**Red Cell Special Interest Group 2018 – Report for Bloodlines**

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The Red Cell SIG meeting provides an annual forum for presentation of UK based red cell research and should be of interest to clinicians, haematologists, biochemists, or anyone involved in red cell research or wishing to learn more about red cells. The Red Cell SIG is usually held on the first day of the BBTS Annual Scientific Meeting, aiming to encourage participation by both the BBTS members and by the wider scientific community. This year, Red Cell SIG was held on Wednesday, 3rd October, at the BBTS Annual Scientific Meeting in Brighton. The meeting comprised three sessions, each with three speakers presenting the very latest developments in our understanding of rare inherited anaemias, disease modification of red cells and improving patient outcomes.

**Session 1: Rare inherited anaemias**

The first speaker of this session, Noemi Roy from the MRC Weatherall Institute of Molecular Medicine in Oxford, described the James Lind Alliance Priority Setting Partnership for rare inherited anaemias. This Priority Setting Partnership (PSP) brought together patients, carers, healthcare professionals, and allied organisations such as charities etc. in order to identify current uncertainties in diagnosis, treatment management and care delivery related to rare inherited anaemias and important to all of the groups. The PSP aimed to address questions which cannot be answered by existing research, in order to better guide future funding. In total, 500 questions were collected, which were then sorted into indicative categories, discarding duplicates. This resulted in 78 questions of which 25 questions were prioritised by a questionnaire. A workshop was held to select the top ten questions and these will be used to decide on future funding priorities.

The next talk was an update on the diagnosis and management of congenital dyserythropoietic anaemias given by Momin Ahmed of the Royal Free London NHS Foundation Trust. Diagnosis of these rare orphan diseases requires first the exclusion of other more common anaemias and where possible the examination of the cell morphology from bone marrow, peripheral blood and reticulocytes. Recent classification by European Network for Rare and Congenital Anaemias lists 10 different types, of which CDAI and CDAII are the most prevalent. CDAI is a macrocytic anaemia, found predominantly in the UK and France, caused by mutations in *CDAN1* and *C15ORF41* affecting nucleosome assembly. Bone marrow examination shows many red cells, binuclear cells, basophilic erythroblasts and nuclear bridging. CDAII is a normocytic anaemia, more common in Italy, caused by mutations in SEC23B affecting perinuclear trafficking. Bone marrow exam shows binuclear cells and double plasma membranes. CDAIII includes the Swedish and sporadic forms. There are also CDA types IV – VII and other even rarer forms. Dr Ahmed illustrated different CDA types through four case studies, two with CDAI, one with CDAII and iron overload and one with a type VI-like CDA caused by a novel mutation in a transcription factor gene.

The third speaker of the morning session was Caroline Scott from the MRC Weatherall Institute of Molecular Medicine in Oxford, who described the use of interferon-α treatment for CDA-I. An early observation was that interferon-α, prescribed to a CDA-I patient to treat a hepatitis C infection, was found to cause a rapid improvement in the anaemia and has since been the main therapy for this disease. However, compliance is a problem due to side effects. The mechanism of interferon-α action is unknown and to help elucidate it, two types of experiments were conducted. Firstly, CD34+ cells from patients were cultured with and without interferon-α to investigate whether the number of abnormal nuclei could be reduced *in vitro* by the treatment. Secondly, the effect of interferon- α on erythroid progenitors was studied. Here they saw the CDA-I patients formed abnormal erythroid clusters with weak expression of CD235 and CD71 and this was improved by treatment with interferon-α. Overall, interferon-α improved proliferation and differentiation of these cells, affecting the erythroid pathway much earlier than previously thought.

**Session 2: Disease modification of red cells**

Can Ince, from Erasmus Medical Center, Rotterdam, The Netherlands, opened this session with his talk on sepsis, hypoxia and erythrocyte dysfunction. Professor Ince proposed that arterial measurement of the efficacy of red blood cell (RBC) transfusions was an unreliable indicator, whilst microcirculation observed as an organ can provide better guide. RBCs must penetrate the microcirculation, where the glycocalyx of endothelial cells sense the deforming RBC and interact for optimal oxygen delivery. RBCs also need oxygen to power the NaKATPase and maintain their hydration and deformability. Older or diseased RBCs are less deformable and less able to deliver oxygen efficiently. In sepsis, RBCs are found to be less deformable and more prone to lysis. In sepsis patients, a correlation was found between mortality and free haemoglobin levels, which should be monitored *in vivo* as an easy indicator. In such patients, transfusion of older lysis-prone RBCs should be avoided and ideally, perfusion of the microcirculation should be monitored in sepsis patients undergoing transfusion. Prof. Ince argued a benefit of choosing higher transfusion triggers for sepsis patients, especially elderly ones.

The second talk of the session on exploring erythrocytes as blood biomarkers for Alzheimer’s disease was presented by Elizabeta Mukaetova-Ladinska from the Institute of Neuroscience, Psychology and Behaviour, University of Leicester. There will probably be 131.5 million people with dementia worldwide by 2050. There are >100 types of dementia and the condition can go on for 30 years. Early symptoms vary, and they include depression, paranoia, anxiety, memory problems, progressing to irritability, poor concentration, hallucination and aggression, and global cognitive impairment. However, diagnosis is usually made only when overt memory problems are detected, and can take 3 – 6 months to establish. The diagnosis is usually done in specialised clinical settings and is based on clinical assessments that include collateral information, physical, neurological and cognitive-behaviour assessments, ECG, chest X-ray and brain scans. Imaging shows cortical atrophy, reduced glucose uptake in the brain, as well as amyloid and tau accumulations. However, neuroimaging can take several hours and many patients cannot cope with remaining still for so long. Lumbar puncture can be used but is invasive. In order to improve early diagnosis, Prof. Mukaetova-Ladinska and her team have identified dementia-characteristic proteins that are also found in the blood. RBCs from people with dementia bind β-amyloid, contain tetramers of α-synuclein (found in neuronal synapses and also in Lewy bodies) and/or higher levels of ubiquitin which could be used as a marker for the disease.

In the last talk of the session, Fiona McQuaid, from the Institute of Immunology and Infection Research, University of Edinburgh, gave an update on red cell rosetting receptors in *Plasmodium falciparum* malaria. There are 200 million cases of malaria per year and 445,000 deaths with no adjuvant treatments available for the most severe cases. Rosetting is the process where uninfected RBCs stick to infected cells, and this is seen more frequently in severe cases of malaria. The infected cells present receptors, including PfEMP1, RIFIN, STEVOR, but these are challenging therapeutic targets because PfEMP1 are highly variable and RIFINa may only be important for blood group A rosetting. Investigation of the host RBC receptors involved in rosetting suggested ABO, CR1, glycophorins A and C and/or heparin sulphate may be involved in the process. Dr McQuaid presented data suggesting that Heparan sulphate is unlikely to be an important rosetting receptor and now hopes to culture a panel red cells lacking proteins of interest, to further test against infected cells.

**Session 3: Improving patient’s outcomes**

The last session of 2018 Red Cell SIG started with a talk on the potential enhancement of red blood cell transfusion compatibility using CRISPR/Cas9-mediated erythroblast gene editing, which was given by Timothy Satchwell, School of Biochemistry, University of Bristol. Alloimmunisation is often a problem for patients receiving multiple RBC transfusions, such as those with thalassemia or sickle-cell disease, and can make it very difficult to find compatible blood to transfuse these patients. This problem is exacerbated in Western countries where the blood donor population is from a different ethnic background from the patients affected by these conditions. To help ameliorate the problem, Dr Satchwell has produced a multiple knock-out cell line as a proof-of-principle demonstration that multi-compatible RBCs could be generated. He utilised CRISPR/Cas9-mediated genome editing of BEL-A cells to generate multiple cell lines deficient in individual blood groups. Eventually, the edits were combined to generate a single cell line deficient in ABO (Bombay phenotype), RhAG (Rhnull), Kell (K0), Duffy (Fynull), GPB (S-s-U-). These antigens are responsible for the most common transfusion incompatibilities. The cell line could be differentiated to produce fully functional, deformable reticulocytes.

Next there was a talk by Janejira Kittivorapart, from Bristol Institute for Transfusion Sciences & School of Biochemistry, University of Bristol on a proteomic approach to discovering predictive biomarkers for transfusion in thalassaemia. Disease severity is varied in patients with HbE/β-thalassaemia and differences in circulating extracellular vesicles (EVs) may contribute to this. EVs were collected from the plasma of 15 patients and controls, quantified and analysed by tandem mass tag labelling mass spectrometry. Unsurprisingly, alpha haemoglobin stabilizing protein was found to be the most increased protein in the patients’ EVs. Catalase, superoxide dismutase, T-complex proteins, heat shock proteins, transferrin receptor, ferritin and cathepsin S were upregulated in patient EVs. Interestingly, haptoglobin and hemopexin were reduced in patients’ EVs reflecting the level of haemolysis that occurs in HbE/β-thalassaemia patients. Western blotting confirmed these results. Monitoring the levels of these plasma proteins may help in the clinical management of thalassemia.

The last talk in this session was given by Peter Smethurst, from the Component Development Laboratory, NHS Blood and Transplant, Cambridge, who presented work on REDJUVENATE – a validation study for the clinical trial. The REDJUVENATE trial plans to test whether outcomes in adult cardiac surgery patients are improved if they receive rejuvenated, instead of standard, RBCs. Rejuvenation of RBCs involves incubation with a solution, Rejuvesol®, containing pyruvate, inosine, phosphate and adenine at 37°C for an hour. The cells must then be washed and reconstituted as a standard unit. This process should restore glycolytic flux and may alleviate some of the storage lesion built up during RBC storage. The study was performed to devise a manufacturing process and validate this as effective and safe. All units remained sterile through the process and showed the expected restoration of cellular metabolites (ATP and 2,3-DPG) whilst supernatant markers of storage age (haemolysis, potassium) were reduced along with rejuvenation-derived purine levels (inosine, hypoxanthine). Rejuvenation did not lead to changes in RBC antigen immunophenotypes.

The 2018 Red Cell SIG meeting was well attended, generating many questions and encouraging audience participation and discussion.