore the OCTAPLAS cannot be determined as the definitive cause of the transfusion reaction. Only one case was considered ‘severe’, with shock and MI, but the underlying disease was oesophageal bleeding and also EC were given. No TRALI or anaphylactic shock related to OCTAPLAS (n=66 625) was reported. The adverse reactions in Jena added up to 236 EC (0.17% of all used EC), 148 TAC (0.3% of all TAC) and 21 SD-Plasma (0.023% of all OCTAPLAS). Only one report regarding 2 units of OCTAPLAS (0.003% of all used OCTAPLAS) did not involve parallel transfusion of other blood components. Our experience in Jena prove that the use of Octaplas fulfils the intention of §1 of the TFG, to supply the patient with a safe blood component.

P 6/8.17 Inherited Macrothrombocytopenia and von Willebrand Disease as a Combined Defect in a Young Patient with Alport Related Symptoms – Perioperative Cochlear Implantation Management

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Introduction: We first report about a rare case of combined congenital platelet function defect. Macrothrombocytopenia and heterogeneously inherited Alport related symptoms (especially high frequency sensorineural deafness) besides typical leukocyte inclusions led to the suspicion of Fechtner syndrome. There was a big variation of antibody reactivities in 64 patients with or without thiaclopride.

Methods: There was a big variation of antibody reactivities in 64 patients with or without thiaclopride.

Conclusion: A rare case of inherited FS macrothrombocytopenia associated with vWB disease was described. Platelet transfusions were successfully performed before cochlea implantation. Since platelet counts using hematoloty analyzers may be hard to interpret in macrothrombocytopenia, testing of platelet function cannot be avoided. We suggest also be aware of congenital/acquired platelet function defects.
Blazek M.

**Objective:** Mild allergic transfusion reactions are very frequent, but their cause remains usually unknown. **Case report:** A 13 years old girl with an osteosarcoma of the rightibia was operated and became pre- and postoperative chemotherapy. At the first transfusion of anaphylaxis platelet concentrate (PC) she showed a mild allergic reaction. The next 5 PCs were given after premedication with an antihistamine and were well tolerated. At the transfusion of the 7th PC she showed again an allergic reaction in spite of premedication with clemastine iv. At the 8th PC the allergic reaction was still stronger although clemastine and decorine were given before transfusion. Therefore the next PC was washed and given after premedication with clemastine and a higher dose of decorine. It was now well tolerated. The last (10th) PC was given 2½ months later. Transfusion was only clemastine, but no reaction occurred. No adverse reactions were seen after transfusion of red blood cell concentrates or virus-inactivated frozen pool plasma.

**Results:** There was no hemolysis or red blood cell minor incompatibility. Sterility testing of the PCs showed no growth. No HLA or HPA antibodies were detected in the patient’s serum. Also the patient had no IgA deficiency. However the patient showed positive results for IgE against celery, carrot, peanut, and hazelnut and less stronger against barley, rye, wheat, almond, potato, tomato, strawberry and pear. When the donor of the 7th PC was questioned at a later point of time, he remembered that he had eaten chocolate and cocoa with hazelnuts the evening before platelet donation.

**Conclusion:** In this case we assume that the allergy of the patient against a lot of vegetable foods was the cause for several allergic transfusion reactions. Therefore we recommend to ask patients for allergies before transfusion of PCs (and FFPs) and to be alert for allergic reactions in such patient who have a positive history especially of food allergies.

**P 7.01 Extracorporeal Elimination of Lipoproteins (26 Years of Treatment in Czech Republic)**

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**Introduction:** We began to treat familial hyperlipoproteinemia (FH) using continual plasmapheresis 26 years ago and it stays rescue method in critical situations. In 1994 we started immunoadsorption treatment. The development headed back to present removing of several pathogenic substances and influencing rheologic function of microcirculation, because blood abides not only Newtons principles.

**Methods:** We have been using plasmapheresis for treatment of hyperlipoproteinemic crises; we remove plasma volume using separators OCS-Spectra (replace solutions: albumin and crystalloids). We have performed 1500 immunopheretic sessions using adsorption columns Lipopak with sheep antibodies. We execute these long-term (8:2;2:96 years) treatment in severe cases of FH resistant to standard therapy. Since 2003, we performed 400 hemophereoses using filters Evaflo, Kuraray. In 2006, we began therapy using adsorbers Lipocollect 300. In present time, we treat long-term 12 patients with FH as only center in Czech Republic. 25 patients were treated using series of hemophereoses. **Results:** LDL-apheresis removed more than 50% of all FH patients in 9 months and reduced subjective symptoms. Intima-media thickness of carotid arteries showed reduction or stagnation in 50% of patients. We use our own software to optimize adsorber performance so immunopheretic sessions can significantly reduce LDL-cholesterol, ApoB and Ln(-) – our target level of LDL after session is 0.5 mmol/L. Using repeated session (after 17.6 + 1.6 days) we can keep median levels of LDL-cholesterol within normal limits mostly. Hemorheopheresis has further rheologic effects- increase of microcirculation blood flow. According to our knowledge, this is important in critical limb ischemia, ARD or acute hearing loss. We have noticed 5.6% of non-significant side effects.

**Conclusion:** Our experience with EE can be therefore summarized: Immunopheresis is successful in FH, lifesaving in homozogous FH. It is well tolerated. Hemorheopheresis improves microcirculation blood flow and ameliorates the course of other diseases mentioned above.

Supported by grant: IGA MZ CR NR-9103-4.

**P 7.02 Complications of Therapeutic Plasma Exchange: A Single Center Experience**

**Altuntas F.1, Kocyiigi1, Kurnaz P.2, Kaynar L.2, Korkmaz U.3, Oztekin M.1, Solmaz M.3, Eser B.1, Celin M.1, Unal A.3, Erciyes University Medical School, Hematology, Kayseri, TR. 2Erciyes University Medical School, Kayseri, TR. 3Erciyes University Medical School/Endocrinology, Kayseri, TR.**

Therapeutic plasma exchange (TPE) is used successfully as a therapeutic option in a variety of diseases. However, it can cause some complications. The aim of this study was to analyze the complications associated with TPE. The type and the number of the complications were retrospectively reviewed in 1375 TPE procedures in 189 patients at the University Hospital from November 2005 to February 2008. The main TPE indications were thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, Good Pasture syndrome, Guillain-Barré syndrome, myasthenia gravis, severe hyperlipoproteinemia and HELLP syndrome. Albumin-saline (30%) and fresh-frozen plasma (FFP) (70%) were used as replacement solutions. All patients received an intravenous infusion of 10% calcium gluconate during the procedure. Central venous catheter was introduced in 151 (80%) patients. Catheter related complications were observed in 16 patients (10%); 5 (3%) catheter dysfunctions, 5 (3%) hematom and 3 catheter related infection (2%). There were 282 (21%) procedure-related complications. Most complications were minor; there were no deaths. Eight procedures (0.5%) were discontinued due to severe complications i.e., hypotension. The most common complications were hypocalcemia (4%), palpitation (4%), fever (2%) and urticaria (1%). Hypocalcemic symptoms resolved after intravenous administration of calcium. Complications were more with FFP compared to albumin (16% vs. 5%). While the most common complications with FFP were urticaria, fever, chills and pruritis, hypocalcemia and hypotension were more common with albumin-saline. In conclusion, therapeutic plasma exchange is a relatively safe procedure. Patients should closely follow-up in terms of complications associated with the procedure, i.e., catheter, replacement solution, and citrate during and after the procedure.
Maly J. EKG, ultrasound examination of heart, arteries and catheterisation. Term follow up of patients. Patients and methods: A group of 12 patients (7 reporting the significance of non-invasive cardiovascular examinations in long-term follow up of patients with severe familial hypercholesterolemia (FH) after other therapeutic approaches have failed. The precise treatment method in the treatment of severe familial hypercholesterolemia (FH) is still under debate. Whether such selective elevation of Lp(a) may need therapeutic intervention. Patients with a selectively elevated Lp(a) without angiographical alterations and clinical symptoms should be examined at regular intervals without treatment, however, if progression occurs or if clinical symptoms are present treatment must be initiated. Diet or drugs have been shown to be ineffective and without clinical value. Alternatively, several apheresis procedures such as heparin precipitation, liposorption, differential filtration and LDL-immune apheresis all lower elevated Lp(a) levels to a certain extent mainly in relation to the initial elevation. According to our experience with patients suffering from advanced coronary heart disease and a selective Lp(a) elevation Lp(a) apheresis is most effective lowering even excessively high Lp(a) values by about 80–85% of the initial value to target values of not more than 30 mg/dl by the end of the treatment. Thus, further progression of the coronary heart disease can be prevented under long term Lp(a) apheresis.

P 7.05 Significance of Non-Invasive Cardiovascular Examinations for the Evaluation of Extracorporeal LDL-Cholesterol Elimination Efficacy

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Introduction: Extracorporeal LDL-cholesterol elimination (EE) is a very successful therapeutic method in the treatment of severe familial hypercholesterolemia (FH) after other therapeutic approaches have failed. The procedures must be effective, but causing minimum discomfort to patients. However, it is very difficult to evaluate the actual activity of atherosclerosis. We report the significance of non-invasive cardiovascular examinations in long-term follow up of patients. Patients and methods: A group of 12 patients (7 males, 5 females) in the age of 47±16.6 were treated during 7.2±2.96 years. Two methods of EE were used: LDL-apheresis and hemopheresis. Both invasive and non-invasive examinations were used in affected body parts EKG, ultrasound examination of heart, arteries and catheterisation. Results: A rapid alleviation of symptoms was observed shortly after initiating EE therapy. Severer arteriosclerotic complications (myocardial infarctions and strokes) did not recur later. Invasive cardiovascular examinations were only necessary at the beginning of EE therapy. Non-invasive investigations (once in 6 months) were sufficient in the following period. The follow-up of the carotids (the media-intima) showed a regression of its thickness in 4 patients, an unchanged state in 3 and worsening was observed in 5 patients. Conclusion: Non-invasive cardio-angiologic examination of patients was sufficient as a follow-up of FH treated by EE. It is of advantage to complement with selected haematological, biochemical and immunological indicators. EE of LDL-cholesterol is an effective method of treatment in severe forms of FH. Supported by the grants IGA MH CZ NR.91034.

P 7.06 Lymphocyto-Plasma Exchange in Ulcerative Colitis – a Case Report

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Introduction: The very positive initial evidence of other published Lymphocyto-plasmapheresis (LCPA) procedures for the treatment of chronic inflammatory bowel disease (IBD) has sparked our interest to consider the innovative treatment. Furthermore, the very low risks and very minimal side effects associated with apheresis are in stark contrast to the risks and immense long-term side effects associated with conventional drug therapy for IBD. Beside genetic and environmental factors, a dysfunction of the immune system is discussed to be pathogenetic for IBD. In particular, cytokines, and lymphocytes are thought to play an important role. Alternative selective adsorber systems are able to bind granulocytes and monocytes. LCPA was more appealing to us in the technologically advanced ability to simultaneously reduce inflammation markers (e.g. cytokines) through performing a plasma exchange while depleting the wider range of activated leukocytes at the same time. Steady state was reached after 3 sessions with a Clinical Activity Index (CAI) of 12 received 5 weekly + 2 bi-weekly treatments as maintenance therapy (7 treatments in total). Each treatment consisted of a simultaneous centrifugal plasma exchange therapy (55–60 % of the patient’s plasma volume that was exchanged with a 5% albumin solution) and a centrifugal leukocyte depletion. Thin bloody stools had completely ceased – Frequency of bowel movement reduced from 3 to 1x/day – Normal texture of bowel – All joints are symptom free (competitive sports were possible again) – Medication necessary – Inflammation marker: Normal (ESR 1 mm/h, CRP < 3) – CAI: 0 points – Normalization of bowel movement and joint problems were seen after the first treatment! Discussion: Through the use of centrifugal apheresis, we were able to achieve fantastic clinical results at lower costs. Although published long-term (>24 months) clinical and histological remissions look promising, we would like to follow this young patient and additional patients long-term. Furthermore, additional well-designed clinical studies will be necessary to show statistical significance and allow for cost effective public-healthcare reimbursement.
documented, as expected, no difference in the prevalence of platelet receptor polymorphisms between vWD patients and healthy individuals (C), using a case-control design. For example, the frequency (%) of HPA-1 alleles (vWD vs. C) was 69.0 vs. 72.3 (1a/1a), 29.9 vs. 25.3 (1a/1b), and 1.1 vs. 2.4 (1b/1b) (p each > 0.05). By contrast, in a case-only design, a trend was observed that prothrombotic platelet receptor variants such as HPA-1b and ez877T are more frequently present in vWD patients with minor bleedings than in those with intermediate or severe hemorrhagic episodes. This association requires confirmation in follow-up studies. Conclusion: The results of this pilot study are indeed supporting our hypothesis that the clinical feature of bleeding diathesis or complications (frequency, severity, site) in patients with prediagnosed vWD is modulated by genetically determined prothrombotic platelet variants and may be also modified by acquired alterations of hemostatic components.

P 9.02
Correlation between Two ADAMTS-13 Activity Assays Based on Different Principles

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Aims: Defects in ADAMTS-13, the von Willebrand Factor (vWF) cleaving protease, are thought to be the main cause for the microvascular thrombotic disorder TTP (thrombotic thrombocytopenic purpura) that is in more than 90% of cases fatal if not treated early and appropriately. Usually this disease is clinically diagnosed, but in recent years the need for rapid and reliable diagnostic tests for ADAMTS-13 levels has increased. We present here the comparison of two commercially available assays for quantification of ADAMTS-13 activity both suitable for routine analysis but based on different principles.

Methods: The two assays differ in their test principle and the readout system as follows: Assay 1 is a fluorogenic assay using a FRETs-vWF73 substrate and a kinetic measurement (TECHNOZYM® ADAMTS-13 ELISA); with this assay ADAMTS13 Antigen can also be determined in a second step. Assay 2 is a chromogenic assay and detects the cleaved vWF73 substrate by a specific monoclonal antibody (TECHNOZYM® ADAMTS-13 Activity ELISA). Results: Citrated plasma of normal donors (n=7), of pooled normal plasma (n=15) and of TTP patients (n=14) were tested in both assays. Results are reported in both assays as percentage of normal activity. The standard for both assays is prepared from a pool of 100 normal donors and defined as 100%. The samples comprised a range from 0.2% up to 107% activity. The overall correlation coefficient between the two different activity assays was 0.96. Five samples were found to have less than 5% activity in both assays. Conclusion: These results show that data obtained by the new TECHNOZYM® ADAMTS-13 Activity ELISA correlate very well with the fluorogenic assay (TECHNOZYM® ADAMTS-13 ELISA) in spite of the fact that these assays are based on very different principles.

P 9.03
Expression of PAR1, Thrombin, Plasmin, and Activated Protein C in the Developing Murine Fetal Liver

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Little is known about the coagulation system in the fetus during intrauterine life. The coagulation system and PAR signalling, however, are known to play important roles in angiogenesis of the fetus. Griffin and colleagues showed that PAR1-deficient embryos die between embryonic days 9.5 and 10.5 due to a defect in hemostatic mechanisms or vascular integrity (Science, 2001, 293, 1666). To further elucidate the time-dependent development of the coagulation system and the PAR receptors in the fetuses we performed micro array analysis in the fetal liver at days 9.5, 11.5, 13.5 post coitum and in adult mice. Fetal liver tissues (n=2) as well as livers from adult BALB/c mice were prepared and total RNA was isolated using the RNeasy kit (Qiagen, Hilden). Hybridization of the samples was performed using the Murine Genome U74A Set Version 2 (Affymetrix, USA) probe arrays representing 12 488 genes/EST sequences. All preparations and hybridizations were performed in duplicate. This resulted in a list of genes that were regulated over the analysed time schedule. On day 11.5 p.c., when the liver becomes the site of extramedullary hematopoiesis, data showed a significant peak in PAR1 and PAR4 expression whereas PAR2 expression slowly decreases from day 9.5 to 13.5 p.c. Expression of thrombin as well as all other analysed coagulation factors (Factor V, VII, IX, X, XII, XIII) showed a common expression profile that is characterized by a moderate increase of expression between day 9.5 and 13.5 p.c. and a ~10-fold increased expression level obtained in the adult mice Levels of plasmin and activated protein C transcripts, also known to be involved in the activation of PAR receptor, steadily increased between day 9.5 to 13.5 p.c. from 1 to 10% respectively, and are highest expressed in the mature liver. Interestingly we found plasminogen activator as well as plasmin inhibitor activator to be maximally expressed on day 9.5 and 11.5 p.c., and decreased to nearly undetectable levels in the adult mice indicating that the plasmin pathway is important at a particular timepoint in the fetal liver. Our results show that PAR1, PAR2 and PAR4 receptors are predominantly expressed at early stages of fetal murine liver development. Expression of PAR1 activators like thrombin, plasminogen and protein C are increasingly expressed during development of the fetal liver but are highest expressed in the adult liver. Increased expression of plasminogen activator as well as plasmin activator inhibitor in the fetal liver suggest that the activity of plasmin is differentially regulated during liver development.

P 9.04
Exclusion of Heparin-Induced Thrombocytopenia by the Particle Gel Immunoassay?

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Heparin-induced thrombocytopenia (HIT) is a prothrombotic condition, characterized by a platelet drop >50% and/or thrombosis with a temporal relationship of 1–2 weeks after initiation of heparin. These surrogate markers are useful for the clinical assessment but are hardly applicable in multimorbid patients with clinical conditions that mimic HIT. Platelet activation assays (heparin-induced platelet aggregation assay, HIPA, and serotonin release assay, SRA) and platelet factor-4-polyanion enzyme immunoassays (PF4-ELISA) confirm HIT. HIPA and SRA are highly specific but laborious requiring selected donor platelets and extended experience. High titer IgG antibodies correlate with clinical HIT, but ELISA is also time-consuming. The alternative Heparin/PF4-antigen platelet gel immunoassay (II-PaGIA) showed positive results within one hour and detects mainly IgG, but also IgM/A antibodies. We evaluated specificity and sensitivity of the PaGIA in relation to HIPA and ELISA (PF4-ENHANCED2, GTI, Waukesha, WI) in 285 patients (median 7yrs, range 1–97, 45% cardiovascular surgery; f/m 56/73; and 55% medical, f/m 78/78) with undetermined likelihood for HIT and 98 controls (median 4yrs, range 26–64, f/m 49/40) to validate it as a rapid assay to exclude HIT. HIPA was positive in 12% of patients. Based on ROC curves (II-PaGIA vs. HIPA), OD-cutoff values for the IgG/A/M- and IgG-ELISA were 0.761 and 0.564, respectively. In controls the OD of IgG-ELISA was 0.066 (0.028–0.500) and lower than from patients’ samples negative by PaGIA and IgG/A/M-ELISA (n=158; 0.074; 0.009–0.339; p=0.017). PaGIA was positive in 70 patients (25%). In both patient groups both ELISA ODs were higher if PaGIA and IgG/A/M-ELISA were positive compared to negative PaGIA but positive IgG/A/M-ELISA (p<0.001). (Data as median; range.) Analyzed by ROC curves (ELISAs vs. HIPA), IgG/A/M-ELISA and IgG-ELISA were significantly predictors for the results of HIPA (p<0.001) and the IgG-ELISA had the highest explained variance (41%). Based on our results the PaGIA can serve as a rapid test to exclude HIT since a negative PaGIA is rarely associated with high titer IgG PF4-antibodies or a positive HIPA. In cases of positive PaGIA results and in patients highly suspicious for HIT but with negative PaGIA, alternative anticoagulation should be considered until results from functional testing are available.
Abstracts

P 9.05 Platelets Express Enhanced CD40L/CD154 in Human Sepsis – Influence of Pre-Analytic Variation on Membrane-Bound and Soluble CD40L

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Background: Soluble CD40 ligand (sCD40L) has been proposed as a biomarker of cardiovascular events. However, pre-analytical problems on surface expression or shedding of CD40L/CD154 are not completely explored. Therefore, we investigated the effect of pre-analytic variation on CD40L/CD154 measurements. Methods: sCD40L levels were measured by ELISA using 4 different blood samples from healthy donors (n = 20), and patients with coronary artery disease (CAD) (n = 40) or sepsis (n = 40) processed as follows: CTAD platelet poor plasma (PPP) and CTAD at 0°C, citrate and serum at room temperature (RT). CD40L/CD154 and P-selectin/CD62P expression was assessed ex vivo and after in vitro stimulation by flow cytometry. Results: Serum samples presented significantly higher sCD40L levels than plasma samples (p<0.05). Elevated sCD40L concentrations in CAD patients were only detectable in sera. Septic patients displayed decreased sCD40L levels (p<0.05) and increased CD154 levels and P-selectin/CD62P surface expression (p<0.05), but not CAD patients. Consistent with enhanced platelet activation, a positive correlation between surface expression of CD40L/CD154 and P-selectin/CD62P (p<0.05, r=0.6) and a positive correlation of both receptors and platelet counts was observed (p<0.05, r=0.8). Moreover, altered release of CD40L/CD154 was detected within in vitro platelet activation in sepsis.

Conclusions: Our data demonstrate the influence of sample processing on CD40L/CD154 determination and suggest that platelet activation is reflected in elevated sCD40L concentrations. Furthermore, the results indicate a complex contribution of CD40L/CD154 to inflammatory processes in sepsis.

P 9.06 Platelet-Free Hemotherapy in Patients with Bernard Soulier Syndrome: Successful Bleeding Prophylaxis with Recombinant Factor VIIa for Dental Extraction

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Background: Bernard Soulier syndrome (BSS) is a hereditary platelet function disorder resulting from partial or full dysfunction of the GP Ib/IX complex. This non-integrin receptor is essential for platelet adhesion onto von Willebrand factor, specifically under high-shear rate conditions. Diseases with BSS present with mucocutaneous bleeding and prolonged bleeding time. Laboratory features also include giant platelets and thrombocytopenia of varying degree. Standard hemotherapy of bleeding episodes or prophylaxis for prevention of hemorrhages during surgical procedures comprises platelet transfusions. However, this treatment can be complicated by antibody formation to the GP Ib/IX-V complex and/or alloimmunization by HLA, thus, limiting the responsiveness to future platelet transfusions. We have therefore evaluated the efficacy and safety of recombinant factor VIIa (rFVIIa, NovoSeven) as first-line therapy in patients with BSS. Methods: Patients, Therapy Protocol and Outcome: Six patients (2 males, 4 females, age ranging from 14 to 34 yrs with prediagnosed BSS were included. Two of them have had platelet transfusions. The patients were now referred for hemotherapy prior to dental surgery. All of them presented without manifest mucocutaneous bleedings but had abnormal closure times (>300 sec), as determined by the platelet function analyser (PFA-100) in response to collagen/epinephrine and collagen/ADP, corresponding to their congenital platelet defect. The therapy protocol included a bolus of rFVIIa (110 μg/kg) administered 15 min prior to surgery, followed by two doses of 90 μg/kg at 120 min intervals postoperatively. During the first 3 days after surgery, rFVIIa (90 μg/kg) was administered every 8 hrs (2 patients) or every 12 hrs (4 patients). Tranexamic acid (Cyklokapron 3 x 1 g per day) was started postoperatively and continued for 10 days in all 6 patients. None of them experienced any intra- or postoperative bleeding episodes. By contrast, hemorrhosis and wound healing were near normal. No platelet transfusions were required in any of the six patients. Conclusion: Perioperative administration of high-dose rFVIIa in combination with antifibrinolytics such as tranexamic acids can be indeed an effective and safe therapeutic alternative for prophylaxis of bleeding episodes in patients with BSS undergoing dental surgery. Although, this is an off-label use at present, administration of rFIIa should be considered instead of platelet transfusions, specifically in young patients with congenital platelet disorders in order to prevent alloimmunization and the risk of refractoriness to future platelet transfusions whenever required.

P 9.07 Combined Analysis in Combined FV and FVIII Deficiency: Novel and Recurrent Mutations and the Ethnical Background of 8 Patients

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Objectives: The number of factor V (FV) and factor VIII (FVIII) deficiency patients undergoing FVIII gene analysis is increasing, especially in the last 10 years. Methods: FVIII/F9D patients were recruited for sequencing. All 8 patients were referred for hemotherapy prior to dental surgery. To date 12 novel different mutations in either of the two genes in about 26 different countries are known. Here we describe the first patient from Azerbaijan with a novel frame shift mutation and seven additional families. Methods: FVIII/FD patients were sequenced on a 650 bases fragment of the FVIII gene. In the seven families the results were compared with an in silico algorithm (PolyPhen v2). Results: The novel frameshift mutation Lys302X identified in Exon 7 of the FVIII gene in a Chinese patient, was transmitted to four patients from China, one patient from French and five patients with Pakistan origin. The variety of Iranian mutations includes rare variants but also a common mutation of Middle Eastern Jews in Exon 1 of the FVIII gene (89-90insG). However the most frequent mutation in Iranian ethnicity is a splice site mutation in the FVIII gene at the last nucleotide of Exon 7 (IVS7-DS-1G>A) that could also be detected in the Iranian female Patient P (FV 9%, FVIII 12%) by analysis of Exon 7 of the FVIII gene. In Poland only the frame shift mutation 841delA in Exon 8 of the FVIII gene was recently identified in one family. Thus, sequencing of Exon 8 of the FVIII gene in the Polish Patient G (FV 23%, FVIII 57%) showed the same mutation. Since Patient H (FV 10%, FVIII 17%) represents the first Azerbaigar FVIII/F9D patient we started screening for mutations originating from Turkey, Iran and Armenia because of the close vicinity to Azerbaijan. Neither the Armenian mutation 1524delA in Exon 13 of the FVIII gene nor another known mutation was found. The complete sequence revealed the novel frame shift mutation Lys230fs in Exon 6 of the FVIII gene. Conclusions: The data suggest apparent correlation between determined mutations and the patients’ descent. Founder mutations in the FVIII gene and F9D gene may lead to repeated occurrence of FVIII/F9D in different countries. Thus, molecular diagnosis in F9D is facilitated by a screening strategy based on patients’ ethnicity may simplify molecular diagnosis of F9D.

P 9.08 Correlation of Platelet RNA Profiles and Platelet Aggregation Response to Arachidonic Acid in Patients with Aspirin-Like Defect

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Objective: The Aspirin-like defect (ALD) is an autosomal dominant inherited platelet disorder associated with mild bleeding symptoms and diminished platelet aggregation response (PAR) to arachidonic acid (AA), very similar to Aspirin effects. The AA pathway was supposed to be involved in ALD pathogenesis. Here, we investigated platelet RNA profiles in ALD patients and controls. RNA signals were correlated with the degree of PAR to AA. Methods: Platelets were isolated from citrated blood of 21
ALD patients and 16 healthy relatives from 11 families. Platelet RNA was processed to Cy3- or Cy5-labelled cRNA, and hybridized to whole genome microarrays (Agilent Technologies). Pearson correlation analysis of expression signals with PAR to AA was determined by light microscopy. Positive correlation (r>0.6) was identified for 5 genes, including thromboxane A2 receptor (TBXAX2R) and arachidonate 5-lipoxygenase (ALOX5). Conclusion: ALD patients revealed significantly lower PAR to AA compared to healthy controls. AA non-responders showed significantly lower RNA signals of TBXAX2R and ALOX5 both included in AA metabolism. Further investigations at the protein level might be performed to characterize the role of TBXAX2R and ALOX5 in the pathomechanisms of ALD.

P 9.09
Identification of the Factor IX Glu-Domain as a Strategy to Improve Hemophilia B Therapy
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Introduction: Engineering of coagulation factors could be an attractive strategy to improve therapeutic proteins, such as factor IX (FIX) in hemophilia B. The Glu-domain mediates membrane binding of FIX and of other vitamin K dependent coagulation proteases and therefore provides an interesting target for protein modification. Substitution of several amino acids in the Glu-domains of protein C or of factor VII have been reported to result in proteins with increased activity.

Methods: In order to generate FIX with higher specific activity, we exchanged residues in the Glu-domain of FIX with corresponding amino acids from other vitamin K dependent coagulation proteases with reported higher affinity to phospholipid membranes than FIX. Mutations were introduced into a FIX expression cassette using site directed mutagenesis. Supernatants of non-transduced cells, or of wild-type FIX and of a FIX variant with decreased protein activity and led to an improved secretion following intramuscular gene transfer. The proteins resulted fully functional.

Results: In our study we included 7 different amino acid substitutions (Y1A, G4Y, V10K, F25Y, N34D, N35D, and R37T). Most of the substitutions resulted in FIX variants with decreased protein activity and none of the single substitutions resulted in a variant with significantly increased activity in our assay system. Since above-mentioned higher activities of protein C and factor VII were only observed when combining the effects of several amino acid substitutions, we also combined amino acid substitutions in FIX with preference to substitutions which did not detrimentally decrease protein activity. Only one of this combination (Y1A G4Y) could at least retain a similar activity (102±13%) compared to wild type FIX (100±20%). Conclusion: In the present study, we could not generate a FIX protein with increased specific activity by modification of the Glu-domain. However, the Glu-domain might still provide an interesting target for strategies to improve protein secretion and release on the cellular or on the supra-cellular level as desired for protein production or following gene transfer.

P 9.10
Comparison of Vitamin K1 and K2 Kinetics of Vitamin K epoxide reductase C1
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Objectives: Vitamin K is a co-factor of γ-carboxylase, an enzyme responsible for functional active blood coagulation factors. Carboxylation requires hydroquinone and results in its conversion to vitamin K epoxide. The vitamin K epoxide is recycled to vitamin K before it can be reutilised. This reaction is catalyzed by the enzyme vitamin K epoxide reductase (VKORC1). Here we present comparative data for VKORC1 kinetics using both substrates - vitamin K1 and vitamin K2 epoxide.

Design and Methods: Gas-liquid chromatography - flame ionization detection was used for quantification of vitamin K1 and K2 epoxides. The enzymatic activity of VKORC1 was measured by estimation of vitamin K1 and vitamin K2 quinone production from HEK transfected cells. Substrates and products were separated on HPLC-RPC18 and detected by DAD at 254 nm. The apparent kinetic constants of K1 and K2 max were calculated. Results: VKORC1 velocity followed the Michaelis-Menten equation. Differences were observed comparing Km and Vmax for the both substrates. Km values of 4.160 and 1.765 μM were received for vitamin K1 and vitamin K2, respectively. Vitamin K2 has a 2.4-fold lower Km so that the binding affinity of vitamin K2 epoxide to VKORC1 appears to be higher. Vitamin K1 epoxide seems to bind in a weaker manner to the enzyme. Differences were also identified when comparing the Vmax - values: 2.0825 nmol x mg⁻¹ x h⁻¹ for vitamin K1 and 2.5549 nmol x mg⁻¹ x h⁻¹ for vitamin K2. A slightly (1.2-fold) faster generation of K2 was observed compared with the formation of vitamin K1.

Conclusions: The apparent VKORC1 kinetic constants for the reduction of vitamin K epoxide indicated a 2.4-fold higher binding affinity to K2 epoxide and a 1.2-fold faster formation of K2.

P 9.11
Evaluation of Prospective Criteria for the Clinical Assessment of Efficacy and Safety of DDAVP (MINIRIN, parenteral)
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Introduction: Although the prescribing description for DDAVP (Minirin parenteral) lists all known side effects, the extent and incidence of side effects that occur during administration of DDAVP in patients with coagulation disorders seem to be not that comprehensively defined. The ultimate goal of this prospective study therefore is to explore – prior to and after the administration of a DDAVP infusion – suitable prospective markers, which might help to identify patients with high probabilities for experiencing side effects. We currently plan to enrol up to 100 patients into this study.

Materials and Methods: Prior to and three days after a standardised infusion of DDAVP (0.3 μg/kg/body weight in 100 ml physiological saline), beside the patient history and the examination, the following laboratory investigations will be performed: general coagulation parameters (PT, aPTT, INR), blood count, multiple serum analysis, von Willebrand analysis (ristocetin cofactor, vWF-Antigen, FVIII-activity) and osmolality of urine.

Results: We were able to include 19 patients into this study (female: 68%, male: 32%, median age: 32.5 years, range: 19–54 years). Patients presenting with potential contraindications for DDAVP were not enrolled into this study. Only 4 patients (21%) reported no side effects suffered from headaches presenting with a ‘pressure-like sensation’ (47%) or regular headaches (40%), fatigue (33%), low blood pressure with vertigo (27%), erythema (20%), ‘body heat’ (13%), nausea (13%), increase of weight (13%) and numbness of the extremities (13%). One patient each complained about an intermittent pre-syncopal status and systremma. Three patients (20%) did experience these symptoms for the period of up to three days after the DDAVP infusion. We did not find a correlation to changes of laboratory parameters nor were we able to identify a specific laboratory reaction towards the DDAVP infusion. Quite too often patients with side effects reported on stress, either due to a problematic state in their private- or business
Molecular Genetic Analysis in Patients with Inherited Factor V Deficiency

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Background: Coagulation factor V (FV) plays an important role in maintaining the hemostatic balance. The gene for F5 is located on chromosome 1q24.2 and consists of 25 exons encoding a 28 amino acids leader peptide and a 2196 amino acids mature protein. FV deficiency represents a rare autosomal recessive bleeding disorder with variable phenotypic expression. Little is known about the molecular basis underlying this disease. In our study we report 35 patients with FV deficiency, due to different types of genetic alterations. Methods and Materials: Automated direct DNA sequence analyses were performed on the 25 exons and intron/exon boundaries for mutation identification. Results and Discussion: In our study we investigated 35 patients with FV deficiency. In 32 cases we identified 35 genetic alterations. In the remaining 3 patients no mutation was found. None of the mutations were previously reported in the F5 database and 20 were novel. Six led to premature termination codons, because of nonsense or frameshift mutations. Fourteen patients exhibited missense mutations. This mutation profile correlates well with data published in the F5 database. The FV activity values, matched with the genetic alterations found in these patients. In three patients with no detected mutation, large deletions or mutations in the 3′UTR (LMAN1) could be considered as possible explanation. Conclusion: Our data substantially contributes to the knowledge of the FV mutational spectrum. Identifying the molecular basis of mutations underlying this rare coagulation disorder will help to obtain more insight into the mechanisms involved in the variable clinical phenotype of patients with FV deficiency.

Role of Microparticles in Thrombin Generation in Patients at Risk for Atherothrombosis

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We have shown recently (Hron et al. JAMA 2006) that measuring thrombin generation can identify patients at low risk for recurrent venous thromboembolism and that peak thrombin is a suitable parameter to differentiate low risk from high risk patients. In this study we used the same assay (Technothrombin® TGA) to analyze patients at high risk of atherothrombosis (a Swiss study of patients with a history of myocardial infarction). Moreover, we investigated the influence of microparticles on thrombin generation in these patients. Thrombin generation was measured using Technothrombin® TGA. The reagent used contained low levels of phospholipid and 5 μM tissue factor. Platelet poor plasma from 178 patients (n=178, age =55.8 years, male/female=157/21) who had a previous myocardial infarction and 79 normal age and sex matched controls (n=79, age = 78.9 years) was analyzed. Microparticle-free plasma was generated from PPP plasma by filtration through a membrane (0.2 μm pore size) using a standardized filtration device (Ceveron® MFU 500, Technoclone). In the control group the mean value for peak thrombin in platelet poor plasma was 252±7.7 nM (mean±SD). A significantly higher (p<0.05) peak thrombin was found in the group of patients (355±49 nM) when patients without medication were analyzed. In patients on aspirin (n=109) the peak thrombin was not significantly different from untreated patients (297±10 nM) while in patients on oral anticoagulation (n=34) peak thrombin was significantly lower (225±27 nM). In the group of patients on oral anticoagulation the lag period was significantly longer as compared to controls or patients without medication (21.9±2 minutes versus 14.8±0.2 minutes) whereas aspirin treatment did not affect the lag period significantly. After removing microparticles from the samples, the peak thrombin values between patient and control group were no longer significantly different. The difference between microparticle free plasma and platelet poor plasma was significantly higher (p<0.05) in the patient groups without therapy (304±39 nM) and in patients on Aspirin therapy (257±23 nM) as compared to the normal controls (182±60.14 nM). These data indicate that thrombin generation is significantly increased in patients with previous myocardial infarction and that this increased thrombin generation is likely due to microparticles contained in these samples.

Incidence and Laboratory Detection of Resistance to Antiplatelet Drugs in a Selected Population of Patients with Arterial Disease

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Background: Evidence of 'resistance to antiplatelet drugs', defined by failure of the agent to hit its pharmacological target, requires specific labora-
tory testing. True resistance to acetylsalicylic acid (ASA), documented by absence of inhibition of cyclooxygenase (COX-1) and determined by serum thromboxane B2 (TXB2) levels, appears to be rare (1–2%). In contrast to ASA, studies to measure pharmacological effects of clopidogrel reveal a broad variety of results, with a significant proportion of subjects (15–30%), who are 'poor responders'. Design and Methods: To evaluate the rate of subjects who have (i) a true resistance to ASA and clopidogrel or (ii) a 'high residual platelet reactivity' while on treatment with ASA, clopidogrel, or both, we have performed a retrospective analysis of 135 orders from 99 consecutive patients whose blood samples were sent between January 2007 and March 2008 to our laboratory for testing under the clinical question 'resistance to antiplatelet drugs'. Pharmacological effects on platelet function were determined by aggregometry in response to epinephrine (5 μM), ADP (5 μM), collagen (0.6 and 2 μg/ml), arachidonic acid (1 μM), by TXB2 levels, and flow cytometric analysis of phosphorylation of the vasodilator-stimulated phosphoprotein (VASP). The results were classified according to the presence or absence of pharmacological effects, i.e. (i) 'resistance', (ii) 'poor response' including 'high residual platelet reactivity', and (iii) 'resistance', i.e. pharmacological inhibition. Results: The blood samples analyzed originated from 66 males and 33 females (patients’ mean age 62±15 yrs) treated with ASA (n=12), clopidogrel (n=15), or both (n=72) for secondary prophylaxis of coronary artery or cerebrovascular disease. Of the 12 patients only on ASA, all were responders, as confirmed by complete inhibition of COX-1. Of the 15 patients only on clopidogrel, 7 showed pharmacological inhibition, whereas 8 were poor responders. Of the 72 patients on combined therapy, 19 were responders (26%), 50 poor responders (69%) and 3 nonresponders (4.2%) with true resistance to both agents. Among the 50 poor responders to both, ASA and clopidogrel, 21 had 'high residual platelet reactivity' (42%). Conclusion: This retrospective study confirms that 'true resistance' to antiplatelet drugs is rather rare. The number of subjects on combined therapy with ASA and clopidogrel was overrepresented in this highly selected patient population, indicative of a subgroup at risk, obviously requiring a more intense antiplatelet treatment. Interestingly, among those, the number of poor responders was unusually high with a significant proportion of patients revealing 'high residual platelet reactivity'. To explore the nature of this phenomenon will be a challenge for future studies.

Plasmapheresis in Thrombotic Microangiopathy

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Introduction: The most prominent diseases associated with thrombotic microangiopathy are the thrombotic thrombocytopenic purpura (TTP) and the haemolytic uraemic syndrome (HUS). These diseases are characterized by thrombocytopenia and haemolytic anaemia in the presence of a microangiopathic haemolytic anaemia. The most effective treatment for TTP is plasma exchange (PE) with either fresh-frozen plasma (FFP) or SD-plasma. In our university hospital FFP and SD-plasma were used for PE in patients with TTP and HUS. In this retrospective study we compared the outcome regarding to the used exchange fluid. Results: A total of 89 patients with TTP (n = 57) or HUS (n = 32) were treated at the university hospital Charité-University Medicine Berlin between Jan 2005 and May 2007. During this time, PE was carried out on 27 patients (26 adults, one child). The mean age of these 27 patients was 47 years (range 16–89). Response was defined by a platelet count of more than 150×109/ l for at least 2 days. For the daily PE-treatment 40 ml/kg body weight were used. 8 patients each received either FFP or SD-plasma. 11 patients received both. The overall mortality was 15% (4/27). In the
surviving patients the average platelet count increased from 56.6 (± 50.3) to 232.7 (± 110). 8/27 patients, who were refractory to PE or in relapse were treated additionally with vincristine or (in one case) with rituximab. Patients who exclusively received SD-plasma (0/8) showed no allergic reactions, whereas a higher number of allergic reactions (4/8) occurred after using FFP for PE. Furthermore, the number of patients, under complete remission was higher in the SD-plasma group (5/8) than in the FFP group (1/8). In contrast, the number of refractory patients was lower in the SD-plasma group (1/8) compared to the FFP group (6/8).

Conclusion: Patients with TTP and HUS were successfully treated with PE. The use of SD-plasma seemed to be more tolerable than FFP due to the reduced number of allergic reactions. The number of patients under complete remission was also observed to be higher in the SD-plasma group. However, due to the small number of patients, further studies are required to confirm these results.

P 9.16 Antioxidant Properties of VKORC1L1
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Objectives: Combined vitamin-K-dependent clotting factors deficiency (VKCFD2) is caused by allelic mutations in VKORC1. The biological function of VKORC1L1, the isozyme of VKORC1, is unknown. It cannot substitute deficient VKORC1 in VKCFD2 despite its ability to reduce vitamin K epoxide to vitamin K quinone. VKORC1L1 is not able to provide g-Carboxyglutamate with vitamin K hydroxynitrone, necessary for g-carboxylation of vitamin K dependent coagulation factors. Starting from the neuroprotective properties of vitamin K, we investigated a possible role of VKORC1L1 in antioxidation. Design and Methods: VKORC1L1 was over expressed by plasmid transfection and silenced by siRNA in HEK cells. Antioxidant activity of VKORC1L1 was determined by CellTiter96® Non-Radioactive Cell Proliferation Assay (Promega), based on the conversion of a tetrazolium dye to formazan by viable cells. Viability of HEK cells was measured after treatment with hydrogen peroxide (0–50 μM) for 18 hours.

Results: Examination of the cell viability under various stress conditions with oxidative agents showed increased resistance of the VKORC1L1 transfected HEK cells. The number of viable cells was 15% to 20% higher compared to non-transfected cells. Silencing the VKORC1L1 gene by siRNA transfection results in higher sensitivity towards oxidative stress and a reduced viability.

Conclusion: Overexpression of VKORC1L1 in vitro shows a significant cytoprotective effect upon exposure to oxidative stress conditions, whereas VKORC1L1 knock down results in sensitivity.

P 9.17 Muscular Compartment Syndrome in Hemophilia A Inhibitor Patients
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Objectives: Inhibitor development (against factor VIII or IX) is a severe complication of haemophilia treatment leading to life-threatening bleedings. Compartment syndrome is a complication of musculoskeletal bleeding. Especially in the forearm, bleeds can cause large increases in tissue pressure in closed muscle compartments leading to alteration of microcirculation and neuromuscular function, thus necessitating acute surgical treatment. Results: We report on 3 inhibitor haemophilia A patients with compartment syndrome of the forearm. Patient 1, age 81 y, spontaneously developed a painful swelling of the left forearm and a compartment syndrome despite FEIBA substituition. Before referring the patient to our institution, an incision was done, but persistent bleeding resulted in a decrease of haemoglobin from 16.2 to 10.2 g/dl and a deterioration of neurological symptoms. A second incision was done in our orthopaedic department while 4000 IE FEIBA were substituted every 6 hours. There was no postoperative bleeding and the intramuscular haematoma resolved. Patient 2, age 6 y, had a fracture of the forearm. He was operated with a FEIBA substitution regimen. Postoperatively, he developed a compartment syndrome of the left forearm with necrosis and was referred to our centre. Repeated operation was done with factor VIII 240 KIE every two hours initially, because of persistent bleeding factor 8 was added. Thereafter bleeding was controlled and haematoma resolved. Patient 3, a diffuse muscle bleeding developed a severe compartment syndrome of the left forearm. He still had an altered recovery of factor VIII and was therefore treated with high dosage of factor VIII. Incision was done at our orthopaedic centre without complications. All three patients had secondary wound healing with a temporary artificial skin substitute.

Conclusion: Compartment syndrome represents a severe complication of forearm bleeding in inhibitor patients. Interdisciplinary management in specialized centers experienced in controlling bleeds with FEIBA and rF.

P 9.18 Evaluation of a Method for Removal and Determination of Thrombogenic Microparticles
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Aims: Circulating cell-derived microparticles play a major role in thrombotic diseases. Currently available methods to analyze microparticles are not easy to standardize, need specialized technical equipment or detect only a subpopulation of microparticles. Here a new method for quantification of circulating thrombogenic microparticles is evaluated (Technoobolin®MP microparticle and Ceveron®MFU-500). Methods: The principle of this method is based on the differences in thrombin generation between platelet-poor-plasma (PPP) and microparticle-free-plasma (MPFP) obtained by filtration through a 200nm filter using a filtration device (Ceveron®MFU-500). PPP was prepared by centrifugation for 15 min at 2,500 g. MPFP was generated by filtration (Ceveron® MFU-500; 0.2 μm low protein binding membrane) or by high speed centrifugation (15,000 xg for 30min). PPP and MPFP samples were analysed for thrombin generation using the Technoobolin®CA method. For calibration, dilutions of purified MP from red blood cells were prepared in PPP and thrombin generation was measured. Recovery of MP from the filter membrane was performed by rinsing the membrane with an equal volume of standard MPFP. Inclusion of ATP and non-filtered substances was analyzed in standard coagulation assays (PT, aPTT, Fibrinogen, FVIII-activity, Lupus-assy).

Results: Peak thrombin from centrifuged (57 nM ±8) or filtered samples (79 nM ±11) was not significantly different (p=0.14) but was significantly lower (p<0.05) than from PPP (71 nM ±2) indicating that MP have a significant effect on thrombin generation and that centrifugation and filtration are equally effective in removing microparticles. The analysis of purified MP diluted in MP free plasma showed that the difference in peak thrombin before and after filtration correlated to the number of microparticles and thus a calibration curve could be established. Significant differences between PPP and MPFP were found for aPTT tests only in one of three reagents tested (PPP 36.0 sec ±3.5, MPFP 38.2 sec ±3.4, p<0.05). The Lupus LCA Index for MPFP (34.8±6) was significantly lower than in PPP (47.4±7; p<0.05). For the other coagulation parameter tested no significant difference was found.

Conclusions: All these results show that circulating microparticles are a major determinant for thrombin generation and that thrombogenic microparticles can easily and quantitatively be analyzed from the difference in thrombin generation between PPP and MPFP obtained by filtration through Ceveron®MFU-500. It is shown that microparticles have a significant effect on thrombin generation and on some standard clotting assays.
sion, the platelet adhesion rate showed no significant gender-related difference, neither at 50 sec-1 nor at 500 or 1500 sec-1 (p>0.05), respectively. The same was true for relative adhesion, defined by the ratio of absolute adhesion after 1 min and at 15 sec after start of perfusion. At low shear (50 sec-1) active adhesion was 4.72±0.2, in men and 3.02±0.215 in women, at high shear (1500 sec-1) corresponding data were 5.39±0.240 and 4.56±0.144 (p each <0.05). Among the female volunteers, no significant difference was observed either with regard to the menopausal status (p>0.05).

Conclusion: This study demonstrates that hormonal differences between men and women have no significant impact on the interaction of platelets with immobilized fibrinogen, neither under venous nor under arterial flow conditions. However, this conclusion is limited to the observations made by an in vitro system. Therefore, it remains to be shown if the same is true in vivo.

P 11 Diagnostic Tool, New Technologies

P 11.01
The Role of PDI and the L-Type Lectin LMAN2L in the Secretion of Full-Length and B-Domain-Deleted Factor FVIII

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The transport of coagulation FVIII from ER to Golgi is mediated by the cargo receptors LMAN1 and MCFD2, which in case of mutations are known to induce FV/FVIII deficiency in patients. But there are still rare homopolymers A cases where no cause is found in the FVIII gene itself or the LMAN1/MCFD2 cargo receptor complex. Therefore we aimed to analyze if the related lectin LMAN2-like protein plays a role for the secretion of B-domain of FVIII in human endothelial cell lines. Furthermore we investigated the impact of phosphodiisulfide isomerase (PDI) on secretion of recombinant FVIII and especially secretion-defective FVIII mutants.

SK Hep-1 and HepG2 cells, representing liver sinusoidal endothelial cells and hepatocytes, were stably transduced with lentiviral vectors carrying a full length (FL) - or B-domain-deleted (BDD) - FVIII transgene or a mutant BDD-FVIII. Point mutations in FVIII domains were chosen by the online database HAMSTeRS and introduced in lentiviral transfer vectors coding for BDD-FVIII by site-directed mutagenesis. Specific knockdown of PDI and LMAN2-like (LMAN2L) protein was induced by siRNA transfection. 24 to 96 h after RNAi-application the amount of active FVIII in comparison to total FVIII Ag was determined in cell supernatants.

We could show that downregulation of LMAN2L protein led to an increase of FVIII:Ag in SK Hep-1 and HepG2 cells expressing FL- or BDD-FVIII. Problematically secreted FVIII in SK Hep-1 cells was mainly inactive, probably due to marked cell toxicity 48h after anti-LMAN2L siRNA application. In contrast HepG2 cells showed less cell toxicity effects and 72h after LMAN2L-knockdown the yield of active and total FL-/BDD-FVIII in the cell supernatant of these cells was increased by more than 100 %. We could also show that anti-LMAN2L siRNA improved secretion of BDD-FVIII mutants in HepG2 cells. Knockdown of PDI had contrary effects on FL- and BDD-FVIII secretion. Whereas PDI downregulation led to strong impairment of BDD-secretion, secretion of active FL-FVIII was slightly increased in both cell lines. An indirect FVIII-ELISA could reveal that up to 2-fold more full-length and full-length FVIII in high density cell lines. Furthermore, we observed an increase in cell toxicity 48h after anti-LMAN2L siRNA application.

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In conclusion, disulfide bonding by PDI is not only necessary for FVIII functionality it also seems to be the bottleneck in secretion of FL-FVIII. LMAN2-like protein could play a chaperone-like role either in ER-retention of FVIII or in degradation of the glycoprotein.

P 11.02
Evaluating Human Parvovirus B19 Sequence Variability in Blood Donors with High Resolution Melt Analysis (HRM)

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Purpose: Human parvovirus B19 (B19) actually forms three genotypes 1–3, where 1 is by far the most prevalent in Europe. Growing information on B19 patients with high resolution melt analysis (HRM) shows that this method might be up to 2000 times faster than standard sequencing, but so far it was only used in research. Aim of the ongoing study is to evaluate whether HRM is a simple and cost effective option to identify, characterize and monitor the presence of different genotypes/relevant mutants of B19.

Methods: Currently available HRM dyes were evaluated in an optimized B19-specific real time PCR. Blood donations from volunteer donors are screened for B19 DNA with the arts parvo B19 LC PCR kit in plasma minipools. Fresh frozen plasma units from donations with viral burden ≥ 10 IU/ml have been identified by resolution analysis of minipools. Viral DNA has been isolated from plasma aliquots with the Qiagen virus kit and amplified by real time PCR (Qiagen HotStart kit) with specific oligos followed by HRM program on the RGH6000 cycery. In parallel amplified targets have been sequenced by standard methods. Alignment and phylogenetic analysis is done with the DNA STAR software.

Results: Plasma from 26 different blood donors of variant regional origin are under investigation. So far we were able to identify a variety of different melting curve profiles. Currently the profiles are compared to the sequence based phylogenetic analysis which will be published in detail in future. As a consequence HRM seems to be a simple and cost effective option to identify, characterize and monitor the presence of different genotypes/relevant mutants of B19.

P 12 Tissue Engineering and Regenerative Medicine

P 12.01
High Efficiency Retro- and Lentiviral Transduction of Blood-Derived Endothelial Progenitor Cells (EPC)

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EPC may play an important role in postnatal vasculogenesis. Enhanced thromboresistance of EPC through overexpression of antithrombotic genes might open new avenues for the treatment of prosthetic and vascular thromboembolic disease. To modify EPC genetically, we have used two culture conditions for isolation and generation of EPC from peripheral blood. Mononuclear cells harvested from healthy volunteers were cultured in En-docult or EBM2-MV medium. Emerging colonies were counted at day 5 or between days 14 and 25. Colony cells were analyzed on day 7, 14, 21, 28 or between days 30 and 60 for leukocyte markers CD45 and CD14, endothelial markers CD31 (PECAM), CD34, CD105 (endoglin), CD141 (thrombomodulin), CD144 (VE-cadherin), CD146 (P1H12), and von Willebrand factor antigen (vWF Ag). The proliferative potential was evaluated by growth kinetics, replating efficiency, and CFSE dilution analysis. Using nonadherent cultured cells, colonies appeared on day 4–5, consisting of central round cells with elongated spindle-shaped cells sprouting at the periphery. The colonies and the spindle-shaped cells slowly disappeared after day 8–10. FACs analysis on day 5, 12, and 21 showed strong expression of CD45 and CD14, weak expression of CD31, but no expression of CD105, CD34, and vWF Ag. Using adherent cultured cells, colonies appeared in 50% of the wells between a median of 20 ±5 days. These cells rapidly replicated to form colonies with a cobblestone-like appearance which later formed a confluent monolayer. After a lag phase of 20 days, cell expanded and detached. By FACs analysis these cells showed strong expression of CD31, CD105, and CD146, intermediate expression of CD41, weak expression of CD34, and no expression of CD45 or CD14. The cells stained positive for VE-cadherin and vWF Ag. An enhanced green fluorescent protein encoding retroviral SFFV vector (cell free derivative from PG13) and a novel lentiviral LeG0 vector expressing the red chorophore tandem dimer (td)Tomato (cell-free derivative from Phoenix GP or 293T using 3rd generation packaging plasmids pMDLg/pRRE and pRSV-Rev; GALV Env; 160/10 virus particles/ml) were used for transduction. EPC were transduced with high efficiency, 62–80% and 91–99%, using murine retroviral and human lentiviral vectors containing the eGFP and tdTomato. Our results suggest that nonadherent cultured cells have a low proliferative potential and display an angiogenic macrophage-like phenotype. In contrast, adherent cultured cells have a high proliferative potential, display an endothelial phenotype without coexpression of leukocyte antigens, and are very efficiently transduced by lentiviral vectors.

Abstracts
P 12.02

Short Term Clinical Scale MSC Propagation in Animal Serum-Free Medium: Minimum Requirements for Sufficient Transplants

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Ex vivo propagation of human multipotent mesenchymal stromal cells (MSC) is currently considered as a prerequisite for MSC therapy. This study was performed to define the minimum requirements for producing sufficient MSC numbers for therapeutic application in a completely animal serum-free standard system from small bone marrow aspiration volumes within clinically acceptable short time.

In compliance to good manufacturing practice we established a time and resource saving efficient procedure for MSC propagation. Bone marrow was seeded without manipulation directly in pooled human platelet lysate (pHPL) and L-glutamine supplemented minimum essential medium without antibiotics. Cells were expanded in serum-free Stem Cell Differentiation medium containing Stem Cell Factor, Insulin-Like Growth Factor I, Dexamethasone and Erythropoietin. At days 1–3 of cultivation, suspension cells were collected to obtain stage 2 cells.

Results: We assessed cell suspensions for the presence of erythrocytic colony-forming progenitors, TER119 (Glycophorin A-associated) antigen positive cells and benzidine-positive cells to detect haemoglobin. Cell numbers amplified approximately 2 logs during EB formation. We detected 15–45 erythrocytic colonies per 30,000 seeded cells, and between 5–38% (CCE) and 2–13% (D3) TER119-positive cells, with similar results in either small scale or mass culture conditions. Moreover, staining for benzidine already revealed hemoglobinization after either step. The maintenance of cells in the second stage was possible only for short-term and resulted in further increase of cell numbers and generation of both BFU-E/CFU-E colonies and Ter119 expressing cells.

Conclusions: Erythrocytic cells of various maturation stages can be derived from murine ES cells using a two-step protocol under small scale and, similarly, mass culture conditions. Their functionality and ability to terminally differentiate remain to be shown. Analogous differentiation of conventional human ES cells or patient-specific pluripotent stem cells may pave the way for substitution of red cells or immediate progenitors useful for transfusion therapy.

P 12.04

Blood Components in the Topical Treatment of Ocular Surface Defects: Regenerative Capacity of Human Albumin versus Serum: An In-vitro Study

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Objectives: Autologous serum has been successfully used for the topical treatment of persistent corneal epithelial defects and severe dry eye. Its positive effect on the regeneration of corneal epithelium is thought to be due to its high content of growth factors, nutritive agents and vitamins. Recently heterologous human albumin (HA) preparations, that are easily available as standard blood products, are advocated as an alternative therapeutic agent in patients with ocular surface disease. In this in-vitro study HA and serum were comparatively tested for their regenerative impact on corneal epithelial cells.

Design and Methods: Serum was prepared from healthy volunteers (n=10) under standardised conditions. HA was purchased as a standard 20% solution originally designated for intravenous application from Baxter. Serum and HA were diluted with Balanced Salt Solution to final concentrations of 100, 50, 25, 12.5 and 6.25%. SV40-immortalized human corneal epithelial cells were incubated with both test substances for 6, 24 and 48 hours. Cell growth was quantified by means of a luminescence-based-ATP-assay. Cell migration was assessed in a colony dispersion assay. The concentrations of EGF, TGF-beta, fibroactin, vitamin A and vitamin E were evaluated with ELISA or HPLC, respectively.

Results: Serum was found to be considerably superior to HA regarding cell growth and migration supporting capacity at all time points and independently of concentration. The best results were obtained with undiluted serum. Concentrations of growth factors and vitamins were higher in serum than in HA: EGF: serum 15.3 ng/ml/+ 52.6 ng/ml, HA 10.6 ± 0.01 ng/ml; TGF-beta: serum 32.0 ± 4.6 ng/ml, HA n.d.; Vitamin A: Serum 3.6 ± 0.6 micromol/l, HA 0.13 ± 0.01 micro mol/l; Vitamin E: serum 23.5 ± 3.7 micromol/l, HA n.d.

Conclusions: Migration and proliferation play a major role in tissue repair. In the cell culture model used, serum was clearly superior to HA in supporting cell migration and proliferation of corneal epithelial cells. This may be due to its higher content of growth factors, fibronecrtin and vitamins. The results of this in vitro study suggest the use of serum eye drops rather than HA in the treatment of patients with ocular surface diseases. For definite proof of the supremacy of autologous serum controlled clinical trials will be necessary.
P 12.05
Differential Effects of Human Alternative Supplements Replacing FCS on Mesenchymal Stem Cells from Different Human Tissues

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Mesenchymal Stromal Cells (MSC) are in focus regarding their clinical potential in cell therapy, tissue engineering and immune regulation. To enable clinical-scale manufacturing, human alternative supplements replacing fetal calf serum (FCS) for MSC expansion are under thorough investigation. We already demonstrated the superiority of pooled human serum (HS) as well as thrombin-activated platelet lysate in plasma (tPRP) on MSC derived from adipose tissue (AT) [1]. Subsequently we investigated their effects on MSC from human bone marrow (BM) and cord blood (CB).

MSC from BM (n=14) were isolated and cultured in basal medium supplemented with either 10% a) FCS (as control), b) AB-HS, c) tPRP or d) human Platelet Lysate (PL). Due to extremely low MSC frequencies in BM, it was not possible to perform paired analysis. Up to now, each two CB units were processed using HS-AB or tPRP and four using PL. In addition three primary CB-MSC cultures established using FCS were subjected to p1 to the four different supplements. For all culture conditions, long-term expansion capacity as well as the differentiation capacity into osteogenic and adipogenic lineage was tested. Cell surface marker expression was analyzed by flow cytometry. Primary isolation and expansion of BM-MSC was possible in all culture conditions. However BM-MSC cultured with AB-HS or tPRP showed reduced proliferation compared to FCS, contradicting the data obtained using AT-MSC. Only medium supplemented with PL yielded clinically relevant cell numbers within the first 2–3 passages. Resting PL in AT-MSC, in vitro gel-formation prevented further expansion in passage cultures. None of 2 CB units tested using AB-HS or tPRP gave rise to MSC, which however barely suggests anything due to the low numbers processed. However, efficiency of 50% (2 from 4 units tested using PL) seems to be encouraging. Strikingly, CB-MSC initially cultivated using FCS, and then subjected to the 4 different supplements, showed absolutely no growth in AB-HS and tPRP, but expansion in PL. For all MSC cultures, quality control assays revealed no impact on viability as well as marker expression. Human MSC derived from the three sources CB, BM and AT respond differently to human alternatives replacing FCS. Identifying the biological basis for the different susceptibility of MSC derived from different tissues will be mandatory to define optimal protocols for clinical-scale manufacturing.

1Kocaoener A, Kern S, Klüter H, Bieback K. Human AB serum and thrombin-activated platelet-rich plasma are suitable alternatives to fetal calf serum for the expansion of mesenchymal stem cells from adipose tissue. Stem Cells 2007;25:1270–1278.

P 13.01
Theraflex UV-Platelets: Influence of UVC-Irradiation on Canine Platelet Concentrates

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Background: The Theraflex UV-System uses UVC light for pathogen inactivation in platelet concentrates. As preparation for preclinical safety studies in dogs, the effects of UVC light on platelet parameters and storage stability on canine platelet concentrates (cPC) were investigated. Materials and Methods: Single cPC in storage medium SSP+ were prepared from 14 donor beagle dogs. The cPC, weight 94 ±4 g, platelet concentration 3.8 ±0.6 x10^11/mL, protein content 20.2 ±2.2 mg/mL, were filled into UVC-transparent plastic bags and irradiated under agitation on a device emitting UVC light (wavelength: 254 nm) at a dose of 0.4 J/cm². Influence of UVC on platelet parameters was investigated the day after UVC treatment and after storage at 22 °C for 6 days. As control, untreated cPC were used.

Results: The influence of the UVC treatment on the examined PC parameters was, with the exception of the p-LCR values, not very pronounced. Statistical analysis by paired t-test between UVC-treated and untreated cPC, however, showed clearly signifi cant differences between UVC-treated cPC and the untreated controls, mainly after a storage period of 4 and 6 days. Like human PC after UVC treatment, UVC-treated cPC showed a higher metabolic activity, indicated by increases in CO2 content, a higher lactate accumulation and a significantly increased collagen-induced aggregation on day 6 (untreated: 22.8% ±1.5, UVC-treated: 30.8% ±3.06). In contrast to human PC, UVC treated cPC exhibited a proportional increase in the percentage of large platelets (p<0.05) after 4 and 6 days of storage if the UVC group (day 6: untreated: 15.1% ±1.8, UVC: 21.3% ±3.1).

Conclusion: The influence of UVC light on in vitro parameters and on storage stability of the treated cPC is minimal up to 4 days after treatment. The UVC effects on cPC exhibit close similarities to those encountered on human PC.

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P 13.02
Blockade of Maternal Anti-HPA-1a-Mediated Platelet Clearance in a in vivo Mouse Model by an HPA-1a-Epitope-Specific F(ab)’2

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Objective: Neonatal alloimmune thrombocytopenia (NAIT) is most commonly caused by transplacental passage of maternal anti-HPA-1a antibodies (mAb) to fetal HPA-1a expressing tissues. Recently, mAb S2Z1 was shown to be effective in preventing HPA-1a alloantibodies from binding to fetal platelets in vitro. Therefore, mAb S2Z1 could be a potential therapy for NAIT through blockade of binding of maternal HPA-1a alloantibodies to fetal platelets. Design and Methods: Resting human platelets from HPA-1a-mothers were injected into the retro-oral plexus of non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. Circulating platelets were exposed to IgG from anti-HPA-1a-sera obtained from NAIT-mothers or AB sera from healthy donors. Purified F(ab)’2 fragment of mAb S2Z1 or control mouse IgG were injected 1 hour prior to human HPA-1a antibodies. Mouse blood samplings were taken over time and analyzed by flow cytometry. The percentage of human platelet circulating in the living mice were determined to evaluate the ability of mAb S2Z1 to prevent anti-HPA-1a-mediated platelet clearance. Results: Human platelets circulated for up to 24 hours in NOD/SCID mice in the absence of platelet antibodies. In contrast, anti-HPA-1a antibodies derived from NAIT-mothers were able to efficiently clear HPA-1a positive platelets from the mouse blood circulation. Administration of S2Z1 F(ab)’2 Fragment prevented anti-HPA-1a-mediated platelet clearance. This fragment did not influence either platelet circulation time or platelet function. Conclusion: In this study, we demonstrated that the F(ab)’2 fragment of mAb S2Z1 could be an effective inhibitor of anti-HPA-1a-mediated clearance of human platelet circulating in an in vivo NOD/SCID mouse model, indicating a new therapeutic approach for NAIT. Further, our NOD/SCID mouse model system allows the study of the effect of human polyclonal antibodies on human cells circulating in a living organism under circumstances similar to those seen in human allo/auto immune diseases.
low and DQ, DQ high resolution matched cohort with a follow-up of 5 years. Transplant related mortality at one and at 5 years (TRM 1 yr, TRM 5yr) as well as overall survival at 5 years (OS 5yr) were analyzed in relation to NOD2/CARD15 genotypes. The individuals were genotyped for the NOD2/CARD15 SNPs rs2066845 (SNP8), rs2066846 (SNP12) and rs7543923 (SNP13). To accomplish genotyping we designed a assay based on "xMap Multi-Analyte COOH (=Beads)" technology from the company Luminex. After establishing a multiplex PCR (MPCR), unsanctioned color-coded beads were specifically linked to each of the SNP through Capture- Oligonucleotides and subsequently hybridized with the MPCR products. Amplification success and specificity of the hybridisation were verified by sequencing in 43 DNA samples from healthy related individuals. Results: NOD2/CARD15 mutations occurred with a frequency of 18.4% in patients and 19.1% in donors. Mutant allele frequencies for SNP 8, 12, 13 were found in 5.4%, 1.7%, 2.5% of donors, and 4.2%, 1.2% and 4.2% of patients, respectively. The frequencies of the NOD genotypes were in Hardy Weinberg equilibrium and confirmed the results of other studies on Caucasian individuals. There were no significant differences in TRM at 1yr, 5yr, as well as OS 5yr for transplants with and without SNPs, SNP12, and SNP13 mutations. The sequencing of the additional 129 tests revealed a 100% match to the results of the in house Luminex assay. Conclusions: The preliminary analysis of our data did not show an independent effect of NOD2/CARD15 mutations in the outcome of unrelated HSCT. With the Luminex technique we established a method for reliable, high throughput NOD2/CARD15 typing.

P 14.02 Quality Control of Leukocyte Reduced Blood Components: Overestimation of WBC Content Due to Nucleated Red Blood Cells
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Background: and Objectives Since several years leukocyte (WBC) filters are introduced in routine blood bank practice. For quality control flow cytometric methods for counting residual WBC are well established and widely used. Especially the commercial avaliable kits are based solely on DNA staining for WBC detection. We hypothesized that nucleated red blood cells (NRBCs) could impact these numbers. Methods: We developed a flow cytometric method for threshold detection of nucleated cells (NC) and leukocyte detection using simultaneously antibody (AB) and DNA nuclei staining. In brief 100 µl sample was stained with CD45 (AB, specific for WBC) and DRAQ5 (DNA stain). Measurement was done using a single platform setting using flow count beads. In parallel a commercial available kit based only on DNA staining (Ph) was used. CD45 positive DRAQ5 positive cells were assumed as WBC. CD45 negative DRAQ5 positive cells were classified as NRBCs, NCs as the sum of WBCs and NRBCs. For proving that these cells are really NRBCs, they were flow cytometric sorted on slides and evaluated using Wright Giemsa staining. Results: We investigated pre storage filtered erythrocyte concentrates (EC, n=128), plasma (FTP, n=5) and platelet concentrates (PC, N=42) as well as 10 whole blood samples. For all samples a good concordance between the commercial kit and the detected NC count was seen. Microscope evaluation of the sorted DRAQ5 positive CD45 negative cells revealed that 95% of these cells were NRBCs whereas the blood products EC 30.5% of all NCs were NRBCs (SD 23.5%), in PC 22.6% (SD 14.1%), in FTP 4% (SD3.8%) whereas in WB only 0.3% (SD0.4%). Conclusion: Solely DNA based counting methods overestimate residual WBC content of leukocyte reduced blood components due the missing discrimination between NRBCs and WBCs.

P 14.03 Dual Anti-Platelet Therapy with Aspirin and Clopidogrel Might Be Ineffective in Cardiologic Patients Regarding the Flow-Cytometric Platelet Reactivity Index
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Objective: Combination therapy with aspirin and clopidogrel is an established treatment for patients presenting with non-ST segment elevation myocardial infarction, or with planned percutaneous coronary stent implantation. In these patients it would be advisable to have a confirmative parameter of the in-vivo platelet function for both to control therapeutic efficacy and to forecast bleeding complications e.g. in cases of emergency or urgent surgery. So far clopidogrel usually has to be stopped at least eight days before the procedure in these cases. Platelet function testing in anti-platelet treated patients would allow a differentiated management of cardiology based on "xMap Multi-Analyte COOH (=Beads)" technology from the company Luminex. After establishing a multiplex PCR (MPCR), unsanctioned color-coded beads were specifically linked to each of the SNP through Capture-Oligonucleotides and subsequently hybridized with the MPCR products. Amplification success and specificity of the hybridisation were verified by sequencing in 43 DNA samples from healthy related individuals. Results: NOD2/CARD15 mutations occurred with a frequency of 18.4% in patients and 19.1% in donors. Mutant allele frequencies for SNP 8, 12, 13 were found in 5.4%, 1.7%, 2.5% of donors, and 4.2%, 1.2% and 4.2% of patients, respectively. The frequencies of the NOD genotypes were in Hardy Weinberg equilibrium and confirmed the results of other studies on Caucasian individuals. There were no significant differences in TRM at 1yr, 5yr, as well as OS 5yr for transplants with and without SNPs, SNP12, and SNP13 mutations. The sequencing of the additional 129 tests revealed a 100% match to the results of the in house Luminex assay. Conclusions: The preliminary analysis of our data did not show an independent effect of NOD2/CARD15 mutations in the outcome of unrelated HSCT. With the Luminex technique we established a method for reliable, high throughput NOD2/CARD15 typing.

P 14.04 Multicenter Study of Roche Cobas TaqScreen MPX Test on the Cobas s 201 Platform for Donor Screening in Pools of 96
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Objective: A multicenter study was done by three German blood banks. Hagen, Springer and Frankfurt, to evaluate the feasibility of routine testing of blood donor samples in pools of 96 using the Roche automated, cobas s 201 platform and the cobas TaqScreen MPX test, which were recently CE-IVD approved. At present, these blood banks test samples in pools of 96 using either a diagnostic or in-house test. Methods: Samples were pooled offline and the pools of 96 were run as individual samples on the cobas s 201 system. The analytical sensitivity for HIV, HCV and HBV was evaluated using WHO International Standards and the 95% LOQ values were determined by Probit analysis. The reproducibility and cross contamination of the system (robustness) was evaluated by testing replicates of low positive samples of HBV, HCV and HIV-1 (10e6 IU/ml for HCV and HIV-1 and 10e7 IU/ml for HBV) and negative samples, respectively. Genome inclusivity was evaluated using genotype panels for HCV, HIV-1 and HBV. To test the clinical performance and specificity, more than 62,000 routine donations were screened in pools of up to 96 donations at each test site, both by the cobas TaqScreen MPX test and the test of record. Results: The analytical sensitivities at all test sites were between1.6 and 3.6 IU/ml, 4.9—10.9 IU/ml and 14.7–26.6 IU/ml for HBV, HCV and HIV, respectively. The robustness of the cobas s 201 system was satisfying with all low positive replicates testing reproducibly reactive and only minor cross contamination rates. According to the panels tested the genotypes 1 to 6 for HCV, A to G for HBV and A to H, N and O for HIV-1 are detected by the cobas TaqScreen MPX Test with the same efficiency for
all genotypes. The clinical performance data showed a specificity of the 96-
pool testing from 99.3% to 100.0% while the sensitivity of the MPX Test
varied from 99.79% to 100.00%. No window cases were detected during the
study but one MPX-only reactive pool sample that tested negative with the routine DiaSorin AmpliPrep/COBAS TaqMan HIV-1 Test. Conclusion: The
cobas 2400 TaqMan MPX system is a fully automated NAT system
suitable for routine blood donor screening. Amplification of HIV-1 is de-
signed in the highly conserved 5’LTR region which might also improve
blood safety. The analytical and diagnostic sensitivity of the cobas TaqMan MPX Test fulfills all requirements of the Paul Ehrlich Institute for
blood donor screening in mini pools up to 96 donations.

P 14.05
Detection of Anti-Lea and Anti-Leb Antibodies in the
Lymphocyte Cytotoxicity Assay

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Objective: Frequently non-HLA-specific cytotoxic antibodies are detectible in lymphocyte cytotoxicity assays (LCT). Whereas HLA-specific antibodies are antibodies of clinical relevance in renal transplantation leading to hyperacute and acute organ rejection the relevance of cytotoxic antibodies with other specificities is still under consideration. Antibodies against MICA- or MICB, angiotensin II receptor type 1 (AT1), vitronectin, 100-kDa antigen, 38-kDa antigen are suggested to be involved in early graft loss, in acute and hyperacute renal graft rejection. Design, Method and Results: In our waiting list for cadaver kidney transplantation we found a patient (serum B) who was positive for non-HLA specific lymphocytotoxic antibodies. His blood transfusion history revealed immunisation against Lewis antigens, with the presence of anti-Lea (titer 32) and anti-Leb (titer 8) antibodies. In the LCT the patient was positive over five successive screening periods (15 month) for cytotoxic antibodies. The peak-PRA value based on LCT was 16% in the second period (month 3–6) and finally decreased to 0% in the fifth period. During the peak-PRA period lymphocytotoxic reactions were present with and without DTT, indicating the presence of IgM and IgG antibodies, whereas in the subsequent screening periods DTT abolished the cytotoxic reactivities. Antibody preabsorption of the sera using Lea and Leb positive erythrocytes significantly reduced the cytotoxic activity with a consistent reaction pattern against a selected donor panel. To confirm the cytotoxic activity of Lewis antibodies we subsequently screened two other Le-positive sera (serum B and serum C) for cytotoxic reactivity. In the investigated sera no HLA-specific antibodies were detected in the ELISA-
screening using LAT Mixed Class I&II screening plates (One Lambda).
Serum B contained Anti-Lea with a titer of 32 and serum C contained a weak titer of Anti-Lea. In the LCT serum B showed polyspecific reactions with a PRA value of 52%, whereas serum C did not show cytotoxic activity. Addition of DTT the serum B became negative in the LCT demonstrating the IgM nature of the anti-Lea antibodies. Conclusion: In conclusion our results indicate that non-HLA antibodies - anti-Lea and anti-Leb antibodies - display cytotoxic reactivity in the LCT. Since clinical investigations suggest that Le-antibodies are related to a reduced long term survival of the renal graft, Le-antibodies detected in the LCT are of clinical relevance. Serum sample exchange and prospective crossmatching of these patients will be a necessary requisite to prevent transplantation of Le+ grafts against these lymphocytotoxic Le-antibodies.

P 14.06
Characterization of New HLA Class I Alleles Using Group-
Specific Sequencing Primers (GSSPs) and HaploType-
Specific Extraction (HSE)

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Objective: This study analyzed the feasibility of GSSP and HaploPrep to
define new HLA-alleles. Design and Methods: During routine tissue typing
the presence of new alleles was initially detected by either reverse hybridiza-
tion or simultaneous sequencing of both alleles in heterozygous samples. In
order to identify and define these new alleles, two different strategies were
applied. Group-specific sequencing primers (GSSPs) were used to obtain
homologous sequence information of the region of interest. Furthermore, the
new alleles were isolated using haplotype-specific extraction (HSE) and
subsequently analysed by sequence-based typing (SBT) covering exons 1 to
5. In addition, the complete sequence information of introns 1, 2, 3 and 4 was
obtained in all cases. Results: 13 new HLA class I alleles (6 HLA-A, 6
HLA-B and 1 HLA-C) were identified using both methods and analyzed
extensively in order to present a comprehensive characterization that enables
a better classification of these new alleles into the HLA system. HLA alleles
can be successfully separated from diploid samples using HaploPrep extraction
prior to HLA typing with sequencing based typing. Therefore, new
alleles of heterozygous samples can easily be characterized by any
additional cloning. Group-specific sequencing primers (GSSPs) serve not
only for resolving genotype ambiguities but can also facilitate the identifica-
tion of HLA alleles with nucleotide exchanges in heterozygous samples.
Conclusion: The combined use of GSSPs and HaploPrep extraction speeds
up the characterization of new alleles since only the ‘new’ allele needs to be
separated and analyzed separately, thus ultimately reducing the number of
HaploPrep extractions and sequencing reactions. The complete sequence
information covering exons 1–5, including introns 1–4, largely defines the
new alleles helping to further characterize the polymorphism of the HLA
system. This knowledge is not only important for scientific questions, but
also for technical aspects of HLA-typing.

P 14.07
Initial Results of the BD Thrombo Count Assay for
Enumeration of Residual RBCs and RBCs in Platelet
Concentrates Using a BD FACSScalibur Flow Cytometer

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Objectives: Enumeration of residual leukocytes (WBCs) and red blood cells
(RBCs) is a mandatory part of the quality control (QC) of platelet concen-
trates (PCs). While flow cytomteric enumeration of residual RBCs (e.g.
using BD Leucocount) is increasingly replacing microscopic counting, RBCs
are counted usually by microscopy. The BD Thrombo Count Assay claims to
offer enumeration of both cell types, WBCs and RBCs, in one test tube by
automated flow cytometry. Our objective was to obtain initial test results of
selected PC samples comparing the Thrombo Count Assay with currently
employed QC methods, microscopic counting of residual RBCs and/or
WBCs, and Leucocount. Design and Methods: Selected PC samples (n=72)
were tested under routine conditions in our quality control lab (Haema AG)
using the BD Thrombo Count Assay. For comparison, residual WBCs were
also enumerated using the BD Leucocount test and manual counting using a
Nagette counter, and residual RBCs enumerated by manual microscopic
counting using a Fuchs-Rosenthal chamber. Flow cytometry and analysis
was done with a FACSScalibur instrument and CellQuest Pro software.
Results: Residual WBC analysis by Leucocount and Thrombo Count
showed a wide cell range of 0–775 WBC/μL. Calculated values resulted in a
fair correlation (r=0.877) and median values of 0.79 vs. 0.81 WBC/μL. In
contrast, values of residual RBCs counted using the Thrombo Count Assay
were considerably higher and did not correlate with the values obtained by
microscopy. The flow cytometry analysis showed different populations of
residual RBCs based on their relative size (FSC) and side scattered light
(SSC). Conclusion: These initial results confirm the utility of the BD
Thrombo Count Assay for enumerating residual cells in PC samples: The
WBC results correlated well with those using the Leucocount test. The BD
Thrombo Count Assay gave higher residual RBC counts compared to micro-
scopic counting that has to be discussed. In further studies we are investigat-
ing the use of commercially available control materials, i.e. the BD Leuco-
count, and manual Control for residual WBCs and an internal QC sample for residual
RBCs.

P 14.08
Performance Validation of BD Thrombo Count Assay for
Flow Cytometric Enumeration of Residual WBC and RBC
in Platelet Concentrates: Single Site Results of a Multi-
Center Study

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Objectives: The BD Thrombo Count Assay has been developed for residual
cell enumeration in platelet concentrates (PCs). As part of a multi-center
study to validate the performance of Thrombo Count, we evaluated PC
samples spiked with residual cells regarding linearity, precision, and accu-
racies according to NCCLS EP10-A and ICH Q2 guidelines. In addition,
sample stability and staining stability were analyzed. Furthermore, selected

Abstracts
Abstracts

P 14.09
Point-of-Care Thrombelastometry – How to Centralize without Loosing Benefits?
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Some studies show that the management of hemostatic therapy with the support of ‘point-of-care’ thrombelastometry (TEM) could significantly decrease the use of red cells and platelets and will have an effect on the use of other blood products in intensive-care units and cardiac surgery. However, the introduction of TEM at ‘points-of-care’ involved problems concerning the local availability of educated personnel, quality control and proper performance of tests. Objectives: Validation and transfer of the test system (ROTEM) to a central blood bank laboratory without substantial loss of immediate availability of test results through rapid sample transport by a blood carrier and online submission of analysis screens. Design and Methods: Measurement system analysis of the TEM-system (ROTEM) through Minitab ‘Gage R&R Study (Nested)’ protocol: three technicians each measured ‘INTEM’ and ‘EXTEM’ of three different samples (healthy blood donors) three times. To represent an extended measurement range we analysed both, undiluted and diluted samples (1+1 and 1+3 with NaCl 0.9%). Influence of the pre-test sample temperature, which could be modified by the transport on the results; one technician measured ‘INTEM’ of 8 samples (duplicates) under varying conditions. Incubation at 22 °C for 30 min was compared to 30 min at 4 °C, 30 min at 4 °C (insulating box) and 30 min at 37 °C (water bath). The results were analysed by ‘Minitab Two sample T-test’. Results: The thrombelastometry system (ROTEM) is able to detect the three analysed samples as different measurement results with a part-to-part % CV of mean values from 10-fold measurements of three selected cell concentration levels (low, medium, high) was calculated. WBC/μL: 0.58 (33%), 5.75 (12%), 10.98 (7.8%); RBC/μL: 633 (2.0%), 5670 (2.0%), 10095 (2.1%). A theoretical CV of ~20% was estimated at ~1.28 cells/μL. In the cell ranges analyzed accuracy ranged from 91–112% for WBC and 87–105% for RBC. In transported PC samples, residual cell counts were found stable after additional 0-24h of storage. Counts of stained samples were found stable for up to 3h. On selected PC samples, values of residual WBCs correlated well with those obtained using the Leucocount test. For RBCs, the values found by Thrombo Count were consistently higher than those obtained by microscopy. Conclusions: The results from this site of the multicenter study verify that the Thrombo Count Assay is a rapid, simple and reliable method for hemostatic enumeration of residual cells in PC samples. In samples spiked with RBCs and WBCs, Thrombo Count values calculated after background correction compared well with expected values and those using the Leucocount test. The fact that the values for RBCs in PC samples were found only higher when using Thrombo Count compared to microscopic counting needs to be further examined, as is being done in a separate investigation.

P 15.01
Blood Transfusion in Europe: Differences and Commonalities Leading to Pan-European Standards and Criteria for the Inspection of Blood Establishments. The EuBIS Project
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The specific objectives of the EU cofunded Project, EuBIS (www.eubis-europe.eu), are to define the actual situation of standards and criteria used for inspections of blood establishments among the participating and collaborative institutions from 19 established, new, applicant and EFTA countries, and to develop a transparent and comparable system of inspection standards for quality systems of blood establishments in place and (B) the current inspection practice. Design and Methods: The Project develops a manual, defining requirements for the structure of quality management systems based on the directive 2002/98/EC and its technical annexes. Based on these requirements, inspection standards and criteria are developed to assist in the independent assessment of quality systems established by individual blood establishments in relation to the requirements of the EU-legislation on blood. Results and Conclusion: Transposition to national laws in EU member states of the Blood Directives has been completed. Following the national diversity generated during the last decades, implementation will need some additional effort. From the survey performed between the Project participants and collaborating partners, the majority of blood establishments responded that the current quality management systems need at least minor improvements or modifications to be adapted to the EU Directive.
Towards an European Specialist in Transfusion Medicine
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**Background:** European directives 93/16/EEC, 2001/19/EC and 2005/36/EC deal with mutual recognition of professional qualifications of European medical doctors. Specialisations listed in the annexes of these directives not only comprise big medical subspecialties like internal medicine, surgery, etc., but also less frequent qualifications like immunology or tropical medicine, not common in all European member states. Transfusion medicine, however, is not represented in these directives.

**Objectives & Methods:** In order to compile the current situation in European countries, a first questionnaire was sent out via the EBA (European Blood Alliance) platform to 15 European countries. The objective of this questionnaire was to evaluate the rate of qualifications in transfusion medicine already established in these countries.

**Results:** Seven out of 15 European countries already have established a clearly defined specialisation in transfusion medicine and immunohaematology/immunology. Since transfusion medicine is not listed in the annexes of the above mentioned European directives, automatic mutual recognition cannot take place even between European member states covering transfusion medicine as a national medical specialisation. A detailed and more complex questionnaire regarding specialisation in transfusion medicine has now been prepared by our group aiming at an analysis of different requirements and constituent parts of qualification in transfusion medicine in different countries. This questionnaire is currently distributed to transfusion medicine societies in all member states of the European Union. A joint working group consisting of members of the European network of transfusion medicine societies (EuroNet-TMS), DGTI and EBA will meet in order to define European standards for qualifications in transfusion medicine. Our final aim is to jointly establish a consolidated European qualification in transfusion medicine and second, to introduce a European specialist in transfusion medicine into annex V of directive 2005/36/EC in order to facilitate automatic mutual recognition of qualifications in transfusion medicine throughout Europe.

Aseptic Manufacturing in Cellular Therapy: Investigation of Environmental Monitoring Programme Excursions
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**Objective:** According to Annex 1 of the EC Guide to Good Manufacturing Practice (GMP) a detailed environmental monitoring programme (EMP) is dealt for the surveillance of aseptic manufacturing of cellular therapy products in a class 100 (A) cleanroom. Here we report an investigation of out of specification (OOS) results and the correcting measures in the absence of any microbial product contamination.

**Design & Methods:** EMP comprises of continuous monitoring the pressure differences and particle counts and the periodical assessment of biological active particles (BAPs) with an air sampler, settle and contact plates. While preparing cells in the cleanroom settle plates are put in the working area and the gloves of the operators are monitored for microbial contamination with contact plates. According to the European Pharmacopoeia standards the plates are incubated, colony forming units (CFU) are counted and bacteria are identified. **Results:** On 7 subsequent occasions average CFU counts on gloves after the preparations were 1.43 (action limit 1.0) and prompted further investigations. Exclusively Bacillus spp. (e.g. Bacillus subtilis resistant spores) were cultured. Ventilation equipment defects were excluded and the alcoholic hand disinfectant dispenser in the cleanroom was changed. In addition, routine EMP was extended by the assessment of BAPs before preparation. During the following 8 episodes average CFU counts on gloves remained elevated before preparation (1.43), but were within the normal range after preparation (0.14). Different lots and sizes of gloves were involved and additional monitoring of the storage cabinet did not reveal significant results. So we changed the hand disinfectant dispenser in the lock at the entrance of the cleanroom. The examination of the alcoholic disinfectant taken through the nozzle of the dispenser revealed 0.34 CFU/ml culturing aerobic spore-forming bacteria. During the subsequent 8 occasions CFU counts on gloves before and after preparation were not elevated. The disinfectant taken from the dispenser in operation showed no microbial growth. Sterility testing of all manufactured cell products detected no microbial contamination throughout the whole period under review.

**Conclusions:** Successful implementation of an EMP according to the EC-GMP Guide is demonstrated by its function as an ‘early warning system’: OOS results in EMP occurred without microbial contamination of all manufactured products. Culturing of spore-forming bacteria from gloves in cleanrooms should prompt investigations of the aseptic process and its dispensers as a potential source for contamination with alcohol-resistant bacterial spores.

Processing of Comments / Complaints by a Central Complaint Management System
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**Purpose:** In order to be able to process the comments and complaints of blood donors more quickly, a central complaint management system was introduced within the scope of quality assurance (QA) in 2001. Whereas the various modes of receiving the comments / complaints (e.g. via letter, phone calls) had previously often resulted in a longer response time, centralizing the operation made it possible to streamline the flow and speed up the replies to the blood donors. **Methods:** All comments/complaints arrive centrally in QA. Using a standardised form, the content of the complaint is recorded, assigned to the competent department and forwarded to the person in charge. Tracking is also carried out by QA. Through a follow-up system a...
check after a specified period of time ascertain whether a reply has been given to the blood donor; if this is not the case, the competent department is reminded of the still pending response. Results: The continuous tracking undertaken through the central complaint management system ensures that blood donors receive an answer to their comments / complaints as quickly as possible. Conclusions: To the blood donor the speedy processing of his or her concern is of great importance. Only satisfied blood donors will come back. The continual improvement in the area of donor satisfaction will support the compliance with our stipulated supply mandate and will increase the commitment of blood donors to the blood donation service.

P 16 Others

P 16.01

Lipids Apheresis, What Can We Say Today?

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The disorders of lipid metabolism usually result in serious pathology conditions, often inheritable. Familial hypercholesterolemia (FH) is associated with the disorder in the function of low density lipoprotein (LDL) receptors. Extravascular LDL elimination - LDL and/or Lp(a) apheresis, can be a treatment of choice for patients with homozygous; drug resistant heterozygous FH; it’s also helpful for the patients with drugs resistant lipids disorders; patients after bypass surgery and also for patients with elevated Lp(a) level. Long-term LDL apheresis with antibodies containing columns ‘LDL Lipopak®’ (POCARD Ltd., Russia) provides significant decrease of LDL-C and increase of HDL-C level compared to baseline. Xanthomas decreases or completely disappears during the first years of treatment, improvement of myocardial function and significant regression of coronary atherosclerosis are observed. We have performed clinical follow up during 25 years of treatment by LDL apheresis. Elevated Lp(a) level more than 30 mg/dl is associated with the development of atherosclerosis in coronary, carotid, peripheral arteries and early vein grafts occlusion after CABG. Patients with increased Lp(a) usually have severe coronary heart disease (CHD). For many patients with elevated Lp(a) and LDL, the LDL level can be effectively controlled by lipid lowering drug therapy but target Lp(a) level can’t be achieved. Currently there are practically no effective drugs for normalization of Lp(a) level. Because of that, we have designed specific Lp(a) apheresis with ‘Lp(a) Lipopak®’ immunosor bent columns. It is an unique approach for the treatment of patients with severe CHD. It’s well known that all available systems for LDL apheresis (such as immunoadsorption, DS, HELP, DALI and other) can reduce Lp(a). But for specific removal of Lp(a) we have developed columns with sheep polyclonal antibodies against human Lp(a) – called ‘Lp(a) Lipopak®’ (POCARD Ltd., Russia). Lp(a) Lipopak can reduced Lp(a) on 80 %; other parameters are practically constant during one Lp(a) apheresis procedure. Our experience with ‘Lp(a) Lipopak®’ columns demonstrated that the removal of Lp(a) from the patient’s plasma up to the normal range by weekly Lp(a) apheresis results in the improvement of the patient’s health status and quality of life. In all cases the progression of disease was terminated. The quantity of daily heart attacks was dropped and exercise tolerance test was improved after 20–30 treatments. The regression of atherosclerotic plaques in some segments of coronary arteries was observed. We postulate that Lp(a) apheresis could be used for some CHD patients with solo elevated Lp(a) to whom routine LDL apheresis can’t be prescribed. We have also proposed another opportunity – combined Lp(a) & LDL apheresis that is a good method for the treatment of CHD patients, with elevated of both Lp(a) and LDL levels. The apheresis procedures with one ‘LDL Lipopak®’ and one ‘Lp(a) Lipopak®’ column for the patient with elevated Lp(a) as well LDL levels were performed. The results show this combined apheresis technique to be effective and helpful for patients resistant to the lipid lowering drugs. Design of new generation of the sorbents for the whole blood perfusion is currently in progress.

P 16.02

Impact of Chronic Hepatitis C Infection in Patients with Haemophilia

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Background: Chronic infection with hepatitis C virus (HCV) has turned out to be a major cause of morbidity and mortality in patients with haemophilia. Approximately 20% of patients with HCV develop cirrhosis over a 20 to 30 year period. As the percentage of haemophiliacs who exhibit end stage liver disease (ESLD) and hepatocellular carcinoma (HCC) is dramatically rising, means to assess those at highest risk for these complications are strongly recommended. Transient elastography by FibroscanTM is a novel technique to estimate the degree of hepatic fibrosis by measuring liver stiffness. Results: In our cohort of patients with haemophilia 315 are anti-HCV positive, indicating ongoing or previous infection with HCV. 61 (20%) have spontaneously cleared HCV. 144 (45%) patients underwent antiviral therapy which was successful in 80 patients. 174 (55%) are still suffering from chronic hepatitis C because of treatment failure (64 patients), because antiviral therapy could not be performed (31 patients) e.g. conflict with other underlying diseases or has not been performed mainly due to fear of side effects, lack of clinical symptoms and expected low efficacy of treatment (78 patients). To date transient elastography was performed in 113 of the chronically HCV-infected patients. 57 (50.4%) of them had no or mild fibrosis (Metavir F0/F1). 18 (15.9%) exhibited moderate fibrosis (Metavir F2). Severe or cirrhotic fibrosis (Metavir F3 and F4) has been diagnosed in 49 (43.6%) of these patients. In the virus negative subcohort 31 (81.6%) patients had no or mild fibrosis and 7 (18.4%) patients showed severe or cirrhotic fibrosis. Conclusion: In order to assess liver fibrosis in our haemophilic patients and identify those eligible for antiviral therapy we used transient elastography which identified an unexpected high proportion of patients with severe or cirrhotic fibrosis of approximately 43%. This finding persuaded a great percentage of them to finally start antiviral therapy. For patients with advanced fibrosis (Metavir F3 and F4) who are not eligible for virus eradication a close-meshed monitoring by ultrasonography and laboratory tests is recommended to prevent a fatal outcome by early therapeutic intervention.

P 16.03

Early Organ Rejection Caused by DP-Antibodies – a Case Report

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Objectives: Relevant histocompatibility markers for kidney transplantation are DR, LA-A, -B, -DR. Humoral rejections are typically caused by antibodies (ab) against these antigens. The clinical significance of DP-antibodies is unclear. Design and Methods: Here we report on a second kidney transplantation in a 49-year-old patient. The first transplant (1998) had lost its function by humoral rejection after 30 months. HLA-ab have been detected by CDC, ELISA, and LUMINEX techniques. Second kidney transplantation was undertaken with a well-matched transplant (mismatch A/B/DR 1/0/0) in 10/2006. The patient has never had ab against that incompatible antigen, even when tested by sensitive techniques. XM with blood and spleen lymphocytes and isolated T-cells were negative. Solely B-cell-XM was positive. Considering the negative T-cell-XM, good organ quality, and good match grade the patient was transplanted. Already the second postoperative day a rejection (Banff 2A) was diagnosed. We retested the donor and found an additional DQ-mismatch. But no donor specific ab against these mismatches could be detected by sensitive Luminex tests. So neither the positive B-cell-XM nor the early humoral rejection signs could be explained by HLA diagnostic tests. Only when tested by the new Luminex single antigen test a DP-antibody (DP11) was detected. The donor was typed for DP and found to be DP11 positive. The ab was also found in sera of the patient prior to the second transplantation. Immunizing event is most likely the first kidney transplantation. In the following course the DP-ab disappeared from the patient’s serum. Conclusions: This case confirms literature and own observations, which show that a positive B-cell-XM may be a risk factor in
Scharf R.E.

including material costs, human resources, administration overhead, costs for financial charges to each part of production (whole blood donation, apheresis expenditures was required. To achieve this goal, a clear assignment of the quality blood products. Therefore, a complete calculation of the actual University Medical Center Düsseldorf, different concepts such as 'mixed typic member of DAMPs released following only necrotic but not apoptotic effect of DAMPs on eosinophils is well demonstrated in vivo and in vitro as well as in our own hands. High mobility group box 1 (HMGB1) is a proto-toxic to tumor cells. In addition oxidation of DAMPs changes their biologic activity. Released IL-8 from eosinophils is a strong chemotactic to granulocytes and may further enhance granulocytes infiltration into tumor tissue. DAMPs-challenged eosinophils decrease their IL-4 and IL-10 release thus paving the way for a Th1 response. We postulate an immune modulating role of eosinophils within tumor microenvironment. Our findings shed some light into the microenvironment of (necrotic) tumor tissue and open perspectives for immunotherapy of cancer.

P 16.04

A Double-Edged Sword between Economics and High-Standard Health Care: Calculating the Production Costs of Stored Blood Components at a University Blood Center

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Objectives: The costs for providing blood have increased as a result of the improved safety standards. On the other side hospitals are obliged to reduce expenditures. Methods: For the University Blood Center the Dept. of Hemostasis, Hemotherapy and Transfusion Medicine at the Heinrich Heine University Medical Center Düsseldorf, different concepts such as ‘mixed calculations’, ‘intramural cost allocation at the university hospital’ and ‘extramural benchmarking analyses’ were prepared to provide the basics for optimized ‘manufacturing’ costs that correlate with the supply of high-quality blood products. Therefore, a complete calculation of the actual expenditures was required. To achieve this goal, a clear assignment of the financial charges to each part of production (whole blood donation, apheresis etc.), a verification of these expenses by the Div. of Controlling and the Div. of Purchasing and Supply, the ascertainment of the real costs for human resources, and the detailed analysis of production data and consumption data were performed. Furthermore, a process flow diagram was developed including all major steps involved in collecting, processing and transfusing blood. With these tools, it was possible to calculate the real entire expenses for donor recruitment and marketing, blood donation, manufacturing and preparation including material costs, human resources, administration overhead, costs for storage, disposition, handling, and transportation. To guide the intramural cost allocation process, it was necessary to differentiate between the various blood products, as their specific manufacturing causes distinct expenses.

Results: Having established these tools, we performed calculations for the production costs in case of transfusing 100% of the blood products and compared these data with the real investment and expenses due to failure rates and expired products. These analyses allowed to identify cost intensive pitfalls and entrapments within the production and operator process flow. For the intramural cost allocation, the real financial charges of the different blood products were presented. In addition, a comparison between real ‘in-house’ and actual ‘market’ prices was provided.

Conclusion: Analysis of blood components expenditures requires both (i) expert knowledge in production processes and their financial charges and (ii) competence in transfusion medicine and clinical hemotherapy.

P 16.05

Eosinophils Enhance Anti-Tumor-Immunity by Sensing and Responding to Necrotic Tumor Cells

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Objectives: Eosinophils are found at increased numbers within necrotic areas of tumor tissues. In case of colorectal cancer the so called tumor-associated-tissue-eosinophilia (TATE) is associated with a favorable prognosis. Nevertheless the pathophysiology of TATE and its impact is almost unknown. Necrotic cell death with subsequent release of damage associated molecular patterns (DAMPs) is a characteristic feature of tumor tissue. DAMPs not only serve as alarmins inducing further tumor proliferation, but also induce a potent immune response including dendritic cell maturation and chemotaxis of leukocytes, specifically eosinophils. The chemotactic effect of DAMPs on eosinophils is well demonstrated in vivo and in vitro as well as in our own hands. High mobility group box 1 (HMGB1) is a prototypic member of DAMPs released following only necrotic but not apoptotic cell death. We sought to characterize the role of eosinophils within tumor microenvironment.

Design and Methods: Human granulocytes were isolated by density centrifugation of whole blood followed by hypotonic lysis of remaining red cells. Eosinophils were negatively separated from other granulocytes using magnetic beads from Miltenyi. Lysates from a colorectal tumor cell line (HCT116) were generated by repeated freeze-thawing. Reconstituent human HMGB1 was purchased from R&D systems. Cytokine measurement in supernatants was assessed using FlowCytomix (Bender MedSystems). Oxidative burst was assessed by flow cytometry using H2DCFDA (Invitro...

P 16.06

Biocompatible Sorbent with Synthetic Ligand for Removal of Anti-A and Anti-B Antibodies from Human Plasma

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The removal of specific antibodies from the plasma of patients is promising method for solving many of transplantology problems. One of the causative factors in the graft rejection, selected by the antigens of histocompatibility, is the incompatibility on the groups of the blood according ABO system. Extracorporeal immunoabsorption for removal of anti-A and anti-B antibodies from patients’ plasma transplantation is considered as a major prophylactic procedure in order to avoid acute rejection and vascular rejection. The synthetic ligands can be very perspective for the development of the new columns for the elimination of immunoglobulins with the therapeutic apheresis techniques.

P 16.07

Perioperative Alterations of Serum Erythropoietin Levels in Patients with and without Compensated Renal Insufficiency

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Introduction: The hormone erythropoietin (EPO) is the main regulator of erythropoiesis and mainly produced by renal cells. Cell loss caused by operative procedures may alter serum levels of the hormone, resulting in an enhanced production, and one may speculate that a reduced increase of the hormone level could be observed in patients with a restricted renal function.

Material and Methods: Blood samples from 12 patients (mean age 63 ±9 years) with a normal renal function (n = 6) and with a compensated renal insufficiency (n = 6) were obtained before and on days 1, 5 and 10 after CABG. sEPO levels were determined by a commercially available ELISA-kit (R&D Systems, USA). Results: Prior to CABG, sEPO (12.6 ±8.3 mU/ml) and Hb (9.3 ±1 mmol/l) were within a normal range in all patients. At day 1 after surgery, sEPO was significantly increased to 67.0 ±0.8 mU/ml. In contrast, sEPO was significantly elevated to 39.7 ±28.5 mU/ml. At day 5 after CABG a significant difference in EPO levels could be observed between patients with normal renal function (35.6 ±19.5 mU/ml) and with compensated renal insufficiency (20.9 ±15.4 mU/ml) whereas at day 10 after surgery patients with compensated renal insufficiency reached again the same EPO levels as patients with normal renal function. Appropriate to the decrease in hemoglobin concentration, clear alterations of serum hemoglobin were observed in the patients with renal insufficiency.
erythropoietin could immediately postoperatively be observed. In patients with compensated renal insufficiency a declined EPO level at day 5 could be observed recovering into a normal range at day 10 after surgery. Therefore the surgical exposure may lead to an aggravation of the restricted renal function especially in the first 5 days after surgery.

P 16.08
Assessment of Blood Products Demand in the Treatment of Adverse Drug Reactions in German Hospitals
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4 Objective: Drug therapies combine progress in medicine with inevitable risks of adverse drug reactions (ADRs). The objective is to examine the demand for blood products in German hospitals induced by ADRs leading to admissions in departments of internal medicine. This paper is one of the first attempts to quantify the ADR-related economic burden and blood consumption in Germany. Methods: 4.444 patient records of ADRs leading to internal medicine hospitalisation were surveyed in 4 regional pharmacovigilance centres in Germany within the years 2000–2007. ADRs must have been induced by at least one drug which causality was qualified as ‘likely’ or ‘very likely’ according to Begaud et al. (1985). Incidence for ADRs was estimated (approx. 3.25%). Market prices for blood products and statistical data on hospitalisation were evaluated by literature and desktop research. Results: Treatment of 741 patients (16.7%) requiring blood products resulted in total consumption of 2,726 × 10⁸ (mean 3.76 × 10⁸ per patient) erythrocyte concentrates (ECs) and 267 (mean 3.38 × 10⁶ per patient) fresh frozen plasma units (FFPs). The mean age of treated patients was 72.9 (SD=12.4) years, 412 (55.6%) were female and 567 (76.5%) were ≥ 65 years old. Drugs most frequently associated with ADRs were acetylsalicylic acid (281), phenprocoumon (234), and diclofenac (118). In most cases, gastrointestinal tract was affected (SOC ‘gastrointestinal disorder’, n=623). Matching statistical data of hospitalisation in Germany with our findings a total demand of 100,934 ECs and 9,882 FFPs units can be estimated resulting in total cost of approx. € 9.1 m per year for all German hospitals. According to a literature review on average 51% of ADRs are preventable [see Grandt et al., Dt. Arztebl 2005; 102]. Prevention of these ADRs could lead to a decreasing annual demand of blood products (ECs: -5.981, FFPs: -5.089 units) resulting in a saving potential of approx. € 4.88 m per year for German hospitals. Conclusions: Blood products are given in one sixth (mainly gastrointestinal bleeding) of all ADRs leading to hospital admissions in departments of internal medicine resulting in serious cost and provision burden for the respective hospitals. Both blood demand and hospital procurement costs can be reduced by half by preventing ADRs. Therefore, improving medication safety is not only essential for the health of the individual patient but should also be one of the essential concerns for medical and public health decision makers in terms of cost reduction.

P 16.09
Thrombopoietin Levels are Different in Plasma and Serum Samples
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Introduction: The hormone thrombopoietin (TPO) is the main regulator of platelet production. Especially in thrombocytopenic diseases TPO seems to be a helpful diagnostic marker. But it should be considered that platelets could influence TPO levels during the preanalytic period by clearing with TPO receptor followed by internalization as well as by release of TPO after platelet activation. Therefore we determined TPO levels under different sampling techniques, storage conditions and platelet counts. Methods: Blood samples of seven healthy blood donors, ten thrombocytopenic patients and eight patients with thrombocytosis were collected in tubes without additives and in EDTA- or citrate-filled tubes. TPO levels were determined by a commercially available ELISA-Kit (R&D Systems). Results: In healthy blood donors and in patients with thrombocytosis TPO levels in plasma samples were found to be in a normal range 64 ±32 pg/ml and 59 ±42 pg/ml, respectively. In contrast, in thrombocytopenic patients TPO plasma levels were elevated to 3024 ±347 pg/ml. In the respective serum samples TPO showed a significant increase at normal and high platelet count to 136 ±56 pg/ml and 145 ±53 pg/ml, respectively, whereas at low platelet counts no significant difference to the plasma values could be observed. Conclusions: Influences of preanalytic conditions on TPO levels could be observed when TPO levels reached a low range (especially in healthy volunteers and in patients with thrombocytosis). In thrombocytopenic patients with TPO at a high range no difference between TPO levels of plasma and serum samples could be observed. These influences should be considered by the determination and evaluation of TPO concentrations under different pathophysiological conditions.

Table 1: Variables related to the apheresis procedure, donor and harvest

<table>
<thead>
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<th>Pre-apheresis</th>
<th>Apheresis procedure</th>
<th>Post-apheresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product</td>
<td>WBC ×10⁹/ml</td>
<td>CD34+ mcl</td>
</tr>
<tr>
<td>Median</td>
<td>47.00</td>
<td>79</td>
</tr>
<tr>
<td>Min.</td>
<td>5.00</td>
<td>3</td>
</tr>
<tr>
<td>Max.</td>
<td>96.00</td>
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Abstracts
P 16.11
Palliative Haemotherapy for Paediatric Patients Outside of the Hospital (OOH) – 2 Years Experience of a Home Transfusion Programme of the University of Muenster

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About 1800 children in Germany aged under 15 are diagnosed to have a malignant disease annually. The number of disease-related deaths up to 5 years after diagnosis in this paediatric population is 300–400 per year. Anemia and thrombocytopenia are relatively common symptoms in particular at the final phase of life of these children. Bleeding, especially when the blood loss is visible is a particularly distressing symptom for both the patient and the family. The discipline of paediatric palliative care has emerged in Germany over the last years. The aim of this development was and still is to enable families to care for their children in their own home. At the University Children’s Hospital Muenster, Department of Paediatric Haematology and Oncology, a multidisciplinary palliative care team was established in the last years. Based on our experience caring for palliative patients at home it is absolutely essential and desirable to have the facility to provide blood products at home.

Although home transfusion programmes are relatively common in the USA and Great Britain, this type of treatment are not realised in Germany even in the palliative setting so far. Since July 1, 1998 a Transfusion Act (‘Transfusionsgesetz’) regulates the clinical use of blood products in Germany in accordance with the guidelines established by the German Medical Association and the Paul Ehrlich Institute, which are regulatory revised and adapted on the basis of current professional knowledge and technology. The administration of blood products, whether in hospital or a doctor’s office, must follow certain quality-assumed rules governing transfusions as stipulated by the Transfusion Act. The University of Muenster now has 2 years experience of a home transfusion programme operating for paediatric patients in palliative care. The transfusion of blood and platelets outside the hospital (OOH) reduce the demand on acute services and the need for hospital admission. The potential risks can be decreased through careful planning and design. For the new field of transfusion of blood and platelets outside the hospital and doctor’s office there is a demand of legal provision.

P 16.12
Good Service and Strong Personal Ties to the Pheresis Unit Are More Important for Many Thrombozytapheresis-Donors than an Allowance – A Follow-up

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Introduction: The transfusion law demands the gratuity of blood donation, however it accepts an allowance orientated at the expenditure for each kind of donation. Therefore the allowance for thrombocytapheresis-donors was lowered clearly and expenditure-referred. The already improved service was maintained. Method: The effect of the lowering was registered by comparison of the donor behaviour 1 year before and 1 year after sinking allowance (phase A and B) and compared to the already published provisional study. Results: At the end of phase B we had lost 36% of the donors registered in phase A. However this loss could be overcompensated by 44% remaining or newly accrued donors. The mean donation frequency remained nearly constant in both phases with 4.7 versus 4.6. However, the number of donated blood products per donor decreased by 0.9 to 7.1 blood products (~12%) versus ~14% in the provisional study. Conclusions: The lowering of the allowance shows one year later mainly a change in the donor population, but no loss of donors at all. Compared to the results of our provisional study the strong personal ties to the pheresis unit and an attractive service are still motivating our donors. An increase of double donations is also confirming this fact.