Value of bone marrow biopsy in the diagnosis of essential thrombocythemia

[haematologica]
2004;89:911-919

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A B S T R A C T

Background and Objectives. Essential thrombocythemia (ET) is a Philadelphia chromosome-negative chronic myeloproliferative disorder (CMPD) whose diagnosis, according to the Polycythemia Vera Study Group (PVSG) criteria, does not include histopathological data. The new WHO classification of CMPD has supplied new diagnostic guidelines which highlight the value of histopathology and facilitate a more precise differentiation of ET from reactive conditions and other CMPD.

Design and Methods. Bone marrow biopsies from 142 adult patients diagnosed with ET according to PVSG criteria were evaluated using the new WHO classification. Megakaryocyte morphology and arrangement, amount of fibrosis and a *clustering index* were studied along with determination of microvessel density (MVD), amount of $CD34^+$ cells and percentage of $MIB\text{-}1^+$ cells and megakaryocytes. The last value, indicated as megakaryocyte proliferation index (MPI), was determined and expressed as a percentage of the counted cells.

Results. According to WHO criteria the 142 biopsies were classified as follows: ET (21%); idiopathic myelofibrosis (IMF) grade 0 (30%), IMF-1 (34%), IMF-2 (10%) and ET/IMF-0 (5%). A significant difference ($p<0.001$) was observed between clustering index values in ET and IMF cases. A peculiar proliferative feature of megakaryocytes, defined *coupling*, was detected in all ET cases. MVD was more pronounced and the number of $CD34^+$ cells higher in cases of IMF than in cases of ET ($p<0.005$; $p = 0.001$, respectively) and MVD significantly correlated with the extent of fibrosis (r=0.861). ET cases showed the lowest values of proliferation; IMF-0 and IMF-1 showed higher values while a decrease of MPI was observed in IMF-2 in accordance with the increase of fibrosis.

Interpretation and Conclusions. In the diagnosis of thrombocythemic disorders, a multidisciplinary approach must include the evaluation of bone marrow biopsies. Some histopathological criteria, along with the use of markers related to activity and proliferation, such as CD34 and MIB-1, underline the biological differences between ET and prefibrotic states of IMF.

Key words: chronic myeloproliferative disease, essential thrombocythemia, bone marrow biopsy.

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Essential thrombocythemia (ET), is a chronic myeloproliferative disorder (CMPD) characterized by a high platelet count. It originates from a pluripotent stem cell. ET generally shows a slight female preponderance (1.5-2.1). The median age at diagnosis is 60 years although there is very wide range of ages in which the disease can appear (18-90 years). Approximately half of the patients are asymptomatic while the other half have vasomotor, thrombotic or hemorrhagic disturbances.[1]

Typical ET is a Philadelphia bcr-abl negative ($Ph1^-$) CMPD with a good prognosis and overall survival,[2,3] although there is controversy over the proper classification of thrombocytosis associated with the pathological *BCR-ABL* gene rearrangement;[4] Ph bcr-abl positive forms with ambiguous clinical significance and prognosis have been described and discussed.[5,6] Besides the typical, indolent form of ET, a heterogeneous spectrum of thrombocythemic disorders exists whose clinical significance is still controversial. This category incorporates forms belonging to the group of unclassifiable CMPDs or, like the recently described ET with ringed sideroblasts,[7] with features of both myeloproliferative and myelodysplastic diseases.

For a long time, the Polycythemia Vera Study Group (PVSG) criteria for the diagnosis of ET have not included histopathological data.[8,9] Bone marrow (BM) histology was used only to exclude previous or other subtypes of Ph- CMPD or myelodysplastic syndromes (MDS). Furthermore, the lack of any cytogenetic or molecular biological markers has made the discrimination between ET and cases of reactive thrombocytosis (RT) without a well known cause[10] as well as the dis-

tinction of ET from the other Ph- CMPD with similar clinical presentation, quite problematic.[11] Moreover, it was demonstrated that a platelet count of 600×10^9/L, generally considered an absolute diagnostic criterion, is not reliable enough, given that platelet counts ranging between 400 and 600×10^9/L can be observed, especially in early stages of the disease.[12]

Recently, a new World Health Organization (WHO) classification for CMPD has been developed, incorporating clinical, laboratory and morphologic data along with fresh knowledge and techniques. This classification once again highlights the concept that correct management of a patient with a CMPD requires a combined, multidisciplinary approach.[13] The new guidelines for diagnosis strengthen the importance of histopathology and emphasize the value of megakaryocyte morphology in the differential diagnosis among the various diseases.[14,15]

In this study, 142 BM biopsies from patients with thrombocytosis > 600×10^9/L, who met the PVSG criteria for the diagnosis of ET, were retrieved from the files of the Institute of Pathology of the University of Palermo and morphologically and immunophenotypically (MIB-1, CD34) evaluated applying the new WHO classification criteria in order to identify positive diagnostic criteria for ET. Prognostic data were assessed, when possible, and the different features useful for discriminating between ET and early idiopathic myelofibrosis (IMF) were studied.

Design and Methods

Patients

This study was based on examination of BM biopsies from 142 adult patients (53 males, 89 females, median age 62 years, range 19-87 years) with thrombocytosis (platelet count >600×10^9/L) seen at our institute between 1995 and 2000 with a clinical and histologic diagnosis of ET according to PVSG criteria.

Although all the cases were diagnosed as ET, in a significant proportion of them further notes, mainly describing the amount of reticulin content considered excessive for ET but not consistent with other diagnoses, were included; in these instances the term *ET developing toward myelofibrosis* was adopted.

Bone marrow trephines

The BM biopsies were fixed in Schaffer's solution, decalcified in EDTA and embedded in paraffin. Routine histologic and histochemical stainings, namely hematoxylin-eosin, PAS Giemsa, silver impregnation according to Gomori, and Perls' iron stain, were performed. Touch imprints were stained with May-Grünwald-Giemsa.

The amount of fibrosis was graded by means of a semiquantitative scoring system, used by other authors in previous studies.[16] In detail, the prefibrotic stage (0) was characterized by a reticulin content less than the double the mean amount; an early fibrotic stage (+1) was defined by less than a three-fold increase in reticulin density compared to that found in normal groups. Fibrosis was graded as +2 when the mixture of reticulin and collagen fibers had a less than five-fold increase in density, while overt fibrosis (+3) was considered to be present when this value was exceeded.

Clustering Index

In order to refine the evaluation of the variable features of megakaryocyte aggregation and distribution, after reclassification, a *clustering index* was assigned to the 142 biopsies and compared in the different classes. A cluster was identified as the aggregation of 3 or more megakaryocytes which may or may not be touching each other. The index was calculated by multiplying the mean number of cluster-forming megakaryocytes (evaluated on the whole biopsy) by the number of clusters in sections of standardized length (10 mm). A quantitative scoring system was chosen to calculate this index instead of a semiquantitative one as it was considered more precise.

Immunohistochemistry

Immunohistochemical preparations were set up using streptavidin-biotin complex (StreptABC) and the monoclonal antibodies: MIB-1 and CD34.

Microvessel density

Microvessel density was estimated independently by two of the authors (AMF, VF) in each BM biopsy by semiquantitative evaluation of CD34 staining. The results were graded on a semiquantitative scale according to the following scheme proposed by Mesa *et al.*:[17] grade 1, scant vessels; grade 2, diffuse moderate increase in vessels; grade 3, areas with marked increase in vessels; and, grade 4, diffuse marked increase in vessels.

CD34$^+$ cells

CD34$^+$ cells were counted at 400× magnification in 10 or more selected fields. Both scattered single cells and cells forming aggregates i.e. groups of 3 or more cells, were counted. The number of CD34$^+$ cells was expressed as a percentage of the whole number of BM nucleated cells.

Proliferation

Proliferating (MIB-1$^+$) cells were visually counted. Slides were evaluated at low magnifications and 10 areas with different percentages of positive cells were

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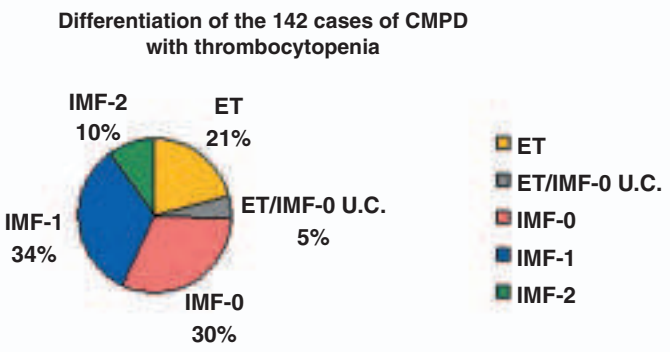

Figure 1. Distribution of diagnosis after histologic evaluation according to WHO criteria. ET (essential thrombocythemia), IMF (idiopathic myelofibrosis) ET/IMF-0 (borderline cases not fitting any other category).

randomly selected. Cells were counted at 400× magnification, the average number of positive cells was calculated and the megakaryocyte proliferation index (MPI) determined and expressed as a percentage of the counted cells. The score was graded in three classes as follows: grade 1 with ≤ 5% of positive cells; grade 2 with 5-10%; grade 3 with ≥10%.

Statistical analysis

Associations between potentially interdependent histological features, such as the grade of fibrosis, the clustering index, the cluster proliferation score and the microvessel density, were studied. Statistical analysis included calculation of the non-parametric Mann-Whitney and Kruskal-Wallis tests to evaluate whether continuous variables differed significantly between CMPD categories. All the statistical tests were manually calculated before computation.

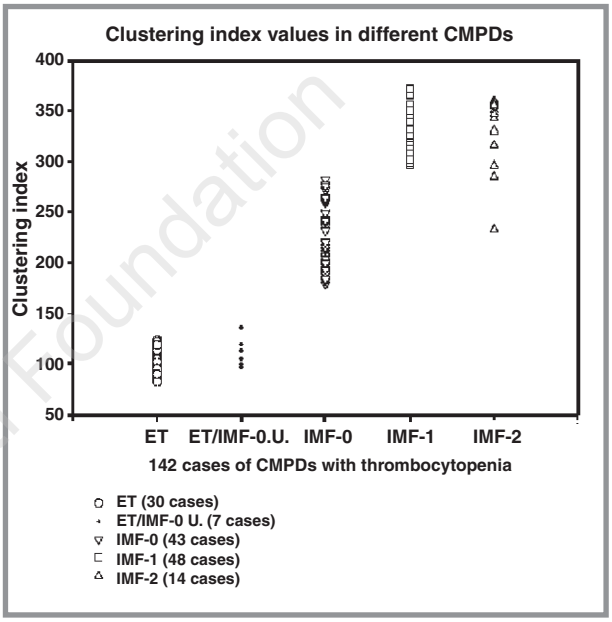

Figure 2. Clustering index values in ET and IMF cases. There is a significant difference ($p<0.001$) between ET and IMF. ET cases, as well as borderline ones (ET/IMF-0 U), show low clustering index values, while in IMF-0, the presence of more frequent clusters with variable numbers of megakaryocytes yields a higher value (mean 225.44, range 179.2-282.2). The highest values (mean 322.1, range 297-372) are observed in cases of IMF-1 and IMF-2 (mean 320.58, range 296-360.3). The latter shows a slight tendency to decrease probably due to the increasing amount of reticulin fibrosis.

Results

Bone marrow trephines

After histologic re-evaluation according to the WHO criteria, the 142 biopsies were reclassified as follows: ET 30 cases (21%); IMF-0 43 cases (30%); IMF-1 48 cases (34%); IMF-2 14 cases (10%); moreover a fifth category, i.e. ET/IMF-0 U was introduced for borderline cases (n=7, 5%) not fitting into any other group (Figure 1).

According to the WHO classification criteria the histologic picture of ET was characterized by the presence of either single or clustered large to giant mature megakaryocytes with deeply lobulated staghorn-like nuclei, normal or slightly increased cellularity, normal neutrophil granulocytopoiesis, normal-sized and located erythroid foci and lack of fibrosis.

Cases interpreted as IMF-0 showed moderate increase of cellularity, more pronounced megakaryocyte clustering with an abundance of abnormal cells ranging from giant to very small, abnormal maturation with hypolobulated cloud-like nuclei and giant bizarre dysplastic cells.

Granulocytopoiesis was moderately increased whereas both the size and number of precursor erythropoietic cells were reduced. Reticulin was not increased. A progressive increase in the number of megakaryocytes showing the abovementioned dysplastic features along with an increase of reticulin content and a more consistent decrease of erythroid precursors were the main morphological features observed in IMF-1 and IMF-2.

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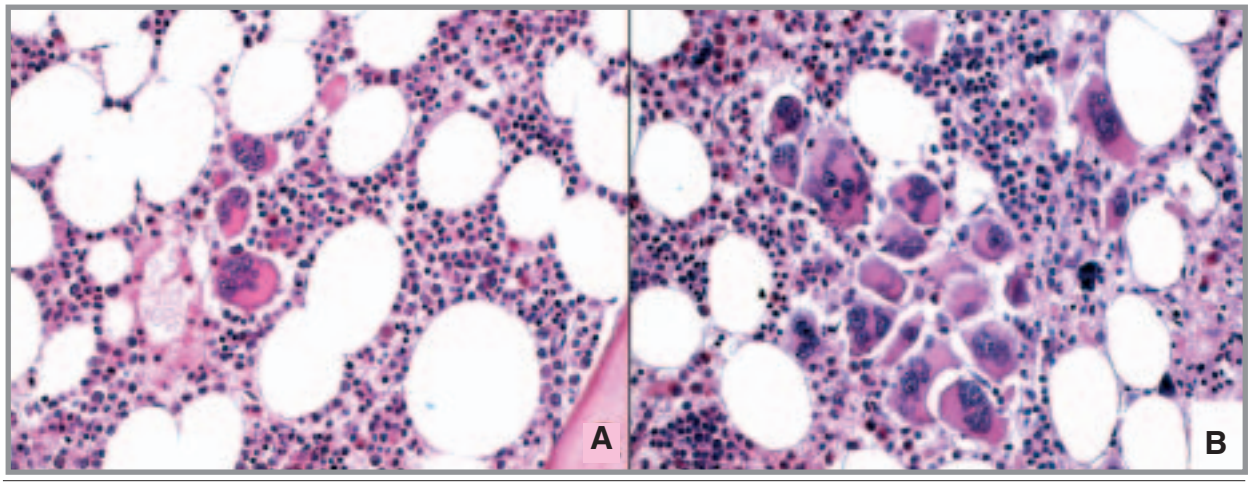

Figure 3. A. Loose cluster of hyperlobulated over-aged megakaryocytes with staghorn nuclei typical of ET. B. Large cluster of megakaryocytes in a case of IMF-1. All cells show bulbous hypolobulated cloudy nuclei (H-E, × 400).

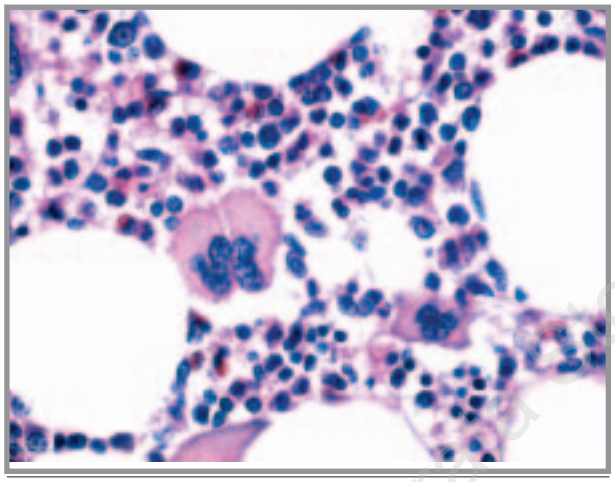

Figure 4. This high-power image clearly shows the megakaryocyte *coupling* i.e. two adjacent cells touching each other and completely surrounded by non-thrombopoietic cells (H-E, × 400).

Table 1. Distribution of microvessel density (MVD) and grading of fibrosis in 142 cases of CMPD after evaluation according to WHO classification criteria.

Grading of fibrosis	*MVD*	*N°*	*Distribution*
0	1	48	ET:23; ET/IMF-0 U: 4; IMF-0:21
0	2	32	ET: 7; ET/IMF-0 U:3; IMF-0:22
1	2	28	IMF-1:28
1	3	20	IMF-1:20
2	3	6	IMF-2:6
2	4	8	IMF-2:8

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Clustering index

A significant difference ($p<0.001$) was observed in clustering index values between cases of ET and IMF cases (Figure 2). ET cases, as well as borderline ones (ET/IMF-0 U), had low clustering index values (mean 100.78, range 83.2-124.4) since there were only rare clusters consisting of a few *over-aged* megakaryocytes. In IMF-0, the presence of more frequent clusters with a variable number of megakaryocytes, yielded a higher value (mean 225.44, range 179.2-282.2). The highest values (mean 322.1, range 297-372) were observed in cases of IMF-1 in which several clusters consisting of many MKCs were detected (Figure 3); cases of IMF-2 showed similar values (mean 320.58, range 296-360.3), although with a slight tendency to decrease probably due to the increasing amount of reticulin fibrosis.

A peculiar aspect of megakaryocytes was what we defined *coupling* i.e. two adjacent cells of the same size touching each other and completely surrounded by non-thrombopoietic cells (Figure 4). This finding, although present even in some cases of IMF (40%), was constantly present in ET (100%).

Microvessel density

The data concerning microvessel density are shown in Table 1.

Vascular proliferation was significantly more pronounced in cases of IMF considered as a whole than in cases of ET ($p < 0.005$) (Figure 5). Twenty-three cases of ET (77%) showed grade 1 MVD while only 7 cases (23%) had a MVD value of 2 and in no cases of ET a MVD value greater than 2 was detected. MVD grades 1 and 2 were equally distributed among cases classified as ET/IMF-0 U or IMF-0 with fibrosis grade 0. The MVD value increased progressively in IMF-1 cases (2-

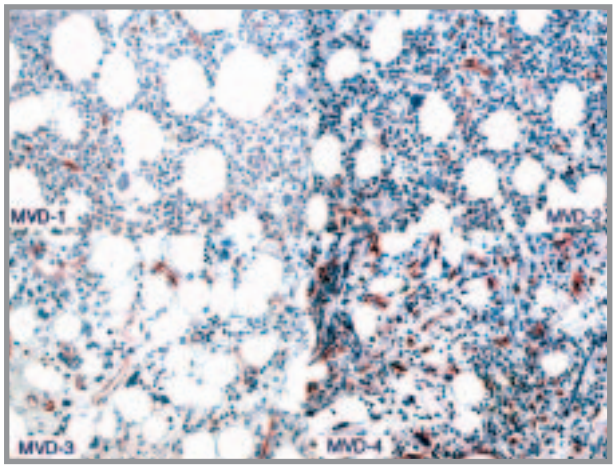

Figure 5. Different degrees of microvessel density. The panel shows MVD-1 (ET), MVD-2 (IMF-1), MVD-3 and MVD-4 (IMF-2) (StreptABC, CD34, ×250).

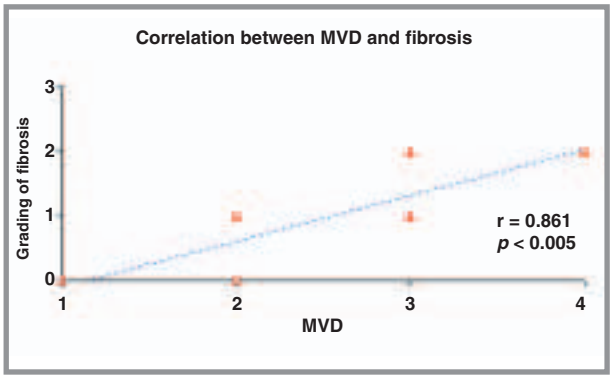

Figure 6. The graph indicates a linear positive correlation between MVD and fibrosis indices. Red spots indicate groups of cases according to the data shown in table 1.

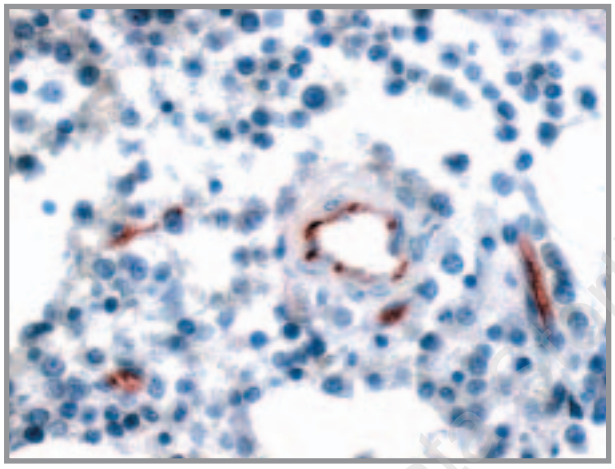

Figure 7. Few CD34⁺ cells, showing a dot-like expression pattern, located around a small vessel in a case of IMF-1 (StreptABC, CD34, ×400).

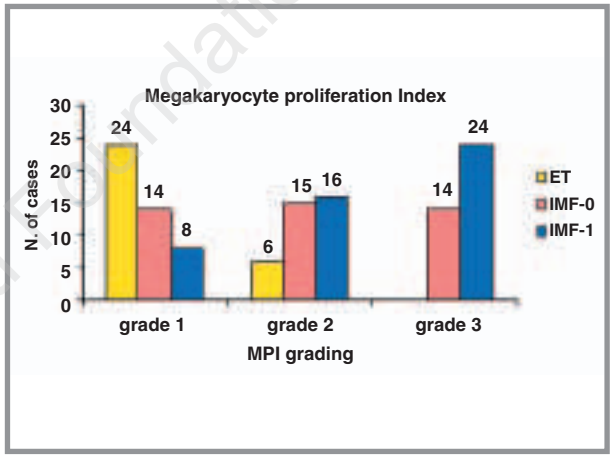

Figure 8. Megakaryocyte proliferation index (MPI) scores in no- or low-fibrotic conditions (ET, IMF-0 and IMF-1). Most of the ET cases (80%) are included in the grade-1 group, while cases of IMF-0 are homogeneously distributed and IMF-1 cases prevalently belong to groups 2 and 3.

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3) reaching highest values in cases of IMF-2. The statistical analysis showed a positive correlation between the MVD and the fibrosis grade. The index of correlation (r = 0.861) was highly significative ($p < 0.005$) (Figure 6) even if some ET and IMF-0 cases, which had the same grade of fibrosis (grade 0), showed different MVD values.

CD34⁺ cells

The few CD34⁺ positive cells showed a double topographic distribution: they were detected both in the periendosteal (paratrabecular) region representing the typical site of hematopoietic precursors and in the BM parenchyma (intertrabecular). The paratrabecular fraction appeared homogenously distributed in both ET and different stages of IMF. As regards the intraparenchymal cells they were, however, scattered, often located around sinusoids or vessels (Figure 7) and their number was minimal in cases of ET while it was significantly greater in cases of IMF (p=0.001). Increasing percentages of positive cells were detected in parallel with disease progression (IMF-0 2.1%, IMF-1 3.3%) except for in cases of IMF-2 particularly with signs of osteosclerosis characterized by a scarcity of CD34⁺ cells (1.2%).

Proliferation

Figure 8 shows the MPI values in the different conditions. MIB-1 immunostaining of megakaryocytes demonstrated significant differences between the so-called *over-aged* cells with lobulated staghorn-like nuclei characteristic of ET, showing very low rates of

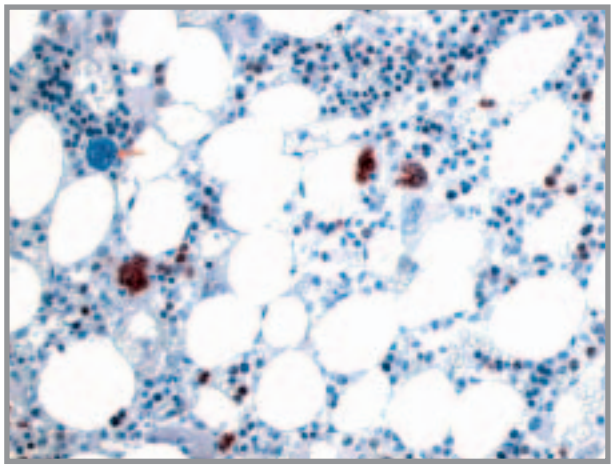

Figure 9. Mib-1 positive cells in a case of IMF-1. Some hypolobulated megakaryocytes show a diffuse nuclear expression while others, with plurilobulated *over-aged* morphology, are negative. A naked nucleus, characteristic of IMF, is visible in the top-left (red arrow) (StreptABC, Mib-1 × 250).

positivity, and dysplastic megakaryocytes with hypolobulated bulbous nuclei often encountered in both in IMF-0 and in IMF-1, which were more frequently positive (Figure 9). The MPI was evaluated according to the above mentioned method. The lowest values of MPI were found in cases of ET (80% grade-1, 20% grade-2), and progressively increased in IMF-0 (33% grade-1, 34% grade-2, 33% grade-3) and IMF-1 (17% grade-1, 33% cases grade-2, 50% grade-3) ($p < 0.01$). Very low values of MPI were detected in IMF-2, in accordance with the progressive increase of fibrosis and advanced phase of the process.

Discussion

CMPD share many biological, clinical and morphological features. Although in the great majority of cases a definite diagnosis is feasible, more challenging and complicated cases occur which do not fit any diagnostic category. The consequence is that even most recent classifications still contain *grey terms* like *chronic myeloproliferative disease, unclassifiable* or *myelodysplastic/myeloproliferative disease, unclassifiable*.

Along with the development of new specific therapeutic strategies for the different diseases, the need for more reliable diagnostic criteria becomes even more compelling.[13] The value of a multidisciplinary approach including the evaluation of a bone marrow biopsy is now well recognized and the diagnostic criteria have, therefore, progressively been changed,[18] yielding the rationale for the formulation of the new World Health Organization (WHO) Classification edited in 2001.[19] It is now fully accepted that a representative BM biopsy is needed for an unequivocal diagnosis of ET with clear-cut separation from other thrombocythemic CMPD.[20,21]

The precise definition of thrombocythemias in the various CMPD (ET, PV, IMF),[22-24] and the identification of the initial (prefibrotic) stages of IMF[14,25] are still controversial but of great importance given the different life expectancies associated with these diseases.[16,26] Clinical criteria alone have proven inadequate thus highlighting the importance of histopathological information such as histotopography and cell morphology for a correct diagnosis.[27] In 1999 the so-called *Cologne criteria* were proposed.[15] These included histopathology among the diagnostic criteria and indicated megakaryocyte morphology as the most crucial point in order to differentiate early prefibrotic IMF from ET and other allied thrombocythemic CMPD,[18] or to identify dysplastic features with more severe prognostic significance.[28,29] Retrospective clinicopathological studies performed on BM biopsies of patients with a prior diagnosis of ET, showed that a certain percentage of these cases, although fitting the PVSG criteria for ET, would be placed more correctly in the categories of prefibrotic or early IMF.[16,18,21,30]

The most useful histopathological criteria for distinguishing the different Ph- CMPDs are number, size and form of megakaryocytes, quality and pattern of density of fibers and increase of blast cells. All of these criteria have been graded from 0 to 3,[31] and together with fiber excess at diagnosis, megakaryocyte involvement is correlated with the risk of myelofibrosis while a blast excess is related to leukemic transformation.[32] This progression, significantly correlated to prognosis and disease development, could be monitored by performing periodic BM biopsies.[33,34]

In our series, the revision of 142 BM biopsies from patients previously diagnosed as having ET according to PVSG criteria, showed that, after careful evaluation of BM pathology using the new WHO criteria, the diagnosis of ET was restricted to 21% of cases; the other cases being re-assigned as IMF-0 (30%), IMF-1 (34%), IMF-2 (10%) and borderline cases (5%).

Interesting morphologic features, not previously studied, were the determination of the clustering index i.e. the tendency of megakaryocyte to form aggregates and the detection of coupling of megakaryocytes. The clustering index was low in cases of ET, increased in cases of IMF-0 and reached its highest values in cases of IMF-1. A slight tendency to decrease, probably due to the increase of fibrosis, was observed in cases of IMF-2. This index might be a further morphological tool useful in differentiating ET from early IMF. As regards the coupling of megakaryocytes, this phenomenon was always detected in ET. In our opinion, this peculiar mor-

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phologic finding, in addition to its diagnostic value, could identify a post-mitotic maturatve phase of two megakaryocytes with a low proliferative rate. This hypothesis fits well with the characteristic morphology and biology of typical ET megakaryocytes opportunely defined *over-aged*, and could explain the constant lack of MIB-1 expression of these cells. It must be underlined that megakaryocytes clustering was never observed in cases of reactive thrombocytosis.[35]

More recently, immunophenotypic studies have provided new data supporting morphologic information. Immunostaining performed with factor VIII was particularly useful in detecting small mononuclear precursor cells,[28] whereas staining megakaryocytes for thrombopoietin receptor, c-Mpl, showed that expression of this receptor was markedly lower in ET than in controls and patients with reactive thrombocytosis.[17] In the same study, vascular proliferation (neo-angiogenesis), considered a diagnostic histologic feature of relevance in many malignancies and documented in CMPD as well,[36] was studied in the BM of patients with ET and found to be increased in this setting compared within BM from healthy controls and patients with reactive thrombocytosis.[17] The importance of the role of angiogenesis in CMPD has been progressively appreciated as recent studies have shown higher levels of MVD in IMF than in ET, PV and CML[37] and angiogenin, an angiogenetic factor produced by neoplastic cells, and host microenvironment, have been investigated.[38]

In our cases CD34 immunostaining showed a significantly higher MVD in IMF than in ET. Interestingly, a significant correlation was demonstrated between the development of angiogenesis and the increase of fibrosis. Neoangiogenesis in CMPD is part of the BM stromal reaction and is due to an enhanced production of vascular endothelial growth factor, an endothelial cell mitogen expressed in magakaryocytes. It can, therefore, be regarded as a marker of disease activity and progressive extramedullary hematopoiesis, thus acquiring a poor prognostic significance.[39] These data are fully in accordance with our results showing a higher degree of vascular proliferation in IMF than ET and a gradual increase according to the progression of the stromal reaction.

Immunophenotypic studies of $CD34^+$ cells in Ph-negative CMPD revealed higher concentrations of this cell subset in IMF than in ET and PV indicating a high proliferative actitvity of the precursor cell pool and raising speculation about its prognostic relevance and the risk of blastic transformation.[40,41,42] We found very low concentrations of BM $CD34^+$ cells in confirmed ET, and rather high values in cases of IMF. These values progressively increased from IMF-0 to IMF-2 reflecting disease progression, while cases with osteosclerotic changes were devoid of $CD34^+$ cells. The strong correlation observed between the number and increase of $CD34^+$ cells and the extent and progression of angiogenesis supports the hypothesis that the latter influences the release of $CD34^+$ cells from the BM and provides a possible explanation for the perivascular distribution of these cells and, by consequence, for the higher number of circulating $CD34^+$cells observed in IMF than in other $Ph1^-$ CMPD.[41] The proliferation index was very low in ET; MIB-1 expression was very scarce and dim in *over-aged* megakaryocytes with staghorn-like nuclei while a stronger expression was detected in megakaryocytes with hypolobulated bulbous nuclei typical of IMF. This difference is likely related to the defect of maturation of megakaryocytes in IMF and the consequent increase of proliferating cells is consistent with the biological and prognostic difference of the two diseases. The difference between the more intense MIB-1 expression observed in the early, hypercellular phases of IMF (IMF-0, IMF-1) and the low values of the advanced fibrotic stages (IMF-2) is noteworthy.

The finding of a high proliferative activity in early hypercellular stages of IMF along with the reduction of proliferation and lower apoptosis rate observed in later stages and in cases with a worse prognosis, suggests that the latter are the result of a progressive failure of hematopoiesis.[43]

A retrospective, clinical analysis performed on 173 patients showed that ET carries a good prognosis with these patients' survival being only very slightly shorter than that of the general population;[44] life-threatening complications such as thrombosis and progression to acute leukemia (AL) were rare.[45]

To date, no consistent biological markers with diagnostic and/or prognostic value for the so-called Ph1 negative CMPD have been identified. Although the incidence of chromosome abnormalities in ET at the time of diagnosis is low (5.5%), trisomy 8 was found at diagnosis in a patient with ET who 35 months later developed ALL,[46] while a case of t(2;3) was reported in a case of blastic transformation of ET after treatment.[47]

Recently, in addition to the standard therapeutic options including the use of alkylating agents and hydroxyurea,[48] anagrelide, an imidazo-quinazolin compound of the quinazolin family, known to inhibit platelet aggregation, has been successfully introduced into the treatment of ET resulting in an overall decrease in megakaryocytic mass.[49] However, platelet function studies demonstrated that the thrombocytopenic effect is not accompanied by correction of platelet dysfunction.[49,50] The study of different growth factors, namely platelet-derived growth factor, transforming growth factor β and basic fibroblast growth factor in patients with ET before and during treatment with anagrelide, suggests that multiple and different

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mechanisms act in the pathogenesis of the disease.[51] Despite the huge amount of emerging knowledge about molecular mechanisms and pathogenesis, thrombocythemic disorders still show biological heterogeneity with different clinical outcomes.[52] The morphological and immunophenotypic data in ET are all consistent with an indolent, slowly developing process with a good prognosis; it follows that the discrimination between ET and prefibrotic states of IMF, an evolutive disease with a more severe prognosis,[34] is of great significance, as is the identification of patients at risk of thrombotic complications or leukemic transformation in order to choose the most specific therapeutic strategy and minimize morbidity;[1] markers related to activity and proliferation, such as CD34 and MIB-1, underline the biological differences between the different diseases. It can, therefore, be said that as ET cannot currently be considered a cytogenetically defined disease, the use of the new WHO classification with the introduction of histopathological criteria, makes the correct diagnosis possible in an ever increasing number of cases.

AMF: main author; VF: conception and design; EI: clinical selection of cases; CT, RP, SI: selection of cases; AMF, VF: revision of bone marrow biopsy; AMF, CT: analysis of immunophenotypical data; CT: assessment of semiquantitative data; AMF drafted the paper; RP and SI performed the immunophenotypical reactions, CT created figure 1 and table 1, VF revised critically and approved the final version of the paper. Supported by a grant of MIUR, Rome (Italy)

Partially presented at the European MPD Workshop on Ph1- MPDs in Rotterdam, May 2004.

Manuscript received April 1, 2004. Accepted June 17, 2004

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