14th Congress of the
European Society for
Haemapheresis and
Haemotherapy

Abstracts

Prague, September 10–13, 2003

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Hemapheresis in the Czech Republic

Short Comments to the 14th Congress of the Interdisciplinary European Society for Haemapheresis and Haemotherapy, Prague, September 10–13, 2003

The 14th Congress of the Interdisciplinary European Society for Haemapheresis and Haemotherapy (ESFH) was held on September 10–13, 2003 in Prague, in association with the 9th Working Days of the Czech Society of Transfusion Medicine and the Slovak Society of Haematology and Transfusion Medicine. It was the first time that the Czech Republic hosted an international congress on hemapheresis.

The standard of health care in the Czech Republic has always been high. After the ‘Velvet Revolution’ and the democratization in 1989, a rapid development of hemapheresis and hemotherapy started. Currently, there are more than 250 cell separators operating in the country, which is quite a high figure in proportion to population. Donor and therapeutic apheresis procedures have grown in number and types, including not only PBPC harvesting and DLI, but also photopheresis and immunoapheresis.

For the hosting Czech Transfusion Society, the 14th congress of the ESFH was a most welcome international forum to draw the attention on their activities and to demonstrate the quality and importance of their research. It was the aim of the International Scientific Committee to present the different aspects of hemapheresis, reflecting research and technical aspects as well as future developments in this rapidly developing field. A main focus of the conference was the emergence and establishment of novel hemapheresis methods and indications in the 2 years since the previous ESFH congress.

The scientific program consisted of 74 oral presentations (36 invited speakers) as well as 71 posters, with contributions not only from hemapheresis specialists, but also from nephrologists, intensive care specialists, and health professionals from adjacent branches of medicine, reflecting the interdisciplinary character of the conference and the ESFH. The congress included also educational programs and a special session for Czech and Slovak technicians. More than 540 physicians and health care specialists (including 113 from the Czech Republic) participated in the 14th ESFH congress.

During the congress, the Hoegman Award, which is announced every 2 years to honor the best young scientist in the field of hemapheresis, was given to Dr. Pavel Zák, Ph.D., from the Department of Internal Medicine II – Hematology, University Hospital in Hradec Králové. Dr. Žák is an active member of research groups dealing with erythrocytapheresis, plasmapheresis, immunoapheresis (LDL apheresis) and photopheresis, and was honored for his optimization of venous access for hemapheresis.

This volume includes a selection of the summaries of the outstanding educational lessons, oral presentations, SAFs (Seminars of Advances in the Field) and posters. For easier orientation, the summaries are numbered in the same way as the papers, oral presentations or posters in the Abstract Book. However, this selection cannot cover the entire spectrum of apheresis presented during the conference. Some copies of the Abstract Book including all contributions of this year’s congress are still available for those who are interested (ISBN 80-903238-8-X, publisher: HK CREDIT, Ltd., Hradec Králové, Czech Republic).

M. Bláha (Congress President), Hradec Králové
Z. Gašová (Congress Vice-President), Prague
1) EDUCATIONAL PROGRAM

Hematopoietic Stem Cell Transplantation
Chairpersons: T. D. Eastlund (USA), A. Gratwohl (Switzerland), J. Malý (Czech Republic)

Hematopoietic stem cell transplantation – current status and trends in Europe
A. Gratwohl (Switzerland)
Non-infectious complications of HSC transplantation
T.D. Eastlund (USA)

Advances in Transfusion Medicine
Chairpersons: A. Weiler-Lorentz (Germany), P. Jarolím (USA), M. Pisačka (Czech Republic)

Recent advances in transfusion medicine
M. Contreras (United Kingdom)
Whole blood versus apheresis platelets – optimization of platelet transfusion
V. Kretschmer, T. Zeiler (Germany)
Testing for bacteria in platelets
D. Mair (USA)

2) PLENARY SESSIONS

Cell Therapy
Chairpersons: W.E. Fibbe (The Netherlands), K. Indrák (Czech Republic), J. Zingsem (Germany)

Therapeutic Hemapheresis
Chairpersons: H. Borberg (Germany), G. Rock (Canada)

Donor Apheresis
Chairpersons: V. Kretschmer (Germany), M. Valbonesi (Italy)

3) PARALLEL SESSIONS

Safety of Hematopoietic Stem Cell Donors
Chairpersons: U. Platzbecker (Germany), D.F. Stroncek (USA), A. Vitek (Czech Republic)

Extracorporeal Photochemotherapy
Chairpersons: F. Heshmati (France), T. Schreiner (Germany), P. Žák (Czech Republic)

Hematopoietic Stem Cells: Collection and Therapy
Chairpersons: M. Contreras (United Kingdom), Z. Gašová (Czech Republic), P. Höcker (Austria)

Studies in Therapeutic Hemapheresis
Chairpersons: T. D. Eastlund (USA), M. Greplová (Czech Republic), B. G. Stegmayr (Sweden)

Therapeutic Plasma Exchange
Chairpersons: A. Bussel (France), A. A. Pineda (USA), P. Kessler (Czech Republic)

Experimental/New Technologies
Chairpersons: M.G. Conlan (USA), S. Filip (Czech Republic)

Immunoadsorption
Chairpersons: S. Fontana (Switzerland), J. Pták (Czech Republic), F. Waagstein (Sweden)

LDL Apheresis
Chairpersons: M. Bláha (Czech Republic), S.N. Pokrovsky (Russia), T. Schreiner (Germany)

Studies in Donor Apheresis
Chairpersons: J. Wollersheim (The Netherlands), R. Moog (Germany), V. Řeháček (Czech Republic)

Hemotherapy/Hemovigilance
Chairpersons: J.C. Faber (Luxembourg), A. Weiler-Lorentz, (Germany), P. Turek (Czech Republic)

4) SEMINARS OF ADVANCES IN THE FIELD

Pathophysiology and Treatment of Thrombotic Thrombocytopenic Purpura
Chairpersons: P. Jarolím (USA), L. J. McCarthy (USA)

Stem Cell Graft Processing
Chairpersons: P. Kobylka (Czech Republic), M. Rinker (Germany)

Autologous Hemotherapy
Chairpersons: V. Kretschmer (Germany), J. Masopust (Czech Republic), M. Valbonesi (Italy)

HLA/Leukodepletion
Chairpersons: A. Brand (The Netherlands), M. Contreras (United Kingdom), E. Matějková (Czech Republic)
Spleen Size Changes in Peripheral Blood Hematopoietic Progenitor Cell Donors Given G-CSF

D.F. Stronccek, S.F. Leitman
National Institute of Health, Dept. of Transfusion Medicine, Bethesda, USA

Background: Granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood progenitor cells (PBPC) are replacing marrow as a source of hematopoietic progenitors for transplantation. PBPC donors given G-CSF experience splenic enlargement and rarely, spontaneous rupture of the spleen. This study evaluated the incidence and time course of splenic enlargement in PBPC concentrate donors and assessed factors affecting size changes.

Methods: Healthy adults were given G-CSF (10 micrograms/kg/day) for 5 days and a PBPC concentrate was collected by apheresis. Ultrasound was used to assess cranio-caudal spleen length prior to giving G-CSF and the day of apheresis. In one group of donors was measured again 3 or 4 days after apheresis (n = 20) and in a second group of donors spleen size was measured again 10 days after apheresis (n = 5). The effects of donor age, gender, race, and changes in blood chemistries, blood counts, and CD34+ cell counts on spleen length change were assessed.

Results: Among donors in the first group spleen length increased in 19 of 20 donors. Mean length changed from 10.9 ± 2.0 cm pre-G-CSF to 12.3 ± 2.1 cm on the apheresis day (p < 0.001). The mean increase in length was 1.5 ± 0.9 cm (13.3 ± 9.1%). Spleen length increased 20% or more in 6 subjects. Three or four days after apheresis the spleen length fell to 11.3 ± 1.8 cm (p < 0.001), but it remained greater than baseline levels (p = 0.03). Spleen length change was not affected by donor gender, race, or age. There was no relationship between changes in spleen length and baseline and apheresis-day blood counts and chemistries and changes in blood counts and chemistries. In the second group of donors, spleen length increased in all 5, but returned to baseline levels 10 days after apheresis. There was no difference in spleen length measured before G-CSF and 10 days after apheresis (10.3 ± 1.2 cm versus 10.0 ± 1.4 cm).

Conclusions: Spleen size increases in almost all PBSC donors. Enlargement is transient and brief but marked in some donors and may place them at risk for splenic rupture.

O 13 Influence of Donor Characteristics and G-CSF-Administration Schedule on the Efficacy of Peripheral Blood Progenitor Cell Mobilisation

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Purpose: CD 34+ cell mobilisation in healthy donors varies to a wide scale. Defining predictive factors of mobilisation efficacy is of great interest to optimise protocols for allogeneic stem cell donors.

Methods: 1474 healthy donors (986 men, 488 women) underwent G-CSF application and PBPC collection at our department between 1/1996 and 8/2002. G-CSF administration was performed in 3 dosages: filgrastim 10 µg/kg/day on 5 days; lenograstim 7.5 µg/kg/day on 5 days; lenograstim 7.5 µg/kg/day on day 1 and 2, 12.5 µg/kg/day on the following 3 days. Leukapheresis was performed at day 5 (and 6, if necessary). CD34+ concentration in peripheral blood (×10^6/L) at day 5 before 1st leukapheresis was analysed for correlation with the following parameters: leukocyte and platelet counts before G-CSF administration, sex, age, body mass index (BMI), nicotine and alcohol consumption of the donors, G-CSF dose and mode of G-CSF mobilisation (single dose versus split dose). CD34+ cell counts on spleen length change were assessed.

Results: The median concentration of CD 34+ cells in peripheral blood at day 5 was 56±1 µl in males and 42±1 µl in females (p < 0.0001). A 2nd apheresis was performed in 34% of males and 53% of females. A significantly positive correlation of CD 34+ cells was found with BMI (p < 0.001) and the schedule of G-CSF application (split versus single dose; p < 0.0001). In a multivariate analysis, the schedule of G-CSF application had the most significant influence on the efficacy of peripheral blood progenitor cell (PBPC) mobilisation. No significant correlation was found with G-CSF dose, donor age, alcohol consumption and smoking status.

Conclusions: In our donor population PBSC mobilisation worked best in male donors with higher BMI. The schedule of G-CSF-administration seems to be very important for the mobilisation efficacy in healthy volunteer donors. Dose splitting of G-CSF should be performed, whenever possible.

O 17 Extracorporeal Photochemotherapy (ECP) in the Treatment of Acute and Chronic GvHD: Possible Relationship with the Number of Treated MNC Cells

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Background: ECP proved its effectiveness in patients either with aGvHD or cGvHD refractory to standard immunosuppressive treatments. Nevertheless, there are still at least two controversial issues: 1) what is the optimal treatment schedule and 2) does a correlation exist between the number of collected/irradiated MNCs (lympho-monon) and clinical response? We retrospectively analyzed our pts series to elucidate this latter issue.

Patients and Methods: 26 pts who underwent ECP for aGvHD (6 pts) or cGvHD (20 pts) were studied. All pts had clinical data (grading, organ involvement, clinical response, etc) were recorded as well as the number of MNC/Kg/bw collected, irradiated and reinfused in each ECP procedure. MNC collection was performed by using a Cobe Spectra device (standard WBC collection set). 8-MOP was added at 200 ng/mL final concentration, UV-A irradiation (2 J/cm2) was performed by using the Uvamatic device. Data are shown as median and range.

Results: 393 ECPs were performed over a 5-year period: 71 in aGvHD pts, who underwent 10 (6–14) ECPs each. Overall aGvHD grading ranged from grade I to grade III, while 16 out of 20 pts had extensive cGvHD. Skin was the major organ involved in 5 out of 6 aGvHD pts and in 18 out of 20 pts with cGvHD; 8 pts from this latter group also had mucosal involvement. Basal pre-ECP WBC count was 2.5 (1.1–15.1) ×10^7/L in aGvHD pts and 5.6 (1.4–15.6) ×10^7/L in cGvHD pts. Clinical response to ECP was as follows: 4 CR, 1 PR and 1 NR in aGvHD pts; 10 CR, 6 PR, 3 NR and 1 NV (two ECPs only) in cGvHD pts. Individuals with cGvHD who showed CR or PR received a double MNC dose compared to NR: 115 ×10^7/Kg vs. 64 ×10^7/Kg. On the contrary no difference was detected in aGvHD pts.

Conclusions: It has been postulated that ECP might trigger a specific APC-mediated response against auto-reactive lymphocytes or induce apoptosis in collected and irradiated MNCs. Our report suggests a possible relationship between MNC dose and clinical response, at least in cGvHD. Further studies on more pts are needed to ascertain the importance of MNC dose in treating GvHD.
finally, UV-A irradiation (21J/cm²) was performed by means of a Uvamatic (Vilber-Lourmat) device.

**Results:** All pts completed the study. MNC collections were performed without clinically relevant side-effects but mild hypocalcemia-related symptoms. Pts were given 5.6–11.1 × 10⁶ irradiated cells /ECP (median dose). PB WBC count did not significantly change all over the study period, as well as CD4⁺, CD8⁺ lymphocyte count and CD4/CD8 ratio. Three pts had no relapses, and two had 3 relapses each, responsive to low dose steroids. In 2 pts steroid total dose tapering was achieved and three did not required steroids all along the study period. EDSS did not worsened in 4 of 5 pts. MS activity evaluated by means of MRI was reduced compared to the pre-ECP period.

**Conclusion:** ECP proved to be feasible and well tolerated in all pts; a substantial lowering or withdrawal of steroid therapy was obtained in all cases. We observed a decrease in the number of relapses, whilst EDSS stabilized in 80% of the pts. Similar findings were observed as to the occurrence of new cerebral lesions in MRI examination after gadolium enhancement. A prospective multicenter study is now required to assess the possible usefulness of ECP in the treatment of RR-MS.

**L 21**

**PBPC Collection Techniques: Is There a Way to Get enough CD 34+ Cells in 'Poor Mobilizers?'**

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**Introduction:** PBPC collection techniques and subsequently the yield of CD 34+ cells play an important role in the effect of autologous and allogeneic transplantation. PBPC are usually collected in the standard or in the large volume leukapheresis regimen (LVL), but a criterion for selection of the optimum method has not yet been defined. The effect of mobilization and consequently the clinical condition of the donor or patient could affect directly the choice of the collection technique.

**Methods:** We evaluated the results of 88 standard and 217 LVL collections in 46 healthy donors and in 149 patients who suffered from hematological oncological diseases. More than 3 total blood volumes of the donor or patient were processed in LVL procedures. The precollection concentration of CD 34+ cells in blood equal or higher than 20 × 10⁹/ml was considered as a criterion of a good effect of mobilizing therapy.

**Results:**

<table>
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<th>Collections</th>
<th>Yield of CD 34+ cells (10⁶/kg)</th>
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</table>
| Standard LVL | Donors well mobilized 4.1 (1–13.6) 6.6 (1.9–13.7)
| Patient well mobilized 3.1 (1.9–43.2) 6.2 (0.9–27)
| Patients weakly mobilized 0.7 (0.2–1.2) 1.4 (0.4–4.4) |

LVL enabled to get a higher yield of CD 34 cells than the standard collections in well mobilized donors, in well mobilized patients as well as in weakly mobilized patients. The yield of CD 34+ cells equal or higher than 6 × 10⁹/kg was obtained from a single LVL procedure (Cobe Spectra) in donors and in well mobilized patients.

**Conclusion:** We can recommend LVL in all donors or in patients who can tolerate it. LVL should also be preferred in weakly mobilized patients where the chance to collect at least a minimum amount of CD 34+ cells for transplantation is possible using only this technique. No serious adverse reactions in LVL have been observed so far.

**O 27**

**Efficacy of Cytoreductive Leukopheresis in Acute Leukemia Patients with Hyperleukocytosis**

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**Objectives:** Therapeutic leukopheresis is used to control the acute symptoms of hyperleukocytosis by cytoreduction. The symptoms of hyperleukocytosis are usually prominent when the leukocyte count is above 75 × 10⁹/L among acute myeloblastic leukemia (AML) patients. Because mortality increases significantly when WBC counts reach higher levels than 100 × 10⁹/L, there is an indication for therapeutic apheresis to prevent tumor lysis and hyperleukocytosis syndrome. Herein we report the procedure based results of 30 acute leukemia and chronic myeloid leukemia (CML) in blastic phase (BP) patients who underwent cytoreductive leukopheresis between January 2000 and September 2002.

**Methods:** The median age was 41 (16–74) years, male to female ratio was 23:7. The diagnosis of the patients were AML-20 (66.7%), acute lymphoblastic leukemia: 5 (16.6%) and CML in BP5 (16.8%). The venous access was supplied by peripheral vein in 80% (n = 24) of patients and by central venous catheter in the remainder (20%, n = 6) Baxter Fennwall CS3000+ (n = 30) and Cobe Spectra devices (n = 1) were used. Leukopheresis was performed in median 1 (1–4) session and median 7L (4.55–9.5) L blood was processed in median 170 (135–220) minutes. Erythrocyte and platelet transfusion were performed if necessary after the procedure.

**Results:** The median leukocyte count, platelet count and hemoglobin levels of patients before and after leukopheresis were 176 × 10⁹/L (93.8–459) – 83.4 × 10⁹/L (58–733), 60 × 10⁹/L (10–750) – 45 × 10⁹/L (11–457) and 8.5 g/dL (6.7–15.7) – 6 g/dL (5–14.2), respectively. The median decrease in the white blood cell count was 45.81% (3.8–81.6). One patient (3.8%) failed to achieve an effective cytoreduction. There was only one hypocalcaemic case who required replacement (n = 1), procedure was ceased because of a life threatening dyspnea and angina in a patient during the third cycle (n = 1), no patient died during the procedure. Leukopheresis is an effective method to achieve a rapid cytoreduction. The administration of hydroxyurea (4 g/day po) accompanying the procedure supposed to be more effective than the procedure alone.

**Conclusion:** In spite of low preapheresis platelet counts, successful apheresis could be performed without major complications. There was no difference in the efficacy of therapeutic leukopheresis between AML, ALL, CML groups. The effects of this procedure in the remission rates and the survival should be investigated and compared retrospectively with the cases whom leukopheresis was not performed.
a WF cleaving metalloprotease in congenital and some acquired forms of TTP.

**Methods**: We have determined outcomes and metalloprotease levels in 50 patients randomly assigned to receive plasma exchange with fresh-frozen plasma (group I) or plasma exchange alone (group II). Plasma exchange was performed every other day until the level of plasma metalloprotease activity in patients receiving plasma exchange was 10% of the initial level. Mortality rate and complications were the main outcomes of this study.

**Results**: Median age, LDH level, platelet nadir and Hb level were not statistically different in two groups. One plasma volume corresponding to 40 ml/kg of body weight was exchanged daily until normal LDH level or normal platelet count were reached. Median five apheresis procedure (1–26) were performed. While maternal mortality rate was 23.1% (6/26) in group I and 4.1% in group II, there was no death in group I. All deaths were in patients Class 1 with low platelets (which were suggested by Martin et al.: platelet nadir <50,000/µl) syndrome with one or more risk factors. Nine clinical trials of IP were conducted. Recovery and lifespan in healthy subjects was shown to be within a therapeutically acceptable range. Prolonged template bleeding times in thrombocytopenic (tcp) patients (pts) were corrected with IP to a similar extent as with conventional plt (CP). Four more trials evaluated therapeutic efficacy and safety of IP in tcp pts requiring multiple plt transfusions (tx).

**Methods**: Two Phase 3 prospective, randomized, blinded trials compared IP processed with a clinical prototype device to CP. In SPRINT, 645 pts received Amicus apheresis IP or CP for up to 4 weeks. In euroSPRITE, 103 pts received tx with buffy coat IP or CP for up to 8 weeks. The 1st endpoint of SPRINT was safety of pts with moderate (WHO Grade 2 bleeding); the 1st endpoint of euroSPRITE was 1-hour plt count increment (CI) and corrected CI (CCI).

**Results**: Demographics and baseline data were well-balanced in both studies.

<table>
<thead>
<tr>
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<th>SPRINT</th>
<th>euroSPRITE</th>
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<tr>
<td></td>
<td>IP</td>
<td>CP</td>
</tr>
<tr>
<td>N</td>
<td>318</td>
<td>327</td>
</tr>
<tr>
<td>% Grade 2 bleeding</td>
<td>58.5</td>
<td>57.5</td>
</tr>
<tr>
<td>% Grade 3 or 4 bleeding</td>
<td>4.1</td>
<td>6.1</td>
</tr>
<tr>
<td>1-hr-24-hr CI (×10²/L)</td>
<td>21±13*</td>
<td>34±22</td>
</tr>
<tr>
<td>1-hr-24-hr CCI (×10³)</td>
<td>11.1±6.7*</td>
<td>16.0±10.1</td>
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<tr>
<td>Aprotinin dose (×10³)</td>
<td>4.6</td>
<td>3.9</td>
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<tr>
<td>Mean total plt dose (×10³)</td>
<td>29.4*</td>
<td>24.1</td>
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<tr>
<td>Mean no. plt tx</td>
<td>8.4*</td>
<td>6.2</td>
</tr>
<tr>
<td>Time between txn (d)</td>
<td>1.9*</td>
<td>2.4</td>
</tr>
<tr>
<td>DURATION plt support (d)</td>
<td>11.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Plt txn reactions (per tx)</td>
<td>5*</td>
<td>4</td>
</tr>
<tr>
<td>RBC txn</td>
<td>4.8</td>
<td>4.3</td>
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</table>

*p<0.05 compared to CP in same study.

By longitudinal regression analysis, 1-hr CI in euroSPRITE was not statistically different between IP and CP Safety was comparable between IP and CP in both studies. No antibodies to S-59 neoantigens were confirmed. Two confirmatory trials in Europe with buffy coat and Amicus IP processed with the final device prototype confirmed these findings.

**Conclusions**: Therapeutic efficacy and safety of INTERCEPT platelets was demonstrated in a series of clinical trials in pts requiring multiple platelet transfusions. These results suggest that INTERCEPT platelets may be used whenever platelet transfusions are indicated.
O 45 Pre-Transplant In Vitro Identification and In Vivo Monitoring of Donor-Derived Alloreactive T Cell Clones and Anti-Leukemia T-Cell Clones

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2Cancer Immunobiology Center, and
3Department of Internal Medicine, Bone Marrow Transplantation Program, University of Texas, Southwestern Medical Center, Dallas, TX,
4Vaccine Research Center, National Institute of Health, Bethesda, MD, USA.

Objectives: Although graft-versus-host disease (GVHD) is usually associated with a graft-versus-leukemia (GVL) effect, GVL can occur in the absence of clinical GVHD. A pronounced GVL effect following allogeneic hematopoietic stem cell transplantation (HSCT) has been documented in animal studies and by clinical observations. However, direct evidence that GVL and GVH are mediated by different clones of T cells is lacking.

Methods: Using irradiated peripheral blood mononuclear cells from patients in remission as target cells for GVH and irradiated leukemia cells from the same patient as target cells for GVL, we have demonstrated that donor-derived T cell clones when mediate GVH and GVL can be different. Primary mixed lymphocyte reactions (MLR) were performed using the recipient’s remission cells to obtain donor-derived GVH-specific T-cell clones. In order to obtain donor-derived GVL-specific T-cell clones, half of cells in the primary MLR were treated with an anti-CD25 immunotoxin to eliminate activated alloreactive T cells. The cells remaining after immunotoxin treatment were then incubated in a secondary MLR with the recipient’s leukemia cells. Activated CD4+ T cells from both the primary and secondary cultures were sorted on a FACS. mRNA was isolated from the sorted cells and a cDNA library was constructed. DNA sequences from the VDJ region encoding the T-cell receptor alpha chain were determined.

Results: In one patient who relapsed after allogeneic HSCT, 2 recipient cell-specific and 2 leukemia cell-specific donor-derived CD4+ T-cell clones were obtained. Both leukemia-specific clones were identical when pre-transplant or relapsed leukemia cells were used as targets in the MLR. One clone recognized both normal and leukemic cells. In a second patient two donor-derived GVH-specific and one GVL-specific T-cell clone were identified. Remaining 8 patients had HLA-mismatched healthy donors only available for in vitro experiments. In all 8 cases the presence of different donor-derived GVH- and/or GVL-specific T-cell clones was clearly demonstrated.

Conclusions: Our recent data demonstrate direct evidence that GVH- and GVL-specific T-cell clones can be separated in vitro and individually monitored in vivo. This strategy is being used to selectively deplete GVH-specific and expand GVL-specific donor-derived T cells prior to donor lymphocyte infusion.

O 46 Controlled-Rate of Freezing vs. Uncontrolled-Rate Freezing of Platelets – What is Better?

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Objective: In an attempt to establish the optimum method for platelet cryopreservation, much work has been carried out. We described our experience with cryopreservation of platelet concentrate derived from buffy-coat (PC-BC) using six different protocols for platelet cryopreservation. Methods: PC-BC units were examined before and after cryopreservation, using uncontrolled-rate and original controlled-rate freezing (with compensation of released heat of fusion) and 6% or 10% dimethyl sulfoxide (DMSO) and 5% DMSO with hydroxyethyl starch (HES), as cryoprotective agents. The platelet count was determined by a flow cytometer Technicon H-3. Morphology of platelets was examined using phase-contrast microscope technique (Polyvar). Four type of platelets were characterized (discoid = 4, spheres = 2, dendrites = 1, balloons = 0 points) to calculate the platelet morphological score (PMS). Platelet ultrastructure was investigated by electronmicroscope (Philips 201C). Cell functions were estimated using tests of hypotonic shock response (HSR) and platelet aggregation with ADP.

Results: Post-thaw platelet recovery in PC-BC groups cryopreserved with 6% DMSO was 91.0 ± 5.5 (controlled-rate freezing) and 85.9 ± 6.5% (uncontrolled-rate freezing), respectively (p < 0.01). The application of controlled-rate freezing resulted in better PMS-recovery (81.8 ± 2.8%) than uncontrolled-rate freezing (75.7 ± 3.9%). The percentage of discoid platelets were higher in controlled-rate setting (57.9 ± 2.6%) than in uncontrolled-rate setting (51.6 ± 3.7%), too. Ultrastructural investigation showed the high frequency of platelets with degranulation, and appearance of pseudopodes and vacuoles when HES and DMSO were used. Finally, the best answer in HSR-test were obtained in cryopreserved with controlled-rate rate of freezing and 6% DMSO (68.0 ± 23.2%). Two of three in the same group compare to control group was 44.8 ± 13.2%.

Conclusion: Our original cryopreservation procedure using controlled-rate of freezing (with compensation of released heat of fusion) and 6% DMSO resulted in highest percent of viable platelets vs. other used freezing methods.

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and laboratory parameters. Conclusions: PAIA neither improves disease activity nor restores ADAMTS13 activity, although circulating ADAMTS13 inhibitors are virtually completely removed. Residual traces of the inhibitor or its re-entry from extravascular sites seem to be sufficient for completely inhibiting newly synthesized endogenous ADAMTS13 and/or the enzyme is produced insufficiently. For future applications to acquired TTP, PAIA should be combined with infusion of exogenous ADAMTS13.

**O 51 Further Experience with Protein A Immunoadsorption in Autoimmune Haemophilia**


**Hematology Central Laboratory, Inselspital, Bern, Switzerland**

**Purpose:** Extracorporeal immunoadsorption to staphylococcal Protein A (PAIA) is a powerful tool in reducing circulating IgG and immune complexes. According to preliminary results, its implementation in the treatment strategy of severe and several severe autoimmune mediated haematological disorders (e.g. catastrophic antiphospholipid-syndrome and thrombotic thrombocytopenic purpura) should be useful. Here we summarize our data on 6 non-haemophilic patients (Ps) with factor VIII autoantibodies (FVIII-auto-AB), which is one of the most promising indications. 

**Methods:** We use a combination of PAIAs, cyclophosphamide (CY), corticosteroids (CST) and immunoglobulins (i.v. as induction cycles (IC)). These are repeated every 3–4 weeks until consistent lowering of the FVIII-auto-AB / increase of FVIII activity. This is followed by a maintenance treatment (MT) consisting of further single PAIAs (on demand) and oral CY or CST. Adjusted individually, the MT is tapered. For PAIA we utilize a cell separator (Spectra, Gambro BCT, USA), staphylococcal Protein A columns and a plasma flow monitor (Imunosorba and Citem). 

**Results:** So far (April 2003), 6 Ps with severe bleedings caused by FVIII-auto-AB were treated with similar protocols with generally excellent results in active bleeding episodes and convincing long term outcomes in 15/17. Our results are in agreement with these reports and offer further support that a combination of PAIA with immunosuppression is an efficient, safe and cost-effective treatment strategy for non-haemophilic Ps with FVIII-auto-AB. However, these results should be confirmed by prospective controlled trials.

**O 54 Long Term LDL Apheresis for Homozygous Familial Hypercholesterolemia**

M. Atassi-Dumont, S. Saheb, H. Zribi, S. Gombert, F. Dairou, E. Bruckert

**Fédération de Biothérapies Cliniques: Hôpital Pitié-Salpêtrière, Paris, France**

**Background:** Homozygous familial hypercholesterolemia (HFH) is characterised by extreme elevation of LDL-cholesterol and consequently development of massive tissue deposits, and complications of atherosclerosis may lead to premature death during the second decade of life. Treatment with LDL-apheresis (LA) has been associated with regression of atherosclerosis and tissue deposits and improved life expectancy. However, age at which needs to be undertaken is still a matter of debate.

**Methods:** We reviewed 18 FH patients treated by LA during more than 6 consecutive years in our institution, between 1977 and 2003. LA were performed biweekly, in a day-care unit. 17/18 patients were treated with lipid-lowering drugs and low fat diet (1/18 patients was not compliant with drug treatment). Clinical symptoms of coronary artery disease (CAD): angina pectoris, myocardial infarction, or angiographic significant stenosis were recorded.

**Results:** Patients were 8 to 44 years old (m = 12.2 ± 8 years). Follow up on LA was 7 to 23 years (m = 11.7 ± 4 years). Observance with the biweekly LA was good in all patients. 4 patients among 18 had CAD prior to LDL apheresis, and 4 patients developed CAD 3 to 11 years after onset of LDL apheresis.

**Conclusion:** The 5 patients who suffered from CAD were 16 to 38 years old (m = 29.8 ± 8.8 years). They started LDL apheresis at 5 to 33 years old (m = 17.8 ± 8.5 years). 1/18 patients had 1 additional risk factor (smoking).

5/8 had clinical and angiographic regression of CAD after onset of LDL apheresis. 1 patient, 38 years old, died from massive myocardial infarction, 18 years after onset of LA (he didn’t take any lipid-lowering drug).

**Asymptomatic Group:** The 10 patients were 8 to 25 years old (m = 18.8 ± 4.6 years). They started LDL apheresis at 2.5 to 13 years old (m = 7.8 ± 3.6 years).

No patient had additional risk factor.

**Conclusion:** Early implementation of LA may prevent onset of clinical symptoms of CAD in Homozygous FH patients, and in our experience, the treatment is feasible in very young children (2.5 years).

**O 57 Successful Retreatment of Patients Intolant to the DALI LDL Apheresis System Using a Modified Reduced Citrate Program**

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**Apheresis Unit, Department of Hematology and Internal Medicine B Department, Hebrew-University Hadassah Hospital, Ein Kerem, Jerusalem, Israel**

**Background and Hypothesis:** The DALI system is a highly efficacious method for removing LDL in patients with hypercholesterolemia. However, some patients develop side-effects and are intolerant to this valuable mode of therapy. Among the serious adverse effects is a syndrome of facial flushing, urticaria, and shortness of breath. This was observed in 6/22 of the patients undergoing DALI treatment in our hospital during the past 3 years (Durst et al, IMAJ 4:677, 2002). This syndrome was always noted in the initial phase of therapy (during the first 2–3 sessions). It was suspected that this syndrome was caused by the high concentration of citrate which is released from the column after priming and abruptly enters the patient’s circulation. Since this parameter was set automatically and was not under the user’s control, we could not test our hypothesis. This syndrome recurred in the 3 patients who agreed to be rechallenged under the same treatment conditions. Therefore, all patients experiencing this syndrome were discontinued from DALI treatment and were treated using other methods.

**Objectives:** To develop an alteration of the DALI system which would A: not cause this adverse reaction and B: elucidate the etiology of this adverse effect.

**Methods:** Because of the awareness of this problem, which had also been reported by other centers, the manufacturer (Fresenius) developed a modification of the system which uses half of the amount of citrate during the initial phase of treatment to prevent clotting in the column. The low citrate DALI system was used by us to treat 3 of the patients who had been tolerant to the original system. Using the modified low-citrate program, with no change in the column or other components of the system, these patients were able to undergo 6 DALI treatment each with absolutely no adverse effects. They are continuing DALI treatment as their regular LDL-lowering treatment modality. Two additional (sensitive but not frankly allergic) patients who had been treated with the high-citrate DALI system were switched to the low-citrate system. They reported feeling subjectively much better after low-citrate DALI sessions.
Conclusion: We conclude that the flushing/dyspnea seen using DALL LDL apheresis system is indeed a function of the high citrate in the return line after priming and it can be successfully prevented using a low-citrate modification of the program.

L 62
Conversion of Platelet Production Method: From Buffy Coat Platelets to Single Donor Platelets – a Feasibility Study

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Objective: Since variant Creutzfeldt-Jakob (vCJD) became a potential threat for the blood supply the Dutch medical advisory board has advised Sanquin to take measures to minimize donor exposure. One of the recommendations was to transfuse only single donor platelets (SDP). Sanquin promoted a pilot study to evaluate technical, logical and economical feasibility of thrombocytapheresis (TPH). To enhance economical feasibility it was decided to try a double platelet products (DPP) system.

Donors and Methods: All A- and O-plasmapheresis donors (PPH) with platelet pre-count of >250,000 and weight >70 kg, who showed previous willingness to become TPH donors, were invited to donate. 100 procedures were performed on Trima® Accel (Gambro BCT), all collected SDP were tested in the QC lab on platelet yield, residual leukocytes, volume, pH, swirl and sterility (bacterial screening). Plasma products were tested on volume and cell counts. Targeted procedure time was 70 min with an exception till 90 min.

Results and Discussion: 58% of participating donors met all the selection criteria. 86.2% of the procedures resulted in a DPP. Mean PLT Yield was 540.4 ± 99.9 × 10^11. WBC content complied with European guidelines. Average procedure time was 64.9 min. 69 of the 100 procedures took at most 70 min, however, 23 procedures took between 70 and 90 min. Simulations on the product revenues per procedure cover largely the costs.

Conclusion: Conversion of buffy coat platelets to single donor platelets appears feasible. Good donor selection and the high collection performance assure high split rates and relatively short procedure times. It further showed that the centre would be auto sufficient (2100 SDP annually) if 203 donors would donate 6 times a year.

O 65
The Content of the LRS Chamber Provides a New Quality Tool for Characterization of the Donor Platelet Profile

Centro Regional de Sangue de Lisboa, Portugal

Objectives: to compare the relative Platelet (Plt) indices and aggregation states of the single dose platelet-derived from Trima with those obtained from the LRS chamber (LRS) and relate them with programmed yields.

Material and Methods: Immediately at the end of collection (n = 25), the LRS was disconnected from the system and the content removed according to a standard procedure. For a quantitative assessment of Plt aggregation and its response to edta, paired samples of edta and citrate were counted with a Cell Dyn 3500 and calculated for dPlt (Pltedta – Pltcitrate) and MPV (MPvedta – MPv citrate). The same approach was used for the products.

Results: on d0 there was a good correlation between donor and product MPV (0.84 versus 8.6; r = 0.84). Mean values of LRS MPV and dMPV (10.8 and dMPV (MPvedta – MPv citrate). The same approach was used for the products.

Conclusion: The Intercept™ treatment causes a platelet loss of appr. 7% which has to be considered for reaching a satisfying platelet dose for transfusion. pH levels are significantly reduced after Intercept™ treatment due to the addition of 15 mL Amotosalen™ (pH = 4.5). The continuing effect of the Intercept™ treatment is reflected by the further decrease of the pH level, higher platelet activation, and larger platelet volumes at the end of storage as compared to untreated platelets. Platelets'
metabolism and their ability to respond to hypotonic shock are not affected by the Intercept™ treatment. The formation of visible aggregates which did not dissolve during storage over night maybe due to the separation technology of the Amicus™ and/or to the addition of InterSol™. Platelets suspended in InterSol™ should not be stored > 5 days because glucose and pH levels are very low at day 7 due to the contents of 60% InterSol™.

**O 67**

**Implemenatation of Concurrent Red Blood Cell and Platelet Collection by Apheresis**

R. Moog
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**Background and Objectives:** New technological developments make it possible to collect red blood cells (RBCs) by apheresis which provides standardised products and has the potential for improved RBC quality. Concurrent collection of RBCs and platelets (PLTs) allows for an increase of the blood supply and reduces costs of laboratory tests. The present study analyses the number of concurrently collected RBC units in plateletpheresis donors and the reasons why donors were deferred from multicomponent collection.

**Material and Methods:** Donors fulfilling inclusion criteria for multicomponent donation underwent concurrent collection of RBCs and PLTs with the single needle procedure of the Amicus blood cell separator. The haemoglobin value prior to RBC collection and of the follow up donation as well as the reasons for deferral were retrospectively evaluated for a period of one year.

**Results:** A total of 404 RBC units were concurrently collected with PLTs. An average of 1.8 RBC units was collected from each donor. The baseline haemoglobin value was almost equal for the first (n = 221), the second (n = 117), the third (n = 54) and the fourth donation (n = 12). Concurrent PLT and RBC collections were well tolerated by most donors. An RBC unit was not collected in 190 aphereses. 39 donors (20.5%) were not accepted for RBC collection due to a donation interval of less than three months. Haemotoma and blood flow problems occurred in 36 (18.9%) requiring a new venipuncture. 33 RBC (17.4%) units were not collected due to low body weight. 24 donors (12.6%) were unwilling to donate an additional RBC unit and 21 RBC (11.1%) units were not collected due to problems with logistics.

**Conclusion:** RBC supply was increased by the implementation of concurrent RBC and PLT collection. The donor eligibility for the procedure has to be taken into account and the logistics of RBC processing (filtration) and RBC and PLT collection. The donor eligibility for the procedure has problems with logistics. The donor eligibility for the procedure has problems with logistics.

**O 69**

**Data Management in Preparative Hemapheresis**

S. Rummelier, D. Barz,
Universitätssklinikum Jena, Institut für Transfusionsmedizin, Germany

**Objective:** Systems that ensure quality in Blood Centres are submitted to Data Management in Preparative Hemapheresis and their ability to respond to hypotonic shock are not affected by the Intercept™ treatment. The formation of visible aggregates which did not dissolve during storage over night maybe due to the separation technology of the Amicus™ and/or to the addition of InterSol™. Platelets suspended in InterSol™ should not be stored > 5 days because glucose and pH levels are very low at day 7 due to the contents of 60% InterSol™.

**Study Design:** 4 combinations of data management systems and hemapheresis devices were compared:
1. Apheressismaster/ComTec,
2. HaemoNet/MCS+,
3. Vista/Trima,
4. Ami-Print/Amicus.

Parameters that can influence the quality of the platelet concentrates can be divided into 2 groups:
1. Procedure related conditions such as: ACD-A consumption (inlet; AC ratio, ACD-A in the product in relation to the expiry and storage of the product), continuity of the blood flow, procedure time (regeneration of platelets from the spleen), alarms, cell concentration, tubing set (length of the set determines the residual set volume, blood loss tracking), ...
2. Donor related conditions: pre-hematocrit, platelet count, mean platelet volume, white blood cell count, ...

The donor and procedure data recorded in the 4 data management systems was compared. We also evaluated the influence of registration and assessment of procedure data on the final quality of the platelet concentrates. Also the obtained messages and alarms were listed. Special attention was given to the statistics these software programs offer, together with the databases used. In this aspect is could be stated that the databases these software programs use, are composed of two interconnected parts. The first one is a donor database containing all donor related info such as name, address, gender, height, weight, previous platelet and hematicrit counts, etc. Databases (like Vista) keep also track of the blood loss of the donor. The second part of the database is the procedure database. Info such as products obtained, run prediction, QC lab data, etc. are kept here.

**Summary:** Although there exist some similarities between the 4 software/device combinations, we could demonstrate clear differences between the systems especially in the field of QC, AC management and blood loss tracking.

**P 3**

**Comparison of the LDP C5 and LDP C2 Protocols for Platelet Concentrates Preparation on Haemonetics MCS+ Separator**

V. Rehacek, H. Samkova
Transfusion Department, Charles University Hospital Hradec Kralove, Czech Republic

**Objective:** The purpose of this study was to compare the quality of platelet concentrates collected with two different software versions on the same cell separator.

**Methods:** 23 plateapheresis procedures were performed using Haemonetics MCS+ separator, protocol LDP – new revision C5. The results were compared with 20 procedures performed on the same MCS+, protocol LDP – revision C2. 994CF-E sets were used in all apheresis. Donor platelet counts, platelet yield, platelet volume, residual WBC count, collection efficiency (CE), and side effects were recorded and analysed.

**Results:** Donor data did not differ significantly. In the C5 group there was slightly higher CE (60.4±6.8%), range 48.8–70.1%), median 61.4%) than in C2 group (59.8±4.5%), range 51.5–70.2%, median 59.5%, p < 0.05). Calculated platelet yield per cycle was 62.4±11.5×10^6, range 48.7–97.5×10^6, median 61.4×10^6 in the C5 group and 61.7±10.5×10^6, range 42.0–88.5×10^6, median 60.2×10^6, in the C2 group (p < 0.05). Calculated platelet yield per hour was (C5): 2.96±0.57×10^10, range 1.95–3.76×10^10, medium 3.03×10^10 and (C2): 2.94±0.49×10^10, range 2.21–4.01×10^10, medium 2.94×10^10 (p > 0.05). No side effects were observed. The WBC contamination (Nageotte chamber) was lower than 1×10^6 in all platelet concentrates.

**Conclusions:** Results of platelet concentrates collected using the protocol LDP – revision C5 and the protocol LDP – revision C2 were comparable. There were no statistically significant differences comparing procedures using these two revisions.

**P 4**

**Results of a Productivity Comparison of Trima® Accel™ with Haemonetics-MCS+ in Platelet Collections**

A. González-Bachs, A.A. Pinacho
Centre de Transfusión i Banc de Teixits, Barcelona, Spain

**Background:** To compare the productivity of two plateletpheresis cell separator platforms (Trima® Accel™ and Haemonetics MCS+) in platelet collections.

**Materials and Methods:** A total of 533 plateletpheresis procedures were available for analysis. 360 procedures were carried out with Trima® Accel™ V5.0 and 173 procedures with Haemonetics-MCS+. Relevant donor parameters (age, sex, total blood volume, hematocrit, platelet count and mean platelet volume), product parameters (volume, predicted yield and real yield) and procedure parameters (time until update of donor parameters, volume of processed blood and procedure time, incidences) were collected. For statistical analysis chi-square and t-test evaluations were used. For correlation use was made of the regression model and correlation factor r of Pearson.

**Results:** Donor characteristics were similar in both groups. With regard to procedures Trima® Accel™ V5.0 was found to process more volume (2.910±0.57×10^10, range 2.21–4.01×10^10, medium 2.94×10^10) whilst obtaining the same final product volume (205 vs. 245 mL; p = 0.416). Correlation between predicted and real yield was
good for both cell separators albeit that the correlation was higher with *Trima*<sup>®</sup> Accel<sup>™</sup> (*r* = 0.87 vs. MCS+ *r* = 0.78). Approximately 40% of the donors showed a variation above 10% between predicted and targeted yield. This was not caused by the time required to update the donor parameters after the start of the procedure (mean time = 11 min, percentiles<sub>25</sub> = 8 y percentiles<sub>75</sub> = 13). A total of 12 infiltrations were observed (

**Table 1.** Abstract P 5

<table>
<thead>
<tr>
<th>TBV (ml)</th>
<th>Hct (%)</th>
<th>Plt-Pre (x 10&lt;sup&gt;11&lt;/sup&gt;/µl)</th>
<th>Predict Yield (x 10&lt;sup&gt;11&lt;/sup&gt;)</th>
<th>Real Yield (x 10&lt;sup&gt;11&lt;/sup&gt;)</th>
<th>Yld / Time (x 10&lt;sup&gt;6/&lt;/sup&gt;min)</th>
<th>Plt Conc (x 10&lt;sup&gt;11&lt;/sup&gt;/µl)</th>
<th>Plm (ml)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (74)</td>
<td>4.899 ± 835</td>
<td>41 ± 3</td>
<td>269 ± 52</td>
<td>5.8 ± 1.3</td>
<td>6.2 ± 2.0</td>
<td>8.5 ± 3.0</td>
<td>1597 ± 320</td>
<td>312 ± 67</td>
</tr>
<tr>
<td>Accel (74)</td>
<td>4.899 ± 825</td>
<td>41 ± 3</td>
<td>261 ± 46</td>
<td>5.6 ± 1.3</td>
<td>5.8 ± 1.7</td>
<td>7.9 ± 2.1</td>
<td>1646 ± 209</td>
<td>172 ± 113</td>
</tr>
<tr>
<td>v4 (74)</td>
<td>4.899 ± 825</td>
<td>41 ± 3</td>
<td>261 ± 46</td>
<td>5.6 ± 1.3</td>
<td>5.8 ± 1.7</td>
<td>7.9 ± 2.1</td>
<td>1646 ± 209</td>
<td>172 ± 113</td>
</tr>
<tr>
<td>Men (43)</td>
<td>4.549 ± 547</td>
<td>43 ± 2</td>
<td>253 ± 34</td>
<td>6.0 ± 1.2</td>
<td>6.4 ± 1.6</td>
<td>8.8 ± 2.0</td>
<td>1607 ± 234</td>
<td>325 ± 75</td>
</tr>
<tr>
<td>Accel (43)</td>
<td>4.549 ± 547</td>
<td>43 ± 2</td>
<td>249 ± 36</td>
<td>6.0 ± 1.2</td>
<td>6.3 ± 1.5</td>
<td>8.5 ± 1.7</td>
<td>1666 ± 161</td>
<td>163 ± 115</td>
</tr>
<tr>
<td>V4 (43)</td>
<td>4.549 ± 547</td>
<td>43 ± 2</td>
<td>249 ± 36</td>
<td>6.0 ± 1.2</td>
<td>6.3 ± 1.5</td>
<td>8.5 ± 1.7</td>
<td>1666 ± 161</td>
<td>163 ± 115</td>
</tr>
<tr>
<td>Women (31)</td>
<td>4.122 ± 452</td>
<td>39 ± 2</td>
<td>291 ± 63</td>
<td>5.5 ± 1.4</td>
<td>5.9 ± 2.5</td>
<td>8.0 ± 4.0</td>
<td>1582 ± 415</td>
<td>29 ± 49</td>
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<tr>
<td>Accel (31)</td>
<td>4.122 ± 452</td>
<td>39 ± 2</td>
<td>278 ± 53</td>
<td>5.1 ± 1.2</td>
<td>5.3 ± 1.7</td>
<td>7.1 ± 2.3</td>
<td>1619 ± 262</td>
<td>184 ± 110</td>
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<tr>
<td>v4 (31)</td>
<td>4.122 ± 452</td>
<td>39 ± 2</td>
<td>278 ± 53</td>
<td>5.1 ± 1.2</td>
<td>5.3 ± 1.7</td>
<td>7.1 ± 2.3</td>
<td>1619 ± 262</td>
<td>184 ± 110</td>
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**Table 2.**

<table>
<thead>
<tr>
<th>Predicted yield 2.8 x 10&lt;sup&gt;11&lt;/sup&gt;</th>
<th>Predicted yield 3.2 x 10&lt;sup&gt;11&lt;/sup&gt;</th>
<th>Predicted yield 4.0 x 10&lt;sup&gt;11&lt;/sup&gt;</th>
<th>Efficiency (%)</th>
<th>CR (plt/min)</th>
<th>Procedure failures</th>
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<td>Tr 1</td>
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<td>n = 50</td>
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<td>n = 10</td>
<td>n = 99</td>
<td>n = 121</td>
<td>n = 9</td>
</tr>
<tr>
<td>2.79 ± 0.49</td>
<td>3.27 ± 0.46</td>
<td>4.13 ± 0.66</td>
<td>59.5 ± 9.8</td>
<td>6.6 ± 0.01</td>
<td>7%</td>
</tr>
<tr>
<td>Tr 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 47</td>
<td>n = 55</td>
<td>n = 4</td>
<td>n = 85</td>
<td>n = 109</td>
<td>n = 3</td>
</tr>
<tr>
<td>2.83 ± 0.38</td>
<td>3.27 ± 0.46</td>
<td>4.32 ± 0.45</td>
<td>60 ± 7.1</td>
<td>6.6 ± 0.00</td>
<td>3%</td>
</tr>
</tbody>
</table>

**P 5**

**Collection of Plasma-Reduced Platelet Concentrates on *Trima*<sup>®</sup> Accel<sup>™</sup>, Compared to Collection of Standard Concentrates on *Trima* Version 4**

R. Verheyden, L. Steenssens  
Blood Transfusion Center Vlaams Brabant-Limburg, Leuven, Belgium

**Background:** In January 2003 one Trima version 4 (v4) automated blood collection system was upgraded to TRIMA ACCEL. Plasmareduced platelet concentrates (PPC) collected on TRIMA ACCEL were compared with normal platelet concentrates (NPC) collected on v4 in terms of yield, productivity and cellular contamination.

**Methods:** Per donor each TRIMA ACCEL procedure (n = 74; all different donors) was compared to the next v4 procedure of the same donor. Collection concentration for TRIMA ACCEL was 4,100 x 10<sup>11</sup>/µl and 1,500 x 10<sup>11</sup>/µl for Trima v4. A prediction-dependent volume of T-sol was added to the PPC within 0.5 hour after collection to reduce the concentration to normal levels. This way, after addition of T-sol PPC had a plasma carry-over of approximately 37%.

**Results:** See table 1 above.

**P 6**

**Trima Validation for Single Apheresis Platelet Products**

A.P. Sousa, E. Vasconcelos, J. Seghatchian, R. Maurício, A.C. Figueiredo, G. Sousa  
Centro Regional de Sangue de Lisboa, Portugal

**Objectives:** to validate the collection and storage characteristics of single dose platelets (plt) obtained by 2 Trimas (Tr1 and Tr2) against CE guidelines.

**Material and Methods:** 224 platepheresis products, obtained from healthy donors (plt > 200,000/µl) were evaluated. Collection was performed according to the manufacturer instructions. The target yields were 2,8, 3,2 and 4 x 10<sup>11</sup> platelets/unit. Regarding pH measurement, on an initial phase 44 products with 1 hour of resting period (rp) were evaluated on day 5 (d5). On a subsequent phase, evaluation was performed on a total of 30 products and included measurements on d1 before shaking, d2 and d5. The rp was 3 and 8 hours for 19 and 11 products, respectively. A Cell Dyn 3500 counter, BD Facsiscalibur flow cytometer, Chiron 340 meter and Bact Alert system were used for plt counting, leucocyte evaluation, pH measurement and bacteriological screening. The overall performance of both machines was assessed by collection rate (CR) and process efficiency.

**Results:** The obtained values of CR, process efficiency and yields are summarized in table 1. There was a correlation of 0.91 between programmed and final volumes. Leucoconversion specification was attained on a 100% basis, as none contained more than 1 x 10<sup>6</sup> leucocytes. 212 products contained more than 2 x 10<sup>9</sup> platelets/unit, which corresponded to a 95% degree of conformance to the CE guidelines. No bacterial contamination was found. No effects on pH were obtained after enlarging the rp (table 2).

**P 7**

**Quality Control in Apheresis Platelet Concentrates: Various WBC Reduction Technologies Lead to Different Leukocyte Depletion**

Clinic for Blood Group Serology and Transfusion Medicine, Vienna University Hospital, Austria

**Objective:** The guidelines of the Council of Europe require the amount of rWBCs in a leucocyte depleted platelet concentrate (PC) < 1 x 10<sup>6</sup>. WBC reduction of apheresis PCs can be performed by preparative technologies (fluidized particle bed technology, elutration principle, interface detector) or by filtration.
Material and Methods: At least four PCs per month of each cell separator are randomly assigned to our QC laboratory. Measurement of rWBCs is performed with the Lexco Count kit (B and D). If a PC contains >1 × 10^8 WBCs, the next two PCs produced by this cell separator also undergo QC. If a second PC > 1 × 10^8 WBCs is detected, the cell separator is eliminated from production till a technical service has been performed. The first PC after reparation is controlled again. Results: A total number of 4,965 PCs have been produced in 2002. QC was performed in 962 PCs (19.4%).

<table>
<thead>
<tr>
<th>Cell separator</th>
<th># PCs</th>
<th># QCs</th>
<th>rWBC (× 10^9) median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amicus SN</td>
<td>803</td>
<td>319</td>
<td>0.04 (&lt;0.02–99.9) 34 (11%)</td>
</tr>
<tr>
<td>Cobe Spectra DN</td>
<td>411</td>
<td>70</td>
<td>0.04 (&lt;0.02–29.3) 1 (1.5%)</td>
</tr>
<tr>
<td>Cobe Trima</td>
<td>425</td>
<td>53</td>
<td>0.04 (&lt;0.02–6.9) 2 (4%)</td>
</tr>
<tr>
<td>ComTec</td>
<td>295</td>
<td>97</td>
<td>0.03 (&lt;0.02–3.9) 11 (11%)</td>
</tr>
<tr>
<td>MCS+</td>
<td>3,030</td>
<td>423</td>
<td>&lt;0.02 (&lt;0.02–364) 38 (9%)</td>
</tr>
</tbody>
</table>

Conclusion: The interface detector which is responsible for WBC reduction with the Amicus (Baxter) and the ComTec (Fresenius) is very sensitive and high rWBCs are found in 11% of the PCs. PCs from the MCS+ (Haemonetics) show the lowest leukocyte contamination in median, but the incidence of filtration failures is 9%. The participle bed technology used with the Cobe has constantly good rWBC results. In some cases failed WBC reduction is donor related: these donors are identified, and they are assigned to another type of cell separator.

P 8 Clinical Efficacy of Plasma-Reduced (PR) Platelet Concentrates from Multi-Component (MC) Collection: A Prospective Study

P. Perseghin, V. Baldini, E. Ellì*, A. Villa*, M. Dassi, A. Bialduzzi*, A. Rovelli*, C. Uderzo*
Servizio Trasfusionale, Ospedale San Gerardo, Monza and *Clinica Pediatrica-CITMO, Univ. Milano-Bicocca

Background: PR-MC platelet collection has been recently introduced in several blood collection units, with both the aims of saving plasma for industry and to possibly reduce the incidence of plasma-protein related side-effects (SE) in patients receiving multiple PLT transfusions (TX). We performed a single center non-randomized prospective study to compare PR-MC platelet units to standard random PLTs and to platelepheresis units.

Patients and methods: Onco-haematological patients who were given PR-MC PLT units. PR-MC PLT units proved their safety and clinical efficacy in children from an onco-haematological setting.

Results: 66 pts received a total of 221 PLT TXs: 92 pts were given R-PLT, 137 pts were given PR-MC PLT and 91 pts received PLT-P units to standard random PLTs and to platelepheresis performed a single center non-randomized prospective study to compare PR-MC platelet units to standard random PLTs and to platelepheresis ob-

Conclusions: Pts who were given PR-MC PLT units showed a satisfactory post-TX CCI and, similarly to those who received PLT-P units, a very low incidence of alloimmunization and blood-borne infections, increasing red blood cell quality and efficacy for patients. Although it seems high cost, the analysis of cost-effect shows normal cost because only one pretransfusion test is performed for double unit red blood cell unit, alloimmunization risk is decreased by using pre storage log 4 leukocyte filters.

P 9 Double Red Blood Cell Apheresis and Clinical Applications

D. Canatan1,2, C. Karadogan1, N. Oguz1, N. Bailta1, R. Coşan1, A. Ozsançak1, H. Dirican1, O. Cengiz1
1Antalya State Hospital, Thalassemia Center and Blood Center-Antalya, 2Suleyman Demirel University, Blood Center-Isparta, Turkey

Objective: Erythroapheresis has two types: donor erythroapheresis including single, double and neocyte and therapeutic erythroapheresis including erythrocytophoresis and erythrocyte exchange. Double Red Blood Cell Apheresis (DRBCA) has many advantages such as maintaining standard red cell volume and hematocrit content, decreasing the risk of alloimmunization and blood-borne infections, increasing red blood cell quality and efficacy for patients. Although it seems high cost, the analysis of cost-effect shows normal cost because only one pretransfusion test is performed for double unit red blood cell unit, alloimmunization risk is decreased by using pre storage log 4 leukocyte filters.

Material and Methods: All 605 voluntary unpaid blood donors were carried out a Double RBC Apheresis by Haemonetics MCS+ including an in-line Pall filter at Antalya State Hospital Blood Center between August 2000 and December 2002.

Results: The mean age of donors (604 male, 1 female) were 33 ± 8 years, their mean pre donation Hb levels: 15.4 ± 1 g/dl, hematocrit levels: 45.7 ± 2%, WBC: 8.1 ± 10^9/L and platelet: 251.2 ± 56 × 10^9/L. The tolerance of all donors were very good and had no adverse reactions. Their blood group distribution were as follows: A+ in 239 (39.5%), O+ in 221 (36.5%), B+ in 71 (11.7%), AB+ in 43 (7.1%), A– in 20 (3.3%), O– in 8 (1.3%) and B– in 3 (0.5%) patients of 1210 RBC units (9.9%) for patients with thalassemia major, 128 units (10.5%) for the patients with malignancy and 962 units (79.5%) for open heart surgery patients at the department of cardiovascular surgery were used.

Conclusions: Double RBC Apheresis is safe and reliable for donors and maintains high quality and safe hemotherapy for all patients.

P 10 Stability of Thawed Packed Red Blood Cells Using the ACP 215® of Haemonetics for Freezing and Thawing

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Background: In the freeze and thaw technology of packed red blood cells (PRBCs) commonly open systems are used. Therefore the shelf life of thawed PRBCs is limited to 24 hours. In this study we evaluated an automatic and functionally closed system for both the glycerolization and deglycerolization process which allows a postthawing storage of 7 days.

Material and Methods: Seventeen PRBCs were collected from healthy male donors using the MCS+ device. All donors met the national and European guidelines for eligibility for cytapheresis donors and gave written informed consent to participate in the study. PRBCs were glycerolized immediately after donation (without adding SAGM) using the high glycerol method. Glycerolization was done with the ACP® device (Haemonetics) at a final concentration of glycerol of 37%. These PRBCs were stored at –80°C for 14 days. Quality was assessed by the measuring of cell counts, free hemoglobin (fHb), K+, LDH, pH, lactate, glucose and intracellular ATP, ADP and AMP immediately after collection, postthawing, after 24 hours, on day 4 and 7.

Results: Rate of hemolysis as determined by fHb, LDH, Hct as well as the increase of K+ content in the supernatant, lactate and consumption of glucose remained within the ranges of conventionally stored PRBCs during their shelf life. Over the storage period pH remained stable above 6.5 and the recovery of intracellular ATP was 60% on day 7. However, we observed a loss of red blood cells and RBC mass after the thawing procedure of 34% and 40% respectively.

Conclusions: With ACP 215® glycerolized and deglycerolized PRBCs meet the quality requirements for transfusion of the European guidelines, but due to the loss of RBC transfusion of 3 PRBCs is necessary to achieve the effect of 2 conventionally stored PRBCs.
P 11\n
Erythrocyte Loss in Case of Hemapheresis Donors: Are the Legal Guidelines Concerning Donor Protection Kept?\n
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Introduction: The guidelines of the BÄK (Bundesärztekammer) concerning the collection of blood and blood components specify the maximum allowed annual RBC donation volume for women up to 1,000 ml and for men up to 1,500 ml. The total donation volume cannot exceed 25 L plasma per year. An interval of at least 8 weeks must be kept between 2 whole blood donations. However, apheresis donors are allowed to donate within much shorter time intervals. The actual apheresis technique permits the traceability of blood component loss. This follow up is so far only possible with VISTA™. This software allows the management of the complete donation process, including donor selection and preparation, donation monitoring and report generation.

Material and Methods: Procedure registration for 40 donors was handled with VISTA™. Donation volumes combined with the RBC loss can typically be calculated as follows: Draw of blood sample for the legally required control testing: approximately 18 ml RBC / 27 ml plasma.

Residual volume of the tubing set with blood-return procedure: approximately 30 ml RBC / 35 ml plasma or without blood-return procedure: approximately 95 ml RBC / 112 ml plasma.

Volume of blood products. Calculation of RBC loss was carried out with a fictitious hematocrit of 40%.

Results:

Table 1. Volume loss female donors (n = 10)

<table>
<thead>
<tr>
<th>No donations per year</th>
<th>Plasma loss (L)</th>
<th>RBC loss (ml)</th>
<th>No incomplete proced./RBC loss (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 19.5</td>
<td>8.955</td>
<td>339.6</td>
<td>1/95</td>
</tr>
<tr>
<td>Min: 17</td>
<td>5.696</td>
<td>765</td>
<td>0/0</td>
</tr>
<tr>
<td>Max: 22</td>
<td>9.590</td>
<td>1,178</td>
<td>4/380</td>
</tr>
</tbody>
</table>

Table 2. Volume loss male donors (n = 30)

<table>
<thead>
<tr>
<th>No donations per year</th>
<th>Plasma loss (L)</th>
<th>RBC loss (ml)</th>
<th>No incomplete proced./RBC loss (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 15</td>
<td>10.435</td>
<td>1,151.8</td>
<td>0.65/61.75</td>
</tr>
<tr>
<td>Min: 27</td>
<td>6.664</td>
<td>1,010</td>
<td>0/0</td>
</tr>
<tr>
<td>Max: 23.2</td>
<td>13.089</td>
<td>1,592</td>
<td>3/285</td>
</tr>
</tbody>
</table>

Conclusion: With VISTA™ it is possible for the first time, to obtain watertight computer supported documentation of the donation procedures, including the blood loss volumes that occurred. The results obtained indicate that, in case of regular donations, 30% of the female and 12% of the male donors lose more RBC volume than permitted by law. Supplementary investigations are necessary because the safety of the donors in relation to the RBC loss is questioned.

P 13\n
Double Dose Plateletpheresis Increases Neutrophil Activation and Platelet-Neutrophil Complex Formation in Volunteer Donors

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Objectives: Platelet-neutrophil complexes (PNC) might play an important role in thrombotic and inflammatory diseases. It has been shown that during extracorporeal circulation, such as haemodialysis and cardiopulmonary bypass, platelets may form heterotypic aggregates with leukocytes via platelet CD62P and leukocyte β2 integrins. There were conflicting results and limited data on the impact of plateletpheresis procedures on PNC formation and neutrophil activation on donors. In recent years, it has been possible to collect double dose platelets by new generation devices and there were no studies concerning the neutrophil activation and PNC formation on donors during double plateletpheresis (DP).

Methods: In this study, we investigated the effects of DP with two different devices (Fresenius AS 204 n = 10 and MCS Plus n = 22) on in vivo neutrophil activation and PNC formation in 22 volunteer donors. Peripheral blood samples were taken immediately before and after apheresis procedure. Changes in PNC formation and neutrophil activation were determined by quantitating the CD42b+ neutrophil counts and the amount of mean fluorescans intensity (MFI) of CD62L, CD54, CD50, CD11b/18, CD42b expressions by using a whole blood method on flow cytometry.

Results: Statistically significant increases were found on 42b+ neutrophil (PNC formation) percentage and counts after apheresis with both machines. CD11b/18, CD50 and CD54 expressions (MFI) on neutrophils did not show any changes after apheresis with both devices. As a marker of neutrophil activation, CD62L expression (MFI) decreased significantly after apheresis with Fresenius machine on the first and seventh days but this was not seen with MCS+ Plus.

Conclusion: Our results show that DP with Fresenius AS.TEC 204 and Haemonetics MCS+ devices results an increase on PNC formation which may be a risk factor for thrombosis and inflammation. However, clinical significance of these findings during apheresis procedures was still not known exactly.

P 18

Successful Mobilisation and Collection of Peripheral Blood Stem Cells (PBSC) after Priming with Chemotherapy and Pegfilgrastim

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Objectives: A successful collection of peripheral CD34+ cells in a 52-year-old patient with mantle cell lymphoma stage II B after priming with chemotherapy and Pegfilgrastim was performed.

Methods: The results were compared to a group of twelve newly diagnosed patients with non hodgkin lymphoma who got daily filgrastim after chemotherapy (7×2–4 g Cyclophosphamid alone, 5× various combination treatments) for priming.

Results: Collection was performed on day 11 after the fourth treatment cycle of CHOP/Mabthera followed by Pegfilgrastim (day 2). At this time WBC count was 5.3 × 10^9/ml and 0.74% CD34+ cells in peripheral blood were detected. After one large volume apheresis (14,400 ml) 14.84 × 10^9 nucleated cells with 2.33% CD34+ cells in the buffy have been collected. In the control group the median WBC count was 10.2 × 10^9/ml, the CD34+ cells were 0.31% in peripheral blood and the nucleated cells in the buffy 10.9 × 10^9 with 1.56% CD34+ cells after a median apheresis volume of 9,000 ml.

Conclusion: Pegfilgrastim was well tolerated and save in our patient and was efficient in combination with chemotherapy as priming therapy for the mobilisation of peripheral CD34+ cells in comparison to the control group.

P 20

Impact of CD34+ Cell Dose on Engraftment in Allogeneic Non-Myeloablative PBSC Transplantations

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Objectives: The number of cells expressing CD34+ and the number of CFU-GM can be used to assess the peripheral blood progenitor cell (PBPC) graft quality and predict engraftment in autologous and allogeneic PBSC transplantation. However, little is known about relationship between the number of transplanted hematopoietic progenitors and time to engraftment after allogeneic non-myeloablative transplantation.

Patients and Methods: Patients (18 males, 12 females) with median age of 53 years (range 27–65) were treated for AML (n = 10), CML (n = 9), lymphoma (n = 5), myeloma (n = 2), CLL (n = 2), MDS, and aplastic anemia (n = 1). Transplantations using non-myeloablative chemotherapy (busulphan, fludarabine, ATG) and sibling HLA matched PBSC were performed between 3/1998 and 12/2002. The relation between the number of transplanted hematopoietic progenitors and time to engraftment after allogeneic non-myeloablative transplantation.

Conclusion: Pegfilgrastim was well tolerated and save in our patient and was efficient in combination with chemotherapy as priming therapy for the mobilisation of peripheral CD34+ cells in comparison to the control group.

and PLT > 50 \times 10^{10}/l was analyzed with Spearman-rank correlation ma-

## P 28

### Red Cell Exchange in Methemoglobinemia Caused by Eugenol (Clove Oil) Intoxication – Case Report

**J. Pták**, **R. Kula**, **M. Carbolová**

1Blood Center, 
2Clinic of Anaesthesiology and Resuscitation, University Hospital Ostrava, 
Czech Republic

**Background**: A case of 20-year-old man intoxicated by parenteral (par-
tially paravenous) application of eugenol (clove oil) in the cubital vein is described. This chemical causes methemoglobinemia, hemolysis and its action in the organism is described as mitochondrial poisoning. Clove oil is used in dentistry as an analgesic, anesthetic and antiseptic, it is also used in perfumery. Methemoglobin is the oxidized form of hemoglobin in which the iron in the heme component has been oxidized from the ferrous to the ferric state. This renders the hemoglobin molecule incapable of effec-
tively transporting and releasing oxygen to the tissues. The episodes of methemoglobinemia and hemolytic anaemia have been due to exposure to aromatic nitro and amino compounds (phenol, naphtalen, antimalaric agents etc).

**Case Report**: The patient was admitted to the hospital with consciousness alteration, cyanosis, dyspnea and high level of hemolysis and myolysis products. Deep necrosis developed in the application site in the cubita. The methemoglobin level was 17% and oxygen saturation was of 66–77%. Controlled ventilation after intubation was started. 8 blood units of packed red blood cells were applied and then therapeutic plasma exchange was performed to eliminate myolysis and hemolysis products. Be-
cause of remaining low oxygen saturation in spite of intensive oxy-
genotherapy and repeated erythrocyte transfusion, it was decided to per-
form red cell exchange (RCE). This procedure has run from 20.00 till 23.30 o’clock. RCE can be able to improve or normalize the oxygen tissue sup-
dering erythrocytes fully functional for the transport of oxygen. The

**Discussion**: Although it is as yet unproven, the efficient and rapid removal of unwanted red blood cells by apheresis might also be helpful in treating rare conditions, whose pathophysiology suggests, that benefit might ensue. In the literature there are described conditions, where for instance using of red cell exchange is used in the treatment for instance malaria, babesiosis and carbon monoxide poisoning. Red cell exchange in above mentioned patient was performed in the situation, when all standard therapeutic measures were used (including methylene blue application) without any suc-
cess. All potentially adverse and undesirable effects associated with the

## P 31

### Therapeutic Approaches in the Management of Oral Cyclosporin A Intoxication


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*Department of Cardiac Surgery, 
*Department of Clinical Pharmacology, University Hospital, Vienna, Austria

**Background**: A 68-year-old male patient who was renal transplanted be-
cause of bilateral end stage kidney disease of unknown origin received a

**Material and Methods**: For WBE a portable cell separator (MCS 3p/Haemonetics®) was used. The exchange medium consisted of 4 irradi-
ated packed red blood cells (PRBCs) and 4 solvent detergent inactivated pooled plasmas (Octaplas®). Because of acute renal failure a continuous hemofiltration with a filtration rate of 2.000 mL/h was applied 2 h after the whole blood exchange.

**Results**: WBE had no immediate effect on serum CsA levels. 1.110 ng/mL were detected after the exchange procedure. 1.022 ng/mL were found in the depleted RBC fraction and 1.257 ng/mL in the depleted plasma fra-
tion of the patient. A rapid decrease of CsA in plasma, however, was seen after starting the hemofiltration. A CsA level of 159 ng/mL was found after 72 h. The patient recovered and was dismissed from the hospital 1 month later.

**Conclusion**: WBE has no immediate therapeutic effect in CsA intoxica-
tion. The decrease of CsA plasma levels after onset of hemofiltration would at first sight suggest therapeutic benefit but when compared to the mean elimination half-life of CsA its effect is doubtful.

## P 39

### Kidney Transplant Rejection Reaction and Plasmapheresis

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**Purpose**: The belief that circulating antibody might be involved in rejec-
tion led to extensive use of plasmapheresis therapy. In hyperacute rejec-
tion of aortic allografts, where therapeutic apheresis would have the firmest the-
oretical footing, the results where uniformly negative. It is apparently not possible to remove sufficient antibody quickly enough to save such grafts.

**Methods**: Between 1994–2002, 22 patients who exhibited symptoms of

**Conclusion**: Plasmapheresis can be applied as supplemental method of treatment for patients following kidney transplantation in cases of kidney trans-
plant rejection reaction. This will allow reduction of immunosuppressant doses and CIC levels in the blood.

## P 41

### Plasma Exchange Therapy in Refractory Myasthenia Gravis (MG) and Guillain Barre (GB) Patients

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*Intensive Care Unit, Hippokration Hospital, Athens, Greece

**Objective**: Myasthenia gravis (MG) is a disorder of neuromuscular trans-
mision characterized by weakness and fatigue. Guillain Barre (GB) is a common polyneuropathy causing disability and respiratory failure. Both

**Results**: A significant correlation between number of transplanted CFU-
GM (median 105.8 × 10^9/kg, range 16.0–347.7) and leukocyte as well as granulocyte engraftment was not found. On the other hand, the CD34+ cell dose (median 7.2 × 10^9/kg, range 2.7–15.4) strongly correlated with leukocyte (r = -0.64, p < 0.001) and granulocyte (r = -0.66, p < 0.001) en-
graftment. A strong positive correlation was found between the number of transplanted CFU-GM as well as with the CD34+ cell dose (r = -0.70, p < 0.001).

**Conclusion**: We can conclude, that the dose of CD34+ cells significantly affected leukocyte, granulocyte, and platelet engraftment after non-myel-
oablative allogeneic transplantation. However, the number of CFU-GM correlated only with platelet recovery and seems to be not useful for en-
graftment prediction contrary to the number of CD34+ cell which could be used for prediction of time to hematopoietic recovery.

**Discussion**: Apheresis is a well established method to remove desired substances from the systemic circulation.
Table 1. Abstract P 39

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>Sex</th>
<th>CIC level in the blood</th>
<th>Amount of removed plasma (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>primary (ext.u.)</td>
<td>during the last procedure (ext.u.)</td>
</tr>
<tr>
<td>1.</td>
<td>28</td>
<td>M</td>
<td>0.263</td>
<td>0.227</td>
</tr>
<tr>
<td>2.</td>
<td>23</td>
<td>F</td>
<td>0.101</td>
<td>0.086</td>
</tr>
<tr>
<td>3.</td>
<td>34</td>
<td>F</td>
<td>0.404</td>
<td>0.386</td>
</tr>
<tr>
<td>4.</td>
<td>48</td>
<td>M</td>
<td>0.247</td>
<td>0.164</td>
</tr>
<tr>
<td>5.</td>
<td>57</td>
<td>M</td>
<td>0.152</td>
<td>0.096</td>
</tr>
<tr>
<td>6.</td>
<td>17</td>
<td>F</td>
<td>0.823</td>
<td>0.108</td>
</tr>
<tr>
<td>7.</td>
<td>27</td>
<td>M</td>
<td>0.028</td>
<td>0.125</td>
</tr>
<tr>
<td>8.</td>
<td>24</td>
<td>M</td>
<td>0.124</td>
<td>0.085</td>
</tr>
<tr>
<td>9.</td>
<td>39</td>
<td>F</td>
<td>0.213</td>
<td>0.130</td>
</tr>
<tr>
<td>10.</td>
<td>32</td>
<td>M</td>
<td>0.3</td>
<td>0.287</td>
</tr>
<tr>
<td>11.</td>
<td>25</td>
<td>M</td>
<td>0.507</td>
<td>0.317</td>
</tr>
<tr>
<td>12.</td>
<td>35</td>
<td>M</td>
<td>0.193</td>
<td>0.203</td>
</tr>
<tr>
<td>13.</td>
<td>36</td>
<td>M</td>
<td>0.115</td>
<td>0.022</td>
</tr>
<tr>
<td>14.</td>
<td>59</td>
<td>M</td>
<td>0.025</td>
<td>0.018</td>
</tr>
<tr>
<td>15.</td>
<td>41</td>
<td>F</td>
<td>0.282</td>
<td>0.161</td>
</tr>
<tr>
<td>16.</td>
<td>45</td>
<td>M</td>
<td>0.031</td>
<td>0.012</td>
</tr>
<tr>
<td>17.</td>
<td>29</td>
<td>F</td>
<td>0.021</td>
<td>0.018</td>
</tr>
<tr>
<td>18.</td>
<td>54</td>
<td>F</td>
<td>0.145</td>
<td>0.052</td>
</tr>
<tr>
<td>19.</td>
<td>32</td>
<td>M</td>
<td>0.012</td>
<td>0.009</td>
</tr>
<tr>
<td>20.</td>
<td>46</td>
<td>M</td>
<td>0.129</td>
<td>0.059</td>
</tr>
<tr>
<td>21.</td>
<td>28</td>
<td>F</td>
<td>0.021</td>
<td>0.018</td>
</tr>
<tr>
<td>22.</td>
<td>53</td>
<td>F</td>
<td>0.145</td>
<td>0.052</td>
</tr>
</tbody>
</table>

ext.u. = External unit – normal < 0.06.

Methods: We studied 4 patients, 2 females and 1 male (mean age 45 years), with MG and 1 40-year-old female with GB who underwent PE after progression while receiving systemic therapy. Two patients had the third relapse of MG, one the second relapse of GB and one had underwent thymectomy after the second episode of MG. All of them at the time of starting the procedure were in a severe healthy condition requiring intensive care and breathing support. We performed PE every other day 5–9 PE for each patient by using MCS+ machine (Haemonetics). One volume of plasma was removed for each procedure and was exchanged with 5% albumin in saline solution. During treatment vital signs such as pulse rate, blood pressure and O2 saturation were monitored and total plasma proteins, coagulation parameters and hemoglobin/hematocrit and rate, blood pressure and O2 saturation were monitored and total plasma. The significant improvement of quality of life is in addition to the therapy with Bosentan (endothelin receptor antagonist for treatment of pulmonary hypertension) a success of immunoadsorption.

Results: ANA and autoantibody (aab) against dsDNA are reduced significantly (ANA: 1:640 to 1:160 and dsDNA-aab: 130.1 IU/ml to 42.3 IU/ml). All patients remain. We performed this study in order to identify the value of plasma supports the patients with the vWF-cleaving protease activity.

Conclusion: The exact cause of TTP has not yet been explained but identification of vWF-cleaving protease has led to a new understanding of the pathogenesis of TTP, and to an understanding the mechanism of the therapy using TPE. TPE is the efficient procedure that helps to patients to overcome the disease. No serious adverse reactions in patients have been observed.

P 48
Therapeutic Plasma Exchange in Patients with Thrombotic Thrombocytopenic Purpura
Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Introduction: Thrombotic Thrombocytopenic Purpura (TTP), first described in 1924, is an increasingly common, potentially fatal, multisystem disease clinically defined by severe thrombocytopenia, microangiopathic hemolytic anemia, neurological abnormalities, renal dysfunction and fever. Combination of the Therapeutic plasma exchange (TPE) and plasma infusion is considered as the most suitable therapy of the disease. TPE helps to remove ULs-vWF multimers and IgG inhibitor, and the addition of plasma supports the patients with the vWF-cleaving protease activity.

Methods: Authors refer their experience with 303 TPE procedures performed in 1999–2003, in 6 patients with TTP who developed heavy neurological symptoms and prolonged thrombocytopenia. The course of the disease was unexpectedly complicated with repeated early relapses. TPE procedures were performed daily by the apheresis technique. The course of the disease was in all 6 patients severe. After a short temporary period of improvement it was complicated with relapses with the significant decrease in the number of platelets, and elevation of LDH levels. Subsequently, it was necessary to continue in TPE procedures and to combine TPE with vincristine and cyclophosphamide. All the patients have recovered and they have no symptoms of the disease until now. The incidence of the adverse reactions like hypocalcemia, allergic reactions, tachycardia and fever were observed in 32 procedures (11%).

Conclusion: The systematic response of PE combined with immunosuppressive therapy is an efficient and safe therapeutic option and can be considered in refractory MG and GB patients.

P 49
Thrombotic Thrombocytopenic Purpura of Adults and Markers of response to Therapy
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Objective: Plasma exchange therapy of thrombotic thrombocytopenic purpura (TTP) improved survival, but a high number of non responders patients remain. We performed this study in order to identify the value of serologic markers of response to plasma exchange (P EX).
Material and Methods: We evaluated 14 TTP patients, 9 females and 5 males with mean age 55 years (range 26–80 years), treated with PEX in our hospital during the last 7 years. According to the treatment protocol a mean 38 ± 5 ml plasma/kg of body weight was exchanged daily until serological evidence of remission appeared, defined as increase of platelet >150,000/mm³ and normal levels of LDH which was defined as response to therapy. The PEX was performed with the machine MCS+ (Haemonetics) and the plasma was exchanged for fresh frozen plasma. To assess the early response to treatment, the decline of LDH and platelet increase from the first to the third cycle was calculated: LDH before the third PEX/LDH before the first PEX (LDH ratio) and platelets before the third PEX/platelets before the first PEX (platelet ratio). Statistical analysis was performed with Wilcoxon test.

Results: We found that 71.5% of the patients responded to therapy with PEX. Four patients were non responders (28.5%). After 2 procedures of PEX none clinical or laboratory marker correlated with outcome. After 2 procedures of PEX only LDH and platelet level had improved in responding patients. The LDH ratio was the best predictive marker of response (p~0.01). Nine of 10 responding patients had an LDH ratio <0.60 and all of the non responders patients had LDH ratio > 0.60.

Conclusion: TTP remains a serious condition and the early identification of patients not responding to PEX might help to modify the therapy with intensive plasma exchange and additional therapeutic interventions. The LDH ratio might be a useful predictive marker for separating responding from non responding patients to plasma exchange.

P 52 Thrombotic Thrombocytopenic Purpura (TTP) and Thrombophilia – Congenital or Acquired? M. Bellia, K. Maragkos, N. Vgontzas, V. Tservenis, E. Nomikou, E. Andrioti 1st Regional Transfusion and Hemophilia Center, Hippokration Hospital, Athens, Greece

Purpose: We investigated our TTP patients for the existence of thrombophilic disorders congenital or acquired contributing to more aggressive and relapsing forms of TTP.

Material and Methods: The last 7 years a total of 21 patients 8 male and 13 female with mean age 46 years and the established diagnosis of TTP were treated in our hospital. Fourteen patients had the relapsing form of the TTP. All the patients entered the protocol with methyl prednisolone and plasmapheresis exchange (PE) by using the machine MCS+ (HEAMONETICS). Twelve out of 21 patients were studied for thrombophilic disorders: FXII activity, Protein C, S, ATIII, APC-Resistance (APC-R), genotyping analysis for FVL/Leiden/FII20210/MTHFR, plasminogen, Anticardiolipin antibodies (ACA) and Lupus anticoagulant (LA). The statistical analysis was performed with χ² test.

Results: We found 7 patients with positive markers of thrombophilia. The characteristics of the 7 patients are shown on the table.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age, years</th>
<th>Relapse</th>
<th>Number of PE</th>
<th>Coexisting disease</th>
<th>Thrombophilic disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>44</td>
<td>yes</td>
<td>19</td>
<td>Infection of the skin</td>
<td>FVL/Leiden</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>63</td>
<td>no</td>
<td>16</td>
<td>Cancer of the bladder</td>
<td>Leiden +</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>58</td>
<td>no</td>
<td>8</td>
<td>–</td>
<td>PrS</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>71</td>
<td>no</td>
<td>18</td>
<td>–</td>
<td>MTHFR</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>50</td>
<td>no</td>
<td>13</td>
<td>Autoimmune hepatitis</td>
<td>ACA IgM +</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>59</td>
<td>yes</td>
<td>7</td>
<td>–</td>
<td>FVL/Leiden</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>22</td>
<td>no</td>
<td>8</td>
<td>–</td>
<td>FVL/Leiden</td>
</tr>
</tbody>
</table>

M = Male; F = female.

The patients 1M with FVL/Leiden and 4F with low levels of PrS and MTHFR needed >15 procedures in order to recover completely. The patient 3M with low levels of PrS and 6F with FVL/Leiden presented DVT (Deep Vein Thrombosis) one week after the last PE. The two patients 2(F), 5(F) had LA and ACA IgM positive and presented thrombosis and infection of their catheter. The patient 7F was homozygote FVL/Leiden, he presented stroke and is on oral anticoagulant therapy without TTP relapse. The other 5 patients with negative thrombophilic tests presented with no signs of thrombosis and milder TTP presentation (p>0.001).

Conclusion: TTP is a syndrome affecting predominantly females (13/8 F:M). The confirmation of thrombophilic disorders may be crucial in therapeutic management due to the possibility of thrombosis and more aggressive presentation. The patients with positive markers of thrombophilia should be monitored closely and anticoagulant or antiplatelet therapy should be administered in cases of thromboembolic events.

P 53 Thrombotic Thrombocytopenic Purpura and Pregnancy M. D. Castella*, M. Pujol*, A. Julia**, I. Massagué**, L. I. Puig*

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Objectives: A series of women with thrombotic thrombocytopenic purpura (TTP) associated with pregnancy is presented. The study analyses the relationships between TTP and pregnancy and maternal and fetal outcome.

Patients and methods: 40 consecutive patients who met the classic criteria for TTP were studied. Among them, 8 patients who were either pregnant or in the postpartum period were identified (20%). All patients were treated with prompt plasma exchange. A number of adjunct therapies also were used in patients not responding to plasma exchange. These included corticosteroids, antiplatelet aggregation drugs, vincristine and splenectomy.

Results: The study population consisted of 9 women, 6 of whom presented an acute single episode associated with pregnancy and 2 having a chronic relapsing form of the disease. These two patients had a total of 18 TTP episodes, 2 of which were related to pregnancy. None of them were diagnosed during pregnancy or in the postpartum period. Another pregnant patient had recurrent disease and neither episode was associated with pregnancy.

Five women were pregnant and 3 were in the immediate post-partum period. In 2 patients the disease developed before midpregnancy. One gestation was successfully terminated with a normal fetus at the 26th week because of worsening of the TTP and the other by an elective abortion at the 16th week of gestation. In 3 patients, the disease manifested in the third trimester and in one of these the gestation was terminated after demonstration of the death of the fetus. The other two mothers gave birth to preterm infants. Fetal mortality was 40% in pregnancies associated with TTP. There was one maternal death. This mortality rate is similar to that observed in the rest of patients of our series (4/32). There were no serious long-term sequelae in the 8 survivors. Mean follow-up of the eight survivors is 6.16 years (range 1.32–11.4 years).

Conclusion: This limited experience suggests that pregnancy could be one of the initiating agents of TTP. However, in those patients where PTT was first diagnosed during the course of a pregnancy, no further episodes were observed. Pregnancy in patients with a previous history of recurrent TTP may increase the risk for recurrence, but it can also be uncomplexed.

As far as survival, pregnancy does not increase the risk for the mother with TTP when compared with the mortality rate in the rest of patients in our series. Preterm delivery and intrauterine fetal death were frequent complications of these pregnancies.

P 54 Patient with Relapsing Severe Thrombotic Thrombocytopenic Purpura and Systematic Lupus Erythematosus – A Case Report Z. Kofista, Z. Fojtik, M. Navrátil

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Objectives: Thrombotic thrombocytopenic purpura (TTP) is a devastating microvascular occlusive disorder associated with an acquired or congenital deficiency of von Willebrand factor – cleaving metalloproteinase (ADAMTS13) which normally prevents the entrance of unusually large VWF multimers into plasma. The acquired form of TTP is caused by autoantibodies against VWFcp, whereas homozygous or compound heterozygous
Results: modification of MNC was performed by the use of 8-MOP (Gerot) and mated mode (v. 5.1, 6). We processed 2 (1.6–2.1) total blood volumes of peripheral blood and in MNC concentrates. The ECP procedures were on levels of T cell subsets (CD 3+, CD 4+, CD 8+, IRI), B cells and NK in respect of a frequently very fast and serious development of this disease, early initiation of the large volume plasma exchange can be a life saving therapeutic approach in these patients.

Conclusion: The acquired form of TTP in SLE patients can be a rare but very serious complication with difficult differential diagnosis, which includes catastrophic antiphospholipid syndrome, disseminated intravascular coagulopathy, haemolytic-uremic syndrome, and activity of SLE. In respect of a frequency very fast and serious development of this disease, early initiation of the large volume plasma exchange can be a life saving therapeutic approach in these patients.

P 55 Extracorporeal Photochemotherapy in Patients with Chronic GVHD

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Introduction: Chronic GVHD is considered as the most common late complication of allogeneic hematopoietic stem cell transplantation. It continues to be a significant cause of morbidity and mortality. Patients are treated by the administration of conventional immunosuppressive therapy over prolonged periods of time. Nevertheless, the therapy causes significant side effects with increased morbidity and mortality due to the infections. The situation may be complicated especially in patients who are refractory to immunosuppressive treatment. New ways of therapy are searched, and promising therapeutic strategies include extracorporeal photochemotherapy (ECP).

Methods: We tried to evaluate the clinical effect of 167 ECP procedures in 6 patients with chronic GVHD. We assessed also the influence of the ECP on levels of T cell subsets (CD 3+, CD 4+, CD 8+, IRI), B cells and NK in peripheral blood and in MNC concentrates. The ECP procedures were performed by means of Cobe Spectra, either in semiautomated or automated mode (v. 5.1, 6). We processed 2 (1.6–2.1) total blood volumes of the patients, which corresponded with 7.1 (6.7–6.7) liters of blood. Photomodification of MNC was performed by the use of 8-MOP (Gerot) and the UV-A irradiator (Psorilux 3070, Heraeus).

Results: The significant improvement of the disease was observed in 4 patients with sclerodermatous skin changes, impaired joint mobility and joint pain. In 2 patients with oral disease only limited and temporary recovery was detected. The levels of T cell subsets, B cells and NK did not show any substantial changes in the course of ECP.

Conclusion: ECP is recently accepted to be the safe and non-toxic method that is able to mediate immune system of the patient without generalized immunosuppression. No increased incidence of infections and no serious adverse reactions in patients have been observed.

P 56 Treatment of Chronic Graft versus Host Disease of the Lung with Extracorporeal Photochemotherapy – Experience in 2 Patients

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Purpose: Photochemotherapy (extracorporeal photochemotherapy, ECP) appears to be an effective treatment for skin and liver manifestations of chronic graft versus host disease (cGVHD) in many studies. Experience in the treatment of pulmonary GvHD with ECP is still limited and controversial.

Methods: We report on 2 patients with severe pulmonary GVHD who were treated with ECP using the UVAR-XTS-device (Therakos Company, Exton). Patient 1 was a 57-year-old woman, diagnosed with AML M1 8/1998. Patient 2 was a 39-year-old man with AML M2, diagnosed 9/1999. Both patients were transplanted from HLA-identical siblings and suffered from severe lung and moderate liver GvHD. ECP was started 12 and 17 months after transplantation, respectively. Additional immunosuppressive treatment consisted of cyclosporin, mycophenolate mofetil and prednisolone (30mg/kg) in Pat. 1 and tacrolimus and prednisolone (30mg/kg) in Pat. 2. ECP treatments were performed on 2 consecutive days every week during the 1st month and every 2nd week thereafter for the following 5 months. Treatment intervals were tapered thereafter individually according to patients condition.

Results: The ECP procedures were well tolerated by both patients without any severe side effects. Technical problems were mostly related to difficult venous access. Patient 1 was treated with ECP for 1 year, during this time pulmonary symptoms improved continuously and liver function parameters normalized. Now she is still in stable clinical condition 2.5 years after completion of ECP treatment. Patient 2 has been treated with ECP for 20 months and therapy is still ongoing. His respiratory symptoms and liver function tests improved substantially. In both patients immunosuppressive medication could be reduced and steroid dosage could be tapered to 5mg. Symptoms of Cushings disease completely resolved.

Conclusion: These 2 cases suggest, that ECP is a feasible and a valuable therapeutic modality for the treatment of lung GvHD. Prospective randomised studies are needed to confirm the efficacy of ECP in these patients.

P 57 Extracorporeal Photoimmunotherapy (ECP) for Treatment of Steroid Refractory Extensive Chronic Graft versus Host Disease (cGVHD): Turkish Experience

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2Istanbul University Medical School Istanbul, Turkey
3Abcoulis Medical School Istanbul, Turkey

Objectives: cGVHD can arise as a late complication after allogeneic hematopoietic stem cell transplantation. Patients with extensive disease to date require intensive early and long-term immunosuppression. We aimed to share the experience of three Turkish hempheresis centers using ECP in this complicated patient population.

Methods: Thirteen patients in three centers were treated with ECP (UVAR-XTS) on 2 consecutive days every 2–4 weeks until resolution of GvHD over a period of 6–15 months. Beyond extensive cutaneous cGVHD, four patients had also bronchiolitis obliterans (BO). Skin scores assessed by an experienced dermatologist. Laboratory and radiological findings after 4 months of ECP were accepted as response criteria. In this almost fully automated system mean 261.4 ml buffy-coat was processed within 193 min using UVADEX sterile solution.

Results: After a median of 12 cycles of treatment, all patients showed a favorable response. ECP was tolerated well only one patient developed Gr4 thrombocytopenia and another patient had a massive GIS bleeding due to

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an esophageal tear. Reduction in cholestatic parameters was observed in 9, improvement in respiratory functions and CT evaluations in 4, and reduction in immunosuppressive agent need in 10 patients. The most impressive result was the reduced need for hospitalization of these patients and improvement of skin lesions. All but one of the skin biopsies scores was also better after ECP. **Conclusion:** As chronic extensive GvHD is a life devastating disorder, every attempt to improve the quality of life should be evaluated carefully. Our findings suggest that ECP is a safe and effective adjunctive therapy for steroid refractory chronic extensive GvHD of the skin. The place of ECP for the treatment of visceral GvHD and additional co-morbid conditions like BO should be further analyzed in large scale trials.

**P 65**
**Hemotherapy in Kidney Transplantation in the Military Medical Academy**


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**Objectives:** A total of 107 kidney transplantations (81 males and 26 females) from living donors (group I) and 14 kidney transplantation (8 males and 6 females) from cadavers (group II) were performed in the period 1996–2002 at the Military Medical Academy (MMA) in Belgrade in the Serbia and Montenegro.

**Methods:** Lymphocytotoxic cross-matching was done before each kidney transplantation. In perioperative transfusion treatment determined quantity of filtered red blood cells (F-RBCs) and/or filtered platelets (F-PLT) were given to recipients according to blood loss.

**Results:** In only 9 (8%) recipients in group I transfusion therapy was not applied perioperatively. An average use of 2.02 units of F-RBCs (942.70 ml) was used intraoperatively in 67 (63%) recipients in group I, an average of 1.9 units of F-RBCs (521 ml) was used before kidney transplantation in 15 (14%) recipients in group I and an average use of F-RBCs (1 208.68 ml) and an average of 0.63 unit of F-PLT were used postoperatively in 61 (57%) recipients. In all recipients from group II used transfusion therapy was applied perioperatively. An average use of 3.4 units of F-RBCs (953 ml) was used intraoperatively. An average use of 4.9 units (1,328 ml) and an average of 1.4 units of F-PLT were used postoperatively.

**Conclusion:** All recipients well tolerated the therapy and no adverse effect of the therapy were observed. The need for transfusion therapy intraoperatively was approximately same in both recipient groups, while in recipients from cadavers need for transfusion support in posttransplantation period was much higher.

**P 68**
**Optimization of Therapeutic Procedure During LDL Apheresis – a Multivariate Computerized Model**

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**Objectives:** LDL apheresis is a very effective method in the treatment of resistant hypercholesterolemia when other therapy (dietary, medicamentous) fails. This is essential and life saving in homozygous hypercholesterolemia, in other cases it might substantially prolong the life without premature occurrence of severe atherosclerotic complications. To maximize efficacy of the usage of LDL absorbers we aimed to create a multivariate computerized model.

**Methods:** LDL apheresis was performed (after separating plasma in a continuous-flow blood separator Cobe Spectra, USA) by absorption-desorption automat ADA (Medicap, Germany) controlling the passage of plasma through a pair of columns containing Sepharose 4b carrying an anti-apoprotein B antibody (Lipopak, Focard). Computerized model was created to control the volume and flow of plasma for optimal adsorption performance. Based on the pilot studies the following data were used for calculation: height, body weight, sex, baseline and expected plasma LDL cholesterol. The principle of calculation is an exponential dependence and suggestion that the absorber eliminates all LDL cholesterol and that the amount of eliminated LDL cholesterol correlates with the binding column capacity. Presumption is even distribution of LDL-cholesterol in plasma, total elimination during passage through column, permanent steady of absorption column and zero neglected release of LDL cholesterol into circulation from tissue reserves during the 4 h of procedure. The software is being tested in a larger number of procedures.

**Results:** Suitable software has been created, using Microsoft Excel, including HTML version. User loads the above mentioned data (see formula) and based on the results of calculation changes optimal plasma flow through separate columns to gain the expected goal. On the contrary to the previously used empirical method a shortening of the procedure has been reached, which has both positive medical and financial consequences.

**Conclusion:** Software for calculation based on the above presumption has been created and validated in the first twenty procedures. It enables to rationalize setting of LDL apheresis, with maximal efficacy of absorption columns. Recently, the software is being tested in a larger number of procedures.

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