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Freie Vorträge
Freier Vortrag 1
A Rare KEL17/IVS3+1g>a Compound Heterozygous Individual, Prone to Anti-KEL11 Immunization
S. Sigurdardottir, C. Engström, B.M. Frey, C. Gassner, Schlieren

Background. The Kell blood group system includes some of the most immunogenic antigens among blood groups known. Among them Kell(KEL1), Kpa(KEL3), and Jsa(KEL6) are well known. The antithetic antigens KEL11/17 further contribute to this list. However, KEL17 is considered as very rare, with an approximate frequency of one KEL17 homozygote among 30'000 Europeans only (Daniels G, Human Blood Groups, 2002). Therefore, anti-KEL11 immunization is rarely observed and may be caused by unusual KEL genotypes, as exemplified here.

Methods. Standard serological methods for antigen- and antibody-detection and specification were used. KEL genotyping was performed using a commercially available test kit “KELplus” (Inno-Train, Kronberg i. T., Germany) and in house KEL11/17 PCR-using Sequence Specific Priming technique (SSP) and KEL gene sequencing.

Results. After standard serological investigation, a 73 year old female presented anti-KEL11 in her serum. Reasoned by the rarity of this observation, molecular confirmation was intended. An in house KEL11/17 PCR-SSP was performed, but resulted in an inexplicable heterozygosity for KEL11/17. Therefore “KELplus” typing was performed and delivered KEL-1,2,-3,4,-6,7 (K, Kpa, Jsa negative), and surprisingly KEL(IVS3+1g>a), for the investigated DNA. Finally, KEL gene sequencing of exons 3 and 8 and adjacent intron sequences confirmed the unusual KEL genotype of the patient: Compound heterozygosity for an expressed KEL1,2,-3,4,-6,7,11,17 and an unexpressed KEL-1,2,-3,4,-6,7,11,-17,(IVS3+1g>a) allele (relevant specificities are displayed in bold and underlined).

Conclusions. KEL(IVS3+1g>a) is the most frequent unexpressed KEL allele, encoding a further exceedingly rare, so called Kell0 phenotype, when present in homozygous, or compound heterozygous form, together with other unexpressed KEL alleles (Koermoecci G et al, Transfusion, 2007). Inherited hemizygously however, unexpressed KEL alleles will allow the second inherited KEL allele to behave as seemingly homozygous, when expressed. Thus explain-
The Luminex method now available provides a better analysis of the anti-HLA situation. This can help to interpret the CDC results particularly in case of positive CDC crossmatches with historical sera in highly immunized patients and to allow transplantation in specific cases.

Freier Vortrag 3
Development and Validation of an In-house High-throughput Blood Group Genotyping System
S. Lejon Crottet, P. Gowland, H. Hustinx, S. Fontana, C. Niederhauser, Bern

Background: Matching blood for patients with irregular antibodies against common or rare blood groups or blood group constellations is demanding. Extended phenotyping of donors is time consuming and expensive, and several serological reagents are rare or not available (e.g. anti-Do(a) and -Do(b)). Our Immunhematology Reference Laboratory runs the rare donor file on behalf of the Swiss BTS SRC and develops strategies for building a broad and more consistent database of broadly typed donors. We have developed and validated an in-house high-throughput blood group genotyping system with electronic data transfer into our and other blood bank databases.

Study design: A specially designed software was used to select donors (criteria: number of donations, blood group, age etc.) and manage the electronic transfer of results. An automated sample preparation and DNA extraction was followed by an in-house multiplex sequence-specific primer PCR (SSP-PCR). The genotyping module includes four multiplexed primer mixes with a total of 22 sequence-specific primer pairs. The SSP-PCR products were analysed by capillary gel electrophoresis and the results were electronically transferred directly to our blood bank database. This system is currently being validated against a series of serologically and/or molecularly typed samples, depending on the availability of reagents. The parameters covered in the validation include sensitivity, specificity, repeatability and robustness.

Results: During the initial validation period three KEL*02 (k) negative, one KEL*04 (Kp(b)) negative, two LU*02 (Lu(b)) negative, three CO*01 (Co(a)) negative and seven YT*01 (Yt(a)) negative alleles were found and all were confirmed serologically. The inclusion of high and low frequency antigens in the search parameters allowed for the identification of donors harbouring rare blood group antigens in conjunction with the broad blood group genotype. After collection of more than 40'000 results in our database, the software showed a correct selection of donors and transfer of results to the blood bank database. We observed a few serological, as well as, molecular inaccuracies, however, the overall concordance remained very high.

Conclusion: This high-throughput in-house blood group genotyping system enables a fast and reliable data collection and transfer of results. Furthermore the method is straightforward and extremely cost effective. Not only are large number of common blood group constellations now available, but also our rare donor file has been significantly extended. The information from this blood group genotyping system permits a rapid crossmatch of compatible blood products for patients.

Freier Vortrag 4
C. Gassner, B.M. Frey, Schlieren, C. Vollmert, Hamburg

Background. MALDI-TOF MS is an accurate, highly automatable and fast technology with the capacity of genotyping more than 150,000 single nucleotide polymorphisms (SNPs) per day. There-fore, genotyping of serologically Dneg, genetically RHD positive individuals, detailing blood donors’ antigenic profiles and screening for blood donors with rare antigenic constellations, summarized as “high-throughput-blood group genotyping (ht-bg-GT), may easily be carried out using this technique.

Methods. A set of SNPs, defining phenotypically relevant polymorphisms of RHD, KEL, JK, FY, MNS and rare alleles such as KEL3, LU1, DI1, DI3, Y2, CO2, KN2, and other (n total = 22), was defined and tested on two selectively compiled and partially genetically pretested donor DNA-panels. SNPs chosen were RHD-specific nucleotides for all 10 exons, and specific nucleotides for categories & partials, weaks, RHDels and unexpressed RHDs (n=29), and RHC, c, e and W (n=5). For KEL, JK, FY and MNS, again, the major alleles and SNPs defining weakly or unexpressed alleles were considered (n=19). Two DNA panels, each including a total of 100 DNAs at a minimum, were genotyped following the standard Sequenom MassARRAY iPLEX® Pro genotyping protocol.

Results. For 10 out of 43 RH specificities tested, no positive control DNAs were included in the tested DNA-panel. However, PCR amplification for those alleles was still carried out in the various multiplex PCRs to emulate realistic amplification conditions. Of the remaining 33 specificities, including specificities for RHC, c, E, e and W, categories & partials, weaks, RHDels and unexpressed RHDs, 5 (e.g. weak RHD type 3), did not give any results, whereas all other (n=28) were correct. Additionally, RHD gene copy numbers could be measured. In KEL, JK, FY and MNS genotyping, of 19 SNPs chosen, 15 could be tested by respective control DNAs and gave correct results for 13 of them. The “rare module” gave correct results for 22 of a total of 24 SNPs tested.

Conclusions. The observed success rate of the MALDI-TOF MS for ht-bg-gt, is highly impressive. The described method is independent of fixed formats like DNA-chips, and users are therefore free to choose and configure modules for their needs. E.g., the presented KEL, JK and FY could represent such a module. This “KEL, JK and FY” and the “RARE module” are currently ready for validation-testing in a serologically predetermined DNA-panel of appropriate size.
Using the air/liquid interface technology, we have developed an in vitro method which closely mimics stem cell differentiation in vivo. Human pluripotent stem cells were initially submitted to neural differentiation on polytetrafluoroethylene membranes floating on appropriate medium. A nervous tissue was generated, including neurogenic niches which produce the mature neural cells including neurons, astrocytes and oligodendrocytes. Its functional properties as well as its high level of cell organization were very similar to human fetal brain, thus providing a new tool for the modeling of human nervous tissues. The introduction of glioblastoma cells in this tissue generated a tumor which closely mimics the in vivo situation, rendering this method very attractive for disease modeling. Because pluripotent stem cells can be potentially differentiated towards blood cells in vitro, we would like to apply this promising system to the modeling of normal and pathological hematopoiesis. However, to achieve this goal, hematopoietic differentiation of pluripotent stem cells still needs to be improved. Based on the known cell diversity of the embryonic structures used to derive pluripotent stem cell lines, we have hypothesized that stem cell cultures could be clonally heterogeneous in their capacity to generate blood cells. We have established by limit dilution methods several human pluripotent stem cell clonal lines and tested them for hematopoietic differentiation using conventional methods. One line was significantly more efficient for hematopoietic differentiation than the others and was further selected. This line will be submitted to hematopoietic differentiation using air/liquid interface system in order to reproduce in vitro a functional hematopoietic niche producing blood cells. As performed for nervous tissue, such a tool is expected to allow modeling of malignant bone marrow diseases (by introduction of malignant cells in the tissue) in order to help understanding pathological blood differentiation.

The combined therapy with Plerixafor and G-CSF has been described in the literature as a successful method in patients with multiple myeloma and lymphoma for mobilization of hematopoietic stem cells. In this retrospective study 13 patients (4 female, 9 male) with a failing stem cell mobilization in the past have been stimulated by a combined therapy of Plerixafor and G-CSF. The demanded cell count ranged between 2,0-4,0x10⁶ according to the patients base disease. Two patients suffered from multiple myeloma and the demanded cell-count was 4,0x10⁶ /kg, 11 patients suffered from lymphoma and the demanded cell-count was 2,0x10⁶/kg. Ten hours from the administration of Plerixafor a stem cell collection with the COBE Spectra Auto-PBSC programme was performed. The predetermined programme data were adapted to the specific patients data. In 77% of the treated patients the mobilization was successful. 8/13 (62%) patients reached the demanded cell count with only 1 collection, 2/13 (15%) needed 2 collections and in 3 patients (23%) a successful collection could not be achieved. This study shows that using Plerixafor with G-CSF in combination with the adapted COBE Spectra Auto-PBSC programme is an effective mobilization and collection method in poor mobilizer.

Chagas disease or South American trypanosomiasis is a parasitic disease caused by Trypanosoma cruzi, endemic in poor rural areas of South and Central America. Infection is usually transmitted from man to man by triatomid bugs, the main insect vectors, but also by blood transfusion, organ transplantation, and congenital infection via the placental route. Migration of population from the endemic area to countries where the disease does not exist may spread the risk of transmission of this disease through blood transfusions. The parasite may be found in the blood of up to 50% of chronic carriers, and asymptomatic Trypanosoma Cruzí positives serving as donors for blood transfusion can be the source of transmission of Chagas disease also in Europe.

A 45 years old regular blood donor of Bolivian origin, already tested for Chagas disease in his country of origin in 2003 (before his immigration in Europe) with negative result, was seen in march 2010 at the transfusion center of HUG, Geneva for his 5th blood donation. During the pre-donation interview he declared to come from a malaria endemic area in Bolivia, and for this reason, the blood was tested for presence of malaria antibodies. Due to indeterminate result of malaria antibodies (ELISA) the sample was sent for confirmation to the Swiss Tropical Institute where the patient was found to be negative for malaria but highly positive for Chagas disease: IFAT 640 react. titre (n.v. below 160), T. cruzi ELISA 1.39 positive. Patient was asymptomatic, but successive investigations before treatment showed ECG abnormalities.

All blood products prepared from this donor were traced; nontransfused products were blocked, recalled and discarded. A look-back procedure was started: 4 units of red cells and 1 unit of fresh-frozen plasma were transfused but no case of transmission of Trypanosoma cruzi infection was found. All blood products were leucodepleted and no platelets were prepared from donor's blood.

A study conducted in Geneva in 2008 (Jackson Y. et al) showed high prevalence (12.8%) of Trypanosoma cruzi infection in 1,012 migrants of South America origin, espe-
cally among Bolivian immigrants (26.2%). The parasite may survive several weeks in blood kept at temperatures 4°C and 22°C and may resist to freezing and thawing, so all labile blood products can transmit the infection.

Chagas disease is not considered a significant public health risk in Switzerland and, for this reason, procedures for donor screening have not been established yet and is not recognized to need compulsory declaration as it is in case of malaria infection. Donors are not questioned on their country of origin in the questionnaire for blood donation.

Preventive strategies should be based on pre-donation risk assessment (donor's country of origin or travelling), implementation of laboratory screening for risk donors followed by permanent exclusion of reactive donors. Pathogen inactivation is the modern possible solution to prevent the risk of transmission of Trypanosoma cruzi infection by blood transfusion.

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**Poster 2**

**Swiss Rare Donor File: a cost-effective strategy for identifying Vel and KEL*04 (Kp(b)) negative donors**

H. Hustinx, E. Münger, S. Fontana, S. Lejon Crottet, P. Golland, C. Niederhauser, Bern

Background: The supply of erythrocyte concentrates for patients with rare blood groups, antibodies against high frequency antigens or multiple antibodies often requires extensive measures. For this purpose, our Immunhematology Reference Laboratory runs the rare donor file on behalf of the Swiss BTS SRC and coordinates efforts with other international partners. Examples of high frequency antigens are KEL*02 (k), KEL*04 (Kp(b)), LU*02 (Lu(b)), Vel, and YT*02 (Yt(a)). KEL*04 (Kp(b)) can be detected with both serologically and molecularly, whereas the molecular background of Vel is not yet known and thus must be identified serologically. In 2010 the Swiss BTS SRC issued a mandate to our institute to screen over 2 years 40'000 donors from Berne (BE) and other regions for the absence of Vel and KEL*04 antigens.

Methods: Regular blood donors from Berne were tested using an in-house sequence specific primer PCR (SSP-PCR) for the presence of the KEL*03 (Kp(a)) in pools of no greater than 24 samples. Those pools positive for KEL*03 were resolved to the single donation and further tested for KEL*04. The KEL*03 positive, KEL*04 negative samples were confirmed serologically. A serological screen was also conducted using the ID Swing automaton in IAT with an in-house anti-Kp(a) antibody to detect the Kp(a) antigen and positive samples were further tested for Kp(b). For the Vel screen bromelised donor erythrocytes were tested on the ID Swing automaton using a 1:20 diluted anti-Vel antibody on neutral cards.

Results: After 1 year of screening, 1 KEL*03 (Kp(a)) positive, KEL*04 (Kp(b)) negative donor was identified from a total of 7'035 donors by molecular methods, whereas 6 were found serologically from a total of 15'449 donors (3 new, 3 previously known). Serologically 2.3% of the screened donors were found to be Kp(a) positive. The overall prevalence of the Kp(a) positive, Kp(b) negative serological tested donors was 1:3'750. The serological screen for Vel identified 10 negative donors for this antigen from a total of 20'006 donors tested (7 new, 3 previously known), which corresponds to a prevalence of 1:2'000.

All these newly identified KEL*04 (Kp(b)) and Vel negative donors were genotyped for a further 22 alleles and have been documented in the rare donor file run by our laboratory.

Conclusion: Simple and efficient screening methods for donors with the rare KEL*04 (Kp(b)) negative and Vel negative phenotype was set-up and conducted over 1 year. The overall costs of this strategy are 4.66 CHF/test/antigen. The ultimate target of 40'000 donors screened for KEL*04 (Kp(b)) and Vel negativity should guarantee the supply of erythrocyte concentrates for patients with these antibodies. The donor pools used in the molecular screening strategy can be used in the future and are a cost-effective tool for additional screening programmes. In our opinion efficient screening programmes for rare donors require a combination of several serological and molecular techniques.

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**Poster 3**

**A NAT-Only HBV Positive Donation: Breakthrough Infection in a HBV Vaccinated Blood Donor?**

A. Glauser, J. Gottschalk, K. Hardegger, B.M. Frey, Schlieren

Objectives: Since the introduction of nucleic acid testing (NAT) for Hepatitis B Virus (HBV) in blood donations, sporadic NAT-only HBV positive donations have been emerged. In general HBV positive donations will be seen as window phase or as occult infection donations. A NAT-only HBV positive donation will be presented, which possibly derives from a breakthrough subclinical infection in a HBV vaccinated blood donor.

Methods: The cobas s201/TaqScreen MPX test (Roche Diagnostics), a multiplex nucleic acid test for blood screening, was implemented at the ZURICH Blood Transfusion Service SRC in April 2008. Routine samples were tested in pools of six. Resolution of positive pools was performed by single donation re-testing. In reactive samples, the individual reactive parameters have to be identified using alternative testing. This was done by the Reference Laboratory of Swiss Blood Transfusion Service SRC.

Results: Since 2008, more than 320'000 donations have been screened for HBV DNA. Three donations have been found to be HBV NAT only positive (negative for HBsAg by conventional ELISA screening). One such donation derives from a HBV vaccinated blood donor. The index donation was repeatable positive by the cobas TaqScreen MPX test but negative with all serological assays including anti-HBc and anti-HBs. Confirmation by the Abbot Real Time HBV assay showed equivocal results with a very low HBV viral titer of 4 IU/ml. Follow up testing two weeks later still showed negative results for HBsAg and anti-HBs. Interestingly, HBV DNA could not be detected anymore and anti-HBs had increased to > 1000 mIU/ml. This finding was confirmed in another follow up sample 3 months after the in-
dex donation with negative results for all but the parameter anti-HBs, which remained on a high level.

Conclusion: These findings suggest a breakthrough subclinical infection of the HBV vaccinated donor, which might have been acquired very shortly before the blood donation. There are anamnestic indications that the partner of the donor is chronically infected by HBV. The strongly boosted anti-HBs might have cleared the virus very rapidly from the plasma. An anti-HBc seroconversion would be expected within the next months.

Outlook: If the chronic HBV infection of the partner can be confirmed, genotypes of both HBV clones shall be compared to prove the recent infection. Furthermore, infectivity of the index donation could be checked in a transgenic mouse model.

Poster 4

**Intravenous immunoglobulin preparations contain free light chains and anti-red blood cell antibodies**
B. Sakem, K. Matuzan, U. Nydegger, L. Risch, Bern

Free light chains (FLCs) and anti-red blood cell (RBC) antibodies may coelute with intact IgG and produce detrimental components within immunoglobulin (Ig) preparations. The absolute and relative percentages of the total IgG concentrations of kappa and lambda FLCs were measured with the Freelite™ FLC assay in five Ig preparations. The kappa and lambda concentrations in these preparations measured as high as 0.36% and 0.20% of the total IgG. The lowest A1-red blood cell (RBC) agglutinating concentrations (mg/ml) varied from 1.95 to 25 in 0.15 molar NaCl (0.9%), from 6.25 to 25 in NaCl + low ionic strength saline solution (LISS), from 4.4 to 25 in phosphate-buffered saline (PBS), and from 3.125 to 25 in PBS plus LISS. The levels of anti-A1, -A2, and -B found in Gamunex® were up to x6 higher. Additionally, non-specified non-ABO-type RBC agglutinating antibodies were found in the lowest reactor of Redimune®. We demonstrate that FLCs and anti-RBCs are present in Ig preparations, but specific assay conditions for the latter require further validation. Our findings suggest a potential risk for iatrogenic hemolysis and/or amyloidosis.

Poster 5

**Additional tests during validation of pathogen inactivation of platelet concentrates (PC) with the Intercept process**
T. Schulzki, Chur

Introduction: Every blood transfusion service in Switzerland was obliged to validate the process of pathogen inactivation (PI) according to the guardbands provided by Cerus Corporation and with the guidance paper of the working party of the SRC Transfusion service. We added some tests for a more thorough understanding of the product quality.

Methods: Thrombocyte aggregation was measured with a thrombostat model 700 aggregometer (device and reagents by Probe&Go, Germany). We used the same procedures established during evaluation of two separators a year ago. Metabolic measurements were done on a Cobas Mira (Roche) with reagents provided by diatools (Switzerland): glucose, LDH, lactate, cholesterol, triglyceride. pH was measured with a glass electrode (Mettlter, Seven Easy). The PCs were produced according to a SOP developed in the prevailation. As additive solution SSP+ (Macopharma) is used. Single (n=7) and double (n=5) products depending on donor capability with a target platelet content of 2.8 resp. 5.6*10^11 plt/unit were prepared. For single units, the target was raised to 3.1 plt/unit for female donors as established some years ago in our centre. Plasma was set to 37%. Double units were split before illumination, only small volume sets were used. For routine cell measurements a sysmex KX 21 (Digitana, Horgen, Switzerland) was used. For platelet count sample were diluted with saline 1+1. A Amicus separator was used.


Results: Since all products passed guardband criteria without problems, only additional measurements are presented. Triglyceride and cholesterol showed normal values, details not shown here.

For double products, significance was tested by Wilcoxon matched pair test. Glucose on day 6 differs significant (p<0.05) from all other groups. Due to the small number, all other differences are non significant. LDH shows no differences. With lactate, only non treated on day 6 differ from day 8 with both kinds of treatment.

Aggregation: For HSR, there were no significant differences during storage, though a trend to lower values was seen (45% day 1 for untreated, 43% for illuminated products, n=5). Even on day 8 the values decreased moderately: 43% for untreated, 33% for illuminated PCs. With all illuminated products(n=12), a 40% mean value on day 8 was achieved.
Sign.diff. (p<0.05, Wilcoxon matched pair test): day 1 non illum. vs. day 1, illum. and vs. day 6, non illum. Since overall significance is 0.24 (Friedman’s ANOVA), this has to be interpreted cautiously.

If all products are taken into account, with no untreated products on day 6 and 8, a high significance (p<0.01) for the non treated PCs on day 1 compared to all others can be shown. There are some hints in the data that it might be better to keep the time until illumination short (data not shown).

pH is somewhat lower in PI-PCs (about 0.1), but never reached critical values (always > 6.8).

Discussion: Due to constraints of the validation, cost and donor, the data shows decrease of product quality. The decrease between day 6 and day 8 seems small, so PCs storage can be prolonged until day 7.

Poster 6
Inactivation of pathogens in whole blood
S. Amar el Dusouqoui, E. Rigal, Geneva

Inactivation of pathogens in platelets has become mandatory in Switzerland using the intercept technology for platelets. Amotosalen is a psoralen derivate used to form irreversible covalent bonds between pyrimidine bases of nucleic acids after UVA illumination. This photochemical reaction blocks nucleic acid replication thus reducing viral, bacterial and parasite reproduction in platelets.

The inactivation using alkylating agent S-303 in packed red blood cells is well under way. Inactivation of all blood components separately will in the future become standard technology in European countries. We do not want to wait for decades before this promising technology is transferring to developing countries. It is clear where blood supply is short and where the highest transmission of blood transfusion transmitted infections occurs. We need to think globally it is our ethical duty to work on the transmission of this technology since the benefit / risk is certainly higher in low resource countries as compared to the low transmission risk of known pathogens in developed countries today.

In developing countries economic aspects matter and we need to work on whole blood pathogen inactivation in a way to limit external energy needs and adapt the kits to be as simple as possible to use. This technology has to be safe and efficient for the patient.

The agent S-303 developed by Cerus will be going into Phase III clinical trials soon after a change in glutathione concentration.

Two previous phase III clinical trials have already been conducted.

It has been proven that S 303 does not affect RBC quality and that primary and secondary endpoints have been reached in an acute anemia phase III study in cardiac surgery patients. No significant difference was noted in matter of adverse events.

Our transfusion centre is in the first phases of whole blood pathogen inactivation planning in relation with teams working on RBC inactivation.

The first phase is conducting studies on plasma coagulation factor inactivation after using the second generation glutathione enriched method and conducting reduction surveillance for the most common pathogens in Africa.

In an article printed in Biologicals in 2009 by N.A. Mufti and al. treatment of whole blood showed effective pathogen inactivation in a study on one single sample. Since that time studies have concentrated on RBCs and have developed. In this article two tables have been published showing In vitro function parameters for RBC, platelet and plasma of pathogen inactivated whole blood after 22 days of storage at 1 to 6 degrees Celsius.

Platelets show higher activation and there is a drop in plasma coagulation factors.

Our studies are aiming to test coagulation parameters (FV,FVIII, FX, aPTT and PT and fibrinogen) using 0.2 mM S 303 and a higher concentration of GSH 20mM (not previously used) and to compare them with thawed fresh frozen plasma in 30 samples and to make sure that they keep haemeostatic efficiency. P selectine expression and platelet microscopic morphology should also be tested in the same number of samples.

At the same concentration as in RBC we are aiming to make sure that reduction of Dengue I – V, Plasmodium falciparum, T. cruzi, HBV, HIV intracellular, Bovine viral diarrhe virus, Bluetongue, S.aureus, S.Marsecens, Y enterocolitica, E.Coli S.epidermidis are sufficiently inactivated.

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