Abstract No. 1

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Title:
CORRECTING IRON DEFICIENCY IN BLOOD DONORS BY SWITCHING FROM WHOLE BLOOD TO PLATELET DONATION

Text:
BACKGROUND: Iron deficiency (ID) is a frequent side effect of whole blood (WB) donation, which can in turn lead to donation deferral due to an inferior hemoglobin (Hgb) level. Rejection for low Hgb levels not only temporarily excludes donors, but also can permanently end the will to donate in otherwise healthy volunteers. In contrast, less red blood cell loss and therefore less iron loss results from plateleth peresis. In an effort to maintain our blood donor pool and counteract ongoing ID in our volunteers, plateleth peresis was offered to WB donors developing ID. STUDY DESIGN AND METHODS: WB donors presenting a decrease in either Hgb or ferritin levels were offered to switch to plateleth peresis with or without iron supplementation. We analyzed the effect of this intervention on deferral rates for an insufficient Hgb level in 168 donors. Further, we assessed how this intervention affected Hgb and ferritin levels, anemia occurrence and platelet (PLT) concentrate yields in the donors who presented at least four consecutive times for thrombapheresis.

RESULTS: Switching WB donors to repetitive plateleth peresis resulted in an increase of median Hgb (+12 g/L, p<0.001) and ferritin (+15.5 ng/mL, p = 0.002) values. Anemia and deferral rates were reduced by 23% (p = 0.004) and 13% (p<0.001). Between high- and low-frequency apheresis donors, no significant differences in Hgb and ferritin levels were found. Similarly, discrepancies in Hgb and ferritin values between donors that adopted iron supplementation and those who did not were insignificant. The median PLT concentrate yield was sufficient (5.43 x 1011 PLTs).

CONCLUSION: Switching iron-deficient WB donors to plateleth peresis was an effective intervention that permitted us to correct low Hgb and ferritin levels while retaining donors in our pool.
Abstract No. 2

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Title:
ROLE OF H-Y MINOR HISTOCOMPATIBILITY ANTIGENS IN PLATELET TRANSFUSION

Text:
H-Y proteins are ubiquitously expressed Y chromosome-encoded minor histocompatibility antigens, which are relevant in the transplantation of hematopoietic stem cells (HSCT) and solid organs. No studies have so far analysed whether H-Y incompatibility influences the outcome of platelet transfusion.

We studied the effect of donor and recipient gender on outcome of 9'038 single donor platelet transfusions. Using standard corrected count increment (CCI) or percent platelet recovery (PPR) calculations, male patients showed inferior recovery rates, irrespective of donor gender. Using an adjusted PPR, which takes into account differences in blood volume between males and females, neither donor nor recipient gender played any role in platelet recovery after transfusion. Similarly, the time to next platelet transfusion was unaffected by both donor and recipient gender. In a subgroup analysis of patients with graft-versus-host-disease after allogeneic HSCT, male recipients of a female allograft - which may carry anti-H-Y T-cells and antibodies - had significantly lower time to next platelet transfusion. However, this occurred after both male donor and female donor platelet transfusions, arguing against an involvement of alloreactivity against H-Y antigens on platelets. In conclusion, this large analysis found no evidence that alloreactivity against H-Y influences the outcome of platelet transfusion.

Abstract No. 3

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Title:
TECHNICAL ADVISOR PROGRAM FOR RESTRUCTURING A NATIONAL BLOOD TRANSFUSION SERVICE IN DEVELOPING COUNTRIES

Text:
BACKGROUND: Blood services in Egypt as part of the Ministry of Health (MoH) used to be compromised by fragmented mostly inefficient blood banks acting independently with almost no standardization. At that point, restructuring of blood services became mandatory in light of epidemiological and demographic changes. A program for restructuring the services was initiated through an agreement between the Egyptian and Swiss Governments.

AIM: The overall aim of this program is to upgrade, enhance and extend the National Blood Transfusion Services (NBTS) of the MoH. The objectives comprises the development of a national blood policy; implementation of national quality management system; provision of laboratory and IT equipment; strengthening of the blood donor program to improve recruitment and retention of voluntary-non-remunerated blood donors from low risk populations; ensuring the appropriate clinical use of blood; training program for capacity building of the NBTS staff; and implementation of Blood Management System.

METHODS: In order to pursue the overall aim and objectives of the project a Technical Assistance Program (TAP) was established covering the following capabilities: General Management, Quality Management, Appropriate Clinical Use of Blood, Laboratory Services, Blood Donor Recruitment, Information Technology and Procurement and Logistics. A technical advisor was assigned to each capability. The technical advisors form the Project Implementation Unit (PIU) that was lead by director general of the NBTS.

RESULTS: The already achieved results are multifold development of a National Blood Policy, centralization of testing, education of appropriate clinical use of blood, implementation of standard procedures and forms across all centers. Furthermore, one of key accomplishment is that the NBTS has a developed and defined professional culture that was developed through the TAP. TAP has also provided a solid foundation (e.g. business process, collaboration between IT, doctors and quality management) for the implementation for the blood management system. Furthermore, the NBTS has proven to have also the right capabilities to respond to crises and emergency as profoundly demonstrated during the Egyptian revolution.

CONCLUSION: The work of the PIU has been proven to be the right organizational measures to develop the required capabilities to reach the aim of the program. Fur-
thermore, it is instrumental in reaching the remaining objectives in a sustainable way.

Abstract No. 4

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Title:
PRESENCE OF DEL261G (O1) AND 803C (B) ON 1 HYBRID ALLELE - A POTENTIAL CAUSE FOR MISINTERPRETATION OF PCR-SSP RESULTS

Text:
BACKGROUND: In blood group determination, discrepancies between pheno- and genotypes are generally an indicator for the presence of unexpected, or unknown, and usually very rare blood group alleles. In the course of routine screening RhD negative donors for the presence of RHD genes, a crosslink-ID-control PCR, specific for the B-allele of the ABO gene, tested positive in one case, although this donor had a record of blood group A at three independent blood donations.

METHODS: Standard serology was used. ABO genotyping was performed using a commercially available test kit “ABO” (Innotrain, Germany) and in house PCR-SSP technique. Allele-sequencing was accomplished by long range PCRs with generic amplifications of exons 1-3 and allele discriminative, nonO1- and O1-specific amplifications for exons 4 to 7, respectively.

RESULTS: PCR-SSP ABO-genotyping at coding nucleotides 261, 802, 803 and 1’061, specific for alleles O1, O2, B, A2 and A, respectively, revealed a BO1 genotype of the investigated sample. However, allele specific sequencing of exons 4-7 resulted in one “regular” A-allele as expected from the serological analysis (blood group A), and a second allele, carrying a G261del deletion, indicative of an O1 allele, and on the same allele the B-specific 803C (Gas-sner et al., 1996). The obtained sequences displayed identity to published ABO hybrid alleles O24 (O1v-B; www.ncbi.nlm.nih.gov) and O41 (O1v-B, tse13), respectively. These alleles have been identified repeatedly in samples from Brazilian blacks and Akans from Ivory Coast (Olson et al., 1997; Roubinet et al., 2004).

CONCLUSION: The definitive name of our observed allele is uncertain, since the most similar O24 lacks sequence information of intron 6, while for O41, sequence of exons 1 to 5 is missing in the respective databases. Alternatively, a new allele, having originated from an independent crossing over event, may not be excluded at this time. However, the ethnic origin of our donor points to Brazilian ancestry and makes presence of one of the yet described alleles, O24, or 41 more likely. Although PCR-SSP technique is a well-accepted method for allele genotyping, the regular A allele in the investigated sample escaped correct identification, due to the pretended independent presence of O1 and B, but which were in fact encoded on one unexpressed allele simultaneously. Direct positive detection of A alleles would circumvent such errors, but is handicapped by the need for a 1.05 kbp intron 6 crossing amplification, resulting in amplifications lengths, unusual for PCR-SSP.
2011), whereas the 20% cPCs generated more than 30% of all PC-related reports. The overall risk for adverse transfusion events in Switzerland is 1:440 transfusions for PI-PCs and 1:150-1:300 for cPCs (2011/2010). Life threatening transfusion reactions occur with a frequency of 1:8’800 after PI-PC and 1:3’300 after cPC transfusion.

CONCLUSION: Although comparability of spontaneously reported data is limited and our evaluation is preliminary, we can conclude that the introduction of PI not only reduces the risk for bacterial TTI’s, but also substantially reduces the risk for platelet related TRs in general and life threatening TRs in particular. These findings are in accordance with previously published international Haemovigilance data pertaining to pathogen inactivated PCs (1, 2). It remains to be seen how this trend towards declining platelet related TR's develops over the next few years when more Haemovigilance Data on pathogen inactivated PCs become available.

References:

Abstract No. 6

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Title: APPLICATION OF STATISTICAL PROCESS CONTROL (SPC) IN THE QUALITY CONTROL MONITORING OF BLOOD PRODUCTS

Text: The use of statistical methods (including statistical process control, SPC) for monitoring quality of blood components is a requirement of EU Directives (2002/98/EC, 2004/33/EC) and of the “Guide to the Preparation, Use and Quality Assurance of Blood Components” (16th Edition 2010, Chapter 1, Paragraph 11). However, practical advice is lacking in these sources. Beckman et al. (Transfusion Medicine, 2009, 19,329-339) provide in their article a practical approach for applying SPC to blood component production. The “process capability” (Cpk) is one important index to judge and predict if a process is reliably capable to meet the specifications. We use this Cpk-index for the evaluation and monitoring of our quality control (QC) data. It is a valuable tool to objectively assess the goodness of a process, but also for recognizing trends in QC data. The index is especially useful to compare different manufacturing techniques leading to the same end product.

Abstract No. 7

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Title: OXIDATIVE STRESS IN STORED RED BLOOD CELLS: SUBCELLULAR QUANTITATION AND IDENTIFICATION OF CARBONYLATED PROTEINS

Text: Erythrocytes, through transfusion-related cold banking, undergo storage lesions, targeting their metabolism, rheological properties, and protein content. Oxidative stress plays an important role in these lesions, among which protein carbonylation appears to be the most representative. Protein carbonylation in stored red blood cells was investigated through the establishment of a subcellular fractionation, allowing to distinguish the soluble hemoglobin fraction, the hemoglobin-depleted soluble fraction, the integral membrane fraction and the cytoskeletal membrane fraction. Additionally to these cellular extracts, the carbonylation was also evaluated in the extra-cellular erythrocyte-derived microparticle extract. Quantitation of protein carbonylation was performed by derivatization of carbonylated proteins with 2,4-dinitrophenylhydrazine (2,4-DNPH), followed by SDS-PAGE, western-blotting and chemiluminescent immunodetection of hydrazide residues. Carbonylated proteins were identified using derivatization with biotin-hydrazide and immunoprecipitation with monomeric avidin-coupled magnetic beads. Eluted proteins and flow through were then separated by SDS-PAGE and proteins were in-gel digested then identified via LC-MS/MS analyses. Protein carbonylation increased in membrane fractions, and particularly between day 29 and day 43 (P<0,01) in the cytoskeletal fraction. Moreover, protein carbonylation within microparticles released during storage showed a two-fold increase along the storage period (P<0,01). Identification of carbonylated content revealed proteins stemming from the cytoskeletal fraction such as spectrin, protein 4.2 and actin. Other membrane (Band 3) and soluble (Peroxiredoxin, hemoglobin) proteins were identified as well. Carbonylation of cytoplasmic and membrane protein fractions differs along storage, and the present study allows explaining two distinct steps in global erythrocyte protein carbonylation evolution during blood banking.
Abstract No. 8

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Title:
EFFECT OF INTERCEPT PROCESS ON PLATELET CONCENTRATES: A PROTEOMIC VIEW

Text:
Pathogen reduction technologies became available and started to be implemented in several countries, with the primary goal to fight against bacterial contamination of blood products. Though pathogen reduction technologies represent a quantum leap in transfusion safety, the biological efficacy of platelets treated with various pathogen reduction techniques has been recently questioned by clinical studies. The gist of the present study was to compare Intercept treated platelets with non-treated ones by proteomic analysis in order to evaluate the effect of Intercept treatment at the protein level.

Platelet concentrates (PC) were prepared from pooleduffy coats. Two ABO-matched PC were pooled and split to yield two strictly identical products, each of those coming from 10 donors. One of these PC was kept in standard blood banking conditions up to 8 days after collection, under agitation at 22°C. The other PC was processed with the Intercept system and stored under agitation at 22°C. Five different preparations were used. Samples were taken at three time points: before treatment (Day 1), after treatment (both in the treated PC and in the control one, Day 2), and after storage at Day 8. Protein extracts were separated on two-dimensional gel electrophoresis and protein spots were analyzed by LC-MSMS on an LTQ Orbitrap XL MS. The proteomic study reveals a relatively low impact on the proteome of treated platelets: it mainly induces modifications ofDJ-1 protein, glutaredoxin 5, and Gl(i)alpha2 protein. Among the proteins identified to be directly altered, both DJ-1 and glutaredoxin 5 point to an oxidative stress-associated lesion. Additionally, the detection of an abundant truncated form of Gl(i)alpha2 in Intercept-treated platelets directly links the Intercept treatment with a potential functional lesion. Moreover, aggregation experiments were run on an APACT 4004 using ADP, TRAP, collagen and arachidonic acid as agonists. The results point out significant differences between day 2 and day 8 of storage. Beyond the limited number of altered proteins, these studies reveal differences on the function of platelets, which suggest that the Intercept treatment of PC may have also an influence on their functions.

Abstract No. 9

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Title:
HIGH-THROUGHPUT BLOOD GROUP GENOTYPING (HTBGG) SHOWS AN UNEXPECTED HIGH FREQUENCY OF FY*X

Text:
BACKGROUND: The Duffy (FY) blood group system is clinically significant in transfusion medicine as FY antibodies have the potential to cause haemolytic transfusion reactions and haemolytic disease of the newborn (HDN). The FY glycoprotein is encoded by the FY(DARC) gene of which there are four major alleles: FY*01 and FY*02, encoding the FY(a) and FY(b) antigens, the silent allele FY*FY, commonly found in blacks, and FY*X (FY*02 M.01), associated with a weak FY(b) expression (FY(b+*)).

The FY*X allele, found predominately in Caucasian populations, is caused by a C271T nucleotide change in the FY*02 allele, resulting in an Arg89Cys substitution of the FY(b) protein. The 271T polymorphism results in an unstable FY(b), thus a quantitative reduction of the protein on the erythrocyte membrane. Furthermore, serological testing for FY(b) in FY(a+b+ w) samples may give negative results. Allele frequencies of FY*X in Caucasian populations range between 0.1-8%, with 2-3.5% being the most common.

METHODS: Our HTBGG programme on randomly chosen blood donations investigates the genotype of 22 alleles (20 clinically relevant antigens) including FY*X. A selection of the FY*02/FY*X positive samples were further analysed by an independent sequence-specific primer PCR (SSP-PCR) for wild type 271C and by DNA sequencing of the FY gene. Selected samples were examined for the qualitative antigen expression of the FY(b) antigen using standard serological techniques.

RESULTS: The molecular screen of 6'167 individual blood donors collected at the Swiss regional blood transfusion service in Berne identified 225 donors carrying the FY*X allele (3.6%). This was divided into 91 FY*X/FY*X positive samples were further analysed by an independent sequence-specific primer PCR (SSP-PCR) for wild type 271C and by DNA sequencing of the FY gene. Selected samples were examined for the qualitative antigen expression of the FY(b) antigen using standard serological techniques.

SUMMARY/CONCLUSION: This is one of the first reports on the FY*X frequency, in which several thousands of samples were analysed. The allele frequency of FY*X in our blood donor population lies surprisingly within the expected upper limit. Several donors with the FY*X/FY*X genotype were previously falsely identified as FY(a+b-). Though the clinical significance of incompatible transfu-
sions due to FY*01/FY*X positive blood products is unclear, a correct FY*01/FY*02 genotyping is surely important to ensure transfusion safety.

Abstract No. 10

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Title:
STEM CELLS ALLOWS THE MODELIZATION OF HUMAN DISEASES IN VITRO

Text:
Because of their differentiation capacities towards multiple cell lineages, stem cells are intensively studied with the goal of cell therapy applications. We propose that stem cells also offer relevant tools to modelize human diseases. A laboratory model reproduce the disease in vitro, with the goal of identifying new biological events and discovering new treatments. To date, there is a lack of relevancy of available models for most human diseases. We have recently developed a model in three dimensions of brain tumor development within a nervous tissue exclusively in humans. For that project, we used tumor cells from patients and the technology of brain-like tissue engineering from stem cells that we recently developed. A tumor is growing in vitro, with a high degree of similarity to the in vivo tumor development in patients. Notably, this system was used to discover previously unknown biological events in brain tumors. Surprisingly, we observed a molecular response that is similar to defense against viruses, despite the fact that no common neurotropic viruses were found. Thus, we have tracked the presence of non-human sequences in tumor biopsies from patients by using next generation ultra-deep sequencing. 8 biopsies were fully sequenced for nucleic acids that can be used as a signature for virus infection (mRNA and miRNA). No known viruses were identified in the disease. However, large (>500bp) non-human mRNA which did not correspond to any known sequences were observed in glioblastoma but not in control biopsies, proposing a virus hypothesis for glioblastoma. Thus, in addition to cell therapy, stem cells offer a promising tool to modelize human diseases in vitro and discover new biological events.

Disclaimer:
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