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Prognostic significance of bone marrow biopsy in essential thrombocythemia

CLAUDIO ANNALORO, GIORGIO LAMBERTENGHI DELILIERS, ALESSANDRO ORIANI, ERMANNO POZZOLI, DANIELA LAMBERTENGHI DELILIERS, FRANCA RADAELLI,* PAOLA FACCINI*

*Centro Trapianti di Midollo and *Servizio Autonomo di Ematologia Diagnostica, Ospedale Maggiore I.R.C.C.S. and University of Milan, Italy*

ABSTRACT

Background and Objective. The diagnostic and prognostic value of bone marrow biopsy (BMB) has been widely investigated in patients with chronic myeloproliferative disorders (CMPD). The present study is based on a review of the results of routine BMBs taken from 93 essential thrombocythemia (ET) patients at the time of diagnosis.

Design and Methods. The common BMB histologic parameters and clinico-hematologic variables were considered for diagnostic and prognostic purposes. Clinico-pathologic correlations were looked for univariately. Moreover, the diagnostic significance of the histologic findings was tested by means of cluster analysis. Overall survival and event-free survival were considered as prognostic endpoints.

Results. There were no correlations between the clinic and pathologic findings, and none of the histologic and clinical parameters was predictive of survival or the occurrence of major clinical events. Cluster analysis of the BMB findings revealed two distinct morphologic patterns: one was clearly myeloproliferative; the other had somewhat dysplastic features. The event-free and overall survival rates in the latter group were significantly worse (p= 0.0377 and p=0.0162 respectively), with major ischemic events accounting for most of the difference in event-free survival.

Interpretation and Conclusions. These results have no clearcut counterpart in the literature, but we feel that dysplastic BMB findings could be included in the definition of ET prognostic scores in order to allow therapeutic strategies to be adapted to the level of risk.

Key words: bone marrow histology, essential thrombocythemia, prognostic factors

Essential thrombocythemia (ET) is a well-characterized chronic myeloproliferative disorder (CMPD), which is usually diagnosed on the basis of the essentially exclusive Polycythemia Vera Study Group criteria.[1] Alternative diagnostic approaches have been proposed, but these have failed to reveal any specific hallmark of the disease.[2] Some investigational groups in Germany have confronted the problem of establishing a precise differential diagnosis using only the results of bone marrow biopsy (BMB).[3-5] Although the idea of being able to recognize each single CMPD variety on histologic grounds alone may arouse some skepticism, the Hannover Classification of CMPD was developed[6] and the reliability of this approach has received further support from comparisons with alternative methods of investigation.[7,8] A more recently raised question concerns the possibility of using BMB histology in the prognostic work-up of CMPD; for this purpose, a scoring system has been designed that takes into account megakaryocyte morphology, reticulum abnormalities and an excess of blasts.[9] The aim of the present study was to evaluate the diagnostic and prognostic value of BMB histologic findings in a series of ET patients.

Design and Methods

The BMBs taken at diagnosis from 93 patients between 1977 and 1994 were reviewed in April 1994. Thirty-five of the patients were male and 58 female; their ages ranged from 10 to 86 years (median 54.5). The samples had been obtained by means of a Jamshidi needle from the posterior superior iliac spine, fixed in aldehyde, embedded in paraffin and stained for morphology and reticulum according to routine techniques (hematoxylin/eosin and Gomori). The diagnosis of ET was established or retrospectively confirmed according to the Polycythemia Vera Study Group criteria.[1]

The treatment plan was as follows: 1) over 60-year old symptomatic patients, and all asymptomatic patients with a history of major thrombotic events, received antiaggregating agents (ASA as first choice) and chemotherapy (hydroxyurea as first choice); 2) mildly symptomatic patients aged less than 60 years and without any previous major thrombotic episodes, and asymptomatic patients with previous major thrombotic episodes received antiaggregating agents alone; 3) under 60-year old highly sympto-

Correspondence: Prof. Giorgio Lambertenghi Deliliers, Centro Trapianti di Midollo, Ospedale Maggiore I.R.C.C.S., via Francesco Sforza, 35, 20122, Milan, Italy.
Phone: international +39-02-55033322 • Fax: international +39-02-55033341 • E-mail: lambert@imiucca.csi.unimi.it

matic patients (angor, transitory ischemic attacks), and similarly-aged mildly symptomatic patients with a history of major thrombotic episodes received both antiaggregating agents and chemotherapy; 4) asymptomatic patients without any previous major thrombotic episodes received no therapy irrespective of their age or platelet level.

The histologic slides were independently reviewed by three observers; the cases of discordance were discussed and classified on the basis of majority opinion. The following BMB histologic parameters were considered for diagnostic and prognostic purposes: 1) overall bone marrow (BM) cellularity was considered normal if hematopoietic precursors occupied 40-60% of the surface of the bone lacunae; 2) reticulum abnormalities, graded as a thickening of reticulin fibers and slight fibrosis; 3) quantitative assessment, and the maturation and dysplastic features of erythroid and myeloid precursors, assessed as decreased, normal or increased by simultaneously taking into account overall BM cellularity and the leukoerythroblastic ratio; 4) a normal maturational direction of the myeloid series from the bone trabeculae to the core; 5) the presence of aggregates of immature (ALIP-like) precursors, further subdived into infrequent (less than 10/ histologic section) and frequent (10 or more/histologic section); 6) the number of megakaryocytes per low magnification field, determined according to routine criteria; 7) the presence and size of megakaryocyte aggregates; 8) the predominant size of megakaryocytes; 9) the predominant nuclear pattern of megakaryocytes; 10) the presence of megakaryocyte mitoses; 11) the presence of megakaryocytes trapped in fibrotic tissue; 12) the number of eosinophils, plasma cells and lymphocytes estimated by eye; 13) the presence of dilated BM sinusoids; and 14) signs of bone remodelling. The breakdowns of the variables considered are summarized in Table 1.

The clinical and hematologic variables considered at diagnosis were: a positive history of major thrombotic events; sex; age; the presence of hepato- and/or splenomegaly; LDH, Hb, WBC, PMN and platelet levels; the leukocyte alkaline phosphatase score; the percentage of immature precursors in bone marrow smears; and cytogenetic analysis performed at diagnosis. The continuous variables were divided into classes. Clinical and pathologic correlations were looked for by means of cross-tabulation. The diagnostic significance of the BMB histologic findings was tested by means of cluster analysis, using the average between-group linkage method, which is included in the SPSS 4.1 package for Macintosh (SPSS Inc., Chicago, IL, USA). Cluster analysis produces hierarchical clusters of items based on the dissimilarity or similarity of one or more variables.

The prognostic value of the individual BMB histologic variables, the clusters identified by cluster analysis, and the clinico-hematologic variables were assessed

Table 1. Descriptive analysis of histologic findings.

Histologic parameter	*Breakdown (No. of patients)*		
Cellularity	Decreased	Normal	Increased
	10	39	44
Reticulum	Normal	Reticulum thickening	Mild fibrosis
	33	21	39
Myeloid series			
quantitative evaluation	Decreased	Normal	Increased
	3	36	54
maturation	Normal	Blockade	
	76	17	
dysplasia	Absent	Present	
	49	44	
maturation direction	Normal	Distorted	
	50	43	
blastic aggregates	Absent	Infrequent	Frequent
	30	36	27
Erythroid series			
quantitative evaluation	Decreased	Normal	Increased
	39	41	13
maturation	Normal	Blockade	
	66	27	
dysplasia	Absent	Present	
	61	32	
Megakaryocytes			
cytoplasmic size	Normal distribution	Predominantly small	Predominantly large
	58	18	17
nuclear pattern	Predominantly up to 4 nuclei	Predominantly 4-8 nuclei	Predominantly 16 or more nuclei
	24	59	10
aggregates	Absent	Predominantly small (up to 10 elements)	Predominantly large (more than 10)
	11	41	41
number per low magnification field	Up to 10	10–20	More than 20
	20	63	10
mitoses	Infrequent	Frequent	
	64	29	
trapping in fibrosis	Absent	Present	
	70	23	
Eosinophils			
quantitative evaluation	Normal	Increased	Greatly increased
	33	37	23
Lymphocytes			
pattern	Normal	Diffuse increase	Aggregates
	42	26	25
Plasma cells			
pattern	Normal	Diffuse increase	Aggregates
	58	15	20
BM sinusoids			
size	Normal	Dilated	
	21	72	
Bone remodelling			
pattern	Absent	Mild	Conspicuous
	63	25	5

using a censoring time of May 31, 1998. Overall survival (OS) and event-free survival (EFS) were considered as prognostic endpoints. Death, blastic crisis, evolution into overt myelofibrosis, the occurrence of secondary neoplasms, and life-threatening hemorrhagic and thromboembolic events were selected as events for the purpose of EFS. The survival curves were calculated according to Kaplan-Meier, and compared by means of the log-rank test. Furthermore, a discriminant function according to Mahalanobis (SPSS 4.1 package for Macintosh; SPSS Inc., Chicago, IL, USA) was calculated in order to predict EFS; the individual histologic and clinico-hematologic variables, cytogenetics and previous chemotherapy were considered in these statistical analyses as possible prognostic factors.

Results

The duration of follow-up for the censored patients ranged from 49 to 238 months (median 109). Nine patients had died: four of thromboembolic events, three of neoplastic progression, and two of other causes. Ten patients had experienced major thromboembolic events, two had developed blastic crises (one with a megakaryocytic and one with a myeloid phenotype), eight myelofibrosis and three second neoplasms; the events occurred 2-169 months after diagnosis. Twelve patients had been lost to follow-up and 59 were alive and event free. Platelet levels at diagnosis were more than $1,000 \times 10^9$/L in 30, and below this value in 63 cases. Eighteen patients had splenomegaly at diagnosis, 12 had hepatomegaly, 13 had both and 50 neither. Cytogenetic analysis showed no Philadelphia chromosome, and a normal karyotype in 86 cases; the observed chromosomal aberrations (one case each) were –Y; +8,+9; +8; 7q–; –5; –21, 11q–; –11, 22q–. The patient with –5 developed a blastic crisis with myeloid phenotype; the other six patients are still event free. The morphologic findings deriving from the reviewed BMBs are summarized in Table 1; the inter-observer concordance at the first independent examination was 89%. Univariate analysis did not reveal any relationship between the BMB histologic and clinicohematologic parameters.

Cluster analysis of the histologic findings allowed two clusters to be identified: one of 40 (Group 1) and one of 53 patients (Group 2). Group 1 showed more frequent reticulum abnormalities (p<0.000001), increased levels of myeloid precursors (p<0.005), dysmyelopoiesis (p<0.005), aggregates of immature precursors (p<0.005), abnormal maturational polarity of the myeloid series (p<0.05), decreased levels of erythroid precursors (p<0.05), no large megakaryocytes (p<0.0005), and the presence of trapped megakaryocytes (p<0.0001). There were no significant relationships between the two histologic groups and their clinical variables, with the exception of a borderline predominance of males in Group 1 (p=0.058).

None of the individual clinical and histologic variables proved to be a predictor of OS or EFS. There was only a slight trend towards a worse OS in the patients with dysmyelopoiesis (p=0.0836) and in those with trapped megakaryocytes (p=0.0804).

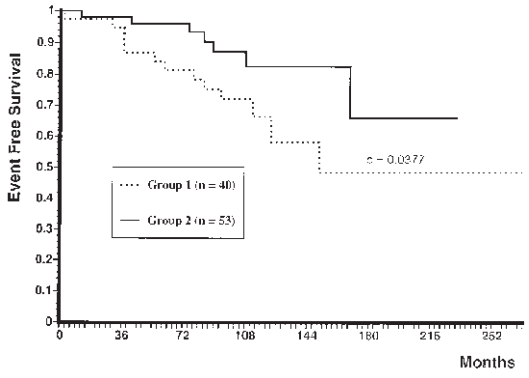

Figure 1. Group 2 patients had a significantly better event-free survival (EFS) than those in Group 1 with dysplastic bone marrow biopsy features.

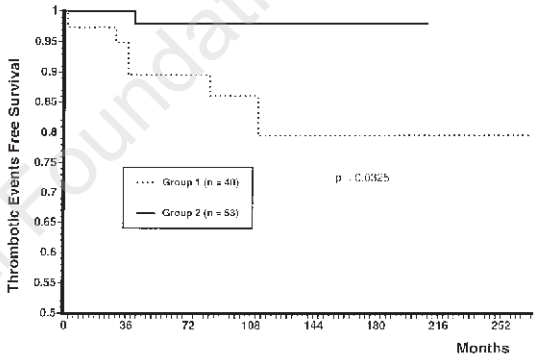

Figure 2. The frequency of thrombotic events was significantly greater in patients with dysplastic bone marrow biopsy features (Group 1).

There were, however, some significant relationships between survival and the groups identified by cluster analysis. Group 1 patients had a significantly shorter EFS (a median of 151 months) than those in Group 2, whose median EFS had not been reached after 238 months (p= 0.0377) (Figure 1). Most of this difference was attributable to thromboembolic events (Figure 2); although the median thrombotic event-free survival had not been reached in either Group, eight of the ten events recorded were in Group 1 (p=0.0325). Median OS had not been reached in either group, but again eight of the nine deaths were in Group 1 and the difference in OS chance was statistically significant (p=0.0162) (Figure 3).

Multivariate analysis allowed a discriminant function for EFS to be calculated (predictive level 77.97)

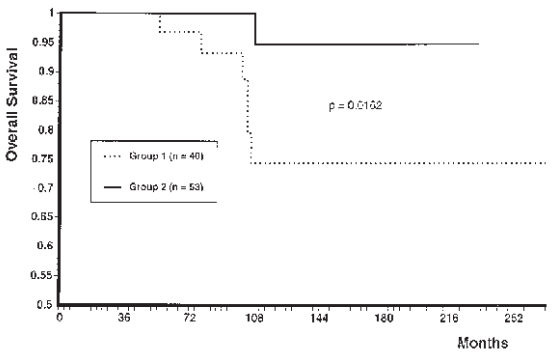

Figure 3. Overall survival (OS) was significantly better in the patients with no dysplastic bone marrow biopsy features (Group 2).

taking into account the following variables: sex, platelet level, splenomegaly, reticulum abnormalities, polarity of myelopoiesis, blastic aggregates, proportion of myeloid precursors, size of megakaryocyte aggregates and megakaryocyte trapping.

Discussion

The described results relate to a single-Institution series of ET patients followed up for a median of about nine years. ET is a CMPD characterized by a usually benign course due to a low rate of blastic crises[9] and what is generally considered to be a not exceedingly high risk of life-threatening thrombotic events;[10] the identification of risk factors therefore requires prolonged observation.

From the diagnostic point of view, the present study tried to find some preferential histologic patterns that may identify possible subsets of ET patients. The two groups emerging from the cluster analysis showed some significant histologic differences whose consistency suggests two extreme patterns possibly encompassed by the diagnosis of ET; to the best of our knowledge there are no equivalent results in the literature with which to compare this finding, since the most frequently pursued objectives of histologic reviews are different.[3-9] The patients in Group 2 (the majority of the patients in our series) had a histologic pattern similar to that commonly described for CMPD in general, and ET in particular.[9,11,12] The most striking findings in Group 1 were reticulin network abnormalities, marked hyperplasia of myeloid precursors, some degree of dysgranulocytopoiesis, a distortion of the normal maturational direction of myeloid precursors, the presence of ALIP-like[13,14] figures, erythroid hypoplasia, the absence of large megakaryocytes and the presence of trapped megakaryocytes; as a whole, these features are reminiscent of myelodysplasia.[13] The present data do not show that about 40% of ET patients have bone marrow dysplasia; we only observed that single dysplastic features can be quite frequently observed in ET, and cluster analysis revealed the tendency of such features to occur simultaneously. As a whole, these dysplastic signs can be regarded as mild since predominantly minor findings are involved, such as abnormalities in the reticulum, in the L/E ratio, and in maturational behavior; in no case did their presence cast any doubt on the original diagnosis of ET. Histologic findings in ET can therefore be regarded as a spectrum ranging from a classical myeloproliferative to a variant dysplastic form. Furthermore, features reminiscent of CMPD have been previously described in myelodysplastic syndromes, particularly chronic myelomonocytic leukemia and those with bone marrow fibrosis.[15] The significance of our findings is enhanced by their independence from common clinical variables.

The prognostic value of the groups was also tested. Since the BMBs were reviewed and the cluster analysis performed in the first half of 1994, the clinical observation was partially prospective. Published papers dealing with prognosis in CMPD recognize differences in risk among the different forms[9] or identify clinical risk factors that are common to all CMPD patients;[10] but they fail to define any prognostic subgroups related to the individual disorders,[10] presumably because of the long EFS and OS expectancies of ET patients, limited therapeutic resources and the low incidence of the disease. In the present experience, the clustering of the histologic findings revealed significant differences in both OS and EFS, particularly in terms of thrombotic rather than neoplastic events; moreover, cluster analysis showed the preferential aggregation of mild dysplastic features and compared favorably with discriminant analysis in predicting EFS. In conclusion, the generalized use of BMB is obviously recommended in the diagnostic and prognostic work-up of ET; furthermore, in order to optimize therapeutic strategies, the dysplastic features in ET could be standardized and included among the parameters suitable for defining a prognostic score.

Contributions and Acknowledgments

CA designed the study, reviewed the bone marrow biopsies, contributed to the interpretation of data and drafted the article. GLD reviewed the bone marrow biopsies, revised the article and gave final approval of the article. AO reviewed the bone marrow biopsies, and contributed to the interpretation of the data and the revision of the article. EP performed the statistical analysis and contributed to the interpretation of the data. DLD contributed to the revision of the article and to the collection of data. FR was involved in the clinical follow-up of the patients. PF was involved in the clinical follow-up and collection of data.

The order of the authors takes into account both the quality of the single contributions offered and the time spent by each contributor.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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