Clinical and laboratory assessment of a patient with thrombocytosis

ABSTRACT

Elevated platelet counts are frequently encountered in hospital medicine and arise from both physiological and pathological mechanisms. Thrombocytosis may be secondary, reflecting an inflammatory state, iron deficiency, recent surgery or point towards an underlying neoplasm. Thrombocytosis may be the presenting sign of solid tumours and haematological conditions.

The discovery of the activating mutations affecting thrombopoiesis led to greater understanding of the pathobiology of essential thrombocythaemia and other myeloproliferative neoplasms. The investigation of suspected primary thrombocytosis has evolved to include testing for these disease-associated mutations.

Therapy for patients with essential thrombocythaemia aims to reduce their risk of thrombotic complications by addressing cardiovascular risk factors, and using antiplatelet agents and, in selected patients, cytoreductive therapy.

This article provides a logical approach to distinguishing reactive or secondary thrombocytosis from thrombocytosis associated with an underlying myeloproliferative neoplasm and gives an overview of the management of essential thrombocythaemia.

Thrombocytosis (platelet count >450x10^9/litre) is a common finding in hospitalized patients, affecting one fifth of trauma patients (Valade et al, 2005) and up to one third of patients admitted to intensive care (Banach et al, 2017). In primary care, thrombocytosis heralds an underlying solid tumour diagnosis in 11.6% of men and 6.5% of women (Bailey et al, 2017). Thrombocytosis triggers 7% of inpatient haematology consultations (Kim et al, 2005).

Elevated platelet counts can be secondary to a range of benign, inflammatory or neoplastic diseases or can arise in the context of a primary haematological disease (*Table 1*). Establishing the correct diagnosis for a particular patient requires insight into the potential causes and appropriate use of laboratory investigations including molecular diagnostic testing.

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Physiology of platelet formation

Platelets are specialized circulating acellular fragments that function to prevent bleeding and minimize vascular injury. Healthy humans produce approximately 1x10^{11} platelets daily in a tightly regulated process.

Megakaryocytes are large, bone marrow-based progenitor cells that specialize in the assembly and release of platelets. Control of platelet count at a physiological level is regulated by thrombopoietin and its receptor on the surface of megakaryocytes and the platelet membrane, c-MPL. Binding of thrombopoietin to c-MPL permits the JAK2 signalling kinase bound to the membrane proximal region of the cytoplasmic domain to dimerize, allowing phosphorylation and subsequent downstream activation of STAT, PI3K, MAPK and ERK1 pathways.

Thrombopoietin hormone is primarily synthesized in the liver, with some contribution from the kidneys and bone marrow (Kaushansky, 2005). Thrombopoietin levels are controlled via an auto-regulatory loop. Circulating platelets express low levels of c-MPL receptor and bind and remove plasma thrombopoietin. With increasing platelet numbers, thrombopoietin is removed from circulation. Conversely a fall in circulating platelet counts allows thrombopoietin levels to rise, delivering increased thrombopoietin to the megakaryocytes and stimulating thrombopoiesis.

Mechanism of reactive thrombocytosis

Causes of secondary thrombocytosis include infection, inflammation, tissue damage (including surgical), haemolysis, solid organ malignancy and iron deficiency. Reactive thrombocytosis is frequently observed in association with elevations in erythrocyte sedimentation rate and/or C-reactive protein level. In one institutional series, 76% of patients with reactive thrombocytosis had an elevated C-reactive protein level (Tefferi et al, 1994), and elevations in erythrocyte sedimentation rate and levels of C-reactive protein are observed in cancer-associated thrombocytosis (Alexandrakis et al, 2003).

Reactive thrombocytosis occurs in response to changes in thrombopoietin levels and pro-inflammatory cytokines. Increases in peripheral thrombopoietin concentrations are found in post-surgical patients, patients with inflammatory bowel disease and those with other inflammatory conditions (Uppenkamp et al, 1998; Hsu et al, 1999). Hepatic thrombopoietin mRNA levels are increased in the presence of the inflammatory mediator interleukin-6 (Kaser et al, 2001).

The relationship between secondary thrombocytosis and thromboembolism is uncertain. The threshold used to define thrombocytosis varies across published studies. In one large, prospective cohort study of 1599 patients admitted to the intensive care unit, 14 patients (0.9%) developed thrombosis. Platelet count on admission to the intensive care unit did not influence the risk of thrombosis during the stay in intensive care. Reactive thrombocytosis (defined in this study as platelets >500x10^9/litre) was observed in 139 (9.6%) patients on recovery. One patient with reactive thrombocytosis and 28 patients without thrombocytosis experienced thrombosis before hospital discharge, a proportion which just reached statistical significance (P=0.048) (Ho et al, 2012). A separate prospective study focused on thrombocytosis in trauma patients admitted to the intensive care unit found no increased risk of venous thrombosis in patients with thrombocytosis (platelets >600x10^9/litre) (Valade et al, 2005).

Thrombocytosis following splenectomy is frequent, affecting three-quarters of patients in historical series (Boxer et al, 1978). The thrombocytosis peaks 21–33 days postoperatively at a median platelet count of 700x10^9/litre before stabilizing after 45 days (Wernick et al, 2017). Normally, one-third of platelets are sequestered in the spleen and loss of the spleen increases the circulating platelet pool. Patients should receive thromboprophylaxis in line with routine postoperative care. Up to half of asplenic patients may have a persistent thrombocytosis for years after splenectomy but cytoreductive therapy to reduce platelet numbers is not required.

Thrombocytosis is reported in up to one third of patients with iron deficiency anaemia but studies of patients with iron deficiency anaemia have reported inconsistent relationships between thrombopoietin levels, erythropoietin, ferritin, interleukin 6 and interleukin 11 and the platelet count.

Paraneoplastic thrombocytosis in non-haematological cancers

Secondary thrombocytosis is seen across a range of solid tumours as a paraneoplastic phenomenon, where elevated platelet counts may precede the diagnosis of malignancy. The malignant cells hijack the physiological process of thrombopoiesis to promote tumour growth and survival. Solid tumours do not usually produce thrombopoietin directly but can overexpress other cytokines (interleukin 1, interleukin 3, interleukin 6 and interleukin 11) associated with platelet generation (Buergy et al, 2012). Interleukin 6 is overproduced in gastrointestinal, renal cell, prostate, ovarian and lung cancers (Kaser et al, 2001).

The malignant tumour may use platelets to promote growth and metastasis via positive feedback loop. Tumour cells activate platelets directly by generating thrombin or indirectly via the tissue-factor pathway. Activated platelets can promote tumour survival, shielding circulating tumour cells from circulating immune surveillance.

Table 1. Differential diagnosis of thrombocytosis

Secondary thrombocytosis	Reactive thrombocytosis	Infection
		Iron deficiency
		Inflammation
		Trauma (including surgery)
		Post splenectomy
		Acute blood loss
	Paraneoplastic thrombocytosis	Lung cancer
		Ovarian cancer
		Cervical cancer
		Renal cell cancer
		Gastric cancer
		Breast cancer
Primary thrombocytosis	Myeloproliferative neoplasms	Essential thrombocythaemia
		Polycythaemia vera
		Myelofibrosis
		Chronic myeloid leukaemia
	Other haematological neoplasms	Myelodysplasia with del(5q)
		Myelodysplasia with ringed sideroblasts

Platelet-derived transforming growth factor-β stimulates proliferation of cancers cells and promotes metastasis (Lin et al, 2014).

Pre-treatment elevations in platelet and leucocyte count are associated with an increased risk of cancer-associated thrombosis and form part of an integrated score to stratify cancer patients into low, intermediate and high risk for thrombosis (Khorana et al, 2008). Cancer-associated thrombocytosis correlates with reduced progression-free survival (Stone et al, 2012). The Khorana Risk Score is currently incorporated into randomized trials of primary thromboprophylaxis for ambulatory outpatients at high risk of cancer-associated thrombosis (Khorana et al, 2017).

Myeloproliferative neoplasms presenting with elevated platelet counts

Myeloproliferative neoplasms is the term given to a group of uncommon clonal haematological diseases characterized by increased production of mature blood cells by clonal haematopoietic precursors and a long-term risk of progression to acute myeloid leukaemia. Myeloproliferative neoplasms are typically divided into chronic myeloid leukaemia (defined by the presence of BCR-ABL fusion gene associated with the translocation t(9;22)) and BCR-ABL negative myeloproliferative neoplasms encompassing

> Myeloproliferative neoplasms are particularly associated with portal, hepatic and other abdominal vein thrombosis.

essential thrombocythaemia, polycythaemia vera and myelofibrosis. While essential thrombocythaemia is characterized by thrombocytosis, persistent polycythaemia is the hallmark of polycythaemia vera. Anaemia, circulating immature red cells and leukocytes, and splenomegaly typify myelofibrosis. All three disorders share an increased risk of thrombosis and a variable tendency for progression to marrow failure or acute myeloid leukaemia.

The commonest myeloproliferative neoplasm is essential thrombocythaemia, with an estimated incidence of 0.38–1.7 per 100 000 per year and a population prevalence of 4–24 per 100 000 in Europe (Moulard et al, 2014). Incidental presentations with isolated thrombocytosis in an asymptomatic patient are common. When symptomatic, patients may experience fatigue, persistent itch, night sweats, bone pain, fever or splenic discomfort. Patients may occasionally present with episodic pain, burning sensation or intense itch affecting the extremities (known as erythromelalgia). These symptoms result from platelet activation and endothelial cell damage within the microvascular circulation and respond to antiplatelet therapy. Patients may have co-existent mild to moderate neutrophil leucocytosis. Significant morphological abnormalities such as leucoerythroblastic appearance on the blood film (circulating nucleated red cells or myeloid precursors) should prompt a search for an alternative diagnosis to essential thrombocythaemia.

Patients with myeloproliferative neoplasms have an estimated 5–7 fold increased risk of both arterial and venous thrombosis compared to the healthy population (Hultcrantz et al, 2014). The cumulative rate of thrombosis is reported at 2–4 thrombotic events per 100 patients with essential thrombocythaemia, 3.8 events per 100 patients with polycythaemia vera per year and 2.2 events per 100 patients with myelofibrosis per year although the authors caution that competing causes of death should be taken into account when looking at the apparently lower rate of thrombosis in patients with myelofibrosis (Barbui et al, 2013). Myeloproliferative neoplasms are particularly associated with portal, hepatic and other abdominal vein thrombosis. Myeloproliferative neoplasms were identified in 40.9% of patients with Budd–Chiari syndrome and 31.5% of patients with non-cirrhotic, non-malignant portal vein thrombosis. Polycythaemia vera accounted for just over half of patients diagnosed with myeloproliferative neoplasm in this study (52.9%), followed by essential thrombocythaemia (24.6%), myelofibrosis (6.7%) and other or unclassifiable myeloproliferative neoplasms (23.5%)(Smalberg et al, 2012).

Essential thrombocythaemia is defined by persistent platelet count >450x10^9/litre, presence of a clonal marker (JAK2, calreticulin or MPL mutation) and absence of BCR-ABL or evidence of another myeloid malignancy (Arber et al, 2016). In the context of typical clinical features and a molecular marker, the British Committee for Standards in Haematology guidelines no longer mandate a bone marrow biopsy to establish the diagnosis of essential thrombocythaemia (Harrison et al, 2010). However, the updated World Health Organization criteria retain the requirement for bone marrow biopsy at diagnosis, as the presence of increased reticulin fibrosis on the marrow trephine has diagnostic and prognostic implications (Arber et al, 2016).

Other haematological neoplasms may occasionally present with thrombocytosis. Polycythaemia vera typically presents with persistent elevated haematocrit. Clinicians should be alert to the patient with thrombocytosis accompanied by hypochromic, microcytic red cell indices but preserved haemoglobin, in whom co-existent iron deficiency masks the clinical phenotype of primary polycythaemia. Iron-deficient patients with polycythaemia vera may present with leucocytosis and thrombocytosis, with the polycythaemic phenotype only coming to light when iron stores are replaced.

Rarely, chronic myeloid leukaemia may present with minimal leucocytosis and a marked thrombocytosis, mimicking essential thrombocythaemia. In view of the clinical consequences of missing a diagnosis of chronic myeloid leukaemia, BCR-ABL testing is recommended for all patients with persistent thrombocytosis lacking JAK2V617F or MPL mutations (Harrison et al, 2013).

In elderly patients, two forms of myelodysplasia may present with anaemia and a normal or elevated platelet count: myelodysplasia with ringed sideroblasts and thrombocytosis, and myelodysplasia with del5q. Careful examination of the peripheral blood smear for dysplastic granulocytes and erythrocytes should avoid confusion with essential thrombocythaemia.

Molecular mechanisms of myeloproliferative neoplasms

In 2005, four groups reported the discovery of somatic gain-of-function mutation in the signalling kinase JAK2 of BCR-ABL negative myeloproliferative neoplasms (Baxter et al, 2005; Jones et al, 2005; Kralovics et al, 2005; Levine et al, 2005). The JAK proteins are kinases linking membrane receptors and intracellular signalling. Normal, wild-type JAK2 is activated in response to haematopoietic growth factors such as erythropoietin and thrombopoietin. Activation of JAK2 by phosphorylation facilitates downstream signalling pathways, notably in the STAT (signal transducer and activator of transcription) family which act as nuclear transcription factors.

The JAK2 mutation consists of a valine to phenylalanine substitution at position 617 and results in autonomous signalling. Mutant JAK2V617F increases cell survival, proliferation and phosphorylation in vitro, even in the absence of haematopoietic growth factors. The

JAK2V617F mutation is found in 95% of patients with polycythaemia vera and over half of patients with essential thrombocythaemia or myelofibrosis. The discovery of the JAK2V617F mutation triggered investigation into other somatic mutations that may be responsible for myeloproliferative neoplasms. An activating mutation in the c-MPL receptor at position c-MPL515 is found in 5–15% of patients with essential thrombocythaemia or primary myelofibrosis (Pardanani et al, 2006). One third of JAK2V617F-negative patients express a mutated, truncated form of calreticulin, which acts as an endoplasmic reticulin chaperone and calcium buffering protein (Klampfl et al, 2013). Two types of calreticulin mutations affecting exon 9 account for the majority of cases; type 1 is a 52 base pair deletion and type 2 is a 5 base pair insertion (Tefferi et al, 2014b).

The type of somatic mutation influences the clinical course of the disease. Patients with JAK2V617F mutated essential thrombocythaemia and polycythaemia vera have a higher incidence of cardiovascular adverse events and progression to myelofibrosis than JAK2 wild-type patients (Vannucchi et al, 2007). Patients with calreticulin-mutated essential thrombocythaemia are predominantly male (59.5% *vs* 31.7% JAK2V617F mutated essential thrombocythaemia), have lower haemoglobin and leucocyte counts and lower risk of thrombosis than other groups of patients with essential thrombocythaemia (Rotunno et al, 2014).

Approach to investigation of thrombocytosis

The degree of thrombocytosis is not helpful in distinguishing primary from secondary causes of thrombocytosis. Platelet counts of >1000x10^9/litre may be observed frequently in patients with solid tumours or inflammatory bowel disease. Conversely, patients with essential thrombocythaemia may present with platelet count 450–600x10^9/litre and patients with other myeloproliferative neoplasms may have normal or even reduced platelet count.

The thrombocytosis must be considered in light of the clinical context in which it is observed. Thrombocytosis in a postoperative patient, especially if a normal platelet count was recorded preoperatively, is likely reactive and merits a repeat full blood count in 4–6 weeks to ensure resolution.

Unexplained thrombocytosis in an older adult requires careful clinical evaluation for the possibility of an underlying solid tumour. Thrombocytosis accompanied by reflux, vomiting, upper abdominal pain or weight loss in an older adult should prompt endoscopic referral for investigation of possible upper gastrointestinal malignancy. Smokers over 40 years old or those with a history of smoking presenting with thrombocytosis should undergo chest X-ray (National Institute for Health and Care Excellence, 2015). In women, a careful clinical history for pelvic pain or abnormal vaginal bleeding should be sought (National Institute for Health and Care Excellence, 2015).

Clinical application of molecular diagnostics for suspected myeloproliferative neoplasm

Testing for molecular markers of myeloproliferative neoplasm is indicated in patients with a persistent thrombocytosis when secondary causes have been excluded (*Figure 1*). Testing for JAK2V617F mutations is performed in regional molecular haematology laboratories by allele-specific polymerase chain reaction or pyrosequencing. In most cases, a 2–10 ml sample of peripheral blood in EDTA is sufficient (Bench et al, 2013).

In most laboratories, the JAK2V617F mutation is tested initially. Only patients with clinical suspicion of underlying myeloproliferative neoplasm and JAK2 wild-type results proceed to testing for MPL mutations and calreticulin mutations. Anecdotally, a minority of centres use next-generation sequencing platforms for identification of myeloproliferative neoplasm-associated mutations (and mutations associated with other myeloid malignancies), which offers the potential to test multiple mutations simultaneously (UK National External Quality Assurance Service, 2017a). The use of sequential *vs* parallel testing algorithms will be evaluated as part of the planned UK National External Quality Assurance Myeloproliferative Neoplasms Gene Panel programme later this year (UK National External Quality Assurance Service, 2017b).

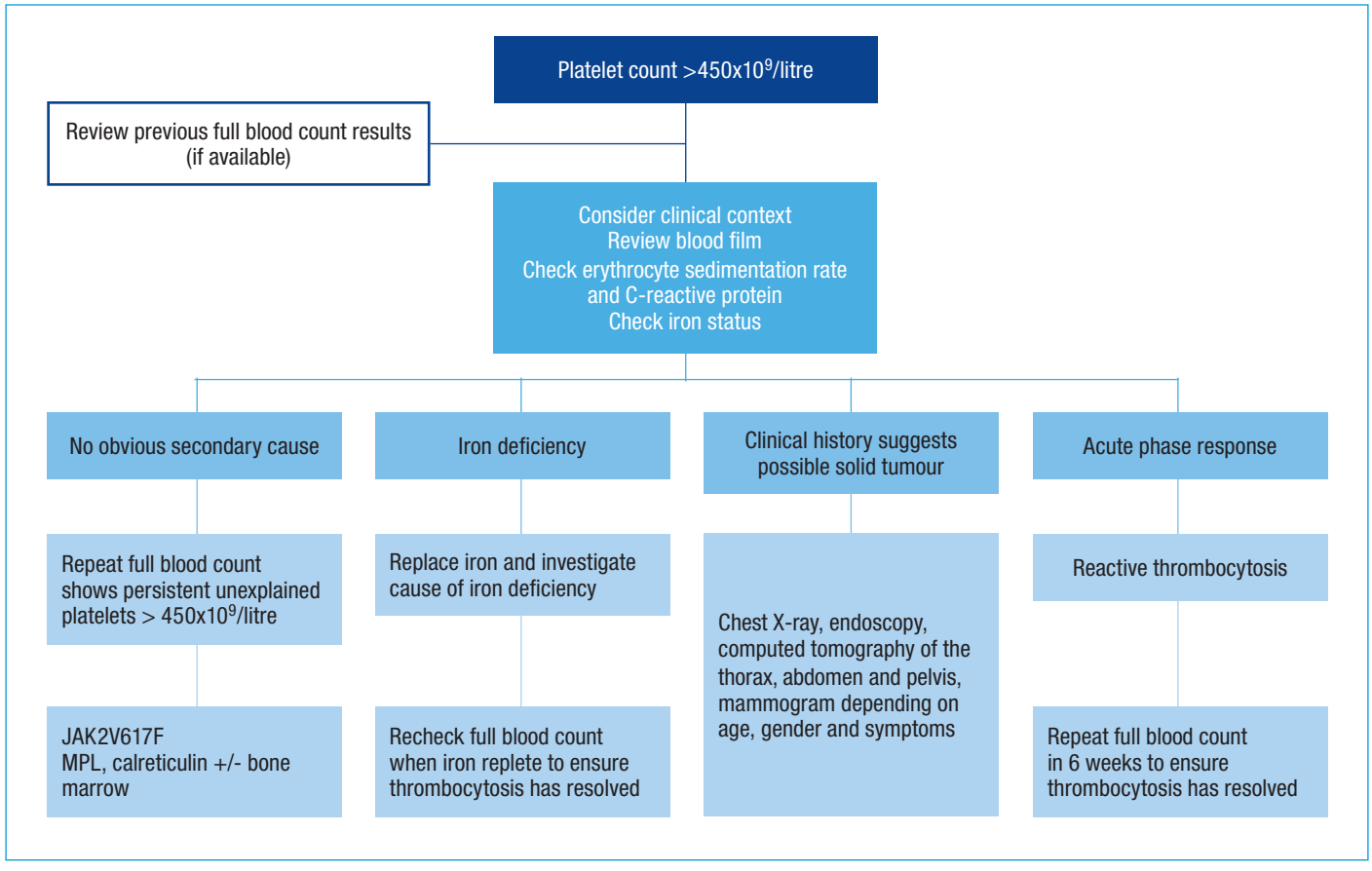
Management of the patient with essential thrombocythaemia

The prognosis for patients with essential thrombocythaemia is favourable, with the median survival for low-risk essential thrombocythaemia 17.5–24.5 years (Gangat et al, 2007; Passamonti et al, 2012; Tefferi et al, 2014a). Patients with essential thrombocythaemia experience greater morbidity, mostly attributable to an increased incidence of venous and arterial thrombosis. The goals of managing essential thrombocythaemia are to reduce the risk of thrombosis, treat symptoms and prevent disease progression.

The International Prognostic Scoring System for Essential Thrombocythaemia criteria offer a validated prognostic tool for prediction of survival and thrombotic complications. Age ≥60 years (2 points), leucocyte count ≥11x10^9/litre (1 point) and prior thrombosis (1 point) are used to assign patients to high-risk (3–4 points), intermediate risk (1–2 points) and low-risk (0 points) groups, translating into a median survival of 13.8 years, 24.5 years and not reached respectively (Passamonti et al, 2012). The degree of thrombocytosis alone is not a reliable predictor of thrombotic risk.

All patients with essential thrombocythaemia should be screened for modifiable cardiovascular risk factors including hypertension, smoking, hypercholesterolaemia and obesity and treated in accordance with national guidelines (Harrison et al, 2010). Aspirin results in a decrease in thrombotic events without increased bleeding for patients with polycythaemia vera and retrospective studies support the use of antiplatelet agents in patients

Figure 1. Algorithm for investigation of a patient with thrombocytosis.



with essential thrombocythaemia (Jensen et al, 2000; Landolfi et al, 2004). One retrospective study suggested observation alone may be adequate for selected low-risk patients with essential thrombocythaemia (Alvarez-Larrán et al, 2010). All patients with essential thrombocythaemia should undergo thrombosis risk assessment when admitted to hospital and, unless contraindicated, receive pharmacological thromboprophylaxis.

Patients over 60 years old and those with a prior history of thrombosis qualify for cytoreductive therapy to reduce the platelet count and thrombotic complications. Hydroxycarbamide (previously known as hydroxyurea) is the recommended first-line cytoreductive agent (Harrison et al, 2010) and the only agent shown to reduce thrombotic events in a randomized trial (Cortelazzo et al, 1995). Patients taking hydroxycarbamide require regular full blood counts to monitor for anaemia and neutropenia and should be educated regarding the potential risk of febrile neutropenia. Long-term use of hydroxycarbamide is associated with dermatological adverse events including an increased risk of squamous carcinomas and patients should be counselled about sun protection. The possible role of hydroxycarbamide in transformation to myelofibrosis or acute myeloid leukaemia remains unclear. Older clinical studies report conflicting results, confounded by inclusion of patients who have received multiple cytotoxic agents. Essential thrombocythaemia itself is associated with an inherent, long-term risk of progression to leukaemia, estimated at 2.1–5.3% at 15-year follow-up (Cerquozzi and Tefferi, 2015). In contrast, long-term use of hydroxycarbamide in sickle cell disease is not associated with an increased risk of leukaemia (Lanzkron et al, 2008).

Anagrelide is a second-line cytoreductive agent which inhibits megakaryocyte differentiation, used in patients in whom hydroxycarbamide is not tolerated or when the response to hydroxycarbamide is inadequate. Adverse effects of anagrelide include headaches, vasomotor symptoms palpitations and arrhythmias. Anagrelide use may be problematic in elderly patients or those with pre-existing cardiac disease. The PT-1 trial compared hydroxycarbamide and aspirin with anagrelide and aspirin for patients at high risk of essential thrombocythaemia and reported that the anagrelide group experienced more arterial thrombosis, bleeding complications and progression to myelofibrosis (Harrison et al, 2005).

Patients with essential thrombocythaemia and extremely high platelet counts (>2000x10^9/litre) can paradoxically experience mucocutaneous bleeding and other haemorrhagic complications as a result of functional von Willebrand factor deficiency (Michiels et al, 2006). At these platelet counts, circulating large von Willebrand factor multimers are lost by proteolysis giving rise to an acquired von Willebrand disease phenotype. Supportive

measures (including direct compression of bleeding sites with or without tranexamic acid) to control bleeding are required. The acquired von Willebrand disease phenotype is reversible once the platelet count returns to the normal range, which usually requires initiation of cytotoxic therapy under specialist haematologist supervision.

Management of essential thrombocythaemia in women of child-bearing potential merits special consideration. Pregnancy increases the risk of thrombosis and essential thrombocythaemia increases specific pregnancy-related complications. The therapeutic strategy is individualized, based on the patient's disease status and obstetric history and should be managed by a multidisciplinary team. In general, aspirin is continued (in the absence of contraindications) and pregnant women should be risk assessed for antepartum thromboprophylaxis with low-molecular weight heparin (Harrison et al, 2010). All women with essential thrombocythaemia should receive low-molecular weight heparin for 6 weeks post-partum. Hydroxyurea is teratogenic and should ideally be discontinued before conception. If essential thrombocythaemia-directed therapy is required during pregnancy, interferon-alpha may be used.

Conclusions

Elevations in platelet count can arise from a range of benign and neoplastic conditions. When investigating thrombocytosis, it is important to consider the clinical context of the patient, especially as unexplained thrombocytosis may herald the presentation of an underlying non-haematological cancer.

The diagnostic approach to thrombocytosis has evolved in parallel with understanding of the mechanisms underlying myeloproliferative neoplasm. The discovery of the JAK2, MPL and calreticulin mutations led to greater understanding of the molecular mechanisms underpinning myeloproliferative neoplasms. Identification of a mutation is consistent with the presence of myeloproliferative neoplasms but requires integration with the clinical features and full blood counts to establish a definitive diagnosis. Understanding how the type and burden of mutation influences the clinical phenotype and disease course may in future offer the potential to refine the prognosis and therapeutic approach for patients with essential thrombocythaemia and other myeloproliferative neoplasms. **BJHM**

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KEY POINTS

- Platelet homeostasis is tightly regulated in a negative feedback loop by thrombopoietin hormone. Reactive thrombocytosis occurs as a result of changes in thrombopoietin concentration mediated by increased levels of inflammatory cytokines, e.g. interleukin-6.
- Thrombocytosis can occur as a paraneoplastic effect in solid organ malignancies, and may pre-date overt clinical signs of malignancy, thus careful history and examination is crucial in patients with thrombocytosis of unclear aetiology.
- Patients with a persistent thrombocytosis in the absence of a clear secondary cause should be investigated for possible myeloproliferative neoplasm with blood film, molecular testing and, if needed, bone marrow biopsy.
- Essential thrombocythaemia carries an increased risk of thrombosis. All patients with essential thrombocythaemia should be assessed for risk of thrombosis using an internationally validated tool and modifiable cardiovascular risk factors. Cytoreductive therapy and antiplatelet agents are indicated for high-risk patients to reduce the thrombotic burden under the supervision of a clinical haematologist.

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