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Megakaryocytes in chronic myeloproliferative disorders: numerical density correlated between different entities

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Summary. A morphometric evaluation of number and grouping of megakaryocytes (MK) in five different groups of chronic myeloproliferative disorders (CMPD) was performed by counting 60 high power fields equaling approximately 14.28 mm^2 of haematopoiesis in each case. Twenty-one up to 29 cases were evaluated for each of five categories of CMPD and one control group; a total of 132 cases of CMPD and 33 control cases were used. The mean number of MK per square millimetre was 15.54 ± 1.53 in chronic myeloid leukaemia of common or granulocytic type (CML.CT), 69.91 ± 5.85 in CML with megakaryocytic increase (CML.MI), 59.59 ± 3.27 in polycythaemia vera (P. vera), 59.85 ± 4.59 in primary thrombocythaemia (PTH), 67.58 ± 4.11 in chronic megakaryocytic granulocytic myelosis (CMGM), and 19.7 ± 3.07 in controls. The distinction between free or isolated MK, and between clustered or grouped MK corresponds to the total cell counts of MK in the various groups of CMPD. Clustering of MK was significantly higher in CMGM and PTH compared to other groups, but the difference between them was not statistically significant. Significant differences in the mean number of MK were obtained between controls and CML.CT on the one hand and all other groups of CMPD on the other. The results further support the histological subclassification of CMPD according to the primary disorders of the Hannover classification (not advanced by sclerosis, fibrosis or excess of blasts, respectively).

Key words: Megakaryocytes – Chronic myeloproliferative disorders – Morphometry – Histology

Introduction

Numerous articles describing the morphology and number of megakaryocytes (MK) in bone marrow (BM) of patients with chronic myeloproliferative disorders (CMPD) have been published since significant differences were observed in these cells between chronic myeloid leukaemia (CML) and the other CMPD (Franzen et al. 1961). In some patients with CML, a reduction in the volume and ploidy of MK was found while the actual number of MK was increased, and elevated platelet counts were observed (Lagerlöf 1972; Branehög et al. 1975). In contrast, patients with polycythaemia vera (P. vera) or primary thrombocythaemia (PTH) showed increased number of MK correlated with increased cellular volume and ploidy (Harker and Finch 1969), which seemed inappropriate with increased platelet counts in peripheral blood.

Diagnostic characterization of the various groups of CMPD should be based upon histopathology from BM biopsies (Georgii 1985; Bartl et al. 1982; Frisch et al. 1984; Burkhardt 1988; Georgii et al. 1990). Early methods to confirm histological classification revealed some correlations with number and morphology of MK (Thiele et al. 1983, 1984). Since descriptive histopathology has yielded two new entities among primary stages, namely CML with megakaryocytic increase (CML.MI) and chronic megakaryocytic granulocytic myelosis (CMGM) as described by the Hannover classification of CMPD (Georgii et al. 1990), it seemed to be of interest to study the number and morphology of MK. The latter are the hallmark of the histopathological diagnosis and presumably the clue to understanding platelet counts in the peripheral blood in all these entities. The purpose of this article is to compare the number and grouping MK in cases from each group of CMPD in primary, untreated stages of these disorders.

Materials and methods

The 132 cases of CMPD selected from our BM registry between the years 1973 and 1989 fulfilled the criteria of (1) being initial biopsies prior to any therapy, (2) excellent technical preparation, and (3) being not smaller than 30 mm in length allowing a reliable determination of the number and distribution of MK.

All cases including controls were either trephines by the Jamshidi and Swaim needle (1971) or myelotomies performed according to the Burkhardt technique (1966). Thirty-three cases from staging

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Table 1. Biopsy cases evaluated by counting the megakaryocytes (MK) and regarding their topographic arrangement in clusters in five groups of chronic myeloproliferative disorders according to the histological system of non-advanced stages of the disease

Hannover classification	Acronyms	No. of biopsies	No. of HPF evaluated (mean)
Chronic myelogenous leukemia			
– common type	CML.CT	29	58.0
– with MK increase	CML.MI	27	63.5
Polycythaemia rubra vera	P. vera	21	62.4
Megakaryocytic myelosis, clinically consistent with primary (essential) thrombocythemia	PTH	28	64.4
Chronic megakaryocytic granulocytic myelosis	CMGM	27	58.3
Controls		33	64
Total		165	

Table 2. Numerical density (n/mm^2) of MK in CMPD and controls: mean (standard error)

CMPD	MK	"Single" MK		MK in clusters		MK nNu
		nu	cf	nu	cf	
Controls	19.70 (3.07)	16.01 (3.03)	3.57 (0.24)	0.08 (0.02)	0.04 (0.01)	0.20 (0.03)
CML.CT	15.54 (1.53)	12.64 (1.21)	2.69 (0.38)	0.14 (0.04)	0.07 (0.03)	0.43 (0.07)
CML.MI	69.91 (5.85)	53.78 (4.04)	11.83 (0.93)	3.67 (1.05)	0.63 (0.18)	1.35 (0.26)
P. vera	59.59 (3.27)	43.78 (2.43)	11.48 (0.64)	3.36 (0.62)	0.97 (0.20)	0.89 (0.24)
PTH	59.85 (4.59)	41.51 (2.79)	11.09 (0.85)	5.82 (1.08)	1.43 (0.24)	1.20 (0.24)
CMGM	67.58 (4.11)	43.45 (2.11)	12.60 (0.81)	9.19 (1.25)	2.34 (0.45)	2.37 (0.32)

MK, Whole number of MK; separate section for "single" MK and MK in clusters; nu, nuclear profile; cf, cytoplasmic fragment; MK nNu, naked nuclei of MK

biopsies, either for non-Hodgkin lymphomas or for metastases in lung cancer were taken as controls. There was no haematological or histological evidence for CMPD in these; however, reactive BM changes could not be excluded completely. The BM cores were fixed in a solution of methyl-alcohol and formalin, embedded in methyl-methacrylate, cut without decalcification in sections of 3–4 µm and Giemsa stained. Histological classification according to the Hannover system (Table 1) was performed by two pathologists familiar with our system, prior to morphometric evaluation (Georgii et al. 1990). Any differences in classification were reconsidered in a panel discussion.

Counting of the numerical density of MK and of its subtypes was performed independently by one experienced and two unexperienced observers. After a training period in a pilot study, the inter-observer variation was found not to be significant. Intraobserver variance was calculated from double determinations performed blindly with an interval of 1–2 months and was found not to be significant.

For counting, slides were moved systematically and only areas of haematopoietic tissue free from bone trabecula were evaluated. This procedure provided identical areas for evaluation. All MK of a field were counted; those reaching over the border of the left half were counted, but those reaching over the border of the right were not. Each observer counted at least 20 high power fields (HPF) per case at a magnification of ×312.5 (objective 25×, intermediate 1.25×, ocular 10×). In the pilot study counts were done with an ocular grid, assuring that the areas counted by each observer were comparable. Counts done with or without grid were not significantly different. Thus the magnification of ×312.5 represented an area of 0.238 mm^2. This is an evaluation of 4.76 mm^2 per observer. The size (>30 mm) of the specimens evaluated guaranteed that each observer counted different areas. The sum of countings from three observers with a total of 60 HPF, a square of 14.28 mm^2, was evaluated for each of the 165 biopsies (Table 2).

Even in those entities of CMPD with high numerical density of MK, in which a high variation of MK counts from one HPF to another was observed, valid results could be obtained by performing countings in the sufficiently representative number of 10–15 HPF, as could be shown by continuous calculation of mean values. Some typical examples of cases with extreme variation of

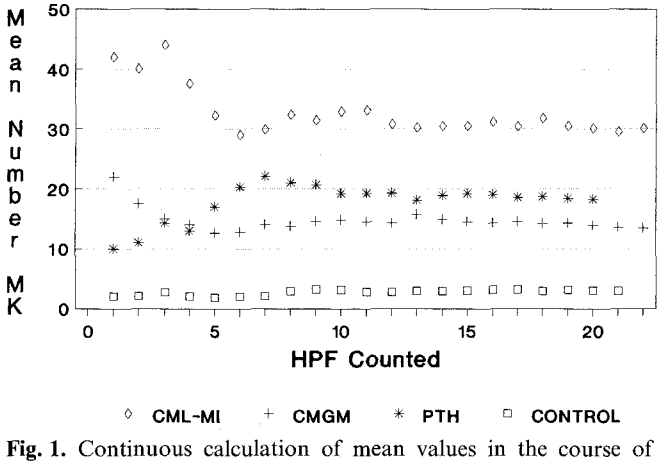

Fig. 1. Continuous calculation of mean values in the course of individual counts of HPF in the cases of CML.MI, CMGM, PTH and controls. For abbreviations, see Table 1

MK numerical density from one HPF to another are given in Fig. 1.

Clusters were defined as three or more MK lying in direct contact with their cytoplasmic membranes. MK lying in clusters were evaluated separately, but additionally were summed in the total number of MK. Furthermore, "naked" nuclei of MK without discernible cytoplasm were recorded separately and not included in the total MK number. MK profiles may appear as cell profiles either with or without a nuclear profile. MK profiles with and without nuclear profiles were counted separately for "single" MK and MK in clusters, respectively. MK profiles without discernible nucleus were designated as cytoplasmic fragments.

Statistical evaluations with Student's t-test were performed by means of the "Statistical Package for Social Sciences" system based on the assumption of a normal distribution of counts. Values of $P<0.05$ were considered to be significant (+), and values of $P<0.01$ as highly significant (++).

Results

The numerical density of MK, evaluated separately for "single" MK and for MK in clusters as well as for MK profiles with and without nuclear profiles, and naked nuclei respectively, generally counted as the number per unit area of 1 mm^2, is displayed in Table 2.

The statistical differences between controls and the different entities of CMPD are shown in Table 3. Highly significant differences in all variables evaluated were found between controls and CML cases with low MK counts, and those entities of CMPD with high MK counts, i.e. CML.MI, P. vera, PTH and CMGM. Highly significant differences exist furthermore between controls and CML.CT in the number of naked nuclei (MKnNu; see Table 3), which is much higher in CML.CT.

Moreover, significant or highly significant differences could be shown between most of the different entities of CMPD in the number of "single" MK, MK in clusters, and/or MKnNu, respectively.

The numerical density of MK in CML.CT is lower than in controls and is almost four-fold lower than in cases of CML.MI. The differences of MK density are illustrated in Fig. 2, where columns represent the percentage of cases within a category of MK density. The initial histological subtyping of CML cases either as CML with low (CML.CT) or with increased numbers of MK (CML.MI) was done on the basis of a subjective diagnosis. As can be seen in Fig. 2, the limit between these two entities was determined at 30 MK/mm^2. While one group of the cases subtyped as CML.CT had MK counts between 5 and 10 MK/mm^2, which are significantly smaller than the counts found in controls, a second group was found with higher counts between 25 and 30 MK/mm^2. In this latter group obvious differences in the distribution of MK among the HPF were displayed, which means a high MK count within one field, whereas virtually no MK were found in others. The same heterogeneity was observed in the group of CML.MI with lower counts of MK, namely 30–50 MK/mm^2. Most of the cases of CML.MI, however, had even higher MK counts between 30 and 90 MK/mm^2. The highest value was obtained in one case with 161.38 MK/mm^2 and was indeed the highest value among all cases of CMPD evaluated in our study.

Table 3. Significant differences between the CMPD and controls according to parameters in Table 2

Compared groups	MK	"Single" MK		MK in clusters		MK nNu
		nu	cf	nu	cf	
Controls – CML.CT	–	–	–	–	–	0.005
Controls – CML.MI						
Controls – P. vera						
Controls – PTH						
Controls – CMGM		All free fields <0.0005				
CML.CT – CML.MI						
CML.CT – P. vera						0.039
CML.CT – PTH						
CML.MI – P. vera	–	0.036	–	–	–	–
CML.MI – PTH	–	0.011	–	–	0.007	–
CML.MI – CMGM	–	0.032	–	0.005	0.001	0.019
P. vera – PTH	–	–	–	–	–	–
P. vera – CMGM	–	–	–	0.001	0.0098	0.003
PTH – CMGM	–	–	–	–	–	0.0012

$P<0.05$ significant, $P<0.01$ highly significant; – = not significant

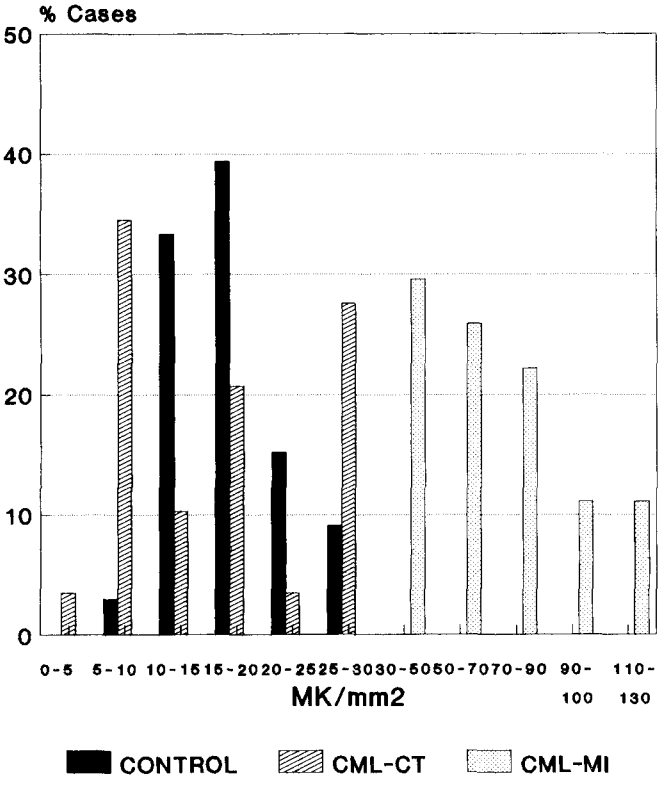

Fig. 2. Frequency distribution of MK per square millimetre in the cases of CML.CT, CML.MI and controls (%). For abbreviations, see Table 1

Discussion

The simple determination of the distribution of MK and its variation in cytology and topography reflects the system of the Hannover classification of CMPD. This is not surprising, because the subjective histological classi-

fication is based upon the striking differences in number and morphology of the MK among the various groups which reflect the haematological designation of diseases. Two groups of CMPD were added by the Hannover system. Philadelphia (Ph^1) positive CML were divided into subgroups according to the number of MK which is a stringent definition, and the diverse group of Agnogenic Myeloid Metaplasia (AMM) was subtyped according to the fibre content. A fibre-free disease was characterized by the conspicuous increase of rather enlarged MK which have a striking morphology and was termed CMGM (Thiele et al. 1977; Georgii et al. 1990). The other subgroups of AMM are defined by their increase of fibres; these are advanced stages and therefore were not taken into consideration. The significant differences in density of MK in CML.CT versus the other groups of CMPD, P. vera, PTH and CMGM, are in excellent agreement with results from evaluating smears or fragments of bone marrow (Franzen et al. 1961; Lundin et al. 1972; Branehög et al. 1975, 1982). The current paper also agrees with the results from comparable histological trials, though the nomenclature of the histopathological findings differs to a certain extent (Harker and Finch 1969; Ellis et al. 1975; Ellis and Peterson 1979; Thiele et al. 1983, 1984, 1985, 1987, 1988, 1989, 1990). However, the results on which this study is based were obtained by a histological technique which is somewhat different from common wax embedding techniques after decalcifying the bone marrow cores (Thiele et al. 1990). It is not surprising, therefore, that the differences are quite more clearly recognizable in the resin technique of undecalcified cores. It is stated that there are no differences between wax- and resin-embedded specimens evaluated by the same authors (Thiele et al. 1982, 1983, 1984, 1988). However, a considerable underestimation of the amount of haematopoiesis and number of MK was found by other comparisons between undecalcified resin and decalcified wax embedding. This is probably due to a higher specimen shrinkage in the latter technique (Kerndrup et al. 1980), a shrinkage which might be due to various factors including fixation, decalcifying methods and loss of water during processing for wax embedding (Wollweber et al. 1981).

The results in density of MK can be compared with those from Lazzarino et al. (1986), since similar histological techniques were applied. Considerably higher averages of MK in CML in their cases compared to ours might be explained by differing histological classifications. In Ph^1-positive CML, a subtype with a predominance of MK as described elsewhere (Georgii et al. 1990) has also to be considered. Cases with megakaryocytic predominance are not included in this study, but will be compared to CML of common type (CML.CT) and CML with simple increase of MK, e.g. CML.MI, in an ongoing evaluation. The finding of two different peaks with respect to the density of MK among the CML.CT implies a heterogeneity of the cases due to a different distribution pattern of megakaryopoiesis. This phenomenon can probably be related to transitional stages of an increase in the megakaryopoiesis between simple CT types and an overt increase in the MI variant. Therefore, those cases should be called overlapping type between CML.CT and CML.MI.

Among the other categories of CMPD there were no significant differences in density of MK. However, the number of MK in clusters is significantly higher in CMGM if compared to P. vera and CML.MI. Although there seems to be a difference of the frequency of clustering in CMGM versus PTH, this was not found to be statistically significant. Finally, the evaluation of naked nuclei from MK is an additional method for distinguishing the various subgroups of CMPD. This may be rather difficult, sometimes especially in overlapping cases, as previously discussed (Bartl et al. 1982; Georgii et al. 1990).

In conclusion, the Hannover classification of CMPD was supported by simple enumeration of the density and of the topographical distribution that is represented by clustering of MK in primary, not advanced stages of the disease.

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