

# The Diagnostic Interface between Histology and Molecular Tests in Myeloproliferative Disorders

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## Introduction

Myeloid malignancies are broadly classified into acute myeloid leukemia (AML) and chronic myeloid disorders (CMDs). The latter include several “myeloproliferative” and “myelodysplastic” subcategories whose specific diagnosis is based primarily on bone marrow histopathology. The term “myeloproliferative disorders (MPDs)” was first applied by William Dameshek (1900-1969) to highlight the phenotypic similarity between chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (CIMF).<sup>1</sup> These four disorders are now referred to as “classic” MPDs and further sub-classified as being either *BCR-ABL*<sup>(+)</sup> (i.e. CML) or *BCR-ABL*<sup>(-)</sup> (i.e. PV, ET, and CIMF).<sup>2</sup> Such classification is now molecularly validated; an activating *JAK2* mutation (*JAK2V617F*) is found in the majority of patients with *BCR-ABL*<sup>(-)</sup> classic MPDs<sup>3-6</sup> whereas it is either absent or occurs infrequently in other myeloid disorders.

In addition to the classic MPDs, chronic myeloid malignancies also include the myelodysplastic syndrome (MDS) and other CMDs that are not classified as either classic MPDs or MDS. According to a recent semi-molecular classification proposal,<sup>2</sup> clinicopathologic entities in the latter category were grouped under the rubric of “atypical” MPD and include chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), chronic neutrophilic leukemia (CNL), chronic basophilic leukemia (CBL), hypereosinophilic syndrome (HES), chronic eosinophilic leukemia (CEL), systemic mastocytosis (SM), and unclassified MPD (UMPD). In contrast, the more formal World Health Organization (WHO) classification system for hematological malignancies considers four categories of CMDs: MDS, MPD, MDS/MPD, and SM.<sup>7</sup> The WHO MPD category includes the four classic MPDs as well as CNL, CEL, HES, and UMPD. The WHO MDS/MPD category includes CMML, JMML, and atypical CML.

Regardless of what one chooses to use for a classification system in MPDs, a gradual transition from clinicopathologic to molecularly-defined disease categorization is becoming evident (Figure 1). In the meantime, however, it is premature to undermine the diagnostic value of bone marrow histopathology, which, when combined with cytogenetic analysis, might also provide useful prognostic information. In the current review, we discuss how molecular tests and bone marrow histopathology are effectively combined in constructing diagnostic algorithms for each one of the three *BCR-ABL*<sup>(-)</sup> classic MPDs as well as atypical MPDs. For the purposes of the current communication, we will focus on *JAK2V617F* when discussing *BCR-ABL*<sup>(-)</sup> classic MPDs, but fully realize the potential role of other molecular markers that are in the process of being described, including *MPLW515L/K* in CIMF and ET and *JAK2D620E* and other *JAK2* mutations in PV.<sup>8-10</sup>

## Polycythemia vera

Because of immediate therapeutic implications, the most important objective in the evaluation of “polycythemia” is to determine the likelihood of a PV diagnosis. It is common knowledge that a seemingly high hematocrit level might (*true polycythemia*) or might not (*apparent polycythemia including relative polycythemia*) reflect a true increase in red cell mass (RCM).<sup>11</sup> Similarly, an increased RCM is not always associated with a hematocrit level that exceeds the “normal” reference range (i.e. *inapparent polycythemia*).<sup>12</sup> Therefore, the possibility of a PV diagnosis should be entertained in the context of both an increased hematocrit level and, regardless of the hematocrit level, the presence of a PV-characteristic clinical feature including large vein thrombosis, aquagenic pruritus, erythromelalgia, or splenomegaly. Accordingly, the best approach to the diagnosis of PV makes use of PV-characteristic molecular, biological, and histological disease markers that are not found in either secondary (SP) or apparent (AP) polycythemia (Figure 2). In this regard, the most helpful diagnostic test is mutation screening for

*JAK2V617F*.<sup>13</sup>

*JAK2V617F* is a G to T somatic mutation of *JAK2*, at nucleotide 1849, in exon 14, resulting in the substitution of valine to phenylalanine at codon 617. It is now becoming evident that *JAK2V617F* is present in nearly all patients with PV<sup>14</sup> but not in other causes of polycythemia.<sup>6,13,15</sup> Therefore, screening for the particular mutation is a reasonable first line test in the evaluation of polycythemia (Figure 2). However, *JAK2V617F* is not specific to PV and is also found in other *BCR-ABL*<sup>(-)</sup> classic and atypical MPDs as well as MDS.<sup>3-6,15,16</sup> Furthermore, *JAK2V617F* mutation detection methods are currently not standardized and the use of either inadequately or overly sensitive assays can produce false negative<sup>3</sup> and false positive<sup>17</sup> results, respectively. Other confounding factors include inconsistency regarding tissue source for test samples and the occasional occurrence of *JAK2V617F*-negative PV.<sup>18,19</sup> Therefore, it is advised to obtain concomitant measurement of serum erythropoietin (Epo) level to overcome the aforementioned shortcomings of mutation screening (Figure 2).

In the presence of *JAK2V617F*, diagnostic certainty for PV, as opposed to other causes of polycythemia, is further enhanced by the demonstration of a low serum Epo level; test sensitivity and specificity for the latter is above 90% and 95%, respectively.<sup>20,21</sup> Therefore, when both mutation analysis and serum Epo results are suggestive of PV, bone marrow examination is not considered essential for diagnosis but is encouraged because of its value in terms of both confirmation of diagnosis and pathological staging that includes accruing baseline information on reticulin fibrosis and cytogenetic analysis (Figure 2).<sup>22</sup> On the other hand, bone marrow examination is necessary for confirmation of diagnosis when the two test results are not in agreement. The bone marrow biopsy findings that are characteristic of PV include hypercellularity for the patient's age that is due to trilineage proliferation ("panmyelosis") with enlarged islands of erythropoiesis and increased numbers of megakaryocytes often found in loose clusters or dispersed throughout the biopsy. The megakaryocytes are variable in size, ranging from small to large or even giant size, but lacking significant atypia in their nuclear configuration or in their nuclear/cytoplasmic ratio.<sup>23</sup> Finally, the possibility of PV, in the absence of both *JAK2V617F* and a low serum Epo level is too low to warrant further PV-directed investigation.

### **Essential thrombocythemia**

Formal diagnostic criteria for ET were first established in the 1970s by the PVSG and have since been revised by the WHO-sponsored cooperative group.<sup>24</sup> In both instances, however, the utilization of a platelet count threshold ( $600 \times 10^9/L$ ) that is significantly higher than the upper limits of "normal" ( $\sim 350 \times 10^9/L$ ) is not biologically sound and could lead to inadvertent oversight of phenotypically-classic ET cases with platelet counts between 400 and  $600 \times 10^9/L$ .<sup>25-27</sup> Regardless, a working diagnosis of ET requires exclusion of both reactive thrombocytosis (RT) and clonal thrombocytosis associated with another myeloid disorder. Usually, patient history, physical examination, peripheral smear evaluation, and measurement of serum ferritin and C-reactive protein levels are adequate to address the possibility of RT.<sup>28</sup> However, a bone marrow examination accompanied by fluorescent in situ hybridization (FISH) or RT-PCR for *BCR-ABL*, mutation screening for *JAK2V617F*, and cytogenetic studies are recommended for the evaluation of clinically-determined, "non-reactive" thrombocythemia in order to confirm the presence as well address the differential diagnosis of an underlying MPD (Figure 3).

The presence of *BCR-ABL*, in the context of a CMD, is diagnostic of CML.<sup>29</sup> In contrast, the presence of *JAK2V617F*, although pathognomonic of an underlying myeloid disorder, is neither ET-specific (the mutation is also found in other MPDs) nor diagnostically essential (*JAK2V617F* is not detected in approximately half of patients with ET).<sup>30</sup> Therefore, at the present time, bone marrow morphological assessment remains the key diagnostic tool, in terms of both confirming an MPD diagnosis and distinguishing ET from other molecularly-undefined causes of clonal thrombocythemia (e.g. MDS, cellular phase CIMF).<sup>31,32</sup> In ET, bone marrow findings are remarkable for the presence of large, even giant, but mature-appearing megakaryocytes with deeply lobulated nuclei that are most often dispersed throughout the biopsy sections, but sometimes also found in loose clusters.<sup>32</sup> Often the bone marrow is normally or only slightly hypercellular for the patient's age, and the panmyelosis that characterizes PV and the granulocytic proliferation and highly bizarre megakaryocytes that characterize the pre-fibrotic

stage of chronic idiopathic myelofibrosis are not found in ET.<sup>33</sup> The presence of dyserythropoiesis, macrocytosis, monocytosis, pseudo Pelger-Huet anomaly of neutrophils or predominance of megakaryocytes with monolobated nuclei suggest MDS or atypical MPD rather than ET.<sup>31</sup> Finally, the presence of clonal cytogenetic lesions in “ET” mandates histological and clinical re-evaluation because such events are infrequent in conventionally-defined ET (< 5%).<sup>34</sup> \

### **Chronic idiopathic myelofibrosis**

The classical clinical presentation in CIMF includes marked splenomegaly and anemia although the specific diagnosis is usually suspected when one encounters the peripheral blood changes of myelophthisis and/or bone marrow fibrosis. However, neither myelophthisis nor bone marrow fibrosis is specific to CIMF and both features can also be seen in a spectrum of clonal and non-clonal conditions. In order to facilitate differential diagnosis, therefore, bone marrow biopsy should be accompanied by FISH or RT-PCR for *BCR-ABL*, mutation screening for *JAK2V617F*, and cytogenetic studies (Figure 4). As stated before, the presence of *BCR-ABL* should be considered diagnostic of CML.<sup>29</sup> The presence of *JAK2V617F* is diagnostic of an underlying clonal myeloid disorder and excludes the possibility of bone marrow fibrosis associated with infections, inflammatory processes, lymphoid disorders, or metastatic cancer.<sup>35</sup> However, the distinction between CIMF (overtly fibrotic or cellular phase), in one hand, and MDS with fibrosis, ET, or acute myelofibrosis (a subtype of AML according to the WHO),<sup>7</sup> on the other, requires careful morphological assessment;<sup>33</sup> *JAK2V617F* is not always present in CIMF (mutational frequency is ~ 50%) and has also been described in ET and in a few cases of MDS and AML.<sup>30</sup>

Histologically, CIMF is characterized by the presence of megakaryocytes that are morphologically more atypical than in any of the other subtypes of MPD. They are often found in sizable loose to tight clusters, and range from small to large with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous or irregularly folded nuclei. Bare megakaryocytic nuclei are common.<sup>36</sup> In contrast, in MDS, the classic morphologic features of myelodysplasia must be demonstrated. The dysplastic megakaryocytes in MDS tend to be small and often have monolobated, hypolobated or widely dispersed nuclei, and are not usually found in clusters as are typical for MPD. On the other hand, the presence of ringed sideroblasts, although seen regularly in MDS, may also be found in some cases of MPD, and therefore lack diagnostic specificity.<sup>37</sup> The distinction between cellular phase CIMF and ET is often impossible from the peripheral blood alone, and therefore the degree of bone marrow cellularity observed in the biopsy (marked hypercellularity in cellular phase CIMF), presence of left-shifted and prominent granulocyte proliferation (typical in CIMF but absent in ET), and megakaryocyte morphology (maturationally-defective with the aforementioned nuclear features in CIMF and giant, mature-appearing megakaryocytes with well-lobulated nuclei in ET).<sup>38</sup> Patients with acute myelofibrosis usually present with severe constitutional symptoms, pancytopenia, mild or no splenomegaly, and feature an increase in blood and/or bone marrow blast count that will often approach or fulfill the required threshold for AML diagnosis. However, in all cases with myelofibrosis, blasts may be difficult to accurately estimate and CD34 assessment by immunohistochemistry may be invaluable in such cases. Finally, although approximately half of the patients with MF display cytogenetic abnormalities,<sup>39</sup> only *del(13)(q12;q22)* and *der(6)t(1;6)(q21-23;p21-23)*<sup>40</sup> are considered specific enough, in the context of a CMD-associated myelofibrosis, to warrant inclusion in operational diagnostic algorithms (Figure 4).

### **Atypical myeloproliferative disorders (WHO: CNL,CEL/HES, UMPD, CMML, JMML, aCML)**

For the purposes of this review as well as in line with a recent communication on the subject matter,<sup>2</sup> we will use the term “atypical” MPDs to refer to the full spectrum of CMDs that are not classified as either classic MPDs or MDS. It should be noted that the WHO classification system incorporates these disorders into three different clinicopathologic categories: MPD (CNL, CEL, HES, UMPD), MDS/MPD (CMML, JMML, atypical CML), and SM.<sup>24</sup> Regardless, the role of molecular testing in atypical MPDs became most evident following the therapeutically-relevant association between activating mutations of the genes for both platelet-derived growth factor receptors  $\alpha$  (*PDGFRA*) and  $\beta$  (*PDGFRB*) and clonal eosinophilia.<sup>41,42</sup> For example, a *FIPILI-PDGFRB* fusion mutation has been

demonstrated in a subset of patients that fulfill conventional diagnostic criteria for HES,<sup>41</sup> SM,<sup>43</sup> or CEL,<sup>44</sup> whereas a *PDGFRB* mutation has been associated with the clinical phenotype of CEL and/or CMML.<sup>45-50</sup> Furthermore, the presence of either one of these two mutations predicts excellent treatment response to imatinib.<sup>51</sup> Therefore, any atypical MPD that is associated with eosinophilia should be screened for both mutations; *FIP1L1-PDGFR*A is karyotypically-occult and is instead detected by FISH or RT-PCR whereas the presence of *PDGFRB*-rearrangement is suggested by conventional cytogenetic studies that show translocations involving chromosome 5q33, e.g. t(5;12)(q33;p13).<sup>45</sup> Of course, CML may also present with marked eosinophilia, and exclusion of this possibility by appropriate genetic testing is also necessary.

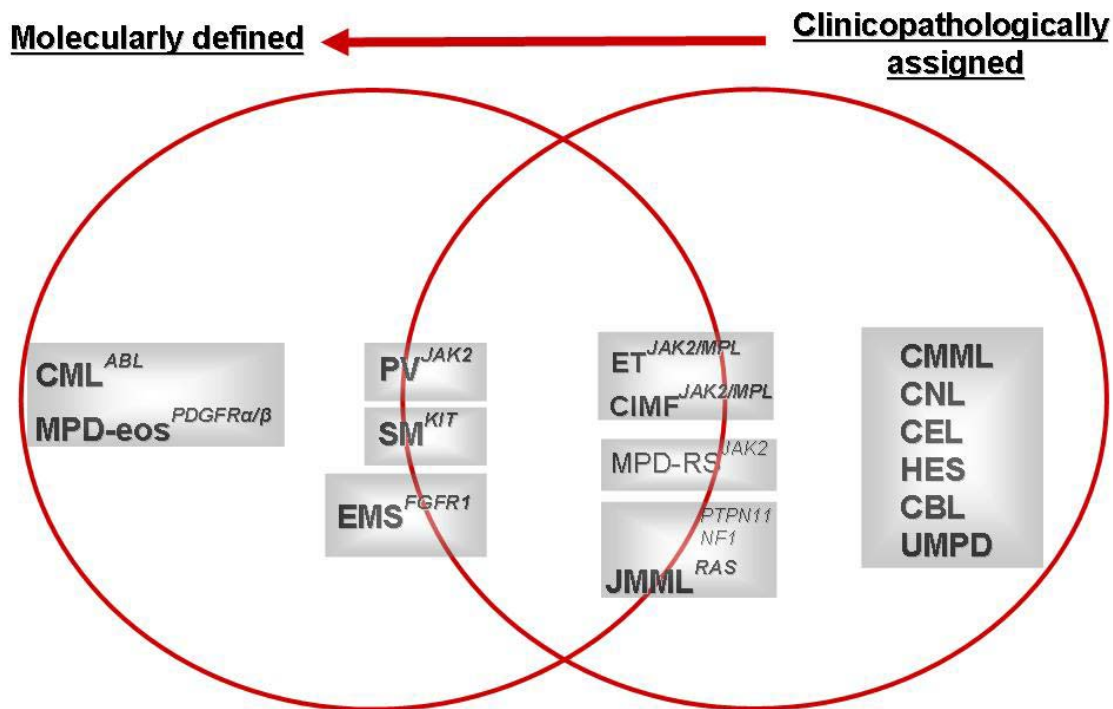
Other eosinophilia-associated atypical MPDs (e.g. HES, molecularly-undefined CEL, 8p11 myeloproliferative syndrome; Figure 5) are usually imatinib-resistant. *8p11* myeloproliferative syndrome harbors *FGFR1*-rearrangement and is characterized by chromosomal translocations involving chromosome 8p11, myeloproliferative and myelodysplastic features, lymphadenopathy and a high incidence of T cell non-Hodgkin's lymphoma with progression to AML.<sup>52,53</sup> Molecularly-undefined CEL is characterized by an “HES” phenotype that also displays either a clonal cytogenetic abnormality or excess blasts in the bone marrow or peripheral blood.<sup>54,55</sup> In contrast, a working diagnosis of HES is made only in the absence of molecular or cytogenetic markers of clonality, bone marrow mastocytosis, monocytosis, or evidence of trilineage myeloproliferation or dysplasia.<sup>56</sup>

Molecular markers have also been described in atypical MPDs that are not always associated with eosinophilia, including SM and JMML (Figure 5). In *FIP1L1-PDGFR*A<sup>(-)</sup> SM, nearly all adult cases might carry the *KIT*D816V or other *KIT* mutations in their mast cells.<sup>57</sup> However, the diagnostic value of mutation screening in this instance is undermined by the relatively low frequency of *KIT* mutation detection from either the peripheral blood or bone marrow that is not sorted for mast cells.<sup>57</sup> Therefore, at present, bone marrow morphological examination remains the primary tool for diagnosis in SM; typical features include clusters of morphologically abnormal, spindle-shaped mast cells that are best evaluated by the use of immunohistochemical stains that are specific to mast cells (tryptase, CD117).<sup>58,59</sup> In addition, we recommend mast cell immunophenotyping in suspected cases in search of aberrant CD25 expression by neoplastic mast cells.<sup>60</sup> In JMML, which is primarily a disease of early childhood, several mutually exclusive mutations affecting one of the Ras signal transduction pathway molecules (e.g. *RAS*, *PTPN11*, *NF1*) have been described but their role in disease diagnosis has not been systematically studied.<sup>61-63</sup> Finally, at present, molecular or cytogenetic studies do not provide additional insight to histological diagnosis for the remaining group of atypical MPDs including CMML, CNL, CBL, or UMPD. The latter category includes both the so called “MDS/MPD histological hybrids” and “atypical CML”<sup>64-67</sup>

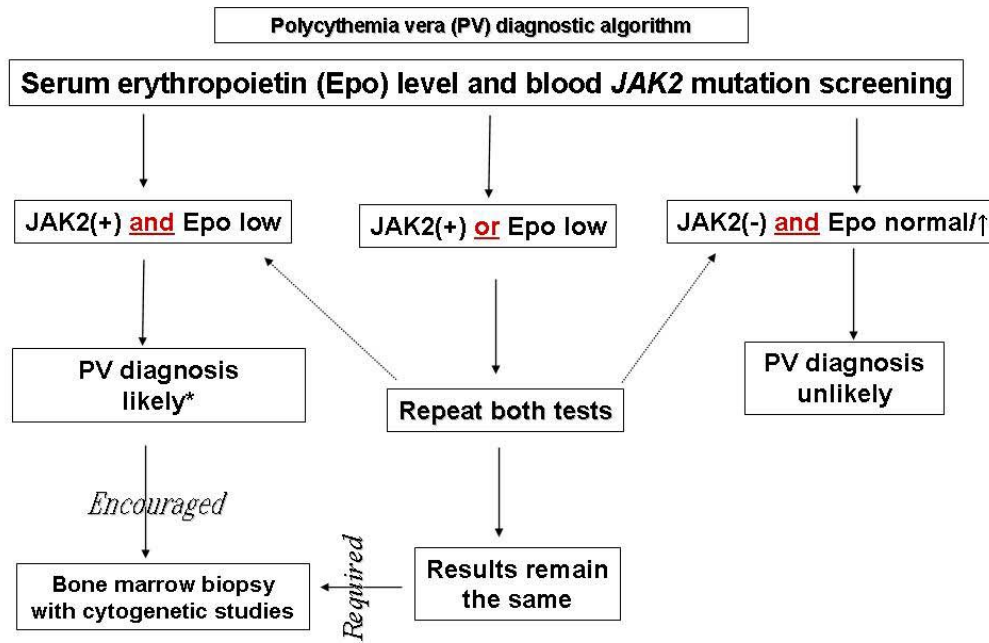
## Conclusion

At present, infallible “gold standards” in MPD diagnostics are rare and limited to instances where a clinicopathologic entity is pathogenetically linked to a specific molecular marker such as *BCR-ABL* in CML. Despite this fact, many investigators continue to use consensus-based diagnostic criteria (e.g. PVSG, WHO) as “gold standards” while evaluating the diagnostic accuracy of novel molecular markers. Such practice has the potential to undermine the diagnostic value of new molecular tests and one should instead utilize the information to re-evaluate the *status quo* in diagnostic methods from the standpoint of both histopathology and consensus-based criteria. For example, do “*JAK2*V617F-negative” patients with PV represent a condition that is biologically different from “PV” or does it imply the presence of another mutation that is pathogenetically equivalent to *JAK2*V617F? Answers to such questions require careful and full utilization of bone marrow histopathology as well as longitudinal clinical assessment.

# Myeloproliferative disorders

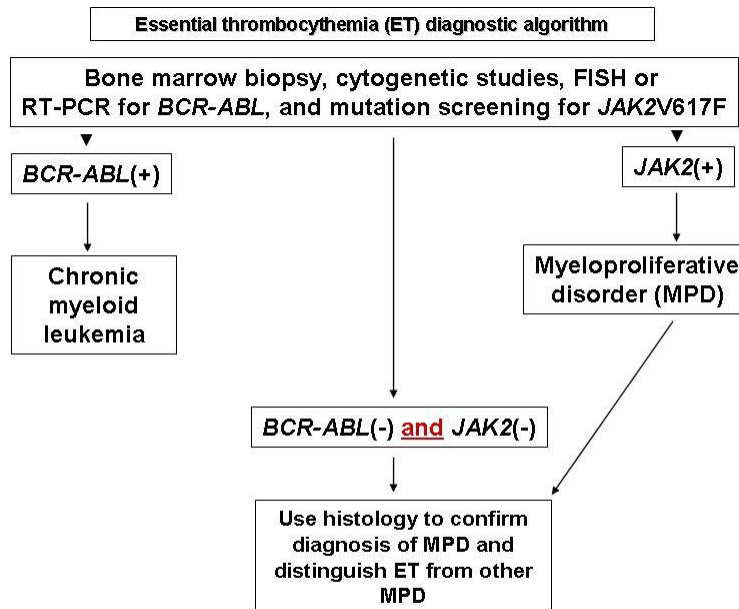


**Figure 1.** A semi-molecular classification of myeloproliferative disorders (MPD). CML, chronic myeloid leukemia; MPD-eos, eosinophilia-associated MPD; PV, polycythemia vera; SM, systemic mastocytosis; EMS, the 8p11 myeloproliferative syndrome; ET, essential thrombocythemia; CIMF, chronic idiopathic myelofibrosis; MPD-RS, myeloproliferative disorder such as ET or CIMF associated with ringed sideroblasts; JMML, juvenile myelomonocytic leukemia; CMML, chronic myelomonocytic leukemia; CNL, chronic neutrophilic leukemia; HES, hypereosinophilic syndrome; CEL, chronic eosinophilic leukemia; CBL, chronic basophilic leukemia; UMPD, unclassified MPD. UMPD includes both MDS/MPD hybrids and “atypical CML”. The far left group (CML and MPD-eos with *PDGFR*-rearrangement) are fully molecularly characterized and the pathogenetic relevance of the associated mutations have been confirmed by effective targeted therapy. In contrast, such therapeutic confirmation of pathogenetic relevance has not been demonstrated in PV, SM, or EMS, despite a near-invariable association with a specific mutation. In ET, CIMF, MPD-RS, and JMML, recurrent mutations occur in approximately half of the patients that suggests molecular heterogeneity and the pathogenetic relevance of the associated mutations remains to be determined. Finally, there is little information regarding molecular pathogenesis for the group of diseases in the far left section of the venn diagram (CMML, CNL, CEL, HES, CBL, UMPD).

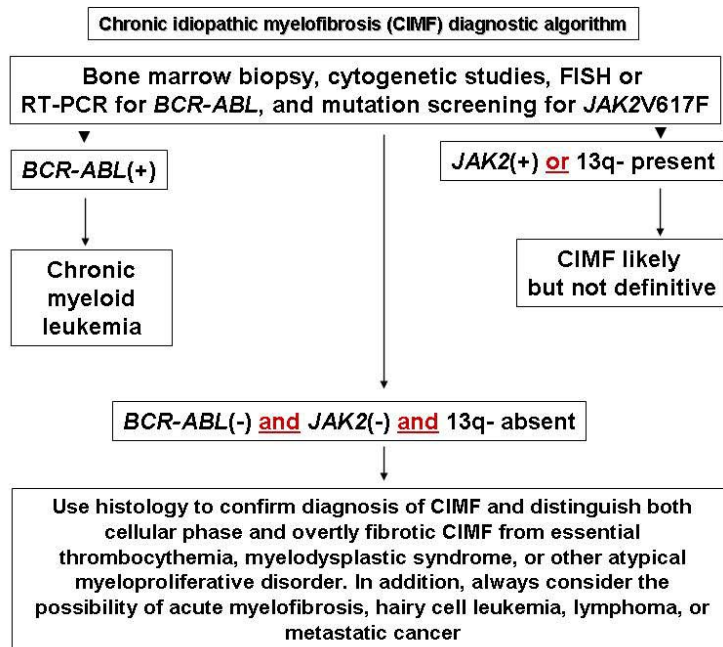


*\*JAK2 mutation analysis does not distinguish PV from other myeloproliferative disorder although homozygous mutation favors PV diagnosis*

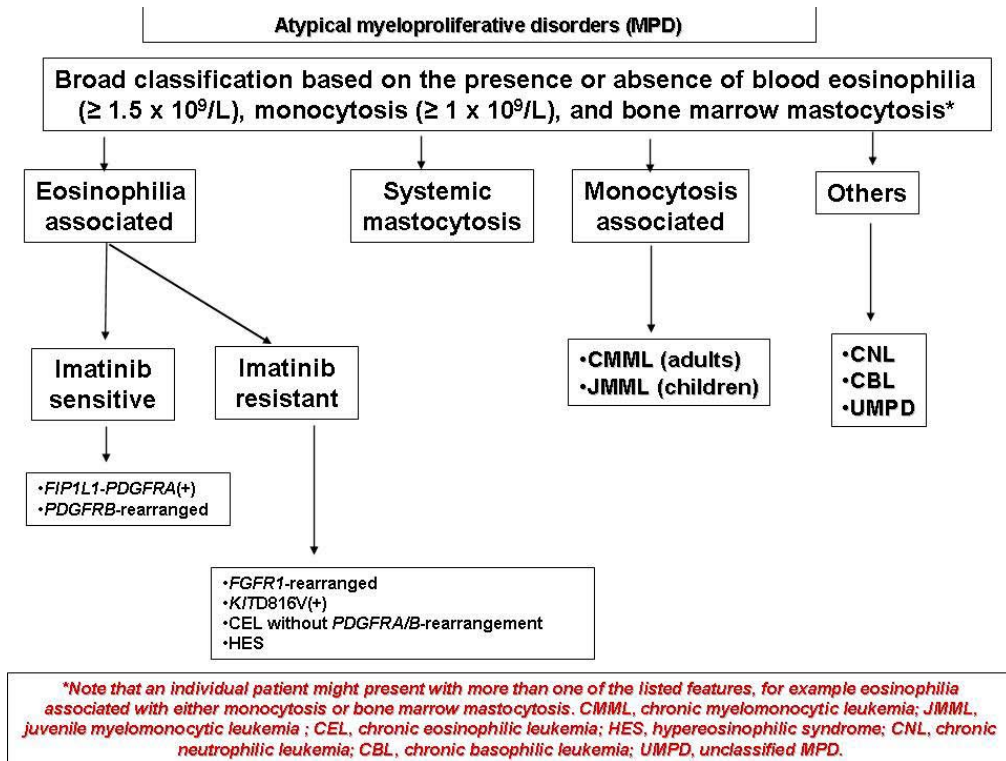
**Figure 2.** A contemporary diagnostic algorithm for polycythemia vera.



**Figure 3.** A diagnostic algorithm for primary thrombocythemia.



**Figure 4.** A diagnostic approach to chronic idiopathic myelofibrosis.



**Figure 5.** A general classification scheme for atypical myeloproliferative disorders.

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