A patient can present with a raised hemoglobin (Hb), raised white cell count (WCC) or leukocytosis, or raised platelet counts and in each case the causes of the abnormality have to be considered and investigated in order to arrive at the diagnosis. Each type of abnormality will be discussed and illustrative cases given. Cases presenting with simultaneous multiple raised cell counts will also be considered.

Erythrocytosis

An absolute or true erythrocytosis is present when the red cell mass is greater than 125% predicted for the patient’s sex and body mass. The presence of an erythrocytosis is indicated by an Hb and/or a hematocrit (Hct) above the upper limit of normal. However, a raised Hb or Hct may not necessarily indicate that a true erythrocytosis is present. It may be necessary to formally measure the red cell mass in order to prove that there is a true erythrocytosis as opposed to an apparent erythrocytosis (where the Hct is raised but the red cell mass is within the reference range) or a relative erythrocytosis (where the Hct is raised but the red cell mass is within the reference range and the plasma volume reduced). In cases where the Hct is above 0.60% in a male or 0.56% in a woman the red cell mass is always elevated and formal measurement of the red cell mass is unnecessary to prove the point.¹

Differential diagnosis of erythrocytosis

An erythrocytosis can be primary where there is an intrinsic defect within the bone marrow compartment or secondary where something usually mediated by erythropoietin (EPO) is driving the red cell mass expansion. In each case the cause can be congenital or acquired.² Table 1.1 lists all the causes of an erythrocytosis.

Initial assessment of an undiagnosed patient who has an erythrocytosis, leukocytosis, or thrombocytosis

Mary Frances McMullin

The patient should be seen and the full blood picture (FBP) repeated to confirm that the abnormality is present. A careful history and examination should then be carried out considering the differential diagnosis.³ Having confirmed the erythrocytosis if a diagnosis of polycythemia vera (PV) seems a possibility, the next investigation indicated is a test for JAK2 mutations which, if positive, will confirm clonal disease.⁴ If PV does not seem likely then the next step would be to measure the EPO level. A low-level EPO suggests a primary defect in the EPO signaling pathway and this should then be investigated further. A normal or elevated EPO level suggests a secondary cause of erythrocytosis and this should then be investigated further, pursuing any suggestions from the history and examination. Figure 1.1 is an algorithm of the suggested investigative route for a patient with erythrocytosis.

Case 1

An 18-year-old woman presented to the maternity unit 10 weeks pregnant. She was well. In her history she stated that her mother had been investigated because “she had too much blood.” On examination there was nothing abnormal of note. Her FBP: Hb 194 g/L, Hct 0.60%, WCC 8.0 × 10⁹/L and platelets (Pts) 151 × 10⁹/L. Her EPO level was 25 mIU/mL (normal range (NR) 2.5–10.5).

As she had an erythrocytosis, with a raised EPO level, she had a secondary erythrocytosis. Because of her young age and strong family history a congenital secondary erythrocytosis was considered. Her globin genes were sequenced first and she was found to have the high-affinity β hemoglobin hemoglobin San Diego β 109 GTG–ATG; Val–Met.
Chapter 1: Investigation of erythrocytosis and leukocytosis

Table 1.1 Patient investigation

<table>
<thead>
<tr>
<th>Erythrocytosis</th>
<th>Leukocytosis</th>
<th>Thrombocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary erythrocytosis</strong></td>
<td><strong>Neutrophil leukocytosis</strong></td>
<td><strong>Primary</strong></td>
</tr>
<tr>
<td><strong>Congenital</strong></td>
<td><strong>Bacterial infection</strong></td>
<td><strong>Essential thrombocytethemia</strong></td>
</tr>
<tr>
<td>Erythropoietin receptor mutation</td>
<td><strong>Inflammation</strong></td>
<td><strong>Polycythemia vera</strong></td>
</tr>
<tr>
<td><strong>Acquired</strong></td>
<td><strong>Tissue necrosis</strong></td>
<td><strong>Primary myelofibrosis</strong></td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td><strong>Any neoplasm</strong></td>
<td><strong>Myelodysplasia (with del5q)</strong></td>
</tr>
<tr>
<td>JAK mutations (congenital or acquired)</td>
<td><strong>Hemorrhage</strong></td>
<td><strong>Refractory anemia with ring sideroblasts</strong></td>
</tr>
<tr>
<td><strong>Secondary erythrocytosis</strong></td>
<td><strong>Hemolysis</strong></td>
<td><strong>Chronic myeloid leukemia</strong></td>
</tr>
<tr>
<td><strong>Congenital</strong></td>
<td>**Drug, e.g. steroids, granulocyte</td>
<td><strong>Chronic myelomonocytic leukemia</strong></td>
</tr>
<tr>
<td>High oxygen-affinity hemoglobin</td>
<td>colony-stimulating factor</td>
<td><strong>Atypical chronic myeloid leukemia</strong></td>
</tr>
<tr>
<td>Bisphosphoglycerate mutase deficiency</td>
<td><strong>Chronic myeloid leukemia</strong></td>
<td>**Myelodysplastic syndrome/</td>
</tr>
<tr>
<td>Methemoglobinemia</td>
<td><strong>Myeloproliferative neoplasms</strong></td>
<td>myeloproliferative neoplasm, unclassifiable</td>
</tr>
<tr>
<td>Oxygen-sensing pathway defects</td>
<td>(polycythemia vera, essential</td>
<td></td>
</tr>
<tr>
<td>VHL gene mutation</td>
<td>thrombocythemia, primary myelofibrosis)</td>
<td></td>
</tr>
<tr>
<td>PHD2 mutations</td>
<td><strong>Smoking</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Acquired</strong></td>
<td><strong>Asplenia</strong></td>
<td></td>
</tr>
<tr>
<td>Erythropoietin-mediated</td>
<td><strong>Eosinophil leukocytosis</strong></td>
<td><strong>Secondary</strong></td>
</tr>
<tr>
<td>Central hypoxia</td>
<td><strong>Allergic disorders</strong></td>
<td><strong>Infection</strong></td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td><strong>Parasitic infections</strong></td>
<td><strong>Inflammation</strong></td>
</tr>
<tr>
<td>Right-to-left cardiopulmonary shunts</td>
<td><strong>Polyarteritis nodosa</strong></td>
<td><strong>Tissue damage</strong></td>
</tr>
<tr>
<td>Carbon monoxide poisoning</td>
<td><strong>vasculitis</strong></td>
<td><strong>Hypersplenism</strong></td>
</tr>
<tr>
<td>Smoker's erythrocytosis</td>
<td><strong>Skin diseases (e.g., pemphigus)</strong></td>
<td><strong>Postoperative</strong></td>
</tr>
<tr>
<td>Hypoventilation syndromes</td>
<td><strong>Metastatic malignancy</strong></td>
<td><strong>Hemorrhage</strong></td>
</tr>
<tr>
<td><strong>High-altitude habitat</strong></td>
<td><strong>Graft-versus-host disease</strong></td>
<td><strong>Iron deficiency</strong></td>
</tr>
<tr>
<td>Local renal hypoxia</td>
<td><strong>Hodgkin's disease</strong></td>
<td><strong>Any neoplasm</strong></td>
</tr>
<tr>
<td>Renal artery stenosis</td>
<td>**Myeloproliferative neoplasms,</td>
<td><strong>Hemolysis</strong></td>
</tr>
<tr>
<td>End-stage renal disease</td>
<td>including mastocytosis**</td>
<td><strong>Drug therapy, e.g., steroids</strong></td>
</tr>
<tr>
<td>Hydro nephrosis</td>
<td><strong>Hypereosinophilic syndrome</strong></td>
<td><strong>Spurious</strong></td>
</tr>
<tr>
<td>Renal cysts (polycystic kidney disease)</td>
<td><strong>Pulmonary syndromes (e.g., Churg–Strauss syndrome)</strong></td>
<td><strong>Microspherocytes</strong></td>
</tr>
<tr>
<td>Post renal transplant erythrocytosis</td>
<td><strong>Basophil leukocytosis</strong></td>
<td><strong>Neoplastic cell fragments</strong></td>
</tr>
<tr>
<td>Pathological erythropoietin production</td>
<td><strong>Myxoe demia</strong></td>
<td><strong>Schistocytes</strong></td>
</tr>
<tr>
<td>Tumors</td>
<td><strong>Smallpox</strong></td>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td>Cerebellar hemangioblastoma</td>
<td><strong>Chickenpox infection</strong></td>
<td><strong>Cryoglobulinemia</strong></td>
</tr>
<tr>
<td>Meningioma</td>
<td><strong>Ulc erative colitis</strong></td>
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<tr>
<td>Parathyroid adenoma/carcinoma</td>
<td>**Myeloproliferative neoplasms,</td>
<td></td>
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<tr>
<td>Hepatocellular carcinoma</td>
<td>including polycythemia vera and</td>
<td></td>
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<tr>
<td>Renal cell carcinoma</td>
<td>chronic myeloid leukemia**</td>
<td></td>
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<tr>
<td>Pheochromocytoma</td>
<td><strong>Idiopathic erythrocytosis</strong></td>
<td></td>
</tr>
<tr>
<td>Uterine leiomyomas</td>
<td><strong>Erythropoietin administration</strong></td>
<td></td>
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<tr>
<td>Exogenous erythropoietin</td>
<td><strong>Androgen administration</strong></td>
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<tr>
<td>Erythropoietin administration</td>
<td><strong>Idiopathic erythrocytosis</strong></td>
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**Neutrophil leukocytosis**

- Bacterial infection
- Inflammation
- Tissue necrosis
- Any neoplasm
- Hemorrhage
- Hemolysis
- Drug, e.g. steroids, granulocyte colony-stimulating factor
- Chronic myeloid leukemia
- Myeloproliferative neoplasms (polycythemia vera, essential thrombocythemia, primary myelofibrosis)
- Smoking
- Asplenia

**Eosinophil leukocytosis**

- Allergic disorders
- Parasitic infections
- Polyarteritis nodosa
- Vasculitis
- Skin diseases (e.g., pemphigus)
- Metastatic malignancy
- Graft-versus-host disease
- Hodgkin's disease
- Myeloproliferative neoplasms, including mastocytosis
- Hypereosinophilic syndrome
- Pulmonary syndromes (e.g., Churg–Strauss syndrome)

**Basophil leukocytosis**

- Myxoe demia
- Smallpox
- Chickenpox infection
- Ulcerative colitis
- Myeloproliferative neoplasms, including polycythemia vera and chronic myeloid leukemia
Chapter 1: Investigation of erythrocytosis and leukocytosis

Diagnosis: Hemoglobin San Diego: high oxygen affinity hemoglobin.

Case 2
A 60-year-old man attended his general practitioner complaining of increasing bleeding from hemorrhoids. He had had hemorrhoids for many years but had increased bleeding over the last 6 months. His FBP: Hb 115 g/L, Hct 0.35%, mean corpuscular volume (MCV) 70 fL, mean corpuscular hemoglobin (MCH) 25 pg, mean corpuscular hemoglobin concentration (MCHC) 29 g/L, WCC 7.5 × 10^9/L, and platelets 380 × 10^9/L. He was referred for gastrointestinal investigations and started on oral iron supplementation. Four weeks later at a follow-up appointment his repeat FBP was Hb 185 g/L, Hct 0.53%, MCV 70 fL, MCH 25 pg, MCHC 30 g/L, WCC 7.5 × 10^9/L, and platelets 380 × 10^9/L. On referral for further investigation of this erythrocytosis, a JAK2 V617F mutation was revealed.

Diagnosis: Polycythemia vera.

Leukocytosis
A leukocytosis indicated by a raised WCC above the normal range can be due to raised neutrophils, eosinophils, or basophils. The differential WCC will reveal the type of leukocytosis and fully elucidate the type of elevated white cells. A monocytosis or lymphocytosis as the cause of the elevated WCC will be revealed and eliminated. White cells are produced and turned over in a very short time frame and a leukocytosis can develop and disappear very rapidly.

Differential diagnosis of a leukocytosis
The causes of a leukocytosis are extensive. A neutrophil leukocytosis can be due to any bacterial infection, particularly pyogenic infections, which will only be sustained if the infection continues. Neutrophilia is also part of the response to any inflammation or tissue...
Chapter 1: Investigation of erythrocytosis and leukocytosis

Increased WCC

Repeat and Confirm

Differential WCC and blood film

Tests for differentials in diagnosis

Full History and Examination

Molecular genetic tests for MPNs

Bone marrow Biopsy

Cytogenetics

Figure 1.2 An algorithm for the investigation of a leukocytosis: WCC, white cell count; MPNs, myeloproliferative neoplasms.

damage, acute hemorrhage or hemolysis. Any neoplasm can be associated with neutrophilia. Many drugs such as corticosteroids can cause neutrophilia and it is the desired response from administration of granulocyte colony-stimulating factor. Myeloproliferative neoplasms, both Philadelphia-positive and -negative, frequently have an associated neutrophilia. Other causes include chronic smoking and asplenia.

An eosinophil leukocytosis has many potential causes, including allergic and parasitic disorders, any metastatic malignancy, systemic disorders such as polyarteritis nodosa, many skin diseases, and vasculitis. Hematological causes include Hodgkin’s disease, many of the myeloproliferative neoplasms, and graft-versus-host disease. There are also a number of specific hypereosinophilic syndromes and pulmonary syndromes which cause eosinophilia.

A basophil leukocytosis is rare but can be caused by viral infections such as smallpox and chickenpox, myxoedema, ulcerative colitis, and in myeloproliferative neoplasms.

A summary list of causes of leukocytosis is presented in Table 1.1.

Initial assessment of an undiagnosed patient with leukocytosis

The patient with a leukocytosis should be seen and have the blood test repeated to confirm that the abnormality remains present. The patient should have a careful history taken, including lifestyle issues and examination carried out with consideration of the differential diagnosis. The differential WCC will give information on the type of leukocyte involved. A blood film needs to be examined. Further investigation then depends upon the information at this point. Any likely cause needs to be further investigated. Molecular testing looking for a clonal disorder (BCR/ABL, JAK2, MPL, and CALR) should be undertaken if there is no obvious cause, to look for a myeloproliferative neoplasm; specific tests such as CSF3R mutations may be considered. Figure 1.2 presents an algorithm to follow for investigation of a leukocytosis.

Case 4

A 62-year-old woman was referred to hematology outpatients as a red flag referral because of a leukocytosis. The referring FBP: Hb 155 g/L, WCC 14 × 10⁹/L and Plts 280 × 10⁹/L. The patient was well with no specific complaints. Her weight was stable. She smoked 30 cigarettes a day and drank moderate amounts of alcohol at the weekends. On examination she was thin and had nicotine-stained fingers. She had a few scattered coarse crepitations in her chest. Repeat FBP with differential WCC: Hb 152 g/L, WCC 13.5 × 10⁹/L and Plts 285 × 10⁹/L, neutrophils 10.5 × 10⁹/L (NR 2.0–7.5), lymphocytes 2.5 × 10⁹/L (NR 1.0–3.5), monocytes 0.5 × 10⁹/L (NR 0.2–0.8), eosinophils 0.4 × 10⁹/L (NR 0.04–0.4), basophils 0.1 × 10⁹/L (NR 0.01–0.1). Chest X-ray showed changes of chronic obstructive pulmonary disease.
Diff erential diagnosis of a thrombocytosis

A raised platelet count can be due to a primary defect in the bone marrow or primary thrombocytosis, or a secondary thrombocytosis where something else drives the increase in the platelet count. There are also a number of reasons where something else may be counted as platelets, erroneously leading to spuriously raised platelets. Bone marrow disorders which lead to thrombocytosis include essential thrombocythemia, where there is an acquired clone in the bone marrow driving the platelet production. The identified clones include JAK2, MPL, CALR, although there are still cases where no clone has yet been identified. Other myeloproliferative neo- plasms, both Philadelphia-positive and -negative, and myelodysplastic syndromes can present with thrombo- cytosis which is primary. There are many secondary causes for thrombocytosis where increased platelets are produced as part of the reaction to the underlying disorder. This includes response to infection, any inflammation or tissue damage, iron-deficiency hemorrhage and hemolysis, and malignancy. Various cellular elements can be counted as platelets by analyzers. Bacteria, schistocytes, and Pappenheimer bodies, for instance, can be counted, and lead to a spurious platelet reading. The causes of thrombocytosis are listed in Table 1.1.

Chapter 1: Investigation of erythrocytosis and leukocytosis

Diagnosis: Neutrophil leukocytosis due to smoking.

This woman may need to be investigated further to ensure that she does not have a lung neoplasm as she is a heavy smoker. However, she has no symptoms and nothing of note on her chest X-ray. Her WCC has been at this level for years. Smoking cessation would be advised.

Case 5

A 65-year-old woman attended her general practitioner for a routine annual check-up. She was feeling very well and had no complaints. Included in the screening bloods carried out was a FBP which showed a raised WCC: Hb 133 g/L, WCC 20.5 × 10^9/L and Pts 275 × 10^9/L, and she was referred to hematology. On arrival she had no further history of note and examination did not reveal any abnormalities. Repeat FBP and differential: Hb 133 g/L, WCC 28.5 × 10^9/L and Pts 271 × 10^9/L, and manual differential neutrophils 16.5 × 10^9/L (NR 2.0–7.5), lymphocytes 5.15 × 10^9/L (NR 1.0–3.5), monocytes 0.57 × 10^9/L (NR 0.2–0.8), eosinophils 0.86 × 10^9/L (NR 0.04–0.4), basophils 0.29 × 10^9/L (NR 0.01–0.1), myelocytes 3.15 × 10^9/L, metamyelocytes 2.0 × 10^9/L. Molecular investigation showed a BCR/ABL rearrangement and no JAK2 mutation.

Diagnosis: Chronic myeloid leukemia.

The presence of the BCR/ABL rearrangement showed a clonal disorder. This is early chronic myeloid leukemia. Further investigation would then include bone marrow examination and cytogenetic examination and/or fluorescent in situ hybridization to confirm the presence of the Philadelphia chromosome.

Thrombocytosis

A thrombocytosis is present when the platelet count is raised above the normal range, greater than 450 × 10^9/L. A markedly and repeatedly raised thrombocytosis is of importance, but the significance of one just above the normal range is difficult to assess.

Initial assessment of an undiagnosed patient with thrombocytosis

As with other patients with an elevated count, the initial assessment is to repeat the count and confirm that there is actually a sustained elevation of the platelet count. The patient then needs to be reviewed and assessed with a careful history and examination. Attention to a search for secondary reactive causes should be included. The next step is to review the blood film and test the iron status (ferritin) and for acute-phase reactants C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR). An elevated acute-phase reactant suggests a reactive thrombocytosis. Iron deficiency should be treated to see if the platelet count decreases. If it does not fall into either of these categories and the elevated platelet count is sustained on repeat, then molecular testing should be carried out, looking for an acquired clone and bone marrow aspirate; trephine and cytogenetic testing should be considered. The algorithm in Figure 1.3 sets out the diagnostic pathway.

Case 6

A 50-year-old woman was referred to hematology because she was found to have an elevated platelet count when attending to donate blood. She was otherwise well. On examination there were no abnormal findings. Repeat FBP done 4 weeks after the initial count was: Hb 135 g/L, MCV 85 fl, WCC 5.5 × 10^9/L, and Pts 820 × 10^9/L. CRP is 0.3 mg/L (NR 0.1–5) and ferritin 30 μg/L (NR 13–300). It was noted also on searching computer records that she had a platelet count of 635 × 10^9/L 2 years ago.
previously when having a minor gynecological procedure. Molecular testing detected a JAK2 V617F mutation. A bone marrow trephine was moderately hypercellular with marked proliferation of megakaryocytes, some of which have hyperlobulated nuclei. Reticulin was grade 0 (out of 4). Chromosomes are normal.

Diagnosis: Essential thrombocythemia.

Case 7
An 80-year-old woman was referred to hematology from rheumatology because of a persistently raised platelet count. She had had rheumatoid arthritis for the previous 20 years. This did not now give her much trouble. Otherwise she was well. On examination she had extensive rheumatoid changes in her hands. There were no other abnormal findings on examination. On repeat her FBP was: Hb 129 g/L, MCV 89 fL, WCC 8.3 × 10^9/L, and Pts 857 × 10^9/L. ESR is 6 mm/h and ferritin 16 μg/L (NR 13–300). A molecular test for JAK2 V617F was positive.

Diagnosis: Essential thrombocythemia.

This woman was presumed to have a reactive thrombocytosis due to her rheumatoid arthritis. However, the referring doctor was suspicious that the rise in platelet count was higher and more sustained than would be expected in a reactive case. Her rheumatoid arthritis was also very quiescent. Molecular testing revealed an acquired clone and therefore she had a primary hematological disorder.

Case 8
A 17-year-old young man attended his general practitioner for review of his mild asthma. A routine blood count revealed an elevated platelet count and he was referred urgently to hematology. At clinic he was well. Asthma was easily controlled with a salbutamol inhaler. On examination he was 181 cm and looked well, including well nourished. There were no abnormalities on examination. His FBP: Hb 147 g/L, RBC 5.34 × 10^12/L, Hct 0.45%, MCV 84 fL, MCHC 343 g/L, WCC 9.7 × 10^9/L, and Pts 1275 × 10^9/L. CRP < 0.3 mg/L (NR 0.1–5) and ferritin 35 μg/L (NR 30–300). Lactate dehydrogenase was 386 IU/L (NR 135–225) and all other biochemistry was within the normal ranges. Blood film showed thrombocytosis and mild poikilocytosis. The bone marrow aspirate was hypercellular with platelet drifts, hyperlobulated megakaryocytes and myeloid hyperplasia, and no excess of blasts. The trephine biopsy was hypercellular with increased hyperlobulated megakaryocytes, disrupted erythropoietic architecture, and reticulin of grade 1 (out of 4). Chromosomes were normal. Molecular testing showed no BCR-ABL1 rearrangement, JAK2 V617F negative, MPL screen negative, and a CALR mutation, exon 9, 5bp insertion.

Diagnosis: Essential thrombocythemia with a CALR mutation.
Initial assessment of an undiagnosed patient more than one cell line elevated

This article has concentrated on the elevation of single cell lines and the diagnostic pathway for each. However, many patients present with elevation of more than one cell line. They need to be investigated in a similar manner with repetition of the blood count to confirm the abnormality and examination of the blood film. Careful history taking and examination are required, looking for reactive and primary or secondary causes, testing to eliminate reactive causes, and then bone marrow and molecular testing for hematological disorders. The cases which follow illustrate where the patient presents with more than one cell lineage elevated.

Case 9

This 33-year-old woman attended her general practitioner for a check-up. She was married 1 year previously. She had had a pregnancy which spontaneously aborted at 12 weeks’ gestation 6 months ago and had not yet conceived again and she was anxious to see if there was any reason for the fact that she had not yet become pregnant. As part of the screening she had an abnormal FBP and so was referred urgently to hematology. When seen in hematology her only complaint was tiredness. On examination she had a pale complexion but her hands were red. There were no other findings on examination. Her repeat FBP was very similar to the original one carried out by her general practitioner and so was referred urgently to hematology. When seen in hematology her only complaint was tiredness. On examination she had a pale complexion but her hands were red.

Case 10

A 50-year-old woman was admitted through accident and emergency with sudden severe abdominal pain. On examination she had diffuse tenderness in her abdomen. Ultrasound of her abdomen showed thrombus in the portal vein and a spleen of 17 cm. She had Budd-Chiari syndrome and was anticoagulated. Her FBP showed: Hb 160 g/L, RBC 5.34 × 10¹²/L, Hct 0.458%, MCV 84 fl, MCHC 338 g/L, WCC 9.7 × 10⁹/L, and Pts 649 × 10⁹/L. Testing for a JAK2 V617F mutation was positive.

Diagnosis: Polycythemia vera presenting with a portal vein thrombosis.

Summary

Patients presenting with an elevated blood count need to be repeated after a period of time to confirm the abnormality. The patient then needs a thorough history and examination considering primary and secondary causes of the abnormality. A blood film should be examined and then further investigation follows a pathway depending on initial results. Bone marrow examination and molecular testing may reveal the hematological diagnosis.

References

Chapter 1: Investigation of erythrocytosis and leukocytosis


Practical approaches to molecular testing and diagnosis of mastocytosis, hypereosinophilia, and MDS/MPN overlap syndromes

Nicholas C.P. Cross, Dimitra Koumaki, Georgia Metzgeroth, and Andreas Reiter

Introduction

Chronic myeloproliferative neoplasms (MPN) comprise diverse disorders, some with proliferative features and others with dysplastic hematopoiesis. In an attempt to improve their classification, the World Health Organization (WHO) has divided them into three principal categories: (1) MPN; (2) myelodysplastic syndrome/MPN (MDS/MPN); and (3) myeloid and lymphoid neoplasms with eosinophilia (MLN-eo) and abnormalities of PDGFRA, PDGFRB, or FGFR1.

The MPN category includes four predominant subtypes: chronic myeloid leukemia plus the three so-called "classical" BCR-ABL1-negative disorders polycythemia vera, primary myelofibrosis, and essential thrombocythemia. In addition, this category also includes the rarer "atypical" entities mastocytosis, chronic eosinophilic leukemia (CEL), chronic neutrophilic leukemia (CNL), and MPN-unclassifiable. The MDS/MPN category comprises chronic myelomonocytic leukemia (CML), juvenile myelomonocytic leukemia (JML), atypical chronic myeloid leukemia (aCML), a "provisional entity," refractory anemia with ring sideroblasts and thrombocytosis (RARS-T), and a "by exclusion" subcategory, MDS/MPN-unclassifiable (MDS/MPN-U).

This review focuses on the molecular diagnosis of atypical MPN, MLN-eo, and MDS/MPN subtypes, many of which are difficult to distinguish in routine practice.

Mast cell disease

Pathogenesis and classification

Mastocytosis is a rare and heterogeneous disease that is characterized by the accumulation of abnormal mast cells in one or more tissues, predominantly skin, bone marrow (BM) and visceral organs, resulting in organ dysfunction in severe cases. It can affect children and adults, but the disease characteristics are very different between different subtypes and the WHO therefore distinguishes several distinct variants of cutaneous mastocytosis (CM) and systemic mastocytosis (SM).

The extent of organ infiltration and subsequent organ damage is the basis for the classification of SM into indolent SM (ISM), SM with associated clonal hematologic non-mast cell lineage disease (SM-AHNMD), aggressive SM (ASM), and mast cell leukemia (MCL). In patients with SM-AHNMD, the SM component can resemble ISM, ASM, or even MCL. Depending on the subtype of SM, cell source (BM or peripheral blood, PB) and assay sensitivity, an acquired mutation in the KIT gene (usually resulting in a D816V amino acid substitution) is detectable in more than 80–90% of adult cases. The proto-oncogene KIT encodes a receptor tyrosine kinase (KIT; also known as CD117) whose ligand is stem cell factor (SCF). SCF/KIT signaling is important for the control of mast cell development/proliferation and the KIT mutations seen in mastocytosis result in
Chapter 2: Diagnosis of mastocytosis, hypereosinophilia, and MDS/MPN

 Constitutive receptor activation in the absence of SCF. Consequently KIT mutations are believed to be the direct driver of aberrant mast cell proliferation, although additional mutations contribute to the precise phenotype and severity of the disease.2,3

Childhood-onset mastocytosis is also a heterogeneous group of disorders. Most cases present during the first year of life and, although not generally considered a hereditary disease, familial cases have been described.4 The course of mastocytosis varies substantially and is influenced by the subtype and the age of onset. Most children have a form of CM, such as urticaria pigmentosa (UP), mastocytoma of the skin, or diffuse CM and the disease usually remains confined to the skin. Lymphadenopathy, hepatomegaly, or splenomegaly is only rarely seen in children with mastocytosis and in most cases blood cell counts and serum chemistry parameters are normal.5

The simplest marker for the diagnosis and severity of mastocytosis is serum tryptase. Tryptases are proteases located in mast cell granules and the median tryptase level in the healthy population is 5 ng/mL. In CM the levels are roughly double that found in healthy individuals but are often much higher, e.g., 50–100 ng/mL or more in SM and as high as >1,000 ng/mL in ASM and MCL. Although a BM investigation is performed routinely in adults with mastocytosis,6 this is not generally undertaken for children unless the serum tryptase level increases over time, the level is massively elevated, or there are other clinical or laboratory signs of systemic disease. A skin biopsy is desirable to confirm the clinical diagnosis of CM, although observation alone may be appropriate in very young children. The histological appearances of the clinical variants of UP are similar; the differences depend on the degree of mast cell infiltration. The mast cells are round or spindle-shaped with abundant eosinophilic cytoplasm and eosinophils are often present.

Although children with mastocytosis generally follow a benign self-limited course with improvement or resolution and no progression to systemic disease, mastocytosis is nevertheless a clonal disease most commonly associated with D816V and other activating KIT mutations. In a large cohort of pediatric patients where the entire KIT sequence was analyzed in DNA extracted from skin biopsies, the D816V mutation was found in 42% of cases and mutations in other exons were observed in 44%.7 It thus appears that the large majority of pediatric patients have alterations in KIT, supporting the idea that childhood mastocytosis is also a clonal disease but with a broader spectrum of KIT mutations than seen in adults. Although important for the diagnosis of adult cases, knowing the KIT mutational status of most children is not currently considered to be of great importance for most children in routine clinical practice.8

Diagnostic procedures

The abnormal clone in mastocytosis is often highly focal in skin lesions or the BM, and is either absent or present at very low levels in PB. Consequently relevant mutations are harder to detect compared to other myeloid neoplasms and it is essential to use a mutation assay with high sensitivity, or to purify mast cells by flow cytometry or laser microdissection.

Conventional Sanger sequencing has a sensitivity of mutation detection of about 20% and thus can only be used on purified mast cells or skin biopsies with high levels of mast cell infiltration. For analysis of BM or PB, it is essential to use an assay with a sensitivity of detection of 0.1–1%. Several such techniques have been developed, as reviewed elsewhere,9 but most centers currently employ quantitative polymerase chain reaction-based techniques to detect KIT D816V in genomic DNA or RNA/cDNA.9,10 For many patients the level of disease, as assessed by the mutational burden, is significantly higher in the BM compared to the PB and consequently BM is the preferred tissue for mutational analysis. It is perfectly acceptable, however, to perform an initial mutation screen on a PB sample and then consider further analysis of the BM if the result is negative. If the BM tests negative for D816V and there is strong evidence for a diagnosis of SM, then Sanger sequencing or next-generation sequencing (assay sensitivity typically 1–5%) of KIT exon 17 should be considered to detect rare activating KIT variants, the most common of which are D816Y and D816H. Due to the complexity of the analysis most centers do not report mutation levels, but emerging evidence indicates that the multilinear involvement of KIT D816V and the KIT D816V allele burden have an important impact on disease phenotype and prognosis.9,10

As indicated above, KIT mutation analysis is not considered to be warranted in children in the absence of indicators of systemic disease. Nevertheless, KIT mutations are detectable in exons 8, 9, 11, or 17 in most cases by Sanger sequencing of DNA extracted from cutaneous biopsies. Further work is required to determine the clinical significance of these mutations.