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Histomorphometric study of megakaryocytes in bone marrow in selected myeloproliferative and lymphoproliferative diseases

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The aim of the study was an estimation of the histomorphometry of megakaryocytes (MK) in bone marrow in selected myeloproliferative and lymphoproliferative diseases. Bone marrow specimens were obtained by trephine biopsies from 41 patients with polycythaemia rubra vera (PV), idiopathic myelofibrosis (MF), chronic lymphocytic leukaemia (CLL), hairy cell leukaemia (HCL) and diffuse large B-cell lymphoma (L). Morphometric evaluation was performed using a standard program set MicroImage (OLYMPUS). The greatest number of typical nucleated MK, "naked" nuclei, anucleated cytoplasmic fragments and the largest area were found in PV. The circular deviation factor of MK and their nuclei increased in all cases. The greatest number of clusters was observed in PV and HCL. A significant increase in the number of dysplastic and "naked" nuclei of MK was noted in all selected haematological diseases. The presence of neoplastic cells in bone marrow increased the morphological changes in MK. Quantitative and morphometrical significant differentiation of MK in separate microscopic field in the same slides confirms the necessity of performing trephine biopsies in each patient with haematological disorders.

key words: trephine biopsy, histomorphometric features, haematological disorders

INTRODUCTION

Megakaryocytes (MK) are the least numerous (around 0.05%) and at the same time the biggest (12–150 μm) of all mononuclear bone marrow cells. Due to their size, they are called the giant cells of the marrow. They are characterised by a big multilobar nucleus surrounded by a double nuclear membrane with frequent gaps. The existence of lobes suggests the multinuclear quality of the cells. The developmental stages, according to Odell et al. [13] and Ebbe et al. [5], include three main developmental stages: stage I — megakaryoblast, stage II — megakaryocyte polychromatophil and stage III — granular megakaryocyte, capable of the production of platelets. The final outcome of the differentiation and maturation of the megakaryocytic system are the platelets. One MK can release to the cavity vessels of the marrow up to several thousand platelets [14]. Taking into account the nuclear/cytoplasmic ratio (N/C) owing to the shrinking of the cytoplasm we can identify mature nuclear forms of MK, the so-called "naked nuclei" which have only a narrow surrounding

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membrane or none at all with cytoplasmic fragments [17]. Each form of the mature MK may create the so-called "cluster forms" which appear especially often in the neighbourhood of the cavity vessels. The cluster forms of MK are closely adjacent in groups of three or more MK [4, 7].

MK are situated mainly in the direct neighbourhood of cavity vessel membranes. Their cytoplasm penetrates into the gaps between the endothelial cells. Fragments of the cytoplasm of MK condition the production and release of the platelets directly into the bloodstream. MK are not only the precursor cells for the platelets but constitute an important element of the blood-marrow barrier which controls the release of other cells of the haematopoietic system into the blood [4, 8, 16]. MK next to the platelets are in a small percentage a physiological element of the peripheral blood [3, 6]. In patients with diseases of the haematopoietic system and the lymphatic system, athero-vascular complications and the presence of the inborn defects of the blood are among the most often encountered complications. They are a result of the disturbances present in the megakaryocytic system of the bone marrow. The aim of the study was the histomorphometric assessment of the marrow MK in selected proliferative disorders of the lymphatic and haematopoietic systems.

MATERIAL AND METHODS

The study was carried out in 41 patients with various haematological disorders: 6 with polycythaemia vera rubra (PV), 8 with idiopathic myelofibrosis (MF), 11 with chronic B-cell leukaemia (CLL), 8 with hairy cell leukaemia (HCL), 8 with malignant B-cell lymphoma (L). Among the patients there were 13 women and 28 men aged from 20 to 87 years, mean age was 58.6 years. The material for morphological assessment was achieved by bone marrow trephine biopsy (TB) with the use of Jamshidi needle from the postero-superior iliac crest. Directly after this procedure, the material was fixed in the so-called "Oxford solution". After fixing (48–72 hours), the oval-shaped fragments of bone marrow from 1 to 2 cm long and of 1 mm diameter were put into paraffin bars. Thin sections at five μm were cut by the use of microtome. Routine staining techniques, such as haematoxylin and eosin for recognition of cellularity, were applied. The identification of the young, immature MK was carried out with the use of immunochemistry with the CD41 antibodies (5B12 clone) defining the platelet glycoprotein IIb (catalogue no M7057, DAKO) and CD61 antibodies (Y2/51clone) defining the IIIa platelet glycoprotein (catalogue no M0753, DAKO). Morphometric tests were conducted using a standard program set MicroImage (OLYMPUS). The achieved results were next analysed using the computer program Statistica Pl. The comparative group (C) was the bone marrow taken during the autopsy of 8 patients who had died because of myocardial infarction.

RESULTS

The greatest mean number of MK in 1 mm^2 of the bone marrow surface was found in patients with PV (50.17), while the smallest mean number was observed in the group C (17.56) and the differences were statistically significant ($p < 0.05$). There were also statistically significant differences in the mean number of MK between the patients with PV and the remaining patients. Similar results and correlations were found when analysing the number of cytoplasmic fragments (CMK). The highest mean number of naked nuclei (NNMK) in 1mm^2 of the bone marrow surface was found in patients with MF (3.29), while the smallest mean number was assessed in the group of patients with z CLL (1.12). Statistically significant differences were also found ($p < 0.05$) in the mean number of NNMK between patients with PV and patients with MF and L. The similar differences were also observed between patients with MF and patients with CLL, HCL and L. The highest mean number of MK area (AMK) in μm^2 was found in patients with MF (357.54), and the smallest in the group C (173.63). Statistically significant differences ($p < 0.05$) of the mean AMK were found between C and other studied groups, and between patients with PV and the remaining patients. Analysing the N/C ratio the differences were also observed between the patients with PV (0.27), and patients with HCL (0.24) and L (0.24). The similar differences were also observed between patients with MF (0.29), and subjects with CLL (0.26), HCL (0.24) and L (0.24). Those differences were statistically significant ($p < 0.05$). Statistically significant differences ($p < 0.05$) of the circular deviation factor of MK (CDMK) and their nuclei (CDNNMK) were also observed between group C and other studied groups. The incidence of clusters was highest in the patients with PV and HCL, while in group C the presence of single clusters was observed only in three cases and those differences were statistically significant (Table 1, 2).

Table 1. Histomorphometric features (means ± standard deviations) of megakaryocytes in the bone marrow of patients in studied groups

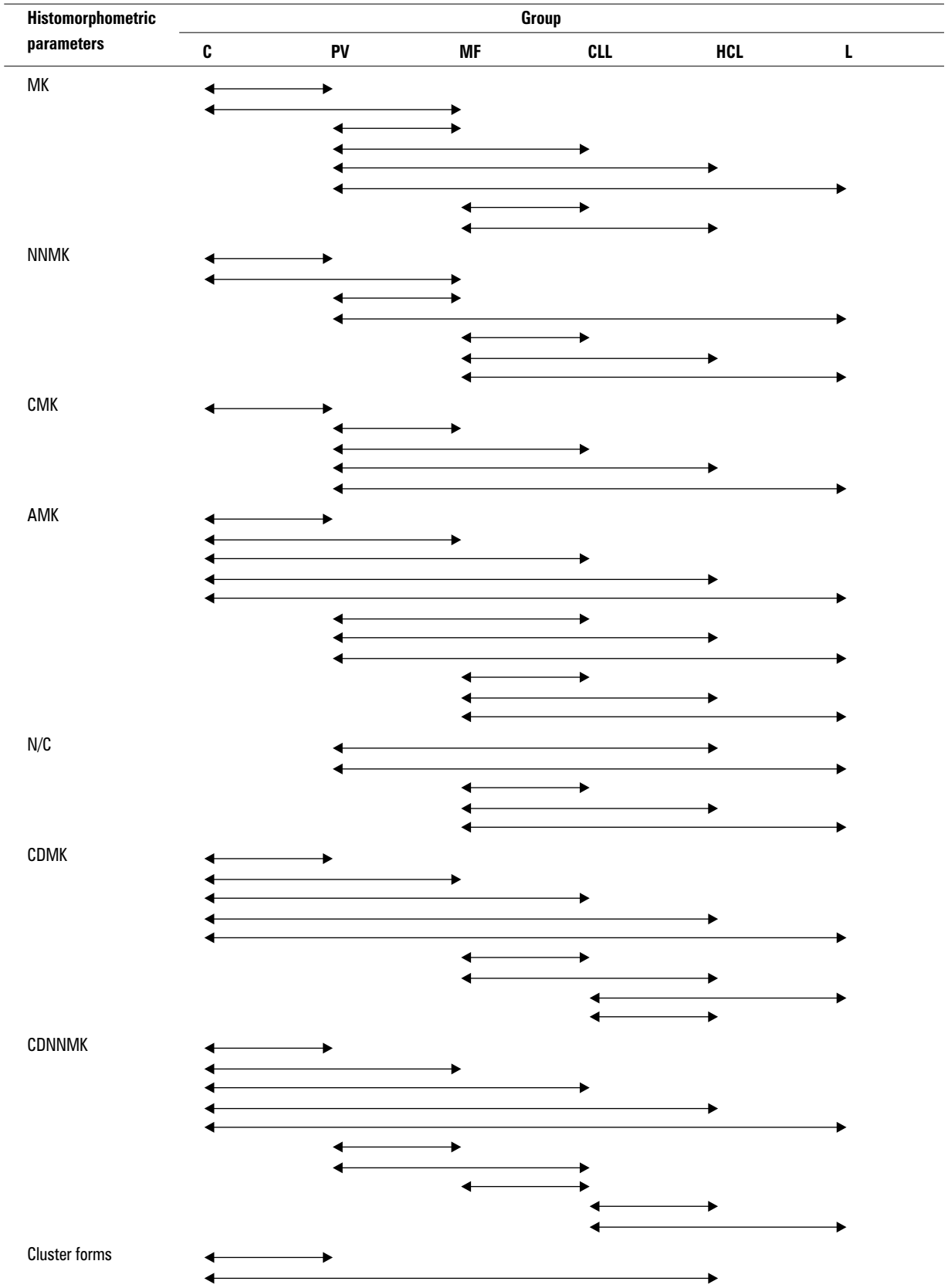
Group	Histomorphometric parameters							
	MK X ± SD	NNMK X ± SD	CMK X ± SD	AMK X ± SD	N/C X ± SD	CDMK X ± SD	CDNNMK X ± SD	Cluster forms X ± SD
C	17.56 ± 6.26	1.25 ± 0.58	1.31 ± 0.7	173.63 ± 73.31	0.23 ± 0.02	0.84 ± 0.02	0.73 ± 0.02	0.25 ± 0.45
PV	50.17 ± 13.51	1.61 ± 0.92	4.89 ± 2.22	327.28 ± 77.62	0.27 ± 0.03	0.75 ± 0.06	0.55 ± 0.07	1.06 ± 0.94
MF	32.25 ± 16.06	3.29 ± 1.71	1.58 ± 1.47	357.54 ± 58.52	0.29 ± 0.04	0.73 ± 0.06	0.57 ± 0.07	1.00 ± 1.53
CLL	20.39 ± 7.66	1.12 ± 1.05	1.45 ± 1.33	245.09 ± 46.84	0.26 ± 0.03	0.78 ± 0.04	0.67 ± 0.06	1.90 ± 1.60
HCL	21.96 ± 8.00	1.25 ± 1.07	1.88 ± 1.39	227.04 ± 56.91	0.24 ± 0.03	0.78 ± 0.07	0.60 ± 0.06	1.80 ± 1.10
L	22.05 ± 7.63	0.95 ± 0.86	1.52 ± 1.08	241.33 ± 57.82	0.24 ± 0.03	0.73 ± 0.06	0.59 ± 0.06	1.50 ± 2.12

DISCUSSION

Histological examination of the bone marrow achieved by TB facilitates morphological assessment of the megakaryocytic system. Qualitative and quantitative changes of the MK, which result from morphological and functional disturbances at every developmental stage of the haematopoietic system, are connected with the production of morphologically and functionally changed platelets or with their significant quantitative disturbance (increase or decrease) in blood. This can be connected with the athero-vascular complications or appearing of the haemorrhagic diathesis. Many morphometric analyses of the marrow in the chronic myeloproliferative diseases (CMPD) are based on the megakaryocytic lineage assessment. In order to make a morphometric analysis of the MK in the marrow, the number of MK in 1 mm^2 was taken into account in order to see if there had been an uncontrolled growth in the megakaryocytic strain, their area, nuclear/cytoplasmic ratio, circular deviation factor and the presence of the cluster forms. Kaloutsi et al. [7] found that statistically significant differences in the number of MK are present in the comparative group and the groups of patients with PV and MF. These differences were noticed when assessing mean values of the MK number in 1 mm^2 in the bone marrow surface with the use of statistical analysis. Nafe et al. [12], making a quantitative analysis of different morphological parameters of MK in CMPD, found that the best general parameter that helps to differentiate between the comparative group and the patients with CMPD (excluding CML, CT-chronic myeloid leukaemia, common type) is the mean number of MK in 1mm^2. They also found that the best differentiating feature between the PV and MF is the number of the "naked nuclei" in MK. In the presented paper we found that there were statistically significant differences in the number of "naked nuclei" of MK, not only in comparison between the comparative group and patients with PV and MF, but also between those last two groups. Elevated numbers of these so-called naked nuclei were observed by Thiele et al. [19]. Changes in the structure of MK, characterised *inter alia* by the presence of the degenerated forms of MK (naked nuclei), are the main differentiating feature in the diseases included as CMPD. This fact is significant due to the release of various cytokines (PDGF, platelet derived growth factor; PF-4, platelet factor 4) from MK, which in turn activates fibrosis. Like Thiele et al. [18], we found a highly uneven area of MK in PV oscillating between 176 and 484 mm^2, as well as very different circular deviations of the nuclei and to a lesser extent the circular deviation of MK. However there were no statistically significant differences between the area of MK in the groups of patients with PV and MF. Simultaneously there was a statistically significant difference of the area of MK in the comparative group and the remaining groups and groups with PV and MF. There were no statistically significant differences between the N/C ratio in the comparative group and groups with PV and MF and between the remaining groups.

In the first stage of the myelofibrosis in the histological study the bone marrow can be rich in cells and may show features of the normal maturation or insignificant dysplastic changes of MK. In the later stage there is an evident domination of MK with the presence of their numerous precursors with different morphological features ranging from micromegakaryocytes and single nucleus forms to giant forms with a hyperchromatic nucleus of the excessive number of lobes. MK creates groups of cells which are called clusters. Around them the number of the reti-

Table 2. Comparison of means values of the histomorphometric features of megakaryocytes between studied groups. The arrow marks statistically significant differences ($p < 0.05$)



culin is moderately increased. In consequent stages reticulin fibrosis dominates over cells of the haematopoietic system and collagen fibres appear. In the later stage the process of fibrosis becomes stronger. The fibrosis of the marrow is focal. The haematopoietic cells which are encountered are mainly MK. In the progress of fibrosis morphological disturbances in the platelet are often observed [9, 15].

In the studied group of patients with MF, fields of the haematopoietic tissue with a small number of cells were found with the domination of MK, which suggests that those were later stages of the disease. In those patients we found disturbances of the CDMK and CDNNMK with the lack of increased number of the cytoplasm fragments. Perhaps single fragments of the cytoplasm were released directly into the lumen of the marrow vessels.

Dysmegakaryocytopoiesis, which is characterised by the presence of micromegakaryocytes, mikromegakaryoblasts, promegakaryoblasts, "naked nuclei" and promegakaryocytes, leads to the production of the functionally damaged platelets, which was noticed by Matolcsy and Majdic in their study of bone marrow in patients with CMPD [10]. They also observed the presence of the agranular, dysplastic or giant forms occasionally with the presence of the nucleus in the platelets, disconnected from the "naked nucleus". They presented different hypotheses connected with dysmegakaryocytopoiesis. It may be a result of the neoplastic transformation of stem cells of the marrow or disturbances in the production of different cytokines by haematopoietic inductive microenvironment cells. In the marrow of subjects with CMPD, Chott et al. [2] observed the presence of MK in groups described as clusters. Kaloutsi et al. [7] also assessed the presence of the clusters through the examination of differences between single so-called isolated forms of MK and their frequency in clusters in five subtypes of CMPDs and the comparative group. The incidence of clusters was increased in patients with MF. However, the difference between MF and the remaining diseases was not statistically significant. In the presented paper, similar results were achieved because the clusters were present in all patients with myelofibrosis but there was no statistically significant difference in comparison to other examined patients. However, a statistically significant difference was found between P and patients with PV and HCL.

In the presented study the disturbances of the MK in bone marrow were found not only in the patients with diseases included as myeloproliferative disorders but also in the group of patients with lymphoproliferative diseases. The histomorphometric assessment of MK in the case of diseases of the lymphatic system is rarely encountered. In the assessment of the marrow MK their localisation is especially taken into account.

In CLL MK may be spread between the cells of other developmental strains especially between lymphocytes [1]. In this study an irregular pattern of MK in the haematological tissue was observed in the group of patients with CLL; moreover, in three cases we found a focal grouping of cells and an increased percentage of clusters.

Increased presence of the clusters in HCL is interesting because we observed a cluster of fifteen MK in this group of patients. We found a statistically significant difference in the incidence of the clusters not only between P and PV but also between P and HCL. This may be connected with the proliferation of the "specific" lymphocytes, which cause the grouping of the MK, and whose physiology has not yet been described [11]. In the presented study, statistically significant differences were observed when assessing the area of MK, circular deviation of MK and their nuclei between group C and patients with CLL, HCL and L. The presence of neoplasm cells in the bone marrow increased the incidence of morphological changes of MK.

In the myeloproliferative disorders and in non-Hodgkin's lymphomas an insignificant increase in the number of dysplastic megakaryocytes and the naked nuclei of MK is observed. High quantitative and morphological differentiation of MK in certain microscopic fields of the same histological material confirms the necessity for conducting TB of the bone marrow in all patients with diseases of the lymphatic and haematopoietic systems. The observed MK changes in the studied groups may influence the presence of disturbances in haemostasis in patients with myeloproliferative and lymphoproliferative diseases.

REFERENCES

1. Brunning RD, McKenna RW (1994) Tumors of the bone marrow. Atlas of tumor pathology. Third Series. Armed Forces Institute of Pathology. Washington, DC.
2. Chott A, Gisslinger H, Thiele J, Fritz E, Linkesch W, Radaszkiewicz T, Ludwig H (1990) Interferon-alpha-induced morphological changes of megakaryocytes: a histomorphometrical study on bone marrow biopsies in chronic myeloproliferative disorders with excessive thrombocytosis. Br J Haemat, 74: 10–16.

3. Dzięcioł J, Dębek W, Chyczewski L, Sulkowski S, Kozielec Z, Kisielewski W (1995) Megakariocyty krążące we krwi w doświadczalnym wstrząsie krwotocznym u szczurów. Acta Haematol Pol, 1: 91–97.
4. Dzięcioł J, Pająk J, Dębek W, Chyczewski L, Sulkowski S, Kozielec Z (1995) Histomorfometryczna ocena megakariocytów szpiku kostnego w doświadczalnym wstrząsie krwotocznym u szczurów. Acta Haematol Pol, 26 (1): 81–89.
5. Ebbe S, Stohlman F (1965) Megakaryocytopoiesis in the rat. Blood, 26: 20–35.
6. Han ZC, Bellucci S, Caen JP (1991) Megakaryocytopoiesis: characterization and regulation in normal and pathologic states. Intern J Haematol, 54: 3–14.
7. Kaloutsi V, Fritsch RS, Buhr T, Restrepo-Specht I, Widjaja W, Georgii A (1991) Megakaryocytes in chronic myeloproliferative disorders: numerical density correlated between different entities. Virchows Archiv A Pathol Anat, 418: 493–497.
8. Lee KP (1989) Emperipolesis of hematopoietic cells within megakaryocytes in bone marrow of the rat. Vet Pathol, 26: 473–478.
9. Maryniak RK (1999) Przydatność badań histopatologicznych szpiku w diagnostyce chorób rozrostowych krwi. Acta Haematol Pol, 30 (Suppl 1): 105–109.
10. Matolcsy A, Majdic O (1990) Circulating megakaryoblasts in chronic myeloproliferative diseases. Acta Haematol, 84: 57–63.
11. Mioduszewska O (1998) Patologia chłoniaków i ziarnicy złośliwej. Pol J Pathol, 49 (Suppl 4): 47.
12. Nafe R, Georgii A, Kaloutsi V, Fritsch RS, Choritz H (1991) Planimetric analysis of megakaryocytes in the four main groups of chronic myeloproliferative disorders. Virchows Archiv B Cell Pathol, 61: 111–116.
13. Odell TT, Jackson CW (1968) Polyploidy and maturation in rat megakaryocytes. Blood, 32: 102–110.
14. Podolak-Dawidziak M (1991) Rozwój, budowa i dojrzewanie megakariocytów. Cz. I. Pol Arch Med Wewn, 85: 128–133.
15. Rzepecki P, Sułek K, Hałka J (1999) Histologiczne klasyfikacje przewlekłych zespołów mieloproliferacyjnych. Acta Haematol Pol, 30 (3): 241–248.
16. Tavassoli M (1986) Modulation of megakaryocyte emperipolesis by phlebotomy: megakaryocytes as a component of marrow-blood barrier. Blood Cells 12: 205–216.
17. Thiele J, Fischer R (1991) Megakaryocytopoiesis in haematological disorders: diagnostic features of bone marrow biopsies. Virchows Arch A Pathol Anat, 418: 81–97.
18. Thiele J, Schneider G, Hoeppner B, Wienhold S, Zankovich R, Fischer R (1988) Histomorphometry of bone marrow biopsies in chronic myeloproliferative disorders with associated thrombocytosis - features of significance for the diagnosis of primary (essential) thrombocythaemia. Virchows Archiv A Pathol Anat, 413: 407–417.
19. Thiele J, Steinberg T, Hoeppner B, Wienhold S, Wagner S, Dienemann, Fischer R (1990) Histo- and immunomorphometry of megakaryopoiesis in chronic myeloid leukemia with myelofibrosis and so-called primary (idiopathic) osteo-myelofibrosis/-sclerosis. Anal Cell Pathol, 2: 215–227.