ORIGINAL PAPER

Differential diagnosis between chronic granulocytic leukemia, polycythaemia vera and essential thrombocythemia using micro- and ultrastructural measurement data performed at the level of megakaryocyte

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Abstract
The common features of chronic myeloproliferative disorders (CMDs) make the differential diagnosis on clinical or paraclinical basis to become more difficult, and validate the requirements for new methods of detailed diagnosis. Besides the cytogenetic methods, the megakaryocyte (MK) morphology is a valuable element of diagnosis included in the recent “en vogue” criteria. The purpose of this paper is to compare different morphological parameters in MKs from patients diagnosed with three CMDs and to establish a differential diagnosis of these disorders. Studies were performed on smears of bone marrow blood — for light microscope analysis —, and bone marrow biopsies — for transmission electron microscope (TEM) analysis — collected from six patients, two diagnosed with chronic granulocytic leukemia (CGL), two with polycythaemia vera (PV), and other two with essential thrombocythemia (ET). On the light microscope images, we observed important differences between the sizes of MKs and of MKs nuclei in CGL, PV, and ET. On the TEM images, we also noted important differences concerning the size and the aspect of nuclei, aspect of mitochondria, the amount and distribution of RER, Golgi apparatus and demarcation membrane system; the most important are the differences recorded in the number, distribution and sizes of vacuoles, alpha-granules and of the dense bodies. This study provides evidence that there are significant morphological differences between the cellular structures in the MKs from the patients with diagnosed with different CMDs, and thus sustains the utilization of this approach for establishing the differential diagnosis of CMDs.

Keywords: megakaryocyte, morphology, ultrastructure, TEM, myeloproliferative disorders, chronic granulocytic leukemia, essential thrombocythemia, polycythaemia vera.

Introduction
Chronic myeloproliferative disorders (CMDs) are a group of hematological diseases, which share a common alteration produced at the level of the marrow hematopoietic stem cell, a multipotent cell, the “mother cell” of all blood cell lines. This cell eventually clonal proliferates and, consequently, increases the number of cells in the peripheral blood, which is the substrate of the dramatic clinical picture, and of the low survival ratio in the absence of an untimely diagnosis and treatment. Description of several CMDs is based on the prevalence of cells’ proliferation in one of the blood lines. However, the common features of these diseases (origin, multi-line proliferation, hepatic and splenic myeloid metaplasia, evolution towards acute leukemia and myelofibrosis) make the differential diagnosis on clinical or paraclinical basis to become more difficult, and validate the requirements for new methods for detailed diagnosis [1]. The discovery of the Philadelphia chromosome [2, 3], and of the BCR–ABL translocation involving chromosomes 22 and 9 [4, 5] characteristic for chronic granulocytic leukemia (CGL), the detection of a point mutation of the Janus Kinase 2 (JAK2) gene identified only in patients with polycythaemia vera (PV), essential thrombocythemia (ET) or myeloid metaplasia with myelofibrosis (MMM) [6–8], as well as other molecular mechanisms involved in their pathogenesis [8] have redraw the algorithm of diagnosis in these diseases. Under these circumstances the megakaryocyte (MK) morphology becomes a valuable element of diagnosis included in the recent “en vogue” criteria [9].

The purpose of this paper is to compare the different morphological parameters at structural and ultrastructural levels in megakaryocytes from patients diagnosed with three CMDs and to determine whether
these parameters can be useful for establishing a differential diagnosis of the CMDs.

Material and Methods

Studies were performed on bone marrow biopsies from six patients hospitalized in the Clinic of Hematology, “Prof. Dr. Ion Chiricuță” Oncology Institute, Cluj-Napoca, of which the informed consent was obtained. The biopsies of the posterior iliac crest were collected under local anesthesia. Two of the six patients were diagnosed with CGL, two with PV, and other two with ET. The samples (of 1 mm³ each) were prepared for transmission electron microscopy (TEM) examination according to the usual protocols [10–13]: prefixation for 2 hours in 2.7% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.4), post-fixation for 1.5 hours in 2% osmium tetroxide (in 0.15 M phosphate buffer, pH 7.4), dehydration with five solutions of acetone of increasing concentrations (between 50–100%, 15 minutes each), infiltration with solutions of Epon 812 in acetone (1:2, 1:1, 2:1, 1 hour each, and in pure Epon overnight), and embedding in Epon 812 (72 hours at 60°C). The ultrathin sections of 70–80 nm thickness were obtained on a LKB 8800 ULTROTOME® III ultramicrotome (LKB, Bromma, Sweden), using a DiATOME diamond knife (Diatome Ltd., Bienne, Switzerland), then stained with uranyl acetate and lead citrate. Sections were examined in a JEOL JEM 100CX II transmission electron microscope (Jeol Ltd. Tokyo, Japan) at 100 kV acceleration voltage and magnifications between 3600× and 19 000×. The most representative images were photographed on 4489 Kodak electron microscope films (Carestream Health Inc., New York, USA), and the films were scanned in an Imacon Flexight X5 film scanner (Hasselblad Imacon, Copenhagen Sweden), using the computer software Imacon FlexColor X5 (Hasselblad Imacon, Copenhagen Sweden).

The blood of the bone marrow, obtained from the patients by bone marrow aspiration within the same examination, was used for preparation of blood smears. The blood smears, stained by the May–Grnwald–Giemsa method [14, 15], were examined in a Nikon Eclipse 80i light microscope (Nikon Corporation, Tokyo, Japan), with objectives of 20× and 40×, using an Olympus Color View 1 CCD camera (Olympus Soft Imaging Solutions GMBH, Münster, Germany).

Morphological measurements on both, electron- and light microscope images, were made with the CellD Olympus computer software (Olympus Soft Imaging Solutions GMBH, Münster, Germany), and the statistical analysis was performed using the Microsoft Office Excel software (Microsoft Corporation, Redmond, USA).

Results

Light microscopy

The three disorders were characterized by uniform distribution of MKs, either grouped into “clusters” or dispersed as isolated cells.

By comparing the largest and the mean diameter (mk d), and the area (mk a) of MKs, we observed important differences between the CMDs studied by us. Thus, we observed the smallest cells in CGL (Figure 1), followed by PV (Figure 2), while in patients diagnosed with ET we found the highest values (Figure 3, Table 1).

Table 1 – The results of morphological sizing of some MK parameters in images of light microscopy

<table>
<thead>
<tr>
<th>D</th>
<th>CGL</th>
<th>PV</th>
<th>ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>mk d [µm]</td>
<td>33.26±7.92</td>
<td>58.83±14.34</td>
<td>71.44±29.80</td>
</tr>
<tr>
<td>(n = 197)</td>
<td>(n = 26)</td>
<td>(n = 47)</td>
<td></td>
</tr>
<tr>
<td>mk a [µm²]</td>
<td>847.46±222.38</td>
<td>2407.61±1124.63</td>
<td>3678.12±2955.22</td>
</tr>
<tr>
<td>(n = 197)</td>
<td>(n = 26)</td>
<td>(n = 47)</td>
<td></td>
</tr>
<tr>
<td>n a</td>
<td>16.57±4.59</td>
<td>37.1±10.19</td>
<td>39.69±15.48</td>
</tr>
<tr>
<td>(n = 197)</td>
<td>(n = 26)</td>
<td>(n = 47)</td>
<td></td>
</tr>
<tr>
<td>n d</td>
<td>186.05±106.62</td>
<td>954.77±512.05</td>
<td>1119.79±791.72</td>
</tr>
<tr>
<td>(n = 197)</td>
<td>(n = 26)</td>
<td>(n = 47)</td>
<td></td>
</tr>
</tbody>
</table>

*D – studied disease. CGL – chronic granulocytic leukemia, PV – polycythemia vera, ET – essential thrombocythemia, mp – measured parameter, mk d – megakaryocyte mean diameter, mk a – megakaryocyte mean area, n d – nuclear mean diameter, n a – nuclear mean area.

Diameter (n d) and area (n a) of the nucleus have the same distribution, with more similar values in PV and ET (see also Table 1).

The nucleo-cytoplasmic ratio is only 0.51±0.01 in CGL (n = 98), while it is the highest in PV, 0.67±0.02 (n = 26), and it is of 0.57±0.02 (n = 48) in ET.

Electron microscopy

In the TEM images, we found different aspects of MKs, which are related to their functional status. A first type is represented by bigger MKs, with a high amount of cytoplasm, also with a very well developed demarcation membrane system, and a high amount of proplatelets (the so-called type III–IV MKs); around such MKs blood, sinuses can be observed and the process of platelets’ detachment is obvious. On the other hand, MKs with a much thinner layer of cytoplasm around the nucleus were observed, with very low amount or even without the demarcation membranes system; near such MKs, sinuses were also identified, all these findings suggesting either the presence of type 1 MKs, or the recent end of the process of platelets releasing from mature MKs.

Examination of the ultrathin sections made on samples collected from patients diagnosed with CGL revealed MKs with small nuclei, with only 2–3 lobes and with a dilated perinuclear space, high amounts of rough endoplasmic reticulum (RER) and Golgi apparatus (3–4 complexes/image), and also oval-shaped mitochondria with transversal cristae. The demarcation membranes system is abundant and MK fragmentation into prospective platelets is obvious at the periphery of cells; in some cases, the fragmentation is more profound inside the cytoplasm, up close to the nucleus. The demarcation membranes have narrow, electron-transparent spaces. These cells have a high number of vacuoles, some of them very big, placed near nucleus and the others, smaller, uniformly distributed in the cytoplasm. The alpha-granules are in a high number and they are placed near the nucleus, but mostly towards the periphery, where they are present in higher amount in
the MKs with a thin layer of cytoplasm (Figures 4 and 5). On the other hand, these granules are uniformly distributed in all the cytoplasm of the MKs of bigger size. In these MKs, we also found few granules with dense core – the dense bodies, spreaded between the membranes of the demarcation system.

In the images obtained on the samples collected from patients diagnosed with PV, we observed MKs with big, lobated nuclei, with a high amount of euchromatin and a perinuclear space with normal aspect. RER is present in a very high amount, the Golgi apparatus is abundant (2–3 complexes/image), and many spherical mitochondria can be observed, without visible cristae and with granular matrix. The demarcation membranes system is poor represented, and in most of the studied cells, the fragmentation of MKs cytoplasm is not so obvious. Membranes of demarcation have small, narrow spaces with electron-transparent regions. These cells also have vacuoles in a low number, and few dense bodies localized in cytoplasm, near the inner region of the demarcation membrane system. The alpha-granules, observed in a high number, are uniformly distributed in all the cytoplasm, from the proximity of the nucleus, up close to the plasma membrane (Figures 6 and 7).

In the images obtained on the samples collected from patients diagnosed with ET we observed MKs with very big, multilobated nuclei, with a very dilated perinuclear space, a low amount of RER and of Golgi apparatus as well (one complex/image), and spherical mitochondria without visible cristae. The demarcation membranes system is present in high amount and the fragmentation of the cytoplasm of MKs is observed only at the periphery of cells. Membranes of demarcation have small, narrow spaces with electron-transparent regions. In the cytoplasm of most of these cells big vacuoles, in high number, have been observed, the biggest being localized close to nucleus. The alpha-granules, of big sizes and in a lower number than in the patients with CGL or PV, are present only to the periphery of the cell and not around the nucleus (Figures 8 and 9). In this disease, we found the higher number of dense bodies, disseminated in all the cytoplasm.

The measurements made on intracytoplasmic structures include the diameter and area of three elements: vacuoles, whose number also characterize the demarcation membranes system, alpha-granules and dense bodies.

Figure 1 – Low-size megakaryocyte in a patient diagnosed with CGL (May-Grünwald–Giemsa stain).
Figure 2 – Megakaryocyte in a patient diagnosed with PV (May-Grünwald–Giemsa stain).
Figure 3 – Big megakaryocyte in a patient diagnosed with ET (May-Grünwald–Giemsa stain).
Figure 4 – General view of a megakaryocyte in a patient with CGL (×2900 original magnification): nucleus with two visible lobes and prominent nucleolus, very well developed demarcation membrane system, high number of vacuoles and alpha-granules. The releasing of platelets is also visible.
Figure 5 – Detailed view of a megakaryocyte in a patient with CGL (×5800 original magnification): nucleus with dilated perinuclear space, very well developed demarcation membrane system, high number of vacuoles and alpha-granules.

Figure 6 – General view of a megakaryocyte in a patient with PV (×4800 original magnification): nucleus with many visible lobes, demarcation membrane system poor represented, low number of vacuoles and relative high number of alpha-granules.

Figure 7 – Detailed view of a megakaryocyte in a patient with CGL (×5800 original magnification): nucleus with nucleolus, demarcation membrane system poor represented, low number of vacuoles and relative high number of alpha-granules; a platelet is released into sinus.

Figure 8 – General view of a megakaryocyte in a patient with ET (×3600 original magnification): nucleus with three visible lobes and dilated perinuclear space, demarcation membrane system in high amount, big vacuoles in high number, and alpha-granules in a lower number.

In CGL and PV, we recorded similar values, distinctly smaller than those of ET did, which again were on the top (Table 2).

Table 2 – The results of morphological sizing of some MK parameters in images of electron microscopy*

<table>
<thead>
<tr>
<th>D</th>
<th>CGL</th>
<th>PV</th>
<th>ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>v d [µm]</td>
<td>33.26±7.92 (n=197)</td>
<td>58.83±14.34 (n=26)</td>
<td>71.44±29.80 (n=47)</td>
</tr>
<tr>
<td>v a [µm²]</td>
<td>847.46±422.38 (n=197)</td>
<td>2407.61±1124.63 (n=26)</td>
<td>3678.12±2955.22 (n=47)</td>
</tr>
<tr>
<td>ag d [µm]</td>
<td>16.57±4.59 (n=197)</td>
<td>37.1±10.19 (n=26)</td>
<td>39.69±15.48 (n=47)</td>
</tr>
<tr>
<td>ag a [µm²]</td>
<td>186.05±106.62 (n=197)</td>
<td>954.77±512.05 (n=26)</td>
<td>1119.79±791.72 (n=47)</td>
</tr>
<tr>
<td>db d [µm]</td>
<td>16.57±4.59 (n=197)</td>
<td>37.1±10.19 (n=26)</td>
<td>39.69±15.48 (n=47)</td>
</tr>
<tr>
<td>db a [µm²]</td>
<td>186.05±106.62 (n=197)</td>
<td>954.77±512.05 (n=26)</td>
<td>1119.79±791.72 (n=47)</td>
</tr>
</tbody>
</table>

Statistical analysis (t-test)

The statistical significance of differences found between the parameters chosen for our morphological measurements is given in Table 3 and discussed below.

Table 3 – The statistical significance of differences found between the parameters chosen for our morphological measurements

<table>
<thead>
<tr>
<th>D</th>
<th>CGL–PV</th>
<th>CGL–ET</th>
<th>PV–ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>mk d</td>
<td>p≤0.001</td>
<td>p≤0.001</td>
<td>p=0.009</td>
</tr>
<tr>
<td>mk a</td>
<td>p≤0.001</td>
<td>p≤0.001</td>
<td>p=0.017</td>
</tr>
<tr>
<td>n d</td>
<td>p≤0.001</td>
<td>p≤0.001</td>
<td>p=0.2</td>
</tr>
<tr>
<td>n a</td>
<td>p≤0.001</td>
<td>p≤0.001</td>
<td>p=0.16</td>
</tr>
<tr>
<td>n-c r</td>
<td>p≤0.001</td>
<td>p≤0.001</td>
<td>p=0.003</td>
</tr>
<tr>
<td>v d</td>
<td>p=0.34</td>
<td>p≤0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>v a</td>
<td>p=0.2</td>
<td>p≤0.001</td>
<td>p=0.001</td>
</tr>
<tr>
<td>ag d</td>
<td>p=0.006</td>
<td>p≤0.001</td>
<td>p=0.001</td>
</tr>
<tr>
<td>ag a</td>
<td>p=0.005</td>
<td>p≤0.001</td>
<td>p=0.001</td>
</tr>
<tr>
<td>db d</td>
<td>p=0.086</td>
<td>p=0.059</td>
<td>p=0.1</td>
</tr>
<tr>
<td>db a</td>
<td>p=0.075</td>
<td>p=0.1</td>
<td>p=0.11</td>
</tr>
</tbody>
</table>


Discussion

CMDs are clonal diseases of the pluripotent or multipotent hematopoietic stem cell. They are characterized by an abnormal accumulation of mature cells, and a chronic evolution [1]. CGL is the only one of this group of maladies, which is accompanied by a “stigma” – the presence of Philadelphia (Ph1) chromosome [2, 3] and/or of BCR/ABL translocation [4, 5]. The diagnosis of Ph1-negative CMDs is often established using “positive” criteria regarding certain clinical, hematological, or biological features, and “negative” criteria as well, namely the exclusion of some cases in which similar hematological abnormalities may occur [16, 17].

However, for the moment, the aspect and distribution of MKs on the stained smears of the bone marrow studied under the light microscope remain among the factors taken into account in order to establish a diagnosis for CMD [18–20]. In these circumstances, the differential diagnosis value of the MK morphology is more recognized in the last decades. Thus, the studies performed using the light microscope proved a different aspect and morphology of the MKs in different disorders [21, 22].

In the images obtained after the examination in the light microscope of the stained smears of the six patients taken into study, the MKs were either observed as isolated cells or grouped in clusters – proving the existence of certain spatial–functional interactions between them. The aspect of MKs and the results of the morphological measurements performed by us correlate with the data reported by other authors [21, 22].

Thus, it can be noted that MKs in patients diagnosed with CGL have the smallest mean diameter, while MKs from patients with ET have the biggest mean diameter. The statistical analysis shows statistical significant differences between the MKs diameters in all the three disorders. The measurement results for MKs area have established the same hierarchy, with statistical significant differences between the MKs in the three diseases – even in the case of PV and ET where the standard deviations (SD) have relatively high values.

Concerning the sizes of nuclei of MKs, we report small nuclei in CGL, while in the other two disorders the mean diameter of nuclei is much higher, the cells with the biggest nuclei being found in the patients with ET. This finding is consistent with data describing MKs with more lobated nuclei in PV and ET, and the size of nucleus correlates in MKs with the polyploidy degree [23–25]. Moreover, Woodruff et al reported an increased proportion of MKs with high ploidy in ET [26]. Statistical significant differences were obtained only comparing the diameters of nuclei in CGL and PV, and CGL and ET respectively. Between the values recorded for the nuclei of MKs in PV and ET there are not statistical significant differences, first of all because of the similar values of the nuclear diameter in the patients with the two diseases, but particularly because of the wider distributions of the recorded values (high SDs). These results (diameter of MKs but especially the MKs diameter of nuclei) reflecting relationships between the general aspect of the cell and its metabolic and functional activity are confirmed by other hematological data such is the number of blood platelets. Thus, in the two patients diagnosed with CGL, we recorded low values for the number of circulating platelets (116 000 and 155 000/mm$^3$), while higher values were recorded in the two patients with PV (263 000 and 717 000/mm$^3$), and very high values in the two patients with ET (908 000 and 1 499 000/mm$^3$). The big difference noted between the values recorded for the patients with the same disorder, reflecting differences in the activity of MKs in those patients, can be also responsible for the high values of the SDs calculated by us for several parameters of MKs (including the MK diameter, and area, nuclear diameter and area, etc.). This high SDs also confirms the accelerated clonal proliferation of MKs, a high number of MKs of very different sizes being observed in our patients, especially in those diagnosed with PV and ET. In the case of the mean nuclear area, the results are similar to those recorded for the nuclear diameter of the cells, but this time, the differences between the nuclei from patients with CGL and the patients with the other two disorders is more pronounced, with statistical significant differences only between CGL–PV and CGL–ET. The statistical non-significant difference recorded between the data from patients with PV and ET can be explained, as in the case of the nuclear diameter, taking into account the high values of SD in the both cases.

The nucleo-cytoplasmic ratio of MKs is the smallest in CGL, bigger in ET and the biggest in PV, with statistical significant differences between the data recorded in the three cases. This changing in the values hierarchy is produced due to a much higher value for the MK mean diameter and area in ET compared to PV,
while in the case of the MK nuclear mean diameter and area, the recorded differences between the two diseases are very small.

Even thousands of studies presenting different ultrastructural aspects of the MK were published, the data concerning the particularities of the MK ultrastructure in the CMDs are only a few and most of them refer strictly to the circulating MKs; among these one of the most cited is the study of Maldonado, from 1974 [27]. However, despite of all these studies, the ultrastructural differences in MKs from bone marrow biopsies of patients with different CMDs have not been described so far. This is why the results of our study can represent an important breakthrough and a new direction in the differential diagnosis of the myeloproliferative disorders must be considered.

On the images recorded after the TEM examination of the ultrathin sections made in the bone marrow biopsies collected from the six patients taken into study, MKs in different functional status [1] have been found, and also the process of platelets releasing was observed in many MKs. As was previously described by other authors, the mature MK have prolongations crossing the endothelium, arrive in sinuses and release the platelets into the blood stream [1, 28–30]. The proplatelets formation involves a reorganization of the cytoskeleton [1, 30, 31], and for the platelets releasing, the reorganization of the extracellular matrix is requested [32]. The development of MK and the production of platelets is controlled by stimulating factors such as interleukin-3 [33], c-kit ligand [34], flt-3 ligand [35], interleukin-6 [36], interleukin-11 [37], NADPH-oxidase 1 [38], and by some inhibiting factors as well: transforming growth factor-β [39], platelet factor 4 and β-tromboglobulin [40], and interferon-α [41].

In our study, the characteristic ultrastructural features of the MKs [1, 30, 32, 42] have been observed in the case of all three diseases of interest for us. Thus, MKs can be observed as giant cells in bone marrow with a single, multilobated nucleus. In their cytoplasm, mitochondria, RER, Golgi apparatus and lysosomes can be observed, like in other eukaryotic cells. Specific for MK are few ultrastructural features, first of all the demarcation membrane system. These membranes are formed by invaginations of the plasma membrane delineating the future platelets, through a system of channels, which are continuous with the extracellular space [42–46]. Because after the release of platelets, parts of this system remain forming the open canalicular system of the mature platelets, it is also called by some authors the open canalicular system of MKs. In the cytoplasm of mature MKs, vacuoles derived from the demarcation membrane system can be also observed [42, 47]. Sometimes, a dense tubular system, derived from the endoplasmic reticulum is present, which serves as a calcium storage compartment [42]. Other structures characteristic for MKs are the future granules of platelets: alpha-granules, secretory organelles containing a large range of proteins with different roles in hemostasis [48–51], and dense core granules or dense bodies, containing serotonin, adenine nucleotides, calcium, pyrophosphate and glycoproteins [51–53]. In our study, we overlooked the discussion concerning lysosomes, even they are important structures in MKs, because they can be differentiate from the other granules only by the using of an appropriate molecular approach.

However, between the MKs of the patients diagnosed with different CMDs, we noted important ultrastructural differences concerning the size and the aspect of nuclei and their components (lobes, chromatin, nucleoli, perinuclear space), aspect of mitochondria, the amount and distribution of RER, Golgi apparatus and demarcation membrane system, and the number and distribution of vacuoles, alpha-granules and of the dense bodies. However, most important, significant differences were recorded after the morphological sizing of the last three common components of MK, as follows.

The mean diameters of the vacuoles in MKs of patients diagnosed with CGL and PV are relatively small and have almost the same value – low SDs must be also noted here. In CGL the number of vacuoles is high, while in PV we recorded a low number of vacuoles; these data correlate with the amount of demarcation membranes system and the MKs fragmentation degree into proplatelets in the patients with the two diseases, but is not consistent with the low number of circulating blood platelets recorded in the patients with CGL. The vacuoles in MKs of patients diagnosed with ET have a mean diameter which is almost three times bigger than in the first two cases, and in the same time display a very high value of SD. The size and the high number of this vacuoles correlate with the high amount of demarcation membranes system, and the fragmentation degree of MKs in ET, and is consistent with the high number of circulating platelets observed in the two patients taken into study. However, statistical significant differences concerning the diameters of vacuoles were noted only between the values recorded in patients with CGL and ET and in patients with PV and ET. The values for the mean area of vacuoles, measured on the ultrathin sections, have the same distribution as those of the mean diameter of vacuoles, with the same statistical significances. However, here, the differences recorded between the data of the patients with ET and with the other two diseases are much higher – more than eight times higher and also with a very high value of SD.

The values of the mean diameter of alpha-granules are smaller than those of vacuoles, and again are smaller in the patients with CGL and PV – the smallest in CGL (with small SDs in both cases), while this value is the highest in the patients with ET – more than two times higher, and with a very high value of SD. Even in the previously published literature data is mentioned a mean diameter for alpha-granules ranging between 200–500 nm [49, 51] we found a lot of much higher values in ET, over 1 μm, with the average of 790 nm. The statistical analysis shows statistical significant differences between the diameters of alpha-granules recorded in all the three disorders. The values for the mean area of alpha-granules, have the same distribution as those of the mean diameter of alpha-granules, with the same statistical significances. The differences recorded between the data of the patients with ET and with the
other two diseases are much higher — more than nine times higher, and also with a very high value of SD.

All the measured parameters in ET, the mean diameter and area of vacuoles, alpha-granules and dense bodies are very high as compared with the values recorded in the other two disorders; besides, very high values of SD are present, suggesting a high diversity of structures, and an intense activity of MKs, which were also found to be in different functional stages. In ET, especially the high values obtained for the mean diameter and area of alpha-granules, and also for the calculated SDs for these parameters, can be explained taking into account the functional activity of MKs: it was proved that the new synthesized alpha-granules are initially small, but, during MK maturation the alpha-granules become very prominent [48, 51]. Therefore, in our images the most MKs observed are of type III and IV, with a relative low number of mature, big alpha-granules. In the literature, alpha-granules in high number were reported in the MKs of animals exposed to pollutants [54].

The mean values calculated for the dense bodies are the smallest in the patients with CGL and the highest in the patients with ET, but there are no statistical significant differences between the values in the three cases; this is due to a very high value of SD in ET, even in CGL and PV small SDs were obtained, and also to a low number of measurements performed in CGL and PV. The values for the mean area of dense bodies have the same distribution as those of the mean diameter of dense bodies, with the same statistical significances, but are almost two times higher in the patients with ET as compared to the values of other patients, and also with a very high value of SD. The number of dense bodies recorded in patients with PV is not consistent with the number of platelets, but because in our case the bleeding time has not been noted, functional problems of the platelets could be considered [55].

In the literature is mentioned a low number of dense bodies in the circulating platelets in patients with several syndromes reviewed by Rendu F et al. [56], Yousssfain T et al. [52], McNicol A and Israels SJ [50], or King SM and Reed GL [49]. However, in all these cases the deficiencies of dense bodies involve also abnormalities of alpha-granules, a totally different situation compared to the results reported here by us. These aspects, together with others such as the differences between the ultrastructural data (high number of vacuoles, and abundant demarcation membrane system in MKs from patients with CGL, or the low number of dense bodies in MKs from patients diagnosed with PV) and the hematological data (the number of circulating blood platelets) can be explained taking into account the differences between the patients with the same disorder, and also as a result of a different turnover of one or more of the above mentioned stimulating or inhibiting factors involved in the normal activity of MKs.

Conclusions

Even on the TEM images, we cannot observe clear differences between some parameters measured in the patients diagnosed with CGL and PV, the different values recorded for the parameters studied under the light microscope allowed us to find morphological differences between the three disorders on which we focused. Thus, the morphological data completed with ultrastructural data can represent for the future a valuable criterion in the differential diagnosis of the CMDs in the conditions in which there are high difficulties in the differential diagnosis of ET and MMM in the prefibrotic stage.

So overall, this study provides evidence that there are significant morphological differences, between the cellular structures in the MKs from the patients diagnosed with different CMDs, and thus sustains the utilization of this approach for establishing the differential diagnosis of CMDs.

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Received: July 23rd, 2009

Accepted: October 25th, 2009