



# Advances in clinical transfusion science

14 & 15 April 2011,  
Amsterdam, The Netherlands  
Venue: Royal Tropical Institute

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# Sanquin Spring Seminars

Advances in clinical  
transfusion science

Conference chair: Prof Anneke Brand MD PhD,  
Leiden, The Netherlands

April 14 & 15 2011

Royal Tropical Institute,  
Amsterdam, The Netherlands

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## Words of welcome

On behalf of Sanquin we welcome you to the fourth biennial Sanquin Spring Seminars. This year's theme 'Advances in Clinical Transfusion Science', puts the focus on our pivotal stake holders: patients and donors.

The program offers a special twist. Following the key note lecture 'Should your research be randomized or observational' we have asked all speakers to – where possible – include their view on methodology of clinical transfusion research. We hope to provoke a discussion on research methodology with transfusion indication and products, which may differ from classical clinical trials with pharmaceutical products.

Many new findings will be presented, possibilities for treatment in the near future as well as developments with impact that lay further ahead. Core of this symposium is the question how to show the effects of new products, new tests, and appropriate indications as well as (unintended) adverse effects in patients. Moreover, what can we ask and is safe to ask from donors.

We are very pleased that so many top scientists agreed to speak during this seminar and share their knowledge and views with you and each other. We are also pleased that again we are able to offer a platform for the younger generation of scientists in this field with a number of selected orals and the poster sessions.

An important part of seminars like this is meeting colleagues in between and after the sessions to stimulate scientific discourse and talk about what interest us all: offering the best of transfusion medicine to patients.

We think that the Scientific Committee succeeded in putting together an exciting scientific program, we hope you will enjoy.

Anneke Brand,  
*Conference Chair*

René van Lie, *Sanquin Executive Board*

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Anneke Brand MD PhD, Conference chair

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Scientific program,  
Thursday, 14 April 2011

09.00:	Registration, coffee & tea	12.10 - 12.45:	The effects of red blood cell storage on innate and adaptive immune responses: lessons from a mouse model <u>James Zimring</u> , Atlanta, United States
10.25:	Opening by Anneke Brand, Conference chair		
<b>Session I: Epidemiology in transfusion medicine</b>		12.45 - 14.30:	Lunch, posters and stands
10.30 - 11.15:	Key note lecture: Should your research be randomized or observational? <u>Ian Vandenbroucke</u> , Leiden, The Netherlands	<b>Session III: Donor health and safety</b>	
		14.30 - 14.50:	External validation of a prediction model for low hemoglobin levels in whole blood donors <u>Mireille Baart</u> , Nijmegen, The Netherlands
<b>Session II: Adverse reactions</b>		14.50 - 15.10:	Safety and vigilance of peripheral blood stem cell donors in The Netherlands, <u>Jo Wiersum</u> , Leiden, The Netherlands
11.15 - 11.50:	The epidemiology of transfusion-related acute lung injury <u>Nicole Juffermans</u> , Amsterdam, The Netherlands	15.10 - 16.30:	A pro-con debate <u>Brian O'Mahony</u> , Dublin, Ireland <u>Cees van der Poel</u> , Utrecht, The Netherlands
11.50 - 12.10:	Red blood cell allo-immunization after transfusion and pregnancy. <u>Henk Schonewille</u> , Leiden, The Netherlands		
		16.30 - 19.30:	Drinks, buffet, posters and stands

# Scientific programme, Friday 15 April 2011

08.45 - 09.30:	Registration, coffee & tea
<b>Session IV: Triggers and targets</b>	
09.30 - 10.05:	Current Status of Granulocyte (Neutrophil) Transfusion Therapy <u>W Conrad Liles</u> , Toronto, Canada
10.05 - 10.25:	The observation of bleeding complications in hemato-oncological patients; results of a pilot study (BOP: Bleeding Observation Pilot study) <u>Paula Ypma</u> , The Hague, The Netherlands
10.25 - 11.00:	Management & outcome in neonates with Rhesus hemolytic disease <u>Enrico Lopriore</u> , Leiden, The Netherlands
11.00 - 11.15:	Coffee break and stands
<b>Session V: Coagulation disorders</b>	
11.15 - 11.50:	Auto-antibodies to ADAMTS13 in patients with acquired TTP <u>Jan Voorberg</u> , Amsterdam, The Netherlands

11.50 - 12.25:	Bleeding, thrombosis and liver transplantation – risk factors and prevention <u>Jacques Pirenne</u> , Leuven, Belgium
12.25 - 12.45:	Glycoprotein IB-Alpha is a mechanoreceptor which clusters upon cold storage and mechanical forces <u>Eelo Gitz</u> , Utrecht, The Netherlands
12.45 - 14.00:	Lunch, posters and stands
<b>Session V: Coagulation disorders (continued)</b>	
14.00 - 14.20:	Uptake of blood coagulation factor VIII by dendritic cells is mediated via its C1 domain <u>Eszter Herczenik</u> , Amsterdam, The Netherlands
14.20 - 14.55:	Platelets: guardians of inflamed vasculature <u>Denisa Wagner</u> , Boston, United States

<b>Session VI: Alternatives for transfusion</b>	
14.55 - 15.30:	Transfusion alternatives in orthopedic surgery – the ‘Transfusie-Op-Maat-study (TOMAAT)’ <u>Cynthia So-Osman</u> , Leiden, The Netherlands
15.30 - 16.05:	Contra-indications of Epo <u>Martin Schipperus</u> , The Hague, The Netherlands
16.05 - 16.15:	Poster award ceremony and closing remarks
16.15 - 17.00:	Farewell reception

Abstracts of Sessions I-VI

Session I Thursday 14 April 2011, 10.30

Jan Vandenbroucke  
Leiden University Medical Centre, Leiden, The Netherlands

Key note lecture:  
Should your research be randomized or  
observational?

## Session II Thursday 14 April 2011, 11.15

Alexander Vlaar, Nicole Juffermans  
Academic Medical Centre, Amsterdam, The Netherlands

## The epidemiology of transfusion-related acute lung injury

This presentation will give an overview of the epidemiology of transfusion-related acute lung injury (TRALI). TRALI is defined as the acute onset of hypoxia with bilateral pulmonary infiltrates and no evidence of left ventricular overload, within 6 hours after transfusion. Two decades ago, TRALI was considered a rare complication of transfusion-medicine. Nowadays, TRALI has emerged as the leading cause of transfusion-related morbidity and mortality. The incidence of TRALI in the general hospital population and in specific patient populations such as critically ill patients will be discussed. Recent studies show that critically ill patients have an increased risk to develop TRALI. This finding may be explained by the two event hypothesis. The first event is the underlying condition of the patient resulting in priming of neutrophils. The second event is the transfusion of a blood product which activates the primed neutrophils, resulting in pulmonary edema. Transfusion factors associated with the onset

of TRALI include the presence of antibodies (human leukocyte antibodies and human neutrophil antibodies) in the transfused blood products and the age of cell-containing blood products transfused. Finally, the prognosis of TRALI will be discussed. Opposed to the traditional view, evidence is accumulating that TRALI has a significant impact on clinical outcome.

**Session II Thursday 14 April 2011, 11.50**

Henk Schonewille  
Dept of Tansfusion Medicine, Sanquin Reserearch and  
LUMC Jon J van Rood Centre for Transfusion Medicine.  
Leiden, The Netherlands

Red blood cell allo-immunization after  
transfusion and pregnancy



## Session II Thursday 14 April 2011, 12.10

James Zimring

**Emory University School of Medicine, Atlanta,  
United States**

# The effects of red blood cell storage on innate and adaptive immune responses: lessons from a mouse model

For decades it has been appreciated that the RBC storage lesion can have profound effects upon the biochemistry of the stored cells. Typically, such considerations have focused on the efficacy of the RBC transfusion from the standpoint of post-transfusion survival and oxygen delivery. However, recent findings in a reductionist animal model demonstrate that storage significantly increases the immunogenicity of stored RBCs. This effect is observed both at the level of activating innate immune responses and enhancing humoral adaptive immunity. In addition to influencing antibody production, the innate immune activation can have profound systemic sequelae, leading to medical morbidity distinct from subsequent antibody production. This presentation will summarize recent findings and present new data in a murine system. In addition, the initial extension of these

studies into the human setting will be described. In aggregate, these studies investigate how the storage lesion and the age of transfused units affects innate and adaptive immune responses to RBC alloantigens.

Session III Thursday 14 April 2011, 14.30

**AM Baart<sup>1</sup>, F Atsma<sup>1</sup>, WLAM De Kort<sup>1</sup>, KGM Moons<sup>2</sup>,  
Y Vergouwe<sup>2</sup>**

*1 - Dept of Donor Studies, Sanquin Research, Nijmegen,  
The Netherlands*

*2 - Julius Center for Health Sciences and Primary Care,  
Utrecht, The Netherlands*

External validation of a prediction model for  
low hemoglobin levels in whole blood donors

Background

Each year, around 5% of whole blood donors is being deferred from donation due to a low hemoglobin (Hb) level. A prognostic model that predicts the risk of low Hb levels has been developed and internally validated in whole blood donors. Before the model can be implemented in the blood bank practice, its ability to produce accurate predictions in a group of independent donors needs to be assessed. In this study, we externally validated the prediction model for low Hb levels in whole blood donors.

Donors and methods

The prognostic model contains the following predictors: sex, seasonality, Hb level measured at the previous visit, difference in Hb level between the previous two visits, time since the previous visit, deferral at the previous visit and the total number of whole blood donations in the past two years. For the external validation, data from 220,946 Dutch whole blood donors were used. Donors donated whole blood in the period 2007-2009. In this population, 12,865 donors (6 %) had a low Hb level. The predictive performance of the model was assessed in terms of calibration and discrimination (area under the receiver operating characteristic (ROC-) curve (AUC).

Results

The calibration slope was 0.95 (ideal value is 1). Predicted risks were in general too low (the calibration intercept was 0.63; ideal value is 0). Discrimination of the model was good. The AUC was 0.84, which is only slightly lower than the AUC after internal validation of the model (0.87).

Conclusion

The previously developed prediction model provided risks of low Hb levels that were too low. A simple adaptation of the model intercept will result in good calibrated predictions. The model

predictions may then be useful to determine whether donors can be invited for a next donation, or whether some interventions such as postponement of the invitation, are warranted. Eventually, the model might be helpful in decreasing the number of Hb deferrals in whole blood donors.

Sessie III Thursday 14 April 2011, 14.50

Jo Wiersum-Osselton

Dept of Tansfusion Medicine, Sanquin Reserearch and  
LUMC Jon J van Rood Centre for Transfusion Medicine,  
Leiden, The Netherlands

Safety and vigilance of peripheral blood stem  
cell donors in The Netherlands

**Session III Thursday 14 April 2011, 15.10**

Brian O’Mahony  
Irish Haemophilia Society, Dublin, Ireland  
Cees van der Poel

Julius Center for Health Sciences and Primary Care,  
Utrecht, The Netherlands

Donor Health and Safety: a pro-con debate

Session IV Friday 15 April 2011, 09.30

W Conrad Liles  
University of Toronto, Toronto, Canada

Current Status of Granulocyte (Neutrophil) Transfusion Therapy

The neutrophil is the initial and most important component of innate host defense against bacterial and opportunistic fungal infections. Neutropenia is the leading factor limiting dose escalation of cancer chemotherapy and represents a major cause of morbidity and mortality in hematopoietic stem cell transplantation (HSCT). The provision of ‘normal’ neutrophils via granulocyte transfusion therapy would appear to be a rational approach to prevent or treat infections in individuals with significant and/or prolonged neutropenia and in individuals with congenital disorders of neutrophil function. The efficacy of granulocyte transfusion therapy appears to relate, at least in part, to both the quantity and quality of neutrophils transfused to the recipient. The potential clinical utility of granulocyte transfusion therapy has been substantially improved by the use of recombinant human granulocyte colony-stimulating factor (G-CSF) to stimulate healthy human donors and increase the

yield of neutrophils obtained by centrifugation leukapheresis. Both related donors and non-related community donors stimulated with G-CSF +/- glucocorticoid have been used in clinical granulocyte transfusion therapy programs. In general, leukapheresis products used for granulocyte transfusion therapy should be transfused as rapidly as possible following collection. However, temporary storage at 10°C has been shown to preserve neutrophil functional properties *in vitro*. Importantly, definitive clinical benefits of granulocyte transfusion therapy remain controversial in the absence of data from a randomized, controlled clinical trial.

**Session IV Friday 15 April 2011, 10.05**

**P. Ypma, O Eissen, R van Wordragen, R Middelburg, M Coccoris, R Fijnheer, E Beckers, L van de Watering, P van der Meer, J van Hilten, JL Kerkhoffs, A Brand**  
Haga Hospital, The Hague & Dept of Transfusion Medicine, Sanquin Research, Leiden, The Netherlands

The observation of bleeding complications in hemato-oncological patients; results of a pilot study (*BOP: Bleeding Observation Pilot study*)

Patients with hemato-oncological diseases receiving myelosuppressive chemotherapy or undergoing autologous stemcell transplantation therapy need platelet transfusions in certain phases of their treatment. The reported percentage of patients experiencing bleeding complications is highly variable, ranging from 10 – 90%. This variability poses a major problem for designing clinical platelet transfusion trials using bleeding complications as a primary endpoint. Recently, a platelet transfusion trial studying the hemostatic efficacy of transfused platelets treated with a novel pathogen reduction technique was initiated (PREPAREs; Pathogen Reduction Evaluation & Predictive Analytical Rating Score). Bleeding complications are

the primary outcome of this study. In the BOP study bleeding complications were noted using a strict bleeding observation strategy. Adjudication of bleeding scores was applied according to the WHO criteria (1-4). Primary objective of this pilot study was the assessment of the percentage of patients with grade 2 or more bleeding (WHO grading).

In total, four hematological centers participated in the study, two academic centres and two top clinical non-academic centres.

Daily assessment of bleeding symptoms was performed by trained- and/or research staff members.

A total of 68 patients were enrolled at four sites, the median follow up consisted of twenty days. The frequency of bleeding of all grades was 87% and of grade  $\geq 2$  bleeding it was 54%. Only 7% of patients suffered from a grade 3 or 4 bleeding and 1 patient died from a bleeding complication. The percentage of days with bleeding of grade  $\geq 2$  for patients on study was 18%. Multivariate analysis showed two independent variables in the occurrence of bleeding complications: the presence of acute leukemia and a low platelet nadir (count) during hospital admittance. The results of the study show bleeding incidences that are comparable to other studies in the 'platelet transfusion literature' using a rigorous bleeding observation strategy. Inter-center variability in our four centres was not statistically significant with regard to bleeding incidences.

Session IV Friday 15 April 2011, 10.25

Enrico Lopriore  
Leiden University Medical Center, Leiden, The Netherlands

Management & outcome in neonates with  
Rhesus hemolytic disease

**Session V Friday 15 April 2011, 11.15**

**Jan Voorberg**  
Dept of Plasma Proteins, Sanquin-AMC Landsteiner  
Laboratory, Amsterdam, The Netherlands

## Auto-antibodies to ADAMTS13 in patients with acquired TTP

## Background

The majority of patients diagnosed with thrombotic thrombocytopenic purpura (TTP) have autoantibodies directed towards the spacer domain of ADAMTS13.

## Design and methods

In this study we explored whether Arg568 and Phe592 contribute to an antigenic surface in the spacer domain. The epitope specificity of anti-spacer domain antibodies was examined using two recombinant patient-derived antibodies, I-9 and II-1, and plasma of 48 patients with acute acquired TTP by means of immunoprecipitation of ADAMTS13 variants containing single or multiple alanine substitutions. Using similar methods, we also determined the presence of anti-TSP2-8 and CUB1-2 domain antibodies in this cohort of patients.

## Results

Anti-TSP2-8 and anti-CUB1-2 domain directed antibodies were present in 17% and 35% of patient samples analyzed, respectively. Analysis of anti-spacer domain antibodies revealed that Arg568 and Phe592, in addition to residues Arg660, Tyr661, and Tyr665, also contribute to an antigenic surface in the spacer domain. The majority of patients (90%) lost the reactivity towards the spacer domain following introduction of multiple alanine substitutions at Arg568, Phe592, Arg660, Tyr661 and Tyr665. Conclusions: IgG directed towards a single antigenic surface comprising residues Arg568, Phe592, Arg660, Tyr661 and Tyr665 predominate in plasma of patients with acquired TTP.



**Session V Friday 15 April 2011, 11.50**

**Jacques Pirenne**  
University Hospitals Leuven, Leuven, Belgium

## Bleeding, thrombosis and liver transplantation

Liver Transplantation (LTx) has become the first line treatment in patients with end stage liver failure. Liver disease is associated to both bleeding and thrombotic disorders. A leading cause of bleeding in patients with chronic liver disease is the portal hypertension and the rupture of esophageal varices. Treatment is local (sclerosis of the varices) and beta blockade to reduce the portal pressure. Chronic partial or complete portal vein thrombosis (PVT) can also be seen in LTx candidates. PVT was an absolute contra indication to LTx but new surgical techniques (thrombectomy, bridge graft, cavoportal transposition, multivisceral transplants) have been developed to allow LTx even in face of PVT. Acute PVT can also develop in patients with liver disease and may require anticoagulation.

LTx remains a major surgical operation with major risk of bleeding, particularly in patients with previous abdominal surgery, retransplants, poor coagulation. Transfusion requirement

during LTx correlates with a poorer outcome. Strategies to reduce bleeding during LTx are thus essential and include: cell saver, meticulous surgery, use of argon coagulation, maneuvers to reduce portal hypertension (TIPS, venovenous bypass, anesthetic management), perioperative correction /support of coagulation (FFP, PPSB, Platelets, Fibrinogene, Anti fibrinolytics, others), use of local biological glues and absorbable hemostatic agents. Bloodless LTx is possible in a substantial percentage of patients. PreTx administration of EPO and even spleen embolization have been performed in some Jehovah witnesses to allow transfusion-free LTx.

Bleeding post LTx is a frequent cause of revision particularly in patients with primary graft poor function. Early hepatic artery thrombosis (HAT) post Tx is a rare (2-5%) but dreadful complication since it frequently leads to graft loss and necessitates reTx. Local repair and local fibrinolysis have been attempted, sometimes successfully. Surprisingly – considering the magnitude of the surgery sustained – general thromboembolic complications (thrombophlebitis, lung embolism) are rarely observed after LTx.

Session V Friday 15 April 2011, 12.25

**E Gitz<sup>1</sup>, H Deckmyn<sup>2</sup>, CA Koekman<sup>1</sup>,  
DJ van den Heuvel<sup>3</sup>, HC Gerritsen<sup>3</sup>,  
JW Akkerman<sup>1</sup>**  
*1 - University Medical Center Utrecht, Utrecht, The Netherlands*  
*2 - Katholieke Universiteit Leuven, Kortrijk, Belgium*  
*3 - Universiteit Utrecht, Utrecht, The Netherlands*

**Glycoprotein IB-ALPHA is a mechanoreceptor which clusters upon cold storage and mechanical forces**

**Background**  
Current protocols for storage of platelet concentrates (PCs) recommend a temperature of 22-24°C and a maximum of 7 days. Problems of the relative high temperature are the growth of bacteria which occasionally contaminate PCs and the platelet viability and function decline known as platelet storage lesion. Improvements are sought in 0°C storage, but this condition changes glycoprotein (GP) Iba, the receptor for von Willebrand Factor (VWF), starting platelet activation and apoptosis. Causes of the GPIbα change might be loss of sialic acid exposing underlying sugars that trigger clustering between adjacent GPIbα

ectodomains, and mechanical forces applied during platelet handling since GPIb is a sensitive mechanoreceptor.

**Methods**  
We labelled 50% of GPIb with Alexa Fluor-488 conjugated Fab-fragments and 50% with Alexa Fluor-594 conjugated Fab-fragments of 6B4 antibody against the VWF binding site and measured Förster Resonance Energy Transfer (FRET) by time-gated Fluorescence Lifetime Imaging Microscopy (FLIM). This technique allows determination of co-localization on a scale of 1- 10 nm.

**Results**  
Fresh platelets showed a FRET efficiency of 1.8±0.9% (n=3), indicating that resting platelets showed little GPIbα-GPIbα interactions. Cold storage (24 hrs, 0°C) increased this value to 10±0.6% which remained constant during subsequent rewarming (37°C). A shear stress found in microcapillaries (10,000 s-1) raised FRET efficiency of fresh platelets to 9.9±0.5%, and of cold-stored platelets to 18.5±0.8%. Coverage of exposed sugars with galactose or N-acetyl-Dglucosamine but not with glucose blocked the increase in FRET efficiency. The change in FRET efficiency occurred without VWF binding or GPIbα shedding.

**Conclusions**  
Cold storage and hydrodynamic forces induce GPIbα-GPIbα associations and both treatments additively increase FRET efficiency. Attempts to improve transfusion with cold-stored platelets should focus on blockade of GPIbα-GPIbα associations.

Session V (continued) Friday 15 April 2011, 14.00

**Eszter Herczenik<sup>1</sup>, Simon D van Haren<sup>1</sup>, Aleksandra Wroblewska<sup>1</sup>, Alexander B Meijer<sup>1</sup>, Luisa Martinez-Pomares<sup>2</sup>, Anja Ten Brinke<sup>3</sup> and Jan Voorberg<sup>1</sup>**

- 1 - Dept of Plasma Proteins, Sanquin-AMC Landsteiner Laboratory and Van Creveld Laboratory, Amsterdam, The Netherlands
- 2 - School of Molecular Medical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, United Kingdom
- 3 - Dept of Immunopathology, Sanquin-AMC Landsteiner Laboratory, Amsterdam, The Netherlands

Uptake of blood coagulation factor VIII by dendritic cells is mediated via its C1 domain

**Background**  
Uptake and processing of FVIII by antigen presenting cells and subsequent presentation of FVIII-derived peptides to CD4+ T cells directs the immune response to FVIII in patients with hemophilia A. Multiple receptors including mannose receptor (MR) and LDL receptor related protein-1 (LRP) have been implicated in FVIII uptake.

**Objective**  
This work studies the involvement of receptor candidates in FVIII uptake by dendritic cells. Furthermore, we explore FVIII residues that mediate endocytosis.

**Methods**  
FVIII uptake was performed with human monocyte derived and murine bone marrow derived dendritic cells. To investigate FVIII endocytosis, competition assays with soluble receptor ligands, binding studies with recombinant receptor fragments, and siRNA-induced gene silencing were performed. Additionally, FVIII targeting monoclonal antibodies KM33 and VK34 were used. To confirm *in vitro* results, hemophilic E17KO mice were pre-treated with antibodies prior to FVIII injections and anti-FVIII titers were determined.

**Results**  
Upon treatment of DCs with mannan or LRP ligand  $\alpha$ 2-macroglobulin, we only observed a minor decrease in FVIII internalization. Additionally, siRNA mediated knockdown of LRP, MR or DC-SIGN expression in MDDCs did not prevent FVIII uptake. Binding studies using Fc-chimeras revealed that LRP, DC-SIGN and MR can bind to FVIII; however we did not observe critical role for these receptors in FVIII uptake. Previous studies

have shown that human antibodies targeting C1 (KM33) and A2 (VK34) domains of FVIII interfere with binding to endocytic receptors. Preincubation of FVIII with VK34 did not influence FVIII uptake; however, KM33 completely inhibited FVIII endocytosis by both MDDC and BMDC. Accordingly, anti-FVIII antibody titers were greatly reduced following pre-administration of KM33 *in vivo*.

**Conclusion**  
Together, our observations emphasize the physiological significance of KM33- targeted residues within the C1 domain in the uptake of FVIII by DCs *in vitro* and *in vivo*.

**Session V (continued) Friday 15 April 2011,**  
14.20

**Denisa Wagner**  
Immune Disease Institute, Boston, United States

## Platelets: guardians of inflamed vasculature

In 2006, we reported that platelets support experimental angiogenesis and that nascent vessels bleed in thrombocytopenia (Kisucka et al., PNAS 2006). Because angiogenesis is accompanied by recruitment of inflammatory cells, we proceeded to study mature vessels at sites of local inflammation in low or normal platelet count. We observed that within minutes of onset of thrombocytopenia microcirculation of the inflamed skin began to bleed. Similarly, inflamed organs began to hemorrhage in thrombocytopenia (Goerge et al., Blood 2008). Platelets deficient in major adhesion receptors could rescue the phenotype and prevent bleeding. These receptors are all crucial in the hemostatic process, indicating that it is a special function of the platelets, different from platelet plug formation, which maintains vasculature during inflammation. Both angiogenesis and inflammation are involved in tumor progression in cancer. In experimental cancer models, we observed that platelet depletion

causes almost immediate bleeding specifically of tumor vessels (Ho-Tin-Noe et al., *Circ Res* 2008). This protective function of platelets was linked to platelet granule release, as degranulated platelets could not rescue the tumor from bleeding. It is the leukocyte recruitment at the site of inflammation or in tumor that causes the hemorrhaging in thrombocytopenia (Ho-Tin-Noe et al., *Am J Pathol* 2009). Recently we found that induction of tumor bleeding by thrombocytopenia makes the tumor more accessible to chemotherapy (Demers et al., *Cancer Res* 2011), resulting in elevated apoptosis of tumor cells and decreased cancer growth.

## Session VI Friday 15 April 2011, 14.55

Cynthia So-Osman

Dept of Transfusion Medicine, Sanquin Research,  
Leiden, The Netherlands

## Transfusion alternatives in orthopedic surgery – the ‘Transfusie-Op-Maat-study (TOMAAT)’

## Introduction

Methods to reduce red blood cell (RBC) transfusions for surgical procedures are increasingly applied. However, how these compare among themselves and with a restrictive transfusion trigger is not known.

## Methods

A randomized, multi-center study in elective knee or hip arthroplasty patients comparing the effect of erythropoietin (Epo), cell-saver (CS) or postoperative drain re-infusion systems (DR) on allogeneic RBC sparing while using a restrictive transfusion policy. Patients were stratified in two groups by preoperative hemoglobin (Hb) level: 10 to 13 g/dL (low Hb) stratum I and randomized for Epo; above 13 g/dL (normal Hb) stratum II and ineligible for Epo.

Both strata were randomized for CS, DR or no blood salvage device. Primary outcome was number of RBC transfusions.

## Results

Transfusion protocol adherence was above 95%. Mean RBC use was 0.3 (SD 1.2) units / patient (n=2442). 11.6% were transfused. In Intention-To-Treat analysis, Epo resulted in a 50 % reduction in the proportion transfused patients (OR 0.5 [0.35 - 0.75]) and a 29% mean RBC reduction (0.71[0.42 - 1.13]; p=0.15). Autologous blood re-infusion did not result in a significant blood-sparing or transfusion-avoiding effect, however, when analyzed as-treated, a reduction from 9.0 to 6.6 % (OR 0.7 [0.5 - 1.0]; p=0.05) in patients with normal pre-operative Hb was found.

## Conclusion

A restrictive transfusion trigger has an important effect on blood-sparing. In anemic patients, Epo showed a significant benefit as a transfusion-avoiding rather than a blood-sparing strategy. In preoperative non-anemic patients, autologous blood re-infusion showed a small effect on transfusion avoidance (Current Controlled Trials number, ISRCTN 96327523; The Netherlands Trial Number, NTR303).

## Session VI Friday 15 April 2011, 15.30

Martin R Schipperus

Dept of Hematology, Haga Teaching Hospital,  
The Hague and Dutch National Hemovigilance Office TRIP,  
The Netherlands

## Alternatives for transfusion in adult patients with cancer: the use of epoetin and intravenous iron

The two alternatives for red blood cell (RBC) transfusion in adult patients with cancer are epoietin and intravenous iron. The use of erythropoiesis-stimulating agents (ESAs) has consistently been shown to reduce RBC transfusions and increase the hemoglobin (Hb) level in patients with anemia that arises during or shortly after myelotoxic chemotherapy. However, awareness has grown of risks associated with ESAs, including increased mortality, venous thromboembolism, tumor progression and stroke. Guidelines such as the recently revised ASH/ASCO guideline and the EORTC guideline address the questions clinicians are faced with such as : 1. What are the defining features of patients with a malignancy who are appropriate candidates for ESA treatment? And 2. What are the optimal approaches for these patients? The

guidelines recommend for patients undergoing myelosuppressive chemotherapy who have a Hb level less than 10 g/dl, that clinicians discuss potential harms (e.g. thromboembolism, shorter survival) and benefits (e.g. decreased transfusions) of ESAs and compare these with potential harms (e.g. serious infections, immune-mediated adverse reactions) and benefits (e.g. rapid Hb improvement) of RBC transfusions. ESAs should be avoided in patients with cancer not receiving concurrent chemotherapy, except those with lower risk myelodysplastic syndromes. Most of the guidelines state that the underlying cause of anemia (e.g. absolute or functional iron deficiency) should be treated prior to ESA use. Therefore, a recent shift in focus towards the use of i.v. iron therapy either alone or in combination with ESAs in patients with cancer has occurred. Randomized controlled trials of i.v. iron in combination with ESAs have demonstrated an improved Hb response rate, reduced need for RBC transfusions, reduced mean weekly ESA dose and improved quality of life in patients treated with i.v. iron compared with those treated with oral iron or those without iron supplementation. Recent data suggest that i.v. iron alone is also effective in patients with cancer and anemia. This supports previous data indicating that i.v. iron therapy alone may be sufficient to correct anemia and reduce the need of RBC transfusions.

Posters

Numbers refer to the poster board,  
abstracts of orals may be found art the sessions section.

Poster number	Title	Presenter
1	Measurement of neopterin levels confirm immune-stimulation by postoperatively salvaged autologous blood transfusion after total knee joint replacement surgery	Nahidul Islam
2	Psycho-cognitive determinants of adverse events and subjective severity at first-time blood donation	Anne van Dongen
3	Regulated effector functions of human IgG through glycosylation and its impact on platelet clearance	Rick Kapur
4 (oral)	External validation of a prediction model for low hemoglobin levels in whole blood donors	Mireille Baart
5	Serum levels of IL-10 ,IL-12 and IL-17a in occult HBV donors	Ali Akbar Pourfathollah
6 (oral)	The observation of bleeding complications in hemato-oncological patients; results of a pilot study (BOP: Bleeding Observation Pilot study	Paula Ypma
7	Evaluation of red cell transfusion practices in patients undergoing elective surgical procedures at a superspeciality center in India	Nitin Agarwal
8	A comparison of the transfusion efficiency of volume-reduced apheresis platelet concentrates with standard apheresis platelets concentrates	Áine Honohan
9 (oral)	Glycoprotein IB-alpha is a mechanoreceptor which clusters upon cold storage and mechanical forces	Eelo Gitz

Poster number	Title	Presenter
10 (oral)	Uptake of blood coagulation Factor VIII by dendritic cells is mediated via its C1 domain	Eszter Herczenik
11	The shear stress-induced transcription factor KLF2 affects dynamics and angiopoietin-2 content of Weibel-Palade bodies	Ellen van Agtmaal
12	Residues Arg568 and Phe592 contribute to an antigenic surface for anti-ADAMTS13 antibodies in the spacer domain	Nicoletta Sorvillo
13	Using BCSI pH1000 to find platelet concentrates with low pH or bacterial contamination	Margriet Dijkstra-Tiekstra
14	Production of platelets at university hospital Brno, Czech Republic	Hana Lejdarová
15	Development of IFN-γ-catch assay after mixed lymphocyte reaction to investigate the mechanism of graft predominance after double cord blood transplantation.	Ben Meijer
16	Establishment of the first who international repository for transfusion relevant bacteria strains-isbt working party transfusion-transmitted infectious diseases (wp-ttid), subgroup on bacteria	Melanie Stoermer
17	Persistent anti-D production is maintained by fetal RhD microchimerism	Esther Verduin

**1. Measurement of Neopterin levels confirm immune-stimulation by postoperatively salvaged autologous blood transfusion after total knee joint replacement surgery**

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**Introduction**  
Following major surgery immunosuppression increases susceptibility to acquired hospital infections. But we showed that this can be reversed by transfusion with autologous salvaged blood (Lancet 2004; 363: 1025–30). Oppositely, transfusion with banked blood significantly increases immunosuppression and consequent threat of infection. In that study, as biomarkers of innate immunity Natural Killer cell precursor (NKpf) levels and Interferon-gamma synthesis by peripheral blood mononuclear cells (PBMC) were used, which though informative but costly and time-consuming. Hence, we hypothesize that levels of a plasma protein named ‘neopterin’, which reflects the activation of macrophages, would be employed as a more practical measure of postoperative immune status.

**Methods**  
40 patients undergoing primary total knee joint replacement gave informed consent for inclusion in this group study. Of these patients, twenty two received autologous salvaged blood transfusion (170-480 ml) within the first 6 hours postoperatively and eighteen received no blood transfusion. Postoperatively salvaged blood was collected into acid citrate dextrose (ACD) as anticoagulant and reinfused using a Dideco 797 recovery device. Not any of the patients received banked allogenic blood transfusions. Blood samples were collected preoperatively on the day of operation prior to anesthetic procedures and 2-5 days postoperatively. Plasma was stored and preoperative and postoperative pairs were measured for neopterin levels using ELISA and expressed as ng/ml. Statistical results were analyzed by SPSS.

**Results**  
The rises in neopterin levels after total knee joint replacement surgery patients ranged from -0.312 to 4.286 (ng/ml). In patients who received salvaged blood transfusion there was a significant increase in mean postoperative neopterin levels from 1.77 to 3.15 ng/ml (p=0.002), with 11 out of 22 (50%) showing increments of greater than 1.00 ng/ml. In patients without any transfusions there was a non significant increase in postoperative neopterin

levels from 2.03 to 2.79 ng/ml (p=0.168) with 3 out of 18 (17%) showing increments of greater than 1.00 ng/ml. These concluding were supposed attributable to alternative causes of macrophage activation such as infections or inflammation.

**Conclusions**  
This current study widens our previous experiments that postoperatively salvaged autologous blood transfusion reverses postoperative immunosuppression. Whereas immunestimulation was showed previously by increased NKpf and IFN-gamma synthesis in PBMC, we display here that this immune-stimulation is accompanied by activation of macrophages. The above results confirm the possible benefits of unwashed salvaged blood transfusion for increasing resistance against postoperative infections.



## 2. Psycho-cognitive determinants of adverse events and subjective severity at first-time blood donation

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## Background

It is known from previous research that experiencing an adverse event reduces retention of blood donors. More importantly, we recently found that the subjective severity of the event has a high impact on maintenance of donation behavior. We studied the psycho-cognitive determinants of adverse events and subjective severity in new blood donors.

## Methods

We sent new blood donors (N=1783) a questionnaire measuring dispositional (e.g. conscientiousness, anxiety) and social cognitive (e.g. intention, attitude) factors before their first donation. After their first donation, we measured adverse events and subjective severity.

## Findings

The results indicate that needle insertion reactions are predicted by anxiety (Beta= 1.272, p=.001). In addition, donors with a lower affective attitude (who think donating regularly will be unpleasant), experience more (pre-)syncope (Beta=.971, p=.002). Finally, donors who are more anxious, have a lower affective attitude or higher conscientiousness found the adverse events they experienced more severe (Beta's = .105 - .137, p's<.05).

## Discussion

Results indicate that anxiety and affective attitude have an influence on the occurrence of adverse events. Moreover, the subjective severity of the event is higher for more anxious, more conscientious donors who think donating regularly will be unpleasant. These results can be used to design interventions to retain new blood donors.

### 3. Regulated effector functions of human IgG through glycosylation and its impact on platelet clearance

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Transfusion reactions caused by alloantibodies are the most important side effect following platelet transfusions. Antibodies against the same target cells formed during pregnancies (Fetal and neonatal alloimmune thrombocytopenia; FNAIT) can lead to life threatening bleeding reactions. Our aim is to determine what signifies a clinically relevant IgG-response against platelets, especially with regards to regulation of antigen-specific IgG-glycosylation. Various biological activities of immunoglobulin G (IgG) including antibody-dependent cellular cytotoxicity (ADCC) can be modulated by the structural feature of the N-glycan in the Fc part. The extent of sialylation, galactosylation, and in particular fucosylation has been described to affect the binding of IgG to Fc receptors (Fc R). By analyzing the Fc-glycosylation of the

pathogenic IgG formed during pregnancy against human platelet antigens (HPA) of the fetus using mass spectrometry, we found markedly decreased levels of core-fucosylation compared to the total IgG1 in the same patients. Other milder differences were also observed with increased levels of galactosylation and sialylation as compared to glycosylation patterns of total serum IgG1 of the same patients. These studies indicate that IgG glycosylation is regulated at a clonal level in patients that influences their ADCC activity which may have a profound effect on disease severity and prognosis. Studies in large patient cohorts will have to be performed to establish such correlations. Moreover, experiments in animal models as well as *in vitro* immunological tests will be needed to unravel the mechanisms regulating IgG Fc-glycosylation.

5. Serum levels of IL-10 ,IL-12 and IL-17A in occult HBV donors

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Occult hepatitis B infected (OBI) can not completely eradicate hepatitis B virus-DNA (HBV-DNA) from their liver and peripheral blood and because this individual is HBS Ag negative it is a problem for transfusion centers. The main aim of this study was to investigate the Interleukin (IL)-10,IL12 and IL-17A serum levels in the donors with OBI. In this observational study, plasma samples of 3700 blood donors were tested for hepatitis B surface antigen (HBsAg) and antibodies to the hepatitis B core antigen (anti-HBc), using enzyme-linked immunosorbent assay (ELISA). The HBsAg-/anti-HBc+ samples were selected and screened for HBV-DNA, using the polymerase chain reaction (PCR). HBV-DNA positive samples were assigned as OBI cases and IL-10 ,IL-12 and IL-17 serum levels were detected using ELISA.

The results demonstrated that, 352 (9.5%) out of 3700 blood samples were HBsAg-/anti-HBc+ and HBV-DNA was detected in 57/352 (16.1%) of the HBsAg-/anti-HBc+ samples. Our

results showed that the IL-10 and IL-17A serum levels increased significantly in the OBI cases in comparison to the controls (P < 0.001) but there was no significant difference of IL-12 in OBI cases comparison to the controls. According to the results of this study the higher level of IL-10 production may suppress the functioning of the immune system against HBV in OBI patients. The elevated IL-17A serum level also indicates a long period of infection in the patients produce enough IL-12 may be contribute in clear of viruses and persistence virus in OHB.

7. Evaluation of red cell transfusion practices in patients undergoing elective surgical procedures at a superspeciality center in India

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Introduction

There is a great tendency in most departments of surgery to request more units of blood for elective procedures than is actually required. Many units of blood routinely ordered by surgeons are not utilized but are held in reserve and thus are unavailable to needy patients. Therefore, it is necessary to streamline blood ordering and transfusion practices. Continuous monitoring of transfusion activity as well as implementation and optimization of transfusion strategies may help to improve transfusion practice and reduce or eliminate this large variability across transfusion practices.

Aim of study

- 1. To assess pattern of red cell transfusion support in patients undergoing elective surgery at our institute.
- 2. To assess blood ordering practices in surgical departments.

Material and methods

This study was an audit of blood usage for patients who underwent elective surgery at our institute between Nov 2007 and Oct 2009. Clinico-pathological and laboratory data for these patients was obtained from the Hospital Information System (HIS) as well as patient’s medical records. C/T ratios, Transfusion Probability & Transfusion Index were calculated for each surgery.

Results

A total of 2217 units were cross matched for 834 patients, of which only 907 (40.9%) PRBC units were utilized while remaining 1310 (59.1%) units were received back unutilized. Cardiac surgeries like CABG, septal repair and valve surgeries used blood optimally without much wastage. Same pattern of blood usage was observed in other surgeries like Gastrectomy, Whipple's procedure, Exploratory laparotomy and Nephrectomy. As per the transfusion indices, blood was not used optimally in surgeries like Thyroidectomy, PCNL, Prostatectomy, Craniotomy and TURP. Surgeries like laparoscopic Cholecystcetomy, Genitoplasty, VVF repair utilized blood inefficiently as indicated by all transfusion indices with TP< 30,TI < 0.5 and CTR>2). A significant number of patients were overtransfused (post transfusion Hct>33).

Conclusion

Enormous variability still exists in the use of blood for surgical procedures and overordering is a common occurrence. There is a great need to develop clinically useful criteria to assess transfusion need and blood should be ordered according to a MSBOS for operations with higher transfusion requirements. Red cell cross matching pattern in surgeries.

8. A comparison of the transfusion efficiency of volume-reduced apheresis platelet concentrates with standard apheresis platelets concentrates

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Background

For more than 35 years our Blood Bank has carried a volume-reduced (VR) platelet product with 15 fold reduced plasma content and 15 fold higher platelet concentration than the standard product, which can be rapidly transfused within 5 minutes with the potential of minimizing the adverse reactions due to plasma. Here we compared the clinical efficiency of VR apheresis platelet concentrates (APC) with that of standard APC's, by corrected counts increments (CCI), time-to-next-transfusion and frequency of adverse transfusion reactions.

Study Design and Method

We performed a single center cohort study among consecutive patients who received either standard APC and or VR-APC HLA/

HPA matched platelets between 1994 and 2008. Using generalized estimating equations 851 transfusions from 68 patients were analyzed for CCI's and 731 transfusions from 64 patients for time-to-next-transfusion. The frequency of adverse reactions was compared between the groups.

Results

The adjusted 1-hour CCI was 23% (95% Confidence interval: 9 to 42) lower and the 24-hr 17% (95% CI: -11 to 59) lower after VR-APC's platelets. The mean time-to-next-transfusion was similar APC's 3.1 days (95% CI: 2.7 to 3.5); VR-APC's 2.8 days (95% CI 2.4 to 3.5). We observed a 48% (95% CI; -52 to 207%) risk reduction in adverse reactions due to volume reduction.

Conclusion

VR-APC show a lower 1 hr. and 24-hr transfusion response than standard-APC which was not reflected in the time-to-next-transfusion. Plasma reduction reduces the incidence of adverse reaction.

## 11. The shear stress-induced transcription factor KLF2 affects dynamics and angiopoietin-2 content of Weibel-Palade bodies

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Endothelial cells contain rod-shaped organelles designated Weibel-Palade bodies (WPBs) that release their content into the vascular lumen following stimulation with agonists such as thrombin or epinephrine. In this study we investigated the effect of the shear stress-induced transcription factor KLF2 on clustering and composition of WPBs using peripheral blood derived endothelial cells. Lentiviral over-expression of KLF2 resulted in a 4.5 fold increase in number of WPBs per cell when compared to mock-transduced endothelial cells. Unexpectedly, the average length of WPBs was significantly reduced. In mock transduced endothelial cells WPBs had an average length of 1.8  $\mu\text{m}$  whereas in KLF2 overexpressing cells WPBs had a average length of 1.4  $\mu\text{m}$ . Overexpression of KLF2 abolished the perinuclear clustering of WPBs observed following stimulation with cAMP-raising

agonists such as epinephrine. We previously hypothesized that perinuclear clustering of WPBs provides a means to limit excessive release of bioactive components from these organelles. We subsequently explored whether storage of P-selectin and angiopoietin-2 (Ang-2) in WPBs is affected by KLF2. P-selectin contributes to the transmigration of leukocytes whereas Ang-2 has been shown to promote vascular leakage and endothelial cell migration thereby contributing to vascular remodeling. P-selectin was readily visualized in both KLF2 and mock-transduced endothelial cells. In contrast, confocal microscopy revealed that WPBs in KLF2-transduced cells did not contain Ang-2. Together our findings suggest show that KLF2 not only regulates the size and dynamics of WPBs but also regulates the contents of this highly versatile storage pool in endothelial cells.

## 12. Residues ARG568 and PHE592 contribute to an antigenic surface for antiADAMT513 antibodies in the spacer domain

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## Background

The majority of patients diagnosed with thrombotic thrombocytopenic purpura (TTP) have autoantibodies directed towards the spacer domain of ADAMTS13.

## Design and methods

In this study we explored whether Arg568 and Phe592 contribute to an antigenic surface in the spacer domain. The epitope specificity of anti-spacer domain antibodies was examined using two recombinant patient-derived antibodies, I-9 and II-1, and

plasma of 48 patients with acute acquired TTP by means of immunoprecipitation of ADAMTS13 variants containing single or multiple alanine substitutions. Using similar methods, we also determined the presence of anti-TSP2-8 and CUB1- 2 domain antibodies in this cohort of patients.

## Results

Anti-TSP2-8 and anti-CUB1-2 domain directed antibodies were present in 17% and 35% of patient samples analyzed, respectively. Analysis of anti-spacer domain antibodies revealed that Arg568 and Phe592, in addition to residues Arg660, Tyr661, and Tyr665, also contribute to an antigenic surface in the spacer domain. The majority of patients (90%) lost the reactivity towards the spacer domain following introduction of multiple alanine substitutions at Arg568, Phe592, Arg660, Tyr661 and Tyr665.

## Conclusions

IgG directed towards a single antigenic surface comprising residues Arg568, Phe592, Arg660, Tyr661 and Tyr665 predominate in plasma of patients with acquired TTP.





**14. Production of platelets at university hospital Brno, Czech republic**

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**Introduction**

University Hospital Brno is the biggest producer of platelets in Czech republic. Due to an extensive oncologic and traumatologic care it is one of the biggest consumers of these products.

**Method**

We monitored platelet production at University Hospital Brno from 2002 to 2010 in the prescribed parameters of quality control - the pH, the concentration and the amount of platelets in transfusion products. From 2002 to 2007 outbalanced production of apheretic platelets. Post storage leukocyte – depletion of apheresis platelets was done in regime on demand before issue of platelets to clinical use. Leucodepleted platelets fluctuated from 43% of whole platelet production in 2002 to 37% in 2007 respectively. 100% pre storage leukocyte - depletion of platelets was adopted in 2008. Accretive requirements from clinical workplaces required implementation of production of platelets from buffy coat resuspended in platelet additive solutions in addition to apheretic platelets. From December 2010 we stopped

apheresis of platelets into plasma. This step accomplished the transition to platelet production resuspended only in platelet additive solution.

**Results**

Measured of platelet concentrations depends on the type of transfusion product. Apheretic platelets had optimal and equable values during a monitored period. Platelets from buffy coat had lower concentrations as expected, but by gradually loading automation into the process of platelet production, its concentration was increasing and its quality too. A rising quality of platelets is best demonstrated by measuring of the pH values. In 2002 they were lower (the average value was 7.02 ) compared to the value in 2010 (7.34). Implementation of areal pre storage leucodepletion of platelets and use of platelet in additive solutions into process of platelet production significantly decreased the number of reported transfusion complications after platelet transfusion (0.85 – 1.13% in the beginning of monitored period and 0.31% in the end).

**Conclusion**

Development of platelets production directed to continual rising of quality and safety of offered products. During of a monitored period was implemented areal pre storage platelet leukocyte –

depletion, areal resuspensation of platelets in additive solutions and NAT testing of infectious markers. That is why a number of reported transfusion complications had a downward trend. Results of monitoring the pH shows a longer survival of platelets in transfusion products and currently the possibility of elongation of storage in the case we are sure there is not a risk of bacterial contamination.

**15. Development of IFN-γ-catch assay after mixed lymphocyte reaction to investigate the mechanism of graft predominance after double cord blood transplantation**

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**Background**  
In double-unit umbilical cord blood transplantation (dUCBT) long-term hematopoiesis is usually derived from a single cord blood unit (CBU). However, predominance of a single CBU is present already within 11-18 days post transplant, when the peripheral blood cells are still very low<sup>1</sup>. The mechanism of predominance of a particular CBU has not been resolved yet. A selective growth advantage of one particular unit as well as an immunological rejection of one CBU has been proposed. The latter is suggested by the observation that effector CD8+ cells, derived from the engrafting CBU, produce interferon-gamma (IFN-γ) in response to the non-engrafting CBU<sup>2</sup>.

We set up an INF-γ capture assay in combination with a mixed lymphocyte reaction (MLR) in order to investigate

whether this technique would be feasible to detect an immune reaction between both CBUs as a possible mechanism of graft predominance.

**Methods**  
Responder thawed post-ficoll or CD34 negative mononuclear cord blood cells or cord blood T cells (1 x 10<sup>5</sup>) were stimulated 5 days with different cell fractions of irradiated HLA mismatched cord blood (1 x 10<sup>5</sup>). After MLR the IFN-γ catch assay (Miltenyi) was performed. In coupled experiments one part was additionally stimulated with anti-CD3/CD28 beads (Dynal beads) for 1 hour to amplify the IFN-γ production. In parallel the luciferase catalyzed ATP-dependent luminescence reaction was used.

**Results**  
After MLR 3.49% ± 5.55 (mean ± SD) of CD8+ cells responded with IFN-γ production (n=12; range 0%-18.0%). After extra bead stimulation the percentage of IFN-γ producing CD8+ cells was 8.17 ± 6.73 times (mean ± SD) increased (n=9; range 2.31-19.66), while the (medium) controls remained negative. Isolated T-cells fraction responded with equal numbers IFN-γ producing cells (0.48%, after bead stimulation 6.91%) compared with the whole mononuclear fraction of the same cord blood (1.85%, after bead stimulation 5.03%). There was no good correlation between IFN-γ

production after MLR and the ATP production, although this was slightly better after extra bead stimulation (correlation coefficient respectively 0.217 and 0.574 (n=8)).

**Conclusions**  
The catch assay measuring the IFN-γ producing responder cord blood cells after MLR seems a feasible tool for detecting low amounts of INF-γ producing cells, which can be amplified with extra CD3/CD28 bead stimulation.

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2. Gutman JA, Turtle CJ, Manley TJ, Heimfeld S, Bernstein ID, Riddell SR, Delaney C, Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. Blood 2010; 115:757-65.

**16. Establishment of the first who international repository for transfusion relevant bacteria strains-ISBT working party transfusion-transmitted infectious diseases (WP-TTID), subgroup on bacteria**

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Bacterial contamination of platelet concentrates (PCs) remains a persistent problem in transfusion. At present, no international transfusion-relevant bacterial strain panel exists for the investigation of blood component-related bacterial contamination. The ISBT Working Party Transfusion-Transmitted Infectious Diseases (WP-TTID), Subgroup Bacteria, organized an international study on blood transfusion relevant bacteria strains to be used as a tool for development, validation and comparison of both bacteria screening and pathogen reduction methods. The bacterial strains were chosen regarding their ability to proliferate in PCs during actual storage which cannot be taken for granted from culture collection strains.

**Methods**

Four blinded bacteria strains (A: *Staphylococcus epidermidis* PEI-B-06; B: *Streptococcus pyogenes* PEI-B-20; C: *Klebsiella*

*pneumoniae* PEI-B-08; D: *Escherichia coli* PEI-B-19) were prepared by the Paul Ehrlich Institute (PEI) as deep-frozen suspensions which are stable, shippable and defined in count of living bacterial cells. Prior to the study they were selected regarding their ability to proliferate to high counts in PCs. Afterwards they were distributed to 14 laboratories from 10 different countries. The participating laboratories were asked to identify the bacterial species, estimate the bacterial count and determine their ability to grow in PCs after low spiking (approximately 0.3 and 0.03 CFU/mL), to simulate contamination occurring during blood donation.

**Results**

Thirteen laboratories sent data for statistical analysis within the deadline. The bacterial strains were correctly identified in 98% of all 52 identifications (1 case reported as *Staphylococcus delphini* instead of the closely related *Staphylococcus epidermidis*). *Streptococcus pyogenes* and *Escherichia coli* grew in PCs in 11 out of 12 laboratories (92.3%), *Klebsiella pneumoniae* and *Staphylococcus epidermidis* replicated in all participating laboratories (100%). The results of bacterial counts were very consistent between laboratories: the 95% confidence intervals were for St. epidermidis: 1.19-1.32 x 10<sup>7</sup> CFU/mL, Str. pyogenes: 0.58-0.69 x 10<sup>7</sup> CFU/mL, K. pneumoniae: 18.71-20.26 x 10<sup>7</sup> CFU/mL and E. coli: 1.78-2.10 x 10<sup>7</sup> CFU/mL.

**Conclusions**

The study was performed in response to the lack of bacterial standards reflecting species and strains relevant to the evaluation of blood components. Furthermore, the study was undertaken as a proof of principle with the aim to demonstrate a) the quality, stability and suitability of the bacterial strains for low-titre spiking of blood components, b) the property of donor-independent proliferation in PCs, and c) their suitability for, and the logistics of, worldwide shipping of deep frozen, blinded pathogenic bacteria. The results of the study demonstrated the stability of the prepared bacterial strains and consistency of results in a large number of transfusion laboratories all over the world. The advancements in this field aiming to establish a recognized standard for worldwide use were discussed during the annual meetings of WHO Expert Committee Biological Standardization (ECBS) in 2009 and 2010. The Committee approved the adoption of four bacteria strains which were included in the international validation study as the first WHO Repository for Transfusion Relevant Bacteria Strains and, additionally, endorsed as a project the addition of nine further bacteria strain preparations suitable for control of PC contamination.

**17. Persistent anti-D production is maintained by fetal RhD microchimerism**

EP Verduin

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**Introduction**

Blood transfusion and fetal maternal hemorrhage (FMH) in pregnancy may lead to maternal red blood cell (RBC) alloantibody formation. During pregnancy, antibodies of the IgG class can cross the placenta and may cause fetal anemia leading to hemolytic disease of the fetus and newborn (HDFN). In more than 80% of cases anti-D is responsible for HDFN. Intrauterine transfusion (IUT) with irradiated RBCs is treatment for severe fetal anemia and fetal survival rate is over 90% nowadays. The LOTUS study (LOng Term follow-Up after intrauterine transfusionS) is conducted by Sanquin Blood Supply and the Leiden University Medical Center (LUMC) departments of Obstetrics, Neonatology and Immunohaematology & Blood transfusion. We aim to identify factors explaining induction, specificity and persistence of alloantibodies against particular RBC antigens in women whose children have been treated with IUTs. We further investigated whether pregnancy (fetus) induced antibodies persisted longer compared to IUT-donor induced antibodies. We postulated that

pregnancy induced D-antibodies may be maintained by long lasting RhD fetal microchimerism.

**Methods**

All women (n=340) and their live offspring (n=395) who have been treated with IUT for HDFN in the LUMC from 1987-2008 were invited to participate and after consent blood or saliva samples were taken. RBC antigen profiles and antibodies were determined. Data on RBC antigenprofiles of IUT donors were drawn from the Sanquin database. For all maternal antibodies the offending antigen (IUT donor or fetus) was assessed. RhD fetal microchimerism was assed by a singleplex RT Q-PCR assay for exon 5 and exon 7. RhD PCR products were confirmed by gelelectrophoresis. Each sample was tested in eight fold in both assays and considered fetal chimeric if in at least 2/16 wells a RhD (either exon 5 and/or 7) product was present.

**Results**

Blood samples of 263 mothers with a total of 546 historical antibodies and with a median follow-up time after the last IUT pregnancy of 8.5 years (0-23.5) were available for analysis. The overall antibody persistence rate was 81%; antibodies responsible for the HDFN persisted in 98% and IUT-donor induced antibodies in 6%. Overall, non-HDFN fetus induced antibodies were 8 times

more likely to persist compared to donor induced antibodies (OR 7.9; 95% CI 3.2-19.4). The median persisting anti-D titer in 205 women was 500 (4-32.000) irrespective of FU. DNA samples from 163 RhD negative mothers with persistent anti-D were available and tested for RhD gene exon 5 and 7. In 43 (26%) women both exons could not be detected and in 35 (21%) women only 1 of 16 wells contained a RhD PCR product. In the remaining 85 (52%) women, ≥2 of 16 wells contained RhD PCR products and were considered microchimeric for RhD.

**Conclusions**

Fetal induced alloantibodies against RBC antigens persist significantly longer than IUT-donor induced antibodies. The high persisting anti-D titer suggests an ongoing alloimmune B cell response, possibly maintained by fetal RhD microchimerism.

# Social Programme

## Drinks and Conference buffet - Thursday 14 April

The drinks and congress buffet will be held in the main hall of the Royal Tropical Institute. This enables you to discuss the sessions and posters during your buffet and enjoy the music by Brisa Latina.

## Farewell drinks - Friday 15 April

On Friday after the last session a farewell reception will be organized in the main hall of the Royal Tropical Institute. You are most welcome to join this reception, as are accompanying persons.

# General Information

(in alphabetical order)

## Accreditation and certificate of attendance

The following societies have rewarded accreditation points. Please, ask at the reception desk for certificates of attendance You may be asked to sign a list of attendance for the society in question.

- Dutch Society for Internal Medicine: 9 points for 2 days
- Dutch Society for Hematology: 9 points for 2 days
- Dutch Society for Social Medicine: applied for
- Dutch Society for Pediatrics: applied for
- Dutch Society for Anaesthesiology: applied for
- Dutch Society for Clinical Chemistry: applied for
- European Accreditation Council for Continuing Medical Education (EACCME). AMA conversion applies, applied for
- Dutch Society for Immunology: Accredited by the Dutch College of Medical Immunologists

## Badges

All participants will receive a personal badge upon registration. You are kindly requested to wear your name badge when attending any meeting or social gathering during the conference.

## Banking Facilities

The official currency in The Netherlands is the Euro. It is recommended that foreign currencies will be converted to Euros at Dutch chartered banks, which are usually open from Monday through Friday from 09.00-16.00 hours. Exchange of foreign money and travellers’ cheques is also possible in most hotels.

## Cloakroom and Luggage

At the Royal Tropical Institute a cloakroom is located near the registration area.

## Electricity

In The Netherlands, electricity is supplied at 220 V - 50 Hz AC.

## Insurance

In registering for the Sanquin Spring Seminars, participants agree that neither the Organizing Committee nor the Seminar Secretariat assume any liability whatsoever. Participants are requested to make their own arrangements for health and travel insurance.

## Language

The official language of the Sanquin Spring Seminars is English.

**Registration Desk**

The registration desk will be open at the following times:

Thursday, 14 April 2011	08.30 - 18.00 hours
Friday, 15 April 2011	08.30 - 16.00 hours

**Shops**

Most shops in Amsterdam are open from 09.00 to 18.00 hours.  
On Thursdays, shops are open till 21.00 hours.

**Taxis**

Numerous taxi stands are located throughout Amsterdam.  
The telephone number of the central taxi service is  
020 - 777 77 77 (**country code 31**).

**Weather**

While April may offer lovely spring weather, it can be quite unpredictable and might be chilly in the evening. Temperatures range from 8 to 14°C. As showers might occur, we advise you to bring raincoat or umbrella.

**WiFi**

Free WiFi is available at the conference venue. The code may be obtained at the registration desk.

## Fifth Sanquin Spring Seminar

Therapeutic Proteins

23 & 24 April 2015  
Amsterdam, The Netherlands

## The 23<sup>rd</sup> Regional Congress of the ISBT

will be co-hosted by Sanquin Blood Supply  
and the Dutch Blood Transfusion Society.

Amsterdam 1 - 5 June 2013  
RAI Conference Center, Amsterdam,  
The Netherlands

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