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Originals

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Chronic Myeloproliferative Diseases on a Pathologist's Desk - a Dilemma of Distinct Entities versus a Clinico-Pathologic Continuum. A Descriptive Study Based on a Material from the Polish Population*

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Chronic myeloproliferative disorders (CMPD) are traditionally diagnosed using criteria based on clinical parameters. A framework for an alternative, trephine bone marrow histology-based approach, was provided by the Hanover group of hematopathologist (the "Hanover classification"). The present study describes a single institution experience with the Philadelphia-BCR/ABL negative CMPD diagnosed on the basis of the histopathology of bone marrow in the three consecutive years (2000 - 2002). Among 246 cases of CMPD (M:F=1:1.6), there were 75 cases of idiopathic myelofibrosis (IMF), 45 of polycythemia vera (PV), 93 of essential thrombocythemia (ET), and 33 cases that were unclassifiable on the basis of morphology (CMPD-U). The clinical profiles of the IMF, PV and ET group emerging from the histological examination correlated with the expected clinical features of these diseases. The ET patients were the youngest (median 51 years) compared to PV (59.5 years), IMF (63.9), and CMPD-U (54.7). In 158 cases (74.3%), the biopsy corroborated the preliminary clinical diagnosis of CMPD, and in the half of these cases it refined the clinical diagnosis of suspected unspecified CMPD placing the disease in a particular specific category (ET, IMF or PV). In the remaining cases the biopsy was done due to an abnormality of unknown origin (usually an accidentally discovered thrombocytosis) or the clinical picture suggesting a disease other than CMPD (11.7%). Some cases of CMPD presented with atypical histological features, such as slight megakaryocytic dysplasia in ET (not justifying the diagnosis of IMF), raising the issue of the subjectivity of histological diagnosis. The trephine bone marrow biopsy provides a useful tool for the diagnosis of CMPD, particularly in the early IMF that may present with a clinical picture undistinguishable from ET, but which carries poorer prognosis and requires more vigorous treatment. A special attention should be paid to the CMPD-U group. Its current nosological status (early phases of IMF/ET/PV or distinct entity or entities?) is still unclear and requires further research.

Introduction

Chronic myeloproliferative diseases (CMPD) encompass a group of bone marrow cancers characterized by effective hemopoiesis and, as the name implies, a clinical course lasting usually from years to decades [12]. Compared to other cancers of the same origin (acute myelogenous leukemias and myelodysplastic syndromes), CMPD are indolent diseases, but nevertheless most of them shorten the lives of the patients, despite a marked progress in the diagnosis and therapy. The most frequent CMPD - chronic myelogenous leukemia (CML) - has its well-established diagnostic standards, containing the inclusion of an abnormal Philadelphia (Ph) chromosome and/or its molecular equivalent - the BCR/ABL translocation. Thus, CML theoretically does not constitute a major problem in differential diagnosis, at least at the level of a referral center. Chronic eosinophilic leukemia/hypereosinophilic syndrome (CEL/HES) and chronic neutrophilic leukemia (CNL) are rare, and especially the latter may be a "once in a career" experience even for a hematopathologist. The three remaining diseases recognized by the recent WHO classification are: essential thrombocythemia (ET), polycythemia vera (PV) and idiopathic myelofibrosis (IMF), known also as idiopathic osteomyelofibrosis, myelofibrosis with myeloid metaplasia, agnogenic myeloid metaplasia or chronic granulocytic-megakaryocytic myelosis. The cumulative incidence of ET, PV and IMF surpasses that of CML [12]. Although not as dramatic as CML in their typical clinical course, all three can be potentially lethal, and require different therapeutic approach. Of the Ph (BCR/ABL)-negative CMPD, IMF is associated with the highest probability of blastic transformation, bone marrow insufficiency and the most significant life loss [20]. By no means is the biggest clinical challenge posed by IMF diagnosed in young subjects, as this group may by considered as candidates for the stem cell

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transplant, either allogenic [11] or autologous [1]. On the other hand, young patients with early ET, who do not demonstrate thrombotic or hemorrhagic complications, may be maintained without cytostatics, on small doses of anticoagulants, or even followed-up without treatment [21]. These clinical features underlie the need for an exact and reliable differential diagnosis between IMF, ET and PV.

For many decades, the diagnosis of Ph (BCR/ABL)-negative CMPD was a domain of hematologists, and was based mostly on clinical parameters backed up by the analysis of smears of blood and bone marrow. The central dogma of human oncological pathology - that a tumor tissue should be seen and classified by a pathologist before starting a more specific treatment - somehow traditionally has not been applied to this group of neoplastic disorders. The insight into the tumor tissue, provided by the trephine bone marrow biopsy and requiring the skills of a pathologist familiar with the peculiarities of bone marrow morphology, was sought only when the aspiration was unsuccessful, or the diagnosis for some other reasons could not have been established. Such an approach was reflected by the Polycythemia Vera Study Group (PVSG) diagnostic criteria for PV [4] (with later modifications [19]), ET [18] and IMF [16]. These criteria, widely accepted by the clinicians, were optimized for the differential diagnosis of CMPD with reactive conditions, and not for differentiating within the IMF/PV/ET group. They largely ignored the histology of the bone marrow or referred to it in a not entirely clear manner ("fibrosis of 1/3 or more of biopsy area" as a prerequisite to diagnose IMF, with no explanation provided what actually it did mean) [16].

The breakthrough came from a group of Hanover pathologists, who in the early 1990' described histological hallmarks of all three Ph (BCR/ABL)-negative CMPD in trephine bone marrow biopsies. According to their seminal paper, in the vast majority of cases not only is it possible to diagnose CMPD on trephine, excluding reactive conditions, but also the differential diagnosis between IMF, ET and PV can be made without the knowledge of clinical data [10]. This of course does not mean that a pathologist should work oblivious of the clinical details, but stresses the specificity and sensitivity of bone marrowy histology in this particular context. According to the data from the Hanover group, approximately 12% of all CMPD, including CML, and 17% of Ph (BCR/ABL)-negative CMPD remained further unclassified, constituting a category of CMPD-U, which required follow-up trephine examinations [9]. A significant contribution to the description of morphology of Ph (BCR/ABL)-negative CMPD was also provided by the group from Cologne, who described very early (prefibrotic) IMF - a clinically valid term, contradictory to the PVSG typology ("idiopathic myelofibrosis without myelofibrosis") [26]. These findings were largely incorporated to the recent WHO classification of CMPD [12]. Currently, the role of a pathologist, reporting a trephine bone marrow biopsy in the cases suspected of Ph (BCR/ABL)-negative CMPD, is well established in most, but, nevertheless, not all hematological centers.

The aim of the present study was to analyze a cohort of Ph (BCR/ABL)-negative CMPD cases diagnosed with a substantial contribution of pathologists in a single academic pathology department. The study explores the role of trephine bone marrow biopsy in this particular context. A special emphasis is placed on the differential diagnosis between IMF, ET and PV, as well as on the significance of the CMPD-U group. The study provides also the first descriptive analysis of the features of Ph (BCR/ABL)-negative CMPD in the Polish population.

Material and Methods

The database of trephine bone marrow biopsies submitted to the Department of Pathomorphology, Collegium Medicum, Jagiellonian University, in three consecutive years (2000 - 2002) was searched for the cases of Ph (BCR/ABL)-negative CMPD, including the instances of IMF, PV, ET and CMPD-U. The present study included the cases with the first representative trephine bone marrow biopsy performed in the 2000 - 2002 period. The investigation did not include cases with non-representative material, a few cases in which the distinction between reactive conditions and CMPD could not have been made, cases of CMPD diagnosed at the stage of a blastic transformation, and four cases belonging to a family with familial thrombocytosis, a condition, whose neoplastic status is doubtful. All the cases were diagnosed by one of three pathologists (ZR, BP and JS), and subsequently re-reviewed by ZR. The positive selection criteria were based on the Hanover (German spelling "Hannover" in the original paper) classification of CMPD, combined with the later contribution from Cologne [24], including the description of early, prefibrotic IMF [10, 26]. These findings are also summarized in the recent WHO manual [12].

Briefly, IMF (chronic megakaryocytic-granulocytic myelosis in the original Hanover nomenclature) was defined as a panmyelosis characterized by hyperproliferation and clustering of megakaryocytes showing significant dysplasia, markedly surpassing that acceptable for other CMPD (Figs. 1A and 1B). The megakaryocytes frequently assume intrasinusoidal or perisinusoidal location. ET was diagnosed on the basis of a markedly increased number of enlarged, deeply lobulated megakaryocytes. The cytoplasm of these abnormal large cells is quite regular, and the degree of nuclear segmentation is proportional to their sizes ("staghorn" nuclei). In contrast to IMF, the pattern of nuclear segmentation remains regular. Apart from these abnormally large megakaryocytes, all other cells belonging to this lineage do not

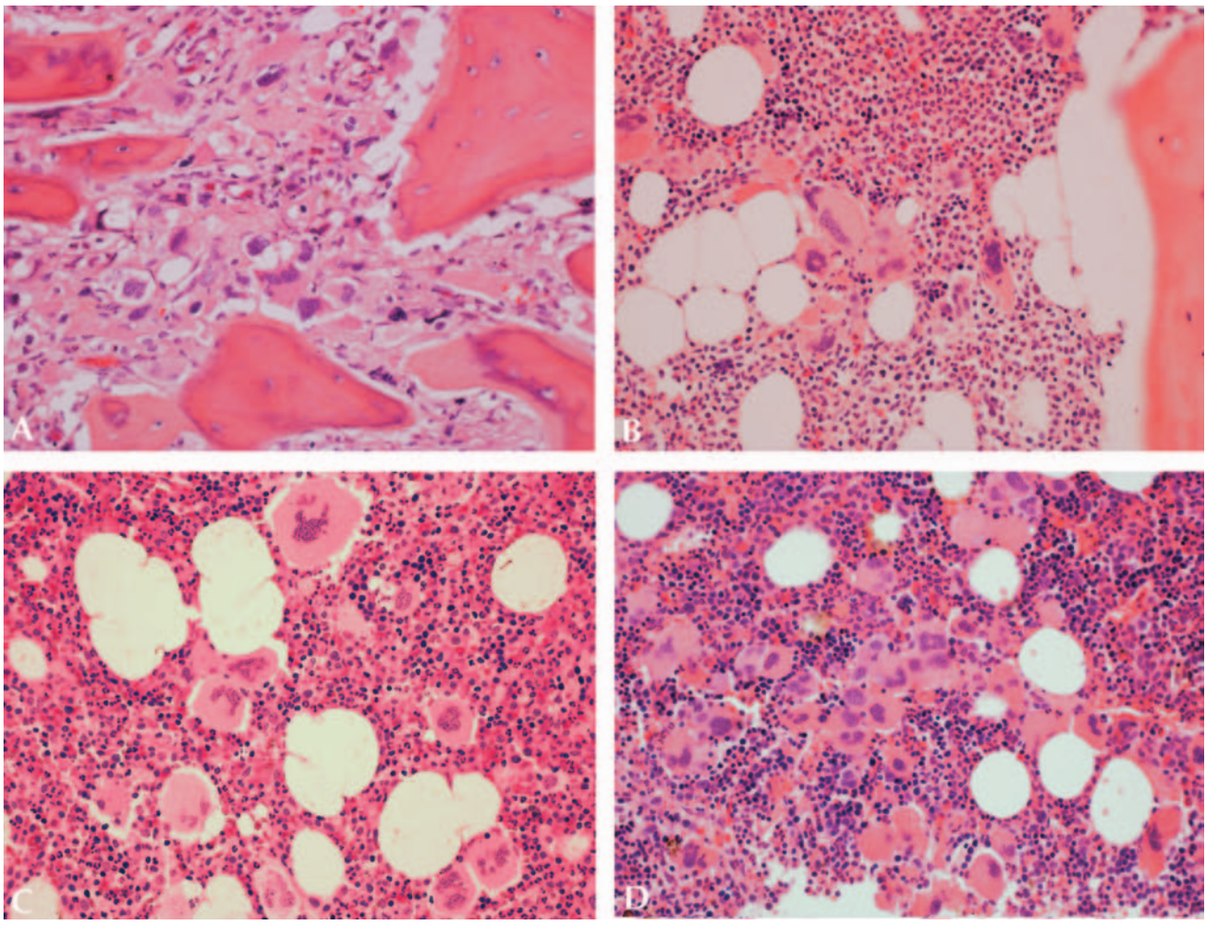

Fig. 1. Typical histology of Ph- (BCR/ABL) chronic myeloproliferative disorders in trephine bone marrow biopsies. Clustering of megakaryocytes constitutes their common histological denominator. All photographs were taken using a 40x-magnifying objective. A. Advanced idiopathic myelofibrosis. Marked dysplasia of megakaryocytes, associated with a relatively poor representation of the remaining hematopoietic lineages. Abnormal bone trabeculae (osteosclerosis). B. Early idiopathic myelofibrosis with higher bone marrow cellularity. Megakaryocytes show obvious atypia, with irregular shapes of both the cytoplasm and the nuclei and an irregular nuclear segmentation pattern. The increased intercellular distances among the hematopoietic cells result from the stromal reticulin fibrosis. C. Essential thrombocythemia is hallmarked by the presence of very large, but regular megakaryocytes, whose nuclear segmentation pattern and N:C ratio resemble normal mature megakaryocytes. The myeloid-to-erythroid ratio remains usually normal and typically there is no or minimal stromal fibrosis. D. In polycythemia vera there is a marked overrepresentation of all major hematopoietic lineages, with an increase in the bone marrow cellularity. Prominent erythropoiesis is a frequent, but not a constant feature. The majority of megakaryocytes do not diverge from the normal maturation pattern. Distended blood vessels with clinging megakaryocytes (visible on the right) can be usually seen.

show major divergences from the normal morphology (Fig. 1C). In PV, presenting characteristically as significant trilineage bone marrow hyperplasia with enlarged and more numerous sinuses, the clusters of megakaryocytes of various sizes (including very large forms) can be observed, but similarly to ET, the megakaryocytes do not show significant dysplasia. Relative erythroid hyperplasia is a frequent feature, however it does not constitute a necessary prerequisite for the diagnosis of PV (Fig. 1D). Increased cellularity of bone marrow and the presence of increased reticulin fibers favor PV over ET. The cases containing unequivocal histological hallmarks of CMPD, namely the clustering of abnormal megakaryocytes with various combinations of other histological abnormalities suggestive of CMPD (an increase in cellularity, distension of sinuses, intrasinusoidal hemopoiesis, sinusoidal tropism of megakaryocytes, increased reticulin fibers), but not fitting the typical picture of ET, IMF or PV, were labeled CMPD-U. While constructing a CMPD-U group, particular attention was paid to exclude the rare conditions morphologically mimicking CMPD, but belonging to other pathological categories. These included the 5q- syndrome, characterized by hyperproliferation and clustering of very typical megakaryocytes with hypo- or non-lobulated nuclei, and the myelodysplastic myeloproliferative diseases (MPD/MDS), encompassing atypical chronic myeloid leukemia and chronic myelomonocytic leukemia,

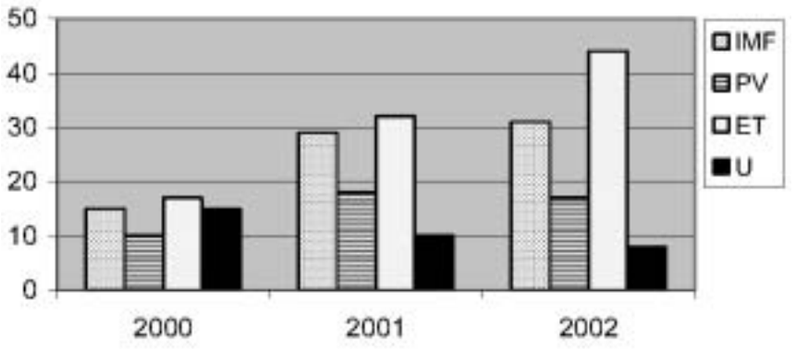

Fig. 2. Distribution of chronic myeloproliferative disorders diagnosed in the Department of Pathomorphology, Jagiellonian University in three consecutive years. The percentage of CMPD-U (unclassified) dropped significantly over the study period (chi-square p=0.004).

in which significant dysplasia affects also the erythroid and/or granulocytic lineages. The diagnosis of 5q-, and MPD/MDS was made based on the WHO criteria that integrate morphology with the clinical data [12].

All the cases were negative for the presence of an abnormal Ph chromosome and BCR/ABL transcript. These tests were performed in the referring clinical institutions.

The trephine bone marrow biopsies were fixed in formaldehyde, decalcified in either saturated EDTA for 2 - 4 days, or later, starting from 2001, in a commercial acid-based medium D-decalcifier (Shandon) for 2 hours. Subsequently, the cores were routinely embedded in paraffin and cut into 2-μm thick sections. The sections were stained with hematoxylin and eosin (2-slides per case), periodic acid-Schiff, Giemsa and Gomori silver methods. Bone marrow fibrosis was assessed semiquantitatively based on the Gomori silver-stained slides on a scale ranging from 0 to +4, according to the scheme developed by Kundel in the modification proposed by Bauermeister [3].

The statistical analysis was performed using the statistical functions incorporated into the Microsoft Excel spreadsheet. The level of statistical significance was set at p=0.05.

Results

Biopsies and diagnoses

The number of trephine bone marrow biopsies submitted to the Department of Pathomorphology, Collegium Medicum, Jagiellonian University, increased after 1999, amounting to 826 in 2000, 1067 in 2001 and 1082 in 2002. Most of the trephines came from two institutions: the University Department of Hematology and the Department of Hematology of the Ludwik Rydygier Memorial Hospital; both of which serve as tertiary community centers for the Kraków area. At that time, the Department did not receive trephine bone marrow biopsies from pediatric hospitals, so the material was limited to adult patients. There were 57 cases of Ph (BCR/ABL)-negative CMPD in 2000, 89 in 2001 and 100 cases in 2002. Of the total 246 cases of CMPD, 75(30.5%) were classified as IMF, 45(18.3%) as PV, 93(37.8%) as ET, and 33(13.4%) as CMPD-U. The distribution of classified CMPD was identical in the three consecutive years, whereas the percentage of CMPD-U dropped markedly in the study period, from 26.3% in 2000 to 8% in 2002 (Fig. 2).

The time from submission of the biopsy to the diagnosis lasted from 2 to 19 days, with the mean of 8 days and median of 7 days, which included, however, also the off (weekend) days. The diagnosis of CMPD-U was the most time-consuming, with the mean of 9.6 and median of 10 days, that differed significantly from the time devoted to diagnosing an average ET case (mean 7.3 days, median 7 days, p=0.01). The time from submission to the final report dropped from the median 11 days in 2000 to median 6 days in 2002, mostly due to the markedly shortened tissue processing at the decalcification step (the change from EDTA to acid-based medium).

Demographic features

The age of the patients ranged from 20 to 82 years, median 59 years, mean 57.5 years, with no significant differences between men and women for the whole group, and for all specific diseases, except ET. Due to the relatively lower number of older men in the ET group, the difference between the mean age of men (47.8 years) and women (55.3 years) was statistically significant, with p=0.01. There were no significant differences in the demographic profile of the patients between two major institutions, each contributing an equal number of cases (Hematology Department, Jagiellonian University - 122 cases, Rydygier Hospital - 122 cases). The distribution of age and sex for the whole group and for particular diseases is presented in Figure 3.

The patients suffering from ET were the youngest (mean 52.8 years, median 51 years), differing significantly from the PV (mean 58.3 years, median 59.5 years) and IMF (mean 63.9 years, median 65 years) groups, with the Student t-test p values 0.039 and 3.8×10^{-8}, respectively. Also the age difference between PV and IMF was significant (p=0.026). The mean (54.7 years) and median (57.5 years) age of the CMPD-U patients did not differ significantly from the well-defined disease categories. However, the CMPD-U cohort showed a slightly different, more flat age distribution (Fig.

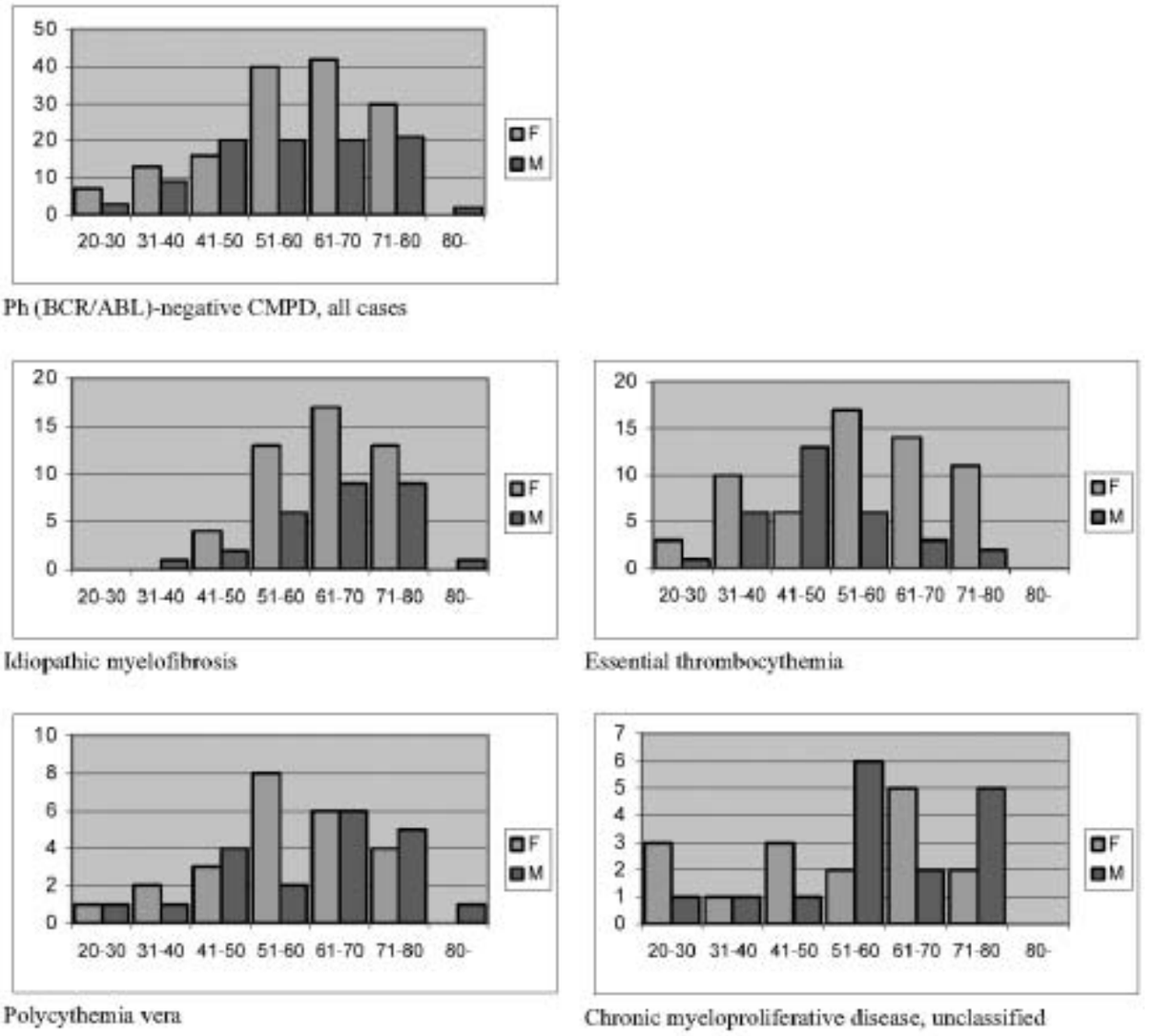

Fig. 3. Distribution of age and sex of Ph (BCR/ABL)-negative chronic myeloproliferative diseases diagnosed in 2000 - 2002 in the Department of Pathomorphology, Collegium Medicum, Jagiellonian University.

3), and an almost equal male:female ratio (16 men, 17 women, 1:1.05). The men-to-women ratio was 1:1.6 for all CMPD. The overexcessive prevalence of women was most pronounced among the patients suffering from ET (31 men, 62 women, 1:2), and to the lesser extent in the IMF (28 men, 47 women, 1:1.68) and PV groups (20 men, 25 women, 1:1.25). Notably, among the relatively young patients, less than 50 years of age, the sex ratios for all the groups were almost equal (Fig. 3), and the marked excess of women was due to their overrepresentation in the sixth, seventh and eight decades of life.

Clinico-pathologic correlations

The profile of clinical data characterizing the basic four groups is summarized in Table 1. Splenomegaly was less frequent in ET compared to IMF ($p=5x10^{-10}$, chi-square), PV ($p=0.0003$), and CMPD-U ($p=0.03$), and in CMPD-U compared to IMF ($p=0.005$), with no statistically significant differences between IMF and PV. Three ET patients were splenectomized prior to the diagnosis, including one in whom the spleen was removed due to a traumatic injury, and one subjected to a radical procedure in peptic ulcer (gastrectomy with splenectomy). No splenectomies were performed in other groups. The only significant difference in the frequency of liver enlargement was that between IMF and ET ($p=0.0017$, chi-square). As could be expected, all three red blood cell parameters of peripheral blood were significantly higher in PV in comparison with all other categories ($p<0.01$ for all of them), despite the inclusion of two cases of the spent phase of PV manifesting anemia. More frequent and deeper anemia was a feature differing significantly IMF from ET

TABLE 1

Clinical data of 246 patients diagnosed with chronic myeloproliferative disorders in the Department of Pathomorphology, Collegium Medicum, Jagiellonian University in three consecutive years

	splenomegaly	hepatomegaly	hemoglobin [g/dL] mean ± SD min - max	packed red blood cell volume [%] mean ± SD min - max	erythrocytes [x10^6/μL] mean ± SD min - max	leukocytes [x10^3/μL] mean ± SD min - max	platelets [x10^3/μL] mean ± SD min - max
IMF	68.9%	40.3%	11.0 ± 2.8 6.4 - 18.9	33.3 ± 8.0 10.0 - 48.6	3.9 ± 1.0 1.0 - 7.5	13.5 ± 11.9 0.9 - 74.0	543 ± 452 25 - 2427
PV	58.1%	30.2%	16.6 ± 3.2 8.8 - 22.1	51.9 ± 8.7 28.0 - 66.9	6.5 ± 2.8 2.8 - 6.7	12.0 ± 6.1 4.0 - 36.3	590 ± 336 117 - 1478
ET	22.6%*	16.5%	13.8 ± 1.8 6.4 - 16.8	42.1 ± 5.6 19.5 - 56.0	4.9 ± 0.9 2.1 - 8.5	10.4 ± 7.6 3.7 - 72.0	998 ± 390 461 - 2466
CMPD-U	43.3%	25.8%	14.2 ± 2.8 7.3 - 18.0	42.5 ± 7.1 26.6 - 54.5	5.0 ± 8.8 2.5 - 6.6	10.2 ± 4.6 2.6 - 25.2	745 ± 397 34 - 1825

* three patients with ET were splenectomized prior to the diagnosis, including one, in whom the spleen was removed due to a traumatic event.

($p<1x10^{-8}$ for hemoglobin concentration, packed red blood cell volume and erythrocytes), and IMF from CMPD-U ($p<1x10^{-4}$ for all three red blood cell parameters).

Thrombocytosis was most prominent in ET, compared to IMF ($p=2.9x10^{-9}$), PV ($p=1.3x10^{-8}$), and CMPD-U ($p=0.003$). A higher platelet count differentiated also the CMPD-U category from IMF ($p=0.023$), but the difference when compared to PV was of a marginal statistical significance ($p=0.053$). Leukocytosis was generally a non-discriminatory feature, with the only statistical difference between slightly higher levels encountered in IMF as compared to CMPD-U ($p=0.04$).

Among the 75 biopsies with histology compatible with various stages of IMF, 26 had been submitted with a preliminary clinical diagnosis of this disease. In 20 cases the clinical diagnosis was CMPD without further specification within this group. In six cases with leukocytosis ranging from 13100 to 74000 per μL, the clinicians suspected CML prior to the trephine. One of these patients, manifesting a typical histological picture of IMF, was followed-up under the label of "chronic phase of CML" for 13 years prior to the first trephine bone marrow biopsy. In five cases the clinical diagnosis was ET. In 11 cases the trephine was performed due to an unexplained abnormality, constituting an established indication for this procedure, such as splenomegaly of unknown origin, unexplained anemia, etc. Finally, in the remaining seven cases, the constellation of clinical signs and symptoms suggested another disease, such as MDS, hairy cell leukemia or other non-Hodgkin's lymphoma. Of note within this group was a case of splenomegaly persisting after the treatment of Hodgkin's disease seven years earlier. The retrospective review of a trephine from 1995, done in another institution for the staging of Hodgkin's disease prior to the treatment, revealed an early IMF concomitant with nodal Hodgkin's disease. The bone marrow was free of the lymphoma. At this time, IMF was overlooked and the issue of persistent splenomegaly remained unexplained and ignored for several years, to be investigated only when the patient started to manifest progressive anemia.

In 21 out of 45 cases with bone marrow histology compatible with the Hanover description of PV, the pre-biopsy clinical diagnosis was identical with the diagnosis based on the trephine or involved the differentiation with a reactive polyglobulia. In 17 cases the clinical data prior to the biopsy were suggestive of CMPD, but without further specification of the disease at the time of the trephine procedure. One patient was biopsied due to pancytopenia of unknown origin, and was diagnosed with the spent phase of PV concomitant with multiple myeloma. In six cases the clinical data favored ET, but the bone marrow histology was indicative of PV. Two of these patients had normal and four marginally elevated peripheral red blood cell parameters.

The histological picture of ET was found in 93 patients, in whom in 38 the clinical diagnosis prior to the biopsy was ET. In 36 patients the clinical diagnosis was further unspecified CMPD and in 17 thrombocytosis of unknown origin. One patient was biopsied due to splenomegaly of unknown origin.

In 19 out of 33 cases diagnosed histologically as CMPD-U, the disease was specified as CMPD based on clinical data prior to the biopsy. Four cases from the CMPD-U group were clinically suspected of PV, two of ET and one of IMF. Four biopsies were performed to explain thrombocytosis of unknown origin, and three for other reasons, including a case followed-up after the treatment of Hodgkin's disease 9 years earlier.

Altogether, the trephine bone marrow biopsy confirmed the preliminary clinical diagnosis of a specific CMPD (i.e.

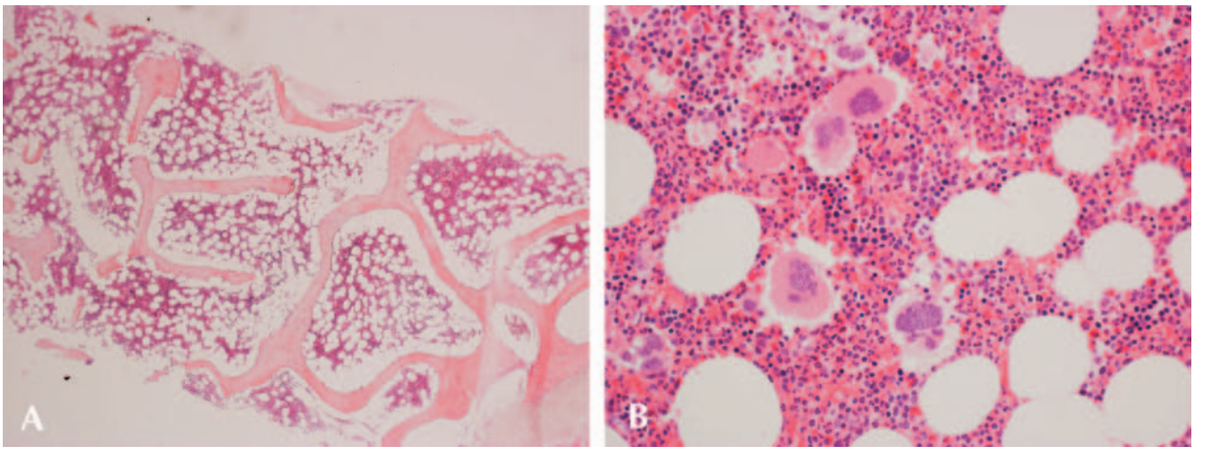

Fig. 4. A case of histological picture most consistent with the diagnosis of essential thrombocythemia, discrepant with the clinical data strongly suggesting polycythemia vera (see Results). A. Relatively low cellularity of the bone marrow in the context of CMPD is usually found in ET, and rare in the course of PV (objective magnification 4x). B. Hyperproliferation of very large, but regular megakaryocytes is accompanied by a proportional representation of myelo- and erythropoiesis. The blood vessels are not dilated.

IMF, ET or PV) in 85 out of 213 CMPD (40%) patients. In further 73 cases (34.3%), the biopsy refined the clinical diagnosis from CMPD to the specific disease within this group. Thirty out of 213 patients (14.1%) owed their histological diagnosis of a specific Ph (BCR/ABL)-negative CMPD to an unexplained sign constituting an established indication for the trephine bone marrow biopsy. Finally, in 25 patients (11.7%), the trephine bone marrow biopsy changed the clinical diagnosis, either from another type of CMPD or from a completely different disease.

Apart from the category of CMPD-U, in a small percentage of cases, in which the histological diagnosis of a specific Ph (BCR/ABL)-negative CMPD was, nevertheless, established, some elements of histology did not fit perfectly the published "archetypal" pictures of IMF, ET or PV. Such features as mild dysplasia of megakaryocytes or marked stromal fibrosis in ET, the presence of "ET-like" large megakaryocytes in IMF or PV and hyperproliferation of erythropoiesis in CMPD other than PV, made the specific histological diagnosis difficult, but not impossible, and were mentioned in the final reports. The most important discrepant histological findings encompassed:

- 10 cases of IMF (13.3%) with only mild dysplasia of megakaryocytes;
- 15 cases of PV (33.3%), including 13 with few large regular megakaryocytes with staghorn nuclei, described as characteristic rather for ET than PV;
- 2 cases with increased dysplasia of megakaryocytes (one later evolved into the typical spent phase of PV in the control biopsy);
- 16 cases of ET (17.2%), including: 10 cases with mild dysplasia of megakaryocytes, raising the issue of differential diagnosis with IMF (of these, five manifested slight (+2) and one marked (+3) stromal fibrosis); one case with morphology of megakaryocytes typical for ET - the presence of large cells with staghorn nuclei and no other features of megakaryocytic dysplasia - but with marked stromal fibrosis (+3); five cases, in which otherwise typical histology of ET was accompanied with significant hyperproliferation of erythropoiesis, of the degree found usually in PV.

Additionally in two women, whose bone marrow specimens demonstrated the classical "Hanover" histological picture of ET, the clinical data could have suggested PV with high platelet levels:

1. platelets: $885x10^3/\mu L$, red blood cells $7.06\ x10^6/\mu L$, packed red blood cell volume 55.8%, hemoglobin 17.9g/dL, splenomegaly (Fig. 4);
2. platelets: $658x10^3/\mu L$, red blood cells $6.7x10^6/\mu L$, packed red blood cell volume 52.5%, hemoglobin 16.8g/dL, no organomegaly.

Such discrepancies between histological and clinical pictures were not found in other CMPD.

Discussion

The methodological approach of the present study differs from many reports on CMPD, as it reflects mostly the perspective of a pathologist on this group of neoplastic disorders. Applying the pathology-based Hanover criteria to the diagnosis may result in a different nosological position of some, particularly marginal or early, cases, as opposed to the diagnosis based solely or mostly on clinical features. Most published reports on CMPD are based on sets of criteria

developed by Polycythemia Vera Study Group (PVSG) [4, 16, 18]. The major drawback of the PVSG approach was the negligence of histology, as the criteria were created mostly by clinical specialists, not only not familiar with the histological morphology of bone marrow, but also feeling autonomous in their diagnostic process, as the aspiration biopsy, performed and interpreted by a hematologist, was at that time perceived as the best and sufficient method of morphological assessment of the hematopoietic tissue. The PVSG were optimized for differential of CMPD against the reactive conditions, and to the lesser extent for distinguishing among particular Ph (BCR/ABL)-negative CMPD themselves. Even though, PVSG approach disregarded such an obvious histological finding as clustering of megakaryocytes, constituting an almost "yes-or-no" hallmark of CMPD other than CML. The strict application of PVSG criteria made it impossible to diagnose ET in subjects, whose platelets were constantly elevated, but did not reach the 600000/μL threshold, what was already pointed out in the literature [17, 22]. On the other hand, many patients with early, particularly prefibrotic, phase of IMF may for several years, or even decades, fulfill perfectly the PVSG criteria for ET, only to show later the "evolution" to fully blown IMF [5, 25, 27]. The differential diagnosis of PV and other CMPD seems to be less controversial, but the clinical picture of this disease may be blurred by co-existent factors leading to a drop in the red blood cell parameters and resulting in anemia or normal red blood cell values in a PV patient. Such patients, when presenting with high platelets (a frequent finding in PV) may once again fulfill the PVSG ET criteria. In a recently published study, in 6.5% of "ET" patients the disease was re-classified as PV during the 5 years of follow-up [13]. Since factors leading to anemia, such as constant blood loss due to gastrointestinal disease or iron deficiency, may be long-lasting or even permanent, and the PV patients are not immune to them, the percentage of misdiagnosed PV patients hidden within the ET or CMPD-U cohorts may be in fact even higher [23]. PV patients may also show apparently normal red blood cell parameters due to the rise in their plasma volume [15]. Another problem, probably not addressed in the literature in a systematic way, is the reactive erythrocytosis in subjects with CMPD other than PV. Analogously to PV patients suffering from anemia, the ET or IMF patients are by no means immune to factors contributing to reactive rise in red blood cells, such as the effect of heavy smoking in some individuals, lung diseases, etc. Reactive polyglobulia in a patient with CMPD may prompt the diagnosis of PV or CMPD-U if the clinical criteria are applied without the morphological control (for instance, according to PVSG hematocrit above 40% and even marginally increased red blood cell mass exclude ET).

The recently published WHO criteria encompass histological findings to the much greater extent, but once again the histology of neoplasia has to be measured against the sets of clinical parameters [12]. From one point of view this approach makes the diagnosis easier in most cases, but on the other hand, a strict application of the WHO criteria makes it impossible to diagnose CMPD in the few patients that have the disease without any doubts - a pathologist actually sees the neoplasm under the microscope and can even name it! - but whose clinical picture is somewhat atypical. The historical domination of purely clinical thinking in neoplastic hematology has led to the situation that would be barely imaginable in other fields. Could we, for instance, accept "four weeks of coughing" as a necessary prerequisite to the diagnosis of a lung cancer? Or perhaps "a 6-month episode of hematuria" as one of the "criteria" necessary to diagnose a renal cell carcinoma?

As we have emphasized, in the present study we have adopted the morphological method to diagnose CMPD. Such a diagnostic approach is quite natural for a pathologist, and implies all the strong and weak points of the histological diagnosis. Obviously, the histological diagnosis is largely independent of the problems resulting from non-homogeneity of the clinical picture of CMPD, as outlined above. On the other hand, the pathologist's eyes and mind comprise a subjective tool, and some borderline cases can not be resolved or are resolved arbitrarily, with a varying contribution of subconscious thinking and under the influence of self-confidence. The hallmark of Ph (BCR/ABL)-negative CMPD - clustering of megakaryocytes - is a very objective morphological finding, as the identification of these cells and identification of their clusters is relatively easy. Contrarily, some other elements of bone marrow biopsy interpretation, particularly the semi-quantitative assessment of reticulin fibrosis or the assessment of megakaryocytic dysplasia, both in terms of their very existence and intensity, are much more problematic. The price paid for this subjectivity is a smaller or larger CMPD-U group, and a possible misclassification of some cases within the particular CMPD groups, detectable at a longer perspective. On the other hand, the issue of differential diagnosis with reactive conditions seems to be marginal, once the pathologic diagnosis is applied.

In the original Hanover material, the CMPD-U group constituted approximately 12% of all CMPD [10]. There are no clinical studies addressing the evolution and prognosis of CMPD-U as a separate category, although the PVSG criteria have had to leave some group of CMPD patients further unclassified. These patients, who demonstrated a prolonged increase in their platelet count, were probably lumped with the ET category. The CMPD-U group in our material showed some exceptional demographic features, although its strict statistical analysis is problematic due to its relatively small size (33 patients) . In contrast to all the other categories, the male:female ratio was almost equal, implying that in CMPD-U males are overrepresented compared to the entire

CMPD group. The age distribution of our CMPD-U patients resembles that of ET, although the percentage of very young subjects (below 30 years of age) - 12.5% - is marginally higher than in ET (4.3%). The CMPD-U category poses a practical clinical dilemma in younger subjects, as the older ones are treated symptomatically. The role of allogenic and autologous stem cell transplantation in the management of young subjects with IMF is still controversial, but it is conceivable that some CMPD-U subjects suffer in fact from an early or atypical IMF, and - if young - could be considered as candidates for a bone marrow transplant or experimental therapies [1, 11]. Currently there is no single marker or panel of markers, be it morphological, immunohistochemical or molecular, fully characteristic for IMF. So far the only way of solving this diagnostic dilemma seems to be based on the strict clinical follow-up accompanied by repeated control trephine biopsies, as in some patients the disease may evolve into a more classical picture of a specific CMPD or possibly CMPD/MDS. It cannot be excluded, however, that the CMPD-U group contains also true "overlap" syndrome(s), not only in the diagnostic, but also in the biological sense of this term. This is corroborated by the overrepresentation of males among the CMPD-U patients, as it would be rather improbable that the trephines originating from males pose more diagnostic problems. Definitely this relatively small, but by no means marginal category requires more pathological and clinical research.

The presence of cases diagnosed as specific CMPD, but demonstrating some relatively minor divergences from classical descriptions of the pathology of ET, PV or IMF, raises two basic issues. The first one concerns the already mentioned subjectivity of the pathological diagnosis. Different pathologists may have different thresholds or individual "working" criteria for morphological alterations of megakaryocytes meriting the designation "dysplasia", which - when significant - favors the diagnosis of IMF. Also how large should a large megakaryocyte typical for ET be, and how much "staghorned" should its nucleus be to call it "staghorn-like", remains a matter of a fairly subjective judgment. Can such cells be observed in an otherwise typical PV, and, if so, what percentage of ET-like megakaryocytes is acceptable? The same applies for quantification of bone marrow fibrosis, semiquantitative assessment of myeloid- to erythroid ratio or derangement of topography of hematopoietic cells. Some of these problems, particularly the issue of megakaryocytic dysplasia, may be addressed by means of objective image analysis. Such a study is currently undertaken in our institution. The second issue associated with the slightly atypical histology of specific CMPD pertains to the possible clinical implications of these findings. ET with dysplasia of megakaryocytes was shown to carry poorer prognosis [2], but these results raise of course the question of differential diagnosis with early IMF. With the exception of the cited report of Annaloro et al. [2], currently there are still no convincing data on the possible clinical implications of atypical histology on the one hand, or on the possible histological connotations of atypical clinical presentation (such as very high platelets in PV, splenomegaly in ET, etc) on the other.

In our material, the atypical histology contributed mostly to the problems in differentiation between IMF and ET, whereas the dilemma of PV vs. IMF was relatively insignificant. Of particular note are two cases with the histology of ET and clinical pictures corresponding to classical PV. Although this discrepancy does not lead to a dramatic clinical impasse, the existence of such cases may be used as an argument for a possible lack of perfection of a purely histological approach.

In the majority of our patients, close to 3/4, the trephine bone marrow biopsy either confirmed or refined the clinical diagnosis of CMPD, pointing to specific entities within this group. The particular strength of the histological examination was illustrated by its role in arriving at the diagnosis in the remaining quarter of subjects. In approximately half of them, the biopsy resulted from a clinical sign (such as splenomegaly of unknown origin) constituting an indication for the trephine. The whole clinical context of these cases prior to the biopsy was unclear, and did not fully justify the diagnosis of a CMPD. Finally, in 11.7% of cases, the clinical picture prompted the suspicion of an entirely different disorder. In these cases the trephine examination completely re-directed the clinical reasoning.

A picture resulting from our methodology can be compared with the published series of CMPD, having in mind that most of them were collected basing on the clinical diagnosis. Our investigation is strictly speaking not a population-based study, as the two institutions contributing the cases do not serve a well-defined geographical area, although taken together, they constitute two major referral centers for South-East Poland. It is also conceivable that some, especially elderly, CMPD subjects are diagnosed and treated in peripheral institutions without being referred to tertiary hospitals, hence in our material we can expect some relative overrepresentation of younger patients. However, the clinico-pathological profile of CMPD from the two major contributing hospitals is almost identical, so we assume that our set constitutes a representative sample for the South-East Poland for the study period.

According to the data published by the WHO, the incidence of ET (1 - 2.5/100000/year) exceeds that of IMF (0.5 - 1.5/100000/year), and PV is the rarest (0.8 - 1.0/100000/year) in Caucasian populations. Similar proportions between the number of cases of particular CMPD were seen in our study. The increase in the number of cases in the three consecutive study years most probably represents a "detection artifact", and not a true rise in the incidence of

CMPD. A marked increase in ET cases diagnosed in the 1990' as compared to 1970' and 1980' was reported in another European population-based study and explained on the basis of an increased use of automated platelet counting [14]. To this we can add the increased use of abdominal ultrasound for various indications, leading to a more frequent detection of marginal splenomegaly, and a factor particularly important for our study - a more extensive use of the trephine bone marrow biopsy by cooperating clinicians. Despite the increase in the number of CMPD per year, the proportions between particular CMPD in the three consecutive years remained constant, with the notable exception of the CMPD-U group. In 2000, the number of cases assigned to the CMPD-U category was comparable to each of the remaining well-categorized groups, whereas in 2002, it dropped below 10%. The marked drop in the number of unclassifiable cases may reflect the impact of control biopsies, an increase in the experience of pathologists (or - and this cannot be altogether excluded - an increase in their level of self-confidence), and the improvement in the quality of the biopsies, both in terms of their collecting in hospitals and processing in the pathology lab. The latter factor - the biopsy quality - should not be neglected and will constitute a subject of a separate study.

Our ET patients are markedly younger (median 51 years, females 55 years, males 48 years, 42% of individuals of less than 50 years of age) than the Dutch patients reported by Jensen (females - median 68 years, males 66, 17% below 50 years of life) [14], and PVSG (median 62.8 years) [18]. The difference, exceeding a decade of age, may have several reasons. Firstly, due to differences in the diagnostic approach, some of the early IMF cases might likely have contaminated the cited series, and the IMF patients are on the average older, even when we take into account the early phase of the disease. Secondly, there is the above-mentioned possible under-representation of older people in our series. Thirdly, the Polish population shows a shorter average life span compared to the Western populations, and the younger age of our patients may to some extent reflect the demographic differences at the population level. For instance, our oldest ET patient was 80 years old, whereas in the Dutch series the oldest subject was 87 [14]. Finally, our series is of a very recent origin, and the younger age of our patents may reflect the current trend for the earlier diagnosis of CMPD, reported also by other authors [6, 14].

Another marked difference found in association with the reported series is the high female-to-male ratio in our IMF group (1:1.68), in contrast to the values close to 1:1 obtained by others [7, 8, 12]. This may be explained by a specific demographic profile of the Polish population, in which females live almost 10 years longer compared to males. Of all CMPD, IMF affects the oldest age groups, and in these age groups in Poland the women markedly outnumber the men.

In conclusion, the trephine bone marrow biopsy is a very useful tool in the diagnosis of CMPD. It allows for categorization of the majority of cases and can resolve some diagnostic problems unsolvable on a purely clinical ground. The weak point of the histological diagnosis of CMPD is a significant contribution of subjective factors to the microscopic assessment of the biopsy. The level of certainty of the pathologic approach may be increased by the development of new methods (such as image analysis) and immunohistochemical or molecular markers of CMPD. The exact meaning of the relatively non-numerous but obvious discrepancies between histological and clinical pictures remains to be established in the course of long-term follow-up of these patients, including the regimen of control biopsies. The same is true for the CMPD-U group that is likely to contain both the cases of well-defined specific CMPD escaping the more specific diagnosis, as well as the true overlap syndromes that need to be better defined.

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