THROMBOSIS IN HAEMATOLOGICAL DISORDERS: TAILORED MANAGEMENT APPROACHES

Summary of presentations from the Alexion-supported satellite symposium held at the 20th Congress of the European Hematology Association (EHA) on 11th-14th June 2015, Vienna, Austria

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Disclosure: Anna Falanga has received speaker fees, consultancy, and research support from Alexion Pharmaceuticals. T. Sakari Jokiranta has received honoraria for lectures from Pfizer, Merck Sharp & Dohme, Alexion Pharmaceuticals, Scopeful, Abbvie, Medac, and Roche, received consultation fees from Pfizer, Merck Sharp & Dohme, and Alexion Pharmaceuticals, and is a shareholder for Scopeful. Anita Hill has received honoraria from Alexion Pharmaceuticals and served on advisory boards.

Acknowledgements: Writing assistance was provided by Dr Saroshi Amirthalingam, apothecom scopemedical Ltd.

Support: The publication of this article was funded by Alexion. The views and opinions expressed are those of the authors and not necessarily of Alexion.

Citation: EMJ Hema. 2015;3[1]:38-46.

MEETING SUMMARY

The meeting commenced with a talk from Prof Anna Falanga on the management of thrombosis in both onco-haematological and non-oncological diseases. Adjunct Prof Sakari Jokiranta gave an overview of the complement system and the interplay between the complement and coagulation systems. Dysregulation of complement and resulting disease states were also discussed. The session was concluded with a presentation from Dr Anita Hill on the management of thrombosis in paroxysmal nocturnal haemoglobinuria (PNH).

Management Options for Thrombosis in Haematological Disorders

Professor Anna Falanga

Haematological disorders can be divided into onco-haematological diseases and non-oncological diseases, both of which have a high risk of thrombosis (Figure 1). Non-oncological diseases include hereditary haemolytic anaemias (HHAs), antiphospholipid syndrome, thrombotic thrombocytopenic purpura (TTP), decreased endogenous anticoagulants, abnormalities of fibrinolysis, and acquired haemolytic diseases such as PNH. Onco-haematological diseases include acute leukaemia, multiple myeloma, lymphoma, and myeloproliferative neoplasm (MPN).

Thrombosis is more prevalent in patients with cancer. In fact, venous thromboembolism (VTE) is a frequent complication of cancer, including haematological cancer, with an estimated risk of 0.6% per year. Treatment with chemotherapy increases the risk of thrombosis by 6.5-fold. Cancer-associated VTE is linked to increased mortality, increased risk of recurrent VTE and bleeding complications, and interruption of chemotherapy, and also has economic implications. Clinical risk factors (such as hypercholesterolaemia, hospitalisation immobility,
previous history of thrombosis, etc.), tumour cells, and host cell response all contribute to increased coagulation activation in cancer patients. Hypercoagulation leads to thrombosis and tumour progression, which in turn promotes hypercoagulation.\textsuperscript{10}

Tumour cells constitutively produce signals that activate coagulation pathways, which is a unique pathogenic mechanism of thrombosis.\textsuperscript{11} The production of tumour procoagulant activities, inflammatory cytokines, angiogenic factors, and the expression of adhesion receptors induces the activation of blood coagulation. This leads to thrombin generation and fibrin formation, resulting in cancer-associated thrombosis.\textsuperscript{11} Different levels of thrombin may be produced depending on tumour types; in an \textit{in vitro} study of human tumour cell lines, promyelocytic leukaemia cells induced the highest levels of thrombin in normal plasma.\textsuperscript{12} Many of the oncogenes commonly dysregulated in cancer drive increased expression of clotting proteins.\textsuperscript{13,14} To treat coagulopathy effectively, it is necessary to understand the underlying mechanisms promoting coagulation. For example, in acute promyelocytic leukaemia (APL), the PML/RAR\textsubscript{α} genetic lesion is associated with overexpression of procoagulant activity, i.e. tissue factor.\textsuperscript{15} Differentiation therapy with all-trans retinoic acid (ATRA) targets the molecular lesion, causing maturation of the affected promyelocytes and a reduction in the procoagulant expression, thus resolving the coagulopathy.\textsuperscript{15} As well as the immediate administration of ATRA, management of coagulopathy in APL consists of platelet transfusion to maintain platelets at $>50 \times 10^9$/l and red blood cell (RBC) transfusion to maintain haemoglobin levels $>8$ g/dl.\textsuperscript{16} If cerebral bleeding is suspected, a computed tomography or magnetic resonance imaging scan should be performed immediately, lumbar puncture should be avoided, and the patient should be transferred to the intensive care unit.\textsuperscript{16} Other treatments have either not shown a conclusive benefit in trials (tranexamic acid, unfractionated heparin), or have not been tested in this setting (low-molecular-weight heparins [LMWHs], pentasaccharide, newer anti-Xa/anti-IIa agents).

No specific guidelines exist for the treatment of VTE in haematological malignancies, making it necessary to adapt guidance for patients with solid tumours. In patients with solid tumours, initial treatment of VTE is LMWH at 200 U/kg/d for 1 month and subsequently 70-80\% of the initial dose for at least 5 months.\textsuperscript{17} For haematological malignancies, expert opinion has suggested adapting the dose according to the platelet count: 70-80\% of initial dose for a platelet count of $\leq 70 \times 10^9$/l or reduced to 50\% if platelets are $\leq 50 \times 10^9$/l. Therapy should be stopped if platelets are $\leq 20 \times 10^9$/l.\textsuperscript{18} The 2014 American Society of Clinical Oncology Clinical Practice Guidelines\textsuperscript{17} recommend LMWH for patients with cancer who have deep vein thrombosis (DVT) and pulmonary embolism (PE), both for the initial 5-10 day treatment and for prolonged secondary prophylaxis of at least 6 months.

\begin{itemize}
  \item Onco-haematological diseases
    \begin{itemize}
      \item Acute leukaemia
      \item Multiple myeloma
      \item Lymphoma
      \item Myeloproliferative neoplasm
    \end{itemize}
  \item Non-oncological diseases
    \begin{itemize}
      \item Hereditary haemolytic anaemias, e.g. sickle cell disease, thalassaemia
      \item Antiphospholipid syndrome
      \item Thrombotic thrombocytopenic purpura
      \item Decreased endogenous anticoagulants
      \item Abnormalities of fibrinolysis
      \item Haemolysis, e.g. paroxysmal nocturnal haemoglobinuria
    \end{itemize}
\end{itemize}

\section*{Figure 1: Haematological disorders with high thrombotic risk.}
MPNs such as essential thrombocythaemia (ET) and polycythaemia vera (PV) have a high thrombotic risk and management of the disease is dependent on the extent of thrombotic risk. Factors that increase the risk of thrombosis in MPNs include an age of >60 years and previous thrombosis. Other risk factors under active investigation include cardiovascular risk factors, leukocytosis, haematocrit in PV, and the V617F mutation in the \( \text{JAK2} \) gene. Recommendations for a risk-adapted treatment approach in treating thrombosis in ET and PV have been released by Tefferi and Barbui, where the treatment regimen is dependent upon the pathophysiology.

Future management of cancer-related thrombosis is likely to focus more on the pathophysiological approach of targeting the oncogenic molecular lesion, while classic anticoagulant and antiplatelet drugs may be considered in a different light.

Non-oncological diseases such as HHAs also have a high thrombotic risk. The most common forms of HHAs are sickle cell disease (SCD) and thalassaemia. Thalassaemia results from a partial or complete lack of synthesis of one of the major \( \alpha \) or \( \beta \)-globin chains of haemoglobin A, whereas SCD is caused by a single amino acid mutation of the \( \beta \)-globin chain. Intravascular haemolysis is a common pathogenic prothrombotic trait in both conditions. Thalassaemia and SCD are caused by the loss of the normal asymmetrical distribution of the RBC membrane phospholipids. Phosphatidylserine is translocated to the external leaflet of the cell membrane resulting in activation of the prothrombinase complex, which facilitates interaction between the RBC and endothelial cells and ultimately leads to a hypercoagulable state. Other factors in thalassaemia contributing to hypercoagulability include reduced levels of nitric oxide (NO) leading to vasoconstriction, increased platelet aggregation, and formation of microparticles from peripheral blood elements.

A high prevalence of thrombotic events (TEs) is observed among thalassaemia patients, particularly those with thalassaemia intermedia. The most notable thrombosis risk factors among thalassaemia patients are advancing age (>35 years old), splenectomy, and serum ferritin \( \geq 1,000 \, \mu\text{g/l} \), as confirmed in the OPTIMAL CARE observational study of over 500 thalassaemia intermedia patients. Optimal preventative strategies are not yet established and the roles of antiplatelets, anticoagulants, fetal haemoglobin induction, transfusion, and iron chelation therapy should be further investigated. OPTIMAL CARE identified haemoglobin levels of \( \geq 9 \, \text{g/dl} \) and transfusion as factors associated with a significantly decreased risk of thrombosis.

Antithrombotics are recommended for treatment of thromboembolism, particularly during acute episodes. The choice of antithrombotic drugs is dependent on the site of thrombosis; aspirin is normally administered for arterial thrombosis and heparin or warfarin for VTE. Regular RBC transfusion has been recommended in thalassaemia patients in order to maintain haemoglobin levels higher than 9 g/dl. For SCD, however, trials of anticoagulants or antiplatelets have been inconclusive. PNH, a further example of non-malignant haematological disease, carries a very high relative risk of VTE compared with other thrombophilic conditions and requires a very specific management approach.

In summary, both non-malignant and malignant haematological disorders can carry a risk of thrombosis. In all cases, the risk factors and underlying pathophysiology must drive the decision-making process for the selection of appropriate and potentially life-saving therapy.

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**Complement-Mediated Thrombosis: A Complex Interplay between Complement and Coagulation**

**Adjunct Professor T. Sakari Jokiranta**

The complement system forms part of the innate immune system. Activation of complement is mediated by >20 proteins circulating in the blood and tissue fluids. In response to a pathogen or to foreign structures, the complement system is activated and results in a sequential activation of proteins and enzymes. This cascade causes the opsonisation of pathogens, which induces a series of inflammatory responses that help fight infection. Complement can be activated via three different pathways: classical pathway, lectin pathway, and alternative pathway. Each pathway is able to cause the activation of C3 and C5 convertases, leading to the activation of a common terminal (lytic) pathway.

The alternative pathway is continuously activated at a low level, with its activity being amplified by various conditions including infection, tissue
damage, surgery, or pregnancy. The alternative pathway is initiated by spontaneous hydrolysis of C3 to form C3(H₂O), allowing generation of fluid-phase C3 convertase which is able to cleave many molecules of C3 to form C3a and C3b. The C3b that is generated is able to attach covalently to the surfaces of host cells or pathogens nearby. C3b that is bound in this way is able to bind complement factor B, which leads to the formation of the alternative pathway C3-convertase (C3bBb) on the target surface, ultimately leading to elimination of the target.

Activation of the complement system results in destruction of the target via three main mechanisms: opsonisation of the pathogen via bound C3b resulting in phagocytosis; generation of C5a, which attracts neutrophils to the site of infection; and creation of pores in the bacterial membrane leading to lysis of the target cell. Damaged cells can activate complement via one or more of the three pathways, and complement-mediated damage can induce further local complement activation.

As the alternative pathway is activated spontaneously, it has the potential to damage host cells if it is not well regulated. Several complement regulatory proteins act to prevent any accidental damage to host cells. Most of these regulators function at the C3 stage within plasma (e.g. factors H and I) or at the cell membrane. A fine balance exists between the activation and regulation of the alternative complement pathway. Impaired regulation, caused by malfunctioning regulators, can cause chronically uncontrolled complement activation leading to organ damage. Similarly, gain-of-function mutations (e.g. mutation in C3 or complement factor B) can result in enhanced activation, which may also lead to organ damage.

Atypical haemolytic uraemic syndrome (aHUS) and PNH are two examples of disease arising from uncontrolled complement activation. aHUS is a rare, life-threatening, systemic disease with a poor prognosis, characterised by microangiopathic haemolytic anaemia, thrombocytopenia, and acute kidney failure. Uncontrolled complement activation causes platelet and endothelial cell activation and damage as well as haemolysis. Multiple genes have mutations associated with the disease, six of which are complement proteins. Three additional genes: THBD, PLG, and DGKE (encoding thrombomodulin, plasminogen, and diacylglycerol kinase epsilon, respectively) are involved in coagulation or fibrinolysis. Patients with aHUS experience complement activation against all cells in contact with plasma, including platelets, leukocytes, RBCs, and endothelial cells. Complement activation on these cells gives rise to a number of clinical consequences, namely platelet consumption, mechanical haemolysis, blood clotting, vessel occlusion, inflammation, and ischaemia, ultimately leading to systemic multi-organ complications and thrombotic microangiopathy (TMA). There is significant clinical overlap with other causes of TMA such as TTP, although TTP is distinguishable through ADAMTS-13 activity: in aHUS it is >5%, whereas in TTP it is always <5%.

Importantly, the complement, coagulation, and fibrinolysis systems are interlinked (Figure 2). Release of C5a, which is a potent anaphylatoxin, acts on endothelial cells and leukocytes, leading to enhanced tissue factor activation and a hypercoagulable state. In turn, the coagulation pathway can lead to complement activation. This can lead to a vicious circle of both pathways activating each other. This continual activation of complement can cause damage to host cells, resulting in organ dysfunction.

PNH is caused by a somatic mutation in the PIGA gene (encoding phosphatidylinositol glycan class A) in haematopoietic stem cells, leading to loss of glycosphingosinol anchor synthesis. This mutation results in the production of abnormal blood cells that lack several cell surface proteins, including complement regulators CD55 and CD59. Absence of CD55 and especially CD59 leads to continual susceptibility of the cells to complement-mediated destruction following spontaneous deposition of C3b on the deficient cells. PNH is caused by a somatic mutation in the PIGA gene (encoding phosphatidylinositol glycan class A) in haematopoietic stem cells, leading to loss of glycosphingosinol anchor synthesis. The high incidence of thrombosis in PNH is due to complement activation on CD59-deficient blood cells, activation and aggregation of PNH platelets, and haemolysis of PNH erythrocytes, leading to reduced NO levels.

The importance of the interplay between complement and coagulation is supported by clinical observations in both aHUS and PNH.
Abnormalities in complement regulation without a known abnormality in coagulation or fibrinolysis leads to microangiopathic thrombosis in aHUS. Furthermore, mutations in genes controlling coagulation or fibrinolysis can lead to complement-mediated pathophysiology. For example, \textit{THBD} mutations have been found in 3-5% of aHUS patients and some aHUS patients have exhibited a plasminogen deficiency. PNH is associated with impaired complement regulation on PNH erythrocytes and platelets, and a significantly increased risk of thrombosis. The best evidence of the interplay of complement and coagulation in these two diseases is provided by therapeutic targeting with eculizumab (ECU) of the terminal complement cascade at C5, as this prevents thrombotic complications in PNH and has a beneficial effect not only in those aHUS patients with a defect in a complement protein but also in those with a \textit{THBD} mutation. ECU functions in these diseases in two ways: first, by preventing formation of C5a, leading to decreased exhibition of tissue factor activity by endothelium and leukocytes; and secondly, the membrane attack complex is not formed on platelets or RBCs, resulting in no hyperactivity of platelets and no intravascular haemolysis or reduction in NO.

Other thrombotic diseases that arise from activation of complement include septicaemia and disseminated intravascular coagulation, ischaemia–reperfusion injury, catastrophic antiphospholipid syndrome, and antibody-mediated rejection. This highlights how chronic, uncontrolled complement activation is involved in the pathogenesis of a variety of serious systemic diseases.

**Management of Thrombosis in Paroxysmal Nocturnal Haemoglobinuria, a Complement-Mediated Disease**

**Doctor Anita Hill**

PNH is a rare, acquired, life-threatening disease characterised by chronic, uncontrolled complement-mediated haemolysis and a prothrombotic state (Figure 3). PNH is diagnosed using high-sensitivity flow cytometry performed on peripheral blood. The disease arises from a mutation in the \textit{PIGA} gene against the background of an underlying bone marrow failure, usually aplastic anaemia. It is thought that the underlying immune attack of normal stem cells in, for example, aplastic anaemia allows the PNH stem cells to expand. Evidence from clinical practice also shows that these two steps may be reversed: a patient with aplastic anaemia who is initially negative for \textit{PIGA} mutation can later...
develop the PIGA mutation, leading to the development of PNH.\textsuperscript{65} For this reason, the British Society for Haematology Guidelines for the Diagnosis and Management of Aplastic Anaemia\textsuperscript{64} recommend testing for PNH upon diagnosis of aplastic anaemia and regularly during follow-up. Other groups of patients who should be considered for PNH testing include those suffering from certain subgroups of myelodysplastic syndromes, patients who develop features of unexplained intravascular haemolysis, and those with unexplained thromboses associated with cytopenias or evidence of haemolysis.

Although PNH is described as a benign disorder, the survival of patients who remain on supportive therapies has remained unchanged over the decades. As many as 35% of patients with PNH die within 5 years of diagnosis despite best supportive care.\textsuperscript{61,65} Unregulated complement activity is the underlying cause of progressive morbidities and mortality in PNH.

A study of ten patients demonstrated how patients can suffer silent complications of uncontrolled complement activation and thrombosis, such as PE and myocardial infarction.\textsuperscript{66} This study highlighted the importance of carefully monitoring patients with high haemolysis (high lactate dehydrogenase [LDH] levels). Importantly, it is recommended that thorough examinations are conducted even in young and fit patients in order to detect silent complications underlying PNH.

Thrombosis is a leading cause of mortality in PNH patients.\textsuperscript{54} Common sites include intra-abdominal and cerebral veins, hepatic veins (Budd-Chiari syndrome), DVT of the lower limbs, and cerebral and coronary arterial thromboses.\textsuperscript{27} The first TE can be fatal and can also increase the risk of death by 5 to 10-fold.\textsuperscript{54} Anticoagulation therapies do not adequately treat thrombosis in PNH.\textsuperscript{27,54} Haemolysis and clinical symptoms can help to ascertain the risk of thrombosis in PNH patients. Multivariate analyses confirmed that LDH \( \geq \)1.5-times the upper limit of normal (ULN) increases the risk of TEs by 7-fold, when adjusted for age, gender, and bone marrow failure.\textsuperscript{67} The combination of elevated LDH with other symptoms such as abdominal pain or chest pain causes a dramatic increase in the risk of a TE.\textsuperscript{67} It is therefore necessary to obtain a thorough understanding of patient symptoms alongside close monitoring of LDH levels. Notably, even patients who have had minimal transfusions have an elevated risk of thrombosis,\textsuperscript{54} indicating that the risk of thrombosis is independent of transfusion history. Similarly, patients on anticoagulation therapy also have a high risk of TEs.\textsuperscript{54} Although PNH is less common than other inherited hypercoagulable states, it has a much higher incidence and relative risk of VTE. The management of the patient changes if PNH is diagnosed in a patient with unexplained thrombosis, hence the recommendation to test.\textsuperscript{68} A high LDH can lead to the suspicion of PNH; however, patients with PNH and normal LDH levels can also suffer from thrombosis.

Figure 3: Clinical presentation of paroxysmal nocturnal haemoglobinuria. Adapted from Hill et al.,\textsuperscript{27} Hillmen et al.,\textsuperscript{67} and Socié et al.\textsuperscript{65}
New oral anticoagulants (e.g. dabigatran, rivaroxaban) are unlikely to benefit patients with PNH as they function at the same points in the coagulation cascade as traditional anticoagulants. In contrast, ECU inhibits the terminal complement pathway by binding to C5 and thus preventing its cleavage into C5a and C5b, thereby impairing prothrombotic mechanisms mediated by the complement system. This mechanism causes a dramatic reduction in the rate of thrombosis: patients treated with a combination of anticoagulants and ECU demonstrated a 94% reduction in TE rate per 100 patient-years versus those treated with anticoagulants alone (p<0.001) (Figure 4).

In the UK, ECU is indicated for transfusion-dependent patients with PNH and transfusion-independent patients who have thrombosis related to PNH, and those who have complications associated with haemolysis, e.g. renal failure, pregnancy, and symptomatic haemolytic PNH. Based on findings from the global PNH registry, the European Medicines Agency has recently updated their approval of ECU to include patients with high disease activity (LDH >1.5 ULN plus one or more specified clinical symptoms), regardless of transfusion history.

In an effort to further our knowledge of PNH, the global PNH registry was initiated several years ago and comprises of anonymised data from more than 3,500 patients from around the world. The main objective of the PNH registry is to collect data to evaluate safety regarding the use of ECU and to characterise the progression of PNH as well as clinical outcomes, mortality, and morbidity in ECU and non-ECU treated patients.

Treatment with ECU appears to impact patient survival, as an analysis of UK data over the last 13 years (up to December 2014) showed that, out of 180 patients treated with ECU, no deaths related to PNH have occurred, which can be compared with the 35% mortality within 5 years seen in patients on supportive therapies.

In summary, the risk of thrombosis in PNH is often underestimated. Patients with unexplained thrombosis with cytopaenia or haemolysis, or recurrent thrombosis despite anticoagulation, should be tested for PNH. Anticoagulation therapy is not sufficient to prevent thrombosis risk in PNH patients; however, ECU therapy has significantly improved survival for patients with PNH.

REFERENCES
4. Levitan N et al. Rates of initial and recurrent thromboembolic disease


