Iron metabolism and anemia

20 - 21 April 2017
Amsterdam, The Netherlands
Dear delegates,

On behalf of Sanquin Blood Supply Foundation it is our great pleasure welcoming you to Amsterdam and this conference in particular. This year’s Spring Seminar is the sixth in a biennial series addressing different themes that are central to Sanquin’s activities. The program of this year’s theme ‘Iron metabolism and anemia’ covers a broad research area from donor to patient and from basic research to novel therapies. We are delighted that so many top-notch speakers, all experts in their field, were able to accept our invitation to present their work and innovative insights.

With the poster session also the younger generation has a platform to show and discuss their work. In six out of seven sessions an abstract was selected for an oral presentation.

We feel that the scientific and organizing committees have put together an exciting program, and we sincerely hope that you will enjoy the talks and will take the opportunity to discuss your work with other delegates during the breaks and the conference buffet.

Marian van Kraaij
Conference chair

René van Lier
Sanquin Executive Board

A WORD OF WELCOME

COMMITTEES

Scientific committee

Marian van Kraaij MD PhD, conference president
Unit director Donor affairs and Transfusion medicine, Sanquin Blood Bank, Amsterdam, The Netherlands

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Manager Dept. Donor studies, Sanquin Research and professor of Donor health care, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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Senior researcher Dept. Blood cell research, Sanquin Research, Amsterdam, The Netherlands

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Hematologist and professor of Translational immunohematology, Academic Medical Center, University of Amsterdam and senior researcher Dept Immunopathology, Sanquin Research, Amsterdam, The Netherlands

Organizing committee

Marian van Kraaij
Odette van Dinteren
Jan Willem Smeenk

Conference support

Martinet Constant
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Registration desk
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Thursday 20 April 2017: 08:30-20:30 hrs
Friday 21 April 2017: 08:00-17:30 hrs

Certificates of attendance
Certificates of attendance will be provide digitally in the week after the conference. The certificate will show the accrediting societies and accreditation points awarded.

Accreditation is awarded by:
Dutch Society for Internal Medicine 12 points
(Nederlandse Internisten Vereniging, NIV)

Dutch Society for Pediatrics 11 points
(Nederlandse Vereniging voor Kindergeneeskunde, NVK)

Netherlands Association for Donor Medicine 12 points
(Nederlandse Vereniging voor Donorgeneeskunde; Koepel Artsen Maatschappij en Gezondheid, KAMG, AbSG)

Dutch Society for Laboratory Medicine 12 points
(Nederlandse Vereniging voor Klinische Chemie en Laboratorium-geneeskunde, NVKC)

Public transport
Amsterdam has a compact city center. Most museums and attractions are within walking distance from the conference venue. Amsterdam has an extended public transport network. Cash payment is not accepted in busses, trams and metro. Tickets may be bought in advance at ticket centers or ticket machines, where most debit and credit cards are accepted.

Shops
Most shops in Amsterdam are open from 09:00 to 18:00 hrs. Evening shopping on Thursday until 21:00 hrs. Within the city center shops may be open longer.

Taxis
Numerous taxi stands are located throughout Amsterdam. Licensed taxis mail be hailed on the street.
Central taxi service: +31 20 777 77 77

Weather
While April may offer lovely spring weather, it can be quite unpredictable and it might be chilly in the evening. As showers might occur, we advise you to bring a raincoat or umbrella.

Wifi
Free WiFi is available at the conference venue. Network: Rode Hoed
Password: rodehoed1987
History of the “Rode Hoed”

Until 1629, a hat maker was established behind the Keizersgracht 102-106 premises. A plaque with a little red hat on the façade of number 104 is all that is left of the craft that lent its name to the Rode Hoed. Since 2010, the ring of canals, within which the Rode Hoed is located, has been inscribed in the UNESCO World Heritage List.

The venue is located at one of the canals in the heart of Amsterdam. The small red hat (rode hoed) on the façade remind us of the time a millinery was located at this address. It was there that in the 17th century a Remonstrant clandestine church was build in the backyard behind the facade of three stately and age-old canal houses. This hidden church remained active until the 1950’s. In 1990 the not-for-profit foundation ‘De Rode Hoed’ was founded as a center for religion, philosophy, music and poetry. At the turn of the century more attention was given to contemporary societal issues in general.

The characteristic organ in the Oosterhuiszaal dates from 1719 and is built by the German Thomas Weidtmann. In 1862, the complete organ had been restored, but it gradually became dilapidated. Since the latest restoration in 2009, however, the organ is in great condition and is played during the regular Clandestine Church Sunday Afternoon Concert Series.

The Jordan Area Tour

Thursday 20 April - 18.30 (till max 20.00)

The venue the Rode Hoed is located in the Jordan area, the liveliest neighborhood of Amsterdam with its many hidden courtyards, streets and picturesque houses! Curious about all the stories about the Jordaan? Discover this during our Jordan Area Tour!

We leave at 18.30 at the reception of the Rode Hoed and we will be back at 20.00. You may collect luggage when you return to the Rode Hoed.

If you want to join, register at the conference desk before the end of the lunch break.

We gladly invite you for a tour
PROGRAM THURSDAY 20 APRIL 2017

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09:35 - 09:40 Introduction
Marian van Kraaij | Conference Chair
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Iron homeostasis and its relationship to erythropoiesis and innate immunity
Tomas Ganz | David Geffen School of Medicine, Los Angeles, USA
10:30 - 11:10 Pumping iron: When monocytes come to the rescue
Filip Swirski | Massachusetts General Hospital, Boston, USA
11:10 - 11:30 Break & posters
11:30 - 12:10 Devil’s dance: heme, iron and hemopexin in hemolysis
Sacha Zeerleder | Academic Medical Centre and Sanquin Research, Amsterdam, The Netherlands
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12:30 - 13:30 Lunch & posters
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Anemia on the ICU, how to fight an ancient foe
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Francesca Aglialoro | Sanquin Research, Dept Hematopoiesis and Landsteiner Laboratory, Academic Medical Center, Amsterdam, The Netherlands
14:25 - 15:05 Interferon gamma and anemia of inflammation
Katherine MacNamara | Albany Medical Center, Albany, USA
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15:30 SESSION III - Hemoglobinopathies
15:30 - 16:10 Genetic and epigenetic regulation of hemoglobin switching
Sjaak Philipsen | Erasmus Medical Center, Rotterdam, The Netherlands
16:10 - 16:25 Dynamics of von Willebrand factor reactivity in sickle cell disease Brenda Luken | Sanquin Research, dept Immunopathology and Landsteiner Laboratory, Academic Medical Center, Amsterdam, The Netherlands
16:25 - 17:05 Trojan horses in sickle cell disease
Karin Fijnvandraat | Academic Medical Center and Sanquin Blood bank & Research, Amsterdam, The Netherlands
17:05 - 19:00 Drinks & buffet
18:25 - 20:00 The Jordan Area Tour
09:30 - 09:35
Welcome
René van Lier
Director Sanquin Research & member executive board Sanquin

09:35 - 09:40
Introduction
Marian van Kraaij
Unit director Donor affairs and Transfusion medicine, Sanquin Blood Bank, Amsterdam, The Netherlands
Conference Chair

Room for notes
Chair
Dorine Swinkels
Radboud University Medical Center,
Nijmegen, The Netherlands

Room for notes
Iron homeostasis and its relationship to erythropoiesis and innate immunity
Tomas Ganz
David Geffen School of Medicine, Los Angeles, USA

The iron-homeostatic system provides sufficient iron for erythropoiesis and other organ requirements, while avoiding the tendency of iron to promote microbial infections and cause tissue injury. The peptide hormone hepcidin, secreted by hepatocytes, controls total body iron content and plasma iron concentrations by inducing the endocytosis of its receptor, the cellular iron exporter ferroportin, thereby suppressing the absorption of dietary iron and the release of recycled iron from splenic and other macrophages and from hepatic stores. Recent studies advanced the understanding of the structural basis of ferroportin function and its interaction with hepcidin.

In response to changing iron needs, hepcidin production is controlled by plasma iron-transferrin concentrations and iron stores in the liver, both of which exert a positive feedback on hepcidin transcription. The key pathway for this response involves the BMP receptor interacting with several iron sensors and adaptors. Genetic studies in mice and humans have led to the detection of the many components of the hepcidin-regulating pathways but the biochemistry of their interactions is complex and under active study.

In response to blood loss or other erythropoietic stimuli, plasma hepcidin concentrations decrease within hours, allowing the compensatory absorption of dietary iron and its release from stores. Here, hepcidin is regulated by erythroferrone, a member of the TNFα superfamily, secreted by erythropoietin-stimulated erythroblasts. Release of erythroferrone mediates early hepcidin suppression after hemorrhage and contributes to the characteristic iron overload in β-thalassemia intermedia, which develops even in the absence of erythrocyte transfusions.

During infections, hepcidin is predominantly controlled by interleukin-6. Within hours after a systemic infection, hepcidin concentration rise and decrease iron concentrations in plasma, an important host defense mechanism most effective against siderophilic bacteria such as Vibrio vulnificus or Yersinia enterocolitica. In hereditary hemochromatosis hepcidin is deficient, making the patients susceptible to life-threatening infection with these microbes. Recent studies implicate non-transferrin iron (NTBI) as the key form of iron promoting infections. It is likely that the host defense role of hepcidin is to limit the concentration of NTBI, and this may affect a broader spectrum of microbes than heretofore realized.

Increasing understanding of systemic iron homeostasis and its disorders is identifying biological targets for diagnostic and therapeutic applications.
Iron is an essential component of the erythrocyte protein hemoglobin and is crucial to oxygen transport in vertebrates. In the steady state, erythrocyte production is in equilibrium with erythrocyte removal. In various pathophysiological conditions, however, erythrocyte life span is severely compromised, which threatens the organism with anemia and iron toxicity. In this talk, I will discuss an on-demand mechanism that clears erythrocytes and recycles iron. I will show that Ly-6Chigh monocytes ingest stressed and senescent erythrocytes, accumulate in the liver via coordinated chemotactic cues, and differentiate to ferroportin 1 (FPN1)-expressing macrophages that can deliver iron to hepatocytes. Monocyte-derived FPN1+ Tim-4neg macrophages are transient, reside alongside embryonically-derived Tim-4high Kupffer cells, and depend on Csf1 and Nrf2. The spleen likewise recruits iron-loaded Ly-6Chigh monocytes, but these do not differentiate into iron-recycling macrophages due to the suppressive action of Csf2. Inhibiting monocyte recruitment to the liver leads to kidney and liver damage. These observations identify the liver as the primary organ supporting rapid erythrocyte removal and iron recycling and uncover a mechanism by which the body adapts to fluctuations in erythrocyte integrity.
Hemolysis is a hallmark of a variety of diseases, such as sickle cell disease, autoimmune hemolytic anemia, paroxysmal nocturnal hemoglobinuria and thrombotic microangiopathies. Hemolytic diseases are characterized by systemic inflammation induced by cell-free heme released upon erythrocyte destruction. Heme toxicity is mediated through the formation of highly reactive oxygen species, which is dependent on TLR4 and induction of the NFκB pathway. In addition, heme induces activation of neutrophils and endothelial cells thereby perpetuating systemic inflammation. Heme released from cell-free hemoglobin and cell-free heme is effectively neutralized by complex formation with haptoglobin and hemopexin, respectively, and subsequent removal of these complexes by the reticuloendothelial system. However, due to scavenger consumption these protective systems are quickly exhausted. Therapeutic administration of hemopexin and/or haptoglobin may therefore be beneficial to neutralize heme toxicity. As a cellular defense line inducible heme oxygenase -1 (HO-1) may in part compensate for the exhausted plasma scavengers by degrading heme into biliverdine, CO and iron, respectively. Upregulation of HO-1 turned out to be a very effective system to protect the host from the heme induced systemic inflammation. Interestingly, these protective effects are not restricted to hemolytic diseases but to other diseases characterized by systemic inflammation, e.g. sepsis.
12:10 - 12:30
Poster pitches
Moderator: Robin van Bruggen
Senior researcher Dept. Blood cell research, Sanquin Research, Amsterdam, The Netherlands

Poster presentations | 13.00-13.30 | odd numbers
1. Michael Wilson | Preoperative iron deficiency in colorectal cancer patients prevalence and treatment
3. Eline Pronk | Identification of transcripts that are differentially translated upon phosphorylation of translation factor eIF2 in erythroblasts
5. Margit Boshuizen | Iron metabolism in critically ill patients developing anemia of inflammation
7. Kristof Van Avondt | Free iron contributes to neutrophil activation in sickle cell disease
11. Eszter Varga | HUMAN Induced Pluripotent Stem Cell Differentiation to red blood cells
13. Marlijn Hoeks | Bone marrow iron overload in transfused acute myeloid leukemia patients
15. Djuna de Back | A method for the biotinylation of red blood cells for clinical research that complies with Good Manufacturing Practice regulation
17. Tiffany Timmer | Associations between SNPs and erythrocyte traits including hemoglobin in humans: a systematic literature review and Donor InSight-III data collection
19. Lisanne Huis in ’t Veld | Risk dependent intervention on the prevalence of low hemoglobin deferral
Chair
Diana Wouters
Senior researcher Dept. Immunopathology,
Sanquin Research Amsterdam, The Netherlands

Room for notes
Anemia is a frequent complication of critical illness and is associated with poor outcomes, particularly in patients with ischemic heart disease. At the same time studies have also reported increased morbidity and mortality in patients who receive blood transfusions. In principle, increasing hemoglobin level is aimed at increasing oxygen delivery (DO2). However, transfusion of packed red blood cells may not have the desired effect on DO2. Moreover, the causes of anemia in critical care patients are numerous, further complicating the decision when and how to increase hemoglobin level. The pathophysiology of anemia in the ICU relates to anemia of inflammation, which is caused by
1) inflammation-induced hepcidin production resulting in iron-restricted erythropoiesis,
2) decreased erythropoietin concentrations and
3) a decreased response of erythroid progenitor cells to erythropoietin.
New therapies are being developed, targeted at hepcidin to allow increased iron absorption and mobilization from iron stores. Furthermore, as a significant part of ICU patients are elective surgery patients, pre-operative optimization of hemoglobin, e.g. by intravenous iron supplementation has received new attention. Finally, decisions to treat anemia must be individualized, taking into account specific patient factors. This approach will ensure that anemia is treated when necessary while avoiding unnecessary exposure to red blood cells.
Background: *Plasmodium vivax* (*P. vivax*) is the second most prevalent parasite species causing malaria in humans and exclusively infects reticulocytes. *P. vivax* Duffy binding protein (DBP) association with Duffy antigen chemokine receptor, DARC is essential for entry. Importantly, DARC expression in erythrocytes and reticulocytes is unchanged and cannot explain *P. vivax* reticulocyte preference. Reticulocytes express CD71 and have residual RNA that can be detected by Thiazole orange (TO) staining, both markers are gradually lost during reticulocyte maturation.

Aim: We hypothesize a small population of immature reticulocytes may display increased association with *P. vivax* DBP potentially explaining the preference of *P. vivax* for aspecific reticulocyte population.

Methods: Reticulocytes were enriched from human peripheral blood by continuous percoll gradient. FACS was used to delineate reticulocyte populations. Western blotting was used to assess expression levels of DARC and other membrane proteins.

Results: CD71/TO double staining of peripheral blood reveals four distinct reticulocyte populations. These are with increasing maturity: CD71high/TOhigh, CD71low/TOhigh, CD71-/TOhigh, and CD71-/TOlow. Binding of Duffy antibodies recognizing the DBP binding pocket as well as DBP itself to CD71high/TOhigh reticulocytes was significantly higher compared to other reticulocyte populations. Interestingly, the expression of DARC did not change significantly during reticulocyte maturation.

Summary/Conclusion: The data suggests an increased epitope exposure of membrane proteins and in particular Duffy epitopes in immature reticulocytes which is probably a key to the preferential binding of DBP to immature reticulocytes and a potential mechanism underlying the preferential infection of reticulocyte subset by *P. vivax*.
Anemia of inflammation is the second most common type of anemia and accompanies many chronic diseases, such as infection, cancer, and autoimmune disorders. Because low levels of hemoglobin are associated with increased mortality in a variety of chronic diseases, understanding the pathophysiology of anemia of inflammation is vital for treating chronically ill patients. In addition, normal aging is associated with an increase in inflammation and is also accompanied by anemia. In contrast to iron-deficient anemia, anemia of inflammation is characterized by inaccessible iron stores and is resistant to erythropoietin. Inflammation significantly impacts iron recycling. While inflammation-induced iron sequestration has evolved to support host defense by depriving invading microbes of this crucial metal, prolonged inflammation leads to iron-restricted anemia. Macrophages play a key role in recycling the majority of iron required for erythropoiesis and resident erythroblastic island macrophages support erythropoiesis in the bone marrow. Moreover, macrophages are targets of inflammatory factors and cytokines, as well as some pathogens. Recent data demonstrate a critical role for bone marrow resident macrophages in regulating hematopoiesis under acute inflammatory conditions and in driving hematopoietic dysfunction and anemia during aging. The impact of inflammatory cytokines on anemia of inflammation will be discussed with a particular focus on how interferon gamma and colony stimulating factors regulate bone marrow resident macrophages and, as a consequence, iron recycling and erythropoiesis.
THURSDAY 20 APRIL 2017 - SESSION III – HEMOGLOBINOPATHIES

Chair
Karin Fijnvandraat
Professor in Pediatric Hematology, Academic Medical Center, University of Amsterdam and Sanquin Blood bank & Research, Amsterdam, The Netherlands

Room for notes
Elevated expression of the fetal γ-globin genes greatly ameliorates the symptoms of β-thalassemia and sickle cell disease, as γ-globin efficiently replaces β-globin in adult erythroid cells. The molecular mechanism of hemoglobin switching involves developmental stage-specific changes in transcription factors and chromatin modifiers. A regulatory cascade involving MYB, KLF1 and BCL11A has been uncovered in recent years. Genome-wide association studies indicate that common SNPs in the BCL11A, HSB1L-MYB and HBB loci account for ~50% of the variation in HbF levels, suggesting that additional factors are involved. In agreement with this notion, LRF was recently reported as an independent repressor of γ-globin, and our lentiviral shRNA screen in human erythroid progenitor cells has yielded ten novel potential γ-globin repressors. Based on the latter results, our current work is focused on functional analysis of protein arginine methylation in hemoglobin switching, using mice as the model system. These animal studies are complemented by analysis of patients with rare variants of known γ-globin modulators, and by genetic and epigenetic analysis of the modulation of γ-globin expression in the HUDEP-2 cell line, a model for adult human erythropoiesis.
Background: Endothelial activation with von Willebrand factor (VWF) release plays a central role in the pathophysiology of vaso-occlusive crisis (VOC) in sickle cell disease (SCD), facilitating adhesive interactions with sickle red blood cells, neutrophils and platelets. However, the precise role of VWF in the pathogenesis of VOC in SCD is unclear.

Aim: To assess the quantity and reactivity of VWF and its protease ADAMTS13 during VOC, and to determine correlations with damage associated molecular patterns (DAMPs) released as a consequence of hemolysis, inflammation, and neutrophil activation.

Methods: In this observational study we obtained sequential blood samples in adult SCD patients during VOC.

Results: VWF reactivity significantly increased during VOC (active VWF, VWF activity and high-molecular weight VWF multimers), whereas platelet count and ADAMTS13 antigen and ADAMTS13 activity concomitantly declined when compared to steady state. Levels of VWF antigen, VWF propeptide and ADAMTS13 specific activity did not change during VOC. VWF reactivity correlated strongly with DAMPs released during hemolysis, inflammation and neutrophil activation, and was inversely correlated with hemoglobin levels and platelet count. In patients that developed acute chest syndrome, levels of VWF were significantly higher, while the ADAMTS13 specific activity was lower than in patients without this complication.

Summary/conclusion: We show that VOC in SCD is associated with increased reactivity of VWF, without ADAMTS13 deficiency. This hyper-reactivity may be explained by resistance of VWF to proteolysis, secondary to processes such as hemolysis, inflammation and oxidative stress. Hyper-adhesive VWF, scavenging blood cells in the microcirculation, may thereby promote VOC in SCD.
Trojan horses in sickle cell disease

Karin Fijnvandraat
Professor in Pediatric Hematology, Academic Medical Center, University of Amsterdam and Sanquin Blood bank & Research, Amsterdam, The Netherlands

Worldwide 300,000 children are annually born with sickle cell disease. Sickle cell disease is a recessive hereditary disorder of hemoglobin. Homozygosity for the HbS allele is the most common genotype, but some patients are heterozygous for HbS and another hemoglobin mutation such as HbC (hemoglobin SC disease) or β thalassaemia (Sβ0 and Sβ+ diseases). The abnormal hemoglobin (hemoglobin S), polymerizes when deoxygenated, changing the normally biconcave shape of the red blood cells into a sickle shape. Sickle cells have a reduced deformability and are easily destroyed, resulting in chronic hemolytic anemia. The other hallmark of the disease is vaso-occlusion: blood vessels are obstructed by a complex process initiated by the sickled red blood cells, involving adhesion of different species of blood cells, chronic inflammation, ischemia reperfusion injury, endothelial damage and dysregulated nitric oxide homeostasis. Vaso-occlusion leads to recurrent painful crises and irreversible damage to all vital organs.

Currently the only curative treatment option for sickle cell disease is stem cell transplantation. For patients that are not eligible for stem cell transplantation, e.g. due to lack of a matching donor, supportive medical treatment is available in the form of hydroxyurea and red blood cell transfusion. Hydroxyurea raises the level of fetal hemoglobin, thereby reducing the sickling of red blood cells. Red blood cell transfusions are required for acute complications such as Acute Chest Syndrome or worsening of the anemia with cardiovascular compromise. Regular red blood cell transfusions are given to prevent stroke in high risk patients. Transfusions are very beneficial in these clinical circumstances. However, one of the complications of multiple infusions is iron overloading, requiring chelation treatment. Thus, although they form a cornerstone of medical treatment in sickle cell disease, transfusions may also be regarded as Trojan Horses, disrupting iron homeostasis.

Room for notes

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Chair
Robin van Bruggen
Senior researcher Dept. Blood cell research,
Sanquin Research, Amsterdam, The Netherlands

Room for notes
Consequences of DMT1 deficiency on erythropoiesis

Monika Horváthová

Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

Divalent metal transporter 1 (DMT1) is essential for intestinal iron absorption and erythroid iron utilization. Homozygous or compound heterozygous mutations in DMT1 are responsible for hypochromic microcytic anemia in human patients and in the mk/mk mouse model. The anemia can be, in part, corrected with high doses of erythropoietin. Hepatic iron overload documented in some DMT1−mutant patients distinguishes them from the mk/mk mice. Reduced hepcidin levels and increased levels of growth differentiation factor-15 (GDF15) and erythroferrone, detected in DMT1-mutant patients and mice, are consistent with the predominant suppressive effect of accelerated/ineffective erythropoiesis on hepcidin production. Comprehensive analyses of the erythroid lineage of DMT1-mutant patient and mice revealed reduced proliferation and differentiation capacity of erythroid progenitors, delayed S-phase progression and mild apoptosis of bone marrow erythroblasts, and reduced life span of mature erythrocytes. This was associated with tissue hypoxia, oxidative damage, endoplasmic reticulum stress, phosphorylation of the alpha subunit of the eukaryotic initiation factor 2 (eIF2α), activation of Chk1-dependent DNA damage checkpoint, and altered metabolism of erythrocytes. In summary, impaired function of DMT1 iron transporter has multiple consequences on the erythroid lineage all contributing to the pathophysiology of the disease.

Acknowledgement: Czech Science Foundation, GA15-13732S; Grant of Palacky University, LF_2016_014
The effect of complement inhibition on erythrocyte destruction in AIHA

Inge Baas
Sanquin Research, Dept Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, Amsterdam, The Netherlands

Baas I1, Reis ES2, Ricklin D2, Lambris JD2, de Haas M3, Zeerleder SS3, Wouters D1
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Background: Autoimmune hemolytic anemia (AIHA) is a rare disease characterized by autoantibodies against erythrocytes. These autoantibodies may activate the classical complement pathway leading to opsonization by complement proteins C3b and C4b, resulting in increased clearance of erythrocytes by phagocytes (extravascular hemolysis). Occasionally, complement activation results in formation of the membrane attack complex resulting in intravascular hemolysis. C3-inhibitor compstatin prevents C3b deposition while no effect on C4b deposition is expected.

Aim: The current aim is to investigate in vitro whether compstatin would be a suitable drug for AIHA treatment.

Methods: Healthy donor erythrocytes were incubated with AIHA patient serum and opsonization was analyzed by FACS using anti-C3-FITC and anti-C4-APC antibodies. To assess the effect of compstatin on uptake of erythrocytes by phagocytes, erythrocytes were fluorescently labeled before opsonization. Opsonized erythrocytes were then incubated with healthy monocyte derived macrophages (M1) and phagocytosis of erythrocytes was measured with flow cytometry or ImageStream after lysing the non-phagocytosed erythrocytes.

Results: Compstatin completely inhibited C3 deposition on erythrocytes, while unexpectedly C4 deposition appeared to be increased. Phagocytic uptake of erythrocytes by macrophages was decreased by compstatin.

Conclusion: Since compstatin inhibits both intravascular and extravascular hemolysis it is an interesting candidate to consider for treatment of AIHA.
Clinical pathological entities and molecular phenotypes in myelodysplastic syndromes

Arjan van de Loosdrecht
Dept. Hematology, VUmc, Amsterdam, The Netherlands

Room for notes
Chair
Wim de Kort
Manager Dept. Donor studies, Sanquin Research and professor of Donor health care, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Room for notes
Blood donation can be viewed as a large clinical study to examine the effects of repeated phlebotomy of approximately 10% of blood volume in otherwise healthy individuals. In the United States about 40,000 people per day donate blood. Donors are from all racial/ethnic backgrounds, range in age from 16 to over 80 years old and can donate every 56 days. In addition, they are altruistic by nature and willing to participate in research studies. This rich population has been used for studies on iron metabolism and red blood cell biology that will be presented. These include a large study of the demographics of anemia, the identification and characterization of ‘superdonors’ and associated genetic variants, a study detailing the recovery of hemoglobin and iron stores following donation and a clinical trial investigating different methods to mitigate iron deficiency in frequent blood donors.
11:10 - 11:25
11% of Finnish blood donor have iron deficiency - sTFR enrolment data from Fin Donor 10 000 study
Pia Niittymäki
Finnish Red Cross Blood Service, Finland

Niittymäki P1, Arvas M1, Mattila P1, Nikiforow N1, Castrén J1, Partanen J1
1Finnish Red Cross Blood Service

Background: Iron deficiency is a known long-term undesirable consequence of blood donation. Only a few studies on blood donor iron stores have measured soluble transferrin receptor (sTFR).

Aim: To estimate the proportion of blood donors with iron deficiency (ID). Methods: Fin Donor 10 000 is an on-going prospective study observing the relationship between health, hemoglobin and iron stores in Finnish blood donors. 1 711 blood donors enrolled the study between 18.5.2015 and 30.6.2016 in the capital region of Finland. Venous hemoglobin (vHb), ferritin and sTFR were measured within 24 hrs. of sampling. Abnormal sTFR value was defined as over 5 mg/l in men and over 4.4 mg/ l in women; abnormal values indicated ID.

Results: 13 % of women and 7 % of men enrolled in the study were found to have ID. All donors with ID had lower ferritin (below 12 mg/l) and lower mean vHb, than those without ID.

Discussion: ID was found in 11 % of participants, which corresponds the rates previously reported in other donor populations. Association between sTFR and ferritin indicates that ferritin measurement alone might be utilized for ID screening in blood donors. In this study donors had longer donation intervals (women, 91 days) and higher cHb threshold (men, 135 g/l), than in some previous studies. Iron supplementation was offered to all women under 50 years and men donating frequently, which may protect donors partially from ID.
Oral iron supplementation (OIS) is a widely used strategy to treat iron deficiency (ID) and iron deficiency anemia (IDA). However, iron absorption from OIS is often low and response is variable. To overcome this, large doses are given but this may reduce compliance due to epigastric discomfort and may have unwanted effects gut microbiota and GI inflammation. Thus, OIS doses should be as low as possible but still efficacious, so absorption should be maximized. In practice, OIS schedules vary widely, and there is no consensus on the optimal dosing regimen. In recently published, short term studies using iron stable isotopes in iron depleted women without anemia, we have shown that single doses of OIS increase hepcidin (PHep) for up to 24h after the dose and sharply reduce the bioavailability of subsequent iron doses given twice daily or on the following day. Recent findings from medium term studies (14 labelled dosages of 60 mg Fe given for 4 or 2 weeks respectively) indicate alternate day dosing to result in a geometric mean (-SD, +SD) Fe absorbed of 131.06 mg Fe (71.4, 240.5), compared to 175 mg Fe (110.3, 278.5) for the daily dosage schedule (P<0.001). Furthermore, the common practice of splitting dosages (bi-daily dosing) does not appear to increase iron absorption compared to administering a large single dose morning dose. These findings emphasize the potential to optimize OIS regimens by defining the response of PHep and other iron biomarkers during supplementation and are of relevance for blood donors exposed to the risk of iron deficiency.
2. Marea van der Rijst | *Unravelling the function of SMIM1 during erythropoiesis and in iron homeostasis*

4. Silvia Hoeboer | *Reactivation of fetal hemoglobin expression: functional analysis of candidate modifiers*

6. Dagmar Pospíšilová | *Hepcidin in newly diagnosed inflammatory bowel disease in children*

8. Sanne Meinderts | *FCYR2C polymorphism associates with protection from red blood cell allo-immunization in sickle cell disease*

10. Jill Dalimot | *Developing red pulp macrophages in vitro*

12. Esther Heideveld | *Modelling human erythroblastic islands*

14. Michael Wilson | *Short-term prognostic value of preoperative intravenous iron in colorectal cancer patients*

16. Jean-Yves Py | *Does hemoglobin level influence donor return?*

18. Femmeke Prinsze | *Distribution of ferritin levels of Dutch donors are ferritin levels proportional to the number of whole blood donations?*
Chair
Marieke von Lindern
Manager Dept. Hematopoiesis, Sanquin Research,
Amsterdam, The Netherlands

Room for notes
13:00 - 13:40
Microcytic anemias due to genetic disorders of iron metabolism or heme synthesis
Dorine W Swinkels
Radboud University Medical Center, Nijmegen,
The Netherlands

Microcytic anemias are primarily caused by nutritional iron deficiency, iron loss resulting from gastrointestinal disease, iron malabsorption, hemoglobinopathies (including some thalassemia syndromes), and severe anemia resulting from chronic disease. For some patients with microcytic anemias, the cause remains unexplained by this categorization. In my presentation I will discuss the pathogenesis, epidemiology, clinical presentation, diagnosis and treatment of rare disorders of microcytic anemia that result from defects in different genes and that lead to genetic disorders of iron metabolism and heme synthesis. These disorders can be classified by their pathophysiology in three groups:
1. Disorders due to low iron availability for erythropoiesis,
2. Disorders due to defects in iron acquisition by the erythroid precursors and
3. Disorders due to defects in the heme and/or Fe-S cluster synthesis.
In these patients, serum levels of ferritin and transferrin saturation may contribute to the diagnosis. Family history, an anemia that is refractory or incompletely responsive to iron supplementation, and features such as neurologic disease and skin photosensitivity may also be indicative of a specific diagnosis. In my presentation I will clarify how pathophysiological mechanisms of these disorders explain the clinical and biochemical presentation and the optimal treatment strategies.
Iron overload in hereditary hemolytic anemia
Stephanie van Straaten

Dept. Clinical Chemistry and Hematology, University Medical Center Utrecht, The Netherlands

Hagens S1, Verhoeven J1, Van Beers EJ1, Van Wijk R1
1Dept. Clinical Chemistry and Haematology, UMC Utrecht
2Van Creveld Clinic UMC Utrecht

Background: Iron overload is an important, yet often overlooked complication in rare hereditary hemolytic anemia. Serum ferritin (cut off 1000ug/L) is often used as screening tool but might underestimate MRI Liver Iron Content (LIC).

Aim/methods: Retrospective interim analysis of iron overload in a Dutch cohort of adult patients with hereditary hemolytic anemia (subgroups: hereditary spherocytosis, β-thalassemia, sickle cell anemia, pyruvate kinase deficiency, hereditary xerocytosis and hexokinase deficiency).

Results: Iron overload (LIC>3mg/g) was prevalent in 39/49 (80%) of patients. Moderate iron overload necessitating treatment (LIC>7mg/g) was observed in 26/49 (53%) of patients. LIC>7mg/g was present in all subgroups and also occurred in patients without history of transfusion. Sensitivity of ferritin >1000ng/ml was respectively 22% for LIC>3mg/g and 33% for LIC>7mg/g (table 1).

Conclusion: Iron overload is prevalent in all types of hereditary hemolytic anemia. Importantly, ferritin levels <1000ug/L do not rule out iron overload.

Table 1: Sensitivity and specificity of ferritin >1000ng/ml for LIC

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<th>LIC&gt;3</th>
<th>LIC&gt;7</th>
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<tr>
<td>Sensitivity</td>
<td>22%</td>
<td>33%</td>
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<tr>
<td>Specificity</td>
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<td>Positive predictive value</td>
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<td>100%</td>
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<tr>
<td>Negative predictive value</td>
<td>26%</td>
<td>58%</td>
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Room for notes
Classical inherited bone marrow failure syndromes are mendelian disorders arising from deficiency of basic cellular pathways. These involve ribosome biogenesis in Diamond Blackfan anemia (DBA) and Shwachman Diamond syndrome (SDS), DNA-repair in Fanconi anemia, mitochondrial function in Pearson syndrome, telomere homeostasis in dyskeratosis congenita, and myeloid differentiation in severe congenital neutropenia. After introduction into general classification of IBMFS, the speaker will focus on molecular and clinical aspects of DBA. This prototypic ribosomopathy arises from heterozygous loss of function mutations in genes coding for ribosomal proteins (RP). Depending on their localization in large (L) or small (S) ribosomal subunit, they can be subdivided in 33 RPS and 47 RPL. Approximately 50-60% of DBA patients carry mutations in 13 RP genes (L5, L11, L15, L26, L35A, S7, S10, S17, S19, S24, S26, S28, S29), and ~10% have large deletions encompassing 8 RP genes. In recent years novel genetic causes were linked to DBA in single patients: ribosome maturation factor TSR2, and the non-ribosomal diseases GATA1-deficiency or deficiency of adenosine deaminase (DADA2). Nevertheless, ~30-40% patients remain with unexplained genetic cause. Phenotypically, DBA is a disease with reduced penetrance and multi-organ syndromic features present in half of patients. Therapeutic hallmarks include glucocorticosteroids, transfusions and stem cell transplantation.
FRIDAY 21 APRIL 2017 - SESSION VII
NEW TREATMENTS OF (CONGENITAL) ANEMIA AND IRON OVERLOAD DISORDERS

Chair
Marian van Kraaij
Unit director Donor affairs and Transfusion medicine,
Sanquin Blood Bank, Amsterdam, The Netherlands

Room for notes
The mutual regulation of iron and erythropoiesis: implications for treatment of iron loading anemias
Clara Camaschella
Vita Salute University and San Raffaele Scientific Institute Milano, Italy

Most body iron is taken up by transferrin receptor and consumed for hemoglobin synthesis in maturing erythroblasts. Iron deficiency and defective iron utilization influence erythroid precursor maturation resulting in microcytic and hypochromic red cells. A novel link between iron homeostasis and the erythropoietic hormone erythropoietin (EPO) is provided by the second transferrin receptor (TFR2), which in the liver activates the expression of the key iron regulator hepcidin while in the bone marrow binds the erythroblast EPO receptor modifying EPO sensitivity. Increased erythropoiesis stimulated by hypoxia, bleeding or erythropoietin treatment releases erythroferrone, which inhibits hepcidin through a still unknown pathway. This mechanism explains the low hepcidin levels coupled to iron overload observed in iron-loading anemias with expanded and ineffective erythropoiesis, as beta-thalassemia. Iron restriction has a paradoxical positive effect on anemia of beta-thalassemia, emphasizing that iron regulates the erythroid maturation. Several approaches that interfere with the iron-erythropoiesis regulation, as the administration of apotransferrin or minihepcidins, the inhibition of the hepcidin inhibitor TMPRSS6 and the deletion of erythroid TFR2 decrease iron overload and ameliorate anemia in murine models of non-transfusion-dependent thalassemia. On the opposite side an activin ligand trap that improves ineffective erythropoiesis (now in clinical trial) decreases iron overload in thalassemia.
Fetal hemoglobin expression in adult erythroid cultures is repressed by CD14+ cells

Steven Heshusius
Sanquin Research, dept Hematopoiesis and Landsteiner Laboratory, Academic Medical Center, Amsterdam, The Netherlands

Heshusius S1, Heideveld E1, Van Dijk TB2, Von Lindern M1, Philipsen S2, Van den Akker E1
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Background: In β-hemoglobinopathies, like sickle cell and β-thalassemia, reactivation of fetal hemoglobin (HbF) expression could serve as a treatment alternative to recurrent blood transfusions. Previously, it has been shown that fetal erythroid cells switch to adult hemoglobin (HbA) production when injected into mice. This suggest the involvement of specific cells that instruct erythroblasts to express beta globin instead of gamma subunits.

Aim: Identify the cells that induce globin switching and the signal transduction in erythroblasts that lays fundament to this.

Methods: Cultured human adult erythroid cells and macrophages were used as a model to study HbF expression regulation (levels fluctuate around 2-10%). Hemoglobin distribution was assessed by flowcytometry of erythroid cultures grown from different source material or culture composition.

Results: Flow cytometry revealed that cultured erythroblasts contain cells expressing HbA only and cells expressing both HbA and HbF. Interestingly, erythroblasts expanded from pure, blood, CD34+ cells contained more HbA/HbF cells compared to erythroblasts from total peripheral blood mononuclear cells (PBMC) cultures. Depletion of the CD14+ cell fraction from PBMC resulted in higher percentage of HbF/HbA cells. Conversely, co-culture of CD34+ cells with CD14+ reduced the HbF/HbA population through cell-cell contact. Sorting stages of erythroid progenitors showed that repression only occurs in co-cultures with hematopoietic stem progenitor cells.

Conclusion: The monocyte/macrophage, CD14+, fraction of PBMC’s actively represses expression of fetal hemoglobin in adult erythroid cultures, through cell-cell contact. This could indicate that inhibition of specific cell-cell interaction can lead to treatment alternatives for β-hemoglobinopathies.
15:55 - 16:35
Large scale culture and differentiation of erythroblasts from different sources including IPSC
Emile van den Akker
Sanquin Research, Amsterdam, The Netherlands

Patrick Burger*,1, Eszter Varga*1, Elina Ovchynnikova1, Steven Heshusius1, Jesse Eernstman1, Tatjana Wust1, Marijke Valkhof1, Esther Heideveld1, Erica Sellink1, Marieke von Lindern1* and Emile van den Akker1*

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* equal contribution

Donor-derived red blood cells (RBC) are the most common form of cellular therapy. However, allo-immunization, blood borne diseases, and donor availability prompt for in vitro cultured, customizable RBC (cRBC). Transfusion units contain 1-2*10¹² erythrocytes, demanding major adaptations to erythroid expansion/differentiation protocols to initiate good manufacturing practice (GMP) and bioreactor scale cultures, a process described here for peripheral blood mononuclear cells (PBMC) and iPSC lines. To control erythroid culture parameters and to reduce culture costs, a customized humanized GMP-grade medium (Cellquin) was generated. This medium allowed the 1*10⁸ times erythroid expansion from PBMCs to pure erythroblast cultures within 25 days, comparable to non-GMP commercial media. Subsequently, specific reticulocyte stabilizing component were identified facilitating erythroblasts differentiation to Band3⁺CD71dim⁺CD235⁺CD44⁺CD34⁻ cRBC, reaching >90% enucleation. In addition, cRBC display, amongst other parameters, deformability comparable to in vivo reticulocytes and correct blood group expression. Upscaling using specific bioreactors now allows us to begin culturing 150*10⁹ cells to initiate future clinical trials. An immortal source to produce in vitro cultured RBCs, such as iPSC would provide an autologous product with absence of immune reactions. PBMC-expanded erythroblasts were re-programmed using OCT4, SOX2, c-MYC and KLF4 polycistronic episomal vectors to iPSC, displaying normal karyotype and pluripotency potential. iPSC were adapted to single cell passage allowing single cell-derived iPSC colony directed differentiation, compatible with upscaling. Using Cellquin, iPSC specification to erythroblasts was followed from the appearance of hemogenic endothelium to hematopoietic specification. Differentiations initiated from 200 iPSC yield ~100*10⁶ CD41⁻CD34⁻CD71⁻CD235⁻CD36⁺ erythroblasts within 25 days. Maturation yielded orthochromatic normoblasts expressing gamma globin chains; fetal hemoglobin. Currently we are adapting the single cell derived iPSC colony differentiations to large scale production scale. In conclusion, we showed that single cell-derived iPSC monolayer differentiation approach is simple, highly controlled, robust and this design is compatible with upscaling. Avoiding virus-integrative reprogramming, feeders and usage of in-house designed GMP-grade media we now aim to maintain a cost effective system moving toward clinical application.

Room for notes
Posters

1. Michael Wilson | Preoperative iron deficiency in colorectal cancer patients prevalence and treatment
2. Marea van der Rijst | Unravelling the function of SMIM1 during erythropoiesis and in iron homeostasis
3. Eline Pronk | Identification of transcripts that are differentially translated upon phosphorylation of translation factor eIF2 in erythroblasts
4. Silvia Hoeboer | Reactivation of fetal hemoglobin expression: functional analysis of candidate modifiers
5. Margit Boshuizen | Iron metabolism in critically ill patients developing anemia of inflammation
6. Dagmar Pospíšilová | Hepcidin in newly diagnosed inflammatory bowel disease in children
7. Kristof Van Avondt | Free iron contributes to neutrophil activation in sickle cell disease
8. Sanne Meinderts | FCYR2C polymorphism associates with protection from red blood cell allo-immunization in sickle cell disease
9. Jill Dalimot | Developing red pulp macrophages in vitro
10. Eszter Varga | HUMAN Induced Pluripotent Stem Cell Differentiation to red blood cells
11. Esther Heideveld | Modelling human erythroblastic islands
12. Marlijn Hoeks | Bone marrow iron overload in transfused acute myeloid leukemia patients
13. Michael Wilson | Short-term prognostic value of preoperative intravenous iron in colorectal cancer patients
14. Djuna de Back | A method for the biotinylation of red blood cells for clinical research that complies with Good Manufacturing Practice regulation
15. Jean-Yves Py | Does hemoglobin level influence donor return?
16. Tiffany Timmer | Associations between SNPs and erythrocyte traits including hemoglobin in humans: a systematic literature review and Donor InSight-III data collection
17. Femmeke Prinsze | Distribution of ferritin levels of Dutch donors are ferritin levels proportional to the number of whole blood donations?
18. Lisanne Huis in ‘t Veld | Risk dependent intervention on the prevalence of low hemoglobin deferral
Preoperative iron deficiency in colorectal cancer patients: prevalence and treatment

Background: In preoperative blood management of colorectal cancer patients, intravenous iron therapy is increasingly used to treat anemia and prevent red blood cell transfusions. However, while iron deficiency is the most common cause of anemia, little is known about the prevalence and type of iron deficiency in this population.

Objective: To investigate the prevalence and type of iron deficiency in colorectal cancer patients and to link these to clinical parameters.

Methods: Preoperative iron status and clinical parameters were retrospectively collected for all newly diagnosed colorectal cancer patients in our institution over a 3-year period.

Results: Iron deficiency was observed in 163 (48.1%) of 339 patients. Of these iron deficient patients, 3.7% had an isolated absolute iron deficiency (AID) and 15.3% a functional iron deficiency (FID), while the rest had a combination of AID and FID. Anemia was present in 66.1% of iron deficient patients. Iron deficiency was significantly associated with right-sided tumors (p<0.001), high ASA classification (p=0.002), advanced tumor stage (p=0.01), and advanced age (p=0.04). In comparing clinical parameters between patients with AID and FID, advanced age was significantly associated with FID (p=0.03), and the presence of anemia with AID (p=0.02).

Conclusion: In preoperative colorectal cancer patients, there is a high prevalence of iron deficiency, including a surprisingly high percentage of patients with a component of FID. As iron deficiency and anemia are associated with impaired physical function and worse postoperative outcome, and as both types of iron deficiency require a different treatment strategy, our results stress the urgency of routinely monitoring preoperative iron status and differentiation between types of iron deficiency.
Unravelling the function of SMIM1 during erythropoiesis and in iron homeostasis

Van der Rijst M1,2, Ligthart P3, Veldhuisen B2-1, Van Alphen F4, Van den Biggelaar M4,
Lissenberg-Thunnissen S2, Ouwehand W5, Van der Schoot CE2, Van den Akker E2
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5University of Cambridge Dept. Experimental Haematology, United Kingdom

SMIM1 is a type II transmembrane protein that harbors the VEL antigen. VEL-negative individuals have a homozygous 17bp-deletion (c.64-80del) in SMIM1, associated with lower red blood cells (RBCs) hemoglobin concentration and decreased ferritin/iron plasma levels. Morpholino knock-down of zebrafish SMIM1 shows a reduction of mature primitive erythrocytes, suggesting a role for SMIM1 in erythropoiesis. SMIM1 function in iron homeostasis was supported by measurement in VEL- individuals showing RBCs with classic hallmarks of iron deficiency anemia. Here we aim to unravel the role of SMIM1 during erythropoiesis. We found that SMIM1 is a multimeric protein existing of a mix of phosphorylated and unphosphorylated molecules. Mass spectrometry of VEL- and VEL+ ghosts shows that VEL-RBCs have higher expression of CD71 and lower expression of the aspartic protease SPPL2A. Interestingly, CD71 contains a SPPL2A consensus site at aa307 (GTGD). We hypothesize that uncut CD71 leads to increased expression of CD71 on VEL- individuals potentially leading to inappropriate iron uptake by RBC and lower plasma iron parameters. Currently we aim to elucidate the interplay of SPPL2A, CD71 and SMIM1 during erythropoiesis and in RBC.
A deficiency of iron reduces the heme concentration in erythroblasts, which leads to activation of HRI (heme regulated inhibitor); one of four specific kinases for eukaryote Initiation Factor 2 (eIF2). The GTPase eIF2 and the associated initiator methionine-tRNA (tRNA\textsubscript{met}) are part of the large complex that scans the 5´end of transcripts for a start codon where translation is initiated. Phosphorylation of eIF2 by HRI, or the related kinases, impairs eIF2 function and reduces mRNA translation. eIF2 is a rate-limiting factor in translation initiation, leading to a general translation block to protect erythroblasts from proteotoxicity of free globin molecules. Interestingly, the presence of upstream open reading frames (uORF) in a transcript may render translation of these transcripts hypersensitive to eIF2 phosphorylation. The concentration of available eIF2 is particularly important if eIF2-tRNA\textsubscript{met} has to reassociate with the scanning complex after the translation of an uORF.

To understand how lack of iron affects erythropoiesis, we first identified the transcripts that are aberrantly translated upon eIF2 phosphorylation. We combined ribosome footprinting, to establish genome wide translation efficiency, with RNA sequencing to determine translation efficiency. In addition, we used ribosome footprinting such that it reveals translation start sites that are used in erythroblasts in presence or absence of eIF2 phosphorylation. This allowed us to select transcripts that are hypersensitive to eIF2 phosphorylation and the uORFs that may control this regulation. Currently we investigate the role of uORFs on translation of downstream targets of eIF2 with luciferase assays.
Reactivation of fetal hemoglobin expression: functional analysis of candidate modifiers

Hoeboer SA1, Van Dijk TB1, Gillemans N1, Philipsen S1
1Dept. Cell Biology, Erasmus Medical Center, Rotterdam, The Netherlands

Background: Beta-thalassemias and sickle cell disease are the most common monogenetic disorders affecting beta-globin function in the human population. It is known that the expression of the fetal gamma-globin genes greatly ameliorates the effects of these diseases. Intense research efforts by us and other groups have led to the identification of the first transcription factors that normally suppress the human gamma-globin genes when the expression of the fetal gamma-globin genes switches. Recently a shRNA screen identified novel potential modifiers which had not yet been subjected to stringent genetic tests.

Aim: The aim of this project is therefore to functionally characterize these novel factors in vivo.

Methods: We will use transgenic mice, carrying the human beta-globin locus, a conditional knockout allele of these potential genes, and we included a Cre knockin allele specific for the erythropoietin receptor locus (EpoR-Cre). In peripheral blood, we will measure standard hematological parameters. In the erythropoietic tissues (fetal liver, bone marrow and spleen), we will perform flow-cytometry analysis to assess the distribution of erythroid progenitors at various maturation stages. Furthermore we will collect DNA, RNA and proteins for further analysis.

Results: We will show here that the recombination of our candidate genes is variable, this depends on both the EpoR-Cre line and the target gene. This is most likely caused by selection of unrecombined cells.

Summary/conclusion: Some of our target genes appear to have a crucial role in erythropoiesis, since apparent selection of unrecombined cells is observed with EpoR-Cre mediated recombination.
Iron metabolism in critically ill patients developing anemia of inflammation
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*These authors contributed equally
1Dept Intensive Care Medicine, Academic Medical Center, University of Amsterdam, The Netherlands.
2Dept Blood Cell Research, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

Abstract: Background – Anemia caused by inflammation (anemia of inflammation, AI) is a major cause of anemia in critically ill patients. AI is characterized by a decreased production of RBC, shortened RBC life span and low levels of transferrin and iron in the blood. Increasing the level of iron in the blood might be an attractive therapy to stimulate erythropoiesis in AI. However, knowledge on changes in levels of regulators of iron metabolism during the course of AI is currently limited, which prohibits development of appropriate strategies to counteract AI.

Aim: To obtain insight in the iron metabolism of critically ill patients during the development of AI.

Methods: A case control study was performed comparing the iron metabolism in ICU patients who develop AI (defined as developing anemia while being septic) with 2 control groups: non-anemic patients with sepsis and non-anemic patients without sepsis. Longitudinal collected blood samples were analysed for levels of serum iron, transferrin (saturation), ferritin, haptoglobin, hepcidin, sTfR and erythroferrone.

Results: In patients with AI, levels of iron, transferrin and transferrin saturation showed an early decrease compared to controls, already prior to the development of anemia. Levels of ferritin and hepcidin were increased in AI compared to controls. In the course of AI development, erythroferrone decreased over time.

Conclusion: In critically ill patients with AI, iron metabolism is already altered prior to the development of anemia. AI is characterized by high levels of hepcidin and ferritin and low levels of iron, transferrin and erythroferrone.
Hepcidin in newly diagnosed inflammatory bowel disease in children

Pospišilová D¹, Karásková E¹, Sulovská L¹, Holub D², Horváthová M³

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Inflammatory bowel disease (IBD) including Crohn’s disease (CD) and ulcerative colitis (UC) are chronic relapsing inflammatory disorders affecting gastrointestinal tract. Anemia is very common in children with IBD and recent data suggest that hepcidin, the master regulator of systemic iron homeostasis, plays a substantial role in the development of anemia in IBD.

In this study we compared hepcidin levels in newly diagnosed non-treated pediatric patients with CD (n=53) and UC (n=23) and evaluated associations between hepcidin and laboratory and clinical parameters of IBD activity. We observed that patients with CD had significantly higher serum hepcidin levels compared to patients with UC (22.6 ng/ml vs. 6.5 ng/ml). CD subjects had also higher levels of serum ferritin, CRP and higher platelet numbers, which may be related to increased IL-6 production. Hepcidin was independently associated with ferritin in both CD and UC patients. A significant positive association between hepcidin and platelet numbers was detected in CD patients. These observed differences in newly diagnosed pediatric patients with CD and UC confirm different contribution of iron deficiency and/or systemic inflammation to anemia in these two IBD subtypes and may help making antianemic treatment decisions.

Grant support: Czech Science Foundation, GA15-13732S.
Free iron contributes to neutrophil activation in sickle cell disease

Van Avondt K\textsuperscript{1}, Schimmel M\textsuperscript{1,2}, Nur E\textsuperscript{2}, Bulder I\textsuperscript{1}, Van Mierlo G\textsuperscript{3}, Van Bruggen R\textsuperscript{3}, Biemond BJ\textsuperscript{2}, Luken BM\textsuperscript{1}; Zeerleder S\textsuperscript{1,2}

\textsuperscript{1}Dept. Immunopathology, Sanquin Research, and Landsteiner Laboratory, AMC, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{2}Dept. Hematology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{3}Dept. Blood Cell Research, Sanquin Research, and Landsteiner Laboratory, AMC, University of Amsterdam, Amsterdam, The Netherlands

Background: Chronic hemolysis is a hallmark of sickle cell disease (SCD). Hemolysis in SCD has been associated with elevated levels of heme in the circulation of human patients and SCD mice. Heme was suggested to trigger neutrophil extracellular trap (NET) formation, as the heme-scavenging protein hemopexin (Hpx) reduced NET release in SCD mice.

Aim: To evaluate the potential therapeutic use of Hpx, we determined whether Hpx would prevent NET formation in human SCD sera \textit{ex vivo}.

Methods: Samples were obtained from 32 incidents of vaso-occlusive crisis (VOC) in 24 adult SCD patients. Moreover, steady state samples were obtained after discharge from the hospital. NET formation by healthy neutrophils was studied with fluorescence microscopy for extracellular DNA.

Results: Hemin activated neutrophils to generate reactive oxygen species and release NETs, which was blocked with plasma-derived Hpx. We observed that circulating free heme levels were elevated in SCD patient serum irrespective of disease state, and serum concentrations of Hpx were reduced in both VOC and steady state compared to healthy donor serum. However, addition of Hpx failed to prevent NET formation in all SCD sera tested. The iron moiety of hemin is required for NET release, and neutrophils formed NETs when exposed to free iron. In addition, the iron-chelator deferoxamine or apotransferrin prevented NET formation in sera of patients during VOC.

Conclusion: In summary, scavenging free iron in SCD sera prevents NET formation. We propose that targeting free iron may be explored therapeutically to prevent or treat VOC development in SCD.
FCYR2C polymorphism associates with protection from red blood cell allo-immunization in sickle cell disease

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Background: Complications in sickle cell disease (SCD) are frequently treated with blood transfusions. With repeated transfusions there is a high risk of allo-antibody formation against minor antigens on donor red blood cells (RBC). Approximately 18-76% of frequently transfused SCD patients develop allo-antibodies. In contrast, in the general population allo-immunization occurs in 10% of the frequently transfused patients. The high prevalence of allo-immunization in SCD patients may be the result of a genetic predisposition. Genetic diversity in members of the Fc-gamma receptor (FcγR) family has already been associated with various antibody-mediated diseases, such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus or Kawasaki disease. Therefore, the genetic diversity in the FcγR family might play an important role in allo-antibody formation and the resulting clinical symptoms in SCD.

Aim: The aim of this study was to evaluate whether polymorphisms in the FCGR2 and FCGR3 gene are associated with RBC allo-immunization in a cohort of SCD patients.

Methods: We genotyped a SCD cohort (n=272) with respect to FcγR polymorphisms and gene copy number variation using multiplex ligation dependent probe amplification.

Results: Using a single variant logistic regression analysis our results show that the so called FCGR2C.nonclassical-ORF polymorphism is strongly associated with protection against allo-immunization in SCD (P=0.003, OR=0.26). Furthermore, our data show that this association is especially strong for the protection against formation of non-Rhesus or Kell allo-antibodies.

Conclusion: We have identified FCGR2C-nonclassical-ORF as a protective marker in allo-immunization in SCD. Moreover, our data indicate that formation of highly immunogenic Rhesus or Kell allo-antibodies is less dependent on the genetic background.
Developing red pulp macrophages in vitro


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Background: Senescent and damaged red blood cells (RBCs) are phagocytosed by red pulp macrophages (RPMs) in the spleen, followed by their degradation. Heme, being a degradation product of phagocytosed RBCs, is the physiologic trigger for inducing Spi-C expression by promoting the degradation of the Spi-C transcriptional repressor Bach-1. The transcription factor Spi-C specifically regulates RPM development and is highly expressed in RPMs.

Aim: The interaction of RPMs with aged and damaged RBCs is essential to elucidate the underlying mechanisms and pathways resulting in RBC degradation. However, access to human spleens from which RPMs are isolated, is limited. Thus, developing RPM-like cells in vitro could be a good approach to study the clearance of aged erythrocytes by splenic macrophages.

Methods: To generate RPM-like cells we virally transduced a human monocytic cell line (THP1) to overexpress Spi-C and induced macrophage differentiation by stimulating cells with phorbol myristate acetate (PMA). Furthermore, we stimulated differentiated THP1 Spi-C cells with hemin to reverse the repression by Bach-1 and enhance the differentiation into RPM-like cells.

Results: As a result, the expression of the scavenger receptor for hemoglobin (CD163), Siglec-1, and heme oxygenase were upregulated in differentiated THP1 Spi-C cells. Moreover, a RPM-like morphology was observed in THP1 cells after hemin stimulation.

Summary/conclusion: These results indicate that THP1 cells can differentiate towards a RPM-like phenotype when SpiC is overexpressed. Furthermore, these RPM-like cells will be further evaluated for their potential to serve as substitute for RPMs to study the phagocytosis and degradation of RBCs.

Room for notes
HUMAN induced pluripotent stem cell differentiation to red blood cells

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Donor-derived red blood cells (RBCs) are the most common form of cellular therapy. The use of iPSCs as an autologous immortal source avoid donor-dependency. The in vitro production of iPSC-RBC has proven to be possible, however there are barriers to overcome prior to clinical application. e.g. virus- and transgene-free iPSCs, xeno-free culturing, scale up cultures (1-2*10^{12} erythrocytes). We generated a humanized GMP-grade medium (Cellquin) to control erythroid culture parameters and to reduce culture costs. This medium allowed the 1*10^8 times expansion of erythroblast (EBL) to PBMCs comparable to non-GMP commercial media. Non-integrative polycistronic episomal vector (O-S-K-M-L) was used to reprogram PBMC-expanded EBLs to iPSC, displaying pluripotency potential and normal karyotype. iPSCs were adapted to single cell dissociation allowing directed colony differentiation using a feeder-free monolayer approach. From day 6 of differentiation Cellquin was applied with lineage-specific growth factors, resulted iPSC differentiation to EBLs which was initiated by the appearance of hemogenic endothelium following hematopoietic specification. Our differentiation resulted ~1*10^5 fold expansion to CD41- CD34- CD71+ CD235+ CD36+ EBLs within 21 days which was reproducible using different iPSC lines. Further maturation of iPSC-EBLs yielded CD71+ CD235+ CD36- pure orthochromatic normoblasts expressing mainly gamma globin chains (fetal ~85%), beta globins (adult <=5%) and epsilon globins (primitive ~10%). Currently we are testing enucleation potential of matured iPSC-EBLs.

In conclusion, we showed that our monolayer approach is simple, highly controlled, robust and compatible with upscaling. Avoiding virus-, integrative reprogramming, feeders and with our GMP-grade media we maintained a cost effective system moving toward clinical application.

Room for notes
Modelling human erythroblastic islands


Background: Erythropoiesis occurs in erythroblastic islands in the bone marrow where central macrophages support erythroblast proliferation, differentiation and enucleation. However, these macrophages have been poorly characterized.

Aim: Characterization of human central macrophages and unravelling the mechanism by which they support erythropoiesis to optimize erythroblast cultures and improve erythropoiesis in anemic patients.

Methods: MACS isolated CD14+ and CD34+ cells from PBMC were cultured in presence of SCF, EPO, and dexamethasone (and mifepristone). Cells were analyzed by flow cytometry, imagestream, cytospin, mass spectrometry and qPCR.

Results: CD14+ cells were differentiated towards macrophages expressing CD163, CD169, mannose receptor, CXCR4 and HLA-DR. Mass spectrometry showed that expression of CD163 and MR was strictly Dex-dependent. Furthermore, it revealed dexamethasone-mediated enrichment of lysosome, endocytosis and endothelial development (e.g. STAB1/IL13RA1/CD81/SLC1A3/FKBP5). Functionally, these macrophages can bind erythroblast and phagocytose nuclei, suggesting the formation of erythroblastic islands. Interestingly, similar macrophages were found in human bone marrow and fetal liver.

Conclusion: We showed that Dex-induced macrophages can be used as a model to unravel the mechanism by which they support erythropoiesis.
Background: Secondary iron overload due to red blood cell (RBC) transfusions is associated with increased morbidity and mortality in various patient groups. However, attention for secondary iron overload and its side effects in hematopoietic patients may need improvement. This may be due to unreliability and/or invasiveness of currently available diagnostic tests to detect iron overload and the possible drawbacks of iron chelation therapy.

Aim: To evaluate the effect of the number of RBC transfusions on bone marrow iron scores.

Methods: This study comprises AML patients in a tertiary treatment center, treated according to current AML treatment regimes. Consecutive bone marrow samples were stained with a standardized Perl's staining. The scoring was performed independently by two experienced researchers according to a pre-specified protocol. The slides were blinded to both researchers to prevent bias. Kaplan-Meier survival analysis was performed to assess the median number of RBC transfusions needed to reach the maximum bone marrow iron score.

Results: Thirty-five patients were included (table 1). At 35 RBC transfusions, 74% of AML patients reached a maximum bone marrow iron score. The median number of RBC transfusions to reach a maximum bone marrow iron score was 23.5 units (95% CI 19.9-27.1), after a mean of 1.64 chemotherapy courses (SD 0.99).

Summary/conclusion: RBC transfusion burden in AML patients is associated with high bone marrow iron scores. Therefore, high bone marrow iron scores may be a valuable indicator of secondary iron overload in transfused AML patients and may guide iron chelation therapy.

Table 1: Patient characteristics

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<th>Mean (±SD)</th>
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<tr>
<td>Age</td>
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<tr>
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</tbody>
</table>

SD: Standard deviation; n = amount of individuals; % = percentage of total cohort (n=35); SCT: Stem cell transplantation
Short-term prognostic value of preoperative intravenous iron in colorectal cancer patients

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Background: Both preoperative anemia and the treatment of anemia, blood transfusions and erythropoiesis-stimulating agents, are associated with increased postoperative morbidity and increased risk of tumor recurrence.

Aim: To assess the efficacy of preoperative intravenous iron infusion in colorectal cancer patients.

Methods: The prognostic value of preoperative intravenous iron was assessed in a retrospective cohort, including all patients who underwent surgery for colorectal cancer between 2010-2016 in a single center hospital. For comparative analyses all anemic patients (at presentation) were divided into 2 groups: usual care (UC) group (i.e. no therapy) and intravenous iron (IV) group (iron infusion <6 weeks preoperative), excluding preoperative blood transfusion and neoadjuvant chemotherapy. For logistic regression analyses, all anemic patients were included. Primary outcome was the change in hemoglobin level; secondary outcomes were the percentage of patients with postoperative complications and blood transfusions.

Results: In total, 758 colorectal cancer patients, eligible for inclusion, underwent surgery, of which 318 (41.9%) were anemic. The IV and the UC group, both excluding blood transfusion, included 52 and 153 patients with mean hemoglobin (Hb) at diagnosis of 6.3 and 6.9 mmol/L, respectively. In IV patients, Hb level was significantly increased (IV=0.65 mmol/L vs UC=0.10 mmol/L, p<0.001), most distinct in patients with advanced anemia and characteristics of absolute iron deficiency, and less peri- (p=0.3) and postoperative blood transfusions (p=0.4), and complications (p=0.03) were observed, as compared to UC patients. In multivariate logistic regression analyses administration of intravenous iron therapy did not affect postoperative blood transfusion and complication rate (OR 0.54, p=0.14 and OR=0.91, p=0.77, respectively).

Conclusion: Based on this retrospective cohort study, implementation of intravenous iron therapy in anemic colorectal cancer patients leads to higher increase of preoperative hemoglobin level, especially in patients with advanced anemia and characteristics of absolute iron deficiency, but this effect did not translate into a significant decrease in peri- and postoperative blood transfusions and complications.
A method for the biotinylation of red blood cells for clinical research that complies with Good Manufacturing Practice regulation


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Background: Biotinylated RBC can be used to independently and accurately measure RBC survival and clearance. This method has several advantages over the standard 51Cr method: 1) study subjects are not exposed to radiation; 2) small blood volumes are required to analyze results, and 3) multiple RBC populations can be measured simultaneously in the same individual. However, so far only experimental non-validated biotin-labeled RBC products have been transfused.

Aim: The goal of this study was to produce a standardized biotin-labeled RBC product in a fast, simple and sterile manner that can be used for clinical research and for the evaluation of new blood products.

Methods: Red Cell Concentrate (RCC) fractions were labeled with two different concentrations of biotin in a closed system (according to GMP), to ensure sterility of the labeled end product. Using a flow cytometric analysis, the reproducibility and robustness of the biotin labeling protocol was assessed, as well as the stability of the labeled (un-)irradiated end product. Additionally, RBC parameters such as phosphatidylserine exposure (PS), Na, K, free hemoglobin, ATP, pH and morphology were determined prior to and after biotin labeling to rule out effects of the labeling procedure on biological activity.

Results: Our data show that RCC can be labeled according to GMP with two different biotin concentrations in a standardized (GMP) manner, without affecting the biological activity.

Summary/conclusion: An easy, rapid (<2 hrs), GMP qualified and robust method was developed to generate biotin-labeled RBC for clinical research.
Does hemoglobin level influence donor return?

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Background: Biotinylated RBC can be used to independently and accurately
Blood donations may worse donor pre-anaemic state, with clinical signs like fatigue.

Aim: To see if such possible signs, presumably linked to donor low haemoglobin level, may impair his return.

Methods: We studied a selection of year 2015 French whole blood donors, born in years 1955, 1965, 1975, 1985, and 1995. Donations without any return were included as “no return”. Other donations were only included if the next event was a spontaneous application. Delay between index donation and the donor return was registered, and considered if less than one year.

Results: 126,442 female and 128,887 male donations were included, with respectively a one year return of 64.7% and 71.8% and a median delay of 163 and 141 days. There is no delay rise with donations near the low hemoglobin thresholds, nor any deterioration of the return rate. By contrast, data rather point to deterioration in case of high level. Donor age does not change these results.

Summary/conclusion: Even if whole blood donation may be immediately less tolerated in pre-anemic donors, it does not affect their return after several weeks.
Associations between SNPs and erythrocyte traits including hemoglobin in humans: a systematic literature review and Donor InSight-III data collection
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Background: Blood donation results in a loss of erythrocytes and therefore new erythrocytes and hemoglobin (Hb) need to be produced. Several genetic loci have been shown to be associated with Hb and other erythrocyte parameters.

Aim: (1) Identify which single nucleotide polymorphisms (SNPs) show associations with Hb/erythrocyte traits in humans, and (2) investigate associations of SNPs with Hb trajectories in blood donors.

Methods: (1) A systematic review on SNPs associated with Hb/erythrocyte traits is ongoing. (2) ,863 donors (with declining and stable Hb trajectories based on latent-class growth analyses and a randomly selected group) have participated in Donor InSight-III (DIS-III). Data-collection consisted of blood sampling for a full blood count, SNP array (UK Biobank Axiom® Array, Affymetrix, CA, USA) and analysis of iron parameters, and questionnaires regarding health, lifestyle and menstruation.

Results: 3,597 titles/abstracts were screened of which 137 full texts are currently being screened on relevance (preliminary results on poster). DIS-III data are currently being prepared for data analyses.

Summary/conclusion: We expect to find known and new associations between SNPs and Hb (trajectories)/erythrocyte traits.
Distribution of ferritin levels of Dutch donors – are ferritin levels proportional to the number of whole blood donations?

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3Dept. Public Health, Academic Medical Center, Amsterdam, The Netherlands

Background: Donation is associated with a loss of iron. To prevent iron deficiency a minimum haemoglobin level is set as eligibility criterion and is measured before each donation. However, ferritin might be a more suitable parameter as it is an indicator of iron stores.

Aim: To map the distribution of ferritin levels in categories of number of donations in the Donor InSight-III population and compare these with Hb measurements in order to develop a better donor screening.

Methods: Donor InSight (DIS) is a cohort study among the Dutch donor population (three questionnaire rounds). In the DIS-III population Hb and ferritin were measured (2015-2016). Data on the number of previous donations were retrieved from EProgesa, the blood bank information system. Per category of donation history mean±SD ferritin and Hb levels were calculated separately for men, premenopausal (≤44) and postmenopausal women (≥45).

Results: A total of 2845 participants were included in these analyses, all had made at least 1 donation. Mean Hb levels were similar for donors with varying donation histories. Mean ferritin levels were approximately 50% lower for donors with more than 50 donations, compared to those with 2-10 donations.

Discussion: Hb levels are relatively stable, ferritin levels drop gradually with the number of donations. However, a stabilization was found at extreme loyal donors (>50 donations), this might be due to donor selection effects. Further research is necessary to disentangle donation history from age effects. Longitudinal data collection from new donors throughout their donor career should clarify patterns of ferritin trajectories.
Risk dependent intervention on the prevalence of low hemoglobin deferral

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2Dept. Social Medicine, Academic Medical Center, University of Amsterdam

Background: Donors with a low hemoglobin (Hb) level are deferred for donation. Although meant to protect donors, these deferrals are demoralizing and increase the risk for donor lapse. Based on previously developed sex specific prediction models for Hb deferral risk, an intervention strategy to decrease the prevalence of donor deferrals was tested.

Aim: The aim of the current study was to conduct an intervention trial for donors with a high risk of Hb deferral, to investigate the effect of prolongation of the donation interval and/or dietary advice on the Hb level and prevalence of Hb deferral on the subsequent visit.

Methods: Donors from 26 blood collection centers throughout the Netherlands with a Hb level lower than 0.2 mmol/L above the gender specific cut-off (total N = 12073) received either no intervention, a prolongation of the donation interval of six or 12 months, dietary advice, or both. The effect of the intervention on Hb levels and deferral rates at the subsequent donation attempt were analyzed using Generalized Estimating Equations.

Results: Young women and donors with lower Hb levels at the first donation attempt were at higher risk of deferral at the second donation attempt. Prolongation of the donation interval by six months significantly reduced the risk of deferral. However, there was no additional benefit of an extra-long 12 month prolongation or dietary advice.

Summary/conclusion: Implementation of a protocol which defers donors at risk for six additional months may reduce the risk of Hb related deferrals in the future.