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SWISSTRANSFUSION
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Abstract No. 1

Authors:
Nicolai Rohner [1], Jakob R Passweg [1], Dimitrios Tsakiris [1], Jörg P Halter [1], Dominik Heim [1], Andreas S Buser [1, 2], Laura Infanti [1, 2], Andreas Holbro

Affiliation:
[1] Division of Hematology, Department of Internal Medicine, University Hospital Basel, Switzerland
[2] Blood Transfusion Service, Swiss Red Cross, Basel, Switzerland

Title:
THE VALUE OF POST THAW CD34 COUNT WITH AND WITHOUT DMSO REMOVAL IN THE SETTING OF ASCT

Text:
This abstract was orally presented at EBMT 2017.

Introduction: CD34 count administered is considered an essential predictor of engraftment in autologous stem cell transplantations (ASCT). Current JACIE standards only require a CD 34 measurement upon cryopreservetion. At our institution, CD34 count is routinely determined after processing and after thawing. The value of post-thaw CD34 count with and without dimethylsulfoxid (DMSO) removal has not been systematically analyzed so far.

Patients and Methods: We included all ASCT patients at our institution between 2008 and 2015 in this retrospective study. The data was collected using the electronic database of our institution and by chart review. All data are expressed as median and the lower 10th and 90th percentile.
Results: Between 2008 and 2015, 237 patients underwent a total of 293 ASCT at our institution. Main indication was Multiple Myeloma, median age at diagnosis was 55 years. Grafts were preserved with 7.5% DMSO and kept at -175°C. Median CD34 count after apheresis and after thawing was 3.96 (2.29 - 11.56) and 2.49 (1.31 - 7.26), respectively. In 97 ASCT (33%), DMSO was removed before infusion. Overall, we found a good correlation between CD34 count after apheresis and after thawing. Additionally, we found no significant association between RBC, PLT and ANC engraftment and CD34 count neither after apheresis or thawing. Washing significantly affected RBC and PLT engraftment, but not ANC engraftment (s. Table). However, RBC, PLT, and ANC engraftment were statistically significantly delayed in patients receiving a graft with a low CD34 recovery (i.e. CD34 < 2.0 x 106/kg). Of 89 patients with a low CD34 count upon thawing, 81 of them were due to a low recovery.
Conclusions: Even though we observed some significant losses in CD34 and a good correlation between lower CD34 doses and longer time to engraftment, we deem these differences not sufficiently important to justify regular, expensive monitoring of post thaw CD34 counts. CD34 count is finally affected by many preanalytic, analytic and single institutional conditions. DMSO washing may affect engraftment and should be balanced against possible infusi on associated side effects.

Table. Engraftment data (days after ASCT: median and the lower 10th and 90th percentile). Numbers (n) indicate numbers of ASCT.

Engraftment	ANC	p-value	PLT	p-value	RBC	p-values
DMSO no removal (n=196)	16 (11-26)		16 (12-28)*		14 (11-27)*	
		0.456		0.047		0.003
DMSO removal (n=97)	15 (11-25)		19 (14-29)*		17 (13-30)*	
CD34 at apheresis						
- Low	18 (15-40)		18 (15-27)		18 (11-29)	
- Median	16 (12-27)	< 0.0001	17 (13-29)	0.007	16 (12-29)	0.146
- High	14 (10-24)		16 (11-27)		15 (11-29)	
CD34 after thawing						
- Low	17 (13-28)		18 (14-31)		17 (12-30)	
- Median	16 (11-25)	< 0.000	17 (13-28)	< 0.000	16 (11-29)	0.002
- High	13 (10-18)		14 (11-21)		14 (11-30)	
CD34 low after thawing but median/high after apheresis	16 (12-27)		18 (13-30)		17 (13-30)	

Low CD34: CD34 < 2x10^6/kg; Median CD34: CD34 ≥ 2 and < 5x10^6/kg; High CD34: CD34 ≥ 5x10^6/kg.
Abbreviations: ANC: neutrophil engraftment; PLT: platelet engraftment; RBC: red blood cell engraftment. D: days; DMSO: Dimethylsulfoxid.
* p-values < 0.05 are considered statistically significant.

Abstract No. 2

Authors:
Henny C, Still F, Lejon Crottet S, Hustinx H, Aeschlimann J, Niederhauser C

Affiliation:
Interregionale Blutspende SRK AG, Murtenstrasse 133, 3008 Bern, Switzerland

Title:
IMPACT OF THE MANDATORY DONOR RHD SCREENING IN SWITZERLAND

Text:
This abstract has been published before at the ISBT congress 2016 (Vox Sanguinis 2016, Suppl.1:1-322, 4C-S24-03).

Background: Serological RhD testing, including indirect antiglobulin test (IAT), is often not sensitive enough to detect very weak RhD variants. However, transfusion of red blood cell concentrates (RBCs) harbouring such RhD variants may cause alloimmunization in RhD-negative recipients as RhD is highly immunogenic. This observation leads to the molecular screening of all serological RhD-negative Swiss donors for the presence of RHD DNA sequences. In order to remain cost-efficient mandatory IAT was discontinued. This RHD screening was declared mandatory in 2013. Results from transition year 2012 were reported (Lejon Crottet S et al.: Transf Apheres Sci (2014) [1]). Herein we summarize the data from the first three years of the mandatory screen (2013 - 2015).
Aim: Detection of RHD variants in RhD-negative donors in order to prevent alloimmunization in RhD-negative recipients.
Methods: In our institute EDTA blood samples from about two-thirds of the Swiss RhD-negative blood donations were pooled in pools of up to 23 donors and DNA extracted as previously described [1]. PCR detection of RHD exon 3, 5 and 10 was done as previously described [1] or since August 2014 by using the RBC-FluoGene D-Screen kit (Inno-Train, Germany). Pools positive for one or several exons were resolved to the single donation and retested. Samples positive for RHD were further characterized using commercially available kits (Inno-Train, Germany and BAGene, Germany) or by exon sequencing and adsorption-elution analysis using polyclonal anti-D pool (in-house) and anti-D clone ESD1 (Bio-Rad, Switzerland).
Results: Between 2013 and 2015 18,537 RhD-negative samples were tested. In total, 154 samples were positive for one or several RHD exons. In 65 of 154 samples RHD variant alleles classified as serologically positive were detected, with RHD*01W.01 (n = 13), RHD*11 (n = 12) and RHD*01W.31 (n = 7) being the most common identified variants. In one sample no mutation could be determined by sequencing, however by adsorption-elution RhD antigens could be revealed. These 65 donors were reclassified as RhD positive. Further 87 donations harboured a RHD variant classified as RhD negative, with the RHD*Ψ allele (n = 27) being the most common, followed by diverse RHD-CE-hybrid alleles (n = 57). In one sample c.53delT was found. This deletion introduces a frameshift at amino acid 18 and premature stop codon at amino acid 38. To the best of our knowledge this variant, RHD* 53delT, has not been reported previously. In addition one sample showed, linked to a deletion of RHD, a mutation in RHCE, reve aling variant RHCE*ceEK. A second sample is under investigation and RhD status could not be confirmed so far.
Summary/Conclusions: Mandatory screening for the presence of RHD in RhD-negative donors revealed 65 donors which must be considered as RhD positive (0.35%). The slightly higher number of RhD positive variants compared to previously (0.15%) [1] is probably due to the discontinuation of IAT as variants such as RHD*01W.01 and RHD*11 are often not detected using standard RhD typing. The chosen strategy reduces further the risk of unnecessary anti-D alloimmunization in RhD negative recipients.

Abstract No. 3

Authors:
Gowland P, Widmer N, Stolz M, Niederhauser C

Affiliation:
Interregionale Blutspende SRK AG, Murtenstrasse 133, 3008 Bern, Switzerland

Title:
CHARACTERISATION OF PARVOVIRUS B19-POSITIVE SWISS BLOOD DONORS

Text:
Previously presented at Joint Congress DGTI & DGI, Nürnberg, 7 - 10 September 2016 (P04-1)

Introduction: Human parvovirus B19 (B19V) a common human pathogen that causes a variety of diseases with outcomes ranging from an asymptomatic or mild childhood disease to severe symptoms, especially in immunocompromised patients. Though transmitted mainly by a respiratory route, B19V transmission via blood and blood products has

been reported. Molecular screening of blood for B19V (NAT) is not required for the release of labile blood products but is performed to prevent a high B19V contamination of plasma manufacturing pools. Our routine B19V NAT of Swiss blood donations began in 2008. Here we present an extensive characterisation of B19V identified between 2008 - 2015.
Methods: B19V NAT is performed in pools of up to 480 donations on a twice weekly basis with an in-house quantitative PCR assay. Donations with viral loads > 1E+6 IU/mL B19 DNA are removed from the transfusion process if not yet transfused. Look-back is conducted when necessary. B19V serology (IgG and IgM) was performed with commercial assays. Viruses were characterized by sequencing and phylogenetic analysis revealing genotypes and novel virus variants.
Results: A total of 70 B19V positive blood donations from 2,119,520 were identified exceeding > 1E+6 IU/mL cut-off in the pool analysed. The overall frequency was 1 : 30,278 donations; in 2013 this increased to 1 : 6,368 donations. The majority had viral loads exceeding 1.0E + 08 IU/mL (median: 2.49E + 10 IU/mL; range: 5.86E + 03 - 3.43E + 12 IU/mL) and > 50% were neither IgM nor IgG positive, indicative of a very recent infection. Most (68) belonged to Genotype 1a, but an equal distribution of variant 1a1 and 1a2 was observed. Two related Genotype 2 viruses were identified in 2013 and 2015.
Conclusions: B19V NAT has been successful in preventing B19V entering the blood supply in Switzerland. The 1 : 30,278 frequency is comparable with that known in other European countries. Seasonal epidemics (e. g. 2013) occurred as reported by others and 2 rare variants were identified.

Abstract No. 4

Authors:
Gowland P, Graber J, Niederhauser C, Henny C

Affiliation:
Interregionale Blutspende SRK AG, Murtenstrasse 133, 3008 Bern, Switzerland

Title:
GENOTYPING FOR HUMAN PLATELET ANTIGENS (HPA-1, -2, -3, -5 AND -15) IN SWISS BLOOD DONORS USING A MULTIPLEX SSP-PCR APPROACH

Text:
Previously presented at Joint Congress DGTI & DGI, Nürnberg, 7 - 10 September 2016 (P02-16)

Introduction: Human platelet antigens (HPAs) are caused by polymorphisms in platelet membrane glycoproteins. Antibodies against HPAs are clinically relevant in immune platelet disorders, such as foetal and neonatal alloimmune thrombocytopenia (FNAIT), posttransfusion purpura (PTP) and multitransfusion platelet refractoriness (MRP). In order to supply these patients with adequate blood products we developed a multiplex-SSP-PCR assay to build up a database of HPA genotyped donors. Here we present the screening data of 1383 donors.
Methods: Both apheresis (platelet and plasma), as well as, whole blood donors were selected for genotyping. Donor screening was performed using published and in-house primers in two multiplex-SSP-PCR reactions targeting HPA-1a, -2b, -3a, -5b and -15a or HPA-1b, -2a, -3b, -5a and -15b respectively. PCR products were analysed using capillary electrophoresis (QIAxcel system, Qiagen, Germany)
Results: 381 apheresis and 1002 whole blood donors were genotyped for HPA antigens. The targets for the two multiplex reactions were chosen according to their expected frequency. Since every reaction yielded a PCR product in at least one allele the incorporation of an internal control proved unnecessary. Rare donors were identified in 27 donors homozygote for HPA-1b (2.0%), 9 donors homozygote for HPA-2b (0.7%) and 15 donors homozygote for HPA-5b (1.1%). The detected frequencies for the different HPA alleles were as follows: 84.4% for HPA-1a, 15.6% for HPA-1b, 90.6% for HPA-2a, 9.4% for HPA-2b, 65.1% for HPA-3a, 34.9% for HPA-3b, 91.9% for HPA-5a, 8.1% for HPA-5b, 48.3% for HPA-15a and 51.7% for HPA-15b.
Conclusions: Genotyping of blood donors for the different HPA alleles revealed allele frequencies comparable to data reported for Swiss and other Caucasian populations (http://www.ebi.ac.uk/ipd/hpa/freqs_1.html).
Multiplex PCR reactions allow fast and cost-efficient typing of a large number of donors. New blood donors with rare HPA allele conformations were found and added to the database. The characterisation of such donors is essential for the adequate supply of patients who are alloimmunized against common HPA-antigens and who often require platelet transfusions.

Abstract No. 5

Authors:
Niederhauser C, Widmer N, Hotz M, Gowland P

Affiliation:
Interregionale Blutspende SRK AG, Murtenstrasse 133, 3008 Bern, Switzerland

Title:
SEROPREVALENCE OF HEPATITIS E VIRUS (HEV) IN THE SWISS BLOOD DONORS: BASIS FOR FUTURE STRATEGY FOR PREVENTING HEV TRANSMISSION TO AT RISK INDIVIDUALS

Text:
Previously presented at 34th Int. Congress ISBT, Dubai, UAE, 3 - 8 September 2016 (P265)
Introduction: Hepatitis E virus (HEV) the etiological agent of hepatitis E is a small icosahedral non-enveloped

single stranded RNA virus that in recent years has become a growing public health concern in many developed countries. HEV infections though often asymptomatic may cause fulminant or chronic hepatitis E, especially in immunocompromised persons and is thus safety concern for hepatologists and the blood transfusion community. There are 4 known genotypes HEV1-4 which are transmitted to humans via contaminated water and meat, via blood transfusion or vertically from mother to child. In industrialised countries HEV genotype 3 has been shown to be the most widespread virus and the route of infection for this virus suspected to be from infected pork/wild game and meat products. Here we present data on the HEV IgG seroprevalence for the Swiss population which will serve as a basis for determining a strategy for preventing HEV transmission to at risk individuals.

Aim: To determine the HEV seroprevalence in blood donors from different regions within Switzerland and to a data base for establish a strategy for preventing HEV transmission to patients at high risk.

Methods: EDTA Plasma samples were collected from blood donors form different regions of Switzerland. The presence of HEV-specific IgG antibodies was determined using the WANTAI HEV-IgG ELISA (Wantai, Beijing, China). The results were analysed to include different geographical regions, age of donor and sex. The data will serve as a basis for a strategy for future blood donor screening programs.

Results: On average 19.5% of the 3,609 blood donations collected from regions above and below the Alps were anti-HEV IgG positive. From each region ≥ 200 samples were included in the analysis and the seroprevalence from these regions ranged considerably between 13% and 34%. A steadily increase in the HEV seroprevalence of donors with age (~7.7%; 18 - 22 years until ~33.9%; 63 - 70 years) was observed. There was no difference between male and female donors.

Summary/Conclusions: At around 20% the current average seroprevalence of HEV in Swiss blood donors is similar to those reported in other middle European countries. This seroprevalence is not constant over the whole of the country but there is a distinct variation, ranging from 13 to 34%, between different regions within Switzerland. Since the seroprevalence increases with the age, new infectious donors must be entering the blood donor pool. These donations pose a threat to high risk donors, such as immunecompromised patients. The data generated in this study were presented to a national working party consisting of hepatologists, blood transfusion and infectious disease specialists that was setup to establish concept for preventing HEV transmission to high risk individuals.

Abstract No. 6

Authors:
Martin Stolz, Peter Gowland, Stefan Pfister, Christoph Niederhauser

Affiliation:
Interregionale Blutspende SRK AG, Murtenstrasse 133, 3008 Bern, Switzerland

Title:
TWENTY MONTHS EXPERIENCE WITH THE COBAS® MPX TEST ON THE COBAS® 8800 SYSTEM IN A ROUTINE SCREENING SETTING

Text:
Previously presented at 34th Int. Congress ISBT, Dubai, UAE, 3 - 8 September 2016 (P265)

Introduction: In January 2015 the cobas® MPX test using the cobas® 8800 system replaced the Procleix® Ultrio assay on the Procleix® Tigris system. The new platform is used for the routine screening of Swiss blood donors for HIV-1, HIV-2, HCV and HBV in an individual donation format (ID-NAT).

Methods: For every blood donation a dedicated 9 mL EDTA plasma tube is taken for the screening with the cobas® MPX test. The samples are tested in an ID-NAT format according to the instructions in the package insert. Confirmation of repeat reactive samples was performed serologically (Abbott Architect) and with an alternative NAT assay (Abbott Real Time PCR Assay).

Results: In total 276,237 donations were tested since January 2015. 483 donations (0.17 %) were reactive in the initial screen: 257 HIV (53%), 140 HCV (29%), 86 HBV (18%). From these, 35 samples (0.01%) were repeat reactive (5 HIV, 11 HCV, 19 HBV) and were confirmed serologically and with another NAT assay. The remaining 448 samples were false positive. Five donations remained inconclusive. 11,120 sample results were invalid (4.0%), the majority (7,622 samples, 2.8%) due to invalid runs (150 from a total of 5,832 runs). Of the remaining invalid samples 3,498 (1.3%) had an invalid test result. Most of these (88.2%) were due to an impaired amplification, 8.8% detected clot and 2.9% various other reasons. 371 of these samples were invalid in repeat runs (3.4%). The invalid runs were void for the following reasons: 68 invalid run controls (45.3%), 34 hardware failures (22.7%), 20 clotting events (13.3%), 15 reagent dispensing problems (10%) and 6 other technical reasons (4.0%). In May 2016 the number of invalid samples was reduced significantly with the introducing of a further centrifugation step prior to the robotic handling of the primary tubes. Since this modification the invalid rate dropped from 1.1% down to 0.29.

Conclusions: The performance of the cobas® MPX test compares with the data provided by the manufacturer and was supported by our own validation data. The rate of initial positives observed, 0.17% with the cobas® MPX test, is higher compared to our previous Procleix® Tigris system (0.11%). In our setting the cobas MPX test has a repeat reactivity rate of 1:13 to 1:14 and a specificity of > 99.83%. Most of the initial reactive samples could not be confirmed and are regarded as “false” positive results. The number of invalid samples is similar to that observed with the former NAT system (4.2% Procleix® Tigris vs. 4.0% cobas® 8800). The majority of the invalid test results were due to an impaired amplification profile and not caused by inhibition events, since dilution steps remain mostly inef-

fective. The introduction further centrifugation of the primary tubes prior to the robotic handling solved most of the underlying problems and improved the invalid rate to 0.29%. The stability of the cobas® 8800 system (2.8%) has surpassed the performance of the Procleix® TIGRIS system (3.6%), which is impressive for such a complex instrument. Although 69% of the invalid results are due primarily to failed test runs, only 22.7% are actually caused by hardware events. Most of the failed runs are caused by invalid run controls mainly the MPX2 (+) C. Improvements in the test algorithm will reduce the number of initially invalid samples due to run controls.

Abstract No. 7

Authors:
Viktoria Reiher, Joerg Sigle, Andreas Holbro, Jakob Passweg, Andreas Buser, Laura Infanti

Affiliation:
Blood Donation Center Basel, Swiss Red Cross, Hebelstrasse 10, 4031 Basel

Title:
PREVALENCE AND SPECIFICITY OF HUMAN PLATELET ANTIGEN (HPA) ANTIBODIES IN HEALTHY PLATELET DONORS

Text:
Background and Objectives: While the occurrence of HLA-antibodies has been described also in males and in females without history of pregnancies, no studies focused on the immunization to human platelet antigens (HPA) in healthy subjects. In particular, the presence of HPA-antibodies in platelet donors and the association of HPA-antibodies in platelet components (PC) with transfusion complications have been scarcely explored.
Study Design and Methods: Serum samples of 161 regular platelet donors of the Blood Donation Center stored between August 1st and December 31st 2009 were tested for HPA-antibodies with a bead-based assay (Pak LxAssay) and evaluated with the Luminex. Donors were part of a cohort of subjects previously evaluated for HLA-antibodies. Donor data (demographics, prior immunizing events, presence of HLA-antibodies) and transfusion reactions in recipients of PC transfused between January 1st 2002 and December 31st 2015 were retrospectively evaluated for correlations with the presence of donor HPA-antibodies.
Results: Positive reactions for HPA-antibodies were found in 13 of 161 tested sera (8%), and were more frequent in subjects with previously detected HLA-antibodies (6 cases; $p = 0.086$). In 8 cases (3 males, 5 females) there was no recognizable prior immunizing event. In all cases the pattern of reactions to platelet glycoproteins (GPs) was indeterminate (anti-Ia/IIa in 5 cases, anti-IIb/IIIa in 4 cases, anti-Ib/IX in 3 cases, anti IIb/IIIa and anti-GP1a/IIa in one case). No increased incidence of transfusion reactions was observed in recipients of PC of anti-HPA positive blood donors compared to those of anti-HPA negative donors (0.7% vs. 1.4%; $p = 0.741$).
Conclusions: Positive reactions in HPA-antibody testing can occur in healthy platelet donors, even without known prior immunizing events and are often associated with HLA-immunization. There was no correlation between positive reactions for HPA-antibodies and donor characteristics such as gender, age or occurrence or type of previous immunizing event. HPA-antibodies in the donors were not associated with a higher risk of transfusion reactions in the recipients of PCs. The clinical significance of these findings both for the blood donors and the recipients of PC remains unclear.

Abstract No. 9

Authors:
D. Wandel, C. Steuer, J.-P. Sigle, L. Bernasconi, R. Egger

Affiliation:
Kantonsspital Aarau, Spitalpharmazie, Tellstr. 25, 5001 Aarau, Switzerland

Title:
TARGETED METABOLOMICS FOR STABILITY TESTING IN AUTOLOGOUS SERUM EYE DROPS AFTER STORAGE OF 9 MONTHS

Text:
Background: Autologous serum eye drops (ASED) are often used for the treatment of dry eye syndrome. The use of ASED comes with uncertainties regarding serum storage times longer than 1 month and any dilution factor.
Purpose: To assess the stability of metabolites in ASED under storage at -20°C up to 9 months and potentially reduce the frequency of blood donations from patients and the logistics involved.
Material and Methods: 450 mL of autologous whole blood was stored for 2 hours at 20 - 25°C and 12 hours at 2 - 6°C. Coagulated whole blood was separated by two sequential 15 minutes centrifugation cycles (6407 x g at 16°C and 3876 x g at 4°C) and manual compression. Unit-dose ASED (1.5 mL) were prepared and stored at -20°C for 9 months. Concentration changes of 76 phosphatidylcholines (PC), 14 sphingolipids (SL), 14 lysophosphatidylcholines (LPC), 21 amino acids (AA), and the combined hexoses were determined on days 0, 28, 81, and 273 by LC-MS/MS using the Absolute/DQ®-p180-Kit (Biocrates Life Sciences) and compared to those in tears of the same person.
Results: Most PC showed a less than 2-fold increase, while PC C30:1/C38:1/C38:2 increased by 5 - 6 fold with most of the increase occurring between days 81 and 273 of storage. The concentrations of all sphingolipids increased by 30% to 80% with most of the change occurring within the first 81 days. On the other hand, the concentrations of

all LPC decreased within 28 days by 50% - 75%. Also primarily within the first month, we observed reductions of AA between 10% (phenylalanine) and 80% (methionine, ornithine). By the end of the test period, the level of hexoses only decreased minimally by about 15%. When comparing to tears, we found that the concentration of hexoses in ASED was similar, while those of all other metabolites, even those that declined, were 10- to 50- fold higher than in tears.
Conclusions: We observed a shift from LPC to PC most likely through the presence of LPC-acyltransferases. The increase in SL may be linked to the release of sphingosine-1-phosphate from platelets during blood clotting. After 9 months, the LPC levels exceeded those in tears by at least 5-fold. The finding that the metabolite concentration in serum is a multitude higher than in tears justifies to dilute the serum 5- fold with a buffered electrolyte/glucose solution to generate ASED. This data also support a storage time of 9 months at -20 °C and thus a less frequent blood donation schedule is possible.

Abstract No. 10

Authors:
Vildana Pehlic, Syzane Rexhepi, Andreas Holbro, Andreas Buser, Laura Infanti

Affiliation:
Blutspendezentrum beider Basel, Hebelstrasse 10, 4031 Basel, Switzerland

Title:
DISCARD OF BLOOD PRODUCTS DUE TO DONOR HYPERFERRITINEMIA AT THE BLOOD DONATION CENTER OF BASEL

Text:
Introduction: Hyperferritinemia is frequent in the general population and in healthy blood donors. Elevated serum ferritin (SF) levels not associated with hereditary hemochromatosis (HH) are mostly due to metabolic causes (overweight, liver steatosis) and do not lead to organ iron overload. According to the Swiss regulations, subjects with HH are accepted as blood donors if their SF values are normal (< 300 ng/mL for males, < 200 ng/mL for females), otherwise blood products must be discarded. No specific regulation is provided for non HH-hyperferritinemia, and uncertainty how to deal with blood components arises in cases with no clear diagnosis. At our Blood Donation Centre, from January 2004 until September 2016, SF values were measured at every blood donation and afterwards in first-time donors and once a year in repeat donors. The number of cases with elevated SF levels and of discarded products due to hyperferritinemia was analyzed.
Methods: SF measurements were available the day following the donation and after processing was completed. Males and females with SF values above the norm who donated whole blood (WB) or 2 red blood cell products (2RBC) were included in the analysis. The number of collected and discarded blood products was analyzed.
Results: From 01.01.2004 up to 01.06.2017 a total of 177,228 WB and 5,327 2RBC donations were performed and 340 subjects with hyperferritinemia were identified: 208 (68.4%) had documented HH, in 55 cases (16%) investigations for HH were negative, and in 77 cases (22.6%) hyperferritinemia remained unclear. The number of RBC and plasma units discarded because of elevated SF levels in the donor was 980 and 816 respectively from 816 WB donations and 82 2RBC apheresis. Assuming that costs for collection, manufacturing and testing are about 200 CHF per unit, the total estimated loss due to blood product discarding in the evaluated period was approximately 360,000 CHF.
Conclusions: Our current policy for handling blood components from donors with hyperferritinemia is meant to warrant quality and safety of blood products. However, published data do not indicate higher infectious risks or lesser quality in components obtained from donors with HH, although evidence is still limited. Furthermore, in cases of non-HH hyperferritinemia, discarding blood products is unnecessary and unethical. Because of these reasons and the considerable financial losses associated with this approach, this procedure may need to be revised.

Abstract No. 11

Authors:
Sonja Sigurdardottir, Stefan Meyer, Claudia Portmann, Beat M. Frey, Christoph Gassner

Affiliation:
Blutspende Zürich, 8952 Zurich-Schlieren, Switzerland

Title:
MNS ANTIGEN MG EXCLUSIVELY APPEARS AS 68C > A MUTANT OF GYPA*02 (N) WITHIN THE ZURICH AREA OF SWITZERLAND

Text:
Background: The human MNSs blood group system is encoded by the genes GYPA and GYPB and is considered as second in complexity to Rh. Mg, encoded by GYPA*11, is a low-frequency antigen located on GYPA. It has repeatedly been described to have a GYP(A-B-A) hybrid structure with a C > A substitution at coding nucleotide (cdnt) 68. Dating back to the 1960ies, Mg had been reported to be found on both GYPA alleles, i.e. M and N, in both cases with virtually undetectable expression of M, or N.
Aims: Mg is very rare, with higher incidences only reported for Swiss and Sicilians, reaching up to one Mg positive individual among 600. A number of seven available GYPA*11 positive cases prompted us to (re)investigate Mg and its molecular background in detail.
Methods: MALDI-TOF MS based blood group MNSs genotyping interrogated cdnt 59C > T of GYPA for MN, and cdnt 143T > C of GYPB for Ss phenotype predictions.

All genotyping results were compared to MNSs phenotypes, obtained by standard-serological methods. All GYP*11 positive samples were identified by original discrepancy of genotype versus existent phenotype, and repetition of genotyping using a commercially available PCR-SSP based method, including testing for GYPA*11 (innotrain GmbH, Kronberg i.T., Germany). All GYPA*11 positive samples and two individuals each of MMSS, MMss, NNSS, and NNss phenotypes were sequenced for GYPA from intron 1 (102 bp), across exon 2 and intron 2 (335 bp).
Results: MALDI-TOF MS based MN genotyping of 11,240 blood donors of the Zurich area in Switzerland delivered seven cases with M + N-serology, but a preliminary GYPA*01/02 (MN) genotype. All genotype repetitions delivered final GYPA*01/11 heterozygous results. Alignments of the investigated sequence did not show any GYPB specific nucleotides on GYPA*11 and exactly corresponded to the GYPA*02 (N) allele, beside its specific cdnt 68C > A point mutation. GYPA*11 allele frequency was calculated to be 0.136%. Consequently, the expected overall frequency of Mg positive individuals is one among 368 in the Zurich area of Switzerland.
Summary: Molecular analysis of seven GYPA*11 (Mg) positive individuals did not deliver any evidences for Mg being encoded by an M-allele. Controversially to reports of Mg, supposedly having a GYP (A-B-A) hybrid structure, results rather suggested presence of a simple point mutation instead. GYP*11, common in Switzerland, seems to be a derivative of GYPA*02 (N) with a simple 1 bp substitution at cdnt 68C > A.

Abstract No. 12

Authors:
Matondo Manzambi Blaise Sumbu [1], Dominique Latinne [2]

Affiliation:
[1] Université de Liège, Faculté de Médecine, Place du 20 Août, 4000 Liège, Belgique
Université de Kinshasa, Faculté de Médecine, Cliniques Universitaires, Banque de sang Kinshasa, République Démocratique du Congo
[2] Service de Biologie Hématologique, Centre de Thérapie Tissulaire et Cellulaire, Université Catholique de Louvain, Bruxelles, Belgique

Title:
FREQUENCIES AND CONSEQUENCES OF THE ANTI-HLA IMMUNIZATIONS: AFTER TRANSFUSION OF LEUKODEPLETED BLOOD PRODUCTS AND TRANSFUSION REACTIONS

Text:
Keywords: HLA, bloods products, HPA, immunization, leucodepletion, transfusion, thrombopenia, Saint-Luc.

Background: In order to estimate the frequency of anti HLA (Human Leucocyt Antigen) immunization in transfusion reactions in the Saint-Luc clinics in Brussels.
Materials and Methods: A retrospective cohort of transfusion reactions was conducted during the period 2010 - 2011. The analysis of transfusion reactions observed repeated the various symptoms and their frequency grouped and classified according to the pathophysiological mechanisms described by MA Popovsky.
Results: The study involved 123 transfusion reactions whose analysis showed the presence of anti HLA antibodies in 42 cases. These reactions were observed in 33 patients 10 to 85 years; they were consecutive transfusion of blood components leukodepleted. 57,024 units of blood were transfused with 39,416 Concentrates of red blood cells, 7,722 Plasma and 9,886 platelet concentrates. A total of 42 anti-HLA Ab was detected in the majority of consecutive cases with transfusion of RBCs 78.6% (33/42). The highest frequency of RT was due to the transfusion of red blood cell concentrates 66.7% (82/123). The overall incidence of transfusion reactions was 2.16 per 1,000 transfusion reactions transfused pockets a frequency of 0.216% (123/57,024). There was a significant association (RR = 1.76, 95% CI [1.2; 2,6]; $p < 0.001$) rates of transfusion reactions according to the transfused blood components. Platelet concentrates were training more reactions than other blood products.
Conclusions: Despite leukoreduction of blood components, an anti-HLA immunization residual risk and transfusion-HPA remains.

Abstract No. 13

Authors:
Suna R., Mihm B., Sigle J.-P.

Affiliation:
Blutspendezentrum SRK Kantonsspital Aarau AG Haus 40, 5001 Aarau, Switzerland

Title:
DTT TREATED CELLS ARE STABLE FOR FOUR WEEKS

Text:
Background: Daratumumab (DARA) is a monoclonal antibody for the treatment of multiple myeloma. Red blood cells (RBC) carry small amounts of CD38 - the target of DARA - on their surface, leading to panreactive panel results in indirect agglutination testing (IAT) of sera from patients treated with DARA. Treatment of RBC with the reducing agent dithiothreitol (DTT) destroys the antigen and prevents binding of DARA [Chapuy et al. (2015) Transfusion]. However, antigens of blood group systems such as Kell and Lutheran are also destroyed. Currently stability of DTT-treated test RBC for IAT at our institution has been validated for 48 hours.

Objectives: To demonstrate that DTT treated RBC from panocel 16 (Immucor, USA) are stable for four weeks after addition of ID-CellStab (Biorad, Switerland).
Methods: ID-CellStab was added to DTT-treated RBC (2% suspension). Antigen profiling and IAT using DARA sera and plasma spiked with alloantibodies was performed at 7, 14, 21 and 28 days after DTT treatment. IAT was performed with Panocell 16 (Immucor) in test tubes and antigen determination with ID-antigen reagents (Biorad).
Results: Antigen profiles (D, C, c, E, e, Cw, Jka, Jkb, Lea, Leb, P1, M, N, S, s) of DTT-treated RBC corresponded to the profiles obtained prior to DTT treatment up to day 28. With regard to Fy antigens, we observed an attenuation of antigen expression right after DTT treatment in some panel RBC; however during the remaining course Fy antigen expression remained stable up to day 28. Anti-C, anti-S, anti-Jka, anti-Jkb, anti-Fya, anti-Fyb antibodies in DARA sera and plasma were detectable on day 7, 14, 21 and 28 in IAT with DTT-treated RBC. DARA sera without antibodies show no reactions with DTT treated RBC in IAT.
Conclusions: DTT-treated test cells treated with ID-CellStab are stable until day 28 with regard to antigen expression and antibody detection in IAT. Due to the stability DTT-treated RBC handling and evaluation of DARA samples in routine laboratory practice can proceed faster.

Abstract No. 14

Authors:
Young-Lan Song, Charlotte Engström,
Adriana Komarek, Nadine Trost, Pietro Peghini,
Inga Hegemann, Sofia Lejon Crottet,
Christoph Gassner, Beat M. Frey

Affiliation:
Blutspende Zürich, Rütistrasse 19, 8952 Schlieren, Switzerland

Title:
CASE REPORT: RARE ANTIBODY AGAINST HIGH FREQUENCY FY5

Text:
Introduction: The Duffy (FY) system consists of 6 antigens. Fy3 and Fy5 are expressed on red blood cells (RBC) of all Fy phenotypes apart from Fy (a-b-), whereas Fy5 is not present on Rhnull cells even if there is a FY gene. This fact suggests that Fy5 is a composite antigen of FY and RH. Both Fy3 and Fy5 are protease-resistant. Anti-Fy5 has been associated to delayed hemolytic transfusion reaction. We report a case of anti-Fy5 made by a 24-year-old African, gravida 2, para 0, with sickle cell disease. The patient presented Fy (a-b-) phenotype with homozygous c.1-67T > C mutation of FY*B alleles. Before, she received multiple transfusions of Fyb positive RBC uneventfully except forming anti-E.
Methods: Standard serological methods for antibody detection and specification were used (gel-card and tube test; BioRad, CH). KEL-, JK-, FY- and MNSs-systems were analyzed by molecular typing (inno-train GmbH, D). Monocyte monolayer assay (MMA) was carried out for assessing the likely clinical significance of RBC antibodies. Paternal serological typing was performed (Erytra®, Grifols, CH) in order to predict the antigen profile of the fetus.
Results The indirect antiglobulin test showed weakly reactive anti-Fy5, which was positive with all test cells, including papainized cells, except Fy (a-b-) and Rhnull Fy (a+b+) cells. The patient's predicted phenotype was R0r, K-k+, Fy (a-b-), Jk (a-b+). Paternal typing revealed the phenotype Fy (a-b+), Jk (a+b-). The MMA of anti-Fy5 on 0rr, Fy (a-b+) RBC showed 0.7% reactive monocytes. There are 4 C-, E-, Fya-, Fyb-, Jka- blood group 0 donors in Switzerland. In total 6 compatible RBC products were transfused during pregnancy and further 2 perioperative when a semi-elective cesarean section was performed because of looming cardiac decompensation in 34th week of pregnancy. The phenotype of the newborn was R0r, K-, Fy (a-b-). Therefore the father must be most likely heterozygous for the c.1-67 T > C mutation of FY*B. Hence, the newborn presented a negative DAT and no signs of HDFN (hemolytic disease of the fetus and newborn).
Conclusions: So far, few cases with anti-Fy5 are reported. In our case, the request of compatible RBC during pregnancy and for cesarean section was challenging. Based on *in vitro* data (MMA) we recommend transfusing Fyb positive RBC if Fy (a-b-) RBC are not available. By doing so, one has to keep in mind the potential risk of delayed hemolysis due to boosting anti-Fy5. By the postnatal results it is impossible to make any point about potential HDFN of anti-Fy5.

Abstract No. 15

Authors:
Young-Lan Song, Stephanie Glaus,
Sonja Sirgurdadottir, Christoph Gassner, Beat M. Frey,
Charlotte Engström

Affiliation:
Blutspende Zürich, Rütistrasse 19, 8952 Schlieren, Switzerland

Title:
AUTO-ANTI-LWA MAY MIMIC ANTI-D AT FIRST GLANCE

Text:
Introduction: Although LW and D antigens are located on different proteins, LW glycoprotein requires an interaction with Rh proteins to exhibit its expression. RhD+ cells express LWa more strongly than RhD- cells. Hence, weak anti-LW may be mistaken for auto- or allo-anti-D. Fortunately, and in contrast to Rh antigens, LW antigens are denatured when treated with dithiothreitol (DTT). We report a case of a 61-year-old female patient with auto-

anti-LWa-antibodies, in need of several peri- and post-operative transfusions, due to a septical infection after implantation and multiple revisions of a knee replacement surgery. Previously, she had received multiple RhD+ transfusions and already formed an anti-E.
Methods: Standard serological techniques for antibody detection and differentiation were applied (gel-card and tube test; BioRad, Cressier, CH). Rhesus pheno- and genotype were analyzed serologically (Erytra®, Grifols, Duedingen, CH) and molecularly using PCR-SSP (inno-train GmbH, Kronberg i. T., D). Additionally, molecular LW was confirmed by PCR-SSP (in-house). Amplicons for RHD sequencing were prepared in house and analyzed externally (Microsynth, Balgach, CH).
Results: Serological analysis showed an 0R1r, K- phenotype. Antibody diagnosis showed an antibody, weakly reactive with all RhD+ RBC in indirect antiglobulin test and on papainized cells. Direct antiglobulin test and autocontrol were positive. Sequencing of the RHD gene confirmed the absence of any mutation possibly explaining the presence of an allo-anti-D. However, there were no reactions with DTT-treated RhD+ and RhD- RBC which confirmed specificity of an anti-LW. In addition, genotyping confirmed the predicted phenotype LW (a+b-), suggesting the diagnosis of an auto-anti-LWa.
Conclusions: Before all diagnostic procedures were finalized, patient was transfused with 16 0rr RBC products. Due to the overall outcome, still presenting auto-anti-LWa, but not showing any hemolysis, we recommended to continue transfusions with RhD+ blood of compatible phenotype (R1r or R1R1). This specifically, since autoantibodies are considered irrelevant for transfusion management. Our approach was proven right, upon resurgery after 3 months and transfusion of 9 0R1r RBC products, followed by 4 0R1r RBC products another two months later, both times without any signs of hemolytic transfusion reactions. Besides, in our case all crossmatches were consistently negative, but it has to be kept in mind, that auto-anti-LWa may cause positive crossmatches with RhD+ RBC.

Abstract No. 16

Authors:
Anna Erickson, Mary Ann Schott, Blake Warbington, Garrett Villegas, Debbie Hanson, Nina Mufti

Affiliation:
Cerus Corporation, 2550 Stanwell Drive, 94520 Concord, CA, USA

Title:
ROBUSTNESS OF THE INTERCEPT BLOOD SYSTEM FOR RED BLOOD CELLS

Text:
Background: The INTERCEPT™ Blood System for Red Blood Cells (RBCs) uses amustaline (S-303) to form adducts with nucleic acids to prevent replication of contaminating pathogens and leukocytes in RBC components. The proposed EU input specifications for the pathogen inactivation process of RBC (PI RBC) are 220 - 360 mL of SAG-M RBC in ACD or CPD, meeting the EDQM requirements for Hemoglobin (Hb) and Hematocrit (Hct) for RBCs in additive solution (AS). Within 48 hours of collection RBCs are added to glutathione (GSH) - in processing solution followed by S-303 addition (20 mM GSH/0.2 mM S-303, based on 280 mL RBC input). After 18 - 24 hours hold at 20 - 25°C, centrifugation and supernatant replacement with SAG-M, PI RBCs can be stored for up to 35 days from collection. *In vitro* function of PI RBC prepared within and outside the range of input specifications, and critical processing parameters were evaluated.
Methods: Whole blood (WB) from apheresis or CPD collections was held at 1 - 6°C or room temperature prior to separation. Input RBCs (n = 160) varied by AS (AS-5 and SAG-M), age, Hb content, Hct, and leukocyte content. Inputs contained 38 - 72g Hb in 216 - 361mL, 57-69% Hct and < 1 × 106 - 2.5 × 109 leukocytes/unit; age range was 1 - 3 days post-collection. Processing parameter variables were GSH and S-303 concentrations, PI hold time, temperature. Input RBCs in AS treated with GSH and S-303 (18 - 29 mM GSH/0.20 - 0.25 mM S-303 final) were held for 16 - 28 hours at 16 - 27°C before centrifugation and replacement of supernatant with SAG-M. PI RBCs stored 35 days at 2 - 6°C were analyzed for *in vitro* parameters and conformity to the EDQM quality requirements for leukoreduced (LR RBCs) in AS, or RBCs in AS after 35 day storage.
Results: All but one unit had Hb values of ≥ 40g [38 - 72g]; the PI RBCs with Hb < 40 g, had an input Hb content of 38 g prior to treatment. The final Hct was 54 - 69%, meeting the 50 - 70% criterion. All PI RBCs stored for 35 days met the hemolysis acceptance criteria of ≤ 0.8% [0.1 - 0.7%]. ATP was > 2µmol/g Hb [2.4 - 5.].
Summary/Conclusions: Robustness of the INTERCEPT treatment was demonstrated within and outside the proposed range of input RBCs and processing conditions. PI RBCs met EDQM guidelines (18th Ed.) for LR-RBCs in AS and show suitability for transfusion. The INTERCEPT Blood System for RBCs is not approved for use.

Abstract No. 18

Authors:
Felicia Santa Maria, Andrew Laughhunn, Peter Bringmann, Adonis Stassinopoulos

Affiliation:
Cerus Corporation, 2550 Stanwell Drive, 94520 Concord, CA, USA

Title:
EFFECTIVE INACTIVATION OF THE NON-ENVELOPED FELINE CALICIVIRUS BY AMUSTALINE/GSH IN RED BLOOD CELLS

Text:

Background: The INTERCEPT™ Blood System for Red Blood Cells (RBC) uses the small molecule amustaline to form covalent crosslinks within double-stranded nucleic acids of leukocytes and contaminating pathogens to prevent replication. The process includes addition of 0.2 mM amustaline and 20 mM glutathione (GSH) and an 18 - 24 hours incubation at RT. Although inactivation is complete after 3 hours, the additional incubation ensures complete decomposition of amustaline. A final centrifugation and exchange of the supernatant with additive solution (SAG-M) provides pathogen-reduced RBC for transfusion. The objective of this study was to evaluate the inactivation of feline calicivirus (FCV) by the INTERCEPT Blood System 1 for RBC prepared in Optisol (AS-5). FCV is a positive-sense, single-stranded RNA virus in the genus Vesivirus within the family Caliciviridae and serves as a model for small, non-enveloped viruses, such as hepatitis E virus.
Methods: For each experiment, a single RBC unit was spiked with FCV to a final concentration of ~ 108.0 pfu/mL and treated with amustaline. The spiked RBC units were mixed with GSH and control samples (Control T = 0) were taken to determine pre-amustaline titers. Each unit was then dosed with amustaline and test samples (Test T = 3) were removed to determine levels of inactivation. Control and test samples were serially diluted and inoculated onto CrFK cells. The plates were incubated for 3 days at 37°C, stained with crystal violet and the plaques enumerated. Log reduction was calculated as the difference between the mean titer in pre-amustaline samples and the mean titer in the 3 hour post-amustaline samples.
Results: Robust inactivation of FCV was achieved as summarized in Table 1.
Conclusions: The non-enveloped feline calicivirus was inactivated to the limit of detection in RBC after treatment with GSH and amustaline. Inactivation of > 6.8 log of FCV was achieved in the CrFK infectivity model.

1 The INTERCEPT Blood System for RBC is not approved for use

Table 1. Feline Calicivirus Inactivation in Red Blood Cells

Replicate	Log Titers (pfu/mL)		Log Reduction per mL
	Control T = 0	Test T = 3	
1	5.8	< -1.0	> 6.8
2	5.9	< -1.0	> 6.9
3	5.7	< -1.0	> 6.7
4	5.9	< -1.0	> 6.9
Mean ± SD	5.8 ± 0.1	< -1.0 ± 0.0	> 6.8 ± 0.1

Abstract No. 19

Authors:
Travis Berry, Patricia Schmidt, Neema Lakshman, Mellissa von Goetz, Anne North, Nina Mufti, Larry Corash

Affiliation:
Cerus Corporation, 2550 Stanwell Drive, 94520 Concord, CA, USA

Title:
RETENTION OF *IN VITRO* HEMOSTATIC CAPACITY OF PATHOGEN REDUCED CRYOPRECIPITATE 5 DAYS POST-THAW

Text:

Background: Cryoprecipitate is formed when frozen plasma is thawed at 2 - 6°C and is enriched for fibrinogen (FB), Factor VIII (FVIII), von Willebrand Factor (vWF), Factor XIII (FXIII), and fibronectin. It is increasingly adopted as a concentrated source of FB in severely bleeding trauma patients in the United States and in some European countries (e.g. UK). Conventional practice involves pooling individual FFP cryo units, which increases the risk of pathogen contamination and transmission thus the limited post-thaw shelf-life of 4 hours. The INTERCEPT™ Blood System enables the treatment of single donor apheresis or pooled whole blood derived (WBD) plasma prepared as Fresh Frozen Plasma (FFP) or frozen within 24 hours (PF24). We developed pathogen reduced (PR) cryo derived from FFP and PF24 with 5 day stability at 22°C.
Methods: Six replicates of type-matched pools of whole blood derived (WBD) and Apheresis (Aph) plasma were split to produce conventional control (225 ± 10 mL) and test components (625 mL ± 25 mL). Test components were PR with amotosalen and UVA light. WBD and Aph FFP were produced by freezing plasma within 8 hours and WBD PF24 within 24 hours. Cryo separated from plasma was frozen at -30°C (Test 62 ± 2 mL, Control 22 ± 2 mL). Test and Control Cryo were thawed at 37°C and characterized immediately post-thaw (T = 0), and after 5 day storage at 22°C, and tested for FB and FVIII function, thromboelastography (ROTEM) and thrombin generation (CAT).
Results: The data for Aph and WBD PF24 cryo is shown in the table below. PR cryo retained sufficient FB and FVIII activity post-thaw and over 5 day at 22°C for hemostatic capacity. ROTEM (EXTEM) showed retention of fibrin formation (α angle) and clot quality (MCF). Thrombin generation was robust despite lower FVIII levels. These parameters were maintained through 5 day storage at 22°C.
Conclusions: PR cryo can be processed from WBD or Aph plasma and stored at room temperature for 5 days. PR plasma provides adequate levels of FB with hemostatic capacity equivalent to control as demonstrated by ROTEM and CAT. Use of PF24 with stability over 5 days can increase the availability of cryo with a reduced risk of transfusion-transmitted infection.

	Apheresis Cryo				WBD PF24 Cryo			
	Test		Control		Test		Control	
	T=0	T=5d	T=0	T=5d	T=0	T=5d	T=0	T=5d
Fibrinogen (mg)	954± 94	1026± 143	406± 106	445± 84	728 ± 113	735 ± 138	333 ± 72	335 ± 68
Factor VIII (IU)	237± 63	212 ± 56	129 ± 32	92± 30	218 ± 32	206 ± 46	167 ± 43	127 ± 26
Peak thrombin (nM)	232 ± 17	236 ± 9	273 ± 15	261 ± 8	167±14.1	180±18	193±18	192±26
ETP (nM)	2,238 ± 175	2,267 ± 89	2,395 ± 130	2,388 ± 201	1,615±119	1,657.7±2 60	1,8125±1 27	1,760±176
MCF (mm)	75 ± 6	78 ± 6	87 ± 5	109±4	69±5	73±67	74±5	79±6
α angle (°)	86 ± 1	85± 2	87 ± 1	86±0.4	85±1	85±1	86±1	80±0.7
A 10 (mm)	71 ± 4	75 ± 6	83 ± 6	84±6	68±4	70±6	71±5	72±7

Abstract No. 20

Authors:
Dania Fischer, Julian Büssow, Patrick Meybohm, Christian Friedrich Weber et al.

Affiliation:
Klinik für Anästhesiologie, Intensivmedizin und Schmerztherapie, Universitätsklinikum Frankfurt am Main, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

Title:
MICROPARTICLES FROM STORED RED BLOOD CELLS ENHANCE PROCOAGULANT AND PROINFLAMMATORY ACTIVITY

Text:
Background: The pathomechanisms of morbidity due to blood transfusions are not yet entirely understood. Elevated levels of red-cell derived microparticles (RMPs) are found in coagulation-related pathologies and also in stored blood. Previous research has shown that RMPs mediate transfusion-related complications by the intrinsic pathway. We hypothesized that RMPs might play a role in post-transfusion thrombotic complications by enhancing procoagulant activity also via the extrinsic pathway of coagulation.
Study Design and Methods: In this laboratory study, blood from 18 healthy volunteers was stimulated with microparticles from expired stored red blood cells. Various clotting parameters were recorded. Flow cytometry, ELISA, and real-time PCR were used to investigate possible mediating mechanisms.
Results: The addition of RMPs shortened the clotting time from 194 to 161 seconds ($p < 0.001$). After incubation with RMPs, there was an increased expression of TF on monocytes and in the plasma. TF mRNA expression increased in a time-dependent and concentration-dependent manner. There was a significant induction of IL-1β and IL-6. After stimulation with RMPs, there was a significant increase in the number of activated platelets, an increased percentage of PAC-1/CD62P double-positive platelets, and an increased number of platelet-neutrophil duplets and platelet-monocyte duplets, indicating enhanced interaction of platelets with neutrophils and monocytes. CXCL-8 and IL-6 were both significantly higher following RMP treatment.
Conclusions: Our results imply that RMPs trigger coagulation via TF signaling, induce secretion of proinflammatory cytokines, and induce cell-cell interaction between platelets and neutrophils. Thus, under certain conditions, RMPs could play a role in post-transfusion complications, via these mechanisms.

Abstract No. 21

Authors:
Adrian Bachofner [1, 2], Tanja Rüfli [2], Andreas Holbro [1, 2], Hein Hustinx [3], Sofia Lejon Crottet [3], Andreas Buser [1, 2], Laura Infanti [1, 2]

Affiliation:
[1] Regional Blood Transfusion Center, Swiss Red Cross, Basel, Switzerland
[2] Division of Hematology, University Hospital Basel, Switzerland
[3] Swiss Immunohematology Reference Laboratory, Interregional Blood Transfusion, Swiss Red Cross, Switzerland

Klinik Hämatologie Unispital Basel, Petersgraben 4, 4031 Basel, Switzerland

Title:
PECULIAR CASE OF A TRANSFUSION REACTION SHORTLY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

Text:
Herein we describe a case of febrile non-hemolytic reaction (FNHTR) in a 64-year-old male 20 minutes after the transfusion of one red blood cell (RBC) unit. Twenty days before he had undergone an allogeneic hematopoietic stem cell transplantation (HSCT) from an unrelated donor with minor ABO-disparity. The patient had been treated for plasma cell myeloma with multiple transfusions in the past, but no transfusion reactions or alloimmunization had been reported.
In the work-up of the transfusion reaction, the antibody screening and the direct antiglobulin test (DAT) before and after transfusion were negative. However, both the crossmatch (indirect antiglobulin test) performed on a pre-transfusion and post-transfusion sample showed incompatibility with the RBC unit administered, suggesting the presence of an alloantibody against a low-prevalence RBC antigen in the patient's serum. A post-transfusion serum sample of the patient and a sample of the involved RBC unit were sent to the Swiss immunohematology reference laboratory for further investigation.
An alloantibody directed against the low-prevalence antigen Dia of the Diego Blood group system was identified and the Dia antigen was detected on the donor RBCs. Furthermore, the monocyte monolayer assay (MMA) result showed 46% of reactive monocytes, suggested a clinical

significance of the alloantibody (> 5% is considered as clinically significant).
Dia antigens are expressed in 0.1% of Caucasians and anti-Dia alloantibodies escape antibody screening, since no Dia-positive RBC are included in the screening cells used for routine testing in Switzerland. The blood donor of the involved RBC unit was a 21-year old Swiss male. Provoking mild clinical manifestations in our case, the anti-Dia alloantibodies are also known to cause severe hemolytic reactions.
FNHTR are frequently observed in patients transfused for hematologic malignancies and after HSCT, although in this setting fever can have several causes. The actual rate of alloimmunization in HSCT is not known, but alloantibodies can be developed despite immunosuppression. In our case, the anti-Dia antibody is the most probable, although uncommon, cause of a FNHTR. However, the mild transfusion reaction could be due to the immunesuppression given to the patient. Even though the Dia positivity in Switzerland is low, the cause of the immunization is still most likely due to a previous transfusion with a Dia positive RBC unit.

Abstract No. 22

Authors:
Charlotte Engström, Stefan Meyer, Young-Lan Song, Adriana Komarek, Alix O'Meara, Claudia Papet, Kathrin Neuschwander, Christoph Gassner, Beat M. Frey

Affiliation:
Blutspende Zürich, Rütistrasse 19, 8952 Schlieren, Switzerland

Title:
TRANSFUSION IN A RARE CASE OF PARA-BOMBAY PHENOTYPE

Text:
Background: Bombay phenotype is characterized by the absence of ABH blood group antigens both on the surface of red blood cells (RBC) and in secretions resulting from silenced mutations in FUT1 (h/h) and FUT2 (se/se) genes. In contrast, para-Bombay phenotype retains some H antigen on RBC either induced from a weakly active (H+ weak/H+weak) or completely silenced FUT1 (h/h). The latter is mandatory linked with an active FUT2 (Se/Se or Se/se). The anti-H in para-Bombay individuals is usually weak and often does not react above 20°C.
Aims: A blood sample from a 61-year-old Thai female with metastatic pancreatic cancer was referred to our laboratory for ABO grouping. We describe the serological and genetic work-up which revealed AB para-Bombay phenotype and subsequent transfusion management.
Methods: Standard serologic techniques were used to detect ABH on RBC (BioRad; Biotest, CH). In addition, a very potent anti-AB serum (Grifols, CH) was used to reveal traces of A and B. Compatibility testing was performed using the indirect antiglobulin test (IAGT) at 37°C. Molecular ABO type was defined using a commercially available test kit (inno-train, D). Sequencing was performed for FUT1 and FUT2.
Results: The routine anti-A, -B and -AB failed to detect the respective antigens and, most notably, no H antigen was traceable. The RBC showed only weak agglutination with the potent anti-AB serum. Only anti-H, but no anti-A or anti-B, was identified in the serum. Initial ABO genotyping by PCR-SSP resulted in AB genotype. To confirm serological H-deficient phenotype sequencing of FUT1 and FUT2 was performed and revealed an active secretor status but homozygosity for the FUT1*01W.09 allele. Latter allows only weak expression of ABH-antigen on RBC.
Summary: In summary, our serological tests were in line with para-Bombay phenotype and confirmed by identification of the homozygous weakening mutation c.658C > T in FUT1. However, if low level of ABH-antigens on erythrocytes is determined by partially active FUT1 or normal secretor status is a matter of debate. Shortly after final diagnostics our patient required transfusion. As we have no access to Bombay or para-Bombay blood in an emergency situation one A1B whole blood unit with negative crossmatch was transfused uneventfully and short-term stabilization was achieved. Due to the malignant disease she died shortly thereafter. In conclusion, we support to transfuse para-Bombay individuals with blood units, compatible by IAGT, when Bombay or para-Bombay blood is not available.

Abstract No. 23

Authors:
Matondo Manzambi Blaise Sumbu [1], Benjamin Longo-Mbenza [1, 2], Steve Ahuka-Mundeke [1, 3], Jérémie Muwonga Masidi [1], Mireille Nganga Nkanga [1], Georges Mvumbi Lelo [1], Donatien Kayembe Nzongola Nkasu [1], Ferdinand Mbayo Kalumbu [1]

Affiliation:
[1] Faculty of Medicine, University of Kinshasa, Kinshasa, DR Congo
[2] Walter Sisulu University, Mthatha, South Africa
[3] National Institute of Biomedical Research (INRB), Kinshasa, DR Congo

Title:
PROFILE OF ABO AND RHESUS (D) BLOOD GROUPS IN BLOOD DONORS AT THE UNIVERSITY CLINICS OF KINSHASA: 2009 - 2013

Text:
Keywords: Blood, group, rhesus, clinics, University, Kinshasa, donor

Background: Blood donation is a response to salve human lives during transfusion. Indeed, this therapy requires compatibility between donor's blood (DB) and that from receiver (BR) as well as ABO and Rhesus (Rh) isogroups because the presence of immunogenic antigen (Ag).
Objective: This study aims to describe the patherns of blood groups ABO and Rh D among Bantu adults.
Methods: This secondary data analysis was carried out at Kinshasa University Clinics of Kinshasa, DR Congo, between 2009 and 2013. Gender, age, category of donors, ABO and Rh (D) were considered. The determination of blood group ABO and Standard Rh was carried out by the method of Beth - Vincent and of Simonin - Michon.
Results: In total, 10,278 records were observed for blood donors aged 34.2 ± 11.2 years for range from 26 years to 35 years and gender ratio of 3 Men: 1 Woman. Family donors were commoner (77.7% n = 7,988) than voluntary and regular blood donors, 57.1% (n = 5,870), 22.3% (n = 2,290), 18.2% (n = 1,870), and 2.4% (n = 249) presented Blood O, Blood A, Blood B, and Blood AB, respectively. Almost all donors (99.5% n = 10,227) presented positive Rh D versus 0.5% (n = 51) donors with negative Rh D.
Conclusions: The majority of donors were young adults, voluntary and regular, Blood O, and positive Rh D as established among Bantu peoples.

Table: Distribution of blood groups ABO per year of collection of the blood donation

	Blood groups % (n)			
YEARS	AB	B	A	O
2009	2.2 (55)	18.5 (455)	22.3 (549)	56.9 (1403)
2010	2.1 (51)	18.3 (439)	22.2 (531)	57.4 (1372)
2011	2.8 (47)	20.0 (322)	22.3 (376)	55.8 (941)
2012	2.3 (36)	18.4 (290)	22.7 (359)	56.6 (895)
2013	2.8 (60)	16.8 (364)	22.0 (474)	58.3 (1259)

Abstract No. 24

Authors:
Andrew Laughhunn, Shanen Conway, Soraya Amar El Dusouqui, Rudolf Schwabe, Cissé Sow, Philippe Grellier, Nina Mufti, Marion Lanteri, Adonis Stassinopoulos

Affiliation:
Cerus Corporation, 2550 Stanwell Drive, CA 94520 Concord, USA

Title:
INACTIVATION OF DUCK HEPATITIS B VIRUS AND PLASMODIUM FALCIPARUM WITH AMUSTALINE/GSH IN WHOLE BLOOD

Text:
Background: Transfusion-transmitted (TT) diseases are prevalent in developing areas of the world, where it is difficult to routinely produce blood components. This illustrates the importance of establishing a robust whole blood (WB) pathogen inactivation system. Two pathogens of great concern in developing nations are hepatitis B and malaria. Plasmodium falciparum (Pf), an intraerythrocytic protozoan parasite, is accountable for nearly all malaria mortality in Africa. In 2015, WHO reported ~ 212 million new cases worldwide, resulting in > 400,000 deaths. Duck Hepatitis B virus (DHBV) is a small, enveloped, dsDNA virus that can be utilized as a model for human hepatitis B virus (HBV). HBV causes a potentially life-threatening liver infection that can become chronic increasing the risk of death from cirrhosis or liver cancer. These studies evaluated the inactivation of Pf and DHBV in WB in support of the Swiss Red Cross Humanitarian Foundation for WB Pathogen Inactivation for Africa. The approach uses the small molecule amustaline to form covalent adducts and crosslinks within nucleic acids of leukocytes and contaminating pathogens and prevent replication. The process includes addition of 0.2 mM amustaline and 2 mM glutathione (GSH) and a 24 hour room temperature (RT) incubation after which the treated WB unit is suitable for storage for up to 7 days at RT.
Methods: For each experiment (N = 4), a single WB unit was spiked with either DHBV or ring-stage Pf-infected red blood cells (iRBC), and treated with amustaline/GSH. A pre-treatment sample was removed prior amustaline addition to determine. Test samples were removed 24 hours after dosing to determine the levels of inactivation. No residual DHBV or Pf was detected after treatments. Log reduction was calculated as the difference between the log titers in pre-amustaline samples and the log titer 24 hours post-amustaline dosing.
Results: Robust inactivation of both Pf and DHBV in WB was achieved to the limit of detection (LOD), with mean inactivation (N = 4) of > 5.3 log10 for DHBV and > 7.5 log10 for Pf.
Conclusions: DHBV and Pf were inactivated to the LOD in WB after treatment with amustaline/GSH, illustrating that the system has potential to mitigate the risk for heptitis and Pf TT in endemic regions that rely on WB transfusions.
The system for WB is not approved for use.

1. Cerus Corporation, Microbiology Department
2. Accuratus Labs
3. Muséum National d'Histoire Naturelle, UMR 7245 CNRS MCAM; Paris, France
4. Cerus Corporation, Global Scientific Affairs
5. Cerus Corporation, Development
6. Swiss Red Cross

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