5

# EVALUATIE GALILEO: VOLAUTOMATISCH SYSTEEM IMMUNOHE-MATOLOGISCHE BEPALINGEN.

H.J.van der Hoek, P.A.W. te Boekhorst, H.H.D.M. van Vliet;
Laboratorium voor Bloedtransfusie- en Hemostase-onderzoek, Hematologie, Erasmus MC, Rotterdam.

Introductie: Recent werd een geautomatiseerde solid phase-techniek geïntroduceerd in de vorm van de bloedgroepautomaat Galileo (Immu-cor). Deze automaat is gevalideerd voor ABO-D bloedgroeptesten, screening op irregulaire antistoffen, antigeenbepalingen en kruis-proeven. De Galileo-resultaten werden vergeleken met Biovue (Ortho) en PEG-IAGT-methode, evenals operationele aspecten zoals bedie-ningsgemak, snelheid en plaatmanagement. Materiaal en Methode: AB0-D volledige test: Galileo vs Autovue. Screening irregulaire antistof-fen: Galileo 4-celpanel (membraan gecoat, Ready-Screen®) vs Biovue 3-celpanel (0,8 %, Surgiscreen®) vs PEG-IAGT 3-celpanel (3%, Pano-screen®). Antigeentypering patiënt: Galileo (Select®/Elisa-plaat) vs buis-jes-methode (direct / IAGT). Kruisproeven: Galileo (Select®) vs PEG-IAGT. Patiënten met antistof(fen) werden gekruist. Operationele aspecten: bedieningsgemak, snelheid, plaat-/ reagensmanagement en storingen werden geïnventariseerd. Resultaten: AB0-D volledige test (n=485): uitslagen goed vergelijkbaar. Bloedgroepdiscrepanties (n=6): in Biovue, anti-M (1); in Galileo, anti-A1(1), g-globuline behandeling (1) en zwakke mixed field (3). Screening irr. antistoffen (n=1045): de gecombineerde resultaten van drie technieken leverden 66 antistof-positieve monsters op die behoren tot het Rhesus-(23),Kell-(10),Duffy-(4),Kidd-(3),Lewis-(4),M-(1) en P-(1) systeem of combinaties (11) ervan. Aspecificiteit aangetoond in 9 gevallen. De specificiteit van Galileo en Autovue t.o.v. PEG bedraagt 98%. De sensitiviteit resp. 79% en 85%. Antigeentyp. patiënt (n=25) : identiek in alle technieken. Kruisproeven (n=150): patiënten met diverse antistoffen getest: 144 identiek, 9 discrepant. Operationele aspecten: overzichtelijke software m.b.t.:opdrachten, reagens/plaat-gebruik en -verbruik, resultaat-historie en -verslaggeving, status monster/batch, aktiviteiten, storingsmelding-en -afhandeling. Het pipetteren van monsters en reagens gaat snel, efficiënt en zonder carry-over. Conclusie: Snelle afhandeling van grote aantallen monsters samen met een grote gebruiksvriendelijkheid en functionele betrouwbaarheid maken de Galileo zeer geschikt voor routinegebruik bij bloedgroeptesten, irregulaire antistofscreening en antigeentypering. De flexibiliteit om cito-monsters te verwerken neemt af bij een toenemend monster-aanbod.Kruisproeftest in Solidphase vs PEG geeft 9/150 discrepanties.

6

# UNEXPECTED RESULTS IN BLOOD GROUP SEROLOGY: THE CHALLENGE OF IMMUNOHAEMATOLOGICAL PUZZLING

B.D. Steetzel1, C. Mouton1, S. Kapteijns2, V.M.J. Novotny1, A.G. van den Bos1
1Department of Blood Transfusion and Transplantation Immunology, 2Department of Paediatrics, Radboud University Medical Centre

Introduction: in daily transfusion practice unexpected negative and/or positive serological results might occur and misinterpreted. We describe a case in which the serological work-up of a common irregular red cell antibody problem resulted in unexpected absence of the red cell antigen involved. The use of a molecular DNA-typing method was helpful to solve the problem.

Case report: prenatal antibody screening of a 36 year old pregnant woman showed presence of anti-K and anti-Fya antibodies. Her red cells were phenotyped K-k+, Fy(a-b+) using standard antisera (Biotest). For reason of possible haemolytic disease of the newborn the father's red cells were phenotyped: K-k+, Fy(a+b+). During pregnancy antibody dependent cellular toxicity test results were < 10% for the anti-Fya antibodies. A healthy girl was born. Laboratory test results indicated no haemolysis. A direct antiglobulin test of the neonate's red cells was negative. However, after elution antibodies were found with anti-K specificity, antibodies with anti-Fya specificity were not detected. The neonate's red cells were serological phenotyped K-k+, Fy(a+b-). Because of the unexpected (repeated) Fy(a+b-) typing results of the neonate's red cells, genotyping (KKD-SSP, Inno-Train) was performed on a neonatal and a maternal blood sample. Interpretation of the KKD-SSP results showed that the genotype of the neonate was FyaFyx, the genotype of the mother was FybFyx. The Fyx allel is associated with weak expression of Fyb.

Conclusions: this case illustrates various pitfalls of standard red cell serology: 1. despite a negative DAT, elution techniques might show the presence of antibodies, 2. antibodies can be eluated from antigen negative red cells due to aspecific binding of antibodies and 3. DNA typing techniques are helpful in cases of conflicting serological results. In this case detection of the Fyx was a coincidence because the parental phenotypes were known and in contrast with the serological observations in the neonate.

7

# ADDITIONAL RED BLOOD CELL ANTIBODIES AFTER BLOOD TRANSFUSION IN A NON-HEMATOLOGICAL ALLOIMMUNIZED PATIENT COHORT.

H. Schonewille, L.M.G. van de Watering and A. Brand
Sanquin Blood Bank, Soutwest Region, Leiden

BACKGROUND: Alloimmunization is a common occurrence in transfused patients. The risk of red blood cell antigen immunization increases with the number of donor exposures, although most antibodies are formed early during the course of transfusion. Because of longer life expectancy of the population and an increased probability of repeat surgery or other diseases requiring blood transfusions, the incidence of additional antibodies becomes of greater clinical relevance. Limited data are available on the frequency of multiple alloimmunization in a non-chronically transfused population. In alloimmunized patients the probability of multiple antibodies increases 3-4 fold with repetitive RBC exposure. We studied additional RBC alloantibody formation in a large non‑hematological transfused RBC alloimmunized patient cohort. STUDY DESIGN AND METHODS: A 20 year retrospective two-center study. Hospital computer data bases were searched for RBC alloimmunized patients who where transfused after the first antibody forming episode. Included were clinically significant alloantibodies against the Rhesus, Kell, Duffy, Kidd and MSs blood group systems. RESULTS: The records of 661 patients revealed 781 antibodies, 15% had multiple antibodies. The median study period per patient was 22 months. Patients received median 6 RBC units in 2 transfusion-events. After blood transfusion 140 patients (21.2%) experienced an additional antibody forming episode, resulting in 33% patients with multiple antibodies. The number and specifity of antibodies at presentation, nor the patient's antigen profile were associated with the incidence of additional antibody formation. Based on the antigen profile of 316 patients, 83% of antibodies could be prevented if extended matching for the C, E, c, K, Fya and Jka should be applied. Considering the current available donors in our region, 1-10% potential donors are available in 37% of patients and >10% potential donors in 63% of patients. CONCLUSION: Patients with RBC antibodies are high responders for additional antibody formation. Extended antigen matching for future transfusions in these patients can prevent more than 80% of additional antibodies and is theoretically feasible.

8

# RED BLOOD CELL ALLOANTIBODIES AFTER TRANSFUSION. FACTORS INFLUENCING INCIDENCE AND SPECIFICITY

H. Schonewille, L.M.G. van de Watering, D.S.E. Loomans and A. Brand
Sanquin Bloodbank Southwest Region, Leiden, The Netherlands

BACKGROUND: Alloimmunization after exposure to red cell alloantigens depends on genetic and acquired patient related factors, dose and route of administration and the immunogenicity of the antigen, but exact kinetics are still unknown. We studied factors possibly influencing antibody incidence and specificity.
STUDY DESIGN AND METHODS: A 5 year retrospective multicenter study with special emphasis on the time interval between transfusion event and antibody investigation. Hospital computer data bases were searched for newly alloimmunized patients with the transfusion history. Included were clinically significant alloantibodies against the Rhesus, Kell, Duffy, Kidd and MSs blood group systems.
RESULTS: Multivariate analysis involving 1710 immunized patients revealed that time interval between transfusion and antibody tests was strongly associated with the antibody specificity. Anti-Jka and anti-Jkb were predominantly found in patients tested within 3 months, while anti-K and anti‑Fya were the most encountered antibodies after more than 5 years following transfusion. Of all immunized patients, new antibodies were detected within 14 days following transfusion in 283 patients (15.9%) and in 1457 patients (82.0%) after more than 14 days. Fifty percent of transfusion recipients were retested for alloimmunization because of a new transfusion indication. Eleven of 2932 patients (0.4%) retested up to 3 days following transfusion had formed new antibodies.
CONCLUSION: The time interval between transfusion and antibody test was associated with RBC antibody specificity. Because RBC antibody tests following transfusion are not routinely performed, many antibodies may (not) be detected at the time of a new transfusion event, posing the transfusion recipient at risk for transfusion delay or a (delayed) hemolytic transfusion reaction. Routine RBC antibody screening 2-4 weeks and 3‑6 months following transfusion would reduce these risks.

9

# MATERNAL RED CELL ALLOIMMUNISATION AFTER INTRA-UTERINE INTRAVASCULAR TRANSFUSIONS FOR HEMOLYTIC DISEASE OF THE FETUS. A REASON TO INTRODUCE EXTENSIVE ANTIGEN MATCHING?

H. Schonewille1, F.J.C.M. Klumper2, L.M.G. van de Watering1, H.H.H. Kanhai2 & A. Brand1,3

1Sanquin Blood Bank, South West Region, Leiden, 2Dept. Obstetrics and 3Dept. Immunohematology and Blood Transfusion, Leiden University Medical Centre, Leiden, the Netherlands

BACKGROUND: Red blood cell (RBC) alloimmunization during pregnancy poses the fetus at risk of developing hemolytic anemia. In severe cases intra uterine transfusion (IUT) therapy is life saving, but maternal additional antibody production due to IUT is a complication. We studied additional antibody production, to explore whether more extensive erytrocyte antigen matching may be warranted.

STUDY DESIGN AND METHODS: A retrospective follow-up study of additional antibodies in all women that received IUTs between 1993 and 2003. Included were clinically significant alloantibodies against the Rhesus, Kell, Duffy, Kidd and MSs blood group systems.

RESULTS: During the 11-year period, 61 of 241 (25%) women produced 74 new additional antibodies after median 3 IUTs. Multivariate analysis revealed that the number of IUTs and the transplacental route of administration were the prominent independent predictors for additional antibody formation. The alloimmunization score, expressed as the number of additional antibodies divided by the number of missing antigens multiplied by the change on incompatible RBC exposure, against clinically relevant antigens (i.e. FY, JK and MS) varied between 5 and 19%. Moreover, the interval between two subsequent IUTs was shortened in case additional antibodies had been formed, posing the fetus at increased risk associated with each IUT procedure. After IUT treatment, 72% of women possess multiple RBC antibodies.

CONCLUSION: Women exposed to IUT are at highest risk for alloimmunization against multiple erytrocyte antigens, with consequences for mother and fetus. Considering, that these antibodies may be formed against antigens of the donor, prevention by more extensive matching of IUTs particularly for FY, JK and MS antigens should be considered.

12

# PREVALENCE & INCIDENCE OF NON-RHD PREGNANCY IMMUNIZATION IN THE NETHERLANDS: RESULTS FROM OPZI STUDY

J.M. Koelewijn1 2 T.G.M. Vrijkotte2, M. de Haas1 3, C.E. van der Schoot1 2, G.J. Bonsel2
1Sanquin Research, Amsterdam, The Netherlands 2Academic Medical Center, Public Health & Epidemiology 3 Sanquin Diagnostic Services, Amsterdam, The Netherlands

Background. Since 1-7-1998 all Dutch women (± 200,000/y) are screened for irregular erythrocyte antibodies (IEA) early in pregnancy. Screening detects pregnancies at risk for hemolytic disease of the newborn (HDN). Early detection and timely treatment may reduce perinatal mortality and morbidity. Evidence for benefits, consequences and costs of screening is still under discussion. The OPZI-study evaluates the programme in a nation-wide study. Key numbers are the prevalence of non-RhD-IEA with clinical relevance (IgG IEA directed against an antigen with fetal expression) ánd the incidence of timely treated serious HDN: need for intra uterine transfusion or transfusion in the first week.

Methods. The coverage of screening approaches 100%. Data are collected from electronic databases, lab records (88 lab's) and obstetric caregivers. Period data collection: 1/9/2002 – 1/9/2004.

Results.We observed 1,565 clinically relevant non-RhD-IEA in 1,348 pregnancies; prevalence 1,348/400,000 (0.34%). IEA were known before pregnancy in 35%, therefore the incidence is 0.22%. Prevalence of IEA-specificities: anti-K 344/1,565 (22 %), anti-c 203 (13 %), anti-E 468(29.9%), anti-C en anti-e 75(4.9%), anti-C(w) 150(9.6%), anti-Fy(a)or(b) 113(7.2%), anti-Jk(a)or(b) 77 (4.9%), anti-S or -s 79(5 %), others 56(3.6.%). If the father is antigen negative, the child is not at risk, essentially false positive screen results from a screening point of view: 559/1,348 (41.5%) of pregnancies, ranging from 4% (anti-c) till 79% (anti-K). In at least 5.7% of these pregnancies new IEA-specificities were detected after screening. 1001 women were included for clinical data collection (response 899/1001 = 90%). The father was antigen-positive in 525/899 (58%). In these women 21 showed serious HDN, which was timely treated. Incidence: 21/525 = 4%; 18 cases were caused by anti-c or anti-K.

Conclusion. Prevalence of non-RhD pregnancy immunization in the Netherlands is 0.34%, incidence 0.22%. Pregnancies at risk for HDN 58.5%. Serious HDN occurs in 4% of the pregnancies at risk.

Compared to other prenatal programs, prevalence and incidence of non-Rh-D-IEA and HDN seem to justify nation-wide screening.

13

# SYMPTOMS OF FATIGUE AND ANEMIA IN AN ORTHOPAEDIC SURGERY POPULATION

C.So-Osman, R.G.H.H.Nelissen, A.Brand and A.Stiggelbout
Sanquin Bloodbank ZW and LUMC, Leiden

Background / aim of the study: in a blood management study on transfusion thresholds we investigated fatigue scores of orthopaedic surgery patients before and after elective total hip- or knee replacement surgery in relation to the patients' hemoglobin (Hb) -levels.
Patients and methods: In three different hospitals patients were randomised for a standard transfusion threshold or a restrictive transfusion threshold according to a blood management protocol. All patients were asked to fill in performance scores (functionality/ADL) and fatigue scores (VAS for fatigue and the fatigue (F:13 items) and non-fatigue (NF: 7 items) subscales of the FACT-Anemia), pre-operatively and up to 14 days post-operatively. At similar time points, Hb values were measured.
Results: overall response rate was 77%(477/619). The VAS scores were highly correlated with the FACT-F score (r = 0.70-0.73). Fatigue pre-operatively correlated with fatigue post-operatively (FACT-F +0.30, Fact-NF +0.39). Also, ADL correlated with the VAS (-0.31) and FACT-F scores (-0.35). Neither ADL nor fatigue scores differed between the two randomised transfusion threshold groups. Hb values (range between 3.8 and 10.8 mmol / L) were not correlated with ADL, VAS or FACT scores at any time.
Conclusions: Surprisingly, in this elective orthopaedic surgery population, symptoms of fatigue and anaemia, as measured by the FACT-Anemia (specifically developed to correlate with Hb values), were not correlated with Hb at all.

16

# AN AUTOANTIBODY WITH KELL SPECIFICITY IN A KELL-NEGATIVE WOMAN

AHL Mulder, HB van der Krift-Grootbod
Ziekenhuis Groep Twente, Hengelo

We present a patient who received multiple transfusions in the course of two years. The patients immunofenotype was Kell negative, but received at least one Kell positive packed cell. As a result she developed an anti-Kell antibody. The antibody was detected in the serum, and at the time of detection of the anti-Kell antibody the DAGT was only weakly positive. The eluate contained the anti-Kell allo-antibody. Since then the patient received Kell negative packed cells only. Eight months after the detection of the anti-Kell antibody the patient presented with a positive DAGT and positive autocontrol. Testing of the eluate showed an antibody with Kell specificity despite the fact that the patient was Kell negative and the fact that she had not received in the last eight months any packed cells from Kell positive donors. No other irregular erythrocyte antibodies could be detected in the eluate. A similar finding has been described for anti-D antibodies in D negative patients (1). In that study the authors concluded that the finding was a result of the elution method used (ELU-kit, Gamma biologicals) and that extensive washing of the erythrocytes preceding the preparation of the eluate resulted in a negative eluate. They concluded that washing of the erythrocytes using the ELU-kit is sometimes not extensive enough and can result in detection of allo-antibody in an eluate. Since our laboratory uses the ELU-kit as well, we repeated the elution with extensively washed erythrocytes, however, the eluate remained positive for anti-Kell antibodies. All our findings were confirmed by an external reference laboratory. (Sanquin Diagnostics, Amsterdam) The patient’s Kell genotype was analyzed and confirmed, and no mutant form of the Kell antigen could be detected. We conclude that this is either an example of the Matuhasi-Ogata phenomenon or that this is an auto-antibody mimicking Kell-specificity. (1) False-positive eluate reactivity due to low-ionic wash solution used with commercial acid-elution kits.

Leger, R et al.. Transfusion 1998, 38:565.

17

# PRODUCTION OF ANTI-A IN PATIENTS (BLOODGROUP A) AFTER TRANSPLANTATION OF BLOODGROUP O KIDNEYS.

Y. Henskens, R. Straat, S. Rouwette, E. Ackermans, C. Jöbses, L. van Pampus.
Transfusion Laboratory University Hospital Maastricht

Background: Haemolytic anaemia due to minor ABO incompatibility occurs infrequently after renal transplantation. Haemolysis is then related to RBC antibodies (often IgG subtype) derived from donor B lymphocytes. An understanding of the cause is essential for determination of the correct blood group when blood components are needed. In order to detect these antibodies, cross matching tests are performed until 3 months after the transplantation.

Aim: We report on the laboratory findings of 7 patients (blood group A) who received a blood group O graft. Type and screen was performed because of a low haemoglobin with or without clinical haemolysis.

Methods:

- DAT/ eluate (eluate prepared by Gamma Elu-kit II).
- Antibody screening and cross matching (DiaMed LISS-IAT).
- Presence of anti-A antibody in blood group typing (DiaMed gel test).
- Follow up of DAT, cross matching and blood group typing.

Results: Antibody screening was negative and DAT was positive (anti-A in eluate) in all patients. Cross matching with ABO identical blood (A) was positive in 4 out of 7 patients. In 3 of these patients the antibody was also present in blood group typing. One of the patients with positive cross matching and presence of anti-A in blood group typing developed severe haemolysis and had a positive DAT 1 year post-transplantation.

Conclusions: 1. Antibody screening fails to detect these donor-derived antibodies since this test is performed with type O RBC’s. 2. Cross matching, compared to the DAT/eluate, does not detect all patients with anti-A. The clinical significance of this finding is unknown. 3. Blood group typing does not detect all antibodies(IgG anti-A and B). 4. Donor-derived antibody production can persist for at least one year after transplantation. 5. Clinical information is essential for the laboratory about patients who received organ transplants.

18

# RIVALRY IN THE BLOOD VESSELS: SURVIVAL OF STORED RED BLOOD CELLS

M. Luten1, B. Roerdinkholder-Stoelwinder1, H.J. Bos1, G.J.C.G.M. Bosman2,
1 Dept. of R&E, Sanquin Blood Bank Southeast Region, Nijmegen, 2 Dept. of Biochemistry, Radboud University Nijmegen Medical Center, Nijmegen

Background Healthy red blood cells (RBCs) have a life span of approximately 120 days in vivo. Donor RBCs are stored for a maximum of 35 days under blood bank conditions. Physicians however prefer to transfuse RBCs stored as short as possible although evidence of a longer survival for short stored RBCs is lacking. In a longitudinal study we determined that during storage under blood bank conditions several parameters of the RBCs in a red cell concentrate (RCC) change (e.g. ATP, 2,3-DPG, pH and Na+ decrease; MCV, K+, hemolysis and LDH increase etc.). The European guidelines state that the 24-hour recovery of transfused RBCs should be at least 75%.Aim Measuring the (differences in) in vivo survival of short and longer stored RBCs after transfusion. Determining the in vivo survival of the currently used RBC products and to establish which of the in vitro parameters might predict the outcome of the in vivo survival. Method Ten hemato(onco-)logic patients will receive each 2 RCC (leukocyte filtrated and radiated). One RCC is stored for a maximum of 10 days, the other RCC is stored for 25-35 days. At several time points after transfusion (1 and 24 hours, 7, 28, 56, 84 and 126 days), blood samples are drawn from the patients. Recovery and survival are calculated based on flow cytometric determination of autologous and transfused RBCs by measuring antigen differences between donor and recipient. Each RCC is sampled before transfusion for measurement of the in vitro parameters (e.g. ATP; 2,3-DPG; pH; hemolysis; MCV).Results At the present time five patients are included in the study. Due to his clinical condition the third patient did not receive a transfusion yet. The fourth patient was excluded because of a transfusion reaction after receiving the first transfusion.

| Patient no. | Storage time (days) | Determined Ag | 1 hr recovery | 24 hr recovery | 7 day recovery |
|---|---|---|---|---|---|
| 001 | 6 | $Fy^a$ | 105% | 95% | 76% |
| | 32 | K | 90% | 83% | 68% |
| 002 | 5 | K | 94% | 81% | 81% |
| | 27 | $Jk^a$ | 75% | 71% | 69% |
| 005 | 9 | K | 117% | 118% | n.d.y.* |
| | 31 | $Jk^b$ | 105% | 107% | n.d.y.* |

*not determined yet

Conclusions The 24-hr recovery of 5 (out of 6) transfused RCC met the European guidelines (¡Ý75%).The lower 24-hr recovery of the longer stored RCC might be explained by the removal of old RBCs in the first 24 hours after transfusion, since longer stored RCC may contain more old RBCs. These preliminary findings suggest that there is a difference of 10-15% in recovery between short (up to 10 days) and longer (25-35 days) stored RCC in favour of the short stored RCC.

20

# RARE ANTI HPA-5a NEONATAL ALLOIMMUNE THROMBOCYTOPENIA CASES

L. Porcelijn, E. Huiskes, S. Hofstede, M. Ruiter, M. Overbeeke
Sanquin Diagnostics (CLB), Platelet/Leukocyte Serology Department

Neonatal AlloImmune Thrombocytopenia (NAITP) occurs in about 1 in 1000 births and results from maternal alloimmunization against platelet antigens present on fetal platelets but absent on maternal platelets. Because of severe thrombocytopenia in utero, in up to 10 percent of cases intracerebral haemorrhage occurs, often leading to severe neurological sequelae or death. In a 13 years period we diagnosed 217 cases of NAITP. Anti HPA-1a was detected in 160 cases (73.4%), anti HPA-1b in 3 (1.4%), anti HPA-3a in 10 (4.6%), anti HPA-5a in 2 (0.9%) anti HPA-5b in 32 (14.7%), anti HPA-15a, 15b, 6bW, 11bW each in 1 (0.5%), anti Tib (private antibody) in 1 (0.5%), anti A in 4 (1.8%) and anti B in 1 case (0.5%).Anti HPA-5a was detected in 2 NAITP cases and by coincidence in one case with a normal platelet count. In one case (father homozygous HPA-5a), a platelet count of 66 x 109/L was detected after preterm rupture of membrane with suspicion of infection. No clinical signs of the thrombocytopenia were present. A following sibling was born with a normal platelet count. In the second case of suspected NAITP the child was born by vacuum delivery and genotyped HPA-5a,b heterozygous. A platelet count of 76 x 109/L, falling in the first hours post partum to 34 was detected. Random platelets were transfused increasing the platelet count to 84, after which the platelet count normalized in 3 days. No haemorrhagic signs were present, the thrombocytopenia was detected after suspicion of asphyxia (Apgar scores 1’ 6, 5’ 7). For the third case an anti HPA-5a was detected after suspicion of a unilateral hydrocephalus at 36 weeks pregnancy which, after birth, was diagnosed as a syndromal structural imperfection. Father was genotyped HPA-5a homozygous. The neonatal platelet count was 260 x 109/L. Although mild to moderate thrombocytopenia was present in two cases, no severe clinical haemorragic problems were seen. Furthermore these two cases were complicated with suspicion of infection and asphyxia.

21

# HEPARIN INDUCED THROMBOCYTOPENIA

L. Porcelijn, L. Terraneo, T. Kocx, E. Huiskes, M. Overbeeke
Sanquin Diagnostics (CLB), Platelet/Leukocyte Serology Department

Heparin Induced thrombocytopenia (HIT) type II is a severe side effect of Heparin use, caused by the formation of antibodies against a Heparin-PF4 complex. A platelet fall of > 50%, a 30% chance of thromboembolic complications, skin lesions and even systemic reactions are the main clinical symptoms. HIT is diagnosed mainly by the presentation of these clinical symptoms during Heparin use and the sort of Heparin used. To support the clinical diagnosis, we perform the Heparin-PF4 ELISA (Asserachrom, Diagnostica Stago). For 127 patients suspected for HIT clinical data were collected and a clinical HIT score was performed. Results were compared with the Heparin-PF4 ELISA results. Although the overall idea is that the Heparin-PF4 ELISA is sensitive but less specific we found the opposite.

| | ELISA pos | ELISA neg |
|---|---|---|
| Type II HIT | 35 (80%) | 5+4 (20%) |
| Unclear | 7 | 12 |
| No type II HIT | 2 (3%) | 62 (97%) |

A specificity of 97% was found. To further confirm this specificity we tested if Heparin alone can give false positive results with 12 inert sera with different concentrations of unfractionated Heparin. No positive results were found in the ELISA test.A sensitivity of only 80% was found. We noticed that of the 9 false negative results 4 were less than 10% under the cut-off value. The sensitivity rises to 89% if these near positive results are included. We now also caution the clinician if the Heparin-PF4 ELISA result is less than 10% under the cut-off value. Furthermore, we are in the process of introducing the Heparin Induced Platelet Activation test (HIPA) as a specific functional test next to the Heparin-PF4 ELISA