Institution: Queen's University Belfast

Unit of Assessment: 1

Title of case study: Identifying Patients with Rare Forms of Erythrocytosis

1. Summary of the impact

Diagnostic tests have been successfully developed for identification of the cause of erythrocytosis, particularly in patients with unexplained forms of this rare disease. A diagnostic service with worldwide reach was developed for the genetic characterisation of patients that carry mutations identified by the Queens's group. It deals with approximately 100 samples per year referred for investigation for this rare disease from the UK, Europe and further afield. Proper diagnosis helps in management of patients with erythrocytosis where the problem is not mutation in one of the familiar causative genes. A pan-European web-based database has been established to collect information on long-term outcomes to inform patient management.

2. Underpinning research

There is a long tradition of haematology research in Queen's, with an exciting team of clinicians and scientists that has included Professor Bridges (Professor of Haematology from 1980 to 1994) and currently Professor McMullin (Professor of Clinical Haematology since 2006), plus scientists such as Professor Lappin (Professor of Haematology from 1997 to 2010) and Dr Percy (an NHS clinical scientist and an Honorary Reader in Queen's since 2009). The group has carried out extensive investigations focussed on the control of erythropoiesis since 1980, which resulted in many high impact publications[1-6].

An erythrocytosis, where there is an increase in the red cell mass and a resulting increase in the number of red cells or erythrocytes in the body, is usually due to the acquired clonal haematological disorder polycythaemia vera (a classical haematological disorder with increased production of all three types of blood cells) or a secondary cause where the hormone erythropoietin is up-regulated for some reason, which causes an overproduction of specifically only red blood cells.

In rare cases, the cause of the erythrocytosis is not clear. This has been a long term focus of the Queens's group. Since 1992 the group has explored the causes of the myeloproliferative blood diseases, in particular polycythaemia vera. They came to recognise that a group of patients did not have polycythaemia vera but had pure erythrocytosis (where there was an increase in red cell production only) of unknown cause. These individuals were investigated and samples were referred from the UK, Ireland and beyond. In 1993 a mutation was discovered in the *erythropoietin receptor* gene in an Olympic cross country skier with extreme erythrocytosis. Our group described the same mutation, arising independently, in 1998[1].

In 2002, a group in the United States discovered that a single mutation in the von Hippel Lindau (*VHL*) gene was the cause of erythrocytosis in a large cohort of individuals from the Chuvash area in the Upper Volga region. Investigation of our cohort of erythrocytosis patients for this mutation demonstrated that a number (not from the Chuvash area) had the same variant[2]. The presence of these mutations in a number of families in various parts of the world led to the justification for their inclusion in the screening programme.

The group then moved on to investigate genes in the oxygen sensing pathway. In 2006, they were the first to identify a mutation in the *PHD*2 gene in one of the families who had been referred for investigation[3,4]. Having identified a potential mutation they then demonstrated with collaborators in the University of Pennsylvania that the mutation did indeed cause erythrocytosis. The Queen's group then discovered the first mutation in the *HIF*2a gene, another gene encoding a protein in the oxygen sensing pathway[5,6], in a family with erythrocytosis in three generations, in 2008. The functional effect of the mutations was elucidated with our US collaborators and subsequently, it was shown that a number of mutations in these genes also cause erythrocytosis.

In summary, this work has established that the mutations identified by this and other groups should be screened for in rare patients with erythrocytosis who neither fulfil the criteria for polycythaemia vera nor have an obvious secondary cause. It formed the basis for the successful development of a diagnostic service.

3. References to the research

1. **Percy MJ, McMullin MF**, Roques AW, Westwood NB, Acharya J**,** Hughes AE**, Lappin TRJ**, Pearson TC. Erythrocytosis due to a mutation in the erythropoietin receptor gene. Br. J. Haematol. (1998) 100, 407-410. doi: 10.1046/j.1365-2141.1998.00550.x (*Cited 31 times. This paper is the first description of a patient with a mutation in the erythropoietin receptor arising independently after the original publication by A la Chapelle in Finland*).

2. **Percy MJ, McMullin MF** Jowitt SN, Potter M, Treacy M, Watson WH, **Lappin TRJ**. Chuvash-type congenital polycythemia in 4 families of Asian and Western European ancestry. Blood (2003) 102, 1097-1099. doi: 10.1182/blood-2002-10-3246 (*Cited 58 times. This paper describes 4 different kindreds with the same mutation that had been discovered in a gene in the oxygen sensing pathway. Before this description this particular mutation had only been seen in the original cohort in the Chuvashia area of Russia. This discovery provided the justification for analysis of erythrocytosis patients with disease of unknown origin for mutations in VHL*).

3. **Percy MJ**, Zhao Q, Flores A, Harrison C, **Lappin TRJ,** Maxwell PH**, McMullin MF**, Lee FS. (joint senior author). A family with erythrocytosis establishes a role for PHD2 in oxygen homeostasis. Proceedings of the National Academy of Science (2006) 103 (3) 654-659. doi: 10.1073/pnas.0508423103 (*Cited 134 times. This paper describes the first ever mutation found in man in one of the Prolyl Hydroxylase genes PHD2, causing erythrocytosis*).

4. **Percy MJ,** Furlow PW, Beer PA, **Lappin TR, McMullin MF**, Lee FS. A novel erythrocytosis-associated PHD2 mutation suggests the location of a HIF binding groove. Blood. (2007) 110 (6) 2193-6. doi: 10.1182/blood-2007-04-084434 (*Cited 64 times. This describes further mutations not previously found in man and their mechanisms of action*).

5. **Percy MJ**, Furlow PW, Lucas GS, Li X, **Lappin TR, McMullin MF**, Lee FS. A gain of function mutation in the HIF2a gene in familial erythrocytosis. New England Journal of Medicine (2008) 358(2) 162-8. doi: 10.1056/NEJMoa073123 (*Cited 85 times. This paper describes the first mutation found in man in one of the Hypoxia-inducible Factor genes, HIF 2a, which was shown to cause erythrocytosis*).

6. **Percy MJ**, Beer PA, Campbell G, Dekker AW, Green AR, Oscier D, Rainey G, van Wijk R, Wood M, **Lappin TR, McMullin MF**, Lee FS. Novel exon 12 mutations in the HIF2a gene associated with erythrocytosis. Blood (2008) 111 (11) 5400-2. doi: 10.1182/blood-2008-02-137703 (*Cited 30 times. This paper describes further HIF 2a mutations accounting for rare cases of erythrocytosis*).

4. Details of the impact

The work of the Queen's group has led to the discovery of rare molecular causes of erythrocytosis. This has led to changes in the clinical guidelines[1,2,3] for testing of patients with these diseases as well as the establishment of a diagnostic service that screens patients for these rare mutations. The service investigations generate income for the NHS.

Around 100 samples per year are received for testing for these rare mutations from the UK, Ireland, many parts of Europe and the USA in a consolidated service framework that has reached a steady state. The direct impact on patients of testing is that in some of the rare cases a diagnosis

can now be made. This benefits the individual patient by guiding clinical management and preventing further futile testing. It reduces health service costs as no more investigations need to be carried out if the abnormality is identified. This is the case in approximately 10% of referrals and 54 patients with these rare diseases have benefited from an accurate diagnosis so far.

The impact of the research on clinical practice has led to national and international guidelines for the investigation and management of these blood disorders. Guidelines incorporate reference to the mutations identified at Queen's and how selected patients should be investigated for the genetic defects. For example, as one of many, the British Committee for Standards in Haematology (BCSH) website guidelines state:

> 'Patients with an unexplained erythrocytosis and low serum EPO levels should be considered for investigation of an EPO receptor mutation. The Chuvash form of erythrocytosis, an autosomal recessive disorder common to a large number of families in central Russia, has been shown to result from mutations in the *VHL* gene. These patients have inappropriately normal or high EPO levels for their Hct'.

The more recent guidance states:

> 'Congenital causes of erythrocytosis include mutations in globin genes giving rise to high oxygen affinity haemoglobin, *BPGM* mutation resulting in bisphosphoglycerate mutase deficiency, mutations in components of the EPO signalling pathway (*EPOR*) and mutations within components of oxygen sensing pathways such as in *VHL*, *EGLN1* (also termed *PHD2*) and *EPAS1* (*HIF2A*). Especially in younger patients, mutations within such genes may identify the cause of the erythrocytosis.' [1]

Although these diseases are rare and the cases caused by unusual mutations are rarer still, they cause ongoing morbidity which is exceedingly costly to health service providers and stressful for the patients. This has led a European Cooperation in Science and Technology action to set up a Network of Experts in the molecular diagnosis of myeloproliferative neoplasms and related diseases. One of the four working groups in this initiative (Working Group 3 chaired by McMullin), is dedicated to the molecular diagnosis of congenital erythrocytosis. This working group has been inspired by the exciting findings of rare molecular causes for erythrocytosis and delivers information on which patients should be investigated and where testing can be done.

An international database is now operational to collate information on individuals with rare forms of erythrocytosis. This provides information on outcomes and a European Congenital Erythrocytosis Consortium has been formed. Several hundred patients are on this database, but an estimated ten times more remain undiagnosed. Furthermore, looking at the sources of samples that the diagnostic service received, makes it clear that many patients in many parts of the world remain uninvestigated.

The European Congenital Erythrocytosis Consortium also organises training schools for clinical scientists on diagnostic methods e.g. the 2nd Training School, in Coimbra, Portugal in 2011, to which McMullin is a major contributor. Participants in training schools[4] are limited to 15 to allow a full interactive hands-on experience and all places are usually taken up. This school was rated excellent or good by all participants with improvement in knowledge, excellent or good rating for laboratory work and comments that 'interactions with participants were very productive'.

5. Sources to corroborate the impact

1. Bench AJ, White HE, Foroni L, Godfrey AL, Gerrard G, Akiki S, Awan A, Carter I, Goday-Fernandez A, Langerabeer SE, Clench T, Clark J, Evans PA, Grimwade D, Schuh A, **McMullin MF**, Green AR, Harrison CN, Cross NC. Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2V617F and other relevant mutations. British Journal of Haematology 2013 160:25-34. doi: 10.1111/bjh.12075

2. Cario H, **McMullin MF**, Bento C, Pospisilova D, Percy MJ, Hussein K, Schwarz J, Astrom

M, Hermouet S. Congenital and acquired erythrocytosis – classification, characterization and consensus recommendations for the diagnostic approach to erythrocytosis in children and adolescents. **Pediatr Blood Cancer 2013 June 14. Doi: 10.1002/pbc.24625 [Epub ahead of print] PMID: 23776154.**

3. **McMullin MF**, Bareford D, Campbell P, Green AR, Harrison C, Hunt B, Oscier D, Polkey MI, Reilly JT, Rosenthal E, Ryan K, Pearson TC, Wilkins B. Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. British Journal of Haematology (2005) 130(2), 174-195. doi: 10.1111/j1365-2141.05535.x (this guideline is reviewed annually and is still current).

4. Examples are: **McMullin**, Idiopathic Erythrocytosis: A disappearing entity, American Society for Hematology, Educational Program New Orleans, USA, December, 2009, Diagnosis and Management of Erythrocytosis, European Hematology Association, Berlin, June, 2009, Erythrocytosis, European Hematology Association, Amsterdam, June 2012.

WEBSITES

http://mpneuronet.eu and

http://impascience.eu/COSTBM0902_net/images/congenital_erythrocytosis.pdf

http://www.erythrocytosis.org/scid/polycythemias_en/

http://www.bcshguidelines.com/4_HAEMATOLOGY_GUIDELINES.html?dpage=1&dtype=General+Haematology&sspage=0&ipage=0#g