**Institution:** University College London

**Unit of Assessment:** 1 - Clinical Medicine

**Title of case study:** Cell membrane biology in haemolytic anaemias: advances in diagnosis and treatment

## 1. Summary of the impact

Research at UCL on human haemolytic anaemias known as the 'hereditary stomatocytoses' has improved diagnosis of these conditions, meaning that patients now avoid unnecessary and potentially life-threatening splenectomies, and inappropriate investigation and treatment for raised potassium levels. Identification of a common single nucleotide polymorphism that causes apparently normal red blood cells to leak salt when cooled (as is normal procedure with donated blood) has raised awareness of this issue in the NHS Blood and Transfusion service, with the result that individuals with this condition have been identified among existing donors, and work is underway to develop a screening method to exclude such individuals from donating blood that cannot be stored safely. Finally, the research has facilitated diagnosis of the recessive metabolic disorder phytosterolaemia by blood count, allowing these individuals to be given appropriate dietary treatment to control their cholesterol levels.

## 2. Underpinning research

The hereditary stomatocytoses are a class of human haemolytic anaemias in which the membrane of the red blood cell 'leaks' the salt atoms sodium and potassium. These 'leaky' cells have a tendency to swell and burst in the circulation. Research over the last 20 years by Professor Gordon Stewart at the UCL Division of Medicine has made novel contributions to the molecular understanding of these diseases, resulting in advances in diagnosis and clinical management.

Prior to work by Stewart, patients with stomatocytosis were commonly diagnosed with a related condition, hereditary spherocytosis. Stewart identified that a crucial feature in diagnosis is the dependence of the increased sodium-potassium permeability as temperature falls. This discovery allowed the clear distinction of a series of phenotypic variants and discriminated each of these variants from hereditary spherocytosis **[1]**. In that condition, removal of the spleen is beneficial; Stewart was the first to demonstrate that splenectomised patients with hereditary stomatocytosis, however, have an increased risk of venous thromboembolism (deep vein thrombosis, DVT), with potentially fatal embolism of clot to the lungs **[2]**.

The research also demonstrated that many of these patients were also being misdiagnosed with hyperkalaemia (high plasma potassium). True hyperkalaemia is a medical emergency, but in these patients the plasma potassium was normal in vivo. The high measured potassium was an artefact of blood sample processing. If a freshly taken blood sample was allowed to cool towards room temperature prior to separation of red cells from plasma during routine blood sampling, potassium leaked from the red cells and gave rise to factitious or 'pseudohyperkalaemia'. These patients were at risk of inappropriate referral to hospital and erroneous emergency treatment for the raised potassium.

In collaboration with Dr Lesley Bruce (NHS Blood and Transplant) Stewart showed that these diseases can be caused by mutations in at least three different genes: *SLC4A1*, coding for the band 3 anion exchanger **[1]**; *RhAG*, coding for the so-called rhesus-associated glycoprotein, a gas channel **[3]**; and *SLC2A1*, coding for the GLUT1 facilitative glucose transporter **[4]**. Salt-leaky mutations in GLUT1 cause recognisable haematological-neurological-ophthalmological syndrome **[5]**.

In the course of this work, Stewart (in collaboration with Dr David Rees, King's College London) also showed that the rare hypercholesterolaemic metabolic condition phytosterolaemia can readily and inexpensively be diagnosed via a specific haematological presentation **[6]**. This is an important

diagnosis, because the condition does not respond to statins, the mainstay of treatment of raised cholesterol.

Among the many families studied by Stewart, that found in Cardiff **[7]** is emerging as important. That family's asymptomatic non-haemolytic condition is caused by a gene change that is already recognised as a single nucleotide polymorphism, present at a frequency of 0.1% in the caucasian population. Red cells from affected donors show a major salt leak at refrigerator temperatures only, and the cells are unsuitable for storage in blood transfusion, as the blood is likely to cause true hyperkalaemia when administered to a recipient.

## 3. References to the research


[1] Bruce LJ, Robinson HC, Guizouarn H, Borgese F, Harrison P, King MJ, Goede JS, Coles SE, Gore DM, Lutz HU, Ficarella R, Layton DM, Iolascon A, Ellory JC, Stewart GW. Monovalent cation leaks in human red cells caused by single amino-acid substitutions in the transport domain of the band 3 chloride-bicarbonate exchanger, AE1. Nat Genet. 2005 Nov;37(11):1258-63. http://dx.doi.org/10.1038/ng1656

[2] Stewart GW, Amess JA, Eber SW, Kingswood C, Lane PA, Smith BD, Mentzer WC. Thrombo-embolic disease after splenectomy for hereditary stomatocytosis. Br J Haematol. 1996 May;93(2):303-10. http://dx.doi.org/10.1046/j.1365-2141.1996.4881033.x

[3] Bruce LJ, Guizouarn H, Burton NM, Gabillat N, Poole J, Flatt JF, Brady RL, Borgese F, Delaunay J, Stewart GW. The monovalent cation leak in overhydrated stomatocytic red blood cells results from amino acid substitutions in the Rh-associated glycoprotein. Blood. 2009 Feb 5;113(6):1350-7. http://dx.doi.org/10.1182/blood-2008-07-171140.

[4] Flatt JF, Guizouarn H, Burton NM, Borgese F, Tomlinson RJ, Forsyth RJ, Baldwin SA, Levinson BE, Quittet P, Aguilar-Martinez P, Delaunay J, Stewart GW, Bruce LJ. Stomatin-deficient cryohydrocytosis results from mutations in SLC2A1: a novel form of GLUT1 deficiency syndrome. Blood. 2011 Nov 10;118(19):5267-77. http://dx.doi.org/10.1182/blood-2010-12-326645.

[5] Bawazir WM, Gevers EF, Flatt JF, Ang AL, Jacobs B, Oren C, Grunewald S, Dattani M, Bruce LJ, Stewart GW. An infant with pseudohyperkalemia, hemolysis, and seizures: cation-leaky GLUT1-deficiency syndrome due to a SLC2A1 mutation. J Clin Endocrinol Metab. 2012 Jun;97(6):E987-93. http://dx.doi.org/10.1210/jc.2012-1399.

[6] Rees DC, Iolascon A, Carella M, O'marcaigh AS, Kendra JR, Jowitt SN, Wales JK, Vora A, Makris M, Manning N, Nicolaou A, Fisher J, Mann A, Machin SJ, Clayton PT, Gasparini P, Stewart GW. Stomatocytic haemolysis and macrothrombocytopenia (Mediterranean stomatocytosis/macrothrombocytopenia) is the haematological presentation of phytosterolaemia. Br J Haematol. 2005 Jul;130(2):297-309. http://dx.doi.org/10.1111/j.1365-2141.2005.05599.x

[7] Gore DM, Chetty MC, Fisher J, Nicolaou A, Stewart GW. Familial pseudohyperkalaemia Cardiff: a mild version of cryohydrocytosis. Br J Haematol. 2002 Apr;117(1):212-4. http://dx.doi.org/10.1046/j.1365-2141.2002.03376.x


## 4. Details of the impact

The underpinning research described above has improved the diagnosis of the hereditary stomatocytoses, avoiding potentially harmful splenectomies in these patients, and inappropriate investigation of artefactual hyperkalaemia. This work has thus established a standard of care for this patient group.

Since 2008, Stewart's team have analysed ~500 blood samples from Europe and North America, successfully identifying hereditary stomatocytoses in 17 pedigrees. The impact of this on patient

care and management has been paramount, facilitating an accurate diagnosis, enabling counselling and avoiding splenectomy for at least 30 individuals in the UK. One patient described the impact of these findings on her family as follows, contrasting those individuals who underwent splenectomy, with those who were able to avoid it as a result of Stewart's research:

> "*Over the years a number of the family have had their spleens removed either here or in Northern Ireland. Our own mother had a splenectomy and she died in 2004 with problems which were associated with blood clotting and Crohn's Disease. My elder sister has had the most problems including a blockage in a vein leading to the liver after her splenectomy and now has problems with bleeding from the gullet ... I have an aunt over in Northern Ireland who had her spleen removed and she had constant problems with her breathing. Those of us who have not had our spleens removed (which includes myself and my younger brother) are well with little problem or complication from DHS [dehydrated hereditary stomatocytosis]. We have another aunt over in Northern Ireland who did not have her spleen removed. She had 12 children without any major problems. We have anaemia, and we need occasional transfusions (not all of us), but none of the breathing problems or other complications which other family members have experienced*" **[a]**.

A further impact of accurate diagnosis is the correct understanding of high plasma potassium levels. In about half of the cases, high plasma potassium levels have been explained on the basis of leaky red cells, removing the need for repeated urgent hospital attendances for repeat potassium measurements. A patient described the impact on her family as follows:

> "*High plasma potassium levels… were a bother to us all as, after any blood test we would be recalled to hospital as an emergency… One thing that has changed our lives is the recognition that our habitually very high plasma potassium levels are due to the abnormality in the red cell membrane. This new understanding has saved us countless late-night hospital visits for urgent repeat potassium measurements*" **[b]**.

This work has been widely cited and included in standard textbooks on this subject **[c]**.

As described above, Stewart's research identified a common single nucleotide polymorphism (SNP) in the Caucasian population ('Cardiff' [ref 7, above]) which causes a milder non-haemolytic anaemia. When blood from these individuals is cooled, the effect on the red cells is substantial, resulting in major potassium leaks. As blood taken from donors is routinely cooled, this raises the potassium in the donated blood from these individuals. When administered to patients this risks unexpected and potentially harmful true hyperkalaemia and the transfusion of damaged or partly lysed red cells, making the transfusion less efficacious. As a result of work by Stewart and Bruce, blood from such donors has been identified in the existing pool of donated blood at the National Blood Transfusion Service (NBTS). In reaction to this finding, the NBTS is now in the process of implementing a screening strategy for this polymorphism on all donated blood in order to avoid this complication. This will have an impact at a national and potentially international level. The work is the subject of a current patent application **[d]**.

Finally, the underpinning research has improved the diagnosis of the rare metabolic condition phytosterolaemia (sitosterolaemia), in which absorption of both dietary cholesterol and plant sterols is unrestricted. This can now be identified quickly by a blood count rather than mass spectroscopy of lipids, enabling diagnosis of the condition to take place via routine haematology, without expensive mass spectrometry. As a result of this work, a number of individuals and families have been identified in the UK (five families in Stewart's study, [ref 6, above]), in China **[e]** and in the US **[f]**. The impact of the correct diagnosis is to enable effective treatment of the condition. Patients with the condition do not respond to the HMG CoA reductase inhibitors (statins), which are currently the mainstay in the treatment of hypercholesterolaemia. Individuals instead must be treated with a diet low in cholesterol as well as plant sterols including sitosterol. With correct dietary treatment, however, cholesterol remains low **[g]**.

## 5. Sources to corroborate the impact

[a] Testimony from Patient CM. Copy available on request.

[b] Testimony from Patient SB. Copy available on request.

[c] For example: Handin R, Lux S, Stossel T, editors. Blood: Principles and Practice of Hematology. Philadelphia: JB Lippincott; 2003. See chapter on Disorders of the red blood cell membrane, p.1709-1858. Copy available on request.

[d] Statement provided by Senior Research Scientist, Bristol Institute for Transfusion Sciences, NHS Blood and Transplant, corroborating effects of cooling on blood from donors; the identification of such individuals among existing donors, and the ongoing development work. Copy available on request.

[e] Wang G, Wang Z, Liang J, Cao L, Bai X, Ruan C. A phytosterolemia patient presenting exclusively with macrothrombocytopenia and stomatocytic hemolysis. Acta Haematol. 2011;126(2):95-8. http://dx.doi.org/10.1159/000327248.

[f] Neff AT. Sitosterolemia's stomatocytosis and macrothrombocytopenia. Blood. 2012 Nov 22;120(22):4283. http://dx.doi.org/10.1182/blood-2012-06-429449

[g] Testimony from patient PP. Copy available on request.