Bone Marrow Histopathology in the Diagnosis of the Early Stage of Chronic Myeloproliferative Disorders

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In the chronic myeloproliferative disorders (MPDs), examination of bone marrow (BM) biopsy specimens is essential to make a correct diagnosis and classification, as well as to monitor progression of the disease over time. In addition, histopathology permits a ready assessment of therapeutic efficacy, assists in risk stratification of patients, and is predictive of prognosis. For this reason, a multidisciplinary approach is required by considering equally clinical and morphological data [1]. This up-to-date concept of clinicopathological evaluation has been strengthened by the WHO classification [2] that includes the major subtypes polycythemia vera (PV), essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (CIMF).

I. Definition and standardization of histopathological features

In accordance with the issues of the WHO classification, the different subtypes of MPDs are characterized by specific histological patterns that are composed of distinctive features and are usually present at diagnosis (Table 1). Although in quite a number of former studies these alterations have been elaborated, a conflict of opinion still exists, whether an untrained pathologist would be able to recognize these features on H&E stained BM sections. Contrasting the determination of age-dependent cellularity and semiquantitative grading of myelofibrosis [3, 4], especially features of megakaryopoiesis may cause

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Th - reactive thrombocythosis, X – major finding, x – additional criterion
significant difficulties concerning definition and consequently recognition among different observers. In this context a systematic evaluation including in particular the arrangement of the megakaryocytes within the marrow space, i.e. histotopography and certain nuclear abnormalities besides maturation defects are crucial. In the normal BM megakaryocytes show a central distribution of single isolated cells [5]. In MPDs, the increase of megakaryocytes is often associated with the formation of small clusters (at least three cells) to extensive groups (more than five cells). These megakaryocyte clusters (Figure 1) may display either a loose or dense arrangement of cells [3]. Moreover, an abnormal dislocation of megakaryocytes towards the endosteal (paratrabecular) border is a highly conspicuous finding that is usually not found in reactive thrombocytosis. Other features indicating a neoplastic process are peculiar nuclear aberrations and maturation defects that imply disturbances of the normal development of megakaryopoiesis [3, 5, 6]. These include an atypical nuclear lobulation (extent and shape of nuclear foldings, i.e. hypolobulation, - often described as cloud-like leading to bulbous (plump, clumsy) nuclei versus hyperlobulation with marked segmentation mimicking a stag-horn-like formation) and anomalies of the chromatin pattern (mostly hyperchromasia). Furthermore, maturation defects include a conspicuous deviation of the nuclear-cytoplasmic ratio or maturation with appearance of bizarre megakaryocytes [3]. It is noteworthy that all these changes may be detectable in megakaryocytes of different sizes (small, median, large, giant) or ploidy status. Finally, so-called naked (denuded, bare) nuclei with condensed chromatin pattern may frequently be shown implicating an enforced cell turnover due to increased thrombocytogenesis. Of the other parameters increase and left-shifting of neutrophil granulopoiesis or erythropoiesis may be a prominent feature as well as reduction in the amount of nucleated red cell precursors depending on disease entity (Table 1).

II. Polycythemia vera (PV)

Unfortunately, the contribution of histopathology of the BM to the diagnosis of PV and to monitoring its progression has not been adequately appreciated. In the original and updated criteria of the Polycythemia Vera Study Group (PVSG), BM findings are not even mentioned [7], and in the WHO classification, they are considered only as a minor criterion in substantiating the diagnosis [8]. Indeed, the major reason why BM morphology has been neglected as a useful tool in the diagnosis of PV is that the disease has been traditionally defined by clinical, laboratory and biologic parameters, which often do suffice to establish the diagnosis [9]. However, when correlated with the clinical findings, a clear pattern of histopathologic features emerge that can be used to confirm the diagnosis in cases in which the clinical data are not so clear-cut. For example, PV is a dynamic, evolving disease process, and early on a number of patients do not fulfill all of the established clinical and laboratory diagnostic criteria, particularly in regard to the red cell mass or hemoglobin/hematocrit values [7]. These patients have often been said to have “latent PV” or “benign erythrocytosis”, and are regarded as a heterogeneous group that may eventually evolve into full-blown. Importantly, some patients have an initial/early prodromal phase of PV that is accentuated by thromboembolic episodes as first manifestation of disease, but in whom a diagnosis is not possible by conventional criteria (Table 1). In these instances, demonstration of the characteristic histologic features of PV could lead to early diagnosis and appropriate therapy. The BM biopsy performed at diagnosis also
is important to establish a baseline against which subsequent specimens can be compared, because histopathology provides the best means to detect various phases of PV, particularly the terminal stages, so-called spent phase, postpolycythemic myeloid metaplasia (PPMM) and blast phase.

The histopathology of initial-early and full-blown polycythemic PV is characterized by a hypercellular BM with trilineage proliferation (panmyelosis) of variable numbers of erythroid and granulocytic precursors, and with megakaryocytic proliferation that has distinctive morphologic features [10]. Some of these findings have more significance than others in establishing the diagnosis of PV and distinguishing it from reactive, or secondary polycythemia (SP) as well as from the other MPDs. For example, although hypercellularity in relation to age-matched hematopoiesis is a common feature of PV (Figure 2a), it may occasionally also be encountered in cases of SP that usually present with a mildly to moderately increased hematopoiesis (Figure 2b). For easy recognition and quantification of neutrophil granulopoiesis versus erythropoiesis, a special stain like naphthol-A-SD-chloroacetate esterase (Figure 2a,b) or myeloperoxidase may be superior to the routine hematoxylin-eosin stain (H&E). Following this staining procedure, it is apparent that in PV the normally small and rounded islets of nucleated erythroid precursors show a conspicuous enlargement and a tendency to merge into sheets (Figure 2a). Although these changes are significantly more pronounced in PV, they may be also expressed in a few cases with

Fig. 2 BM histopathology in PV versus SP. a PV with tri-lineage proliferation and hypercellularity including prominent sheets of erythroid precursors and megakaryocytes of different size. b SP with slight increase in cellularity in a senescent patient with large islets of erythropoiesis. c Pleomorphous aspect of megakaryocytes in PV with sizes ranging from small to large - see also a. d Uniform small to medium-sized megakaryocytes in SP. e SP with coarse iron deposits in macrophages (arrow). f Prominent perivascular deployment of plasma cells and adjacent eosinophils in SP (arrow). g In SP (arrow), deposits of cell debris are regular findings (arrows)
severe SP and therefore, this is not an entirely reliable diagnostic parameter [11]. A similar situation may be observed regarding the neutrophil cell lineage, because an increase in pro- and metamyelocytes (left-shifting) is frequently displayed in PV and in SP. On the other hand, the features of the megakaryocytic proliferation in PV are characteristic and have been acknowledged to enable a distinction between PV, the other subtypes of MPDs, and SP [10]. Previous studies reported by the PVSG showed that 95% of biopsies from patients with PV have an increase in the number of megakaryocytes, but others have observed that aside from the increase in numbers, the cytological appearance of this cell lineage exerts a discriminating impact. It has been repeatedly emphasized that megakaryopoiesis in early as well as in full-blown PV displays a pleomorphic aspect, i.e., small, medium sized, large and giant megakaryocytes are either dispersed or loosely clustered [11]. In particular, the mature megakaryocytes that have hyperlobulated nuclei but that fail to show other gross nuclear abnormalities, such as deviation from nuclear-cytoplasmic maturation may serve as diagnostic hallmark (Figures 1, 2c) because they are in contrast to the small to medium-sized megakaryocytes found in SP (Figure 2d). The finding of the specific histological pattern of a left-shifted (immature) erythroid and granulocytic proliferation (Figure 2a) associated with a megakaryopoiesis displaying a striking variety of cell sizes is in contrast to the uniformly large to giant size of the megakaryocytes in ET, which are usually not accompanied by significant proliferation in the other cell lineages (Figure 2e,d). Discriminate analysis of standardized BM features [3] in erythrocytosis reveals that in addition to the peculiar appearance of the megakaryocytes, certain constituents of the stroma compartment also enable a clear-cut distinction between PV and SP. Iron-laden macrophages are rarely observable in PV, and are found in about 6% of patients, which is opposed to the frequent occurrence of this phenomenon in SP (Figure 2e). An increase in reticulin fibers is never encountered in SP. Usually, SP shows an inflammatory reaction with prominent perivascular plasma cells (Figure 2f), many scattered eosinophils and small accumulations of cell debris ingested by macrophages (Figure 2g). The latter features are most prominent in so-called smokers polycythemia associated with recurrent bronchopulmonary infections [11-13]. In summary, Table 1 describes the results of an independently performed study that includes discriminant analysis of standardized BM features in a large cohort of patients with sustained erythrocytosis.

III. Essential thrombocythemia (ET)

According to the widely recognized standards, the diagnosis of ET was usually established by the PVSG criteria, i.e. by demonstration of thrombocytosis and the exclusion of other diseases, particularly full-blown PV [14]. However, a critical evaluation of these diagnostic guidelines, that up to now have been applied in all relevant clinical trials, reveals that they do not permit a clear-cut distinction of ET from the prefibrotic and early stages of CIMF that are often associated with thrombocythemia [15]. In contrast, by using a different diagnostic approach, the WHO classification does enable a separation between CIMF and (true) ET [16, 17], a substantial change concerning the spectrum of this entity has to be realized. These significant differences are predominantly due to the inclusion of histopathology in the WHO classification, because evaluation of BM specimens derived from patients with the diagnosis of ET based on the PVSG criteria reveal a striking heterogeneity [15, 18]. A wealth of data has accumulated concerning the role of BM pathology in the differential diagnosis of thrombocythemia in MPDs with the aim to define more clearly histological patterns that characterize (true) ET. In contrast to prefibrotic CIMF with accompanying thrombocythemia - false ET (Figure 3a), in true ET, neither a relevant increase in cellularity nor a significant left-shifted neutrophil granulopoiesis is observable (Figure 3c). The most conspicuous differences are related to megakaryopoiesis (Figure 1), because in true ET gross disturbances of histotopography (dense clustering) are not remarkable, but a more or less random distribution of megakaryocytes within the BM is prevalent (Figure 3d). In (true) ET, there is a predominance of large to giant mature megakaryocytes with extensively lobulated (staghorn-like) nuclei [15, 18, 19] surrounded by a correspondingly mature cytoplasm (Figure 4b). These features are clearly distinguishable from the abnormally hypolobulated (cloud-like) and hyperchromatic nuclei of megakaryocytes in false ET (prefibrotic- early CIMF) with their striking maturation defects (Figure 1) that result in a marked anomaly of nuclear-cytoplasmic maturation, i.e., megakaryocyte dysplasia (Figure 4a). Finally, at presentation, there is no substantial increase in reticulin observable in true ET, a finding that contrasts with the allowance of some degree of fibrosis according to the criteria of the PVSG [14]. Using standardized features of BM assessment that include histopathology [3], a definitive differentiation between ET and those entities mimicking this disorder may be easily achieved (Table 1). This distinction of ET according to the PVSG [14] versus the WHO classification [16] implies significant consequences, regarding
complications like fibrosis, therapeutic strategies and outcome [20], because only a fraction of those diagnosed by the PVSG criteria, ranging between 20% to 30%, may be regarded as true ET.

IV. Chronic idiopathic myelofibrosis (CIMF)

CIMF initially presents with a hypercellular BM characterized by megakaryocytic myeloproliferation (Figure 3a) but no increase in reticulocyte or mononucleated neutrophil (often left-shifted) and megakaryocytic lineages, with a concomitant arrest of the nucleated erythroid precursors [21] was previously termed chronic granulocytic myelosis (CMGM) [22-25]. Most conspicuous, however, is the megakaryopoiesis (Figure 3b) characterized not only by a disturbance of BM histotopography (loose to dense clustering and translocation to the endosteal borders), but also by striking abnormalities of maturation (Figure 3b). Significant anomalies of megakaryocytes do not only consist of variations in size that range from small to giant forms (Figures 3a, b), but also of an aberration of the nuclear cytoplasmic ratio created by bulbous and hyperchromatic cloud-like nuclei (Figure 4a). Furthermore, apart from their disorganized nuclear lobulation there are many naked (bare) megakaryocytic nuclei detectable [2, 17]. Overall, the megakaryocytes in CIMF regularly are marked by a more atypical (dysplastic) appearance than in ET.

Fig. 3 BM histopathology in CIMF (false ET) versus (true) ET. a Prefibrotic CIMF with hypercellularity composed of prominent granulocytic and megakaryocytic proliferation revealing dense clusters of small to giant megakaryocytes with hypolobulated (bulbous) nuclei. b In routinely stained specimens clustering and abnormal endosteal translocation of megakaryocytes and the prominent granulopoiesis are easily recognizable. c ET with age-matched cellularity and a predominant proliferation of large to giant mature megakaryocytes showing extensively lobulated nuclei and a lack of significant proliferation or left-shifting of the other cell lineages. d Dispersed or loosely clustered large to giant mature megakaryocytes are the diagnostic hallmark of ET.
Fig. 4 BM histopathology in CIMF versus ET. a Abnormalities of megakaryocytes in CIMF are consistent with hyperchromatic and hypolobulated (cloud-like) nuclei. b Large to giant megakaryocytes in ET with deeply lobulated (staghorn-like) nuclei and no conspicuous maturation defects.

other subtype of the MPDs significantly contrasting those seen in ET (Figures 4a,b). This is one of the characteristic features discriminating false ET (i.e. prefibrotic-early stage CIMF) from true ET. Although progression of CIMF is unpredictable, increasing megakaryocytic maturation defects are associated with a more rapid transition from the prefibrotic into overt fibrosclerotic stages (dysplasia), although this transition is not dependent on the platelet count. As has been elucidated in different studies, there is a more than a 65% probability of progression from a prefibrotic-early stage to a full-blown CIMF or MMM conforming with the classical diagnostic criteria. This peculiar feature of disease process is accompanied by relevant changes in clinical findings. Patients that initially present with early stage CIMF are prone to develop increasing anemia, splenomegaly and a leuko-erythroblastic blood picture in the course of disease evolution merging finally into MMM.

In contrast to the prefibrotic-initial stages of CIMF (CIMF-0), the more advanced and fibro-osteosclerotic lesions of disease (CIMF-3) conforming with MMM are characterized by coarse bundles of collagen fibers in the BM. There is usually an optional plaque-to-budd-like osteosclerosis (endophytic bone formation) detectable and areas of patchy hematopoiesis, revealing progressive hypoplasia. Comparable with the initial stages, atypical megakaryocytes are again a most prominent feature including naked (denuded, condensed) nuclei and dilated marrow sinuses containing groupings of intraluminal hematopoiesis, especially megakaryocytes. It is noteworthy that even without prior cytoreductive therapy mild to moderately expressed myelodysplastic changes may occur in the natural course of the disease process, occasionally indicating an insidious transition into an acceleration and blastic crisis (terminal stage). Finally, although in the past decade many groups were engaged in the study of risk stratification and prognosis of CIMF patients, a comparison of these data reveals an extreme heterogeneity [26, 27]. This result may be significantly influenced by including patients with PPMM into the corresponding
calculations and the failure to recognize the prefibrotic and early stages of this disorder. On the other hand, it is reasonable to assume that survival may be related to stages in which CIMF is diagnosed. In univariate analysis, the prodromal phases show a more favorable survival in comparison with more advanced stages (MMM). However, in multivariate calculations, evolution of BM fibrosis displayed no significant influence on survival [26, 27].

V. Summary

Chronic idiopathic myelofibrosis (CIMF), essential thrombocythemia (ET), and polycythemia vera (PV) are characterized by specific patterns of histopathology. When considered in the context of clinical findings, the histologic features not only discriminate these entities from each other (Figure 5), but also provide valuable information that can identify various risk groups, predict prognosis, and assess therapeutic efficacy. Histopathology is especially crucial to distinguish between myeloproliferative diseases in which the clinical and laboratory features overlap, but the prognosis and therapeutic implications are significantly different. For example, prefibrotic CIMF is frequently associated with thrombocythemia and thus, easily misdiagnosed as ET, from which it differs markedly in terms of development of fibrosis, blast transformation and survival. Yet the histopathology of these two diseases is considerably different at onset and usually permits an accurate diagnosis. It is estimated that between 70% to 80% of patients entered into clinical studies on ET, actually present early-stage CIMF with marked thrombocythemia (false ET). Similarly, the prodromal phase of PV, which may also mimic ET, cannot be recognized by conventional criteria, but has a characteristic histopathology pattern. Of course, the dynamics of the disease process must always be taken into account when following individual patients, particularly in PV and CIMF, and follow-up studies with repeated BM biopsy specimens readily allow monitoring of disease progression.
**Fig. 5** Schematic description (flow-sheet) of prominent histological features exerting diagnostic relevance to differentiate chronic myeloproliferative disorders associated with an evaluated platelet count from reactive lesions in bone marrow biopsy specimens.

**Cellularity**

- **Age-related**
  - Increase
  - **PV/CIMF-0/-1**
  - Normal
  - **ET/RTh**

**Reticulin**

- Normal

**Megakaryocytes**

- Maturation defects
- (dysplasia)
- Bulbous and naked nuclei

- CIMF-0/-1

**Granulopoiesis**

- Increase
- Normal

**Erythropoiesis**

- Increase and normal to reduced

**Megakaryocytes**

- Large to giant size hyperlobulated nuclei
- Small to medium size

**VI. References**