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# The Role of Morphology, Cytochemistry and Immunohistochemistry in the Diagnosis of Chronic Myeloproliferative Diseases

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**Abstract**

Chronic myeloprolifeative diseases (CMPD) are clonal hematopoietic stem cell disorders characterized by excessive proliferation and production of one or more of the myeloid cells and are subclassified according to the predominant cells, such as chronic myelogenous leukemia (CNL), chronic eosinophilic leukemia (CEL), polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). This brief review focuses on the characteristic morphology of each clinical entity and the useful cytochemical (including leukocyte alkaline phosphatase, myeloperoxidase, butyrate esterase, chloroacetate esterase and cyanide-resistant peroxidase) and immunohistochemical (including von Willebrand factor/CD61, keratin, tryptase, CD117, CD68 (PGM-1), c-Mpl and bFGF) stains for differential diagnosis.

## 1. Introduction

Chronic myeloproliferative diseases (CMPD) are clonal hematopoietic stem cell disorders characterized by excessive proliferation and production of one or more of the myeloid cells are subclassified according to the predominant cells, such as chronic myelogenous leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic (CIMF). All of the CMPD have the potential to undergo clonal evolution and a stepwise progression into accelerated phase with myelofibrosis or in effective hematopoiesis and acute blast phase [1].

CML was the first disorder found to be associated with recurrent chromosomal translocation, which resulted in the formation of the BCR/ABL fusion gene. This fusion gene was also recognized as giving rise to an abnormal fusion protein with tyrosin kinase activity (important in signal transduction and regulation of cell growth); fundamental in the pathogenesis of the disease. Unfortunately, there is no specific genetic defect yet discovered for any of the remaining CMPD entities. For these, the diagnosis still rests upon the careful correlation of morphologic findings from peripheral blood and bone marrow specimens, with other laboratory findings and with clinical observations. The diagnostic criteria for each CMPD entity has been well summarized in various textbooks and also in a recently published monograph, entitled "Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues," in the series of WHO Classification of Tumours [1]. This brief review will focus on the characteristic morphology for confirming each clinical entity, the importance of excluding other disorders closely mimicking CMPD, and utilizing the appropriate cytochemical and immunohistochemical stains useful in making the difficult differential diagnosis (Table 1).

## 2. Characteristic Morphology

### *2.1. Chronic Myelogenous Leukemia (CML)*

In chronic phase, the peripheral blood smear shows left-shifted neutrophilia, basophilia and eosinophilia in some patients. The bone marrow is usually markedly hypercellular with expansion of the paratrabecular cuff of immature neutrophils. Blasts usually account for fewer than 5% of the narrow cells, and eosinophils may be substantially increased in some patients. In accelerated phase, along with cytogenetic evidence of clonal evoluation, myeloblasts may increase to 10-19%, basophilia increase to $>$20% and persistent thrombocytopenia or thrombocytosis with prominent proliferation of small dysplastic megakaryocytes in large clusters associated with reticulin fibrosis.

### *2.2. Polycythemia Vera (PV)*

In the polycythemic stage, besides erythrocytosis, neutrophilia and basophilia are commonly seen in the peripheral blood smear. Thrombocytosis is found in more than 50% of patients. In the bone marrow, erythroid, megakaryocytic and granulocytic proliferation (panmyelosis) account for the increased cellularity for the patient's age. Megakaryocytes are conspicuous and may be seen in clusters. In accelerated or "spent" phase, along with decreasing red cell

**Table 1.**

Usual features of CMPD at diagnosis and major difeerential dianosis.

| | CML | CNL | CEL | PV | ET | CIMF |
|---|---|---|---|---|---|---|
| CBC | ↑WBC | WBC ≥25x10$^9$/L<br>Neutrophils > 80% | Eosinophils > 1.5x10$^9$/L | Hgb > 18.5 g/dL (M), >16.5 g/dL (F)<br>WBC > 12x10$^9$/L<br>PLT > 400x10$^9$/L | PLT ≥600x10$^9$/L | Anemia<br>WBC and PLT<br>low, normal or elevated |
| Other Lab | Ph chromosome [t9;22)(q34;q11)]<br>BCR/ABL | No Ph chromosome or BCR/ABL | No Ph chromosome or BCR/ABL | No Ph chromosome or BCR/ABL<br>Normal arterial $pO_2$<br>Low erythropoietin | No Ph chromosome or BCR/ABL | No Ph chromosome or BCR/ABL |
| Morphology | Immature neutrophils<br>Basophilia<br>Eosinophilia<br>BM with marked granulocytic hyperplasia | Immature granulocytes (< 10%)<br>Myeloblasts < 1%<br>BM with marked neutrophilic hyperplasia, myeloblasts < 5% | BM with marked eosinophilia; myeloblasts < 20% | Hypercellular marrow with panmyelosis<br>Absent stainable iron | Normal to slightly hypercellular marrow with proliferation of large megakaryocytes in loose clusters | Leukoerythroblastosis<br>Dacrocytes<br>Bone marrow with prominent megakaryocytic proliferation in clusters<br>Myelofibrosis<br>Osteosclerosis<br>Sinusoidal hematopoiesis |
| Exclusion | Leukemoid reaction<br>CMML<br>aCML<br>Unclassified CMPD | Reactive neutrophilia<br>Other CMPD: PV, ET, CIMF<br>CMML<br>aCML | Reactive eosinophila allergy, parasitic<br>Neoplasms: T-cell lymphoma, Hodgkin lymphoma, ALL, mastocytosis<br>T-cell clone with abnormal cytokine production<br>Other CMD or AML | 2° erythrocytosis<br>hypoxia<br>↑$O_2$-affinity Hgb<br>inappropriate EPO production by tumor<br>Familal erythrocytosis | Reactive thrombocytosis<br>inflammation<br>neoplasm<br>splenectomy<br>Other CMPD<br>MDS, esp. del(5q) | Metastatic Ca<br>Accelerated phase of other CMPD<br>Atypical MDS with myelofibrosis<br>Mastocytosis |
| Useful Cytochemistry | LAP (low)<br>BE/CLE Myeloperoxidase CD41 | LAP (high)<br>BE/CLE Myeloperoxidase | Cyanide-resistant peroxidase CLE | LAP (high) | | |
| Useful Immuno-histochemistry | CD68<br>c-Mpl<br>bFGF | | | c-Mpl<br>bFGF | c-Mpl<br>bFGF | b-FGF<br>Keratin & vWF<br>Tryptase & CD117 |

mass, leukoerythrocytosis and dacrocytosis appear in the peripheral blood smear due to the development of post-polycythemic myelofibrosis and myeloid metaphases (PPMM) with features similar to CIMF.

### *2.3. Essential Thrombocythemia (ET)*

The most striking abnormality of the peripheral blood smear is marked thrombocytosis with anisocytosis. The bone marrow is usually normocellular or slightly hypercellular (for the patient's age) with marked proliferation of large megakaryocytic nuclei and micromegakaryocytes are also common findings. The bone marrow biopsy usually shows fibrosis along with atypical megakaryocytic proliferation in clusters. The bone marrow cellularity and the degree of reticulin fibrosis vary considerably, depending on the stage of disease encountered. In the prefibrotic stage (cellular phase), the reticulin fibrosis is minimal, and the marrow is hypercellular for the patient's age with an increase in the number of neutrophils and of atypical megakaryocytes in clusters. In the fibrotic stage, the marrow is usually normocellular or hypocellular. Increased numbers and dilatation of marrow sinuses with intrasinusoidal hematopoiesis are characteristic. Atypical megakaryocytes are often the most notable marrow elements and occur in sizable clusters or within dilated sinuses. Sometimes the marrow may be almost devoid of hematopoietic precursors situated within the marrow sinuses. Osteosclerosis may be observed in some cases. Increased numbers of blasts to 10-19% in the blood or marrow indicates an accelerated phase of the disease. The most common sites of extramedullary hematopoieses (EMH) are the sleen and liver. The splenic EMH have three distinct histologic patterns of infiltration by myeloid precursors in red pulp, namely diffuse, nodular, and a predominance of immature granulocytes. The pattern of immature granulocyte predominance was found to be associated with decreased survival [2].

### *2.4. Chronic Myeloproliferative Diseases, Unclassifiable (CMPD, U)*

The designation, CMPD, U, should be applied only to cases that have definite features of a myeloproliferative disease but that fail to meet the criteria for any of the specific CMPD entities or present with features that overlap two or more of the categories. These may include the cases encountered at very early stage of disease or in the late stage of disease without previous history.

## 3. Differential Diagnosis: Exclusion of Disorders That Closely Mimic CMPD

The major manifestations of CMPD are excessive proliferation of myeloid cells resulting in peripheral cytosis (*i.e.* erythrocytosis in PV, thrombocytosis in ET, granulocytosis in CML, neutrophilia in CNL, eosinophilia in CEL) and abnormal proliferation of megakaryocytes resulting in myelofibrosis and myeloid metaplasia. In the differential diagnosis, besides the recognition of morphologic characteristics of each disease entity, it is important to be familiar with other clonal and nonclonal disorders that may be associated with cytosis and/or myelofibrosis and may closely mimic CMPD. It is important to exclude these disorders in order to achieve accurate diagnosis. The major disorders, which may closely mimic each entity of CMPD, are summarized in the table, under the heading of exclusion. Because of wide variation in the clinical and morphologic characteristics in various stages of disease in each entity, especially in the very early stage or late stage, additional cytochemical or immunohistochemical characteristics may be helpful in differential diagnosis [3].

**CML:** The most common cause of neutrophilia, which may mimic CML, is a leukemid reaction secondary to infection or other neoplasm. Characteristic high leukocyte alkaline phosphatase (LAP) score and absence of basophilia are helpful in distinguishing from CML with characteristic

low LAP score and basophilia. However, it is important to remember the high LAP score and absence of basophilia are also characteristic for CNL. Fortunately, the CNL is very rare disorder and requires extensive clinical workup to exclude any underlying infection, neoplasm, or other myeloproliferative disorders for the diagnosis. In consideration of CEL, combined cytochemical stains for cyanide-resistant peroxidase and chloroacetate esterase are useful in differentiating abnormal neutrophils with unusually large granules from eosinophils and identifying abnormal eosinophils with aberrant expression of chloroacetate esterase. The presence of significant monocytosis in chronic myelomonocytic leukemia (CMML) is the major characteristic distinguishing from CML. However, it can be difficult to distinguish abnormal monocytes and immature granulocytes, especially in the bone marrow. Cytochemical studies that aid in the identification of monocytes such as -naphthyl butyrate esterase (BE), used along or in combination with naphthol AS-D chloroacetate esterase (CLE) are very helpful in confirming the diagnosis of CMML rather than CML. Dysplasia of neutrophils (*i.e.* hypogranularity, hyposegmentation of nuclei, dual esterase staining, partial myeloperoxidase or partial chloroacetate esterase deficiency) are commonly seen in atypical chronic myeloid leukemia and are absent in CML. Currently, the demonstration of Philadelphia chromosome t(9;22)(q34;14) and/or BCR/ABL fusion gene is the gold standard for the diagnosis of CML and distinguished from other CMPD and reactive cytosis.

**CIMF:** Myelofibrosis as major characteristic seen in CIMF is generally considered to be secondary to abnormal megakaryocytic proliferation [4]. There is wide variation in the morphology of abnormal megakaryocytes in various stages of disease and sometimes can be difficult to distinguish from other diseases associated with fibrosis, including metastatic carcinoma and mastocytosis. Immunocytochemical staining of CD41 on smear or immunohistochemical staining of von Willebrand factor or CD61 are very useful for identifying abnormal megakaryocytes in confirmation of CMPD [3,5]. On the other hand, immunohistochemical staining of keratin is helpful in identifying abnormal epithelial cells in metastatic carcinoma rather than intrinsic marrow disorder such as CIMF. In addition, the immunohistochemical staining of tryptase and/or CD117 are useful in identifying abnormal mast cells in mastocytosis as a possible differential diagnosis [6].

## 4. Immunohistochemical Expression of Cytokines in Marrow Cells and Their Practical Application in the Diagnosis of Chronic Myeloid Disorders

Various cytokines, including transforming growth factor β (TGF-β), platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), are known potent regulators of hematopoiesis, fibrogenesis and angiogenesis. Immunohistochemical study of normal bone marrow has clarified expression pattern of these cytokines and their receptors in myeloid cells and stromal cells including histiocytes, fibroblasts and endothelial cells [7]. Immature erythroid precursors strongly express PDGF-B, PDGFRβ, TGFβR1, TGF βR2, FGFR1, FGFR3, FGFR4 and VEGF. Immature myeloid precursors strongly express PDGFRβ, $TGF\beta_1$, $TGF\beta_2$, $TGF\beta_3$, TGFβR2, FGFR3 and FGFR4. Megakaryocytes stained primarily for PDGF-B, PDGFRβ, $TGF\beta_2$, $TGF\beta_3$, TGFβR2 and weakly expressed bFGF, TGFR1 and VEGF. HistIocytes displayed intense $TGF\beta_1$, bFGF and FGFR2 expression. Plasma cells also displayed intense PDGF-B, PDGFRβ, $TGF\beta_2$, TGFβR1, TGFβR2, bFGF, FGFR1, FGFR2, FGFR4 and VEGF expression. Fibroblasts and endothelial cells carried receptors for all of the aforementioned cytokines. The last two cell types also expressed the ligand cytokines to varied degrees. These findings are consistent with the known autocrine and/or paracrine regulatory function of these cytokines in physiologic and clonal hematopoiesis. PDGF is known to stimulate erythropoiesis and megakaryopoiesis and abrogates the inhibitory effect of TGFβ suppresses hematopoiesis in general. On the other hand, bFGF stimulates hematopoiesis and abrogates the inhibitory effect of TGFβ. The cytokines receptor profile in marrow endothelium and fibroblasts supports the functional role of the corresponding ligands in angiogenesis and fibrogenesis. The fibroblasts in myelofibrosis with myeloid metaplasia (MMM) are known to be polyclonal and are secondary stimulated by megakaryocyte-derived cytokines to proliferate and produce collagen [4].

Similar immunohistochemical studies of chronic myeloid disorders have also demonstrated decreased c-Mp1 (thrombopoietin receptor) expression of megakaryocytes in all chronic myeloid disorders and decreased bFGF expression of histocytes in CMPD but not in myelodysplastic syndromes (MDS) [8-11]. These immunohistochemical staining patterns may complement the morphologic distinction between CMPD and MDS and between reactive and clonal myeloproliferation (*i.e.*, PV, ET, etc.). It may also suggest that the CMPD are bFGF independent myeloproliferations.

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