Philadelphia-negative Myeloproliferative Neoplasms: Diagnostic Insights, Genetic Factors and Vascular Impact on Survival

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Percy, Waiting for Ricky

Your friend is coming, I say
to Percy and name a name

and he runs to the door, his
wide mouth in its laugh-shape,

and waves, since he has one, his tail.
Emerson, I am trying to live,

as you said we must, the examined life.
But there are days I wish

there was less in my head to examine,
not to speak of the busy heart. How

would it be to be Percy, I wonder, not
thinking, not weighing anything, just running forward.

Mary Oliver, Dog Songs

List of Papers

This thesis is based on the following Papers, which will be referred to in the text by their Roman numerals.

I. Genetic variation in *IL28B* (I*FNL3*) and response to interferon-alpha treatment in myeloproliferative neoplasms.
Lindgren M, Samuelsson J, Nilsson L, Knutsen H, Ghanima W, Westin J, Johansson P L, Andréasson B.
European Journal of Haematology. 2018;100:419-425

II. Highly reduced survival in essential thrombocythemia and polycythemia vera patients during follow-up.
Ahlstrand E, Samuelsson J, **Lindgren M**, Pettersson H, Liljeholm M, Ravn-Landtblom A, Scheding S, Andréasson B.
European Journal of Haematology. 2020;104:271-278

III. Survival and risk of vascular complications in myelofibrosis: A population-bases study from the Swedish MPN-registry.
Lindgren M, Andréasson B, Samuelsson J, Pettersson H, Enblom-Larsson A, Ravn-Landtblom A, Scheding S, Bentham C, Ahlstrand E.
European Journal of Haematology. 2022;109:336-342

IV. Myeloproliferative Neoplasm-Unclassifiable (MPN-U): Diagnostic and prognostic insights from a population-based study in Sweden.
Lindgren M, Andréasson B, Ravn-Landtblom A, Chaireti R, Enblom-Larsson A, Peter Johansson P L, Samuelsson J, Arvidsson M, Scheding S and Ahlstrand E.
Manuscript

Contents

List of Abbreviations

CMML	Chronic myelomonocytic Leukemia
CHR	Complete hematologic remission
CR	Complete remission
DOAC	Direct oral anticoagulants
EFS	Event free survival
ELN	European Leukemia Net
ET	Essential thrombocythemia
HU	Hydroxyurea
IFN-α	Interferon alpha
IFN-λ	Interferon lambda; the protein
IFNL	Interferon lambda; the gene
LMWH	Low-molecular weight heparin
MDS	Myelodysplastic syndrome
MF	Myelofibrosis
MPN	Myeloproliferative neoplasms
MPN-AP	Myeloproliferative neoplasm accelerated phase
MPN-BP	Myeloproliferative neoplasm blast phase
MPN-U	Myeloproliferative neoplasms unclassifiable
MR	Molecular remission
ORR	Overall response rate
OS	Overall survival
PrePMF	Prefibrotic primary myelofibrosis
PMF	Primary myelofibrosis
PR	Partial response
PV	Polycythemia vera
VAF	Variant allele frequency
VKA	Vitamin K antagonist
VTE	Venous thromboembolism
WBC	White blood cell

Introduction

The Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs)—polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF)—are clonal stem cell disorders characterized by the overproduction of mature blood cells[1,2]. These diseases are driven by acquired somatic mutations, with *JAK2*V617F being the most prevalent driver mutation in PV, ET, and PMF. Other common mutations, such as *CALR* and *MPL*, are predominantly associated with ET and PMF, while *JAK2* exon 12 mutations are found in a minority of PV patients[3]. These genetic discoveries have significantly advanced our understanding of the molecular mechanisms underlying these diseases and have improved diagnostic accuracy. MPNs originate from a single mutated hematopoietic stem cell, initiating clonal hematopoiesis, with the driver mutations typically being mutually exclusive and playing distinct roles in disease phenotype, prognosis, and thrombotic risk. Beyond these, additional somatic mutations contribute to disease progression and prognosis[3-5].

MPNs are associated with a considerable risk of vascular complications, including thrombosis and bleeding[6,7], which are leading causes of morbidity and mortality in affected patients[8]. Vascular events in uncommon sites, such as the splanchnic veins[9], present unique diagnostic and prognostic challenges. In addition to these complications, MPNs carry a variable risk of progression to myelofibrosis or leukemic transformation to an accelerated phase (MPN-AP) or blast phase (MPN-BP), making disease management a delicate balance between mitigating risks and managing symptoms[10].

Diagnostic challenges are a recurring issue in MPNs, particularly for patients who do not meet established criteria for PV, ET, or MF. The category of myeloproliferative neoplasm-unclassifiable (MPN-U) exemplifies the difficulties that arise in interpreting clinical, laboratory, and morphological findings[11-13].

Traditional treatment for MPNs has focused on normalizing blood counts, reducing the risk of thrombotic complications, and alleviating symptoms such as fatigue and splenomegaly. Interferon-alpha (IFN-α) has emerged as a promising treatment with disease-modifying potential[14]. Studies have demonstrated that IFN-α can reduce clonal burden and induce molecular remission[4], though its use is often limited by tolerability issues and its mode of administration. More recently, targeted therapies such as JAK inhibitors have revolutionized the treatment landscape, particularly for patients with myelofibrosis[4].

The studies in this dissertation address key aspects of MPNs. The first study examines genetic variations in *IL28B* (*IFNL3*) and their influence on the response to IFN-α treatment. The second and third studies investigate survival outcomes and the impact of vascular complications, focusing on different patient groups with essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF), using data from the Swedish MPN registry. Lastly, the fourth

study analyzes diagnostic and prognostic challenges in patients with myeloproliferative neoplasm-unclassifiable (MPN-U) to improve classification and management of this heterogeneous group.

Literature Review

Epidemiology of MPNs

In Sweden, approximately 600-700 individuals are diagnosed annually with myeloproliferative neoplasms (MPNs), corresponding to an incidence of around 6 cases per 100,000 person-years, according to data from the Swedish MPN Registry, which has maintained near-complete national coverage since its establishment in 2008[15]. Among the MPN subtypes, polycythemia vera (PV) and essential thrombocythemia (ET) account for approximately 75% of all MPN diagnoses, while primary myelofibrosis (PMF) remains less common. Registry data indicate a slightly higher incidence of PV and PMF in men, whereas ET is more frequently diagnosed in women.

The prevalence of MPNs is substantially higher than their incidence due to the chronic nature of these disorders, with PV and ET comprising the majority of cases. The observed prevalence reflects both the chronicity of the conditions and advances in treatment that have improved survival rates over time.

In Sweden, MPNs most commonly present in individuals approximately 70 years of age, although cases are observed across all age groups, including younger adults and, rarely, children[15].

Genetic predisposition has been documented in the pathogenesis of MPNs. Germline mutations and familial clustering indicate an inherited component in some cases, with relatives of MPN patients exhibiting a 6- to 8-fold higher risk of developing the disease[16]. However, the penetrance of inherited predisposition is generally low and requires the acquisition of somatic driver mutation for the development of MPN. The *JAK2* 46/1 haplotype is one of the most studied germline variants is associated with a 2- to 6-fold increases risk of MPNs[17-19]

Historical perspectives on MPNs

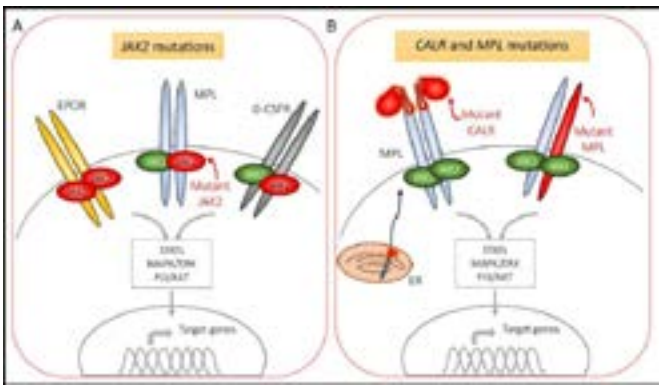
The Philadelphia chromosome-negative MPNs – PV, ET and PMF - have long captured the interest of hematologists due to their shared features of clonal hematopoietic proliferation and diverse clinical expression. The foundational perspective on these disorders was introduced in 1951 by William Dameshek in his editorial in Blood titled ”Some Speculations on the Myeloproliferative Syndromes”. In this article, Dameshek suggested that PV, ET, and PMF were different manifestations of a shared underlying mechanism driven by an unknown stimulus affecting bone marrow proliferation [20].

The first breakthrough in understanding this underlying mechanism came in 2005 with the discovery of the *JAK2*V617F mutation., a somatic gain-of-function mutation in the JAK2-kinase. This discovery was independently reported by four research groups and was based on its role in driving unregulated hematopoiesis[21-24]. The groundwork for this finding was laid in 2004 by William Vainchenker and colleagues, who demonstrated that JAK2 inhibitors suppressed erythropoietin-independent colony formation in PV [25]. This discovery was subsequently followed by the identification of somatic mutations in *MPL* in ET and PMF [26,27], *JAK2* exon 12 mutations in *JAK2*V617F-negative PV patients [28,29] and *CALR* mutations in ET and PMF in 2013 [30,31]. These findings have enhanced diagnostic accuracy and complemented the long-established role of bone marrow histology in classifying MPNs [1,2].

It is now well established that many patients with MPNs harbor additional somatic mutations beyond the driver mutations in *JAK2*, *CALR*, and *MPL*[32]. These co-occurring mutations, often in genes involved in epigenetic regulation, splicing, or DNA repair contribute to disease progression, prognosis, and treatment strategies, reflecting the genetic complexity and heterogeneity of MPNs.

Genetic and molecular mechanisms in MPNs

The pathogenesis of myeloproliferative neoplasms (MPNs) is driven by mutations that result in constitutive activation of the JAK-STAT signaling pathway. These mutations primarily affect receptors for erythropoietin, thrombopoietin, and granulocyte colony-stimulating factor (G-CSF), causing persistent signaling activity[33].



Mutations in JAK2, CALR, and MPL drive excessive myeloproliferation via constitutively active signaling downstream of JAK2[33].

MPNs originate from a single mutated hematopoietic stem cell, initiating clonal hematopoiesis. The three main driver mutations in MPNs - *JAK2*, *CALR* and *MPL*- are typically mutually

exclusive and play distinct roles in disease phenotype, prognosis, and thrombotic risk. These mutations can be acquired long before the development of overt MPN disease, a state referred to as clonal hematopoiesis of indeterminate potential (CHIP)[34]. Progression to clonal expansion and overt MPN typically requires additional factors such as genetic predisposition, additional mutations, inflammation or aging[4].

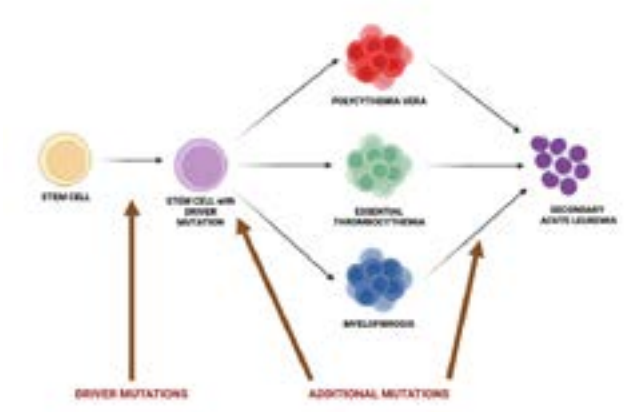


Figure 1. A simplified picture of clonal evolution from hematopoietic stemcell via driver mutations to overt MPN, via additional mutations to secondary acute leukemia.

Driver mutations

JAK2 (Janus kinas 2)
The *JAK2* V617F mutation occurs in exon 14 and is found in ~95% of PVcases and 50–60% of ET and PMF cases [22-24]. This mutation results in a valine-to-phenylalanine substitution at position 617, leading to constitutive activation of the JAK-STAT pathway, independent of ligand binding to the erythropoietin receptor (EpoR), thrombopoietin receptor (TpoR), or G-CSF receptor. *JAK2* exon 12 mutations, found in 2–3% of *JAK2* V617F-negative PV cases, also lead to constitutive JAK-STAT signaling. These mutations are associated with higher hemoglobin levels and lower platelet counts compared to *JAK2* V617F-mutated PV [28,29,35].

CALR (Calreticulin)
CALR mutations are frameshift mutations in exon 9, occurring in ~20–35% of patients with ET and PMF [30,36]. The two most common variants are type 1 (52-base-pair deletions) and type 2 (5-base-pair insertions). Mutated *CALR* lacks the C-terminal KDEL sequence, leading to altered binding to TpoR and ligand-independent activation of JAK-STAT signaling. *CALR* mutations are associated with a more favorable prognosis compared to *JAK2* or *MPL* mutations [37,38].

MPL (Myeloproliferative Leukemia virus oncogene)
MPL mutations are present in approximately 5-8% of PMF and 1-4% of ET [26,27]. These mutations lead to ligand-independent activation of TpoR, resulting in constitutive JAK-STAT pathway activation.

Triple-negative disease
Approximately 10% of PMF patients lack mutations in *JAK2*, *CALR* or *MPL*, referred to as triple-negative (TN) disease. PMF TN patients typically have a worse prognosis, with significantly shorter survival and higher rates of leukemic transformation compared to patients with driver mutations [39].

An estimated 10-15% of ET patients are also triple-negative[40]. In a recent report by Godfrey et al [41], these patients were predominantly younger females, and in contrast to PMF presents as very-low risk by the revised IPSET thrombosis score and comprehensive genomic sequencing often failing to identify recurrent genomic lesions[32,42-44]. Although the diagnosis of TN ET requires fulfillment of histologic bone marrow criteria for ET, emerging evidence suggest that at least some cases are polyclonal [45,46] . Additionally, recent data indicate that polygenic germline variation may influences platelet parameters in these patients[47].

Prognostic and co-occurring mutations

Beyond driver mutations, additional somatic mutations contribute to disease progression and prognosis. These mutations can be classified into high-molecular-risk (HMR) mutations and other additional mutations.

High-Molecular-Risk (HMR) mutations
HMR mutations include alterations in *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, *IDH2*, and *U2AF1* which are associated with reduced overall survival (OS) and increased leukemic transformation risk in PMF [5,38,48]. The presence of a single HMR mutation reduces median OS to ~7 years, compared to 12 years for those without HMR mutations. Patients with two or more HMR mutations have a significantly worse prognosis, with a median OS of ~3 years [38]. HMR mutations are crucial for risk stratification, particularly in identifying candidates for allogeneic HSCT.

Additional mutations
Beyond the HMR category, other mutations influence disease heterogeneity and progression but are not classified as HMR. These include mutations in splicing factors (*SF3B1, ZRSR2*), epigenetic regulators (*TET2, DNMT3A*), and tumor suppressors (*TP53*). Although *TP53* mutations are not considered HMR, they are strongly associated with leukemic transformation and poor outcomes [5].

The identification of driver and co-occurring mutations is essential for diagnosis, risk stratification, and management of MPNs. Incorporating HMR mutations into prognostic models such as MIPSS70+ Version 2.0, enhances predictive accuracy for overall survival and leukemic transformation, guiding therapeutic decisions, particularly for HSCT.

Advances in diagnostics

The classical MPNs—PV, ET and PMF (including prefibrotic and overt stages), and MPN-U are clonal hematopoietic stem cell disorders, characterized by increased proliferation of the myeloid lineages in the bone marrow. While MPN-U is considered a distinct subcategory [49], theses MPNs share overlapping clinical, morphological, and molecular features, with their diagnosis requiring the integration of clinical parameters, bone marrow morphology, and molecular genetic findings for accurate classification and risk stratification. The WHO classification has evolved over time to incorporate these elements.

Clinical parameters

Clinical parameters provide the initial clues for an MPN diagnosis. Peripheral blood abnormalities such as erythrocytosis (PV), thrombocytosis (ET and prePMF), or anemia and cytopenia (PMF) are fundamental diagnostic indicators. Organ involvement, particularly splenomegaly, along with constitutional symptoms such as weight loss, night sweats, and fever, can also be characteristic features of MPNs.

Bone marrow morphology

Bone marrow morphology remains a cornerstone in the diagnosis of MPNs. PV is characterized by hypercellularity with trilineage proliferation, dominated by erythropoiesis. ET features an increased number of large, mature megakaryocytes with hyperlobulated nuclei and minimal or absent fibrosis. Prefibrotic PMF displays atypical megakaryocyte clustering with atypical nuclear morphology, increased age-adjusted cellularity, and decreased erythropoiesis but without significant fibrosis (reticulin grade ≤1). In fibrotic PMF, reticulin or collagen fibrosis is the dominant feature, accompanied by megakaryocyte atypia, and osteosclerosis in advanced stages[1,2].

Although prePMF shares clinical similarities to ET, their bone marrow features are distinct. Differentiating ET from prePMF is of clinical importance, as prePMF is associated with shorter survival, higher rates of leukemic transformation, and a greater likelihood of progression to overt myelofibrosis compared to ET[50].

Myelofibrosis can occur as a primary disease (PMF) or as a secondary progression from PV (post-PV) or ET (post-ET). These secondary forms of MF were initially classified according to IWG-MRT criteria[51] but has since been included in the ICC classifications.

Molecular genetics

Molecular genetic findings are central to MPN diagnostics, confirming clonality and aiding subtype classification. *JAK2*V617F, present in ~95% of PV cases and 50–60% of ET and PMF cases, is the most common driver mutation. *CALR* and *MPL* mutations are seen in 20–25% and 5–10% of ET and PMF cases, respectively[52]. Triple-negative cases, where these mutations are absent, require expanded molecular testing to identify alternative clonal marker mutations associated with myeloid neoplasms, such as *ASXL1, IDH1, IDH2, SF3B1, SRSF2*, TET2, to

support the clonal nature of the disease[1,2]. These additional markers also provide insights into disease prognosis and progression.

Evolution of classification systems

The World Health Organization (WHO) introduced the first standardized classification system for MPNs in 2001, emphasizing bone marrow morphology as a key diagnostic component[53]. In 2008, the classification was updated to include *JAK2* V617F as a diagnostic marker, improving diagnostic precision [54] Bone marrow biopsy was also established as essential for distinguishing between ET and PMF, with a focus on megakaryocyte morphology and fibrosis grading. Additionally, hemoglobin and hematocrit thresholds for PV diagnosis were lowered.

In 2016, the WHO criteria were refined to incorporate *CALR* and *MPL* mutations for the diagnosis of ET and PMF[49]. For PMF, the presence of "another clonal marker " was introduced as a diagnostic criterion in cases where *JAK2*, *CALR*, and *MPL* mutations were absent[55,56]. Prefibrotic PMF (prePMF) was formally recognized as a distinct clinical entity, allowing for better differentiation from ET and overt PMF.

The 2016 update also further lowered diagnostic thresholds for hemoglobin and hematocrit to facilitating detection of masked PV [49,57]. The diagnostic framework for MPNs in the 2022 WHO classification closely aligns with the 2016 edition and is paralleled by the International Consensus Classification (ICC) introduced the same year[1,2]. Post-PV and post-ET myelofibrosis continue to be classified according to the criteria developed by the International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) [51] which are also included in the ICC 2022.

Building on these principles, the WHO classification and IWG-MRT establish specific diagnostic criteria for PV, ET, MF (prefibrotic, overt and secondary), and MPN-U, integrating clinical, morphological, and molecular findings. Summaries of these criteria, including tables from WHO 2016 and IWG-MRT, follow.

Table 1. Summary of diagnostic criteria for PV and post-PV MF according to WHO 2016 and IWG-MRT.

PV	Post-PV MF
Major criteria 1. Elevated hemoglobin concentration or elevated hematocrit or increased red blood cell mass 2. Bone marrow biopsy showing age-adjusted hypercellularity with trilineage proliferation (panmyelosis), including prominent erythroid, granulocytic, and increase in pleomorphic, mature megakaryocytes without atypia Presence of *JAK2* V617F or *JAK2* exon 12 mutation *Minor criterion* • Low serum EPO levels	*Required criteria* 1. Previous established diagnosis of PV 2. Bone marrow fibrosis of grade 2 or 3 *Additional criteria* 1. Anemia or sustained loss of requirement of either phlebotomy or cytoreductive treatment for erythrocytosis 2. Leukoerythroblastosis 3. Increase in palpable splenomegaly of >5 cm from baseline or the development of a newly palpable splenomegaly 4. Development of any 2 of the following constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)
The diagnosis of PV requires either all 3 major criteria or the first 2 major criteria plus the minor criterion	The diagnosis of post-PV MF is established by all required criteria and at least 2 additional criteria

Table 2. Summary of diagnostic criteria for ET and post-ET MF according to WHO 2016 and IWG-MRT.

ET	Post-ET MF
Major criteria 1. Platelet count ≥450 × 10^9/L 2. Bone marrow biopsy showing proliferation mainly of the megakaryocytic lineage, with increased numbers of enlarged, mature megakaryocytes with hyperlobulated staghorn-like nuclei, infrequently dense clusters; no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis; no relevant bone marrow fibrosis. 3. Diagnostic criteria for BCR:ABL1-positive CML, PV, PMF, or other myeloid neoplasms are not met 4. *JAK2, CALR*, or *MPL* mutation *Minor criteria* • Presence of a clonal marker or absence of evidence of reactive thrombocytosis.	*Required criteria* 1. Previous established diagnosis of ET 2. Bone marrow fibrosis of grade 2 or 3 *Additional criteria* 1. Anemia and a >20 g/L decrease from baseline hemoglobin concentration 2. Leukoerythroblastosis 3. Increase in palpable splenomegaly of >5 cm from baseline or the development of a newly palpable splenomegaly 4. Elevated LDH level above the reference range 5. Development of any 2 of the following constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)
The diagnosis of ET requires either all major criteria or the first 3 major criteria plus the minor criteria	The diagnosis of post-ET MF is established by all required criteria and at least 2 additional criteria

Table 3. Diagnostic criteria for prefibrotic and fibrotic PMF according to WHO 2016 criteria.

PMF; prefibrotic phase	PMF; fibrotic phase
Major criteria 1. Bone marrow biopsy showing megakaryocytic proliferation and atypia, bone marrow fibrosis grade <2, increased age-adjusted bone marrow cellularity, granulocytic proliferation, and (often) decreased erythropoiesis 2. *JAK2, CALR,* or *MPL* mutation or presence of another clonal marker or absence of reactive bone marrow reticulin fibrosis. 3. Diagnostic criteria for BCR:ABL1-positive CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms are not met. Minor criteria • Anemia not attributed to a comorbid condition • Leukocytosis ≥11 x 109/L • Palpable splenomegaly • LDH level above the above reference range	Major criteria 1. Bone marrow biopsy showing megakaryocytic proliferation and atypia, accompanied by reticulin and/or collagen fibrosis grades 2 or 3 2. *JAK2, CALR,* or *MPL* mutation or presence of another clonal marker or absence of reactive bone marrow reticulin fibrosis 3. Diagnostic criteria for ET, PV, BCR:ABL1-positive CML, myelodysplastic syndrome, or other myeloid neoplasms are not met Minor criteria • Anemia not attributed to a comorbid condition • Leukocytosis ≥11 x 109/L • Palpable splenomegaly • LDH level above the above reference range • Leukoerythroblastosis
The diagnosis of pre-PMF or overt PMF requires all 3 major criteria and at least 1 minor criterion confirmed in 2 consecutive determinations	

Table 4. Diagnostic criteria for MPN-U according to WHO 2016 criteria.

MPN-U
Morphological, laboratory, molecular, and/or clinical data indicative of an MPN.
Absence of WHO criteria for specific MPN entities, including CML, MDS, or MDS/MPN.
JAK2, CALR, or *MPL* mutation or presence of another clonal marker or absence of reactive bone marrow reticulin fibrosis
Exclusion of secondary effects due to treatment, other diseases, or the natural progression of other myeloid neoplasms.
The diagnosis of MPN-U requires all 4 criteria

Prognostic models in MPNs

Risk stratification in MPNs is an essential aspect of patient management, used to guide treatment decisions and predict outcomes. The stratification models evaluate several risks, including overall survival, vascular complications and disease progression. Over the years, several clinical scoring systems have been developed, with more recent models incorporating molecular risk factors.

While PV and ET models primarily focus on vascular events and transformation risks, PMF models are oriented toward overall survival and disease progression. including the selection of candidates for HSCT.

For PV and ET, the risk of vascular complications, including thrombosis and bleeding, is a primary consideration in the risk scoring models. In PV risk stratification relies on two clinical factors: age ≥60 years and a history of thrombosis. These parameters classify patients as either low-risk or high-risk. In ET, a more detailed risk stratification is used, with the revised IPSET-thrombosis model including age, history of thrombosis, *JAK2* mutation status, and cardiovascular risk factors[58]. Patients are classified into low-, intermediate-, or high-risk groups with annual thrombosis risks of 1%, 2.35%, and 3.56%, respectively. The revised IPSET thrombotic model is also validated for risk prognostication of thrombotic events in prePMF [59].

PMF is associated with reduced survival and an increased risk of progression to accelerated and blast phase MPN, compared to PV and ET. Multiple models have been developed to predict survival and disease progression. DIPPS and DIPPS-plus model incorporate clinical factors such as age > 60 years, anemia(Hb < 100 g/L), leukocytosis (>25 x 10^9/L), constitutional symptoms, and peripheral blood blasts (≥1%)[60,61]. DIPSS-plus includes additional factors including cytogenetic abnormalities and transfusion dependency. MIPPS-70 and MIPPS-70+ Version 2.0 add molecular factors, including *ASXL1, EZH2*, *SRSF2*, *IDH1/2* and *U2AF1* mutations and unfavorable karyotypes, to improve the precision of risk stratification and identification of patients who may benefit from more intensive therapies, including allogeneic HSCT[62,63].

No specific risk models are validated for MPN-U due to its heterogeneity. Individualized assessments based on clinical and molecular findings guide management strategies, often paralleling those used for classified MPN subtypes[64].

Vascular Events in MPNs

Thrombotic events represent a major cause of morbidity and mortality in patients with MPNs. Both arterial and venous thrombosis occur significantly more frequently in individuals with MPNs compared to the general population, with incidence rates varying across studies [65,66]. A Swedish registry study by Hultcrantz et al, reported a markedly increased risk of venous thrombosis in patients with MPNs, as well as an elevated risk of arterial thrombosis at diagnosis. The age-standardized incidence for thrombosis was 4,45 per 100 000 patient years, but incidence

increasing with age and risk remaining elevated over time [67]. Similarly, the ECLAP study, the largest epidemiological investigation of PV involving 1 638 patients, highlighted the high thrombotic burden in this population[68].

Thrombotic complications are often a primary presenting feature, revealing the diagnosis in approximately 20% of MPN patients. The overall prevalence of thrombosis is highest in PV (28,6%) followed by ET (20,7%) and PMF (9,5%), with arterial events being more common than venous[69]. Following diagnosis , the risk of thrombosis remains significantly elevated compared to matched controls.

After diagnosis, thrombotic events remain significantly more common in patients with MPNs compared to matched controls[67,70]. In PV, the incidence of thrombosis is 1.36 per 100 000 patient-years, with venous events occurring twice as frequently as arterial events[71]. In PMF, the incidence ranges from 1.65 to 2.23 per 100 000 patient-years, arterial and venous events occurring in equal numbers[72]. Cardiovascular complications in PV are also the primary contributors to reduced survival, resulting in a lower median survival (14.1 years) than that of an age- and sex-matched population[70,73,74]

Thrombosis in MPNs arises from multifactorial causes involving both clonal and patient-related factors[75]. Clonal factors, specific to the malignant hematopoietic clone, include hyperviscosity, elevated blood counts, and endothelial dysfunction, particularly in PV[76]. The presence of a JAK2 mutation further amplifies the thrombotic risk, with *JAK2*-mutated ET and PV exhibiting roughly double the thrombosis risk compared to *CALR*-mutated ET[77]. *JAK2*-mutated clonal cells, including erythrocytes, leukocytes, and platelets, contribute to a prothrombotic phenotype. Activated platelets from the malignant clone form aggregates with leukocytes, releasing cytokines and enhancing thrombogenesis, a process referred to as thrombo-inflammation[78].

A critical component of thrombo-inflammation in MPNs is the formation of neutrophil extracellular traps (NETs), a process known as NETosis. NETs, composed of DNA fibers and antimicrobial proteins, are produced by neutrophils as part of the innate immune response and play a dual role in hemostasis and host defense. During immunothrombosis, a term introduced by Engelmann and Massberg in 2013, platelets cooperate with neutrophils to promote coagulation and thrombus formation[79]. However, dysregulation of immunothrombosis, as seen in J*AK2*V617F MPNs, can lead to pathological thrombosis, including arterial and venous events. NETs are significantly increased in MPN patients and contribute directly to thrombus formation, as demonstrated in studies by Guy et al. and Schmidt et al. [75,80]. Experimental models in mice confirm the central role of NETs in MPN-associated thrombosis [81,82] Importantly, activated platelets in *JAK2*V617F MPNs promote NET formation, while aspirin-mediated platelet inhibition has been shown to reduce both NETosis and thrombotic risk.

Additionally, preactivated leukocytes in MPNs are an independent risk factor for thrombosis[83-85]. In a prospective PV cohort, leukocyte counts $>11 \times 10^9$/L were associated with a higher risk for thromboembolism[71].

Preventing thrombosis in MPNs involves controlling hematologic parameters and addressing individual risk factors. Classical risk factors include age 60 or older and a history of thrombosis[86-88] For ET, the revised International Prognostic Score of Thrombosis for ET (IPSET) incorporates classical risk factors along with the presence of *JAK2*V617F mutation and cardiovascular risk factors[58,89]. The revised IPSET model has also been evaluated for prePMF[59].

Preventing both the occurrence and recurrence of thrombotic events in MPNs involves a combination of antithrombotic and cytoreductive therapies, along with the assessments and management of cardiovascular risk factors. Treatment goals for PV include maintaining hematocrit levels below 45%, as established ty the CYTO-PV study[90]. This study demonstrated that patients with a hematocrit target of <45% experienced significantly lower rates of cardiovascular death and major thrombotic events compared to those with a target of 45-50%. Although no definitive evidence exists for a specific platelet threshold, a platelet count below 400 x 10^9/L is commonly targeted. Additionally, maintaining leukocyte counts below 12 x 10^9/L has been observed to have a protective effect in PV[71].

For PV patients the ECLAP study demonstrated efficacy of low-dose aspirin in preventing thrombotic complications[91]. However, the efficacy of low-dose aspirin in ET has not been evaluated in randomized trials. Current evidence for aspirin use in ET relies on extrapolations from the ECLAP study and retrospective analyses, recommendation for high-risk or low-risk with *JAK2* V617F-mutation or cardiovascular risk factors[92,93]. However, in patients with *CALR*-mutated ET, low-dose aspirin has not shown a benefit in thrombosis prevention and is associated with increased bleeding risk. For patients with high platelet turnover, twice-daily aspirin has demonstrated potential benefits in reducing thrombotic events, as supported by the ARES study[78].

Cytoreductive therapy is essential for managing hematologic parameters in MPNs and cytoreductive therapies like hydroxyurea or interferon are used to normalize platelet and leukocyte counts, reducing thrombotic risk[94]. However, the optimal choice of cytoreductive therapy for preventing thrombotic complications remains unclear.

Acute thrombotic events are managed with standard anticoagulants. Increasing evidence from studies on cancer-associated thrombosis suggests that in patients with MPN and VTE, direct oral anticoagulants (DOACs) can be initiated directly[95,96]. Vitamin K antagonists (VKAs), such as warfarin, are reserved for cases of severe renal impairment and remain the only option in patients with mechanical heart valve or antiphospholipid syndrome. Cytoreductive therapy is also initiated or intensified to control hematologic parameters and prevent recurrence. Specific thrombotic scenarios, such as hepatic vein thrombosis (Budd-Chiari Syndrome) and sinus thrombosis require prolonged anticoagulation due to high recurrence risks[97,98].

Bleeding events are more common in MF and ET than in PV[69]. Risk factors include advanced age, prior major bleeding events, renal or liver dysfunction, thrombocytopenia, uncontrolled hypertension, gastrointestinal disease, and prior stroke[95]. Extreme thrombocytosis (>1,500 × 10^9/L) is a key risk factor, as it can lead to acquired von Willebrand Syndrome (VWS), where depletion of von Willebrand multimers causes functional deficiency and increased bleeding

risk[99-101]. Platelet dysfunction, characterized by impaired granule release and reduced receptor function, further exacerbates bleeding tendencies [101,102]. Dual therapy with aspirin and anticoagulants is generally avoided due to the heightened bleeding risk [99,100]. In cases of acute bleeding, anticoagulants should be discontinued, and blood counts normalized with cytoreductive therapy. Tranexamic acid may be administered to improve hemostasis, and in severe cases, platelet transfusions or von Willebrand factor concentrate may be required[101].

Therapeutic approaches

Hydroxyurea

Hydroxyurea (HU) is a cytoreductive agent with extensive evidence supporting its use in the treatment of myeloproliferative neoplasms, including PV and ET. Its efficacy in reducing the risk of cardiovascular events has been demonstrated in several randomized controlled trials and epidemiological studies. The CYTO-PV study showed that maintaining hematocrit levels below 45% with treatment, including hydroxyurea, significantly reduces thrombotic events in patients with PV [90]. For ET, studies like Haider M, et al. using the IPSET-thrombosis model, demonstrated how HU effectively reduces thrombotic risk, particularly in high-risk patients[89].

HU works by inhibiting DNA synthesis, making it effective in controlling elevated blood counts. Although there has been theoretical concern regarding an increased risk of leukemic transformation with HU use, prospective studies and a large Swedish registry study have not shown a significant increase in leukemia risk, provided the patient has not been previously treated with other cytotoxic agents[86,103,104]. In the treatment of younger patients, IFN-α is recommended as the first-line option, and HU should be avoided during pregnancy.

Interferon-alpha

Interferon-alpha is a cytokine with diverse biological properties, including antiviral, antiproliferative and immunomodulatory effects. In MPNs IFN-α induces hematologic remissions by inhibiting the proliferation of hematopoietic progenitors and normalizing blood counts[105-107]. It also exerts disease-modifying effect by selectively targeting hematopoietic stem cells carrying *JAK2* V617F driver mutations, achieving molecular remissions[4,108-111]. In early forms of MF, IFN-α has also shown the potential to normalize bone marrow histology and reverse fibrosis[112].

IFN-alpha has been used off label in clinical practice for decades, with the first study by Silver et al in 1988 demonstrating its efficacy in PV and ET, followed by subsequent studies in MF[113-116]. Early studies focused on non-pegylated forms of IFN-α. Non-pegylated forms were later succeeded by trials on pegylated forms with improved pharmacokinetics, reduced dosing frequency and a more acceptable toxicity profile. Most recently, the development of monopegylated ropeginterferon alpha-2b (ropegIFN) represented an advancement, becoming the first IFN approved for treatment based on the Phase 3 PROUD-PV and CONTINUATION-PV trial by Gisslinger et al [117].

Current guidelines recommend IFN-α as first- or second-line treatment for PV and ET, and particularly for younger patients, high-risk individuals and those intolerant to HU[92,118-120]. It is also the only cytoreductive therapy considered safe during pregnancy for managing blood counts and reducing pregnancy-related complications[121,122].

Clinical trials in , including the PROUD-PV and CONTINUATION-PV study, demonstrated that ropegIFN achieved a higher rate of CHR and greater reductions in *JAK2*V617F allele burden compared to HU. These responses were associated with improved event-free survival which included thromboembolic events, disease progression to MF or acute leukemia and death[117]. In ET, pegIFN-α has shown efficacy comparable to HU, with meta-analysis reporting a CHR rate of approximately 59% and a low incidence of thromboembolic events[123].

IFN-α has a well-documented toxicity profile, including flu-like symptoms, musculoskeletal pain, and autoimmune or psychiatric complications, with the development of pegylated and monopegylated formulations appearing to improve tolerance, likely due to improved pharmacokinetics[113,117].

JAK inhibitors

JAK inhibitors are an established treatment option for MF, particularly for managing constitutional symptoms, reducing spleen size, and improving quality of life. Their use has been extensively studied in clinical trials, beginning with ruxolitinib, a JAK1/JAK2 inhibitor approved in 2011. The COMFORT-I and COMFORT-II trials demonstrated significant reductions in spleen size and improvements in constitutional symptoms compared to placebo or best available therapy (BAT) [124-126]. Long-term follow-up suggested improved survival in the ruxolitinib-treated group, though this finding remains under discussion[127,128]. Common adverse effects include anemia, thrombocytopenia, and an increased risk of opportunistic infections.

Fedratinib, a selective JAK2 inhibitor, is approved for intermediate-2 and high-risk MF, including patients who are resistant or intolerant to ruxolitinib. The JAKARTA and JAKARTA-2 trials showed that fedratinib effectively reduced splenomegaly and improved symptom burden[129-131]. Monitoring thiamine levels is recommended due to the risk of Wernicke encephalopathy, and prophylactic antiemetic treatment may mitigate gastrointestinal side effects.

Momelotinib, another JAK inhibitor, has been studied in patients with MF and anemia in the SIMPLIFY-I, SIMPLIFY-II trials and MOMENTUM trials[132-134]. The trials demonstrated its efficacy in reducing splenomegaly and transfusion dependency, though its symptom control was less pronounced compared to ruxolitinib. It is considered a treatment option for patients with anemia where other JAK inhibitors may be less suitable.

Pacritinib is indicated for patients with MF and severe thrombocytopenia (platelet counts $<50 \times 10^9$/L). The PERSIST-1, PERSIST-2, and PAC203 trials showed that pacritinib effectively reduces spleen size and symptom burden in this subgroup[135,136]. Its additional FLT3 inhibition may provide further therapeutic benefits beyond JAK inhibition.

Although JAK inhibitors provide symptomatic relief and disease control for many patients, responses are often time-limited, and cytopenias remain a frequent challenge during treatment. The need for effective therapies in the post-ruxolitinib setting continues to be an area of clinical focus.

Hematopoietic stem cell transplantation (HSCT)

Hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for patients with myelofibrosis (MF) and other advanced myeloproliferative neoplasms (MPNs). It is generally reserved for patients with intermediate-2 or high-risk disease based on prognostic scoring systems such as the Dynamic International Prognostic Scoring System (DIPSS), the mutation-enhanced international prognostic scoring system (MIPSS) or MIPSSv2 (the karyotype-enhanced MIPSS70)[60,62,63].

Disease progression in MPNs: accelerated and blast phase

Accelerated phase MPN (MPN-AP) is defined by the presence of 10–19% blasts in the peripheral blood or bone marrow, while blast phase MPN (MPN-BP) is characterized by ≥20% blasts. The risk of transformation varies across subtypes, with rates reported as 2.3–7% in PV, 5.8% in prePMF, and 10–20% in MF within 10 years of diagnosis. In contrast, the risk In ET is <1%. Risk factors for transformation include advanced age, complex karyotype, *TP53* mutation, additional cytogenetic abnormalities, and the sequential use of multiple cytoreductive agents. However, HU alone has not been conclusively linked to an increased risk of leukemic transformation[104,137].

The prognosis of MPN-AP/BP is dismal, with a median OS of 2-5 months after transformation. Induction chemotherapy, while potentially effective in achieving remission, is associated with significant toxicity, including a treatment-related mortality of 33%[138].

The management of MPN-AP/BP is challenging, and treatment strategies vary based on patient eligibility for curative or palliative approaches. HSCT remains the only potentially curative treatment. Outcomes are better in patients who achieve a complete remission prior to transplant[139]. Patient selection is crucial and is based on factors such as age, performance status, comorbidities, psychosocial considerations, and pre-transplant response.

Hypomethylating agents (HMA) such as azacitidine (AZA) and decitabine are widely used, particularly in patients who are not candidates for HSCT. The overall response rate (ORR) with AZA monotherapy is approximately 52%, with a median OS of 11 months[140]. Combining HMAs with ruxolitinib has shown improved response rates, achieving an ORR of 53% and a median OS of 7.9 months in patients with post-MF transformations[141,142]. The combination of HMAs and venetoclax has demonstrated higher ORR of 50–53%, though this approach is associated with significant toxicities[143,144]. Induction chemotherapy has limited efficacy and significant toxicity, with a reported ORR of 41% and a median OS of 4 months. However, it may serve as a bridge to HSCT in select patients[145]. For patients ineligible for intensive treatments or transplantation, a

palliative strategy is recommended. This includes HMAs with or without JAK inhibitors to manage splenomegaly and constitutional symptoms[142].

Given the poor prognosis associated with MPN-AP/BP, early referral to a transplant center is essential to evaluate candidacy for HSCT and to optimize treatment planning. Bridging therapies, including HMAs with or without JAK inhibitors, can be used to control disease and reduce marrow blasts prior to transplant[139,145].

Criteria for Treatment Response in PV, ET, and MF

Standardized response criteria for PV and ET were introduced by the ELN in 2009 to improve the interpretation and comparability of clinical studies[146]. These were revised in 2013 by ELN and IWG-MRT, partly due to the discovery of the *JAK2*V617F mutation, which shifted treatment goals from solely focusing on cytoreduction and thrombosis risk to broader disease modification[147]. The discovery of the *JAK2*V617F mutation in 2005 also prompted further revisions to the response criteria. Despite this, the primary aim remains the assessing antiproliferative activity, without explicitly incorporating molecular response.

Key elements of the 2013 response criteria for PV and ET include:

- Platelet count < 400×10^9/L.
- Hematocrit < 45% without phlebotomy (PV).
- Bone marrow remission: Resolution of megakaryocyte hyperplasia and the absence of reticulin fibrosis > grade 1.
- Absence of disease progression and thrombotic or hemorrhagic events.
- Symptomatic improvement assessed via the MPN-SAF TSS [148].

Routine monitoring of bone marrow morphology is uncommon in national care programs [92,120] and is primarily used to assess disease progression.

For MF, response criteria were introduced by the IWG-MRT in 2006[149] and updated in 2013 [150] with new categories to capture clinical improvement:

- Anemia response: Hemoglobin improvement without transfusion.
- Spleen response: ≥ 35% reduction in spleen volume or palpable spleen size.
- Symptom response: Reduction in MPN-SAF TSS.

Additional response definitions include cytogenetic remission and molecular remission, though their role in routine clinical practice is still under investigation.

Aims

The overarching goal of the work presented in this thesis is to deepen our comprehension of MPNs, with a particular focus on diagnostic challenges, treatment responses, survival rates, and associated vascular complications. By addressing these aspects, the four articles aim to contribute to the existing knowledge base on MPNs, encompassing issues related to classification, occurrence, prognosis and treatment approaches.

The long-term goal is to improve the diagnosis, treatment, and outcomes of MPN patients. Through investigations into genetic variations, survival patterns, vascular complications, and diagnostic challenges, the articles aim to contribute to the foundation for personalized treatment strategies, improved prognostic indicators, and better guidelines for monitoring and intervention.

Each article has specific aims:

1. Genetic Variations in *IL28B* (*IFNL3*) and Response to Interferon-Alpha Treatment in Myeloproliferative Neoplasms:
   Aim: Examine how genetic variations in the *IL28B* gene influence the effectiveness of interferon-alpha therapy in patients with myeloproliferative neoplasms.
2. Highly Reduced Survival in Essential Thrombocythemia and Polycythemia Vera Patients with Vascular Complications:
   Aim: Assess the survival rates of patients with essential thrombocythemia and polycythemia vera patients who experience vascular complications compared to those who do not, highlighting the prognostic importance of these complications.
3. Survival and Risk of Vascular Complications in Myelofibrosis – A Population-Based Study:
   Aim: Analyze survival rates and evaluate the likelihood of vascular complications in individuals diagnosed with myelofibrosis. using data from a population-based study.
4. Myeloproliferative Neoplasms Unclassifiable:
   Aim: Investigate the prevalence of MPN-U in Sweden, analyze survival data, explore diagnostic challenges, and examine the incidence of thrombotic events to enhance understanding and management of this heterogenous condition.

These specific aims collectively contribute to the broader objectives of advancing knowledge, improving diagnostic accuracy, enhancing prognostic tools, and optimizing therapeutic approaches for patients with myeloproliferative neoplasms.

Materials and methods

The Swedish MPN Registry

The Swedish MPN Registry is a web-based national quality register established in January 2008 to systematically document and monitor the diagnosis of myeloproliferative neoplasm (MPN). The registry was initiated by the Swedish representatives of the Nordic MPN Study Group and is managed by the Swedish MPN Group in collaboration with the Regional Cancer Center (RCC) Stockholm-Gotland. Funding is provided by the Swedish Associations of Local Authorities and Regions (SKR). The registry is integrated within the Swedish Blood Cancer Registry and, as of 2020, includes the classical MPN diagnoses of polycythemia vera (PV), essential thrombocythemia (ET), prefibrotic myelofibrosis, and myelofibrosis (both primary and secondary). Additionally, it includes MPN unclassifiable (MPN U) and the rare diagnoses of chronic eosinophilic leukemia and chronic neutrophilic leukemia.

Since 2013, the registry has implemented structured follow-up registrations every three years for patients diagnosed from 2010 onward, allowing for longitudinal tracking of clinical and treatment outcomes. Data is continuously updated and presented via an interactive web-based reporting system and an annual PDF report [15]. The coverage rate is high, with approximately 97% of cases reported in comparison to the Swedish Cancer Registry, although there is a noted time lag in reporting.

To evaluate the reliability of the registry, a systemic validation was conducted in 2022. This validation compared registry-reported data with source records for the three most common MPN diagnoses (PV, ET, and myelofibrosis) for cases diagnosed between 2017 and 2018. The results confirmed a high degree of consistency between the reported and the original source records.

The registry includes patients aged 18 years or older. Key variables cover demographic, diagnostic, clinical, and treatment-related data. At diagnosis, information is recorded on the date and method of diagnosis (e.g., cytology, histopathology, molecular driver testing), adherence to WHO criteria, and prior MPN diagnoses. Data on thromboembolic and hemorrhagic complications are captured, including type and timing of events, alongside other complications such as malignancies and infections. Laboratory data at diagnosis includes blood status (e.g., hemoglobin, leukocytes, platelets), peripheral blast count, lactate dehydrogenase (LD), and erythropoietin levels. Clinical assessment documents constitutional symptoms (fever, night sweats, unintentional weight loss) pruritus and palpable splenomegaly. Molecular profiling detects MPN driver mutations (*JAK2, CALR, MPL*), non-driver mutations, and cytogenetic abnormalities when analyzed.

Treatment-related data include classification as treatment-requiring and planned treatment strategies. Possible treatments include venesectio, cytoreductive agents (HU, IFN-α, JAK2

inhibitors, anagrelide, busulfan, phosphorus-32), antiplatelet and anticoagulant therapies (aspirin, clopidogrel, vitamin K antagonists, DOAC, LMWH), transfusion therapy, corticosteroids, erythropoiesis-stimulating agents (ESA), and immunomodulatory drugs (IMiDs). Eligibility for stem cell transplantation evaluation is also recorded.

Follow-up every third-year tracks vital status (date and cause of death), complications (including bleeding, thromboembolic events, and progression to acute myeloid leukemia), changes in diagnosis, ongoing treatment, and new complications. Laboratory parameters are updated along with constitutional symptoms and palpable splenomegaly, ensuring consistency with baseline data.

Quality indicators are tracked to ensure the effective care. These include the proportion of patients reported to the registry within 12 months (>90%), use of bone marrow histopathology for diagnosis (>90%), PV patients receiving antithrombotic treatment (>90%), and high-risk patients receiving cytoreductive therapy (>90%). Long-term targets include hematocrit control (EVF <0.45) in PV patients (>80%) and platelet control (TPK $<400 \times 10^9$/L) in high-risk ET patients three years post-diagnosis (>80%).

Study populations and outcomes

Paper I

The study included patients diagnosed with PV, ET or MF between 1987 and 2012 from nine hematology centers in Sweden and Norway, according to the 2008 WHO criteria. Eligible patients had received IFN-α treatment for at least three months. Patients with incomplete records, insufficient follow-up, or missing genetic data were excluded. The primary outcome was the hematologic response to IFN-α treatment, classified according to the European Leukemia Net (ELN) criteria. Secondary outcomes included molecular response, assessed by changes in the JAK2 V617F allele burden, the incidence of treatment-related adverse events, and vascular complications during treatment. Responses were analyzed in relation to genetic polymorphisms in the *IL28B* gene (rs12979860, rs8099917, and rs12980275).

Paper II and III

Patients for Papers II and III were identified from the Swedish MPN Registry and included those diagnosed between 2008 and 2018, according to the 2008 WHO diagnostic criteria. Paper II focused on PV and ET, while Paper III included MF. Patients with incomplete records or missing critical variables were excluded. The primary outcome in both studies was the incidence of vascular complications, including thrombotic and hemorrhagic events, and their impact on survival. Secondary outcomes examined the influence of clinical and hematologic parameters, such as white blood cell count, hemoglobin levels, and treatment modalities, on vascular risk and survival.

Paper IV

The study included patients initially classified as MPN-U in the Swedish MPN registry between 2008 and 2018. The primary outcome of the study was to determine how many patients met the diagnostic criteria for MPN-U after a reclassification process based on the 2016 WHO criteria. Additionally, the study aimed to describe the clinical, morphological, and molecular characteristics of these patients, as well as analyze the frequency of vascular events and survival within this group.

Statistical methods

The studies included in this dissertation employed a range of statistical methods tailored to their respective objectives, including cohort and nested case-control designs, as well as analyses of risk and outcomes.

Descriptive statistics were used in all studies to summarize baseline data. Continuous variables were presented as means, medians, and standard deviations, while group differences were assessed using Chi-squared or Fisher's exact tests for categorical variables and Mann-Whitney U or Student's t-tests for continuous variables, depending on data distribution.

Survival analyses in Papers II, III, and IV were conducted using the Kaplan-Meier method to estimate survival probabilities, with log-rank tests employed to compare groups. Cox regression models were applied in Papers II and III to estimate hazard ratios (HRs) with 95% confidence intervals (CIs), facilitating the analysis of risk factors for vascular complications and their impact on survival. In addition, multivariate logistic regression was used in Paper II to identify interacting risk factors.

Nested case-control designs in Papers II and III were employed to investigate associations between vascular complications and risk factors, utilizing both univariate and multivariate analyses.

Ethical considerations

All four sub-studies in this thesis are observational studies based on data from patient records and the Swedish MPN registry. The research was conducted in compliance with ethical principles and approved by the relevant ethical boards.

Paper I involved a retrospective analysis of data collected from multiple centers in Sweden and Norway. Ethical approval for this study was granted by the Regional Ethical Review Boards in both Sweden (Dnr 1050-11) and Norway. Written informed consent was obtained from all participating patients for the use of their clinical and genetic data. The genetic data analyzed were targeted, without containing general genetic information that could lead to broader implication or require interventions.

Papers II, III and IV utilized data from the Swedish MPN Registry. Ethical approval for Paper II and III was provided by the Regional Ethical Review Board in Gothenburg (Dnr 389-18), while Paper IV was approved by the Swedish Ethical Review Authority (Dnr: 2021-06127-01).

Once collected, all data from patient records and the registry were de-identified prior to analysis, ensuring that the processing of results did not involve personal identifiers. Data access was strictly limited to authorized researchers, and all datasets were securely stored in compliance with established standards for safeguarding sensitive information. No intervention was conducted at any stage of the studies, except for the sample collection in Paper I.

The findings of these studies contribute valuable knowledge to the field of myeloproliferative neoplasms and have or will be published in peer-reviewed journals, ensuring transparency and contribution to the scientific community. All manuscripts provide appropriate acknowledgment of ethical approvals and data sources, reflecting adherence to the ethical standards throughout the research process.

Results

Paper I: Genetic Variation and Interferon-Alpha Response

The study aimed to evaluate the impact of constitutional genetic variations within the *IL28B* (*IFNL3*) gene on the response to IFN-α treatment in patients with MPN. IFN-α is a cytoreductive therapy with potential disease-modifying properties for MPNs, but its efficacy and tolerability vary among patients. Challenges in assessing treatment response, particularly in terms of disease-modifying effects, and the requirement for prolonged treatment duration highlight the need to better understand genetic factors that may influence treatment outcome. A key objective was to determine whether specific constitutional genetic variants involved in inflammatory pathways could predict treatment response, thus supporting more personalized treatment approaches.

A total of 100 patients (PV: 47, ET: 43, MF: 10) were included in the study, with a median treatment duration of 34 months on IFN-α. Hematologic response was evaluated according to ELN criteria [146]. Among patients with PV, 60% achieved a complete response (CR) and 4% a partial response (PR). In patients with ET, 70% achieved CR and 30% PR. For myelofibrosis, responses were assessed according to the IWG-MRT and ELN consensus criteria [150], with a response observed in 90% (9/10) of patients. Of these 6/10 had an aquired response during treatment, while 3/10 maintained a response from prior therapy.

Analysis of the three SNPs in the *IL28B* gene revealed that the CC genotype at rs12979860 was strongly associated with a higher rate of complete hematologic response in the PV group (79%, p=0.036) compared to patients with the non-CC genotype (46%). A similar trend was observed in ET, where 84% of patients with the CC genotype achieved CR compared to 63% in those with the non-CC genotype, although this difference did not reach statistical significance (p=0.174).

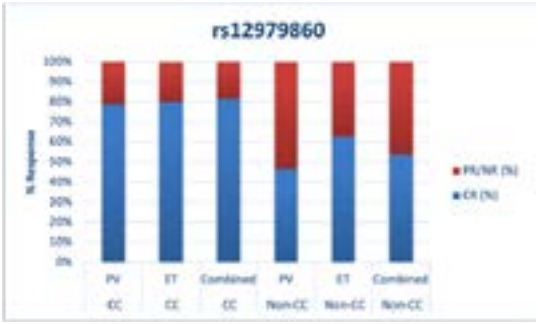
For rs8099917, the TT genotype demonstrated a trend toward improved CR compared to the PV group (69% for TT compared to 44% for non-TT), but the difference was not statistically significant (p=0.130). Similarly, in the combined PV and ET cohort, the TT genotype achieved CR in 71% of cases compared to 57% for the non-TT genotype (p=0.255).

The AA genotype at rs12980275 exhibited a statistically significant association with CR in both the PV group (79%, p=0,036) and the combined PV and ET cohort (80%, p=0.014) compared to the non-AA genotype, which was associated with lower CR rates (46% in PV and 54% in the combined PV and ET cohort). These findings indicate that genetic variations in *IL28B* may play a role in predicting response to IFN-α therapy.

Among patients treated with HU prior to IFN-α therapy, no significant associations between *IL28B* genotypes and treatment response were observed. In this subgroup, the CR rate was 60% in PV and 64% in ET, with a combined CR rate of 62%. This lack of correlation suggests that the

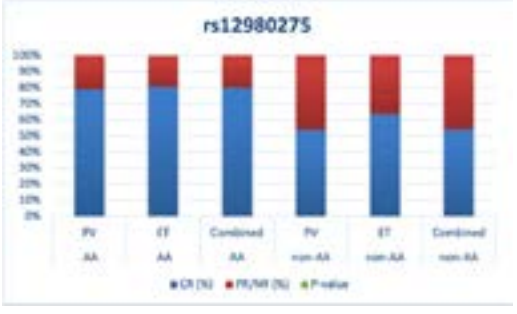
predictive value of *IL28B* polymorphisms is specific to IFN-α therapy and does not extend to cytoreductive treatments in general.

For the MF group, the results were limited to clinical improvement (CI) and could not be included in the statistical analysis due insufficient data on bone marrow morphology.



Response to IFN-α based on rs12979860 (CC vs non-CC) in PV, ET, and the combined cohort.

Response to IFN-α based on rs8099917 (TT vs non-TT) in PV, ET and the combined cohort.



Response to IFN-α based on rs12980275 (AA vs non-AA) in PV, ET and the combined cohort.

Table 5. Association between IL28B genotype and response to IFN-α treatment: Odds ratios (OR) and 95% CI

Genotype IL28B	CR	PR/NR	OR	95% CI
rs12979860 CC	31	7	4.43	(1.57 - 12.50)
rs12979860 Non-CC	28	24	1.17	(0.43 - 3.16)
rs8099917 TT	39	16	2.44	(1.02 - 5.84)
rs8099917 Non-TT	20	15	1.33	(0.54 - 3.30)
rs12980275 AA	32	8	4.00	(1.40 - 11.40)
rs12980275 Non-AA	27	23	1.17	(0.45 - 3.06)

Table 6. Association between IL28B genotype and response to hydroxyurea: Odds ratio (OR) and 95% CI.

Genotype IL28B	CR	PR/NR	Odds Ratio	95% CI
rs12979860 CC	9	4	2.25	(0.51 - 9.95)
rs12979860 Non-CC	7	6	1.17	(0.27 - 5.06)
rs8099917 TT	10	7	1.43	(0.36 - 5.64)
rs8099917 Non-TT	6	3	2.00	(0.43 - 9.26)
rs12980275 AA	8	5	1.60	(0.40 - 6.42)
rs12980275 Non-AA	8	5	1.60	(0.40 - 6.42)

Table 7. Comparison of complete response (CR) rates between IFN-α and hydroxyurea by IL28B genotype: Odds ratios (OR), 95% confidence intervals (CI), and p-values.

Genotype	CR in IFN-alpha (%)	CR in HU (%)	OR (IFN-alpha vs HU)	95% CI	p-value
rs12979860 CC	82	60	2.33	1.1–5.2	0.036
rs12979860 Non-CC	46	62	0.74	0.4–1.6	0.688
rs8099917 TT	69	71	0.97	0.5–2.2	0.255
rs8099917 Non-TT	44	50	0.85	0.3–2.0	0.512
rs12980275 AA	79	62	1.90	1.0–3.9	0.014
rs12980275 Non-AA	54	55	0.98	0.5–2.1	0.954

Paper II: Vascular Complications and Survival in ET and PV

This study evaluated the impact of risk factors, treatments and blood counts on the occurrence of vascular complications and their influence on survival in patients with ET an PV. The analysis was based on a nested-control study within the Swedish MPN-registry. A total of 922 ET patients

and 763 PV patients with a mean follow-up time of 46 moths for ET and 37 months for PV, corresponding to 3533 and 2383 patient-years, respectively.

During follow-up, 71 ET patients (8%) and 81 PV patients (11%) experienced at least one vascular complication. The incidence rates were 2.0 and 3.4 events per 100 patient-years in ET and PV respectively. Among ET patients thromboembolic events accounted for the majority of events, with rates of 1.4 thromboembolic and 0.6 hemorrhagic events per 100 patient-years. For PV, the rates were 2.5 thromboembolic and 0.9 hemorrhagic events per 100 patient-years.

A total of 71 ET cases with vascular complications were matched to 71 ET controls without complications. Similarly, 81 PV cases were matched to 81 PV controls. The matching was based on MPN subtype, age and sex.

At the time of diagnosis, no significant differences were observed between cases and controls in either the ET and PV groups regarding blood counts (hemoglobin, hematocrit, WBC count, and platelet count), *JAK2*V617F mutational status/allelic burden or EPO-levels. The frequency of vascular complications prior to diagnosis was also similar between cases and controls.

Table 8. Baseline Characteristic for ET and PV Patients

	ET (n=922)	ET Cases (n=71)	ET Controls (n=71)	PV (n=763)	PV Cases (n=81)	PV Controls (n=81)
Age at diagnosis (Mean±SD)	65 ± 16	73 ± 13	73 ± 13	68 ± 13	73 ± 11	73 ± 11
Hemoglobin (g/L)	138 ± 15	136 ± 15	136 ± 17	173 ± 22	172 ± 23	175 ± 24
Hematocrit (%)	42 ± 4	42 ± 5	42 ± 5	53 ± 7	54 ± 6	55 ± 7
WBC ($x10^9$/L)	9.1 ± 5.5	9.1 ± 5.0	7.6 ± 3.4	12.7 ± 11.5	13.5 ± 9.6	9.6 ± 4.9
Platelets ($x10^9$/L)	817 ± 312	845 ± 279	815 ± 291	554 ± 277	530 ± 285	517 ± 241
JAK2 V617F (%)	61.6 (n=568)	70 (n=50)	65 (n=46)	nd	nd	nd
JAK2 V617F allelic burden (%)	nd	nd	nd	74.6 (n=568)	60 (n=49)	65 (n=46)

At the time of the vascular event, mean WBC levels were significantly higher in PV cases compared to controls (13.5×10^9/L vs. 9.6×10^9/L, $p < 0.001$). However, this difference was not significant in multivariate analysis, suggesting that the protective effect of cytoreductive therapy could account for the observed difference. In ET, there was also a trend toward higher WBC in cases (9.1×10^9/L vs. 7.6×10^9/L, $p= 0.063$), but this did not reach statistical significance. No significant differences were observed for hemoglobin, hematocrit, or platelet counts between cases and controls in either ET or PV.

The use of cytoreductive therapy was a significant protective factor, especially in PV. At the time of vascular events, only 51% of PV cases were treated with cytoreductive therapy compared to

79% of controls (p<0.001). In multivariate analysis, cytoreductive therapy was associated with a significantly reduced risk of vascular complications (odds ratio 0.22, CI 0.096-0.50, p<0.001). Anti-thrombotic treatment was also more frequent in PV controls (99%) compared to cases (86%), and this difference was statistically significant (p=0.007). In multivariate analysis, anti-thrombotic therapy remained a significant protective factor for PV cases (odds ratio 0.085, CI 0.009-0.79, p=0.03).

For ET, 75% of cases and 82% of controls received cytoreductive therapy (p = 0.416), and the use of hydroxyurea was consistent between groups. Anti-thrombotic therapy was widely used in both ET cases (93%) and controls (93%), with no difference between groups (p = 1.000).

TABLE 5 Multivariate analysis comparing cases and controls regarding blood counts and concomitant treatment at the time of vascular complications

	ET		PV	
	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P
Age	1.01 (0.97-1.04)	.596	1.00 (0.97-1.04)	.896
Sex	1.06 (0.51-2.2)	.881	1.28 (0.62-2.66)	.509
Hematocrit ≥ 45	–[a]	–	1.01 (0.98-1.03)	.610
WBC > 11	1.48 (0.54-4.05)	.446	1.44 (0.64-3.2)	.374
Platelets > 400	1.08 (0.51-2.3)	.842	0.57 (0.25-1.3)	.571
Anti-thrombotic therapy	0.88 (0.22-3.6)	.866	0.085 (0.009-0.79)	.03*
Cytoreductive therapy	0.53 (0.20-1.5)	.220	0.22 (0.096-0.50)	<.001*

Note: Sex; male/female, Hematocrit; %, WBC and Platelets; *10^9/L.
Abbreviation: CI, confidence interval.
[a]Too few cases for analysis.
*P< .05.

Vascular complications had an impact on survival in both ET an PV. In ET, the 5-year survival was significantly reduced in patients with vascular complications (65% vs. 81%, p= 0.020). For PV, the impact was even more pronounced, with a 5-year survival rate of 60% for cases compared to 85% in controls (p< 0.001).

Paper III: Vascular Complications and Survival in MF

The study included 392 patients with MF from the Swedish MPN registry. The median follow-up time was 65 months, corresponding to a total of 2123 patient-years. During follow-up, 58 patients (15%) experienced at least on vascular complication, resulting in an incidence rate of 2.8 events per 100 patient-years. The median time from diagnosis to the first vascular event was 25 months (range 2-103 months).

Among the 58 vascular events, 60% (n=35) were thrombotic and 40% (n=23) were hemorrhagic. The most common thrombotic events were cardiac and cerebral events, while gastrointestinal and cerebral hemorrhages were the most frequently observed bleeding events. The incidence rate of thrombotic events was 1.7 events per 100 patient-years, while the incidence rate of hemorrhagic events was 1.1 events per 100 patient-years. At the time of thrombotic

complication, 70% of patients were receiving antithrombotic treatment, while for hemorrhagic events, only 23% of patients were on such treatment.

A nested case-control analysis, was conducted, including 58 cases with vascular complications were matched to 58 controls without vascular complications. Baseline characteristics of the study population are summarized in Table 9. No significant differences were observed at diagnosis between cases and controls regarding blood counts, *JAK2*V617F mutation status, spleen size, or history of prior vascular events. However, there was a significant difference in the distribution of IPSS risk categories, with a larger proportion of cases (52%) classified as intermediate-2 or high-risk compared to controls (33%, p=0.04).

Table 9 Baseline characteristics for MF cases and controls

	MF Cases (n=58)	MF Controls (n=58)	p-Value
Age (years)	72.2 ± 11	72.6 ± 11	0.82
Gender (female, %)	41	40	0.85
Hemoglobin (g/L)	109 ± 20	116 ± 20	0.062
Hematocrit (%)	36 ± 5	36 ± 7	0.72
WBC count (×10^9/L)	13.0 ± 9.6	11.5 ± 8.9	0.41
Platelets (×10^9/L)	480 ± 364	494 ± 307	0.81
JAK2 V617F (%)	67	59	0.83
Prior vascular event (%)	34	19	0.093
IPSS INT-2 and HR (%)	52	33	0.04

At the time of the vascular event in cases and the corresponding matched time point for controls, there were no significant differences in terms of blood counts, DIPPS risk, or use of cytoreductive or antithrombotic therapy.

Survival was significantly reduced in patients with vascular complications compared to both their matched controls and the entire cohort om MF patients without vascular complications.

1. Case control analysis: The median survival time from diagnosis for patients with vascular complications was 48 months, compared to 92 months for controls (p=0.013, log rank test).
2. Entire cohort analysis: In the full cohort, survival was significantly shorter in patients with vascular complications (48 moths) compared to without vascular complications (104 months, p<0.001).

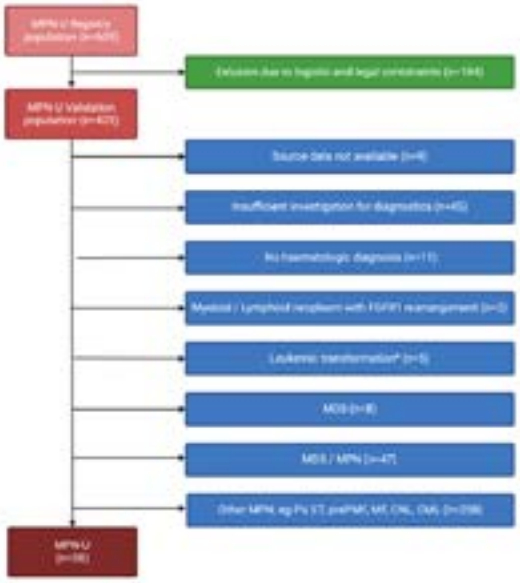
A multivariate Cox regression model was conducted for the case-control analysis to assess the impact of vascular complications, IPSS risk and *JAK2*V617F mutation status on survival. In this model, only IPSS risk category remained a significant explanatory factor for survival. The results showed that patients with Intermediate-2 and High-risk IPSS had significantly shorter survival compared to those in Low and Intermediate-1 risk groups. The impact of vascular complications observed in univariate analysis was no longer significant after adjustment for IPSS risk (HR = 1.4, p= 0.24).

For the entire cohort analysis, survival was also affected by IPSS risk group and *JAK2* V617F mutation status. The median survival for patients with *JAK2*V617F mutation was 80 months,

while median survival was not reached for patients without the mutation (p<0.001). Survival analysis by IPSS risk category revealed a clear gradient, with median survival times of not reached for Low risk, 89 months for Intermediate-1, 58 months for Intermediate-2, and 37 months for High risk.

Paper IV: MPN-Unclassifiable – Diagnosis and Prognosis

Between January 1, 2008, and December 31, 2018, 609 patients with a diagnosis of MPN-U were identified in the Swedish MPN registry. Of these, 425 patients (69.8%) were included in the analysis as they were located in predefined geographical regions. Following a comprehensive review of patient medical records, only 38 patients (8.9%) were confirmed as meeting the 2016 WHO criteria for MPN-U. The remaining 387 patients (91.1%) were reclassified as other hematologic diseases.



Flow chart illustrating the validation process for 609 patients initially registered with a diagnosis of MPN-U in the Swedish Registry between 2008 and 2019. Reasons for exclusion from the final diagnosis of MPN-U are detailed on the right-hand side of the chart.

The reclassification revealed that the majority of cases (61%) were reassigned to classical MPN subtypes, including ET: 86 patients (20%); PV: 60 patients (14%); PrePMF: 31 patients (7%) and Overt MF: 75 patients (18%).

Other reclassified diagnoses included chronic neutrophilic leukemia (CNL) in 4 patients (1%) and chronic myeloid leukemia (CML) in 2 patients (0.5%). Additionally, 58 patients (14%) were identified as having a myelodysplastic syndrome (MDS) or MDS/MPN overlap syndrome, with chronic myelomonocytic leukemia (CMML) being the most common reclassification in this group. 45 patients (11%) had insufficient clinical, molecular, or histopathological data to support a reclassification, while in 11 cases, no hematological neoplasm could be confirmed.

The 38 patients with confirmed MPN-U presented with distinct clinicopathological characteristics related to blood counts and bone marrow findings. The median age at diagnosis was 61 years (range 24–89), and 68% of patients were female. Hematological parameters at diagnosis showed a predominance of thrombocytosis (61%) with a median platelet count of 597 $\times 10^9$/L (range: 140–1563 $\times 10^9$/L), while leukocytosis was present in 32% of patients, with a median WBC count of 10.3 $\times 10^9$/L (range: 3–27.6 $\times 10^9$/L). Peripheral blood blasts were detected in two patients, but at low levels. Bone marrow examination revealed hypercellularity in 82% of cases, and megakaryopoiesis was increased in 95% of patients. Clustering of megakaryocytes was observed in 87% of patients. Fibrosis grade >1 was detected in only 3 patients. Most cases (92%) had fibrosis grades of 0 or 1. Iron staining data were available for 55% of the patients, with evidence of iron deficiency in 50% of these cases.

Vascular events prior to the diagnosis of MPN-U were reported in 58% of patients (n=21). The most common vascular event was splanchnic vein thrombosis (SVT), occurring in 33% of patients. Other vascular complications included: cerebrovascular events: 6 patients (16%), cardiac events: 6 patients (16%), venous thromboembolism (VTE): 1 patient (3%). No bleeding events were reported before diagnosis.

Mutation analysis revealed that *JAK2*V617F was the most common mutation, detected in 82% of patients (31 of 38). Of the 7 patients without *JAK2* mutations, 3 carried a *CALR* Type 1 mutation, while the remaining 4 were either triple-negative or had incomplete mutational data. No mutations were detected in *JAK2* exon 12, *MPL*, or *CALR* Type 2 in this cohort.

Most patients received a combination of antithrombotic and cytoreductive therapy. Antithrombotic therapy was administered to 35 patients (92%), including antiplatelet agents (n=21) and/or anticoagulants (n=14). Cytoreductive therapy was prescribed for 34 patients (89%), primarily hydroxycarbamide (n=17) and interferon-alpha (n=9). JAK inhibitors were used in 2 patients, while 1 patient underwent allogeneic HSCT.

Over a median follow-up time of 6.2 years (range 4.6–7.8 years), no leukemic transformations were observed. The 5-year overall survival (OS) for the 38 confirmed MPN-U patients was 68.2% (95% CI: 49.5–81.2%). Median survival was not reached during the study period.

Discussion

Genetic variation and interferon-alpha response

Paper I examined the relationship between genetic polymorphism near the IL28B gene and hematologic response to IFN-α in patients with MPNs, focusing on PV and ET. Using a hypothesis-targeted testing approach, the analysis identified significant associations between specific polymorphisms and the likelihood of achieving a complete hematologic response (CR), particularly in patients with PV.

The rs12979860 CC genotype strongly predicted CR, with 79% of PV patients achieving CR compared to 46% of non-CC carriers. In the combined PV and ET cohort, CR rates were 82% for CC carriers versus 54% for non-CC. A similar trend was observed for the rs12980275 AA genotype. These findings suggest that rs12979860 and rs12980275 may serve as predictive biomarkers for IFN-α therapy, particularly in PV. The shared impact of these variants is likely explained by linkage disequilibrium [151], a phenomenon where genetic variants close to each other on a chromosome are inherited together more frequently than expected by chance. The rs8099917 TT genotype showed a weaker association with treatment response, achieving 69% CR in PV compared to 44% for non-TT carriers, with non-significant trends in the combined cohort, suggesting that rs8099917 may play a less prominent role in response to IFN-α therapy.

The differing impact of polymorphisms between PV and ET may be attributed to differences in ELN response criteria [146]. In PV, binary response definitions such as hematocrit normalization without phlebotomy create clear distinctions between response levels, whereas ET relies on continuous measures of platelet and leukocyte levels. This distinction may have contributed to the more pronounced genetic associations observed in PV.

The study also included a HU control cohort. Although the limited size of the HU cohort (n = 26) posed challenges, it provided an essential comparison group to evaluate whether these genetic associations were specific to IFN-α. The lack of significant genetic associations in the HU cohort underscores the specificity of IFN-α signaling pathways in influencing treatment outcomes. However, the small size of the HU cohort limited statistical power and necessitates expansion in future research.

The rs12979860 polymorphism, previously referred to as "*IL28B*," is located within intron 1 of the *IFNL4* gene on chromosome 19 and is more accurately termed IFNL4 rs12979860 [151,152].

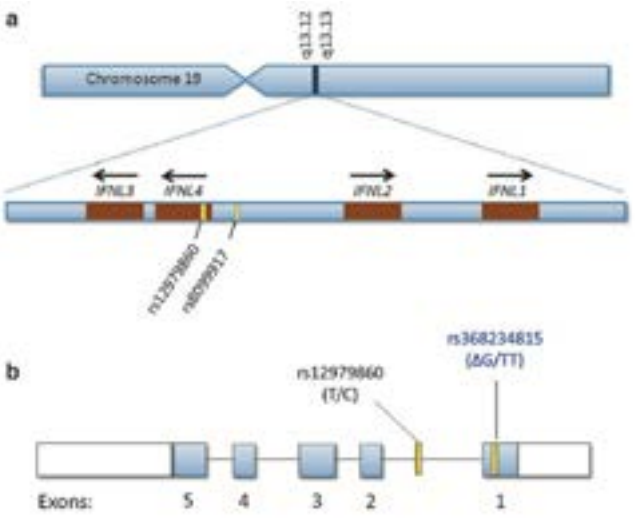


FIG. 1. Location of the interferon (IFN)-λ gene family and genome-wide association study markers rs12979860 and rs8099917 on chromosome 19 (**a**); exonic structure of *IFNL4* with the location of the *IFNL4* rs12979860 ("*IL28B*") and *IFNL4* rs368234815 (*IFNL4*–ΔG) polymorphisms (**b**).

Genomic location of the interferon lambda gene family on chromosome 19, including genome-wide association markers rs12979860 and rs8099917 (a). The exonic structure of the IFNL4 gene is shown with the locations of rs12979860 ("IL28B") and IFNL4 rs368234815 (ΔG/TT) polymorphisms highlighted (b)[152].

IFN-λ proteins, including IFN-λ1, IFN-λ2, and IFN-λ3, encoded by genes near *IFNL4*, activate the JAK-STAT signaling pathway. This pathway promotes the expression of interferon-stimulated genes (ISGs), which exert antiproliferative and immune-modulatory effects. Variants associated with reduced IFN-λ4 production, such as the CC and AA genotypes, likely alleviate negative regulation of IFN-α signaling, enhancing therapeutic [153]. This mechanism aligns with findings in hepatitis C studies, where similar polymorphisms predict interferon efficacy [154].

However, IFN-α and *IFNL4* signaling mechanisms differ. IFN-α directly targets *JAK2* V617F-mutated hematopoietic stem cells via the IFNAR receptor, exerting a cell-autonomous effect, whereas *IFNL4* signals through IL10R2 and IFNLR1, primarily expressed on epithelial and immune cells, suggesting an indirect role in immune modulation [110].

A study published in 2021 by Jäger et al. investigated *IFNL3/4* polymorphisms in 122 PV patients from the PROUD-PV and CONTINUATION-PV trials, treated with ropeginterferon alfa-2b for 36 months[155]. An initial GWAS analysis found no significant associations with molecular response (MR), likely due to the limited statistical power of the cohort. However, targeted analyses of *IFNL4* variants, including rs12979860, rs8099917, rs368234815, and rs117648444, revealed strong genotype correlations with MR rates.

Notably, the rs12979860 CC genotype was associated with higher MR rates, consistent with its predictive value for complete response (CR) in this study. The rs368234815 TT/TT genotype, leading to no IFN-λ4 production, had an MR rate of 89.3%, while carriers of the functional IFNL4-P70 double G allele showed significantly lower MR rates (43.3%). The rs117648444 variant, causing impaired IFN-λ4 activity, produced MR rates comparable to the rs368234815 TT/TT genotype, linking reduced IFN-λ4 function to better treatment outcomes

Haplotype analysis further demonstrated that loss-of-function or impaired-function *IFNL4* variants were associated with higher MR rates, while functional variants corresponded to diminished responses. Importantly, no relationship was found between baseline *JAK2*V617F variant allele frequency (VAF) and MR, indicating an independent effect of *IFNL4* variants on treatment outcomes.

The findings highlight the impact of linkage disequilibrium (LD) between *IFNL4*-DG haplotypes and rs12979860-T in Asian and European populations, complicating the identification of causal variants. Strong LD suggests that genetic effects are closely intertwined, making it challenging to disentangle their individual contributions [156,157].

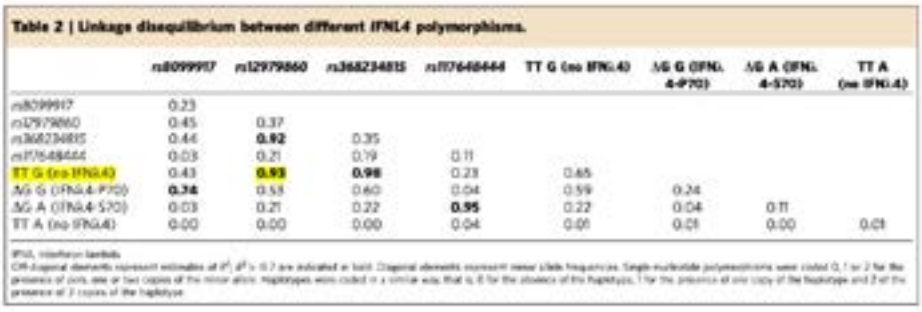


Table 2 | Linkage disequilibrium between different IFNL4 polymorphisms.

	rs8099917	rs12979860	rs368234815	rs117648444	TT G (no IFNL4)	ΔG G (IFNL4-P70)	ΔG A (IFNL4-S70)	TT A (no IFNL4)
rs8099917	0.23							
rs12979860	0.45	0.37						
rs368234815	0.44	**0.92**	0.35					
rs117648444	0.03	0.21	0.19	0.11				
TT G (no IFNL4)	0.43	**0.93**	**0.98**	0.23	0.65			
ΔG G (IFNL4-P70)	**0.34**	0.53	0.60	0.04	0.59	0.24		
ΔG A (IFNL4-S70)	0.03	0.21	0.22	**0.95**	0.22	0.04	0.11	
TT A (no IFNL4)	0.00	0.00	0.00	0.04	0.01	0.01	0.00	0.01

IFNL, interferon lambda.

Linkage disequilibrium between different IFNL4 polymorphisms[156]. Linkage disequilibrium (LD) is the non-random association of alleles at different loci, often due to their physical proximity on a chromosome, which reduces recombination between them.

Chen et al. emphasized the importance of population-specific studies, as rs12979860 and rs368234815 variants are less common in Asian populations compared to Europeans, reducing their predictive value in certain cohorts [158]. Furthermore, most research on these polymorphisms originates from Western countries, limiting the generalizability of findings to diverse populations. These discrepancies underscore the need for validation across broader ethnic groups and exploration of alternative genetic predictors tailored to regional differences.

MPNs are not only defined by the driver mutations *JAK2*, *CALR*, and *MPL* but also by the presence of concomitant somatic mutations in several genes[32]. Some of these genetic alterations hold prognostic value, aiding in assessing the risk of disease progression or predicting potential phenotypic manifestations. However, the predictive role of these co-existing mutations in response to cytoreductive therapy remains unclear, and their dynamic changes under IFN-based therapy in MPNs continue to be an area of active research. The DALIAH trial, a randomized phase III study comparing IFN-α (including IFNα-2a and IFNα-2b) to HU in MPN patients, has provided insights on this topic. Genomic profiling via next-generation sequencing (NGS) was conducted on paired pre- and post-treatment samples from both IFN-α and HU cohorts. While treatment-emergent *DNMT3A* mutations were more frequently observed in patients treated with IFN-α, particularly those who did not achieve complete hematologic remission (CHR), the study

did not identify any specific role for non-driver mutations in predicting response or resistance to IFN treatment[159].

In addition, emerging evidence from studies such as the Andean-enriched NFKB1 haplotype research presented by Song et al, highlights the role of inflammation and genetic factors in predicting hematologic responses[160]. This haplotype modulates NFκB activity, reducing pro-inflammatory and pro-thrombotic gene expression, and has been associated with higher response rates to ropegIFN (58.6% vs. 33.3%, $p<0.0001$). These findings suggest an evolving landscape of genetic predictors that influence not only disease progression but also therapeutic outcomes.

Paper I employed a hypothesis-driven approach using TaqMan allele discrimination to investigate genetic associations. This method efficiently identified associations with known single nucleotide polymorphisms (SNPs) but was limited in its ability to detect novel variants. TaqMan assays are well-suited for targeted testing of known SNPs due to their reliability and precision, making them effective for hypothesis-driven research. However, unlike genome-wide association studies (GWAS), they do not allow for the agnostic identification of new genetic associations. GWAS enables comprehensive exploration of genetic variability across the genome and has the potential to uncover novel variants associated with treatment response, although it requires larger cohorts and stringent statistical corrections to address the increased risk of false positives. Both approaches have strengths and limitations, with TaqMan assays providing focused testing of known SNPs and GWAS offering broader discovery across the genome.

The study's reliance on a small HU control cohort (n = 26) limited the ability to detect smaller effect sizes or confirm trends observed in the IFN-α cohort (n = 100). Sample size calculations suggest that a minimum of 40–70 HU patients would be necessary to achieve sufficient statistical power. Expanding this control cohort in future studies would strengthen comparisons and improve the robustness of findings. Grouping partial response (PR) with non-response (NR) addressed statistical constraints but may have obscured nuanced differences in response levels. Additionally, reliance on CR as a proxy for molecular remission (MR) was necessitated by limited molecular data, with *JAK2*V617F allele burden follow-up available for only 17% of patients.

Hardy-Weinberg equilibrium (HWE) is important in genetic association studies to verify that allele frequencies follow expected patterns under random mating, with no mutation, migration, or selection in a sufficiently large population. Deviations from HWE can suggest genotyping errors, bias, or biological effects, making it a useful quality control step. This study did not assess HWE, which limits the ability to identify potential genotyping errors or population-specific influences. As recommended by STREGA (Strengthening the Reporting of Genetic Association Studies), including HWE analysis could have improved the study by addressing possible biases affecting the results.

Vascular complications and survival

Paper II and III evaluates the incidence and impact of vascular complications in MPNs using data from the Swedish MPN registry. Median follow-up times were 46 months for ET and PV, and 65 months for MF. The incidence of vascular events was 2.0, 3.4, and 2.8 events per 100 patient-years for ET, PV, and MF, respectively.

Thrombotic events predominated in ET and PV, while MF exhibited a higher proportion of hemorrhagic events, reflecting subtype-specific vascular risk. These complications significantly influenced survival: MF patients with vascular events had a median survival of 48 months compared to 92 months for matched controls without vascular events. Across the broader MF cohort, patients without vascular events demonstrated a median survival of 104 months. In PV and ET, thrombotic and hemorrhagic events were associated with shortened survival.

Elevated WBC counts were a predictor for vascular events in PV but not conclusively in ET. In MF, vascular events were more common in older patients with lower hemoglobin levels and a higher prevalence of *JAK2*V617F mutations. However, multivariate analysis identified age and hemoglobin levels as significant predictors, while *JAK2*V617F status did not retain statistical significance after adjustment.

Cytoreductive therapy and antithrombotic treatment showed a protective effect, particularly in PV. Patients with PV who were undertreated had worse outcomes. In ET, the high proportion of patients receiving treatment made it more challenging to identify statistically significant differences in treatment effects. Furthermore, the lower incidence of vascular complications in ET compared to PV may contribute to less distinct results when analyzing underlying risk factors and treatment outcomes.

Prognostic models, such as the International Prognostic Scoring System (IPSS) for MF, are valuable for predicting disease progression and survival[161]. Although vascular risk is not explicitly included in the IPSS, our multivariate analysis identifies IPSS risk categories as the primary predictor of survival. The parameters included in the IPSS, such as age, hemoglobin levels, and WBC counts capture aspects of disease severity. These parameters may indirectly reflect vascular risk, suggesting that IPSS may encompass elements relevant to both overall disease burden and vascular complications, even if not directly calculated.

Our study builds on data from the Swedish MPN registry, as previously described by Abdulkarim et al. which included 1,105 patients with polycythemia vera (PV) and 1,284 with essential thrombocythemia (ET) [162]. Among PV patients, 37% experienced vascular complications prior to diagnosis. Multivariate analysis identified hemoglobin levels below the median concentration as the only significant risk factor for vascular complications in PV. In ET, 35% of patients experienced vascular complications, with age >65 years, leukocyte counts (WBC) $>12 \times 10^9$/L, and *JAK2*V617F mutations identified as significant risk factors. Combined data for ET and PV revealed a 35% prevalence of vascular complications, of which bleeding events constituted a minority (12.5%).

Earlier observations of vascular events in MPNs [163-166] have yielded inconsistent results regarding the role of leukocytosis as a risk factor for vascular events. A systematic review and meta-analysis concluded data inconclusive due to a variability in WBC thresholds[167]. The REVEAL study, a prospective observational study in U.S. clinical practice, analyzed 2271 patients with PV over a median follow up of 44.7 months[71]. This study reported 142 vascular events in 106 patients, corresponding to an incidence of 1.36 events per 100 patient-years. Hematocrit levels > 45% and WBC counts > 11 x 10^9/L were significantly associated with vascular events. Subgroup analysis further demonstrated that WBC count > 12 x 10^9/L was associated with vascular events even when hematocrit was well controlled (≤ 45%).

Recent data from Enblom-Larsson et al. included 3 141 ET and 2 604 PV patients from the Swedish MPN registry matched 1:5 with controls based on gender, age and geographic region[168]. The study demonstrated increase rates of VTE, major bleeding, all-cause mortality in ET (0.63, 0.79 and 3.70 per 100 patient-years, respectively) and PV (0.94, 1.20 and 4.80 per 100 patient-years, respectively). Additionally, arterial events and all-cause stroke were more prevalent in PV. Comorbidities such as hypertension, ischemic heart disease, prior ischemic stroke were strongly associated with new vascular events and mortality and diabetes mellitus was identified as a novel risk factor for major bleeding, particularly in PV patients.

The work by Carobbio et al.[169] and Barbui et al.[170] provides additional insights into the mechanisms linking thrombotic events to survival. Carobbio et al. emphasize the concept of competing risks, demonstrating that vascular complications are part of a dynamic interplay with disease progression. Using a multistate model, they illustrate how transitions between disease states, such as thrombosis or progression to advanced stages like overt MF or blast phase, affect overall survival. These transitions reflect the complexity of disease trajectories in MPNs and align with our findings, which highlight the significant mortality associated with thrombotic complications.

Barbui et al. provide a detailed analysis specific to PV, showing that thrombosis accelerates disease progression and doubles the risk of death within the first decade after diagnosis. Arterial thrombosis, in particular, is a strong predictor of mortality, with a hazard ratio of 1.74. These findings underline the importance of effective thrombotic risk management as component of MPN care.

An important methodological consideration is the potential value of including an external control group, comprising individuals without ET or PV, to better assess the absolute and relative risks of vascular complications. While the nested case-control design within the cohort allows for detailed comparisons among MPN patients, it does not provide a broader reference for evaluating the disease-specific risk relative to the general population. A matched control group from a population-based registry, adjusted for age, sex, cardiovascular risk factors, and treatment of such factors, could enhance the understanding of how ET and PV influence vascular risk beyond the inherent variability within the MPN cohort. Such an approach would allow for direct quantification of risk increases attributable to ET and PV compared to individuals without MPN, strengthening the epidemiological relevance of the findings.

In addition, the matching criteria used in this study—age, sex, and MPN entity (ET, PV, or MF)—were relatively coarse and did not account for cardiovascular risk factors such as hypertension, diabetes, dyslipidemia, or smoking. While there was an intention to capture cardiovascular risk factors through chart reviews, such information was often incomplete, reflecting a common challenge when supplementing registry data with retrospective chart analysis. Treatment of cardiovascular risk factors, including antihypertensive, lipid-lowering, antidiabetic, and antiplatelet or anticoagulant therapies, were also not accounted for, despite their likely significant impact on vascular risk. The indication for antiplatelet agents—whether due to MPN itself, primary prophylaxis for known cardiovascular risk factors, or secondary prophylaxis following a thrombotic event—was also not consistently documented.

These findings are derived from population-based registries, ensuring comprehensive data coverage and validity. However, several limitations warrant consideration. The reliance on supplemental chart review introduces potential biases, including underreporting of vascular events and variability in clinical documentation. Furthermore, distinctions between prefibrotic and overt MF were not consistently available, potentially introducing heterogeneity into the data. Sample sizes for some subgroups, particularly MF patients with detailed vascular event data, were relatively small, limiting statistical power. Despite these challenges, the observational design provides valuable real-world insights. Future studies should adopt prospective designs to validate these findings, reduce reporting biases, and standardize criteria for vascular event documentation. Incorporating molecular markers into analyses may further enhance the reliability of results.

MPN-Unclassifiable

This study highlights the complexity of diagnosing myeloproliferative neoplasm-unclassifiable (MPN-U) and its impact on patient outcomes. By reevaluating 425 cases registered as MPN-U using the 2016 WHO criteria, only 8.9% retained the MPN-U classification. This high reclassification rate underscores the challenges of diagnostic ambiguity and the importance of comprehensive diagnostic workflows. Splanchnic vein thrombosis (SVT) was found to be a common clinical feature, occurring in 33% of patients with confirmed MPN-U. Additionally, the cohort's 5-year survival rate of 68.2% exceeded previously reported data for MPN-U, suggesting that stringent diagnostic criteria positively impacted survival outcomes. Notably, no leukemic transformations were observed during follow-up, diverging from prior registry reports.

The findings are based on evolving diagnostic criteria for myeloproliferative neoplasms (MPNs). The revisions introduced by the 2016 WHO classification narrowed the scope of MPN-U by refining criteria for other MPN subtypes, such as ET and prePMF. However, variability in bone marrow biopsy reporting remains a significant challenge in the accurate diagnosis of MPNs. A study by the Australasian Leukaemia & Lymphoma Group (ALLG) revealed significant inconsistencies in bone marrow pathology reporting, with 26% of patients not undergoing biopsies and 33% showing discordant findings due to incomplete descriptions of megakaryocyte morphology and fibrosis grading [171]. Similarly, a study by Gorak et al. on MDS found a reclassification rate of 33%, highlighting the variability in interpreting bone marrow morphology

and dysplasia[172]. These findings emphasize the need for standardized reporting practices to improve diagnostic accuracy, align with WHO criteria, and ensure reliable data collection for registries and clinical trials.

In this study, adherence to stringent diagnostic protocols resulted in an incidence rate below 5%, aligning with recent literature[11]. The availability of next-generation sequencing (NGS) allows for molecular findings to support the diagnosis of related myeloid disorders, reducing reliance on bone marrow histology alone. Both the Australasian Leukaemia & Lymphoma Group and Gorak et al. emphasize the critical role of molecular diagnostics and centralized pathology review in improving diagnostic accuracy and guiding treatment decisions[171,172].

Accurate diagnosis of MPNs remains challenging due to the complexity and variability in bone marrow morphology, including megakaryocyte features and fibrosis grading. Inter- and intra-observer variability further complicates the evaluation, particularly in cases such as triple-negative ET. The subjective nature of WHO and ICC criteria adds to diagnostic inconsistency, emphasizing the need for objective approaches [171,173-176].The integration of advanced tools such as artificial intelligence (AI) could offer potential solutions. AI-driven image analysis can standardize assessments of megakaryocyte and fibrosis, reducing variability and improving diagnostic accuracy[177-181].

The study underscores the importance of thorough diagnostic evaluations, including bone marrow biopsies and molecular profiling, to accurately classify MPN-U. Misclassification can lead to suboptimal management, as evidenced by the high rate of reclassification to other MPN subtypes and myelodysplastic syndromes.

The study's strengths include its population-based design, high coverage rate, and comprehensive re-evaluation of medical records. However, some limitations must be acknowledged. Retrospective data may introduce selection biases, such as the exclusion of patients with less complete diagnostic documentation. Additionally, the lack of centralized pathology review and limited availability of advanced molecular profiling may have influenced the reclassification outcomes. While the geographic focus covered 75% of Sweden's population, the results may not be fully generalizable to regions with differing healthcare structures or resources.

Conclusions

Paper I demonstrates that specific genetic variations in the *IL28B* (*IFNL3/4)* gene, particularly rs12979860 CC and rs12980275 AA genotypes, are associated with improved hematologic response to interferon-α therapy in MPNs. The findings suggest the potential of these genetic markers as predictors for treatment efficacy, particularly in PV.

Paper II establishes that vascular complications, including thrombosis and bleeding, significantly reduce survival in patients with ET and PV. Cytoreductive and antithrombotic therapies are identified as protective factors. These finding contribute to the understanding of vascular factors as major determinants of prognosis in ET and PV and underline the importance of early assessment for vascular risk and appropriate therapeutic interventions.

Paper III demonstrates that vascular complications are associated with a significant reduction in median survival among patients with MF. However, multivariate analysis identifies IPSS risk categories as the primary predictor of survival. These findings indicate that IPSS remains a reliable prognostic tool, even though vascular complications are not explicitly included as a risk factor in its calculation.

Paper IV determines that most patients initially classified as MPN-U can be reclassified into established MPN subtypes or other hematologic diseases based on updated WHO criteria. Among the patients confirmed with MPN-U, distinct clinical characteristics are identified, including a high prevalence of thrombocytosis and splanchnic vein thrombosis.

Populärvetenskaplig sammanfattning

Myeloproliferativa neoplasier (MPN) utgör en grupp sällsynta kroniska blodcancersjukdomar inkluderande polycytemia vera (PV), essentiell trombocytemi (ET), primär myelofibros (PMF). Dessa sjukdomar orsakas av förändringar i blodets stamceller, vilket leder till överproduktion av blodceller och ökad risk för komplikationer som blodproppar och blödningar.

Genetiska mutationer spelar en central roll i sjukdomsutvecklingen. Mutationer i gener som *JAK2*, *CALR* och *MPL* är drivande faktorer som aktiverar signalvägar kopplade till sjukdomens progression. Den första studien undersöker genetiska variationer i *IL28B* (även kallad *INL3/4*) som kan påverka immunreglering och svar på behandling. Studien fokuserar på hur dessa genetiska variationer påverkar behandlingsresultatet med interferon-alfa, en behandlingsmetod som visat förmåga att minska både den hematologiska sjukdomsaktiviteten och de drivande mutationernas påverkan.

Utöver sin genetiska komplexitet är MPN-sjukdomar starkt förknippade med en ökad risk för hjärt- och kärlkomplikationer. I den andra och tredje studien, baserade på data från det svenska MPN-registret, framkommer att patienter med MPN har högre risk för både arteriella och venösa blodproppar. Dessa komplikationer påverkar inte bara livskvaliteten utan också överlevnaden.

En särskild utmaning är diagnosen av patienter som inte passar in i de klassiska MPN-kategorierna, kända som oklassificerad MPN (MPN-U). Den fjärde studien analyserar diagnostiska och prognostiska utmaningar hos dessa patienter i syfte att bidra till en förbättrad klassificering och behandling.

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METHODS

Registry

The Swedish Myeloproliferative Neoplasm (MPN) registry[13] is a national quality record within the Swedish blood cancer registry, aimed at monitoring MPN diagnosis, treatment, complications, survival and to assess compliance with the national care program[14], in accordance with predefined quality indicators. Established in 2008, the registry encompasses all Philadelphia-negative myeloproliferative neoplasms including polycythemia vera, essential thrombocythemia, primary myelofibrosis, chronic neutrophilic leukemia, chronic eosinophilic leukemia not otherwise specified and myeloproliferative neoplasm unclassifiable. Since 2010, patients have been subject to prospective follow-up by the registry every three years. The diagnostic standards for registry inclusion adhere to WHO criteria and have undergone periodic updates to ensure consistency, notably incorporating the diagnosis of prefibrotic myelofibrosis in 2020. In the time period from 2008 to 2018, the overall coverage rate of the MPN Registry, in relation to cases reported to the mandatory population-based nationwide Swedish Cancer Registry stands at 98,9%[13]. A systematic validation of data reported by the Swedish MPN registry against primary source data was conducted in 2022[13].

Study Population and Data Collection

In this retrospective study, patients with a diagnosis of MPN-U were identified from the Swedish MPN registry, covering the period between January 1, 2008, and December 31, 2018. Data cut-off for data collection was 24th of January 2022. Clinical and laboratory data, including bone marrow morphological characteristics at the time of diagnosis, were systematically collected through comprehensive reviews of patient medical records. All reviews were conducted by haematologists with specific expertise in MPN to assure consistency and accuracy in data abstraction.

To address logistical and geographical considerations while ensuring comprehensive population coverage, data collection was restricted to specific validated regions across Sweden. These regions included Region Skåne (population 1,402,425) in the South; Regions Östergötland (population 469,704), Jönköping (population 367,064), and Kalmar (population: 247,175) in the Southeast; Region Stockholm (population 2,415,139) in Stockholm-Gotland; Regions Örebro (population 306,792) and Uppsala (population 395,026) in the West; and Regions Västerbotten (population 274,563) and Norrbotten (population 249,693) in the North. Together, these regions represent approximately 75% of Sweden's population (7,842,440 out of 10,452,326 inhabitants). (SUPPLEMENTARY SECTION)

All data were analysed and interpreted according to the 2016 World Health Organization classification of haematolymphoid tumours[7], with patients reclassified if they did not meet the defined criteria for MPN-U.

The study was approved by the Swedish Ethical Review Authority, Dnr 2021-06127-01.

Statistical Methods

A descriptive analysis was performed, and patient- and disease-related variables were expressed as median and range for continuous variables and frequencies for categorical variables. Duration of overall survival was defined as date from initial diagnosis to the date of death of any cause or date of censoring. Survival estimates were calculated by Kaplan-Meier analysis

Statistical analysis was performed using IBM SPSS Statistics Version 29.0.

RESULTS

Reclassification

Between January 1, 2008, and December 31, 2018, the Swedish MPN registry identified 609 patients with MPN-U. Of these, 425 were located within the predefined geographical regions outlined in the methods section, representing 69,8% of the total MPN-U cohort. Comprehensive reviews of medical records were conducted to systematically validate the diagnoses of these 425 patients.

Upon evaluating the clinical, morphological, and molecular features of the 425 patients recorded as MPN-U in the Swedish MPN registry, 38 patients (8,9%) were found to meet the diagnostic criteria for MPN-U based on the 2016 WHO classification (Figure 2).

The majority of cases (n = 258, 61%) were reclassified as other specific myeloproliferative entities. This included the classical MPN diagnoses essential thrombocythemia (ET) in 86 patients (20%), polycythemia vera (PV) in 60 patients (14%), prefibrotic myelofibrosis in 31 patients (7%) and overt myelofibrosis in 75 patients (18%). Chronic neutrophilic leukemia (CNL) was diagnosed in four (1%) and Philadelphia chromosome-positive chronic myeloid leukemia (CML) was identified in 2 (0,5%) patients.

Additionally, 58 patients (14%) were identified as having a neoplasm belonging to the myelodysplastic spectrum: This included eight patients (2%) who fulfilled the criteria for myelodysplastic syndrome (MDS) and 49 patients (12%) who were classified as MDS/MPN overlap syndromes. Among the latter, the majority were diagnosed with chronic myelomonocytic leukemia (CMML).

Progression to accelerated or blast-phase disease was observed in five patients (1%); all of whom lacked an initial definitive diagnosis of a primary myeloid neoplasm. Furthermore, two patients were found to have myeloid/lymphoid neoplasm with FGFR1 rearrangement.

In 45 cases (11%), the absence of bone marrow biopsies for adequate histopathological assessment, combined with incomplete molecular data in some instances, prevented any definitive reclassification or fulfillment of diagnostic criteria. Additionally, source data were

unavailable for 9 patients, and 11 patients were found not to have any hematological neoplasm upon further evaluation.

Patient Characteristics in Reclassified MPN-U

The clinicopathological characteristics of the 38 patients meeting the 2016 WHO criteria for MPN-U are summarized in Table 1. The age at diagnosis ranged from 24 to 89 years, with a median age of 61 years. The majority of patients were female (68%). At diagnosis, the median hemoglobin level was within the normal range, at 137 g/L (range: 105-191 g/L). Thrombocytosis was observed in 61% of patients, with a median platelet count of 597 x 10^9/L (range, 140-1563 x 10^9/L) and leukocytosis was present in 32%, with a median leukocyte count of 10.3 x 10^9/L (range, 3 – 27.6 x 10^9/L). Peripheral blasts were detected at low levels in two patients.

A total of 10 patients presented with normal blood counts across all parameters. Splenomegaly was observed in 9 patients, with 1 patient excludes due to prior splenectomy for trauma. Pruritus was reported in 3 patients, and constitutional symptoms, including weight loss, were noted in 1 patient. Bone marrow examination revealed hypercellularity in 82% cases, with increased megakaryopoiesis observed in 95%, clustered in 87% of cases. Fibrosis grades ≥ 2 was detected in 3 patients, while most (92%) had grades 0 or 1. Iron staining data were available for 55% of patients, of whom 50% showed evidence of iron deficiency.

Vascular events prior to the diagnosis of MPN-U were documented in 21 patients (58%), with splanchnic vein thrombosis (SVT) being the most common event (33%, n=11). Other vascular events included cerebrovascular incidents and acute myocardial infarction (both 16%, n=6), and venous thromboembolism patient (3%, n=1). No bleeding events were reported before the diagnosis.

Genetic Profile of MPN-U

*JAK2*V617F mutation status was assessed in all patients, with the mutation detected in 31 (82%). Among the 7 patients negative for *JAK2*V617F, 3 carried *CALR* type 1 mutations. Of the remaining 4 patients, 2 were triple-negative (negative for *JAK2*, *CALR*, and *MPL* mutations), while mutational data for *CALR* and *MPL* was unavailable. Cytogenetic analysis was performed in 8 patients, all of whom exhibited a normal karyotype.

Therapeutic strategies in MPN-U

Anti-thrombotic therapy, including anti-platelet agents and/or anticoagulants, was prescribed to 32 patients (91%). Cytoreductive therapy was administered to 27 patients (79%), predominantly featuring hydroxycarbamide (n=17) and interferon-alpha (n=9). JAK inhibitors were used in 2 patients, and 1 patient received busulfan. One patient underwent allogeneic hematopoietic stem cell transplantation for myelofibrosis transformation.

Outcome of MPN-U

Over a median follow up of 6.2 years (range: 4.6-7.8), 8 thrombotic or bleeding events occurred (thrombotic: n=5; bleeding: n=3). Two patients progressed to overt myelofibrosis during follow-up. The median overall survival (OS) was not reached, and the 5-year estimated OS was 68,2% (95% CI: 49,5-81,2%).

Uni- and multivariate analyses across patient- and disease-related variables did not identify any significant factors impacting overall survival.

DISCUSSION

Diagnostic Challenges and Reclassification

This population-based study highlights the significant diagnostic challenges associated with MPN-U. Systematic application of the 2016 WHO criteria revealed that only 8,9% of patients initially registered as MPN-U retained this diagnosis upon re-evaluation, underscoring the diagnostic ambiguity within this category. Most patients (61%) were reassigned to the classical MPN subtypes, including essential thrombocythemia, polycythemia vera, prefibrotic and overt myelofibrosis. This shift may reflect the refinements introduced in the 2016 revision[4] including the lowering of haemoglobin and haematocrit thresholds for diagnosing polycythaemia vera (PV), the recognition of prefibrotic myelofibrosis (pre-PMF) as a distinct entity with specific morphological bone marrow features, and the reduction of minor criteria for PMF. These changes align with earlier reports where reclassification similarly reduced the proportion of MPN-U cases[5,6,8].

Bone marrow biopsy serves as a cornerstone for establishing the diagnosis of MPN-U. However, in 11 % of cases, the diagnosis of MPN-U was applied despite insufficient diagnostic work-up, often due to the absence of bone marrow biopsies needed to evaluate morphological features, combined with a lack of molecular analysis of driver mutations in some instances. In some cases, advanced age or patient refusal to undergo invasive procedures contributed to these diagnostic gaps. Nonetheless, bone marrow examination was performed in most cases, although limited to aspiration and cytological assessment rather than comprehensive histopathological analysis. These findings emphasize the need for greater awareness of the essential diagnostic components required for accurately diagnosing MPN-U.

Incidence and Population Estimates

Our findings indicate that the observed incidence of MPN-U, initially reported at approximately 10% of all MPN cases in the Swedish registry, was significantly reduced when applying the 2016 WHO criteria, with only 8.9% of cases retaining the MPN-U classification. Although our study did not encompass the entire population of MPN-U patients in Sweden, the results from the validated regions, which represent 75% of the population, suggest that the true incidence of MPN-U is likely lower. Extrapolating from our findings, we estimate that MPN-U constitutes approximately 1–2% of all MPN cases nationwide when strict diagnostic

criteria are uniformly applied. This estimation assumes that the observed patterns in our study population reflect the characteristics of the unexamined portion of the registry.

Clinical and pathological features of MPN-U

Patients who retained the MPN-U diagnosis exhibited distinct characteristics. The median age median age was 61 years, with a predominance of thrombocytosis and frequent vascular events prior to diagnosis. Splanchnic vein thrombosis (SVT) was the most common vascular complication, occurring in 33% of patients before diagnosis, aligning with previous studies that identify SVT as a potential presenting feature of MPN-U[8,12,15]. Notably, no SVT events were observed during follow-up, possibly reflecting the protective effect of antithrombotic therapies initiated after diagnosis.

Bone marrow analyses revealed hypercellularity with increased and clustered megakaryopoiesis in most cases, while reticulin fibrosis was limited to grades 0-1 in 92%. These finding align with the report by Deschamps et al., supporting the hypothesis that MPN-U predominantly represents an early, pre-fibrotic stage[8]. Other similarities include a predominance of female patients and frequent thrombocytosis. However, leukoerythroblastosis was consistently absent in our cohort, and the majority of patients carried mutations in MPN driver genes. The prevalence of the *JAK2V617F* mutation was higher, while the fraction of triple-negative was considerably smaller.

A subset of patients (14%) reported as MPN-U were reclassified as MDS or MDS/MPN overlap syndrome, suggesting that expanded myeloid molecular panels at diagnosis could have improved classification accuracy. Histopathological reports in these cases frequently noted expanded and left-shifted granulopoiesis with presence of dyserythropoesis, dysgranulopoiesis or monocytosis. Clinical parameters such as cytopenia, peripheral blast presence, or peripheral monocytosis were frequently observed in these reclassified entities but were rare among the 38 patients retained in the MPN-U category.

In a recent study, Crane et al. examined 94 cases of MPN-U to identify potential prognostic markers[16]. Their findings highlighted bone marrow blast count and the DIPSS-plus score as significant predictors of overall survival in multivariate analysis. Their cohort included a notable proportion of patients with advanced-stage MPN-U with features such as cytopenia in over 25% of patients, a lower prevalence of thrombocytosis even in early-stage disease, and frequent leukoerythroblastosis, particularly in advanced-stage cases. Additionally, nearly half of their patients exhibited reticulin fibrosis grades 2–3 (46% overall; 23% in early stage, 92% in advanced stage).

Crane et al. also reported a higher proportion of triple-negative cases (14%) compared to our cohort. Among the 37 patients who underwent expanded molecular profiling, 70,3% were found to have secondary mutations, 43,2% of which were classified as high-risk mutations (HMR). This high prevalence of secondary mutations contrasts with findings by Deschamps et al, where mutations outside the driver genes were found in a minority.

Given the significant clinicopathological differences between our cohort and that of Crane et al., we remain cautious regarding the prognostic utility of the DIPPS-plus score or other PMF-validated scoring systems in MPN-U. However, incorporating complementary NGS at the time of initial diagnosis could be advantageous in ensuring accurate placement within the appropriate diagnostic category across the spectrum om myeloid neoplasms.

Survival and Disease Outcomes

The observed 5-year survival rate of 68,2,5% for patients with confirmed MPN-U in this study contrasts with the registry-reported 5-year survival rate of 57% för MPN-U during the same period. This difference likely reflects the impact of reclassification, where a substantial proportion of patients initially registered as MPN-U were subsequently reassigned to other myeloid entities associated with a poorer prognosis [17,18]. Notably, no leukemic transformations were observed during the median follow-up of 6.2 years in this cohort, diverging from earlier Swedish registry reports.

The absence of leukemic transformation in our cohort may be attributed to the stringent reclassification criteria applied in this study, excluding myeloid disorders such as myelofibrosis, MDS and MDS/MPN overlap – conditions with a higher propensity to leukemic transformation.

While our study lacks data to fully elucidate these differences in survival and transformation rates, the frequent reclassification of cases to myelofibrosis, MDS, or MDS/MPN likely contributed to the observed disparities. Additionally, it is possible that incorrect initial diagnoses resulted in suboptimal treatment for some patients, further affecting survival outcomes. This underscores the critical need for accurate diagnostic approaches, particularly at the time of initial evaluation, to ensure appropriate management and prognostic assessment.

Implications for Diagnosis and Management

This study underscores the critical importance of adhering to comprehensive diagnostic workflows to refine the classification of MPN-U and improve patient outcomes. The significant reclassification of patients initially diagnosed with MPN-U highlights the limitations of incomplete diagnostic evaluations, including the lack of bone marrow biopsies or molecular data in a subset of cases. This finding reinforces the necessity of combining clinical, morphological, and molecular assessments to achieve diagnostic accuracy. Advanced molecular profiling, such as next-generation sequencing, could play a role in detecting non-driver mutations and distinguishing MPN-U from other myeloid neoplasm, especially MDS and MDS/MPN overlap syndromes.

The observed heterogeneity in management strategies reflects the absence of clear guidelines for MPN-U. In our cohort, most patients received antithrombotic and cytoreductive therapies, largely extrapolated from established approaches for classical MPNs. A valuable management framework for MPN-U has been proposed by McLornan et al., emphasizing an individualized,

patient-centred approach tailored to clinical presentations, including disease progression, thrombosis, constitutional symptoms, and proliferative blood counts[16].

Aligned with McLornan et al.´s recommendation for dynamic reassessment, the findings from this study also highlight the importance of ongoing diagnostic re-evaluation for patients initially classified as MPN-U. Regular follow-up focusing on changes in clinical presentations and laboratory parameters, complemented by repeat morphological and molecular diagnostics when indicated, may facilitate improved classification over time. This dynamic approach allows for adjustments in management strategies, ensuring that patients receive appropriate care tailored to their evolving disease profile. Furthermore, the term MPN-U should be reserved for cases with sufficient diagnostic data to avoid the risks associated with misclassification and suboptimal treatment.

Limitations

This study has limitations inherent with its retrospective design and reliance on registry data. While comprehensive medical record reviews were conducted, bone marrow biopsies were not re-evaluated by a centralized pathology panel. Additionally, the study´s geographic focus on regions covering 75% of Sweden's population may limit the generalizability of the findings.

Conclusion

This study provides valuable insights into the diagnostic challenges and clinical characteristics of MPN-U. By applying stringent diagnostic criteria, we demonstrate a markedly lower incidence of MPN-U compared to previous estimates. Regular reassessment of MPN-U diagnoses emerges as a critical step for ensuring accurate classification and facilitating appropriate treatment strategies over time.

Our findings highlight the need for standardized workflows that integrate clinical, morphological and molecular data. These approaches, combined with ongoing refinement of classification systems, are essential to improving diagnostic accuracy and optimizing patient outcomes. Additionally, the development of clear guidelines for monitoring and reassessment remains a priority.

AUTHOR CONTRIBUTIONS

Marie Lindgren, Björn Andréasson and Erik Ahlstrand performed the study design, analysis and were involved in drafting the paper. All other co-authors contributed data.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

Table 1 Demographics and Baseline Disease Characteristics (n=38).

Characteristics	n (%)	Median
Age, years, range	38 (100)	61 (24–89)
Sex	38 (100)	
Male, %		12 (32)
Female, %		26 (68)
Year of diagnosis, range	38 (100)	2014 (2008–2018)
Full blood count	38 (100)	
Haemoglobin g/L, range		137 (105–191)
WBC count, 10^9/L, range		10.3 (3–27.6)
Platelet count, 10^9/L, range		597 (140–1563)
Peripheral blood blasts, %, range		0.1 (0–1)
Lactate dehydrogenase, UI/L, range	28 (74)	3.9 (2.3–6.7)
Palpable splenomegaly, %	36 (95)[a]	9 (25)
Thrombosis, %	36 (95)	21 (58)
Splanchnic venous thrombosis, %		12 (33)
Cardiac, %		6 (17)
Cerebrovascular, %		6 (17)
Venous thromboembolism, %		1 (3)
Other malignancy, %	38 (100)	4 (11)
Molecular analyses		
JAK2 V617F, %	38 (100)	31 (82)
JAK2 V617F allele burden (%)	15 (48)	26 (4–72)
JAK2 exon 12	[b]	0
CALR Type 1	[b]	3
CALR Type 2	[b]	0
MPL W515L	[b]	0
Triple negative	[b]	2
Cytogenetics	8 (21)	
Normal, %		8 (100)
Complex, %		0 (0)

[a] Missing data for one patient due to splenectomy prior to diagnosis. [b] Excluded due to small sample size caused by sequentially performed molecular diagnostics and missing data for patients diagnosed prior to the discovery of the CALR mutation.

Table 2 Bone Marrow Features of 38 Cases of Myeloproliferative Neoplasms Unclassifiable.

Morphologic variables	Value
Bone marrow cellularity, % (range)	65 (30–100)
High[a], n (%)	31 (82)
Normal[a], n (%)	7 (18)
Reduced[a], n (%)	0 (0)
Megakaryopoesis, n (%)	
Increased	36 (95)
Clustered	33 (87)
Pleiomorphic	25 (66)
WHO fibrosis grade, n (%)	
0	22 (58)
1	13 (34)
2	3 (8)
3	0 (0)
Iron staining, n (%)	21 (55)
Positive	11 (52)
Negative	10 (48)

[a] *age-adjusted bone marrow cellularity*[3,19]

Table 3 Therapy Received in 38 Cases of Myeloproliferative Neoplasm Unclassifiable

Treatment	n (%)	Total
Antithrombotic therapy, any	35 (92)	32
Antiplatelet therapy		21
Anticoagulants		14
Novel oral anticoagulants		7
Vitamin K-antagonist		3
Low molecular weight heparin		2
Cytoreductive therapy	34 (89)	27
Hydroxycarbamide		17
Interferon alpha		9
JAK inhibitor (e.g. ruxolitinib)		2
Busulfan		1
Allogeneic bone marrow transplant	38 (100)	1

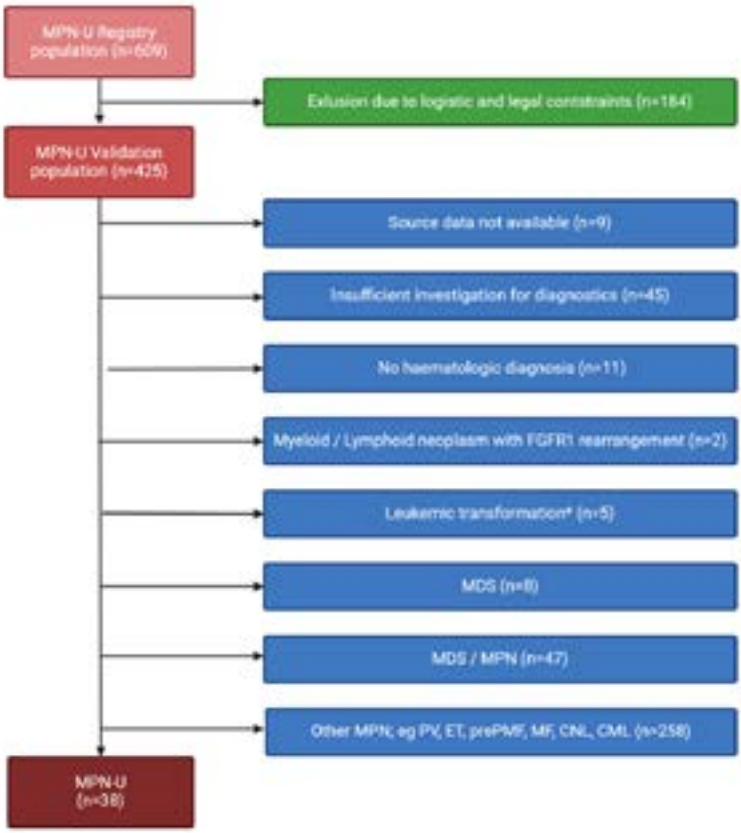
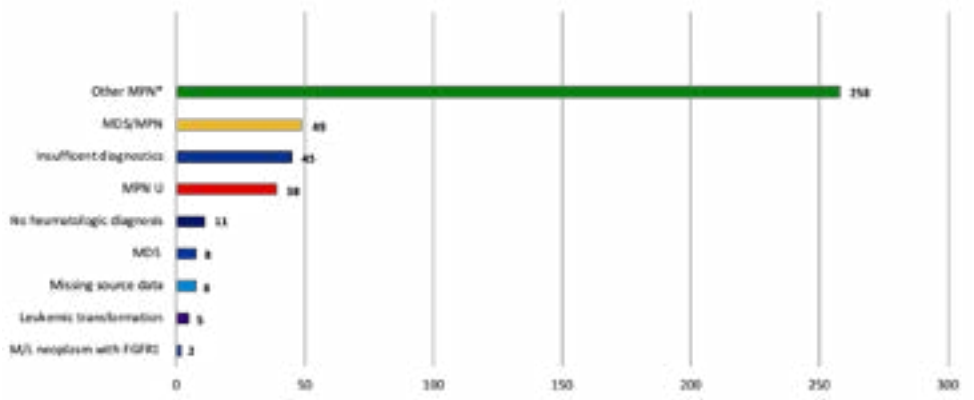


Figure 1 Flow chart illustrating the validation process for 609 patients initially registered with a diagnosis of MPN-U in the Swedish Registry between 2008 and 2019. Reasons for exclusion from the final diagnosis of MPN-U are detailed on the right-hand side of the chart.



Polycythaemia Vera, Essential thrombocythemia, Prefibrotic and Primary Myelofibrosis, Chronic Neutrophilic Leukaemia

Figur 2 Reclassification of 425 patients initially registered with a diagnosis of MPN-U in the Swedish MPN registry during the period 2008 – 2018, according to the WHO 2016 criteria. The number of patients in each diagnostic category is presented. Abbreviations: MPN-U: myeloid neoplasm unclassifiable; MDS/MPN: myelodysplastic syndrome / myeloproliferative neoplasm, MDS: myelodysplastic syndrome; M/L neoplasm with FGFR1: Myeloid / Lymphoid neoplasm associated with fibroblast growth factor receptor 1 rearrangement.

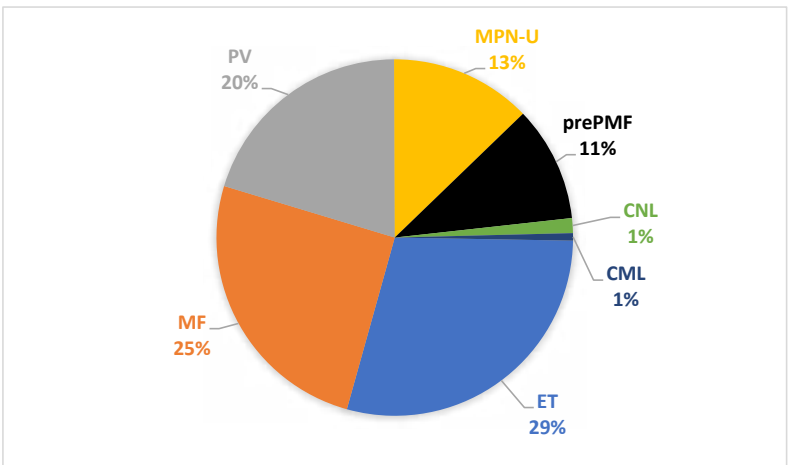
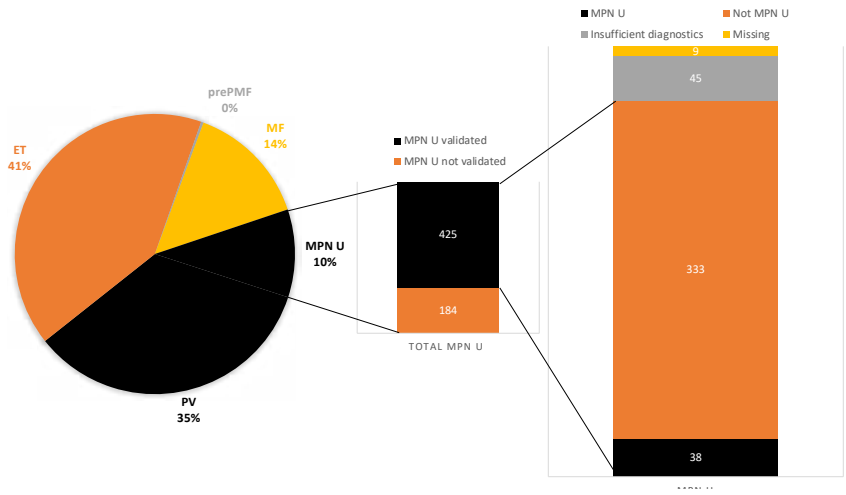


Figure 3 Result of the reclassification of MPN-U within the category of myeloproliferative neoplasms, detailing specific subcategories. Abbreviations: CML: chronic myeloid leukaemia; CNL: chronic neutrophilic leukaemia; ET: essential thrombocythemia; MF: myelofibrosis; MPN-U: myeloproliferative neoplasms unclassifiable; prePMF: prefibrotic primary myelofibrosis; PV: polycythaemia vera



a) b) c)

Figure 4 Flowchart summarizing key findings: a) the proportion of MPN-U among all MPN diagnoses reported to the Swedish MPN Registry between 2008 and 2018. b) the number of validated (n=425) and not validated (n=184) MPN-U cases; and c) the outcomes of the reclassification process. Abbreviations: ET: essential thrombocythemia; MF: myelofibrosis; MPN U: myeloproliferative neoplasms unclassifiable; prePMF: prefibrotic primary myelofibrosis; PV: polycythaemia vera

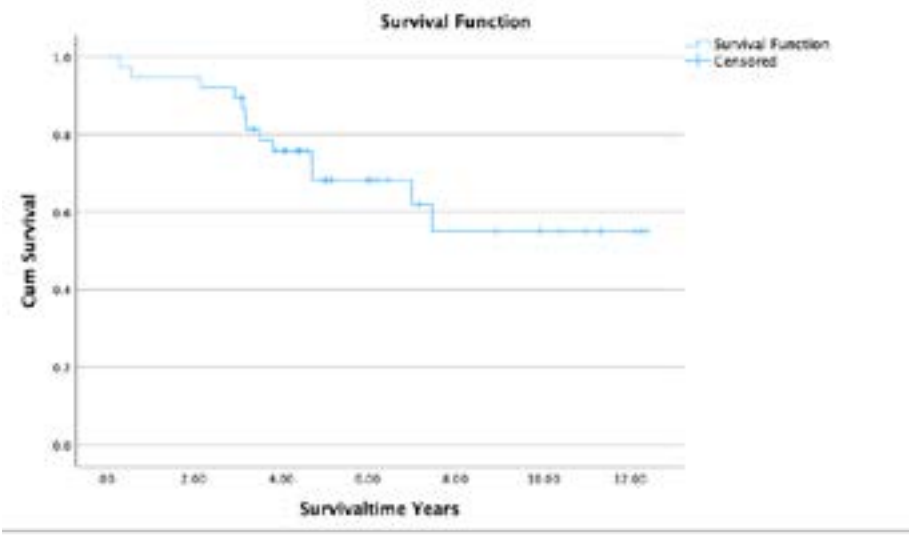


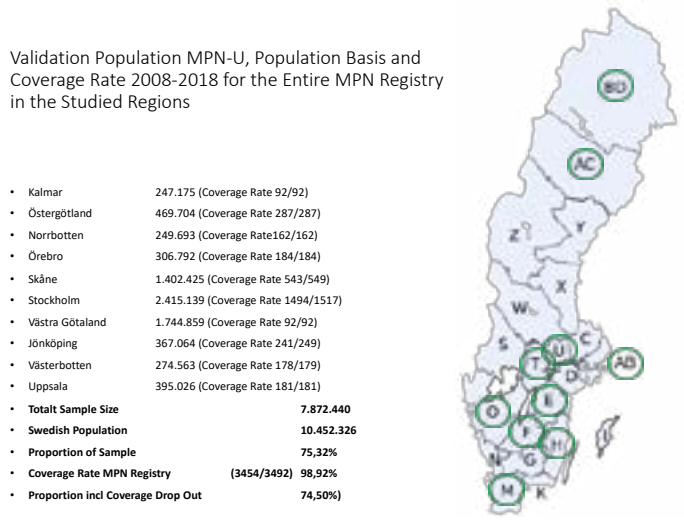
Figure 6 Kaplan-Meier survival analysis of 38 patients reclassified as MPN-U. The median overall survival was not reached during the follow-up period.

SUPPLEMENTARY SECTION

Table 1: Swedish MPN-registry; data on number and subentity of classical MPN and MPN-U reported to the registry between 2008 – 2018.

		Diagnos					
Sjukvårdsregion	**Diagnosår**	**PV**	**ET**	**pre-PMF**	**MF**	**MPN UNS**	**Totalt**
Mellansverige	2008-2018	348	402	2	148	164	1064
Norra	2008-2018	201	239	0	41	76	557
Södra	2008-2018	281	301	2	129	79	792
Stockholm-Gotland	2008-2018	497	691	8	244	131	1571
Sydöstra	2008-2018	261	268	3	79	56	667
Västra	2008-2018	472	560	2	211	103	1348
RIKET	2008-2018	2060	2461	17	852	609	5999

Figure 1: Validation Population MPN-U, Population Basis and Coverage Rate 2008–2018 for the Entire MPN Registry in the Studied Regions



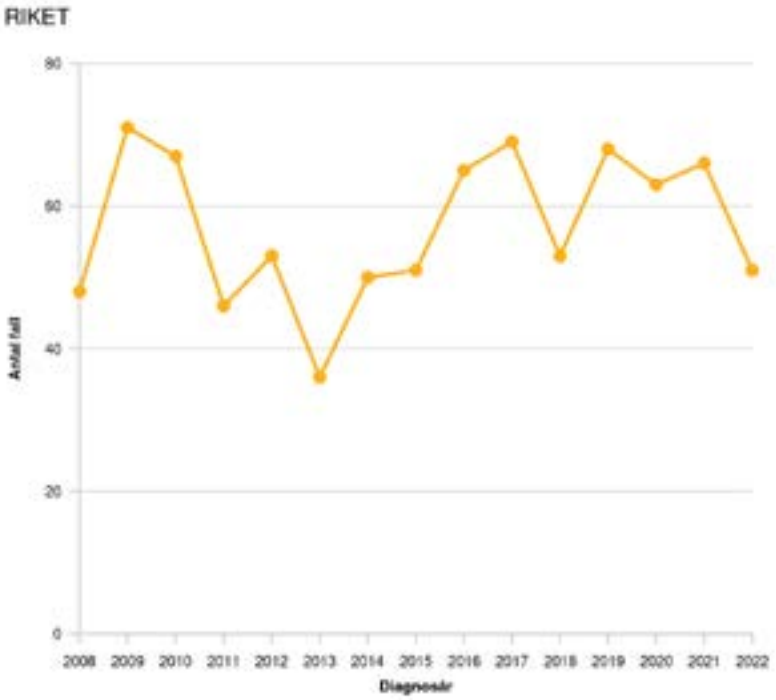


Figure 2: Incidence of myeloproliferative neoplasm unclassifiable in Sweden 2008–2022; number of patients.

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