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Case Report

Clinical Discernment, Bone Marrow, and Molecular Diagnostics Are Equally Important to Solve the Phenotypic Mimicry among Subtypes of Myeloproliferative Neoplasms

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Abstract: The 2016 WHO classification integrates clinical, bone marrow (BM)-morphology, and molecular features to define disease entities. This together with the advancements in molecular detection and standardization of BM features enable an accurate diagnosis of myeloproliferative neoplasms (MPN) in the majority of patients. Diagnostic challenges remain due to phenotypic mimicry of MPN, failing specificity of BM-morphology, and the fact that phenotype-driver mutations, such as *JAK2*V617F, are not exclusive to a particular MPN, and their absence does not preclude any of these. We present a series of cases to illustrate themes to be considered in complex cases of MPN, such as triple-negative (TN)-MPN or MPN-unclassifiable (MPN-U). Eleven patients labelled as TN-MPN or MPN-U were included. Serum tryptase and NGS were part of a systematic/sequential multidisciplinary evaluation. Results were clustered into four categories based on diagnostic entities and/or how these diagnoses were made: (A) With expanding molecular techniques, *BCR-ABL1* and karyotyping should not be missed; (B) systemic mastocytosis is underdiagnosed and often missed; (C) benign non-clonal disorders could mimic MPN; and (D) NGS could prove clonality in some "TN"-MPN cases. The prognostic/therapeutic consequences of an accurate diagnosis are immense. In TN-MPN or MPN-U cases, a multidisciplinary re-evaluation integrating molecular results, BM-morphology, and clinical judgment is crucial.

Keywords: triple-negative myeloproliferative neoplasms; MPN-unclassifiable; mastocytosis; molecular diagnostics; next-generation sequencing

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1. Introduction

In the 2016 edition of the World Health Organization (WHO) classification system of tumours of the hematopoietic and lymphoid tissues, chronic myeloid neoplasms are classified into myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), MDS/MPN overlap, and myeloid/lymphoid neoplasms with eosinophilia and recurrent rearrangements of *PDGFRA*, *PDGFRB*, and *FGFR1* or *PMC1-JAK2*. The category of MPN includes the three major *JAK2/CALR/MPL* mutation-related MPNs (i.e., polycythaemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF)) as well as four other clinicopathologic entities: chronic myeloid leukaemia (CML), chronic neutrophilic leukaemia (CNL), chronic eosinophilic leukaemia, and not otherwise specified (CEL-NOS) and MPN unclassifiable (MPN-U). The latter subgroup includes MPN-like neoplasms that cannot be clearly classified as one of the other six subcategories of MPNs. Of note, mastocytosis is no longer classified under the MPN category [1]. Integrating clinical, bone marrow

(BM) findings, and molecular features is the most suitable attempt to define disease entities. This together with the advancements regarding the standardization of morphological bone marrow (BM) features enables an accurate diagnosis and differentiation of MPN subtypes in the majority of patients.

Yet, in routine clinical practice, diagnostic challenges remain. First, a multidisciplinary discussion at the highest level is a prerequisite for managing the phenotypic mimicry among MPNs with other myeloid neoplasms and even benign hematopoietic disorders [2]. Second, there is the issue of the failing specificity of BM morphology for differentiation of some MPN entities and their separation from MDS/MPN overlap syndromes or MDS-particularly in clonally undefined PMF [3,4]. Indeed, the reproducibility of the BM characteristics as described in the WHO classification remains a debate issue [2]. Thirdly, phenotype-driver mutations, such as *JAK2*, *CALR*, and *MPL* mutations, are not mutually exclusive and not exclusive to a particular MPN and their absence does not preclude any of these neoplasms. A positive mutation assay establishes the presence of a clonal haematopoiesis and not its identity, and the absence of a specific mutation does not rule out MPN. In fact, up to 20% of ET and 10–15% of PMF patients have no canonical *JAK2*, *MPL*, or *CALR* driver mutations and are currently referred to as "triple-negative" (TN) [5,6]. Despite the above-mentioned challenges, it needs to be remembered that the diagnosis of MPN is a multidisciplinary task requiring consideration of the presenting clinical features, morphological assessment of the peripheral blood and bone marrow aspirate (cytology) and biopsy, standard laboratory parameters, and, ever increasingly, the underlying acquired genetic status. Actually, the treating physician holds the reins, as all diagnostic pillars are available to her/him. An accurate diagnosis distinguishing between the different subtypes of MPN and their separation from MDS/MPN and systemic mastocytosis (SM) is of utmost clinical importance, as treatment options and outcome for the different subtypes vary significantly.

In this work, a cohort of clinical cases are presented to underscore essential points that need to be integrated in the diagnostic work-up when faced with complex cases of MPN, such as ill-defined TN- and/or treatment-refractory MPN or MPN-U.

2. Patients and Methods

A cohort of eleven patients referred to the University Hospital Halle in 2019 because of treatment-refractory TN-MPN or clonally ill-defined MPN-U is included. Canonical mutations in the *JAK2*, *CALR*, and *MPL* genes by standard polymerase chain reaction (PCR) were already excluded by the treating physicians. Systematic re-evaluation consisted of:

A. A thorough history and clinical examination, blood picture with a differential count, and blood chemistry (liver and renal function tests as well as LDH);
B. A serum tryptase level, which is part of our routine diagnostic work-up for hematologic diseases (reference range < 11 μg/L). Further laboratory tests were done as indicated;
C. The presence of a molecular analysis for *BCR-ABL1* and classical karyotyping was checked; and
D. Fresh BM aspirates for cytology and biopsies were re-evaluated by the haematologist and pathologist at our institution, respectively.

If a definite MPN entity could not yet be clearly defined:

A. Molecular testing for *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2*—even if eosinophilia is not present—was conducted [1,7];
B. Deep sequencing by next-generation sequencing (NGS) was done. Currently, the molecular Panel of NEO New Oncology GmbH, Cologne, Germany is used by our institution (Figure 1); and
C. Finally, cases were presented and discussed together with haemato- and molecular-pathologists in the multidisciplinary Molecular Tumour Board of the Krukenberg Cancer Center of the University Hospital Halle.

A) Coverage of exome hotspot regions

Gene Name	Chromosome	Start	End	Coverage	Reads	Region
ABL1	chr9	133738226	133738465	1901	5704	Exonic_hotspot
ABL1	chr9	133750226	133750466	2245	7328	Exonic_hotspot
ABL1	chr9	133748215	133748455	709	2248	Exonic_hotspot
ABL1	chr9	133747437	133747677	553	1783	Exonic_hotspot
CSF3R	chr1	36933372	36933612	4261	13930	Exonic_hotspot
ETNK1	chr12	22811897	22812137	1179	3562	Exonic_hotspot
FLT3	chr13	28592544	28592784	2299	7717	Exonic_hotspot
IDH1	chr2	209113078	209113198	3047	6564	Exonic_hotspot
IDH2	chr15	90631849	90632078	1611	4075	Exonic/UTR_hotspot
IDH2	chr15	90631728	90631848	1235	3016	Exonic_hotspot
JAK2	chr9	5069831	5069951	782	1748	Exonic_hotspot
JAK2	chr9	5073621	5073861	1377	4547	Exonic_hotspot
JAK2	chr9	5069952	5070150	899	1791	Exonic_hotspot
KIT	chr4	55599176	55599416	2166	7335	Exonic_hotspot
KRAS	chr12	25398148	25398388	1056	3181	Exonic/UTR_hotspot
KRAS	chr12	25380237	25380396	1358	2285	Exonic_hotspot
MPL	chr1	43814962	43815121	2693	4274	Exonic_hotspot
MPL	chr1	43818296	43818495	3179	7349	Exonic/UTR_hotspot
MPL	chr1	43805142	43805270	2283	3122	Exonic_hotspot
NPM1	chr5	170837449	170837569	610	1609	Exonic_hotspot
NRAS	chr1	115258614	115258854	3024	9657	Exonic/UTR_hotspot
NRAS	chr1	115256490	115256689	2259	5103	Exonic_hotspot
SRSF2	chr17	74732948	74733068	1652	3429	Exonic/UTR_hotspot

B) Point mutations, small insertions, and deletions

Gene	Investigated Exons	Transcript
ABL1	1–11	NM_007313
ASXL1	11, 12	NM_015338
BCOR	2–14	NM_001123384
CALR	8, 9	NM_004343
CBL	8, 9	NM_005188
CSF3R	14, 17	NM_000760
DNMT3A	2–23	NM_175629
ETNK1	3	NM_018638
ETV6	1–8	NM_001987
EZH2	2–20	NM_004456
FLT3*	14, 15, 20	NM_004119
IDH1	4	NM_005896
IDH2	4	NM_002168
JAK2	12, 13, 14, 15	NM_001322194
KIT*	8, 17	NM_000222
KRAS	2, 3	NM_004985
MLL1 (KMT2A)	1–36	NM_001197104
MPL	4, 10, 12	NM_005373
NPM1	1–11	NM_002520
NRAS	2, 3	NM_002524
RUNX1	2-9	NM_001754
SETBP1	4	NM_015559
SF3B1	13, 14, 15, 16	NM_012433
SRSF2	1	NM_003016
STAG2	3–35	NM_001042749
TET2	3–11	NM_001127208
TP53	2–11	NM_000546
U2AF1	2, 6	NM_001025204
WT1	7, 9	NM_024426

Figure 1. *Cont.*

C) *Translocations*

Gene	Investigated Exons	Investigated Introns[1,2]	Transcript
ABL1		1-2	NM_007313
BCR	8, 13-14	1,5,8,13,14,19	NM_004327
CBFB		4-5	NM_001755
ETV6		4-5	NM_001987
EVI1 (MECOM)		3	NM_004991
FGFR1		10	NM_001174067
JAK2	11, 12-15	8,10,11,16,18	NM_001322194
MLL1 (KMT2A)		6-14	NM_001197104
MLLT2 (AF4, AFF1)	5	4	NM_001166693
MYH11	29-31, 33	28-33	NM_001040114
PCM1		26, 36	NM_001315507
PDGFRA	12	11	NM_001347829
PDGFRB	2-23	8-11	NM_002609
PML	6	3, 6	NM_033238
RARA		2	NM_000964
RPN1		1	NM_002950
RUNX1		6	NM_001754
RUNX1T1		2	NM_175634

Figure 1. The current myeloid next-generation sequencing panel. (**A**) Coverage of exome hotspot regions. (**B**) Point mutations, small insertions, and deletions. (**C**) Translocations.

3. Results

The diagnostic work-up of the eleven patients was clustered into four categories based on diagnostic entities and/or how these diagnoses were made. A summary of the patients, initial referral diagnoses, main clinical features, the diagnostic hint that ultimately lead to the final diagnosis, and the therapeutic options can be found in Table 1.

Table 1. Patients, initial referral diagnosis, diagnostic clue, final diagnosis, and treatment.

Case	Gender	Age (y)	Referral Diagnosis	Clinical Characteristics	Somatic Gene Mutation per NGS	Diagnostic Hint	Final Diagnosis	Therapy
#1	F	65	TN prefibrotic MF	Platelets >1 million/µL	Not done	fusion gene *BCR-ABL1*	CML	Nilotinib
#2	F	66	TN ET	RBC-TD Platelets >1 million/µL	*BCOR*	Cytogenetics: MDS del(5q)	MDS del(5q)	Lenalidomide
#3	M	63	TN prefibrotic MF	RBC-TD, Ascites, wasting	*cKIT*D816V	Tryptase >200 ng/mL	ASM	Midostaurin, HCT
#4	F	55	MPN-U	RBC-TD, wasting, dysphagia (PEG)	*KRAS* *cKIT*D816V	Tryptase 49 ng/mL	ASM	Midostaurin, HCT
#5	F	52	TN PV	Leucocytosis	*NPM1*	Tryptase >200 ng/mL	SM-AHN (AML)	Chemo-therapy, Imatinib, HCT
#6	M	63	MDS/MPN-overlap	Pancytopenia, splenomegaly	*EZH2*	Tryptase 34 ng/mL	SM-AHN (MDS)	Midostaurin, HCT
#7	F	69	MPN-U	Pleural effusions, splenomegaly	*NRAS*	Tryptase >200 ng/mL	SM-AHN (MPN)	Midostaurin

Table 1. *Cont.*

Case	Gender	Age (y)	Referral Diagnosis	Clinical Characteristics	Somatic Gene Mutation per NGS	Diagnostic Hint	Final Diagnosis	Therapy
#8	M	31	TN PV	retinal vein thrombosis	Not done	rare heterozygous variant in the beta-globin-chain (point mutation in exon 2 (c.119A > C)	congenital erythrocy-tosis	Phlebotomy, ASA
#9	F	74	TN ET	RBC-TD, platelets >3 million/µl	None	Raynaud disease	cold agglutinin hemolytic anemia	Steroids
#10	M	56	TN PMF	RBC-TD, wasting	*JAK2* c.3323A > G p.N1108S; (exon 25) *ASXL1* *RUNX1*	sequencing	PMF	Ruxolitinib, HCT
#11	F	41	TN MPN-U	Fatigue, splenomegalie	*JAK2* c.3188G > A, p.R1063H (exon 25)	sequencing	PMF	Ruxolitinib

AML, acute myeloid leukaemia; ASM, aggressive systemic mastocytosis; ASA, acetylsalicylic acid; *ASXL1*, additional sex combs-like 1; *BCOR*, B-cell-lymphoma-6-co-repressor; *BCR-ABL1*, breakpoint cluster region-Abelson murine leukaemia; *c-KIT*, V-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; CML, chronic myeloid leukaemia; ET, essential thrombocythemia; *EZH2*, enhancer of zeste, drosophila, homolog 2; F, female; HCT, hematopoietic stem cell transplantation; *KRAS*, V-KI-RAS2 Kirsten rat sarcoma viral oncogene homolog; M, male; MDS, myelodysplastic syndrome; MF, myelofibrosis; MDS, myelodysplastic syndrome; µL, microliter; MPN, myeloproliferative neoplasm; MPN-U, myeloproliferative neoplasm unclassifiable; Nb., number; ng/mL, nanogram per millilitre; NGS, next-generation sequencing; *NPM1*, nucleophosmin 1; *NRAS*, neuroblastoma rat sarcoma viral oncogene homolog; PEG, percutaneous endoscopic gastrostomy; PMF, primary myelofibrosis; PV, polycythaemia vera; RBC, red blood cell count; *RUNX1*, runt-related transcription factor 1; SM-AHN, systemic mastocytosis with an associated haematological neoplasm; TD, transfusion dependent; TN, triple negative; y, years.

3.1. Molecular Testing for BCR-ABL1 and Classical Karyotyping Are Essential, Cost-Effective Elements in the Diagnostic-Work-Up of Myeloid Malignancies

Patient #1: A 65-year-old woman was referred as a TN prefibrotic myelofibrosis refractory to hydroxyurea, which she received for the last 14 months. Clinically, there was marked thrombocytosis with leukopenia and a left shift. Testing for *BCR-ABL1* and classical karyotyping were not done. *BCR-ABL1*-positive CML could be diagnosed through a positive PCR for *BCR-ABL1* combined with the detection of t(9;22)(q34.1;q11.2) by classical karyotyping. Evaluation of the BM confirmed the chronic phase of the disease. Therapy with the tyrosine-kinase inhibitor (TKI) nilotinib was started, and the patient achieved a hematologic, cytogenetic, and eventually a major molecular remission.

Patient #2: A 66-year-old female with transfusion-dependent (RBC-TD) anaemia and marked thrombocytosis. She was diagnosed as TN-ET and treated with hydroxyurea and anagrelide for the last 16 months. Reassessment revealed the lack of classical karyotyping. A bone-marrow examination revealed large and pleomorphic megakaryocytes with numerous mononuclear marginal and single lobed nuclei. Classical cytogenetics revealed a 5q-minus in 19 of 22 analysed metaphases.

The diagnosis of a 5q-minus syndrome was made, and therapy with lenalidomide was initiated. Normalization of platelet and haemoglobin values along with a complete cytogenetic remission was achieved.

3.2. Awarness of Systemic Mastocytosis as an Underdiagnosed Entity Must Be Upsurged

Patient #3: A 63-year-old male was referred with a diagnosis of TN-prefibrotic myelofibrosis. The patient suffered from a high disease burden (sweating, 20 kg weight loss, and

fatigue). In addition to massive splenomegaly, marked ascites and RBC-TD anaemia were present. Three BM biopsies over 12 months were performed.

Re-evaluation revealed a serum tryptase >200 µg/L (reference range <11 µg/L). BM examination showed a hypercellular marrow with delayed myelopoietic maturation, a normal blast count, and minimal reticular fibrosis. Multifocal clustering of >15 mast cells (MCs) co-expressing CD2, CD25, CD117, and mast cell tryptase were detected. BM cytology could not be performed because of dry tap. An activating mutation *KIT*D816V was present. A diagnosis of SM was made. Due to the presence of C-findings (RBC-TD anaemia and ascites), the criteria for aggressive SM (ASM) were fulfilled [8]. Treatment with the TKI midostaurin was started. Clinical improvement was fast and striking. After six months, the patient received a planned allogeneic hematopoietic cell transplantation (HCT).

Patient #4: This severely ill 55-year-old female referred with a diagnosis of MPN-U was previously published [9]. Again, the first clue to ASM was an elevated serum tryptase level of 49 ng/mL. Mast cells typical for mastocytosis were seen in the BM re-evaluation at our institute The subsequent molecular analysis revealed a classical activating p.D816V point mutation of the *cKIT*-gene and a mutated *KRAS*-gene. Treatment with midostaurin lead to a dramatic clinical improvement within three months.

Patient #5: A 52-year-old female was seen with a diagnosis of TN-PV with progressive leucocytosis and splenomegaly. Treatment consisted of phlebotomy, acetylsalicylic acid, and interferon alpha. Progression to AML with *NPM1* and *CDKN2A* mutations occurred a few months later, and the patient was referred to us. The serum tryptase level was >200 ng/mL. BM revealed a diagnosis of SM with an associated haematological neoplasm (SM-AHN). An activating point mutation in *cKIT* could not be detected. Induction chemotherapy with daunorubicin and cytarabine yielded blast clearance but persistence of MC aggregates. Therapy with the TKI Imatinib as bridging to HCT was initiated. Hepatosplenomegaly resolved, and HCT was performed. The patient is in complete remission since then.

Patient #6: A 63-year-old male presented with a diagnosis of MDS/MPN overlap. Clinically, pancytopenia and marked hepatosplenomegaly were present. The serum tryptase was 34 ng/mL. BM aspiration was a dry tap. BM biopsy showed a dysplastic haematopoiesis with 7% blasts in the bone marrow along with morphological criteria of SM. A *cKIT*D816V could not be detected. The diagnosis of SM-AHN (MDS with excess blasts-1 (MDS-EB1)) was made. Additionally, *EZH2* and *IDH2* mutations were found by NGS. The TKI midostaurin was initiated followed by HCT.

Patient #7: A 69-year-old female with a diagnosis of MPN-U was referred to us with a history of multiple liver abscesses caused by a Listeria monocytogenes infection. She was severely ill and complained of severe bone pain and weight loss. Clinically, there were pleural effusions and splenomegaly. Osteolytic bone lesions were detected by CT scan. Again, serum tryptase was >200 ng/mL. Classical cytogenetics revealed a monosomy 7, and NGS detected a mutation in the *NRAS* gene. *cKIT*D816V was absent. Again, BM aspiration was a dry tap. BM histological assessment revealed atypical mast cell infiltrates and MPN features enabling a diagnosis of SM-AHN, with MPN-U being the associated hematologic malignancy. The initiated therapy with imatinib had to be switched to midostaurin because of lack of efficacy. Azacidine was later added to control the progressive increase in blasts. Allogeneic HCT could not be performed because of ill health, and the patient eventually died.

3.3. Benign Acquired and Hereditary Disorders Could Be the Cause of Reactive Bone Marrow and/or Peripheral Blood Abnormalities

Patient #8: This 31-year-old patient referred with a diagnosis of TN-PV was previously published [10]. In addition to the unlikely diagnosis of TN-PV, the young age of the patient and a positive family history of polyglobulia were hints for a possible hereditary cause of erythrocytosis. Indeed, genetic work-up revealed the presence of a rare heterozygous point mutation in the beta-globin-chain (exon 2 (c.119A > C) leading to a change in codon 40 (CAG > CCG)). This variant belongs to the high-oxygen-affinity hemoglobinopathies.

Patient #9: A 75-year-old female with TN-triple-negative ET was seen. RBC-TD anaemia and refractory thrombocytosis (>3 million per µL) were predominant. The patient was treated with hydroxyurea, anagrelide, and cytarabine. When first seen, the patient described seasonal symptoms suggestive of mild Raynaud's phenomenon. A monoclonal serum immunoglobulin was not identified by protein electrophoresis, but an active autoimmune haemolytic anaemia, which was complement mediated by IgM cold agglutinins, was detected. Re-evaluation of the BM showed hyperplastic erythropoiesis that was initially misinterpreted as ET. There was no evidence of an underlying disease, such as aggressive lymphoma, other overt malignancies, or specific infections. Panel sequencing presented no mutations, and B-cell clonality analysis showed a physiological pattern. A diagnosis of primary chronic cold agglutinin disease (CAD) was made, and the patient was started on 50 mg prednisolone per day. Although only occasional patients are reported to respond to steroids [11], the response in the patient was dramatic, with normalization of haemoglobin and platelets within four weeks. Rituximab was given later to maintain the response and taper off steroids.

3.4. Deep Sequencing Is Able to Detect Non-Canonical Somatic or Germline JAK2 or MPL Mutations in TN-MPN Patients

Patient #10: A 56-year-old male was diagnosed with TN-PMF. Clinically, the patient presented with pronounced wasting, RBC-TD anaemia, and splenomegaly. Among others, a missense germline atypical *JAK2*-mutation in exon 25 (c.3323A > G; p.N1108S) and a somatic *ASXL1* mutation were detected [12]. Treatment with the JAK inhibitor ruxolitinib was started. After achieving a symptomatic response, an allogeneic HCT was conducted six months later.

Patient #11: A 41-year-old female presented with TN-MPN-U. Clinically, she had severe constitutional symptoms, such as fatigue as well as splenomegaly. Through a critical re-evaluation of the BM histology, a diagnosis of PMF was made; deep sequencing by NGS revealed an atypical mutation in exon 25 of the *JAK2* gene (c.3188G > A, p.R1063H). Therapy with ruxolitinib led to a fast and durable clinical response.

4. Discussion/Conclusions

As proposed by the WHO, the clinical cases underscore the importance of a multidisciplinary diagnostic approach that integrates sound clinical judgment, BM aspiration (cytology and karyotyping), and biopsy as well as molecular genetic features as partners of equal weight. This is the most suitable attempt to reach accurate diagnoses and to define disease entities, particularly in clonally ill-defined TN-MPN or MPN-U.

The identification of distinct, disease-driving mutations within the *JAK2*, *CALR*, or *MPL* genes has revolutionized the diagnostic and management landscape of classical *BCR-ABL1*-negative MPN. Deep sequencing allows the search for other non-driver "most frequent" mutations, e.g., in *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, and *SF3B1* [13,14].

Further, whole or targeted exome-sequencing approaches in TN-MPN have identified numerous novel somatic or germline mutations that occur in alternative exons of both *JAK2* and *MPL*. Many of these mutations result in a gain of function by inducing ligand-independent JAK2-STAT5 signalling [5,6]. Such non-canonical mutations could even be detected in around 10% of patients with classical *JAK2*V617F and *MPL*W515L-positive myelofibrosis [12].

Yet, in this illuminating era of expanding and extensive molecular diagnostic techniques, it is of utmost importance not to ignore or overlook well-established standard karyotyping and simple molecular testing for *BCR-ABL1* in patients labelled with a diagnosis of TN-MPN because of the immeasurable diagnostic, prognostic, and therapeutic consequences, as was the case in patients #1 and #2.

At this point, it must be emphasized that phenotype-driver mutations, such as JAK2, CALR, and MPL mutations, as well as the other non-driver "recurrent" mutations are not mutually exclusive and not exclusive to a particular MPN. This is most likely also true

for non-canonical *JAK2* and *MPL* mutations. A positive mutation assay establishes the presence of a clonal disorder but not its identity, as was the case in patients #10 and #11.

In all instances, sufficient BM aspiration should be secured in order to allow for an appropriate cytological evaluation and screening for driver and other mutations as well as classical cytogenetic analysis. The continual challenge remains in how to incorporate the molecular discoveries into the ever-evolving MPN molecular diagnostic algorithm.

With regard to BM findings, particular attention should be paid to BM morphology, as morphological features remain a central distinguishing feature in the 2016 edition of WHO classification system [1]. Yet, controversies concerning the BM morphology of different MPN subtypes is ongoing [2]. Although the overall BM evaluation shows a high degree of reproducibility, the identification of rare but specific morphological features displays a more limited reproducibility among different haemato-pathologists [4,15–17]. Further, poor-quality BM specimens, staining artefacts, treatment-induced BM modifications, and less experienced examiners might, in part, account for these shortcomings.

Certainly, advancements in the characterization and standardization of morphological BM features yielded an improvement in the differentiation of MPN subtypes, and educational workshops for haemato-pathologists can improve the integration of all histological characteristics into meaningful, reproducible subtyping of MPNs. Integrating cytological findings from a BM aspiration with BM biopsy histology is of utmost importance. Further, providing clinical data to the pathologist is an effective and simple approach to an improved diagnosis. Indeed, the histologic consensus of 53% among haemato-pathologists when BM evaluation was performed blinded to all clinical data increased to 83% when clinical data were taken into consideration with a concordance of 71% with the clinician's diagnoses [15].

Thus, a multidisciplinary discussion between clinicians, haemato-, and molecular-pathologists is the most powerful key to establishing a valid diagnosis in daily practice.

This is particularly true for patients with SM, which is, unfortunately, often overseen by both clinicians and less experienced pathologists. This was the case in patients #3 to #7. In fact, SM is the most frequent diagnostic revision we make in "real-world" practice in patients referred to the University Hospital Halle because of treatment-refractory TN-MPN or ill-defined MPN-U. For a phenotypic heterogenous disorder like SM—a true chameleon of internal medicine—routine screening with serum tryptase, although only a minor diagnostic criterion, is a simple and cheap clue for an eventual underlying SM and increases the awareness of physicians and pathologists to this disorder. In contrast to routine molecular screening for a *KIT* mutation, which is absent in 15% of patients with SM—as was the case in patients #5 to 7—serum tryptase is elevated in the majority of patients with SM [8].

Finally, benign acquired and hereditary non-clonal disorders as a possible cause of reactive bone marrow and/or peripheral blood abnormalities need to be excluded. Scanty information is available regarding the wide spectrum of reactive BM abnormalities which could be seen in the "real-world" setting. Benign hereditary or acquired non-clonal conditions might imitate MPN phenotypically, and the reactive BM abnormalities might be misinterpreted by less experienced examiners as surrogate features for MPN. Frequently, a thorough history and clinical examination provide helpful hints for an underlying benign disease and guides the further diagnostic work-up, as was the case in patients #8 and #9. Of note is that for some patients with TN-MPN, particularly TN-ET, there is no evidence of clonal haematopoiesis by X chromosome inactivation patterns. Whether these cases truly have a clonal malignancy is also questionable, as a polyclonal haematopoiesis is a feature consistent with a hereditary disorder [5,6]. In this work, we tried to highlight some aspects that accompany being faced with seemingly complex cases of MPN in everyday clinical practice, such as the importance of multidisciplinary, the fact that a correct diagnosis does not always require sophisticated and expensive techniques, and that the clue might be found in a thorough history, clinical examination, or simple and available tests, such as

that for *BCR-ABL1* and classical karyotyping. In addition, the awareness of the possibility of an underlying rare disease, such as SM, could be of value.

Yet, the report reflects a monocentric experience, and we could not claim to be exhaustive in the listing of possible pitfalls. The actual incidence of other underlying disorders in ill-defined MPNs or MDS/MPN overlap could only be answered in a prospective, well-designed, multicentre trial. In addition to the diagnostic endpoints of such a trial, scientific programs such as the analysis of bone-niche dysregulations and the identification of a possible cross talk between bone niche and immune system, which may contribute to propagating disease progression and mediating drug resistance, could be pursued [18,19].

In conclusion, an accurate diagnosis through distinguishing between the different subtypes of MPN and their separation from MDS/MPN overlap and SM is of utmost clinical importance, as treatment options and outcome for the different subtypes vary significantly. In clonally ill-defined TN-MPN or MPN-U, a multidisciplinary re-evaluation where molecular genetic features and BM findings along with a sound clinical judgment are integrated as partners of equal weight is crucial.

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