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Review

Myelodysplastic/Myeloproliferative Neoplasms with Features Intermediate between Primary Myelofibrosis and Chronic Myelomonocytic Leukemia: Case Series and Review of the Entity

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Abstract: Diagnosis of myeloid neoplasm is currently performed according to the presence of a predetermined set of clinical, morphological, and molecular diagnostic criteria agreed upon by a consensus of experts. Even strictly adhering to these criteria, it is possible to encounter patients who present features that are not easily ascribable to a single disease category. This is the case, e.g., of patients with de novo myeloid neoplasms with features intermediate between primary myelofibrosis (PMF) and chronic myelomonocytic leukemia (CMML). In this study, we retrospectively searched the pathological database of IRCCS Humanitas Research Hospital to identify cases of chronic myeloid neoplasm with monocytosis with a driver mutation of classic myeloproliferative neoplasms (MPN) and showing morphological MPN features. For each case, we assessed all epidemiological, clinical, histopathological, and molecular data. Then, we carried out a literature review, searching for cases with features similar to those of our patients. We retrieved a total of 13 cases presenting such criteria (9 from the literature review and 4 from our institution); in all of them, there was a coexistence of clinical, histopathological, and molecular myelodysplastic and myeloproliferative features. To date, according to current classifications (World Health Organization and International Consensus Classification), given the presence/absence of essential features for PMF or CMML, these patients should be formally diagnosed as myelodysplastic/myeloproliferative neoplasm unclassified/not otherwise specified (U/NOS). This review aims to summarize the features of these difficult cases and discuss their differential diagnosis and their classification according to the novel classifications and the existing literature on overlapping myeloid neoplasms.

Keywords: MPN; MDS/MPN; PMF; CMML; JAK2; dysplasia; bone marrow

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1. Introduction

Primary myelofibrosis (PMF) and chronic myelomonocytic leukemia (CMML) are two of the most severe and prognostically unfavorable conditions among the chronic neoplasms of the hematopoietic stem cell [1–3]. Conceptually, they are easily differentiable: while PMF is (mainly) characterized by a hyperproliferation of the granulopoietic and megakaryopoietic lineages and stromal bone marrow remodelling driven by specific mutations (i.e., involving JAK2, CALR, and MPL genes), CMML's hallmarks are represented by a granulocytic hyperproliferation with prominent monocytic differentiation, frequently associated with mutations in SRSF2, TET2 and ASXL1 genes [1,4–6]. Both conditions are rare, with an incidence of, respectively, 0.5–1.5 cases per 100,000 individuals for PMF and 0.35 cases per

100,000 population in CMML, and typically occur between the sixth and eighth decades of life, with CMML patients being slightly older than PMF ones [1–4]. Furthermore, PMF and CMML patients experience similar biological evolution, with progressive bone marrow failure and deaths related to either a consequence of cytopenia or leukemic evolution [3,4,6].

PMF is categorized within the myeloproliferative neoplasms (MPN) as it frequently features peripheral leukocytosis and splenomegaly, both being diagnostic criteria for the entity, whereas CMML is included in myelodysplastic/myeloproliferative neoplasm (MDS/MPN) because it presents with co-existing myeloproliferative findings (e.g., peripheral monocytosis) and features of myelodysplasia (notably cytopenia, as the epiphenomenon of ineffective hematopoiesis) [1,5].

Interestingly enough, since at least the 2001 WHO classification of hematopoietic tumors, emphasis was given to the need to distinguish between de novo MDS/MPN and MPN developing myelodysplastic stigmata during the disease [6]. This distinction underscored the fact that classical MPN (i.e., essential thrombocythaemia, polycythemia vera, and PMF) might show a peculiar evolution among myeloid neoplasms characterized by a progressive increase in bone marrow fibrosis and osteosclerosis, with consequent development of bone marrow failure, leukoerythroblastosis, and (splenic) extramedullary hematopoiesis [7,8]. Such an evolution is more rarely observed in either myelodysplastic syndromes (MDS) or MDS/MPN, where clonal evolution, progressive impairment of hematopoietic progenitors function, and leukemic progression embody the natural history of the categories [6,7].

Fibrotic phases of classical MPN (i.e., post-essential thrombocythemia, and post-polycythemia vera myelofibrosis and overt PMF) may also develop MDS-like features such as cytopenia and even morphological dysplasia of the hematopoietic lineages. Still, they have been shown to benefit from different therapeutic strategies compared to de novo MDS/MPN and generally have relatively longer survival rates. Thus, their differentiation is clinically meaningful [9–11].

The diagnosis of myeloid neoplasms is currently performed according to the 2022 World Health Organization (WHO) classification and the 2022 International Consensus Classification (ICC) [1,5]. In both systems, the diagnostic categories are identified according to the presence or absence of a series of peculiar clinical, morphological, and genetic diagnostic criteria that are relatively similar across the two classifications. Only from the integration of these sets of data at the clinical presentation is it possible to assign any given patient to a disease group (e.g., MPN, MDS, MDS/MPN) and a specific entity and then employ the best therapeutic algorithm for his/her management [1,6].

Strictly adhering to all these principles, it is still possible to encounter patients who present with clinical-pathological syndromes that are not easily ascribable to a single disease category. For this reason, both 2022 WHO and ICC classifications list "unclassified" (U) or "not otherwise specified" (NOS) entities that are needed to include patients that fail to fulfill the criteria for a specific entity, still bearing typical characteristics of a disease group (e.g., MPN-U/NOS or MDS/MPN-U/NOS) [4,5]. While MDS/MPN-U/NOS is a diagnosis of exclusion, and thus a basket category, MPN-U/NOS has been conceived to include early stages of classical MPNs that still do not have a fully developed and recognizable phenotype, as well as those cases presenting with unexplained portal or splanchnic vein thrombosis but without specific features of a specific classical MPN entity [12].

Of importance, from the explorative study of divergent cases included in the U/NOS categories, it is possible to recognize novel myeloid disorders or forms of disease evolution, as exemplified by myelodysplastic/myeloproliferative neoplasm with thrombocytosis and SF3B1 mutations and myelodysplastic/myeloproliferative neoplasm with ringed sideroblasts and thrombocytosis, NOS [13–15]. After its first description in the late 1990s, the entity was accepted as a provisional entity in the 2001 WHO classification and formalized in later editions as part of the MDS/MPN group. A similar situation has been highlighted in more recent years studying patients diagnosed as de novo PMF presenting with clinical and histopathological features of CMML and vice versa [16,17].

In this work, we aim to thoroughly analyze cases of myeloid neoplasms with features intermediate between PMF and CMML (MNIPC), present novel data from our experience, and retrieve further cases from a review of the medical literature. Our goal is to critically revise such an entity, providing an up-to-date view of its biological, diagnostic, clinical, and prognostic features, as well as a useful tool to face the differential diagnosis between PMF, CMML, and other overlapping conditions, according to the current classifications of myeloid malignancies.

2. Materials and Methods

2.1. Case Selection

A retrospective search was carried out in the pathology database of IRCCS Humanitas Research Hospital to identify cases of chronic myeloid neoplasm with (1) persistent monocytosis (>500× U/mmc) for at least six months, (2) bearing a driver mutation typical of classic MPNs and (3) showing morphological stigmata of MPN including megakaryocyte hyperplasia, clustering and atypia, and/or bone marrow fibrosis grade ≥ 2. Cases diagnosed between January 2019 and December 2023 that fulfilled the above-mentioned criteria were included in the study.

For each included case, all epidemiological, clinical, laboratory, molecular cytogenetic, and follow-up data were retrieved. Moreover, the initial bone marrow biopsies were extracted from the archive and reviewed by four experienced hematopathologists (AB, DR, SU, SF).

2.2. Morphologic and Immunophenotypical Assessment

Hematoxylin eosin and Giemsa stains were used to evaluate bone marrow biopsies. Masson's trichrome and reticulin stains were used to assess marrow fibrosis according to WHO and ICC criteria. The presence of different morphological features was specifically investigated while reviewing the bone marrow biopsy samples (i.e., bone marrow cellularity, evidence of lineage maturation, dyserythropoiesis, dysmegakaryopoiesis, megakaryocytes clustering, left-shifted granulopoieisis, architectural displacement of erythrons, megakaryocytes and/or adipocytes, increase of microvessel density, presence of intrasinusoidal hematopoiesis, increased bone marrow collagen and/or reticulin fibrosis and osteosclerosis) [6,18].

Immunohistochemical evaluation of the trephine biopsies was also carried out using the automated platform Dako Omnis Envision Flex, with antibodies against CD3, CD14, CD16, CD20, CD34, CD61, CD68R/PGM1, CD117, CD163, E-Cadherin, Myeloperoxidase (MPO), and p53 (DAKOCytomation, Glostrup, Denmark).

2.3. Genetic Assessment

Genomic DNA (gDNA) was isolated from bone marrow aspirate or blood. gDNA was extracted with a silica membrane-based purification method from blood or bone marrow using the QIAamp DNA MiniKit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA quantification was performed using the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Library preparation was performed with the "SOPHiA DDM™ Dx Myeloid Solution" kit that covers 30 relevant genes (10 with complete coding sequences) associated with myelodysplastic syndromes, myeloproliferative neoplasms, and leukemia. Libraries were prepared using 200 ng of gDNA diluted in IDTE. The manufacturer's protocol was followed for all steps. Briefly, gDNA was enzymatically sheared, the end repaired, and adenosine was added to the 30 end. Dual barcoded adapters were ligated to the DNA fragments, and a dual-size selection of products was subsequently performed using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). Libraries were amplified by PCR, cleaned up with Agencourt AMPure XP magnetic beads, and quantified using Qubit (Thermo Fisher Scientific). A total of 150 ng per library was pooled and lyophilized, and targets were hybridized with probes. Probe-target duplexes were bound to streptavidin beads, pulled down, and washed. A post-capture amplification

and cleanup were performed. Final libraries were quantified using Qubit, and quality and size were assessed using a Bioanalyzer (Agilent, Santa Clara, CA, USA). Paired-end sequencing of samples was performed using the MiSeq Reagent Kit V3 (600 cycles) on the MiSeq sequencing platform (Illumina, San Diego, CA, USA). Data-driven medicine (DDM) software (version n°5.10.53.1) was used for the analysis of the data derived from MYS; reads were mapped to the hg19 reference. Statistics and coverage of the samples were calculated; variants with a variant allele frequency (VAF) < 1%, known as common dbSNPs, were filtered out in addition to those variants not predicted to cause amino acid change or a splice site effect.

Cytogenetic analysis for all four cases was performed on bone marrow aspirates non-stimulated cultures using fluorescence Q-banding. According to European guidelines, a minimum of 20 metaphases were analyzed if no abnormality was found [19]. Anomalies were considered clonal when at least 2 metaphases had the same aberration, except for clonal monosomy, which had to be present in at least 3 metaphases. A karyotype was considered complex when $\geq$3 aberrations were found within one clone. The karyotype was described following the latest version of the International System for Human Cytogenetic Nomenclature (ISCN2020) and reported in Table 1 for all patients. For case with complex karyotype (#13, Table 1) was performed interphase FISH analysis with a specific probe for the genes KMT2E (7q22), EZH2 (7q36), KMT2A (11q23) and ETV6 (12p13) (MetaSystems probes, Altlussheim, Germany) allowing the confirmation of the cytogenetic results, particularly the recurrent translocation in myeloid neoplasms t(12;22)(p13;q12) MN1::ETV6 for the presence of ETV6 rearrangement.

2.4. Literature Review

A literature review was also carried out on PubMed, searching for articles or reviews describing cases of myeloid neoplasms with clinical-pathological features similar to those included in the present series, and specifically including PMF patients with monocytosis and MDS/MPN cases with megakaryocyte hyperplasia, fibrosis and/or JAK2 mutations. The last search was performed in March 2024.

Table 1. Clinical and molecular features, treatment, and outcome of the cases analyzed in this work (including data from the review of the literature).

#	1st Author	Sex	Age	Hemoglobin (g/dL)	Leukocytes (U/mmc)	Monocytes (U/mmc)	Mono %	Platelets (U/mmc)	LDH (U/L)	Splenomegaly (cm)	MPN Driver Mutation	VAF	Additional Mutations	Karyotype Anomalies	Therapy	HSCT	Status	FU (mo)
1	Chapman #1	M	73	11	24,200	970	4.0	145,000	N/A	Yes	JAK2V617F	48	ASXL1 (42%), SRSF2 (40%), TET2 (46%)	none	Ruxolitinib, Alisertib	N	SD	40
2	Chapman #2	M	76	9.6	6100	2440	40.0	145,000	N/A	Yes	JAK2wt		N/A	none	Azacitidine	N	DOD	8
3	Chapman #3	F	75	14.4	19,700	2460	12.5	226,000	315	No	JAK2V617F	60	TET2 (47%), TET2 (44%)	none	Ruxolitinib	N	SD	54
4	Chapman #4	M	58	12.4	17,900	1400	7.8	66,000	N/A	Yes	MPL	44	ASXL1 (43%), NRAS (25%), SRSF2 (46%), TET2 (48%), TET2 (42%)	none	Ruxolitinib	Y	DOC	12
5	Chapman #5	M	74	13.6	15,000	3700	24.7	498,000	N/A	Yes	JAK2V617F	60	KRAS (33%), TET2 (48%)	none	Observation	N	SD	24
6	Chapman #6	M	63	7	35,100	2850	8.1	263,000	N/A	Yes	JAK2V617F	N/A	TET2	none	N/A	N	LFU	2
7	Hu #1	M	67 *	9.2 *	70,000 *	2300 *	18 *	42,000 *	2594 *	Yes	JAK2V617F	47	N/A	46,XY,−6, del(7)(q22q34), +r[20] **	Hypometilating agent, Rigosertib	N	DOD	22
8	Hu #2	M	67 *	9.2 *	70,000 *	2300 *	18 *	42,000 *	2594 *	Yes	JAK2V617F	30	SRSF2	47,i(X)(p10),+13, del(13)(q12q14)x2 [20] **	Hypometilating agent	N	DOD	29
9	Hu #3	F	67 *	9.2 *	70,000 *	2300 *	18 *	42,000 *	2594 *	Yes	JAK2V617F	39	N/A	47,XY,+8[9] **/45,XY,add(4)(q27),−12, add(17)(p11.2)[4] **/ 46,XY [7] **	Hypometilating agent	N	DOD	52
10	Present work #1	M	60	9.9	37,100	1900	5.1	280,000	397	Yes (15)	JAK2V617F	84	ASLX1 (48%)	46,XY, del(7)(q22q36)[4] **/ 46,XY[24] **	Ruxolitinib, Fedratinib	Y	DOD	55
11	Present work #2	F	65	13	8650	1300	15.0	99,000	570	No	JAK2V617F	35	DNMT3A, IDH2	46,XY,-7[23] **	Azacytidine	Y	CR	14
12	Present work #3	M	66	11.8	31,930	780	2.4	54,000	N/A	Yes (18)	JAK2V617F	38	CBL (27%), IDH2 (47%), SRSF2 (44%), TET2 (24%)	none	Azacytidine, Ruxolitinib	N	PD	24

Table 1. *Cont.*

#	1st Author	Sex	Age	Hemoglobin (g/dL)	Leukocytes (U/mmc)	Monocytes (U/mmc)	Mono %	Platelets (U/mmc)	LDH (U/L)	Splenomegaly (cm)	MPN Driver Mutation	VAF	Additional Mutations	Karyotype Anomalies	Therapy	HSCT	Status	FU (mo)
13	Present work #4	M	56	6.6	55,000	1800	3.3	41,000	1360	Yes (23)	CALR	43	TET2 (6%)	46,XY,add(6)(p23), t(7;11)(q22;q13), t(12;22)(p13;q12)[8] **/47,idem,+add(6) (p23)[5] **.nuc ish(D7Z1,KMT2E, EZH2)x2[100] **, (KMT2Ax2)[100] **, (ETV6x2) (3′ETV6 sep 5′ETV6x1) [70/100] **	HU, Vyxeos, Azacytidine, Venetoclax	N	PD	158

CR: complete response; DOD: dead of disease; DOC: dead of other cause; F: female; FU: follow-up; mo: months; HSCT: hematopoietic stem cell transplant; LDH: lactate dehydrogenase; M: male; mo: months; MPN: myeloproliferative neoplasm; N: no; N/A: not available; PD: progressive disease; SD: stable disease; VAF: variable allele frequency. * median value calculated on the sum of the values of the complete blood counts of the patients included in the article; ** Numbers in square brackets refer to metaphases/nuclei counting.

3. Results

Between 2019 and 2023, we performed 694 novel diagnoses of myeloid neoplasms at Humanitas Research Hospital, including 165 novel diagnoses of MPN (38 of which were PMF), 207 novel diagnoses of MDS, and 103 novel diagnoses of MDS/MPN (49 of which were CMML and 11 MDS/MPN-U/NOS). A total of 4 patients fulfilled the criteria for MNIPC.

3.1. Epidemiological and Clinical Features

The four included patients (3 males, 1 female) had a median age at diagnosis of 62.5 years (range 56–66 years).

All but one patient clinically presented with splenomegaly (range 15 to 23 cm), at least one cytopenia, and one peripheral cytosis. Specifically, anemia was present in 3/4 patients (median Hb value: 10.85 g/dL, range: 6.6–13 g/dL), while leukocyte count was elevated (mean 34,500× U/mmc, range 8600–55,000× U/mmc), as was monocyte count (mean: 1550× U/mmc, range: 780–1900× U/mmc). Monocytes represent between 2 and 15% of total leukocytes, being ≥10% only in one patient. All but one patient was also thrombocytopenic (median platelet count: 76,500× U/mmc, range: 410,00–280,000× U/mmc). Moreover, in most patients, an elevated LDH was also detected (median: 570 U/L, range: 397–1360 U/L) (Table 1).

3.2. Histological and Immunohistochemical Features

Cellularity was roughly 90% in all patients except one, who had bone marrow diffusely fibrotic and only sparsely cellulated. All patients had trilinear maturation defects and dysplasia (including morphological dyserythropoiesis and displacement of erythroid cells close to bone trabeculae) and augmented myeloid-erythroid ratio, paired with atypical megakaryocyte proliferation (including giant megakaryocytes with multilobated, cloud-like or stag-horn like nuclei), either diffuse or grouped in clusters. Of interest, dense clusters as specified in the 2022 ICC classification (i.e., at least six closely packed megakaryocytes) were generally absent, even though clusters of two to four cells were commonly observed. Three cases also showed architectural displacement of adipocytes. All patients were negative or had less than 2% p53 positive cells at immunohistochemistry. CD34+ cells, were equal or less than 2% in all cases. Clusters of plasmacytoid dendritic cells CD123+ were never detected. All cases presented a prevalence of classical CD14+/CD16− monocytes, which was consistent with flow cytometry findings. Microvessel density increased in all patients, while two cases also showed intrasinusoidal hematopoiesis. The bone marrow biopsy showed some degree of reticulin fibrosis in all patients, with a Grade 3 or a Grade 2 in one case each and a Grade 1 in two cases. Collagen fibrosis was detected in one case, whereas all patients had some degree of osteosclerosis (Figures 1–4, Tables 2 and 3).

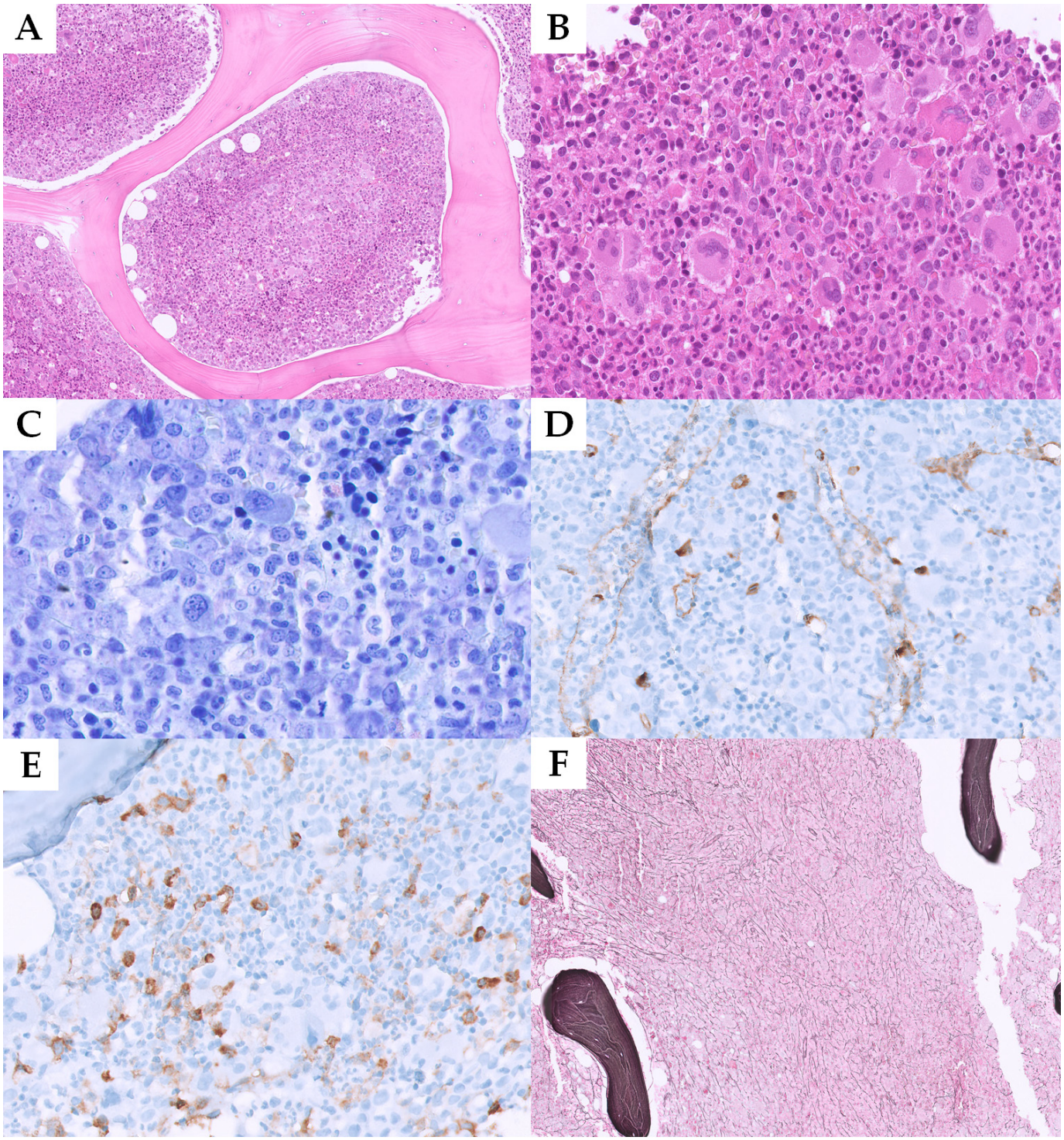

Figure 1. Histopathological features of case #1. The bone marrow biopsy was hypercellular ((**A**), HE 50×), with myeloid expansion and an increased number of megakaryocytes, often in clusters ((**B**), HE 200×), and morphological dyserythropoiesis ((**C**), Giemsa 200×). At immunohistochemical evaluation, despite a low number of CD34+ precursors. The same staining revealed an increase in microvessel density. ((**D**), CD34, 200×), The number of CD14+ monocytes was also increased ((**E**), CD14, 200×). Bone marrow fibrosis grade 1 was also observed ((**F**), Gömöri, 50×).

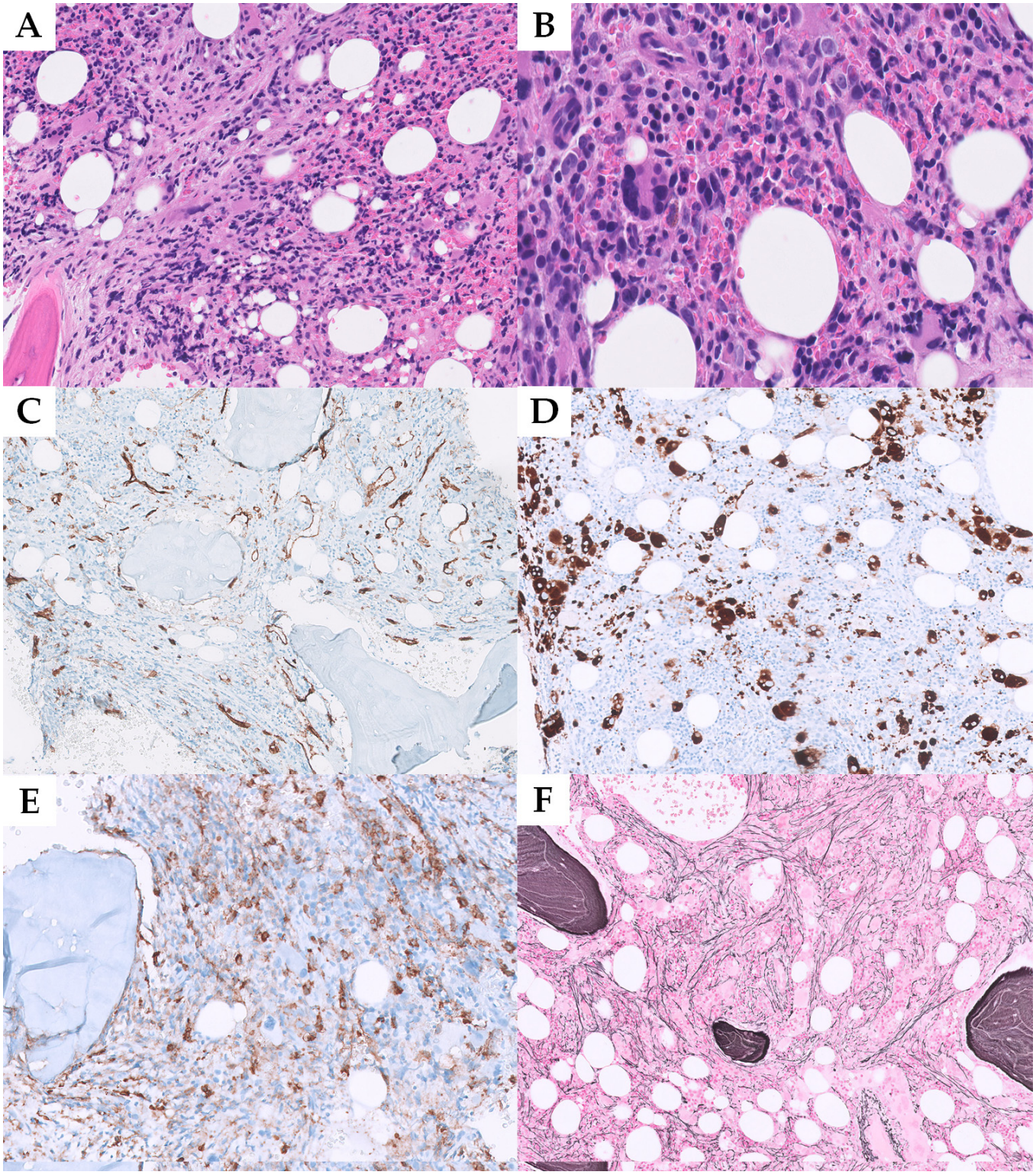

Figure 2. Histopathological features of case #2. The bone marrow biopsy showed hypercellularity ((**A**), HE 100×), with atypical megakaryocytes, ((**B**), HE 400×). At immunohistochemical evaluation, the microvessel density was highly increased ((**C**), CD34, 50×). Megakaryocytes were small, with hypolobated nuclei, and often in clusters ((**D**), CD61, 50×), and the number of CD14+ monocytes was high ((**E**), CD14, 200×). Bone marrow fibrosis grade 1 was observed ((**F**), Gömöri, 50×).

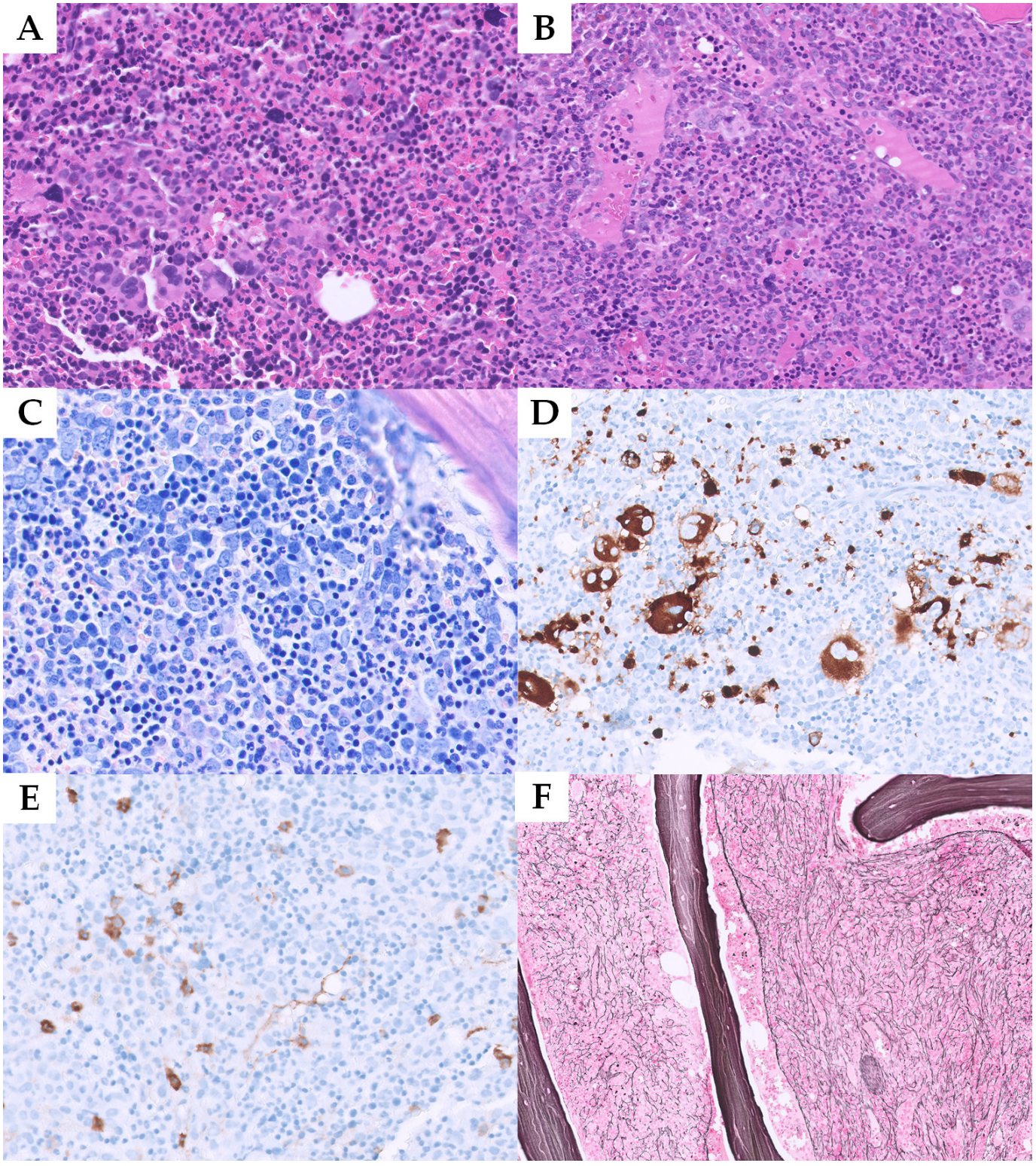

Figure 3. Histopathological features of case #3. The bone marrow biopsy showed a 100% cellularity ((**A**), HE 100×), with myeloid hyperplasia with left-shifting and enlarged blood vessels with intrasinusoidal hematopoiesis ((**B**), HE, 50×). Giemsa stains revealed marked morphological and topographic dyserythropoiesis ((**C**), 400×). Immunohistochemistry highlighted the presence of clusters of atypical megakaryocytes ((**D**), CD61, 100×), while the number of CD14+ monocytes was only minimally increased ((**E**), 100×). Reticulin fibrosis was grade 2 ((**F**), Gömöri, 50×).

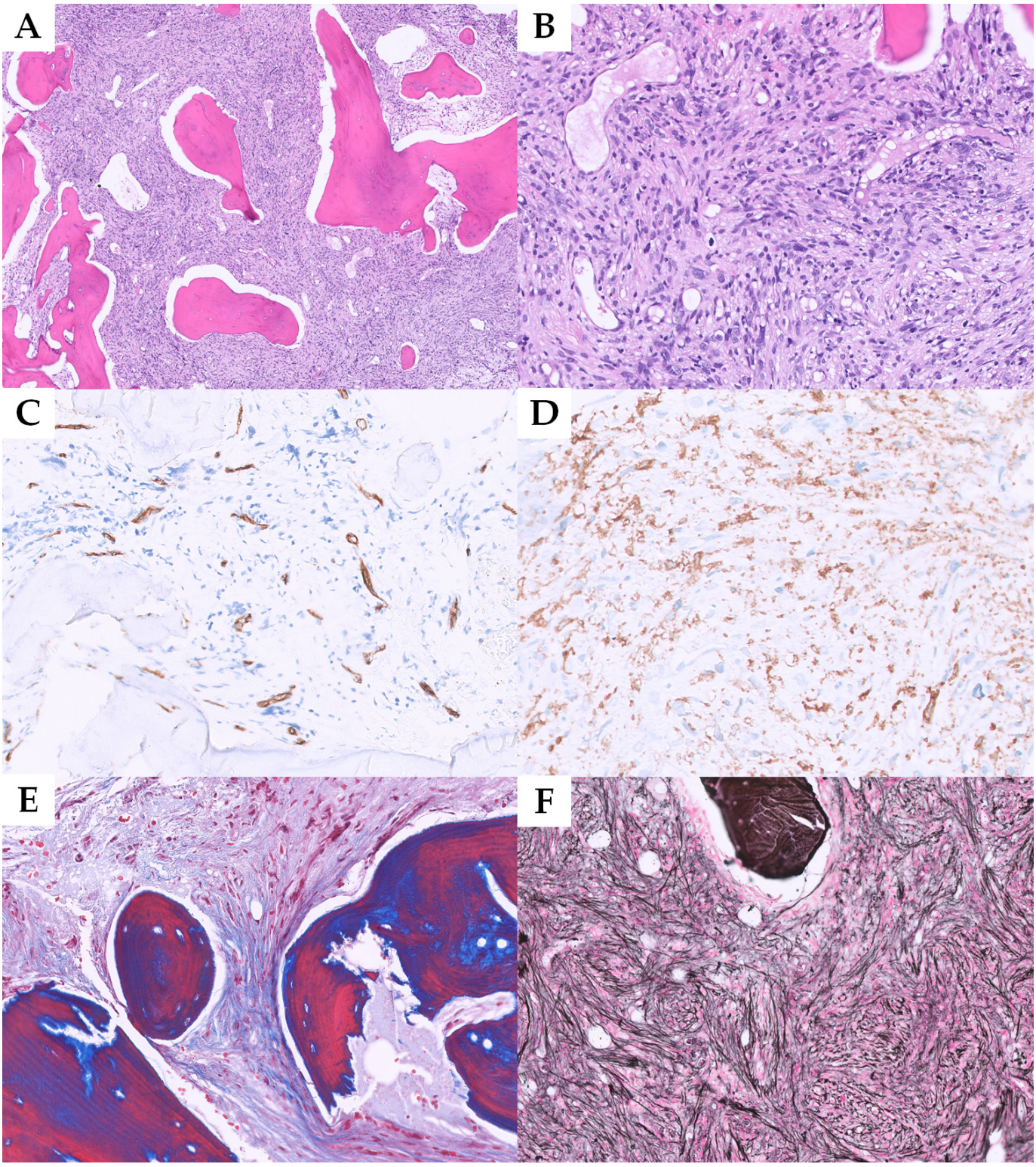

Figure 4. Histopathological features of case #4. The bone marrow was highly hypercellular with prominent osteosclerosis ((**A**), HE 50×) and enlarged sinusoids ((**B**), HE, 100x). CD34 stains revealed a normal number of precursors ((**C**), 50×), while CD14 showed a marked increase of monocytes ((**D**), 50×). Masson trichrome highlighted a collagen fibrosis grade 2 ((**E**), 100×) while reticulin fibrosis was grade 3 ((**F**), Gömöri, 100×).

Table 2. Histological features of the cases analyzed in this work (including data from a review of the literature).

#	1st Author	BM Cellularity	Morphological Erythroid Dysplasia	Topographic Displacement of Erythrons	Left-Shifting Granulopoiesis	Hyperplastic Megakaryopoiesis	Megakaryocyte Morphology (Myeloproliferative, Atypical, Hypolobated)	Dense Clusters of Megakaryocytes	M:E Ratio	Bone Marrow CD34+ Cells (%)	Paratrabecoular Adipocyte	Microvessel Density	Intrasinusoidal Hemopoiesis	Bone marrow CD14+ cells (%)	Bone Marrow Fibrosis (Grade)	Collagen Fibrosis (Grade)	Osteosclerosis
1	Chapman #1	95%	Y	N/A	Y	Y	M, A	Y	N/A	N/A	N/A	N/A	N/A	N/A	2	N/A	N/A
2	Chapman #2	95%	N	N/A	Y	Y	A	N	N/A	7	N/A	N/A	N/A	30	3	N/A	N/A
3	Chapman #3	95%	N	N/A	Y	Y	M, A	N	N/A	N/A	N/A	N/A	N/A	15	1	N/A	N/A
4	Chapman #4	95%	Y	N/A	N	Y	M, A, H	Y	N/A	3	N/A	N/A	N/A	N/A	3	N/A	N/A
5	Chapman #5	80%	Y	N/A	N	Y	M, H	N	8:1	N/A	N/A	N/A	N/A	15	2	2	N/A
6	Chapman #6	100%	Y	N/A	Y	Y	M	Y	6:1	4	N/A	N/A	N/A	60	3	N/A	N/A
7	Hu #1	95%	Y	N/A	Y	Y	M, A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1	N/A	N/A
8	Hu #2	95%	Y	N/A	Y	Y	M, A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2	N/A	N/A
9	Hu #3	95%	Y	N/A	Y	Y	M, A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2	N/A	N/A
10	Present work #1	90%	Y	N	Y	Y	H	Y	10:1	2	Y	Y	Y	10	1	0	1
11	Present work #2	90%	Y	N	Y	N	H	N	4:1	1	Y	Y	N	15	1	0	1
12	Present work #3	100%	Y	Y	Y	Y	A	N	6:1	2	Y	Y	Y	6	2		1
13	Present work #4	80%	N	Y	N	N	H	N	4:1	1	N	Y	N	1	3	2	2

A: atypical; BM: Bone marrow; H: hypolobated; M: myeloproliferative; M:E: myeloid:erythroid; N: no; N/A: not available; Y: yes.

Table 3. Comparison of PMF and CMML, WHO and ICC diagnostic criteria with analysis of cases of myeloid neoplasms with features intermediate between PMF and CMML (MNIPC) described in the present work.

Feature/Diagnostic Criterion	PMF WHO 2022	PMF ICC	CMML WHO	CMML ICC	MNIPC
1-Clinical/Laboratory					
Cytopenia				Essential	11/13 (84%)
Anemia	Minor	Minor			8/13 (62%)
Leukocytosis: WBC > 11,000 U/mmc (PMF) WBC > 13,000 U/mmc (CMML)	Minor	Minor	CMML, myelo-proliferative subtype		11/13 (84%)
Monocytes $\geq$ 500 U/mmc			Essential	Essential	13/13 (100%)
Monocytes $\geq$ 1000 U/mmc				Needed in case of no evidence of clonality	11/13 (84%)
Monocytes $\geq$ 10% of WBC			Essential	Essential	7/13 (54%)
Abnormal partitioning of peripheral monocyte subsets			Desirable	Needed in case of no evidence of clonality	N/A
Increased LDH	Minor	Minor			7/7 (100%)
Leukoerythroblastosis	Overt PMF only	Overt PMF only			N/A
Splenomegaly	Minor	Minor			11/13 (84%)
2-Histopathological					
Hypercellularity				Essential	13/13 (100%)
Hyperplastic granulopoiesis	Major	Major			13/13 (100%)
Hypoplastic erythropoiesis	Major	Major			N/A
Dysplasia			Desirable	Needed in case of no evidence of clonality	11/13 (84%)
Megakaryocytic hyperplasia	Major	Major			11/13 (84%)
Megakaryocytic atypia	Major	Major			8/13 (62%)
Dense clusters of megakaryocytes		Major			4/9 (44%)
Increased monocytes				Essential	6/8 (75%)
Reticulin fibrosis grade 0–1	Major	Major			4/13 (31%)
Reticulin fibrosis grade 2–3	Major	Major			9/13 (69%)
Absence of reactive bone marrow fibrosis	Major	Major			13/13 (100%)
Peripheral and/or bone marrow blasts >2/5%, <20%				In CMML with excess blasts	1/7 (14%)
Peripheral and/or bone marrow blasts <20%			Essential	Essential	13/13 (100%)

Table 3. *Cont.*

Feature/Diagnostic Criterion	PMF WHO 2022	PMF ICC	CMML WHO	CMML ICC	MNIPC
3-Genetic					
Clonal marker	Major	Major	Desirable	Essential	12/13 (92%)
JAK2 mutation	Major	Major			10/13 (77%)
JAK2 mutation VAF					44%
MPL or CALR mutation	Major	Major			2/13 (15%)
Absence of Philadelphia chromosome	Major	Major	Essential	Essential	13/13 (100%)
Absence of tyrosine kinase fusions	Major	Major	Essential	Essential	13/13 (100%)
4-Other criteria					
Exclusion of other myeloid neoplasms	Major	Major	Essential	Essential	13/13 (100%)

CMML: chronic myelomonocytic leukemia; De: desirable; Es: Essential; ICC: international Consensus Classification; Ma: major criterion; Mi: minor criterion; N/A: not available; PMF: primary myelofibrosis; WHO: World Health Organization; WBC: white blood cells.

3.3. Genetic Characterization

At sequencing analysis, all patients presented with two or more mutations. MPN driver mutation occurred in JAK2(V617F) in three cases (VAF range: 35–84%) and in CALR (VAF: 43%) in the last one. TET2 and IDH2 mutations occurred in two patients each, also in combination. Other mutated genes were SRSF2, ASXL1, CBL, and DNMT3A (Figure 5). The median VAF of non-driver gene mutations was 35.5%, ranging from 6% to 48%. Of importance, no disease-defying gene mutation or fusion was detected at NGS and FISH analyses, except for case #13 with complex karyotype that includes the t(12;22)(p13;q12) resulting in MN1::ETV6 gene fusion, confirmed by the presence of a balanced ETV6 gene rearrangement with a locus-specific break-apart probe.

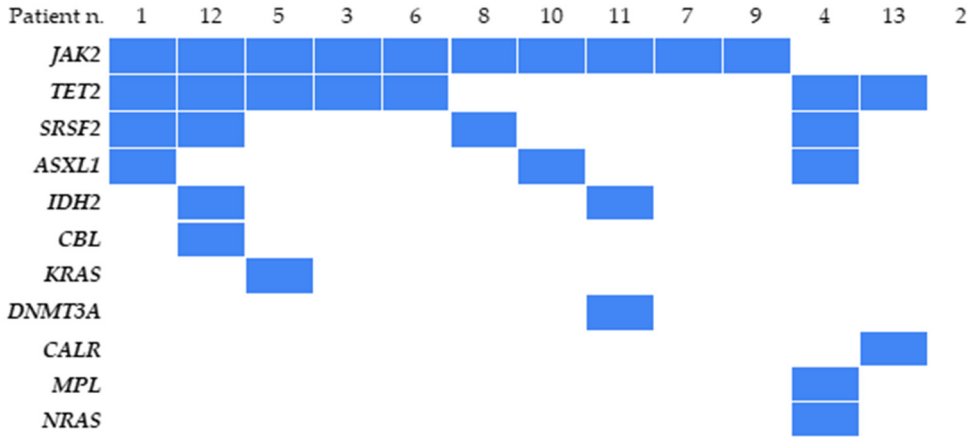

Figure 5. Oncoplot of recurrently mutated genes in patients with myeloid neoplasms with features intermediate between PMF and CMML with our series and are described in the literature [16,17].

Cytogenetic analysis showed a del(7q) in one case (#10) and a monosomy 7 in a second case (#11), a complex karyotype with add(6)(p23), t(7;11)(q22;q13) and t(12;22)(p13;q12) in another case (#13) and a normal karyotype in the last patient (#12, Table 1).

3.4. Treatment and Outcome

The treatment was variable among the patients. Patients failed to respond to the used drugs, including Azacytidine, Fedratinib, Ruxolitinib, Vyxeos, and/or Venetoclax. A complete remission was obtained in one patient, undergoing a hematopoietic stem cell

transplant, while two patients experienced a progressive disease, and the last one died of disease. The median follow-up was 39.5 months, ranging from 14 to 158 months (Table 1).

3.5. Literature Review

Reviewing the literature, we found descriptions of nine additional cases fulfilling the criteria mentioned above in two different papers [16,17]. The clinical, histopathological, cytogenetic, and molecular features of these patients are summarized in Tables 1 and 2.

Most patients were males (77%), with a median age at diagnosis of 73.5 years (range 63–76 years). All but one patient clinically presented with splenomegaly, and seven cases showed at least one cytopenia and one peripheral cytosis. Anemia was present in 7/9 patients (median Hb value: 11.7 g/dL, range: 7–14.4 g/dL), while leukocyte count was elevated in all but one case (mean 18,800× U/mmc, range 6100–70,000× U/mmc), as was monocyte count (mean: 2450× U/mmc, range: 970–3700× U/mmc). Monocytes represent between 4 and 40% of total leukocytes, being ≥10% in six patients. Regarding platelet count, six cases were thrombocytopenic, whereas one had thrombocytosis (median platelet count: 185,000× U/mmc, range: 42,000–498,000× U/mmc).

Regarding histopathological data, all cases showed some degree of reticulin fibrosis, with a Grade 3 in three cases, a Grade 2 in four, and a Grade 1 in two cases. In one case, there was the availability of data regarding collagen fibrosis (Grade 2), whereas data about osteosclerosis were not reported. Cellularity increased in all patients (range 80–100). They all had trilinear maturation defects and dysplasia, paired with atypical megakaryocyte proliferation, with the presence of dense clusters in three patients.

In molecular analysis, MPN driver mutation occurred in JAK2(V617F) in seven cases (VAF range: 30–60%) and in MPL (VAF: 44%) in one. TET2 mutations occurred in five patients, whereas no IDH2 mutations were found. Other mutated genes were SRSF2, ASXL1, NRAS, and KRAS. Cytogenetic analysis showed a complex karyotype in three patients (Table 1).

The treatment of choice was hypomethylating agents in four cases and Ruxolitinib in three. One patient underwent a hematopoietic stem cell transplant, but he died of other causes. In three cases, the disease remained stable; however, four patients died of the disease. The median follow-up was 24 months, ranging from 2 to 54 months (Table 1).

3.6. Survival Analyses

Survival analyses were carried out on both our patients and those retrieved from the literature. As a whole, patients' median overall survival was 52 months (Figure 6).

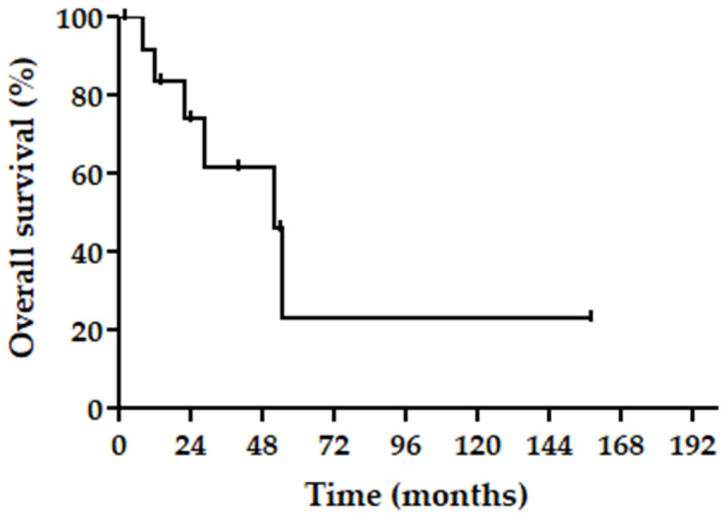

Figure 6. Kaplan-Mayer plot or patients with myeloid neoplasms with features intermediate between PMF and CMML followed in our center and retrieved from the literature [16,17].

Clinical, histopathological, genetic, and prognostic features of all the patients included in the article are further summarized in Table 4.

Table 4. Median values and ranges of the most relevant clinical, molecular, histopathological, and prognostic features of myeloid neoplasms with features intermediate between PMF and CMML (MNIPC) cases described in our series and in the literature.

	Median	**Min**	**Max**
Male sex/Total, (%)	10/13 (77)		
Age at diagnosis	67	56	76
Hemoglobin (g/dL)	9.9	6.6	14.4
Leukocytes (U/mmc)	31,930	6100	70,000
Monocytes (U/mmc)	2300	780	3700
Relative monocytes (%)	12.5	2.4	40
Platelets (U/mmc)	99,000	41,000	498,000
LDH (U/L)	1360	315	2594
Splenomegaly/Tot, (%)	11/13 (85)		
JAK2 mutation n., (%)	10/13 (77)		
Molecular driver VAF	44	30	84
Bone marrow cellularity	95%	80%	100%
Erythroid dysplasia n., (%)	10/13 (77)		
Left-shifting granulopoiesis n., (%)	10/13 (77)		
Hyperplastic megakaryopoiesis n., (%)	11/13 (85)		
Myeloproliferative or atypical megakaryocytes n., (%)	10/13 (77)		
Dense clusters of megakaryocytes	4/13 (31)		
M:E ratio	6:1	4:1	10:1
Bone marrow CD34+ cells (%)	2	1	7
Bone marrow fibrosis (grade)	2	1	3
DOD n., (%)	6 (46)		
Follow-up (mo)	24	2	158

DOD: dead of disease; LDH: lactate dehydrogenase; M:E: myeloid:erythroid; Max: maximum; Min: minimum; N: number; VAF: variable allele frequency.

4. Discussion

Persistent monocytosis for more than six months is the hallmark and one of the WHO and ICC diagnostic criteria for CMML. In the novel WHO and the ICC classification, the cut-off for monocytosis has been lowered from 1000 to 500× U/mmc to specifically include those cases with myelodysplastic CMML presenting with a low leukocyte count, and with less than 1000× U/mmc peripheral blood monocytes. Importantly, persistent monocytosis may be observed in myeloid neoplasms other than CMML [20]. Indeed, the differential diagnosis of myeloid neoplasms with monocytosis may be problematic, given the prognostic and therapeutic differences among the different categories of diseases [21].

Specifically, the main differential diagnoses in patients with myeloid neoplasms with monocytosis are CMML, juvenile myelomonocytic leukemia, and PMF, chronic myeloid leukemia, or polycythemia vera with monocytosis [20]. Furthermore, a small fraction of patients diagnosed with chronic neutrophilic leukemia or myelodysplastic/myeloproliferative neoplasm with neutrophilia, as well as most subsets of myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (except for cases with PDGFR-alpha rearrangements and ETV6::ABL1 fusion) may present with monocytosis, even though much rarely [1,5,20].

As the differential diagnosis of myeloid malignancies is characteristically a multidisciplinary one, the diagnostic criteria, according to both WHO and ICC classification, encompasses a series of clinical, cytological, histopathological, and genetic features. The evaluation of all these features together generally allows the ruling out of most of the differential diagnoses in myeloid neoplasms presenting with monocytosis [21–24]. Possibly, the most problematic of these differentials is between CMML and PMF. Indeed, the degree of clinical, morphological, and molecular overlap of the two conditions is higher if compared

with other entities [17,25]. For this reason, the literature dealing with this subject is more abundant but also very heterogeneous, given that various scientific groups focused their work on different diagnostic features shared by the two diseases, e.g., the presence and VAF of JAK2 mutations, the increase in reticulin fibrosis, or bone marrow cytology and histopathology.

According to the 2022 WHO and ICC diagnostic criteria, CMML and PMF share some of their diagnostic criteria, and besides these, they also have many clinical, histopathological, and molecular features in common (Table 3) [1,5]. As an example, some of the clinical criteria for PMF (i.e., leukocytosis, splenomegaly, and increased LDH) are also frequently observed in CMML, even though they are not listed among the diagnostic criteria of this latter. Similarly, monocytosis represents the main diagnostic criteria for CMML but is also observed in a fraction of PMF patients. Histopathologically, both neoplasms feature hypercellular bone marrow with increased granulopoiesis and less than 20% of bone marrow blasts, while genetically, they both require the presence of a clonal marker to be diagnosed [6,18]. Around 90% of PMF patients feature mutations in JAK, MPL, or CALR genes (so-called MPN driver genes), while CMML typically bears a mutation in SRSF2 and TET2 genes, showing, however, JAK2 mutation in around 10–20% of cases [26,27] Eventually, the presence of dysplasia in at least one hematopoietic lineage is required by the WHO classification for the diagnosis of CMML, but it may also be observed in PMF (e.g., in megakaryocytes) [5–7]. More specific diagnostic criteria are represented in PMF by the presence of megakaryocytic hyperplasia with dense clustering, proliferative morphology, and atypia, features that are not observed in CMML. In overt PMF (ICC)/PMF fibrotic stage (WHO), reticulin fibrosis grade 2 or more and leukoerythroblastosis represent two additional diagnostic criteria [1,5]. Accordingly, WHO and ICC classification suggest considering histopathological and molecular features to solve difficult differential diagnoses. Specifically, the ICC classification suggests privileging the diagnosis of PMF in patients developing myeloid neoplasms with JAK2 mutation and monocytosis if the JAK2 V617F VAF is high and/or in the presence of a history of myeloproliferative neoplasm [1,5].

The medical literature generally approached these ambiguities by analyzing specific subentities, i.e., PMF with monocytosis, CMML with bone marrow fibrosis, and CMML with JAK2 mutations [16,17,25,28]. Monocytosis is described in up to 17% of patients diagnosed with PMF, and it is developed in a median of 42 months after the initial diagnosis [29,30]. Such patients have been demonstrated to display a higher age at diagnosis, level of leukocytosis, number of circulating blasts, and rate of KRAS mutations with respect to PMF cases without monocytosis. Moreover, they experience leukemic evolution more frequently, and in fact, they were initially compared with patients with PMF in the accelerated phase [29,30]. Accordingly, the prognosis of patients with PMF with monocytosis is worse, with a median survival of 45 months, vs. around 60 months in those without monocytosis [21,22,29,30]. On the other hand, CMML patients present with reticulin fibrosis grade 2 or 3 in around 3% of cases [28]. These cases differ from those with typical CMML for having higher median leukocyte and absolute monocyte counts, as well as higher LDH levels and JAK2 mutation rates (50%). Median overall survival in this group is lower compared with either CMML or PMF, even though not significantly. Eventually, a second divergent subgroup of CMML patients, accounting for 10–20% of cases, is characterized by the presence of JAK2 mutations [16,26]. As with those of the previous group, these patients also present with myeloproliferative features such as higher leukocyte count but have comparable absolute monocyte count and survival compared with typical CMML ones. Importantly, the VAF of JAK2 mutation is considered in the ICC classification as a differential feature between JAK2-mutated CMML and PMF. Whereas in the former, the median VAF is 17%, in the latter, it is as high as 43% [16]. Intriguingly, taking into account the two subgroups of CMML considered above, while fibrosis grade ≥ 2 is present in around 30% of JAK2-mutated CMMLs, 50% of patients with CMML with fibrosis grade ≥ 2 bears a JAK2 mutation, thus likely representing the same subgroup of patients analyzed from different perspectives [16,28].

Instead of dissecting the differential issue just discussed, some authors advocated the existence of a "hybrid"/"gray-zone" de novo MNIPC. Emphasis should be given to the fact that both PMF and CMML may acquire specific features of other myeloid neoplasms during their clinical course, but according to the general principle of WHO classifications, a revised diagnosis must be avoided [21]. Therefore, a case can be considered MNIPC only when presenting ab initio with clinical-pathological features fulfilling the WHO/ICC criteria for PMF, monocytosis and/or CMML-associated genetic alterations (i.e., SRSF2, TET2, ASXL1 mutations), or fulfilling CMML's diagnostic criteria, and showing megakaryocytic hyperproliferation with atypia, significant reticulin fibrosis and mutation in MPN driver genes. The existence of MNIPC was initially conceptualized by Chapman et al. in 2018, and to the best of our knowledge, 9 cases of such overlapping entities are currently described in the English medical literature [16,17]. Despite sharing some of the WHO and ICC diagnostic criteria with PMF and CMML, MNIPC also displays an overlap with these entities in the clinical, histopathological, and molecular features that are not included in WHO and ICC diagnostic criteria.

In this work, we report four more cases of MNIPC. Considering together these novel cases and the previously described ones, it should be noted that in all of them, myelodysplastic (e.g., cytopenia and morphological dysplasia) and myeloproliferative features (e.g., peripheral cytoses and splenomegaly) co-exists. Therefore, such cases are to be categorized as MDS/MPN according to the current WHO and ICC classifications (Table 4) [1,5,6]. Indeed, MNIPC patients are mostly anemic, with very high leukocyte counts, increased monocyte counts (median value: 2300 U/mmc) with relative monocytosis (median 12,5%), and thrombocytopenia. They also show high levels of LDH, and most of them (85%) are splenomegalic.

Histopathologically, MNIPC patient presents with an increased bone marrow cellularity, erythroid dysplasia, left-shifted granulopoiesis with increased myeloid-erythroid ratio, and markedly increased megakaryopoiesis with MPN-like and atypical megakaryocytes and frequent detection of fibrosis grade $\geq$ 2 [5,6]. In our series, we also assessed the presence of other stromal alterations, and we could demonstrate the presence of an increase in collagen fibrosis in one patient and an osteosclerosis grade $\geq$ 1 in all of them. Similarly, we also observed other features that are commonly present in bone marrow biopsies of MPN patients, such as paratrabecular dislocation of adipocytes, an increase in microvessel density, and the presence of intrasinusoidal hematopoiesis. At the immunohistochemical evaluation, CD14 revealed an increase in monocytes (as observed in CMML), while CD34 demonstrated that blast count was below the cut-off for a PMF in the accelerated phase (i.e., $\geq$10%).

Molecular characterization of MNIPC cases (Figure 5) revealed a prevalence of mutation typically observed in CMML (i.e., *TET2*, *SRSF2*, *ASXL1*), mostly co-occurring together with *JAK2*V617F mutation [26]. Of the patients with known cytogenetic anomalies, three presented deletion/monosomy of chromosome 7 (3/6, 50%), a common finding in different subsets of myeloid neoplasm, with a poor prognostic impact [31]. Only one case (#13) with complex karyotype includes the known t(12;22)(p13;q12) resulting in MN1::ETV6 gene fusion, observed in several myeloid neoplasms and more recently reported in a case of AML with also erythroid differentiation [32].

Even though treatment regimens were relatively inhomogeneous, it may be observed that an MDS-like approach (e.g. azacytidine) was more frequently employed, compared with MPN-like regimens (e.g., JAK1/2 inhibitors, hydroxyurea).

As mentioned above, the 2022 WHO and ICC clinical and pathological diagnostic criteria for PMF and CMML include features that are shared by both entities. To verify whether such diagnostic criteria allow us to formally include the cases we studied into one diagnostic category, we compared the WHO and ICC diagnostic criteria for PMF and CMML and calculated how frequently each feature was also present in patients with MNIPC. Not surprisingly, most diagnostic criteria for either PMF or CMML were present in most patients (Table 3). The only major diagnostic criterion that was described in less

than half of patients was the evidence of dense clusters of megakaryocytes (a specification included in the major criteria of the 2022 ICC classification only), which was lacking in 56% of cases. Similarly, the presence of relative monocytosis ($\geq$10% of total leukocyte count) was observed in only 54% of patients. This last feature represents the essential diagnostic criteria for CMML in both WHO and ICC classification. Accordingly, Valent et al. included MNIPC in the CMML subset "CMML with a concomitant myeloid neoplasm" in their revision, stating that "the presence of additional (chronic) myeloid, mast cell, or lymphoid neoplasms does not exclude a diagnosis of CMML" as "Co-existing myeloid neoplasms and CMML may be derived from the same original founder clone". The reason for their view is that "CMML clone is dominant, and the additional sub-clone is [. . .] usually not relevant clinically, even if these smaller clones express certain driver mutations [. . .]" [33].

Despite being very interesting, this concept is not fully shared by the experts' community. Moreover, formally, given the impossibility of ruling out either PMF and CMML, the co-occurrence of cytopenia, peripheral cytoses, and morphological features of MPN and the lack of specific gene rearrangements/fusions, patients with MNIPC should be better diagnosed as MDS/MPN-U/NOS [1,5].

Limitations of the Present Studies

This study has some limitations that are mostly related to its subject. At first, despite being the second largest and possibly the better-characterized series of MNIPC, this condition is very rare, and thus the number of analyzed patients is low. This is also true when considering ours together with all other patients described in the literature. A second limitation is linked to the heterogeneity of the clinical, histopathological, molecular, and follow-up information available for the whole cohort, and especially for the patients retrieved in the literature. A third limitation of our work is linked to the very high complexity of MNIPC diagnosis and to the fact that in real life, these patients are—in the best scenario—classified as MDS/MPN-U/NOS, PMF with monocytosis, or CMML with bone marrow fibrosis: for this reason, we believe that MNIPC cases are generally not recognized, and thus also underreported and under characterized.

5. Conclusions

In this work, we summarized for the first time the complete phenotype of patients with MNIPC. Despite being rare, these cases are meaningful as they embody the biological fluidity of clonal myeloid proliferation and the difficulties of pigeonholing all cases according to specific and reproducible criteria.

MNIPC represents an entity in which different prognostically unfavorable features (e.g., monocytosis and bone marrow fibrosis) co-exist since patients' clinical presentation, and future studies on larger cohorts of patients are needed to determine whether these patients consistently differ in their outcome from other myeloid neoplasms currently considered in the 2022 WHO and ICC classification.

Even more importantly, it should be determined if these patients may benefit from a tailored therapeutic approach, mirroring that employed in CMML, PMF, or other conditions.

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