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Workup of the Abnormal Complete Blood Count: Thrombocytosis

First recognized as a unique blood component in 1878, the origin and function of platelets remained obscure for some time thereafter.¹ Various proposed to be either red cell precursors or debris from white cell breakdown,² we now know that platelets are anucleate cytoplasmic fragments, derived from bone marrow megakaryocytes, essential for hemostasis and coagulation. Automated platelet counts are now one of the most common laboratory values reported.

Thrombocytosis (usually defined as a platelet count over 400,000) is a very common incidental laboratory finding and often poses a difficult diagnostic challenge. The vast majority of cases will represent reactive, or secondary, thrombocytosis, frequently due to iron deficiency, infection, splenectomy or chronic autoimmune disease (see Table 1). Importantly, reactive thrombocytosis is not associated with thrombotic or hemorrhagic complications.³ A significant minority of patients, however, will have primary, or clonal, thrombocytosis due to essential thrombocythemia, another chronic myeloproliferative disorder or, rarely, acute leukemia or myelodysplasia. Clonal thrombocytosis, in contrast to reactive thrombocytosis, carries the risk of potentially devastating thrombosis or bleeding, often necessitating potentially toxic drug treatment. Thus, given the important clinical and treatment implications, differentiation between primary and reactive thrombocytosis is essential. Unfortunately, in the occasional patient this distinction may be exceedingly difficult to make.

Table 1 - Causes of Thrombocytosis
Secondary (Reactive) Thrombocytosis

- Infection
- Postsplenectomy
- Inflammatory Diseases
- Idiopathic inflammatory bowel disease
- Collagen vascular diseases
- Malignancy
- Acute Hemorrhage or hemolytic anemia
- Iron deficiency anemia
- Trauma
- Response to vigorous exercise
- Rebound from thrombocytopenia
- Response to drugs

Primary (Clonal) Thrombocytosis

- Chronic myeloproliferative diseases
 - Essential thrombocythemia
 - Polycythemia vera
 - Chronic idiopathic myelofibrosis
 - Chronic myeloid leukemia
- Myelodysplastic syndrome
- Acute myeloid leukemia

An unexpectedly high platelet count should first be confirmed by careful examination of the corresponding peripheral smear,

as various particles in peripheral blood may be erroneously counted as platelets by automated hematology analyzers. Many such causes of spurious thrombocytosis have been documented: white cell fragments in the setting of severe infection or tumor lysis syndrome; red cell fragments in a microangiopathic process, as with severe burns, thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS); red cell inclusions, such as Pappenheimer bodies; microorganism, including bacteria and yeast; and cryoglobulins.^{4,5} In addition to confirming the platelet count, examination of the peripheral smear may also yield other important diagnostic clues, such as Howell-Jolly bodies and target cells in the setting of asplenia, microcytic red cells with iron deficiency, or basophilia in the setting of a chronic myeloproliferative disorder. The degree of platelet count elevation is not particularly helpful in the differential diagnosis: while some have advocated a platelet count of 1 million as a threshold between reactive and clonal thrombocytosis, there is too much overlap to make this a good discriminator.⁶ The duration of thrombocytosis, however, is important, as chronic unexplained thrombocytosis in the absence of prior splenectomy should raise the suspicion of clonal thrombocytosis. Hepatosplenomegaly is also suspicious for neoplastic thrombocytosis. While an elevated fibrinogen or C-reactive protein is helpful in documenting an acute phase reaction in the setting of an occult inflammatory or malignant process, normal levels are not informative.⁷

Similar to adults, thrombocytosis in children is associated with infection, postoperative states and iron deficiency anemia. There is a predictable rise in platelet counts following splenectomy, peaking at levels that may exceed 1 million 1 to 3 weeks following surgery; in some cases, years may be necessary for platelet counts to return to normal.⁸ Other conditions that have been associated with pediatric thrombocytosis include Down's syndrome, congenital adrenal hyperplasia, Kawasaki disease and gastroesophageal reflux disease. Certain drugs, especially some antibiotics, have also been associated with elevated platelet counts in children, and neonatal thrombocytosis has been associated with some maternal drug exposures.⁹

Essential thrombocythemia (ET), first described in 1934, may be the most common chronic myeloproliferative disorder and is usually diagnosed in patients between 50 and 60 years of age. Compared with other MPDs, females and younger patients are over-represented, likely contributing to the superior overall survival in patients diagnosed with ET. As no specific biologic mark-

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ers exist, essential thrombocytosis is ultimately a diagnosis of exclusion, requiring that reactive thrombocytosis and other causes of clonal thrombocytosis be ruled out (see Table 2).

Table 2 - Diagnostic Criteria for the Diagnosis of Essential Thrombocythemia¹⁶

Positive Criteria

1. Sustained platelet count of $\geq 600 \times 10^9/L$
2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged mature megakaryocytes.

Criteria of Exclusion

1. No evidence of polycythemia vera
2. No evidence of chronic myelogenous leukemia
3. No evidence of chronic idiopathic myelofibrosis
4. No evidence of myelodysplastic syndrome
5. No evidence that thrombosis is reactive due to
 - underlying inflammation or infection
 - underlying neoplasm
 - prior splenectomy

Symptoms of ET are usually attributable to microcirculatory disturbances. Microvascular thrombosis of the digits may lead to erythromelalgia, characterized by intense burning or throbbing pain in a patchy distribution in the hands and feet. Vascular disturbances in the central nervous system produce headaches, dizziness, and visual and acoustic symptoms. Essential thrombocythemia is also associated with a hemorrhagic diathesis, manifested as superficial "platelet-type" bleeding in the skin and mucous membranes. Paradoxically, bleeding risk is proportional to the platelet count, and attributed to enhanced removal by platelets of large von Willebrand multimers.¹⁰ Large vessel arterial and venous thrombosis are an important source of morbidity and mortality, with deep venous thrombosis and pulmonary embolism occurring with some frequency.

In most patients with ET, the platelet count exceeds 1 million and the platelets characteristically display striking variation in size. Anemia may be present if there has been hemorrhage, but most patients have a normal hemoglobin level. The white blood cell count is usually normal, although granulocytosis may be seen. In striking contrast to other myeloproliferative disorders, basophilia is not a feature of ET.¹¹ The bleeding time, prothrombin time and activated partial thromboplastin time are usually normal. Platelet function studies, while often abnormal, are not usually performed.

If the possibility of ET or other primary thrombocythemic disorder is entertained, bone marrow examination should be considered. The bone marrow biopsy in ET characteristically displays conspicuous hyperplasia of large to giant megakaryocytes. Bone marrow aspiration also provides material for cytogenetic testing and, while there is no

recurring or specific cytogenetic abnormality in ET, it is essential to exclude the presence of the Philadelphia chromosome indicative of chronic myelogenous leukemia. Bone marrow aspirate can also be now be submitted for mutational analysis of the Janus kinase-2 (JAK2) gene, which represents a promising recent advance in the study of myeloproliferative disorders.¹²

Janus kinase 2 (JAK2) is a protein kinase involved in cytoplasmic messaging via signal transducers and activators of transcription (STAT) proteins which, in turn, affect genes involved in cell proliferation and survival (these kinases include two back-to-back proteins, reminiscent of Janus, the Roman god who looked simultaneously in two directions). A recently described point mutation, resulting in the substitution of valine for phenylalanine at codon 617 (JAK2^{V617F}), reportedly leads to enhanced activity of the JAK2 protein,¹³ and is present in up to 55 percent of patients with ET.¹⁴ Also present in up to 97 percent of patients with polycythemia vera and up to 57 percent of patients with idiopathic myelofibrosis, the JAK2 mutation cannot discriminate between ET and these disorders, but if present would argue strongly against reactive thrombocytosis. JAK2 mutational analysis may also be performed on peripheral blood.

Essential thrombocythemia is considered to be an indolent disorder and, indeed, in a recent Mayo Clinic study of 322 patients, there was no difference in survival, in comparison to controls, during the first decade after the diagnosis. Life expectancy, however, worsens beginning in the early to middle second decade of the disease, with increased mortality due to thrombotic events or to delayed transformation into acute leukemia, myelodysplasia, polycythemia vera or myelofibrosis. Independent predictors of poor survival include age at diagnosis greater than 60 years of age, leukocytosis, tobacco use and diabetes mellitus.¹⁵

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