Platelet antibody and antigen testing

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Discrepancy of HPA-1a phenotype and genotype in 11 patients reveal 5 separate mutations in exons 2, 3, 4 and 11, and one mutation in the splicing area of intron 6 of GPIIIA

Osnes L[1], Tjeldhorn L[1], Hauge R[1], Skogen B[2], Kjeldsen-Kragh J[1], Randen I[1]
1Dept. of Immunology and transfusion medicine, [1]Ullevaal University Hospital, Oslo
[2]University Hospital of Northern Norway, Tromsø

Blood samples from 11 women with a discrepancy between genotype and phenotype for HPA-1a were investigated. All women were HPA-1a negative by flow cytometry using the monoclonal antibody SZ21 at a dilution of 1:100. At the genomic level the HPA 1a/b polymorphism was determined using hybridization probes and melting curve analysis. To investigate detected discrepancy between phenotype and genotype we sequenced GPIIIa and GPIIb. Blood samples available from four patients were assessed for expression of GPIIb/IIIa by flow cytometry using mAb directed against other epitopes than HPA-1a. Five separate mutations in GPIIIa (all heterozygous) were detected in six patients. Two patients had a replacement mutation at different locations in exon 2. Two patients shared the same mutation in exon 3. One patient had a mutation in exon 11 and one had a 'out of frame' deletion in exon 4. Three additional patients were heterozygous for a point mutation in intron 6 within the 5'-splicing site. No mutations were discovered in GPIIb. One of the patients with a mutation in exon 2 and the patient with an exon 11 mutation had no significant reduction in expression of GPIIb/IIIa as judged by quantitative analysis (MESF-units) of the receptor with mAb directed against GPIIb (clones: 5B12 and P2) and GPIIIA (clone: Y2/51) compared with normal controls. The cells from one of the patients with an exon 3 substitution showed reduced binding of Y2/51 and 5B12 by approximately 1/3 compared to controls, whereas binding of mAb P2 was unchanged. In two patients no mutations were detected in either GPIIb or GPIIIa and in one of these the expression of GPIIIa and GPIIb was reduced by approximately 50%. Three mutations resulting in amino acid substitution in the regions involved in the HPA-1a epitope formation were discovered. These probably result in conformational changes of the epitope disrupting binding of SZ21. Disulfide binding between the N-terminal domain and the core domain (aa 423 to 622) of GPIIIa/IIb are probably important in normal HPA-1a formation. Amino acid substitution in exon 11 may induce conformational changes by interfering with disulfide binding in this region. The deletion in exon 4 results in a stop codon and premature termination. The consequence of this is probably missing expression of the allele carrying the HPA-1a gene. The detected mutation in intron 6, affecting three patients, may interfere with recognition of the splicing site leading to alternative splicing, and thus changed conformation and /or expression of the GPIIb/IIIa.

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A new platelet alloantigen, Swi^a, located on glycoprotein Ia (α2 integrin subunit) identified in a family with fetal and neonatal alloimmune thrombocytopenia

Kroll H[1], Köhl K[2], Zwingel C[2], Hoch J[3], Bald R[4], Santoso S[2]
[1]Red Cross Blood Transfusion Service NSTOB, Institute Dessau, Germany [2]Institute for Clinical Immunology and Transfusion Medicine, University Giessen, Germany [3]Institute for Transfusion Medicine and Experimental Hemostaseology, University Bonn, Germany [4]Clinics for Gynecology and Obstetrics, Leverkusen, Germany

We describe a new low frequency alloantigen, Swi^a, which was identified in a family with fetal and neonatal alloimmune thrombocytopenia. The first pregnancy was uneventful and the child born with normal platelet count. The second child was lost due to prenatal intracranial hemorrhage. During the third pregnancy, the fetus was treated with repeated intraumbilical platelet transfusions. The newborn required one further transfusion and recovered completely. Analysis of maternal serum in MAIPA assay with paternal platelets showed reactivity with integrins αIIbβ3 and α2β1, but not with α5β1 and α6β1, indicating anti-HPA-1a and an additional alloantibody against α2 (anti-Swi^a). Extended family and population studies showed that 4 of 10 members of the paternal family but none of 500 unrelated blood donors were Swi^a carriers. By immunochemical studies, the localization of the Swi^a antigen on α2β1 could be confirmed. Analysis of paternal α2 cDNA showed a C3389T bp substitution in exon 28 resulting in a Thr-Met amino acid substitution. Reanalysis of family members by RFLP using Msl I endonuclease showed perfect correlation with phenotyping. There was no relation of the Swi^a antigen to HPA-5 or -13. A Swi^a allele-specific cDNA construct in the mammalian expression vector pMPSV was generated by site-directed mutagenesis and transfected into CHO cells. Expression of the recombinant Swi^a antigen on CHO cells was confirmed by immunoprecipitation and MAIPA assay. Swi^a platelets showed a normal aggregation response to collagen and other standard agonists. Adhesion of CHO cells expressing recombinant Swi^a to immobilized collagen was not impaired compared to wild type controls. Furthermore, anti-Swi^a did not influence the adhesion onto collagen. B-cells from a Swi^a individual were immortalized by EBV transfection. In summary, we report the third alloantigen on α2, Swi^a. Inclusion into the HPA-nomenclature is proposed.

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A new Particle-agglutination (paGIA) assay for the Antigen-Specific Detection of Platelet Antibodies

Meyer O[1], Agaylan A[1], Bombard S[2], Kiesewetter H[1], and Salama A[1]
[1]Charité, Universitätsmedizin Berlin, Institut für Transfusionsmedizin, Augustenburger Platz 1, 13353 Berlin, Germany [2]DiaMed AG, Cressier sur Morat, Switzerland

The clinical significance of platelet antibodies is reflected by their association with autoimmune thrombocytopenia, alloimmune thrombocytopenia, drug-induced immune thrombocytopenia, and neonatal alloimmune thrombocytopenia. Until now, differentiation between the causative antibodies has been too technically demanding and infeasible for most routine laboratories. Here, we describe a novel antigen-specific particle assay (ASPA) for platelets similar to that of red blood cells. Platelets were solubilized and then incubated with red-dyed polystyrene particles coated with monoclonal antibodies to various platelet glycoprotein complexes. These particles were directly tested for coating with autoantibodies (n = 8), indirectly tested for serum autoantibodies (n = 33), or alloantibodies against HPA-1a (n = 4) or HPA-5b (n = 5). Serum samples from healthy blood donors (n = 100) served as negative controls. Negative reactions were clearly distinguishable from positive reactions, and the results of the particle assay were in concordance with those obtained by the standard monoclonal antibody-specific immobilization of platelet antigen assay (MAIPA) in all cases with alloantibodies. In three patients, only the ASPA was able to detect autoantibodies that were completely undetectable by the MAIPA. In contrast, in only one patient, the MAIPA detected autoantibodies that the ASPA failed to detect. In general, the new test appears to be more sensitive than the standard MAIPA for the detection of platelet autoantibodies. Furthermore, the specifity of the ASPA compared with the MAIPA is similar to the specifity of antigen-specific assays, which has been shown to be very high. Most importantly, screening for serum antibodies to the most relevant platelet GPs can be completed within 30 minutes at any time when monoclonal antibody-coated particles and solubilized platelets or GPs are already available. It seems possible to use lyophilized or recombinant GPs for coating onto the particles. In our opinion, the new antigen-specific particle assay is reliable, yet less complex and time-consuming than the currently available assays, and it can be implemented in any routine laboratory.

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Real-time analysis of HPA-1a antigen-antibody interaction by surface plasmon resonance technology

Andrei-Selmer C[1], Socher I[1], Bein G, Kroll H[2], Santoso S[1]
[1]Institute for Clinical Immunology and Transfusion Medicine, Giessen, Germany
[2]German Red Cross Blood Service NSTOB, Institute Dessau, Germany

Neonatal alloimmune thrombocytopenia (NAIT) is induced by maternal immunization against a fetal human platelet alloantigen (HPA). In about 75% of serologically verified NAIT cases alloantibodies (alloabs) against HPA-1a are responsible. However, in a certain number of cases that are clinically suspicious for NAIT, no maternal HPA-1a alloabs are detectable. Here, we studied the real-time binding characteristics of different HPA-1a alloabs by surface plasmon resonance (SPR) technology (Biacore 2000). In comparison to most other techniques, SPR allows direct measurement of antigen-antibody interaction without any washing procedure. Integrin αIIbβ3 was purified by affinity chromatography from platelets from HPA-1aa or -1bb donors, and immobilized onto CM5 biosensor chip by amino coupling. Monomorphic monoclonal antibody (mab) AP3 (anti-αIIbβ3) or polymorphic mouse and human anti-HPA-1a mabs (SZ21 and 2E10) were injected serially into the reaction chamber. Real-time antigen-antibody binding characteristics (association-dissociation) were recorded and analyzed by Kinject software (Biacore). Mab AP3 showed similar binding characteristics with HPA-1a and -1b isoforms (control experiment). In contrast, mab SZ21 dissociated more rapidly from HPA-1b than from HPA-1a, indicating its prefential binding to HPA-1a. Similarly, human mab 2E10 (originally derived from a NAIT mother) bound to HPA-1a, but not to HPA-1b. Furthermore, we tested various HPA-1 alloabs from mothers with NAIT and from patients with PTP. Anti-HPA-1a alloabs from NAIT patients bound to HPA-1a antigen with similar binding characteristics as shown by mab 2E10. No association with HPA-1b was detected with these sera. Vice versa, a HPA-1b alloab which derived from a NAIT mother interacted specifically with HPA-1b. Interestingly, one HPA-1a alloab from a case with typical NAIT failed to react in antigen capture assay (MAIPA) but showed a specific interaction with the HPA-1a antigen in the SPR assay. Analysis of HPA-1a from PTP patients showed specific

binding to HPA-1a, but not to HPA-1b. These results indicated that SPR technology may represent a useful technique for (1) the direct analysis of platelet alloab binding characteristics (2) the detection of platelet alloabs non-reactive in MAPA assay. This information may help to improve the diagnosis of antibody mediated thrombocytopenia and the understanding of the pathomechanism of this disease.

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Direct comparison between elisa and maipa in the measurement of anti-HPA-1a (in IU/ml and au/ml) in neonatal alloimmune thrombocytopenia

Bessos H[1], Matviyenko M[3], Killie MK[3], Urbaniak SJ[1,2]
[1]*Scottish National Blood Transfusion Service, National Science Laboratory, Edinburgh, UK* [2]*Academic Transfusion Medicine Unit, The University of Aberdeen, Scotland, UK* [3]*University Hospital of Northern Norway, 9038 Tromsø, Norway*

Most severe cases of neonatal alloimmune thrombocytopenia (NAIT) in the Caucasian population occur when mother and baby differ in their HPA-1 allotype. The relationship of anti-HPA-1a antibody quantity to the severity of NAIT remains uncertain. However, recently three studies have shown a correlation between antibody level in the mother and the severity of thrombocytopenia in the newborn whereas one study has not reported such a correlation. Two different methods for quantification of antibodies were employed: an enzyme linked immunosorbent assay (ELISA) in the latter study, and monoclonal antibody immobilisation of platelet antigens (MAIPA) assay in the other three studies. The aim of this study was to compare these two assays in the quantification of anti-HPA-1a antibodies.

Methods: Twenty-nine samples obtained from mothers at various stages of pregnancy giving birth to babies with normal or moderately reduced platelet count, or severe thrombocytopenia, were tested blind by ELISA and MAIPA. The ELISA was based on the method described recently by Bessos *et al.* (Immunohematology, 21, 109, 2005) where a polyclonal antibody standardised against the recently established WHO/NIBSC standard 03/152 (100 IU/mL) was used to generate a standard curve. The MAIPA was based on a modified method originally described by Kiefel *et al* (Blood 70:1722, 1987) where antibody quantity was determined both in IU/mL (samples 1–17) and AU/mL (samples 18–29). The polyclonal standards were assayed in replicates in 8–10 serial doubling dilutions starting at 1:1, while the test samples were assayed in replicates in 1–5 serial doubling dilutions starting at neat (1:1 in ELISA) to 1:64.

Results: The polyclonal standards in the ELISA and the MAIPA were standardised as 145 IU/mL and 15 IU/mL respectively. Although similar anti-HPA-1a antibody amounts (IU/mL) were obtained by the ELISA and MAIPA in some samples, in other samples different amounts were obtained. Despite the observed individual variations, there was a significant correlation between the two assays ($R^2 = 0.695$ $P < 0.01$; range of antibody amount: 0.01–177.7 IU/mL). In addition, there was a significant correlation between the ELISA (range 2–67.4 IU/mL) and the MAIPA (range 23–9080 AU/mL) for samples 18–29 ($R^2 = 0.969$, $P < 0.001$).

Conclusion: This is the first direct comparison of anti-HPA-1a antibody quantities in IU/mL and AU/mL employing ELISA and MAIPA methods. Although considerable variation between the two assays in anti-HPA-1a antibody measurement exist in some cases (the reasons for which remain to be discerned), the correlation between the two assays remains significant.

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Measurements of *in vitro* platelet quality parameters in photochemical treated and gamma-irradiated single-donor platelet concentrates during storage

Apelseth TO[1,2], Bruserud Ø[3,4], Wentzel-Larsen T[5], Bakken AM[1], Hervig T[1]
[1]*The Blood Bank, Haukeland University Hospital* [2]*Gades Institute, University of Bergen, Bergen, Norway* [3]*Section for Hematology, Department of Medicine, Haukeland University Hospital* [4]*Institute of Medicine, University of Bergen, Bergen, Norway* [5]*Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway*

Background: Photochemical treatment (PCT) prevents replication of pathogens in platelet concentrates (PCs) by cross linking nucleic acids. PCT affects all cells containing DNA/RNA, including normal cells. An initial platelet storage lesion evolved during preparation is important for platelet quality during storage. High levels of platelet activation may decrease response to agonist stimulation and lower survival of transfused platelets. *In vitro* platelet function parameters may thereby be helpful when assessing impact of changing preparation techniques on PCs. In this study we compare platelet quality parameters in photochemical treated, gamma-irradiated and untreated single-donor PCs during storage.

Materials and Methods: Double-dose single-donor leukoreduced PCs (n = 14) were split in two identical units, each component fulfilling requirements for standard platelet concentrates. Two Study arms were created; Study Arm A consisting of seven PCT PCs with corresponding untreated PCs, and Study arm B consisting of seven PCT PCs with corresponding gamma-irradiated PCs. PCs added PASIII (Intersol), were photochemical treated with Amotosalen and Ultraviolet A-light. Corresponding control PCs, added PASII (T-sol), received no treatment or were gamma-irradiated before storage. Aliquots were drawn and *in vitro* quality parameters measured after 1, 5, 7 and 11/12 of storage. Platelet activation parameters were analyzed by flow cytometry using RPE-conjugated CD62P and CD42b-specific MoAb. The proportion of $CD61^+$ microparticles (platelet fragments formed due to activation during preparation and storage) were gated by size from total $CD61^+$ population by use of 1 ?m polystyrene microspheres. Cytokines were investigated by enzyme-linked immunosorbent assay.

Results: Photochemical treatment significantly lowered the day 1 platelet dose, the platelet concentration and the PC volume, and these differences persisted during storage for up to 12 days. pH (22°C) decreased during storage and were significantly lowered for PCT PCs after 5 and 7 days. Higher glucose consumption and lactate production rates were observed after PCT in both study arms. Significantly higher rate of lactatdehydrogenase (LDH) release and $CD61^+$ microparticle formation were observed in PCT PCs. Proportion of $CD61^+$ microparticles and $CD62P^+$ platelets were significantly increased, whereas the proportion of $CD42^+$ platelets were significantly lowered by PCT. Levels of beta-thromboglobulin were comparable between PCT and control PCs throughout the storage period, but significantly higher accumulation rates were observed in PCT PCs when corrected for platelet concentration at time of sampling.

Conclusions: Photochemical treatment may affect *in vitro* platelet quality parameters. Findings may indicate higher levels of platelet activation and platelet destruction in PCT PCs during storage.

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A comparison of two ELISA tests for the detection of hit antibodies

Dovč Drnovšek T, Rožman P
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

Background: Heparin-induced thrombocytopenia (HIT) is a common immune-mediated complication of heparin therapy, caused by antibodies against the PF4: heparin complexes. Several enzyme-linked immunosorbent assays (ELISA) are commercially available for the detection of HIT antibodies. We compared two of them, aiming especially at their diagnostic values and special feature – the inhibition of antibodies binding by excess heparin.

Material and Methods: Twenty samples from patients with suspected HIT were tested with PF4 Enhanced assay (GTI, Brookfield, USA) and Asserachrom HIPA (DIAGNOSTICA STAGO, Asnieres, France), according to the manufacturer' instructions. Beside the detection of HIT antibodies (Ab), the procedures included a confirmatory test (CT) with the inhibition of Ab binding by excess heparin, which is recommended by the first but not by the second manufacturer.

Results: Among 20 samples, 10 (50%) were positive with the GTI (Ab-pos/CT-pos). Of these, six (6) were also Ab-pos/CT-pos with the STAGO assay, two (2) were Ab-indeterminate/CT-pos, and two (2) were Ab-neg/CT-pos. The next four (4) out of 20 samples (20%) were indeterminate for HIT antibodies when tested with the GTI assay (Ab-pos/CT-neg), whereas in the STAGO assay, one of them was Ab-pos/CT-pos, one was Ab-pos/CT-neg, 1 was Ab-neg/CT-pos, and one was Ab-neg/CT-neg. The next four (4) samples (20%) were Ab-indeterminate/CT-pos when tested with the GTI assay, however, with the STAGO assay, two of them were Ab-pos/CT-pos, one was Ab-indeterminate/CT-pos, and one was Ab-neg/CT-pos. The last 2 out of 20 samples (10%) were completely negative with the GTI test (Ab-neg/CT-neg). When tested with the STAGO assay, one of them was Ab-neg/CT-neg, but the other sample was Ab-neg/CT-pos.

Conclusion: The sensitivity of the PF4 Enhanced, GTI ELISA assay was generally higher as compared with the Asserachrom HIPA, DIAGNOSTICA STAGO. The results of the two tests neither fully overlapped nor complemented each other, which in certain cases (25% of samples) contributed to a diagnostic pitfall. The inclusion of confirmatory tests even worsened the resolution of both tests. Future studies are therefore needed to evaluate the prognostic value of both tests based on the clinical evaluation of corresponding patients.

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Determination of human platelet apoptosis induced by shear stresses, thrombin and calcium ionophore A23187

Leytin V, Mykhaylov S, Allen D, Lyubimov E, Freedman J
Division of Transfusion Medicine, Department of Laboratory Medicine, St. Michael's Hospital, Toronto, Ontario, M5B 1W8, CanadaDepartment of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

Apoptosis, or programmed cell death, is appreciated as the main physiologic mechanism that regulates cell life-span and serves for controlled deletion of unwanted cells. Since its discovery, apoptosis was long attributed exclusively to nucleate cells. It took more than twenty years to recognize apoptosis in enucleated cells cytoplasts and anucleate platelets. During the following years, apoptosis has been demonstrated in platelets treated with natural and artificial agonists, in platelet concentrates aged during storage

under standard blood banking conditions, and in animal models of suppressed thrombopoiesis and thrombocytopenia. Other studies documented that mechanical forces (shear stresses) stimulate platelet activation and signalling in the absence of exogenous chemical stimuli. In this work, we analysed whether shear stresses can trigger platelet apoptosis. Using a cone-and-plate viscometer, we exposed human platelet-rich plasma to different shear stresses, ranging from physiologic arterial and arterioles levels (10–44 dynes/cm^2) to pathologic high levels (117–388 dynes/cm^2) occurring in stenosed coronary, peripheral or cerebral arteries. We found that pathologic high shear stresses trigger apoptosis events, including mitochondrial transmembrane potential depolarization, caspase-3 activation, phosphatidylserine exposure, and platelet shrinkage and fragmentation into microparticles, whereas physiologic shear stresses are not effective. Platelets subjected to pathologic shear stresses are characterized by impaired platelet function as shown by the absence of ADP-induced platelet aggregation. Apoptotic changes were also induced by the treatment of platelets with calcium ionophore A23187 (10 μM) and thrombin (1 U/mL). Thus, in the present work, we have demonstrated that platelet apoptosis can be induced by chemical stimuli and by mechanical rheological forces (pathologic high shear stresses). Most of shear-induced apoptosis events occur inside of the platelet, including depolarization of mitochondrial inner membrane potential, activation of cytosolic enzyme caspase-3, and translocation of phosphatidylserine from the inner to the outer plasma membrane leaflet. These data suggest that the effects of shear stress on platelet apoptosis are mediated by mechanoreceptor(s) that transmit apoptosis signals to the cell interior. The platelet paradigm of apoptosis induced by chemical agonists and shear stresses suggests that apoptotic cytoplasmic machinery may function without nuclear participation.

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Megakaryocyte hypoplasia and platelet transfusion refractoriness due to recipient derived HPA1a antibodies post unrelated donor stem cell transplantation

Massey E, Masurekar A, Culliford S, Calvert A, Green A, Lucas G, Marks D
Bristol Childrens Hospital and National Blood Service, Bristol, UK

A 51-year old woman, who had previously had two children, was diagnosed with refractory anaemia with trilineage dysplasia and normal cytogenetics. In view of progressive symptoms she was treated with FLAG chemotherapy but management was complicated by upper GI bleeding and platelet transfusion refractoriness associated with HLA class I antibodies against HLA-B13, -B60, -B27, -B41, -B62, -B71, <-B35. Despite transfusion of HLA class I selected platelets her one hour platelet count increment remained in single figures. HPA-1a antibodies were detected in her serum and the patient was typed as HPA-1b1b, HLADRB3*0101 positive. The transfusion of HPA1a negative, HLA selected platelets resulted in satisfactory increments. The patient received a 10/10 antigen matched unrelated donor transplant with reduced intensity conditioning (Fludarabine 30 mg/m^2/day D-7 to D-3, Busulphan 4 mg/kg/day D-3 to D-2. CAMPATH 20mg/day D-6 to D-4). Mycopenolate Mofetil was used for Graft versus host disease (GvHD) prophylaxis. 5.29 $\times$ 10^6 /kg CD34+ peripheral blood stem cells were infused. The donor genotyped as HPA1a1a. The patient achieved neutrophil engraftment (persistently >0.5 $\times$ 10^9 /l) by D+14 and had full donor chimerism on analysis of peripheral blood CD3+ and CD15/45+ cells by D28. Despite continued 100% donor engraftment she remained platelet transfusion dependent six months post transplant with detectable HPA-1a antibodies. HLA class I specific antibodies became undetectable by Luminex and LCT seven weeks posttransplant. Bone marrow aspirate and trephine showed normal erythropoiesis and myelopoiesis but an absence of megakaryocytes. B cell chimerism appeared to show 100% donor engraftment but absolute numbers were low. The patient continued to obtain satisfactory increments to HPA-1a negative HLA selected platelets but developed gastrointestinal bleeding due to biopsy confirmed GvHD of the stomach and duodenum. Intravenous Methylprednisolone was initiated followed by tapering doses of oral Prednisolone. No improvement in platelet counts or platelet transfusion requirements were achieved following steroids or the infusion of high dose Intravenous immunoglobulin (0.8 g/kg on 2 consecutive days). Recipient derived HPA-1a antibodies can persist for at least 6 months post allogeneic transplant despite the disappearance of other recipient derived antibodies and apparent 100% donor engraftment. The persistence of these antibodies is associated with lineage specific delayed megakaryocytic engraftment and can result in significant patient morbidity.

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HPA genotyping – where are mistakes made?

Metcalfe P[1], Allen D[2], Kekomaki R[3], Kaplan C[4], de Haas M[5], Ouwehand W[6]
[1]National Institute for Biological Standards and Control, Potters Bar, UK [2]National Blood Service, Oxford, UK [3]Finnish Red Cross Blood Service, Helsinki, Finland [4]INTS, Paris, France [5]Sanquin Research, Amsterdam, The Netherlands [6]University of Cambridge & National Blood Service Cambridge, UK

HPA genotyping has been included in the Platelet Immunology Quality Exercises organised by NIBSC since 1998 and currently 29 laboratories participate in this part of the exercise. Every six months laboratories are sent four coded whole blood samples and asked to genotype for HPA-1, -2, -3 -5 & (since 2003) HPA-15. Most laboratories use PCR-SSP, either with in-house protocols (14) or commercial kits (10), and the remainder use QPCR (5). A total of 12,780 individual results have been assessed and the overall error rate since 1998 is 0.65%. This compares favourably to other quality schemes where the accuracy of SNP genotyping has been tested. However, the errors are not distributed equally between the various HPA systems – most errors occur with HPA-3 & -5 genotyping. False negative reactions (52% of errors) are more common than false positive reactions (33%) or no result (15%). This scheme has shown that despite the apparent reliability of molecular techniques, mistakes do occur. It also highlights the need for careful selection of techniques and the use of in-house DNA controls.

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Subcutaneous application of anti-D given in patients with autoimmune thrombocytopenia

Meyer O[1], Kiesewetter H[1], Hermsen M[2], Petriedes P[3], Rose M[4], Seibt H[5], Salama A[1]
[1]Institut für Transfusionsmedizin, Charité, Universitätsmedizin, Berlin, Germany [2]Evangelisches Krankenhaus, Klinik für Kinder- und Jugendmedizin, Hamm, Germany [3]Hämatologisch-onkologische Praxis, München, Germany [4]Klinikum Minden, Klinik für Kinder- und Jugendmedizin, Minden, Germany [5]Hämatologisch-onkologische Praxis, Berlin, Germany

Intravenous (i.v.) anti-D is still used in the treatment of ITP. This treatment may results in fever, chills, headache, nausea, dizziness, intravascular haemolysis, and disseminated intravascular coagulation. To avoid these reactions, we decided to give anti-D subcutaneously. Until now, a total of 22 Rh D-positive patients (three children and 18 adults) have been treated. The patients received 50 μg/kg anti-D (Rhophylac® or Rhesogam®, ZLB Bioplasma, Bern, Switzerland) within 3–5 min. subcutaneously. The platelet count increased in 17 (77%) of the treated patients within one week after s.c. injection of anti-D. Three patients had previously been treated with i.v. anti-D and developed acute adverse reactions. These patients tolerated s.c. anti-D, and the effect was comparable to that observed with i.v. anti-D. . The administered volume of anti-D was about 0.33 mL/kg, and was not associated with any local complication, and did not lead to a clinically relevant haemolysis in a single case. In addition, s.c. treatment was repeatedly effective in all patients who required retreatment. Although s.c. anti-D may take one or two days longer to reach maximum effect, a clinical effect seemed to take place within the first two days. This was supported by the fact that bleeding in affected patients completely vanished within two days, and by the fact the platelet count also began to increase within two days. Most importantly, patients treated with s.c. anti-D must not be monitored as closely for signs and symptoms of acute adverse reactions as those treated with i.v. anti-D.

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Testing for Heparin/Platelet Factor 4 Antibodies by the particle gel immunoassay and by testing for specific IgG antibodies by ELISA in surgical compared to medical patients

Schallmoser K[1], Macher S[1], Drexler C[1], Panzer S[2], Lanzer G[1]
[1]Department for Blood Group Serology and Transfusion Medicine, Medical University of Graz, Austria [2]Department for Blood Group Serology, Medical University of Vienna, Austria

Background: Heparin-induced thrombocytopenia type II (HIT II) is caused by antibodies (ab) against Heparin/platelet factor 4 (HPF4) complex. Commercially available ELISAs detect ab of IgG-, IgM- and IgA-class without differentiation whereas it is assumed that only IgG ab are responsible for typical clinical sequels. Aim of our study was to compare detection of IgG ab in patients with surgical and medical diagnoses tested for HPF4 ab with two different serological assays.
Materials and Methods: Serum samples of 26 surgical (19 male; mean age 64.4 ± 16.9 years) and 56 medical patients (28 male; mean age 67.2 ± 16.1 years) with clinically suspected HIT II were tested by a gel particle test (ID-PaGIA HPF4, DiaMed) and by ELISA (GTI PF4 HAT45, Diagast). Samples with positive results in the ELISA were additionally tested with an anti-IgG-conjugate (Diagast).
Results: Twenty-two surgical patients (22/26; 84.6%) were positive in the ELISA; whereas 15 were positive for IgG ab (15/22; 68.2%) and 14/15 (93.3%) had a positive reaction in the gel particle test. In the group of medical patients 47/56 (83.9%) had HPF4 ab in the ELISA, 22/47 of those (46.8%) had IgG ab but only 13/22 (59.1%) were also positive in the gel particle test ($P < 0.05$). Compared to the medical patients the surgical patients had a significantly higher rate of positive reactions in the gel particle test when the ELISA was positive (14/22, 63.6% vs. 13/47, 27.6%; $P < 0.05$) supposing a higher incidence of clinically relevant HPF4 ab. Six of 26 (23.1%) surgical and 7/56 (12.5%) medical patients had HPF4-ab in the ELISA but no IgG ab and a negative gel particle test suggesting non-specific reactions in the highly sensitive ELISA ($P = 0.5$).

On the other side 1/15 surgical (6.7%) and 9/22 medical (40.9%) patients had a negative gel particle test although IgG ab were detectable by ELISA ($P < 0.05$). This may indicate that the solely use of the gel particle test is not sufficient as rapid screening test for clinically relevant HPF4 ab of IgG class.
Conclusion: Serological testing for HPF4 ab to confirm clinical diagnosis of HIT II seems to depend on using a variety of test systems. Differences in surgical and medical patients concerning occurrence and detection of relevant IgG ab may exist and have to be evaluated in prospective studies.

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Heterogeneity of HPA-3 alloantibodies: Consequences for the diagnosis of fetal and neonatal alloimmune thrombocytopenia

Socher I[1], Zwingel C[1], Schallmoser K[2], Hundhausen T[3], Santoso S[1], Kroll H[4]
[1]Institute for Clinical Immunology and Transfusion Medicine, Justus Liebig University, Giessen, Germany [2]University Clinic for Blood Group Serology and Transfusion Medicine, Graz, Austria [3]German Red Cross Blood Service NSTOB, Institute Oldenburg, Germany [4]German Red Cross Blood Service NSTOB, Institute Dessau, Germany

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by maternal alloantibodies (alloabs) against a fetal human platelet antigen (HPA). Immunization against HPA-3a residing on the αIIbß3 integrin is responsible for at least 1–2% of cases and can cause severe FNAIT. Recently, HPA-3a alloabs reacting only with whole platelets have been described. Since such antibodies could be overlooked by standard antigen capture assays the relevance of HPA-3 in FNAIT may be underestimated. Here, we investigated the reactivity of anti-HPA-3a (n = 11) and anti-HPA-3b (n = 1). Initial studies in the MAIPA assay showed that most sera reacted markedly stronger with fresh HPA-3 homozygous platelets than with platelets from heterozygous donors. Furthermore, four anti-HPA-3a and one HPA-3b sera completely failed to react with heterozygous platelets. Therefore, we only used homozygous platelets for further studies. The influence of the age of test platelets on alloab reactivity was analyzed. Whereas, all sera reacted with αIIbß3 integrin from fresh platelets in MAIPA assay, a continuous reduction of reactivity during a storage period of 14 days was observed. We found three reaction patterns: (a) complete loss of reactivity (n = 4), (b) considerably weakened reaction (≥70% reduction; n = 4), and (c) minor reduction of reactivity (≤40% decrease; n = 3). The HPA-3b alloab was non-reactive on day 14 (pattern a). Anti-HPA-1a alloabs used as control remained stably reactive. When platelets that had been stored in liquid nitrogen were used in MAIPA assay, 9 of 11 anti-HPA-3a and one anti-HPA-3b alloabs were reactive. There was no correlation between the reaction pattern and antibody titer. We then asked weather the HPA-3 epitopes are heterogeneous. Interestingly, two different reaction patterns of HPA-3a alloabs could be distinguished by immunoprecipitation. Whilst the majority of HPA-3 alloabs recognized the αIIbß3 complex, two sera precipitated preferentially the αIIb subunit. This finding could be confirmed by immunoblotting as only the two latter sera were reactive. Thus, HPA-3a alloabs seem to react with different epitopes on the αIIbß3 integrin. This heterogeneity, however, did not correlate with the stability of HPA-3a reactivity during platelet storage. In conclusion, a considerable heterogeneity of HPA-3 alloabs may hamper the serologic diagnosis of FNAIT. Fresh or nitrogen stored platelets from homozygous donors should be used in antigen capture assays. Further studies addressing the molecular nature of the epitope heterogeneity are required.

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MCS+ apheresis platelets: 7-day storage is feasible

Vrielink H, Meer PF van der, Pietersz RNI
Sanquin Blood Bank North West region, Amsterdam, The Netherlands

Objective and Design: Whole blood buffy coat derived, WBC reduced platelet (PLT) concentrates can be stored at least 7 days after donation provided that bacterial screening is performed. In this study we evaluated the laboratory quality of apheresis derived PLT concentrates with a storage period of 8 days after donation.
Methods: After receiving consent from the donors, 12 apheresis procedures were performed with the MCS+ (Haemonetics; disposable 994CFE next generation, software version C.5).WBC reduction is achieved by the procedure. The volume, the numbers of PLT, WBCs and RBCs of the product were tested on day 0 after donation, as well as pH, glucose, lactate, CD62p expression and swirling effect on days 0 and 8. A sample for bacterial cultivation was taken at day 8 after donation.
Results: All products complied with the requirements as mentioned in the national guidelines. Bacterial cultivation of all products showed no growth.

	Mean ± SD
PLT ($\times 10^9$/unit)	382 ± 45
Volume (mL)	311 ± 19
PLT concentration ($\times 10^9$/unit)	1.2 ± 0.1
WBC ($\times 10^6$/unit)	0.2 ± 0.3
RBC ($\times 10^6$/unit)	0.9 ± 0.9

	Day 0	Day 8
	Mean ± SD	Mean ± SD
PH	7.1 ± 0.03	7.1 ± 0.1
Glucose (mmol/L)	22.0 ± 1.3	15.5 ± 1.7
Lactate (mmol/L)	1.3 ± 0.5	12.0 ± 1.9
CD62p (%)	8.8 ± 4.0	24.7 ± 6.7
Swirl	3+	3+

Conclusion: At day 8 after donation, all apheresis platelet concentrates are within the specifications of the national guidelines. We conclude that 7-day storage of MCS+ derived WBC reduced apheresis platelet concentrates is feasible.