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Major bleeding, thromboembolic complications and mortality in patients with myeloproliferative neoplasms

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To Noah, Adam and Niklas

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Abstract

Background: Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are Philadelphia-negative myeloproliferative neoplasms (MPN). These relatively rare, chronic hematological malignancies are characterized by clonal hematopoiesis with an excess production of blood cells and various degrees of bone marrow fibrosis. Patients with MPN have an increased risk of vascular events, including both arterial and venous thrombosis, as well as bleeding. Disease progression, leukemic transformation, and thromboembolic complications lead to reduced quality of life and inferior survival in patients diagnosed with MPN compared to the general population. The aims of the first study were to assess how frequent elevated blood values occur in patients diagnosed with thromboembolic events and if the underlying condition impact the risk of recurrent events. Study II-IV investigate treatment patterns, rates and risk factors of arterial and venous events, major bleeding and all-cause mortality (ACM) in patients diagnosed with PV, ET, and PMF.

Methods and material: All adult patients in the county of Norrbotten diagnosed with acute myocardial infarction (AMI), ischemic stroke, transient ischemic attack (TIA), peripheral arterial thromboembolism, pulmonary embolism (PE), deep venous thrombosis (DVT), and abdominal thrombosis during 2017 and 2018 were included in study I.

The cohort used in paper II-IV consists of all adult patients diagnosed with PV, ET, and myelofibrosis (MF) included in the Swedish MPN Register from 1 January 2008 to 29 December 2021, and a matched control population randomly selected from the Population Register by Statistics Sweden. By using multiple Swedish health care registers data on outcomes, treatment patterns and mortality were retrieved.

Results: In study I, we found that of the 3931 patients diagnosed with a thromboembolic event, 30.4% (n=1195) had elevated blood values fulfilling the 2016 revised WHO criteria for PV and ET. Out of these, 411 had no obvious underlying condition causing erythrocytosis or thrombocytosis and therefore should be investigated further to rule out an underlying MPN. Patients with unexplained thrombocytosis and secondary erythrocytosis experienced the highest rates of recurrent events.

Among the PV and ET patients included in study II, the rate of venous events, major bleeding, and ACM per 100 treatment years was significantly increased compared to corresponding controls during follow-up. Additionally, the PV patients exhibited higher rates of arterial events and all-cause stroke than the controls.

The rates of thromboembolic events, major bleeding and ACM in patients diagnosed with myelofibrosis (MF) in study III were all significantly increased compared to the controls. We also found that patients with ongoing JAK inhibitor (JAKi) therapy had higher rates of major bleeding, arterial and venous events and ACM compared to patients with no ongoing symptoms-directed therapy or hydroxyurea (HU). Use of JAKi, low-molecular weight heparin (LMWH), previous arterial or venous event and advanced age were all identified as independent risk factors for a new event. A potential explanation for the observed increased risk of thrombosis with JAKi is that Swedish guidelines restrict their use to high-and intermediate 2-risk patients. The use of LMWH in special circumstances such as postoperative care and during immobilization may partly explain the association with thrombosis. A previous venous event, a higher leukocyte count at diagnosis and treatment with JAKi were associated with an increased risk of major bleeding.

Analysis of treatment patterns among PV and ET patients in study IV, demonstrated that high-risk patients, to a large extent, were managed in accordance with established guidelines. Quite unexpectedly, we also observed that a large proportion of patients categorized as low-risk at diagnosis were treated with cytoreductive therapy during follow-up. Higher age and leukocytosis at time of diagnosis were established as predictors of thrombotic complications, major hemorrhage and ACM in both PV and ET. Treatment with HU and interferon (IFN) was associated with a reduced risk of ACM, and additionally, HU therapy had a protective effect against thromboembolic events and major bleeding.

Conclusions: Elevated blood values are frequently found in patients diagnosed with thromboembolic events and finding the underlying cause of the abnormal blood tests is of importance to reduce risk of recurrent thrombosis.

Patients with PV, ET and MF experience significantly higher rates of thromboembolic events, major bleeding and ACM compared to matched controls, and the rates of the outcomes diverges in the different treatment groups. Multiple risk factors for vascular complications, major

bleeding and ACM in PV, ET and MF have been identified, and integrating these risk factors into clinical decision-making can optimize treatment selection for each individual MPN patient, ultimately aiming to improve quality of life and overall survival.

List of scientific papers

This thesis is based on the following papers, which are referred to by their Roman numerals throughout and are included in full at the end.

I. Larsson AE, Andréasson B, Holmberg H, Liljeholm M, Själander A. Erythrocytosis, thrombocytosis, and rate of recurrent thromboembolic event-A population-based cohort study. Eur J Haematol. 2023 Jun;110(6):608-617. doi: 10.1111/ejh.13938. Epub 2023 Feb 12. PMID: 36725666.

II. Enblom-Larsson A, Renlund H, Andréasson B, Holmberg H, Liljeholm M, Själander A. Thromboembolic events, major bleeding and mortality in essential thrombocythaemia and polycythaemia vera-A matched nationwide population-based study. Br J Haematol. 2024 May;204(5):1740-1751. doi: 10.1111/bjh.19337. Epub 2024 Feb 13. PMID: 38351734.

III. Enblom Larsson A, Renlund H, Andréasson B, Holmberg H, Liljeholm M, Själander A. Thrombosis, major bleeding, and mortality in 1079 patients with myelofibrosis: a matched population-based study. Blood Adv. 2025 Jun 10;9(11):2783-2793. doi: 10.1182/bloodadvances.2025016247. PMID: 40117492; PMCID: PMC12167804.

IV. Enblom Larsson A, Renlund H, Andréasson B, Holmberg H, Liljeholm M, Själander A. Polycythemia Vera and Essential Thrombocythemia: A Nationwide Population-Based Study on Treatment Patterns, Vascular Complications and Survival – *Manuscript*

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Abbreviations

ACM	All-cause mortality
aCML	Atypical chronic myeloid leukemia
AMI	Acute myocardial infarction
AML	Acute myeloid leukemia
ASA	Acetylsalicylic acid
AvWS	Acquired von Willebrand syndrome
CDR	Cause of Death Register
CI	Confidence interval
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
DIPSS	Dynamic International Prognostic Scoring System
DOAC	Direct oral anticoagulants
DVT	Deep venous thrombosis
EPO	Erythropoietin
ESA	Erythropoiesis-stimulating agents
ET	Essential thrombocythemia
Hb	Hemoglobin
Hct	Hematocrit
HR	Hazard ratio
HU	Hydroxyurea

IFN	Interferon
IMiDs	Immunomodulatory drugs
IPSET	International Prognostic Score of Thrombosis in Essential Thrombocythemia
IPSS	International Prognostic Scoring System
JAKi	Janus kinase inhibitors
JAK-STAT	Janus kinase-signal transducer and activator of transcription
LMWH	Low-molecular weight heparin
MDS	Myelodysplastic syndromes
MF	Myelofibrosis
MPN	Myeloproliferative neoplasms
MPN-SAF	Myeloproliferative Neoplasm Symptom Assessment Form
NGS	Next generation sequencing
NPR	National Patient Register
PDR	Prescribed Drug Register
PE	Pulmonary embolism
PMF	Primary myelofibrosis
PrePMF	Prefibrotic primary myelofibrosis
PV	Polycythemia vera
P32	Radioactive phosphorus
RCT	Randomized controlled trial
SE	Secondary erythrocytosis

SMF	Secondary myelofibrosis
SVT	Splanchnic vein thrombosis
TIA	Transient ischemic attack
TPO	Thrombopoeitin
VTE	Venous thromboembolism
WBC	White blood cell

Populärvetenskaplig sammanfattning

De myeloproliferativa neoplasierna (MPN) är en grupp av relativt ovanliga blodsjukdomar som karaktäriseras av förhöjda blodvärden och olika grader av bindvävsomvandlad benmärg, så kallad fibros. Den kliniska bilden vid polycytemia vera (PV) domineras av förhöjda röda blodkroppar, och vid essentiell trombocytemi (ET) är det främst blodplättarna som är ökade. Förhöjda vita blodkroppar förekommer vid båda sjukdomarna, och även vid myelofibros (MF) som också ingår i gruppen av MPN. Vid MF har den normala benmärgen ersatts av bindväv, vilket leder till minskad produktion av röda och vita blodkroppar och blodplättar. Vid alla typer av MPN finns en risk för att sjukdomen ska ändra karaktär och övergå i en akut leukemi. Patienter som diagnosticeras med MPN har också en ökad risk att drabbas av blodproppar, vilket kan påverka både livskvalitet och livslängd.

I den första studien i den här avhandlingen undersöker vi hur många av de patienter som drabbas av någon form av blodpropp under 2017 och 2018 i Norrbottens län som också har förhöjda, avvikande blodvärden. Vi undersöker också om den bakomliggande orsaken till de höga blodvärdena påverkade risken att drabbas av en ny blodpropp.

I de tre efterföljande studierna analyseras riskfaktorer och frekvens av olika typer av blodproppar, stora blödningar och överlevnad hos MPN patienter som finns registrerade i svenska MPN-registret. Vi beskriver också vilka behandlingar som används vid dessa sjukdomar och om dessa läkemedel skiljer sig åt när det gäller risken för blödningar, blodproppar eller dödsfall.

I den första studien kunde vi se att en tredjedel av de 3931 patienter som drabbades av någon form av blodpropp i Norrbotten under 2017-2018 hade förhöjda blodvärden vid ett eller flera tillfällen. Hos 411 patienter kunde man inte hitta någon tydlig förklaring till de förhöjda blodvärdena, och därför borde dessa patienter utredas vidare för att utesluta bakomliggande blodsjukdom. I samma studie kunde vi också visa att patienter som hade oförklarligt förhöjda blodplättar och patienter med förhöjt blodvärde på grund av hjärt- eller lungsjukdom hade den högsta frekvensen av återkommande blodproppar.

Hos PV- och ET-patienterna som ingick i studie II kunde vi se att antalet venösa blodproppar, större blödningar och död var signifikant högre än

hos normalbefolkningen. Patienterna med PV hade också högre frekvens av arteriella tromboser (till exempel hjärtinfarkt och stroke) jämfört med kontrollpersonerna.

I studie III kunde vi se att patienter med MF hade en klart ökad risk för både blodproppar, blödningar och död jämfört med kontrollgruppen. Pågående behandling med JAK-hämmare (ett läkemedel som används vid MF), lågmolekylärt heparin (blodförtunnande medicin), tidigare blodpropp och högre ålder visade sig vara riskfaktorer för att drabbas av ny blodpropp. En tidigare venös blodpropp, förhöjda vita blodkroppar i samband med diagnos och behandling med JAK-hämmare var faktorer associerade med ökad risk för stor blödning hos MF-patienterna.

Av resultaten i studie IV kunde vi se att de svenska patienter med PV och ET som löper störst risk att drabbas av blodproppar, i stor utsträckning behandlas i enlighet med internationella rekommendationer. Något förvånande kunde vi också konstatera att en relativt stor andel av de patienter som bedömdes ha en låg risk att drabbas av komplikationer vid diagnostillfället fick benmärgshämmande behandling under uppföljningstiden. Hög ålder och förhöjda vita blodkroppar vid diagnos identifierades som riskfaktorer för att drabbas av blodproppar, större blödningar och död. Pågående behandling med hydroxyurea och interferon (båda dessa är benmärgshämmande läkemedel som används vid PV och ET) hade en skyddande effekt mot död, och hos patienter med PV hade hydroxyurea också en skyddande effekt mot tromboser och blödning.

Introduction

A historical glance

In 1903, William Osler, professor of medicine at the Johns Hopkins University described a 44-year-old man with diffuse cyanosis, vertigo, moderate spleen enlargement, and elevated red blood cells. The patient had no heart disease or emphysema, and Osler suggested that this may be a new disease although further investigation was needed (1). This is not the first written presentation of polycythemia vera, eleven years earlier, the French physician Vaquez published a paper on a 40-year-old male with vertigo, whistling in the ears, vomiting, splenomegaly, cyanosis, and elevated red corpuscles (2).

Half a decade later, in 1951, William Dameshek found resembling characteristics in chronic granulocytic leukemia, polycythemia vera, thrombocythemia and myeloid metaplasia (now known as myelofibrosis) and classified them as the myeloproliferative syndromes (3).

The discovery of the Philadelphia chromosome by Nowell and Hungerford in 1960, first observed in two male patients with chronic myeloid leukemia (CML) and later in nine additional CML patients in 1961 (4), distinguished CML from the other myeloproliferative neoplasms (MPN), which lacked this chromosomal abnormality and were consequently classified as Philadelphia-negative MPN.

A major breakthrough in uncovering the origins of MPN came in 2005, when four research teams almost simultaneously published the finding of the *JAK2* V617F mutation, a mutation that leads to constitutively activated JAK2 kinase, which induces erythrocytosis (4-7).

Since 2005 several mutations associated with MPN have been discovered: *JAK2* exon 12 (8), *MPL* (9) and *CALR* (10, 11). These genetic aberrations explain some of the diverging characteristics of the diseases and also provide a possible target of therapy.

Epidemiology

The MPNs are relatively rare hematological malignancies, with an estimated incidence of 4.45 per 100 000 person-years. Among the subtypes, ET exhibits the highest incidence at 1.60 per 100 000 person-years, followed by PV at 1.48. PMF is the least prevalent, with an

incidence of 0.52 per 100 000 person-years. PV and PMF occur more commonly in men, whereas ET is more frequently diagnosed in women (12).

The MPN are diseases that predominantly affect elderly people, with the highest incidence in the ages between 70 and 79 years (12), and the median ages are 65, 68 and 70 years in PV, ET, and PMF, respectively (13). Although unusual, the diseases do occur in younger ages and a population-based study from Sweden reports an incidence of MPN of 0.67 per 100 000 person-years among individuals aged 15 to 39 (12).

Pathogenesis

The group of Philadelphia-negative MPN are all characterized by clonal hematopoiesis leading to an overproduction of blood cells. As described earlier, research during the last decades has found several driver mutations contributing to the development of the disorders, however the underlying cause of why these genetic aberrations occur remains unclear.

The JAK-STAT signaling pathway

The JAK-STAT (Janus Kinase-Signal Transducer and Activator of transcription) signaling pathway is an intracellular cascade from the cellular membrane to the nucleus, regulating hematopoiesis, immune responses, inflammation, and cellular regeneration among other functions (14).

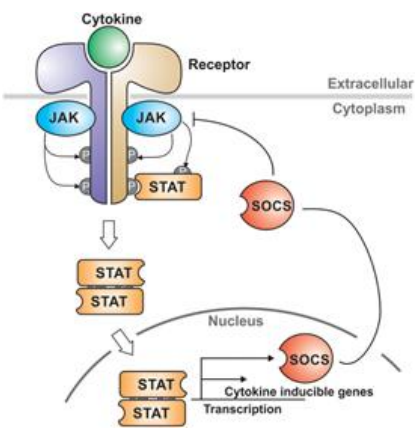

Figure 1 *The Janus Kinase-Signal Transducer and Activator of transcription signaling pathway (15)*

The normal JAK-STAT pathway in hematopoiesis includes a number of steps (figure 1). First erythropoietin (EPO) binds to the receptor, which leads to receptor dimerization and activation of JAK (Janus kinase), an enzyme on the intracellular part of the receptor, which leads to phosphorylation of the receptor. The phosphorylation recruits STAT proteins, which dimerize and are subsequently transported into the cell nucleus, where they bind to DNA and activate gene transcription, ultimately driving blood cell production. Loss of function or mutations in the JAK-STAT pathway have been identified in MPN, other malignancies and autoimmune disorders (14).

JAK2 mutations

The *JAK2* V617F mutation, identified in 2005, is a point mutation located in exon 14 within the pseudo kinase domain of the *JAK2* gene. Under normal conditions, this domain functions to suppress JAK2 activity (5). Acquisition of a *JAK2* V617F mutation results in constitutive activation of the JAK-STAT signaling pathway, driving uncontrolled hematopoiesis (16). A *JAK2* mutation is present in over 97% of all patients diagnosed with PV (17), and in 50-60% of all patients with ET and PMF (18).

When searching for additional disease-related mutations in PV patients not harboring *JAK2* V617F mutations, researchers in 2007 found acquired mutations in *JAK2* exon 12, causing a distinct increase in red blood cell production (8). The *JAK2* exon 12 mutation occurs in approximately 1-2% of all PV-patients and is not present in patients with ET (8, 19). The *JAK2* exon 12 mutation gives rise to clinical features which deviates from the characteristics seen in patients with *JAK2* V617F mutations; the patients are significantly younger and present with isolated erythrocytosis without abnormalities in the megakaryocytic or granulocytic lineages (8).

MPL mutations

Gain-of-function mutations in the *MPL* (myeloproliferative leukemia virus oncogene) gene encoding the thrombopoietin receptor (also known as the MPL receptor) result in aberrant signaling that promotes increased megakaryocytic proliferation and leads to thrombocytosis (20). The normal activation of the MPL receptor includes thrombopoietin (TPO) binding to the receptor, causing a dimerization, which triggers activation of the downstream signaling that leads to megakaryocyte proliferation. A reduction of circulating levels of TPO leads to inactivation of the MPL receptor, thereby suppressing

thrombopoiesis (21). Acquired mutations in c-*MPL* genes at W515 and S505 are seen in 5-8% of patients with ET and MF (9). These mutations induce conformational alterations in the MPL receptor, triggering downstream intracellular signaling pathways resulting in increased production of platelets (16).

CALR mutations

In 2008 the first complete genetic code of a human using next-generation sequencing (NGS) was published in Nature (22), a breakthrough discovery that enabled the identification of genomic variations potentially linked to disease. Using NGS, two research groups in 2013 found somatic mutations in the gene *CALR* in 60-90% of ET and MF patients with unmutated *JAK2* or *MPL* (10, 11). The *CALR* gene encodes the protein calreticulin which functions as a chaperone in the endoplasmic reticulum. The mutated calreticulin interacts with the MPL receptor and thereby causing a constitutive activation of JAK2 and downstream signaling leading to thrombocytosis (23).

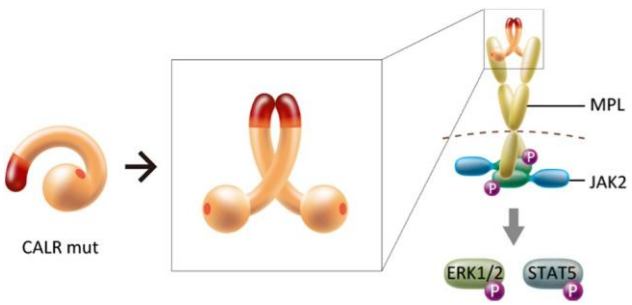

Figure 2 *Mutant calreticulin binds to the MPL receptor, triggering dimerization and activation of the MPL-JAK2 complex. This, in turn, initiates intracellular signaling that leads to uncontrolled platelet production* (23).

CALR mutations are most frequently seen in ET and MF but occurs in other hematological malignancies, i.e., myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia (CMML) and atypical chronic myeloid leukemia (aCML)(10, 11). Studies have shown that *CALR* mutations do not occur in PV (10, 11), although Girodon et al. presented two cases of PV with *CALR* mutations. Nevertheless, the presence of other genetic abnormalities contributing to erythrocytosis in these patients could not be fully ruled out (24). The majority (85%) of *CALR* mutated cases have a type I mutation, a 52-base pair deletion, or type II, a 5-base pair insertion (25).

Other mutations

Lymphocyte-specific adaptor protein (LNK or SH2B2) is a protein that modulates the JAK-STAT signaling pathway by binding to the JAK2 complex and suppressing downstream activation and hematopoiesis through a negative feedback mechanism. In animal models, LNK deficient mice developed splenomegaly, extramedullary hematopoiesis, leukocytosis, and thrombocytosis (26), classical features seen in MPN. In 2010 Oh et al. discovered mutations in *LNK* in one patient with PMF and one patient with ET (27). *LNK* mutations occur in 2%, 2-6% and 3-6% of patients with PV, ET, and PMF, respectively (28).

CBL mutations have been found in 5-10% of MF patients, but also occur in other hematological malignancies such as CMML, AML, aCML and MDS (28-30). Wild-type *CBL* negatively regulates tyrosine-kinase activity and thereby inhibits proliferation of blood cells. Somatic mutations in *CBL* lead to prolonged intracellular signaling and excessive hematopoiesis. *CBL* mutations are associated with MPN characterized by a more aggressive clinical course and inferior prognosis compared to other mutations (30).

In addition to above-mentioned driver mutations several other genetic aberrations contributing to disease progression have been identified (table 1).

Table 1 *Other somatic mutations occurring in MPN.*

Mutation	Normal function of wild-type gene	Frequency in MPN	Clinical implications
DNMT3A	Permitting the differentiation of hematopoietic stem cells (31)	5.7% (32)	Resistance to IFN in *JAK2* V617F-positive MPN (33)
PPM1D	Negatively regulates DNA damage-response signals, such as p53 and CHK1, allowing the cell cycle to restart (34)	1.9% (35)	
TET2	Controls proliferation and differentiation of myeloid cells (36)	15.1% (32)	Loss of *TET2* leads to myelomonocytic proliferation and clinical characteristics similar to CMML (37)
ASXL1	Modulates the transcription of genes involved in cell proliferation and differentiation (38)	19.0% (32)	Correlates with inferior survival and increased risk of leukemic transformation in MF (39)
EZH2	Controls cell cycle progression, autophagy and apoptosis, DNA damage repair (40)	4.6% (32)	Accelerates development of myelofibrosis (41), associated with leukemic transformation and inferior survival (42)
SRSF2	Controls DNA transcription and repair DNA damage (43)	6.4% (32)	Associated with leukemic transformation and inferior survival (39)
IDH1, IDH2	Cellular protection of oxidative stress (44)	1.3%, 2.0% (32)	Increased risk of progression to acute leukemia (39)
U2AF1	Regulates mRNA splicing (28)	6.2% (32)	Correlates with impaired overall survival (45)
SF3B1	Regulates mRNA splicing (19)	4.7% (32)	Impaired overall survival and increased risk of progression to MF (46)
ZRSR2	Controls cell cycle progression and differentiation of hematopoietic cells (47)	2.4% (32)	
TP53	"Guardian of the genome"(48) – induces cell cycle arrest, activates DNA repair, triggers apoptosis if irreparable damage (49)	3.3% (32)	Presence of *TP53* mutations in MF is associated with an extremely poor prognosis and in ET increased risk of blast transformation(50)

CMML chronic myelomonocytic leukemia, *ET* essential thrombocythemia, *IFN* interferon, *MF* myelofibrosis, *MPN* myeloproliferative neoplasm

Clinical characteristics

Symptoms correlated to microvascular disturbances are common in both PV and ET and include headache, visual symptoms, dizziness, and erythromelalgia (episodic painful sensations combined with warmth and erythema in peripheral extremities) (51, 52).

An enlarged spleen may cause abdominal discomfort and early satiety. Splenomegaly is most frequently seen among patients with myelofibrosis but also occurs in PV and ET (53).

Pruritus is a common symptom among patients with MPN (54), with aquagenic pruritus representing a distinct subtype most commonly associated with PV, though it can also occur in ET and MF. This condition is marked by severe itching without visible skin lesions, triggered by contact with water at any temperature, and may persist for an hour or longer (55).

Constitutional symptoms such as night sweats, fever and weight loss can be present in all MPN subtypes, although most frequently in MF patients (53, 56).

In an online survey completed by 699 patients diagnosed with MPN, the most frequent and severe symptom was fatigue, affecting 64%, 45% and 54% of the ET, PV, and MF patients, respectively (57). The same study reported reduced quality of life irrespective of low- or high-risk classification of the disease and a considerable proportion of patients reported significant impairment in their capacity to work and perform daily activities (57).

A Swedish study assessing symptoms in recently diagnosed MPN patients using the MPN-SAF (MPN Symptom Assessment Form) found that PV patients reported higher levels of inactivity, pruritus, and depression, as well as lower overall quality of life, compared with ET patients. In PMF, patients at diagnosis showed fewer symptoms than those in later disease stages, with constitutional complaints, abdominal discomfort, fatigue, and reduced quality of life becoming more pronounced over time (56).

Diagnosis

Bone marrow morphology

In PV, bone marrow biopsy typically demonstrates age-adjusted hypercellularity with trilineage proliferation involving erythropoiesis, granulopoiesis, and megakaryopoiesis. An increase of normoblastic erythropoiesis and expansion of megakaryocytes are the most prominent features, with megakaryocytes appearing in clusters and frequently showing morphological abnormalities such as variation in size and irregular nuclear lobulation (58, 59).

The bone marrow in ET is often normocellular with a significant proliferation of megakaryocytes. The megakaryocytes are enlarged in size and scattered in loose clusters throughout the bone marrow. Reticulin fibers may be minimally increased, but more extensive fibrosis will exclude an ET diagnosis and instead support a diagnosis of prefibrotic primary myelofibrosis (prePMF), which in peripheral blood can resemble ET (52, 59).

In advanced PMF the peripheral blood smear displays leukoerythroblastosis (immature leukocytes and nucleated red blood cells) and teardrop-shaped red cells. The bone marrow shows increased reticulin staining with prominent fibrosis (grade 2-3). In prePMF the bone marrow is characterized by a less prominent fibrosis (grade 0 or 1) and can be hypercellular whereas the bone marrow in overt myelofibrosis is normo- or more often hypocellular. The megakaryocytes in PMF appears atypical compared to other types of MPN, with ballon-shaped nuclei and occurring in large dense clusters in close contact with bone trabeculae or vascular sinuses (59, 60).

Diagnostic criteria

The MPN diagnosis is based on clinical parameters, blood counts, genetic analysis, and bone marrow examination. The diagnostic criteria of PV, ET, prePMF and MF currently used in Sweden is the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia presented in table 2-5 (61).

The thresholds for hemoglobin (Hb) were lowered in the 2016 revision of the criteria for PV, and hematocrit (Hct) were added to the diagnostic criteria compared to previous versions from 2001 and 2008 (62, 63). This modification was made in order to be able to diagnose patients with masked PV, i.e., patients with *JAK2* mutation and bone marrow morphology consistent with PV but not fulfilling the Hb criteria. These changes raised concerns among clinicians who feared that many healthy subjects will undergo unnecessary and costly investigations regarding PV. A study from Brazil showed that among almost 250 000 persons, 6.48% of the men and 0.28% of the women had Hb or Hct levels exceeding the thresholds in the diagnostic criteria of PV, which would drastically increase the annual incidence of individuals requiring a diagnostic work-up to exclude PV (64). To prevent unnecessary investigations, secondary erythrocytosis (SE), which is more prevalent than primary erythrocytosis (65, 66), should first be excluded by performing a thorough medical history, physical examination,

pulmonary function evaluation, and oxygen saturation measurement. Molecular testing should be reserved for patients with persistently elevated blood values accompanied by symptoms or clinical features indicating PV (67).

Table 2 *WHO criteria for polycythemia vera (61)*

Major criteria
1. Hemoglobin >16.5 g/dL in men, hemoglobin >16.0 g/dL in women
or,
Hematocrit >49% in men, hematocrit >48% in women
or,
increased red cell mass (RCM)*
2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
3. Presence of *JAK2*V617F or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion†

* More than 25% above mean normal predicted value.
† Criterion number 2 (bone marrow biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5 g/dL in men (hematocrit, 55.5%) or >16.5 g/dL in women (hematocrit, 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a bone marrow biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

Table 3 *WHO criteria for essential thrombocythemia (61)*

Major criteria
1. Platelet count ≥450 × 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for *BCR-ABL1*+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR*, or *MPL* mutation
Minor criterion
Presence of a clonal marker or absence of evidence for reactive thrombocytosis
Diagnosis of ET requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion

Table 4 *WHO criteria for prefibrotic myelofibrosis (61)*

Major criteria
1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for *BCR-ABL1*+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,* or absence of minor reactive bone marrow reticulin fibrosis†
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis ≥11 × 10^9/L
c. Palpable splenomegaly
d. Lactate dehydrogenase (LDH) increased to above upper normal limit of institutional reference range
Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion

* In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (e.g., *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.
† Minor (grade 1) reticulin fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Table 5 *WHO criteria for overt primary myelofibrosis (61)*

Major criteria
1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3
2. Not meeting WHO criteria for ET, PV, *BCR-ABL1*+ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker*, or absence of reactive myelofibrosis†
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis ≥11 × 10^9/L
c. Palpable splenomegaly
d. Lactate dehydrogenase (LDH) increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis
Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

*In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (e.g., *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.
†Bone marrow fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Differential diagnosis of erythrocytosis

Elevated Hb and/or Hct is a common cause of referral to a hematology specialist. To confirm the underlying cause of persistently raised blood values knowledge on both polycythemia vera and secondary causes of erythrocytosis is needed (table 6). Primary erythrocytosis originates from an inherent abnormality in erythroid progenitor cells, while secondary erythrocytosis is driven by external stimuli that increase red blood cell production, predominantly through elevated EPO levels (67). EPO, which is secreted by the kidneys, is the primary regulator of erythropoiesis. An analysis of the EPO levels can help distinguish whether the erythrocytosis is primary or secondary, but it should be noted that patients with PV may present with normal EPO levels (68).

Primary erythrocytosis

Primary erythrocytosis can be classified as either congenital or acquired. The acquired forms include polycythemia vera and idiopathic erythrocytosis, the latter being a diagnosis of exclusion made when all other known causes of erythrocytosis have been ruled out (69).

Congenital causes of primary erythrocytosis are exceedingly rare and include mutations in the Epo receptor and *SH2B3* (*LNK*). Genetic testing for these mutations should be considered in patients with long-standing erythrocytosis, a positive family history of elevated hematocrit, and subnormal EPO levels (66, 70).

Secondary erythrocytosis

SE is most commonly caused by conditions causing hypoxia, for example cardiovascular diseases with cardiac failure or right-to-left shunts, chronic obstructive pulmonary disease, sleep apnea, or obesity hypoventilation syndrome (67).

Renal artery stenosis, end-stage renal failure, hydronephrosis and polycystic kidney disease can induce local hypoxia in the kidneys, thereby stimulating EPO production and leading to a subsequent erythrocytosis (71).

Several tumor types, for example hepatocellular carcinoma, renal cell carcinoma, and cerebellar hemangiomas, have been associated with abnormal EPO production and erythrocytosis (67).

Drug-induced erythrocytosis may occur in patients receiving erythropoiesis-stimulating agents (ESAs) and elevated Hct is frequently observed in men with ongoing testosterone treatment; however, the precise mechanisms by which androgens influence erythropoiesis remain incompletely understood (71, 72). Use of diuretics lead to reduced plasma volume and consequently a higher concentration of red blood cells. The use of SGLT-2 (sodium-glucose cotransporter 2) inhibitors in patients with diabetes, chronic heart failure or chronic kidney disease has also been associated with erythrocytosis. At the introduction of these agents, the rise in Hct was attributed to hemoconcentration due to their mild diuretic effect. Subsequent studies, however, demonstrated increased reticulocyte counts and reduced levels of hepcidin, a hormone regulating iron metabolism (73), and thereby suggesting other mechanisms causing erythrocytosis (74). Further studies propose that SGLT-2 inhibitors induce local renal hypoxia which lead to erythrocytosis (71). A Danish study on patients with heart failure with reduced ejection fraction treated with SGLT-2 inhibitors showed that 207 (3.3%) of the 3138 included patients developed erythrocytosis, but the erythrocytosis was not associated with an increased risk of thrombosis (75).

The congenital causes of SE is as previously described rare entities. Since the 1970s erythrocytosis has been known to be an endemic disorder in the region of Chuvash in Russia (76). In 2002 it was established that Chuvash polycythemia was caused by a mutation in the von Hippel Lindau (*VHL*) gene (77). The *VHL* gene normally encodes a protein that degrades hypoxia-inducible factor alfa (HIF-α), a key transcription factor controlling EPO production. The mutated *VHL* gene leads to accumulation of HIF-α, which subsequently drives increased EPO synthesis (78). Adult patients with Chuvash polycythemia typically exhibit a characteristic clinical profile, including elevated Hb and EPO levels, lower blood pressure, lower BMI, and decreased white blood cell counts (79). Since 2002 other mutations causing defects in the oxygen-sensing pathway have been discovered; *EGLN1* (80) and *EPAS1* (81).

The oxygen-hemoglobin dissociation curve describes the relationship between arterial oxygen tension and Hb saturation and illustrates the mechanisms of oxygen loading in the lungs and unloading in peripheral tissues. The affinity for oxygen depends on the Hb and there are variants of Hb with high affinity for oxygen, leading to hypoxia at tissue level and thereby inducing EPO production and erythrocytosis. The first high oxygen-affinity Hb variant, Hb Chesapeake, was described in 1966 where an 81-year-old man with Hb 199g/L and 15 of his relatives exhibited a

left-shifted dissociation curve and an abnormal band on the Hb electrophoresis (82). Other rare causes of erythrocytosis are methemoglobinemia, bisphosphoglycerate (2,3-BPG) mutase deficiency and mutations in the *PIEZO1* gene (67).

Table 6 *Causes of erythrocytosis (67, 83)*

Classification	Causes	Erythropoietin level
Primary erythrocytosis		
Congenital	EPO receptor mutation	Low
	SH2B3 (*LNK*) mutation	Low
Acquired	Polycythemia vera	Low
	Idiopathic erythrocytosis	Low, normal, or high
Secondary erythrocytosis		
Congenital	*Defects in oxygen sensing pathway*	High or normal
	Chuvash polycythemia	
	Mutations in *EGLN1* or *EPAS1*	
	Left-shift of Hb-oxygen dissociation curve	High
	High oxygen-affinity hemoglobin	
	Methemoglobinemia	
	2,3-BPG deficiency	
	PIEZO1 mutations	
Acquired	*Central hypoxic process*	High
	Chronic lung disease	
	Right-to-left cardiopulmonary vascular shunts	
	Hypoxic states (sleep apnea/altitude)	
	Local renal hypoxia	High
	Renal artery stenosis	
	End-stage renal disease	
	Hydronephrosis	
	Renal cysts (polycystic kidney disease)	
	Tumors	High
	Hepatocellular carcinoma	
	Renal cell cancer	
	Cerebellar hemangioblastoma	
	Parathyroid carcinoma/adenoma	
	Uterine leiomyoma	
	Pheochromocytoma	
	Meningioma	
	Drug-associated	
	Erythropoietin	High
	Use of androgens	High or normal
	Diuretics	Normal
	SGLT-2 inhibitors	High or normal
	Alcohol excess	
	Post-renal transplant erythrocytosis	

BPG bisphosphoglycerate, *SGLT-2* sodium-glucose cotransporter 2

Relative polycythemia

A reduced plasma volume will lead to an apparent elevation of HCT although the number of circulating red blood cells remain normal, a condition called relative polycythemia, pseudopolycythemia, Gaisböck's syndrome or stress polycythemia (84).

Differential diagnosis of thrombocytosis

Thrombocytosis is a common finding in both in-patient and out-patient care. Up to one third of patients admitted to the intensive care unit present with thrombocytosis (85), and the most common causes are infection, inflammation or tissue damage in trauma or surgery leading to reactive thrombocytosis. Primary thrombocytosis consist of MPN and some variants of MDS (table 7).

Table 7 *Causes of thrombocytosis (86)*

Classification		Causes
Primary thrombocytosis		
	Myeloproliferative neoplasms	Essential thrombocythemia Polycythemia vera Myelofibrosis Myeloproliferative neoplasm unclassified (MPN-U) Chronic myeloid leukemia
	Myelodysplastic syndromes	MDS with isolated del 5q MDS/MPN with ring sideroblasts and thrombocytosis
Secondary thrombocytosis		
	Reactive	Infection Inflammation Iron deficiency Trauma (incl. surgery) Post splenectomy Bleeding Malignancy

MDS myelodysplastic syndromes, *MPN* myeloproliferative neoplasms

Reactive thrombocytosis

As listed above, a variety of clinical conditions may present with elevated platelets, and the increase in megakaryopoiesis is mediated by cytokines, for example IL-6. Thrombocytosis in patients with iron deficiency is a common finding, although the underlying mechanisms remain incompletely understood (87).

Complications

Thromboembolic events

Patients with MPN, regardless of subtype, have an increased risk of arterial and venous events compared to the general population (88). The highest frequency of thrombotic events occur around the time of diagnosis (88, 89), however, many patients with MPN experience arterial or venous events prior to their diagnosis (90). Thrombosis in atypical sites is frequently seen in MPN patients especially splanchnic vein thrombosis, SVT (89). Previous studies based on the Swedish MPN register conclude that vascular events, to a large extent, impact life expectancy in PV, ET, and MF (91, 92).

Patients with PV appear to have a higher risk of thrombosis compared to ET and PMF patients (93). A medical history including a previous thrombosis and age over 65 years is well-known risk factors for thrombotic events in PV (94, 95). In a randomized trial, Marchioli et al. demonstrated that PV patients that maintained a Hct level below 45% had a significantly reduced incidence of fatal cardiovascular events and thrombotic complications compared with those whose Hct was maintained between 45–50% (96). Additional risk factors for arterial and venous events in PV have been established; hypertension, diabetes, hyperlipidemia, leukocytosis, and major hemorrhage (97, 98).

In ET patients, age over 60 years, presence of *JAK2* mutation, male gender, previous arterial event and hypertension, have been identified as risk factors for arterial thrombosis. A previous venous thrombosis, male gender, and presence of *JAK2* mutation are associated with an increased risk of future venous thromboembolism (VTE)(99, 100).

Risk factors for thrombotic events in PMF has not been studied as extensively as in PV and ET, but presence of *JAK2* V617F mutation, prior thrombotic events, hypertension and being classified as intermediate-2 or high-risk category according to the International Prognostic Scoring System (IPSS) are all associated with an increased risk of thrombotic events (101, 102).

Bleeding

Patients with MPN are at increased risk of bleeding, attributable to disease-specific factors such as acquired von Willebrand syndrome (AvWS), thrombocytopenia, and/or the use of antiplatelet or anticoagulant drugs (103). AvWS can occur in patients with extreme

thrombocytosis (platelet count >1000 x 10^9/l) and is caused by a reduction of the von Willebrand factor multimers (104). A retrospective study on 829 MPN patients revealed that 17.2% of the patients were affected by a bleeding complication and out of these 143 events, 47 (32.9%) were classified as major bleeding (transfusion dependent anemia, retroperitoneal hemorrhage, central nervous system involvement, or other life-threatening bleeding) (105).

Patients with PMF have higher rates of bleeding complications than patients with PV and ET. Previous thrombosis, age, use of anti-platelet agents or anticoagulants, leukocytosis, or platelets >1000 x10^9/L at diagnosis, previous bleeding episodes, male gender, and atrial fibrillation have been pinpointed as risk factors of bleeding in MPN patients (89, 99, 100, 105, 106).

Disease progression

Secondary myelofibrosis

Both PV and ET carry an inherent risk of progression to secondary myelofibrosis (SMF). In PV 4.9-6% of the patients experience transformation into SMF during the first ten years of the disease, while the cumulative risk of fibrotic transformation at 15 years is estimated at 6–14%. Age above 60, bone marrow fibrosis, splenomegaly and *JAK2*V617F allele burden >50% are associated with an increased risk of post-PV MF. Progression to SMF occurs at a lower frequency in ET than in PV, with a cumulative 10-year and 15-year risk of 0.8-4.9% and 4-11% respectively. Risk factors for fibrotic progression in ET include advanced age, anemia, *MPL* mutation, bone marrow hypercellularity and reticulin deposition (107).

Leukemic transformation

Leukemic transformation (or blast phase MPN, BP-MPN) can occur in all three subtypes of MPN, and the diagnosis is confirmed when the blast count in the bone marrow or peripheral blood exceeds 20%. An accelerated phase (AP-MPN) can precede BP-MPN and is characterized by a blast count between 10-19% (108). In a large study from Italy and the United States 2.8%, 4.9% and 12.4% of the included patients with ET, PV, and PMF, respectively, developed acute myeloid leukemia (AML) during follow-up (109). Risk factors for leukemic transformation is described in table 8. The median time from diagnosis to BP-MPN in ET, PV and PMF was 11, 10 and 3 years, respectively (93).

Table 8 *Risk factor for leukemic transformation in PV, ET, and PMF (107, 110, 111)*

Risk factors	PV	ET	PMF
	Age	Age	Age
	Leukocytosis	Leukocytosis	Male sex
	Reticulin fibrosis	Anemia	Anemia
	Splenomegaly	Reticulin fibrosis	Circulating blasts ≥3%
	Abnormal karyotype	Thrombosis	Constitutional symptoms
	ASXL1	Platelets ≥1000 x10^9	IDH1
	Use of pipobroman	*TP53*	ASXL1
	Use of busulfan	*RUNX1*	SRSF2
	Use of P32	Use of melphalan	Very high-risk karyotype
	Use of chlorambucil		

ET essential thrombocythemia, *PMF* primary myelofibrosis, *PV* polycythemia vera, *P32* radioactive phosphorus

Prognosis

Patients with MPN have an impaired survival compared to the general population due to thromboembolic complications and leukemic transformation (112, 113). The 5-year survival for ET, PV and PMF has been reported to be 88.7%, 88.3% and 44.9% respectively, and the median survival time for the same patient groups has been estimated to 12.1 years, 11.9 years, and 4.0 years (114).

Age constitutes the most significant prognostic factor for overall survival in PV, ET, and PMF (51, 52, 60), and other risk factors associated with inferior survival are presence of leukocytosis (PV, ET, PMF), abnormal karyotype (PV), thromboembolic events (PV, ET), anemia (ET, PMF), presence of constitutional symptoms (PMF) and circulating blasts >1% (PMF) (109, 115, 116).

Risk stratification

Over the past decade, several scoring systems have been developed to stratify the risk of thrombotic events and disease progression in MPN. In PV, the main goal of treatment is to reduce risk of thrombotic complications, and patients with a previous thrombosis or age over 60 years are considered high-risk for developing a thrombosis, while patients aged under 60 years and no previous thromboembolic event are classified as low-risk (51).

The Revised International Prognostic Score ET (IPSET)-thrombosis is a four-tiered stratification model to predict thrombosis in ET. Patients aged under 60 years, with no previous thrombosis and *JAK2* unmutated are classified as very low-risk and low-risk patients include patients younger than 60 years, no medical history of thrombotic events but presence of *JAK2* mutation. Patients aged 60 years and older, no previous thrombotic event and *JAK2* unmutated are considered to have intermediate risk of thrombosis. The high-risk group consist of patients with previous thromboembolic event or age over 60 years combined with *JAK2* mutation (117).

In 2009 the International Working Group for MPN Research and Treatment developed the International Prognostic Scoring System (IPSS) to predict the prognosis in PMF, including five independent risk factors for inferior survival: age above 65 years, Hb <10 g/dl, leukocyte count $>25 \times 10^9$/L, circulating blasts ≥1% and presence of constitutional symptoms (115). The same group later designed a scoring system to predict survival in PMF at diagnosis as well as during follow-up, DIPSS (Dynamic International Prognostic Scoring System), which includes the same variables but Hb below 10g/dl assigned two adverse points instead of one. The low and intermediate-1 categories include patients with 0 points and 1 point, respectively. In the study on which the DIPSS scoring system was based, the median survival time was not reached in the low-risk group, whereas it was 14.2 years in the intermediate-1 group. Patients classified as intermediate-2 (3-4 points) had a median survival of 4 years, while those in the high-risk category (5-6 points) had a median survival of only 1.5 years (118).

To identify patients eligible for allogeneic stem cell transplantation more advanced scoring systems have been developed (MIPSS70 and MIPSSv2) and these include mutational status, presence of high-risk mutations (*ASXL1, EZH2, SRSF2* and *IDH1/2*) in addition to peripheral blood values, circulating blasts and constitutional symptoms (60).

Treatment

Polycythemia vera

Current treatment strategy in all PV patients regardless of risk category include phlebotomy to maintain Hct below 45% and low-dose acetylsalicylic acid (ASA) unless contraindicated (51). For patients categorized as high-risk of developing thrombosis, cytoreductive therapy is recommended. HU is considered the first-line treatment in older

patients, whereas interferon (IFN) is an option for younger patients, particularly in women of reproductive age (51). Among low-risk patients who presents with substantial symptoms, such as pruritus, splenomegaly, constitutional symptoms, or a frequent requirement of phlebotomy, cytoreductive treatment may be considered (51). Low-risk patients with a medical history including hypertension, diabetes mellitus or ischemic heart disease or persistent leukocytosis >15 x 10^9/l, extreme thrombocytosis could also benefit from cytoreductive therapy (58). In case of HU intolerance or resistance, ruxolitinib, a JAK inhibitor has shown to be effective in maintaining Hct below 45% as well as reducing PV-related symptoms (51, 58). Busulfan is a therapeutic option in cases of HU intolerance or resistance; however, there has been concerns regarding increased risk of leukemic transformation, and the use of busulfan is generally restricted to patients with limited life expectancy (58).

Essential thrombocythemia

In very low-risk ET patients who are *CALR*-mutated or triple-negative (lacking driver mutations) and without cardiovascular risk factors, observation alone is recommended according to the Swedish Care Program of MPN. In the presence of cardiovascular risk factors, low dose ASA is advised. Low-risk patients, i.e., patients younger than 60 years, with *JAK2* or *MPL* mutation but no previous thrombosis, are recommended treatment with ASA. In intermediate-risk patients with cardiovascular risk factors and in high-risk patients cytoreductive therapy is indicated, and as in PV the first-line treatment is HU and in younger patients IFN (119). All high-risk patients should be treated with antiplatelets agents or if the medical history includes venous thrombosis, anticoagulants is warranted. In cases of extreme thrombocytosis (platelets > 1000 × 10^9/L), ASA should be used cautiously due to the risk of AvWS. If platelet counts exceed 1500 × 10^9/L, ASA should be avoided, and cytoreductive therapy should be initiated instead. Third-line treatments in ET consists of anagrelide and busulfan (119).

Primary myelofibrosis

The Swedish Care Program for MPN recommends observation only in asymptomatic low-risk patients with myelofibrosis (119). For low-risk PMF patients with symptomatic splenomegaly, leukocytosis, or thrombocytosis, HU is the preferred first-line therapy, whereas ruxolitinib may be considered as a second-line option in patients refractory to HU (60). IFN can be used in selected cases of PMF, particularly those presenting with proliferation and low-grade bone

marrow fibrosis (119). Allogeneic stem cell transplantation remains the only potentially curative therapy for PMF and should be considered in high- and very high-risk patients, after evaluating age and comorbidities (60). ESAs may be beneficial in patients with symptomatic anemia (119).

Aims of the thesis

The overall aim of this doctoral project is to advance understanding of the risk of thromboembolic complications, major bleeding, and mortality in patients with elevated blood values as well as in those diagnosed with MPN.

Specific aims:

Paper I

To determine the frequency of elevated blood values among patients with thromboembolic events, to evaluate how many of these should undergo further investigation for a potential myeloproliferative neoplasm, and to assess whether the risk of recurrent events is influenced by the underlying condition.

Paper II

To evaluate the incidence of arterial and venous events, major bleeding, all-cause stroke, and all-cause mortality in Swedish patients with ET and PV in comparison with matched controls.

Paper III

To evaluate the incidence of arterial and venous events, major bleeding, all-cause stroke, and all-cause mortality in patients diagnosed with MF compared to the general population, and to investigate whether different types of symptom-directed therapies influence the frequency of complications and overall mortality.

Paper IV

To characterize real-world treatment patterns, quantify the incidence of thromboembolic and major bleeding events during treatment, and identify clinical and therapeutic factors associated with thrombosis, bleeding, and all-cause mortality in patients diagnosed with PV and ET.

Materials and Methods

Study I

Study population and outcomes

This retrospective cohort study included all adult patients in the county of Norrbotten who were diagnosed with an AMI, ischemic stroke, TIA, peripheral arterial thromboembolism, PE, DVT, portal vein thrombosis, or other abdominal thrombosis during 2017 and 2018.

Elevated blood values, as defined by the 2016 revised WHO criteria for PV and ET (Hb >16.5 g/dL in men, Hb >16.0 g/dL in women, Hct >49% in men, Hct >48% in women, or platelet count ≥450 × 10^9/L), were extracted from the patients' medical records at three time points: at the first thromboembolic event, and both before and after the event. Data on MPN diagnosis, date of diagnosis, conditions associated with secondary erythrocytosis or reactive thrombocytosis, recurrent thromboembolic events and death were also collected. The inclusion period was from 1 January 2017 to 31 December 2018, with study follow-up ending on 1 September 2021.

Statistical methods

Continuous variables were summarized using mean, median, and range. Differences in proportions between categorical variables were assessed with Pearson's Chi-square test or Fisher's exact test.

The study outcomes included recurrent thromboembolic events which was reported as recurrence rates and event-free survival across subgroups. Time under risk started at the time of the including event and in the event-free survival analysis, only the first recurrent thromboembolic event was considered. Restricted mean survival time was used to summarize survival over the defined time period where death was used as the endpoint. We used restricted mean survival time since it does not rely on the proportional hazards assumption like the Cox proportional hazard model and it also provides an interpretable measure of average survival up to a specified time point (120).

Survival distributions were compared using the log-rank test. Survival analyses were performed in R version 4.1.3 and visualized with Kaplan-Meier plots.

Study II-IV

Included registers

Study II-IV are based on data retrieved from multiple Swedish Health Care Registers.

1. The Swedish Blood Cancer Register

   In 2007 the Swedish Blood Cancer Register was established with the aim to systematically monitor diagnosis, treatment, follow-up, quality of diagnostics, care, and survival of patients with malignant blood cancer or lymphoma. The Blood Cancer Register is a part of the Swedish Cancer Register, and since 1958, Swedish law has required mandatory reporting of all newly diagnosed cancer cases to the register, leading to a very high level of coverage. The high coverage is also a result of a dual reporting system, introduced in 1984, in which both the pathologist and the responsible clinician submit reports for each cancer case (121).

2. The Swedish MPN Register

   The Swedish MPN Register was founded in 2008 as a part of the Swedish Blood Cancer Register, and since 2023 the MPN Register is an independent quality register containing data on PV, ET, MF, prePMF, chronic eosinophilic leukemia and chronic neutrophilic leukemia. The register includes data on diagnosis, laboratory values at diagnosis and every third year of follow-up, mutational status, treatments, complications, and death. The MPN register captures 98% of all diagnoses, with follow-up coverage of 93% at 3 years and 89% at 6 years. (122).

3. The National Patient Register

   The National Patient Register (NPR), maintained by the Swedish Board of Health and Welfare, contains data on inpatient care (since 1987) and outpatient care (since 2001) and includes diagnoses classified using the International Classification of Diseases, Ninth and Tenth Revisions (ICD-9 and ICD-10), along with information on hospital admissions, surgical procedures, and discharge dates (123).

4. The Stroke Register (Riksstroke)

   The quality register on stroke care in Sweden, Riksstroke, was founded in 1994, and since then the register collects data on diagnoses and hospitalization of patients diagnosed with ischemic stroke, TIA, intracerebral hemorrhage, and subarachnoid hemorrhage. Riksstroke has a coverage of 94% and demonstrates high validity for stroke diagnoses, as it records only the index stroke and captures the exact date of diagnosis rather than relying on ICD-10 codes from hospital discharge records (124).

5. The National Prescribed Drug Register

   The National Prescribed Drug Register (PDR) has, since 2005, recorded all prescribed drugs dispensed at pharmacies, with the primary purpose of improving patient safety in pharmacotherapy. It is updated on a monthly basis and maintains complete nationwide coverage (100%) (125).

6. The National Cause of Death Register

   The Cause of Death Register (CDR) contains data on all deaths occurring in Sweden, with the primary purpose of describing causes of death and monitoring mortality trends at a national level. For every deceased individual, a physician is required to complete both a death certificate and a cause-of-death certificate, the latter of which is submitted to the National Board of Health and Welfare to be incorporated in the CDR (126).

7. The Total Population Register

   Statistics Sweden (SCB) is responsible for the Total Population Register, which includes data such as identity, gender, age, registered residence, and personal identity number (127).

Study population and outcomes

Paper II

All adult patients with a diagnosis of PV and ET who were recorded in the MPN register between 1 January 2008 and 29 December 2021 were included in the study, and for each patient five controls were randomly selected from the Total Population Register by Statistics Sweden. The

controls were matched by gender, geographical region, and age at the time of PV or ET diagnosis.

The outcomes assessed were arterial and venous events, major bleeding, all-cause stroke, and ACM during follow-up. The stroke-related outcomes were retrieved from the Stroke Register and the other outcomes from the NPR and CDR.

Paper III

The study population in study III comprised all adult MF patients registered in the MPN register from 1 January 2008 to 29 December 2021, and as in study II matched controls were randomly selected from the Total Population Register.

Frequency and risk factors of arterial and venous events, major bleeding, all-cause stroke, and ACM were investigated, and these outcomes were also correlated to ongoing therapy. As in paper II the outcomes were collected from the Stroke Register, NPR, and CDR.

Treatment data were obtained from the NPR, and the duration of each treatment episode (both cytoreductive therapy and treatment with antiplatelet agents and anticoagulants) was estimated based on standardized dosing regimens. A treatment period started on the date a prescription was dispensed at the pharmacy and was assumed to extend for 92 days, with the duration prolonged upon subsequent dispensations. Busulfan, which is prescribed for fixed, time-limited courses due to its prolonged pharmacological effect, was assumed to have an effect for 365 days. Because HU and warfarin dosing varies substantially between individuals, each prescription was assumed to correspond to 183 days of treatment. Treatments recorded in the MPN register (IFN, phlebotomy, and P32) were considered to begin when the therapy was documented as ongoing in the register and to end when it was recorded as discontinued at follow-up.

Paper IV

Study IV was based on the same patient cohort as paper II, and the outcomes assessed were thrombotic and bleeding complications as well as ACM during treatment of PV and ET. Outcome data and information on therapy were collected following the same procedures as described in study II and III.

Statistical methods

Baseline characteristics were summarized using continuous variables (median and interquartile range) and categorical variables (expressed as percentages). Differences in baseline characteristics in Paper II were evaluated using the Kruskal-Wallis test for continuous variables and the Chi-squared test for categorical variables.

The outcomes were presented as rates (events per time, in which time was measured in 100-year units) and hazard ratios (HR) with 95% confidence intervals (CIs). HR were calculated using a Cox regression model adjusting for sex, age, and calendar year of diagnosis. To compare the rates of the outcomes among cases and controls, log-rank test were performed and to visualize the results of the Cox regression model Kaplan-Meier curves were generated. To identify risk factors for the outcomes, multivariable analyses were conducted, adjusting for sex, age, laboratory values, mutational status, prior events, and different therapies.

Ethical considerations

All studies included in this thesis are observational studies based on data from patient records (study I) and data from multiple health care registers including the Swedish MPN Register (study II-IV). The research was performed according to the Declaration of Helsinki.

Paper I

The first study is a retrospective cohort study using data collected from medical records in the county of Norrbotten. The data collected from the medical charts were documented in files only accessible to the research group. All information retrieved was de-identified, and a paper code key was created and retained by the investigator. The study was based on a review of medical records from which only data on thromboembolic event, laboratory values, underlying condition that may cause elevated blood values and time of death were extracted. The absolute majority of patients were considered not to be affected by the retrieval of this data, and due to that fact, informed consent was not obtained. A relatively large number of patients included were also deceased at the time of collecting data, an informed consent would therefore have resulted in a more selected inclusion. The study was approved by the Swedish Ethical Review Authority (Dnr 2020-04631).

Paper II-IV

The three following studies were based on data retrieved from several health care registers in Sweden. In study II and III cases from the MPN register were matched with randomly selected controls by Statistics Sweden (SCB), and all data were subsequently transferred to the National Board of Health and Welfare (in paper IV no matched controls were included). The data from the MPN register and SCB were then merged with information from the NPR, the Stroke Register, the PDR, and the CDR. Prior to being provided to us, all material was de-identified by the National Board of Health and Welfare, which retained a code key for three months after delivery to enable correction of any potential errors in the released dataset. Access to the data was restricted only to the authorized researchers, and all datasets were securely stored in accordance with established standards for the protection of sensitive information. As stated in the ethics application and approval of study II-IV informed consent was waived because all data were obtained from registers and no contact with, or influence on the included cases and controls were made. Study II-IV were approved The Swedish Ethical Review Authority (Dnr 2021-02227).

Results

Paper I

During 2017 and 2018, 3931 patients in Norrbotten county were identified having an arterial or venous event. The study cohort had a mean age of 73.5 years, and 56.7% were male.

A total of 13.2% (518/3931) of the included patients had elevated Hb or Hct levels meeting the first major criterion of the 2016 WHO classification for PV. Elevated platelet counts (>450 $\times 10^9$/L) were found in 17.6% of patients (694/3931). Concurrent elevations in Hb/Hct and platelet counts were observed in 17 patients. Increased Hb/Hct and platelet counts were present at the time of the thromboembolic event in 4.1% (163/3931) and 1.3% (51/3931) of patients, respectively. Only 1.3% (50/3931) of the patients had elevated Hb/Hct at all three time points (before, at the time of, and after the thromboembolic event), and one of these patients was diagnosed with an MPN. Platelets >450 x 10^9/L at all three time points were observed in 0.3% (11/3931) of the patients and out of these, two were diagnosed with an MPN.

When evaluating underlying causes of elevated blood values, SE was the most common explanation of high Hb/Hct, which was identified in 26.6% (138/518) of patients. An MPN diagnosis accounted for 9 cases (1.7%). The remaining 371 patients had no clear etiology of the elevated Hb/Hct and needed further investigation to exclude MPN. Among the 694 patients with thrombocytosis, a reactive cause was identified in 90.9% (631/694), most commonly due to infection and after surgery. An MPN was diagnosed in 2.3% (16/694) of the patients, while 47 patients (6.8%) had no underlying condition that could explain the elevated platelet counts and these patients should therefore undergo additional investigation for possible MPN (table 9).

Table 9 *Underlying causes of elevated hemoglobin, hematocrit, and platelet levels (128).*

Affected blood values	Classification	Causes
Elevated hemoglobin/ hematocrit n=518		
	Primary erythrocytosis n=9	
		Polycythemia vera n=8
		Essential thrombocythemia n=1
	Secondary erythrocytosis n=138	
		Cardiopulmonary disease n=54
		Use of androgens n=4
		Dehydration n=80
	Unexplained erythrocytosis n=371	
Elevated platelets n=694		
	Primary thrombocytosis n=16	
		Essential thrombocythemia n=11
		Polycythemia vera n=5
	Reactive thrombocytosis n=631	
		Infection n=304
		Inflammation/malignancy n=60
		Post-surgery n=233
		Iron deficiency/gastrointestinal bleeding n=34
	Unexplained thrombocytosis n=47	

A recurrent thromboembolic event occurred among 11.0% (434/3931) of the patients. The highest rate of a new thrombotic event were observed in the group of patients with unexplained thrombocytosis with a rate of 8.87 per 100 person years (95% CI 2.96-14.78).

Patients with unexplained thrombocytosis had the most inferior event-free survival of 3.71 years (95% CI 3.25-4.18), whereas the patients with unexplained erythrocytosis displayed an event-free survival of 4.41 years (95% CI 4.32-4.50). Analysis of restricted mean survival time across subgroups showed that patients with unexplained erythrocytosis had the most favorable outcome, 3.91 years (95% CI, 3.75-4.07), whereas those with SE had the poorest, 2.70 years (95% CI, 2.35-3.04, figure 3).

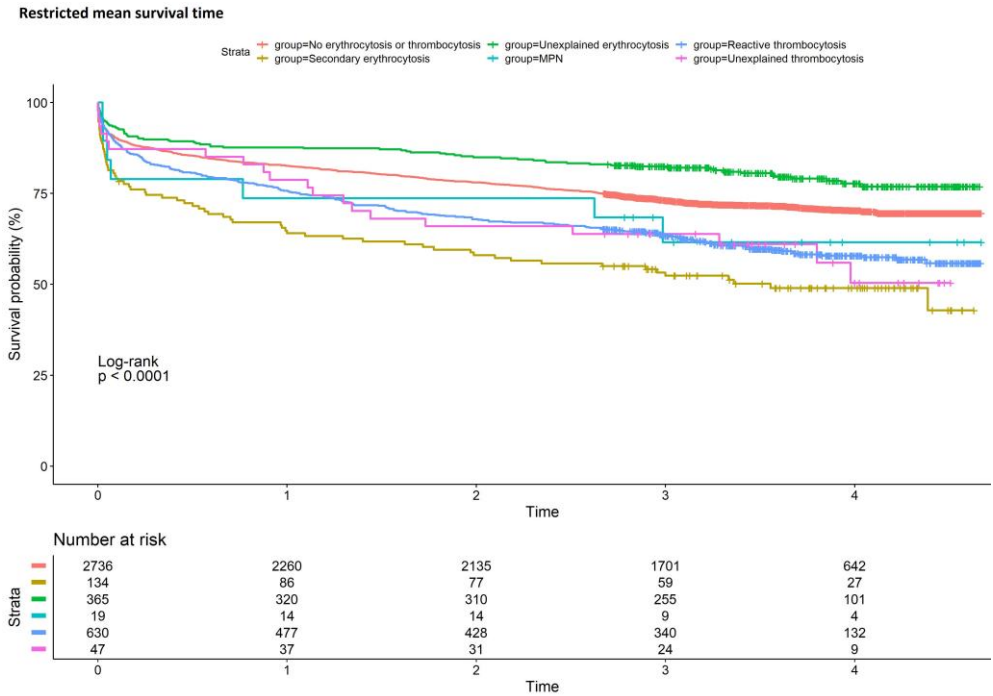

Figure 3 *Restricted mean survival time in the different subgroups of erythrocytosis and thrombocytosis. The restricted mean survival time summarizes survival from time 0 to the end of follow-up (128)*

Paper II

In total, 3141 patients with ET and 2604 patients with PV were included and matched to 15705 and 13020 controls, respectively. The median age of the ET and PV patients at time of diagnosis were 69.1 years and 70.4 years, and 58.6% and 50.6% were females. In total, 22.3% and 25.2% of the ET and PV patients had a medical history including arterial event prior to the MPN diagnosis, and a venous event before the MPN diagnosis were recorded in 6.7% of the ET patients and 9.4% of the PV patients. A splanchnic vein thrombosis (SVT) was more commonly seen in ET and PV patients before diagnosis (0.96% in ET and 1.8% in PV versus controls 0.045% and 0.031%). A history of major bleeding was significantly more common in ET patients than in their matched controls (5.6% versus 4.6%), whereas in PV patients and controls the frequency of major bleeding were comparable (5.8% versus 5.2%).

Among patients with ET, 328 arterial or venous events were recorded during follow-up. VTE (all subtypes combined), PE, and SVT occurred significantly more frequently in ET patients than in matched controls (table 10).

Table 10 *Rates of first event in cases with essential thrombocythemia (n=3141) and corresponding controls (n=15705). Rate is event/time and time is measured in 100-year units (129).*

Event	**Rate** Patients	**Rate** Controls	**HR** (95% CI)	***P*-value**
Arterial event or VTE	1.80	1.40	1.19 (1.05-1.35)	**0.0054**
Arterial event*	1.20	1.10	1.01 (0.88-1.17)	0.85
Ischemic stroke	0.49	0.47	0.93 (0.74-1.17)	0.56
Myocardial infarction	0.72	0.66	1.03 (0.85-1.24)	0.79
Peripheral arterial disease	0.07	0.05	1.34 (0.71-2.51)	0.36
VTE†	0.63	0.29	2.00 (1.61-2.49)	**<0.001**
Pulmonary embolism	0.52	0.24	2.03 (1.60-2.59)	**<0.001**
Deep venous thrombosis	0.11	0.06	1.65 (1.0-2.73)	0.052
Splanchnic vein thrombosis	0.04	0.02	2.85 (1.18-6.88)	**0.020**
Cerebral venous thrombosis	0.01	0.001	9.80 (0.89-108.05)	0.062
Major bleeding‡	0.79	0.42	1.76 (1.46-2.13)	**<0.001**
Intracranial bleeding	0.23	0.14	1.44 (1.02-2.03)	**0.041**
Gastrointestinal bleeding	0.60	0.28	1.98 (1.58-2.48)	**<0.001**
All-cause stroke	0.70	0.61	1.04 (0.86-1.26)	0.67
All-cause mortality	3.70	2.52	1.22 (1.12-1.33)	**<0.001**

95% CI 95% confidence interval, *HR* hazard ratio, *VTE* venous thromboembolism
* Arterial event: ischaemic stroke, myocardial infarction and peripheral arterial disease
† VTE: pulmonary embolism, deep venous thrombosis, splanchnic vein thrombosis and cerebral venous thrombosis
‡ Major bleeding: intracranial bleeding and gastrointestinal bleeding
Bold *p*-values are those of statistical significance.

Long-term follow-up demonstrated a significantly higher cumulative incidence of VTE, major bleeding, and ACM among ET patients compared to controls (Figure 4).

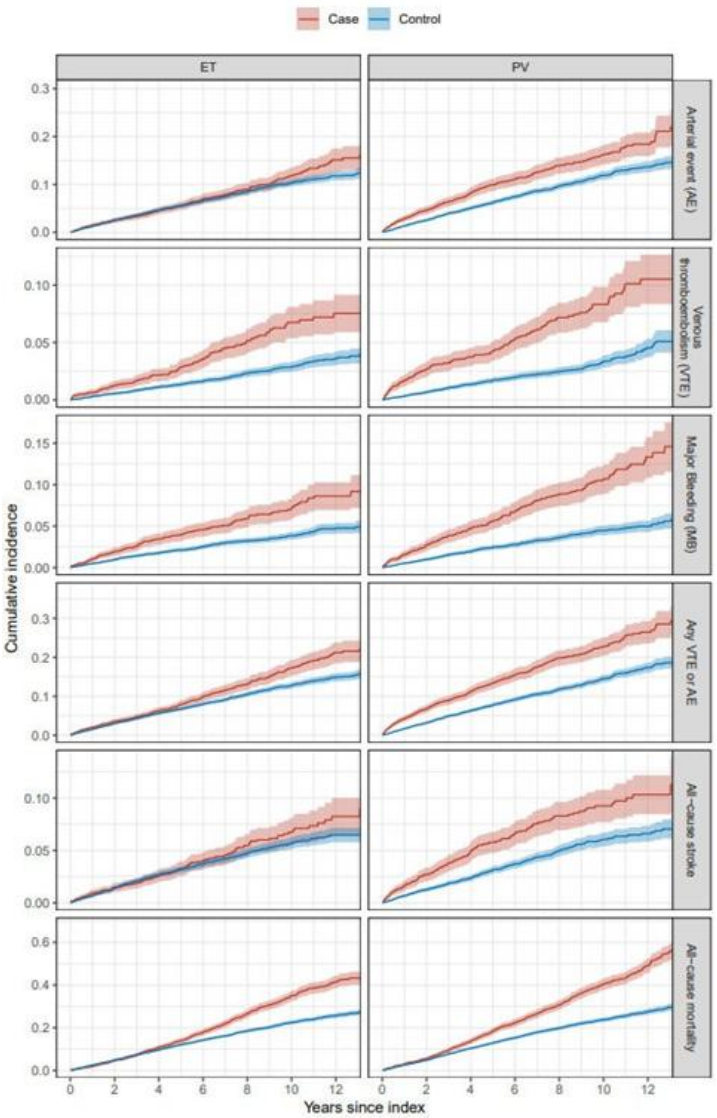

Figure 4 *Kaplan-Meier curves illustrating outcomes in essential thrombocythemia (ET) and polycythemia vera (PV) over a 12-year follow-up period. Cases are shown in red and controls in blue, with shaded areas indicating 95% confidence intervals (129).*

In patients with PV, 405 arterial or venous events were observed, corresponding to an event rate of 2.80 per 100 person-years, with a HR of 1.65 (95% CI 1.47-1.85, $p<0.001$) when comparing to the controls. With the exception of cerebral venous thrombosis, all outcomes assessed were more common in PV patients than in their corresponding controls. The highest HRs were observed for SVT (HR 17.63, 95% CI 7.15-43.51, $p<0.001$) and DVT (HR 2.29, 95% CI 1.47-3.59, $p<0.001$, table 11).

Table 11 *Rates of first event in cases with polycythemia vera (n=2604) and corresponding controls (n=13020). Rate is event/time and time is measured in 100-year units (129).*

Events	**Rate** Patients	**Rate** Controls	**HR** (95% CI)	***P*-value**
Arterial event or VTE	2.80	1.60	1.65 (1.47-1.85)	**<0.001**
Arterial event*	1.90	1.30	1.43 (1.25-1.64)	**<0.001**
Ischemic stroke	0.78	0.50	1.44 (1.17-1.78)	**<0.001**
Myocardial infarction	1.10	0.75	1.42 (1.19-1.69)	**<0.001**
Peripheral arterial disease	0.16	0.05	3.04 (1.82-5.06)	**<0.001**
VTE†	0.94	0.35	2.56 (2.08-3.14)	**<0.001**
Pulmonary embolism	0.64	0.27	2.20 (1.72-2.80)	**<0.001**
Deep venous thrombosis	0.19	0.08	2.29 (1.47-3.59)	**<0.001**
Splanchnic vein thrombosis	0.14	0.01	17.63 (7.15-43.51)	**<0.001**
Cerebral venous thrombosis	0	0.0014	-	-
Major bleeding‡	1.20	0.47	2.36 (1.97-2.83)	**<0.001**
Intracranial bleeding	0.31	0.13	2.25 (1.59-3.17)	**<0.001**
Gastrointestinal bleeding	0.88	0.35	2.38 (1.93-2.94)	**<0.001**
All-cause stroke	1.07	0.62	1.62 (1.35-1.94)	**<0.001**
All-cause mortality	4.80	2.72	1.49 (1.37-1.62)	**<0.001**

95% CI 95% confidence interval, *HR* hazard ratio, *VTE* venous thromboembolism
* Arterial event: ischaemic stroke, myocardial infarction and peripheral arterial disease
† VTE: pulmonary embolism, deep venous thrombosis, splanchnic vein thrombosis and cerebral venous thrombosis
‡ Major bleeding: intracranial bleeding and gastrointestinal bleeding
Bold *p*-values are those of statistical significance.

In long-term follow-up, PV patients exhibited a significantly increased cumulative incidence of arterial and venous events, major bleeding, all-cause stroke, and all-cause mortality compared with controls (Figure 4).

Subsequent analysis of outcomes by age group revealed that patients with ET and PV aged 18-50 years experienced a significantly higher incidence of arterial or venous events compared to their respective control groups. Across all age groups, all-cause mortality was increased in both ET and PV patients compared with controls, and among PV patients older than 50 years, the incidence of all studied outcomes (any arterial or venous event, all-cause stroke and all-cause mortality) was

increased relative to controls, with the exception of all-cause stroke in the oldest subgroup (80-100 years).

When examining the effect of age on outcomes, we found a significant interaction between age group and HR for both arterial or venous events (p<0.001) and ACM (p=0.012). The risk of events and mortality increased disproportionately with age in ET and PV patients, especially over the age 60, when the risks escalated faster than in younger groups. In ET, patients aged 70 had nearly twice the risk for arterial events or venous events, all-cause stroke, and ACM compared with those aged 60. Similarly, in PV, 70-year-old patients had higher risks of major bleeding, all-cause stroke, and ACM, roughly doubling the risks observed at age 60.

A medical history including ischemic heart disease and hypertension were in multivariable analysis associated with an increased risk of arterial events in patients with PV and ET, while a prior VTE emerged as an independent predictor of recurrent VTE in both groups.

Paper III

In study III we included 1079 patients with MF and 5395 controls, with a median age of 72 years at MF diagnosis. When classifying the MF patients according to subgroup, 939 were identified as PMF, 29 as prefibrotic myelofibrosis (prePMF) and 108 as SMF. An arterial or venous event prior to MF diagnosis were seen in 24.8% and 8.0% of the patients, respectively, whereas 8.5% of the patients had a medical history of major bleeding before diagnosis.

During follow-up HU was the most frequently prescribed treatment, in total 672 patients received HU therapy for an accumulated time of treatment of 2040 patient-years. JAKis were administered to 238 patients throughout 450 patient-years. IFN and immunomodulatory drugs (IMiDs) were used in 82 and 55 patients, respectively. Patients treated with HU had the highest mean age, 73.9 years, compared to patients in the IFN-treated group, 55.7 years (table 12).

Antiplatelet agents were prescribed to 757 of the patients and direct oral anticoagulants (DOAC), warfarin and low molecular weight heparin (LMWH) were used in 176, 115 and 201 patients, respectively. For a total time of 2010 patient-years, 1019 patients had no ongoing antithrombotic therapy (table 12).

In total 125 arterial and 51 venous events occurred among the MF patients, with corresponding rates of 2.59 and 1.06 events per 100 person-years. In the control group the rates of arterial and venous events were 1.51 and 0.38, respectively, resulting in HRs of 1.73, 95% CI 1.40-2.12 and 2.75, 1.95-3.90, both $p<0.001$ (table 12). The rates of thrombotic events were higher among patients with SMF than in patients with PMF. Among MF patients four events (rate 0.083) of SVT occurred, compared to 2 events (rate 0.0089) in the control group.

Table 12 *Rates of events in different treatment periods. Treatment periods are defined from the registry and pharma data for the case group, but each control gets the same period put in for comparison. Treatment periods are not exclusive, e.g. the time on one therapy can overlap another treatment. (123).*

		AE rate	HR (95% CI)	*p*-value	VTE rate	HR (95% CI)	*p*-value	MB rate	HR (95% CI)	*p*-value	ACM rate	HR (95% CI)	*p*-value
All time	MF n=1079	2.59	1.73	**<0.001**	1.06	2.75	**<0.001**	2.55	3.78	**<0.001**	11.01	3.92	**<0.001**
	Controls n=5395	1.51	(1.40-2.12)		0.38	(1.95-3.90)		0.68	(2.98-4.79)		2.81	(3.50-4.40)	
No symptom-directed treatment	MF n=720	2.15	1.49	**0.031**	0.79	2.85	**<0.001**	2.32	3.31	**<0.001**	11.81	4.31	**<0.001**
	Controls n=3517	1.45	(1.04-2.15)		0.32	(1.53-5.31)		0.71	(2.22-4.94)		2.76	(3.57-5.20)	
Hydroxyurea	MF n=672	2.35	1.47	**0.020**	1.27	3.17	**<0.001**	2.05	3.14	**<0.001**	10.00	3.29	**<0.001**
	Controls n=3319	1.59	(1.06-2.03)		0.40	(1.92-5.21)		0.66	(2.12-4.64)		3.01	(2.75-3.95)	
Interferon	MF n=82	0.37	0.54	0.56	0.37	1.66	0.66	1.11	14.97	0.019	2.22	3.68	**0.016**
	Controls n=408	0.67	(0.069-4.30)		0.77	(0.17-15.98)		0.075	(1.56-143.95)		0.77	(1.28-10.61)	
JAK inhibitors	MF n=238	4.67	3.59	**<0.001**	1.56	4.10	**0.0064**	5.33	8.08	**<0.001**	14.67	5.81	**<0.001**
	Controls n=1134	1.29	(2.03-6.35)		0.38	(1.49-11.31)		0.67	(4.18-15.63)		2.54	(4.05-8.34)	
IMiDs	MF n=55	2.17	1.15	0.90	0	-	-	4.35	3.22	0.20	41.30	23.26	**<0.001**
	Controls n=270	1.82	(0.13-10.29)		0.89			1.36	(0.54-19.27)		1.82	(7.91-68.38)	
ESA	MF n=308	3.13	1.75	0.066	0.84	1.01	0.99	3.75	4.85	**<0.001**	19.17	5.25	**<0.001**
	Controls n=1501	1.80	(0.96-3.17)		0.78	(0.34-3.00)		0.78	(2.50-9.41)		3.69	(3.89-7.08)	
No anti-thrombotic treatment	MF n=1019	2.04	1.66	0.0053	0.94	2.50	**0.0012**	3.18	5.06	**<0.001**	14.73	6.33	**<0.001**
	Controls n=5056	1.23	(1.16-2.37)		0.38	(1.43-4.36)		0.63	(3.56-7.20)		2.33	(5.32-7.53)	
Anti-Platelet agents	MF n=757	2.53	1.63	**0.0017**	0.63	1.76	0.072	1.67	2.60	**<0.001**	6.51	2.14	**<0.001**
	Controls n=3760	1.57	(1.20-2.21)		0.36	(0.95-3.27)		0.65	(1.74-3.88)		3.04	(1.75-2.61)	
DOAC	MF n=176	4.00	1.83	0.080	4.33	5.83	**<0.001**	3.67	4.02	**<0.001**	15.00	4.37	**<0.001**
	Controls n=812	2.18	(0.93-3.58)		0.75	(2.55-13.30)		0.90	(1.77-9.12)		3.38	(2.89-6.61)	
Warfarin	MF n=115	5.33	2.29	**0.0070**	2.00	3.02	**0.036**	2.67	3.00	**0.016**	14.00	3.43	**<0.001**
	Controls n=561	2.31	(1.25-4.19)		0.67	(1.07-8.48)		0.89	(1.23-7.34)		4.10	(2.29-5.12)	
LMWH	MF n=201	7.14	4.64	**<0.001**	9.29	60.52	**<0.001**	7.14	12.10	**<0.001**	17.14	7.58	**<0.001**
	Controls n=965	1.56	(1.93-11.16)		0.16	(7.91-462.8)		0.63	(3.80-38.59)		2.34)	(3.98-14.46)	

ACM all-cause mortality, *AE* arterial event, *DOAC* direct oral anticoagulants, *ESA* erythropoiesis-stimulating agents, *HR* hazard ratio, *IMiDs* immunomodulatory drugs, *LMWH* low-molecular weight heparin. *MB* major bleeding, *VTE* venous thromboembolism

Major hemorrhages were seen at a rate of 2.55 compared to 0.68 in controls (HR 3.92, 2.98-4.79, $p<0.001$) and the rate of bleeding were higher in patients with SMF (3.10) than in patients with PMF (2.14).

The rate of ACM was significantly increased in MF patients (11.01) in comparison to controls (2.81), with a HR of 3.92 (3.50-4.40, $p<0.001$, table 12). In the group of patients with SMF the rate of ACM was higher (15.22) than the rate observed among PMF patients (10.57).

During follow-up patients with no ongoing symptom-directed therapy experienced lower rates of arterial events (2.15) compared to patient treated with HU (2.35) and JAKi (4.67). Only one arterial event occurred among IFN-treated patients (rate 0.37). The highest rates of venous thrombosis and major bleeding were seen in patients receiving JAKi (1.56 and 5.33) whereas IFN-treated MF patients had the lowest rates of VTE (0.37 and 1.11). Patients treated with IMiDs experienced the highest rates of ACM (41.30) in contrast to IFN treated patients who had the lowest rates of ACM, 2.22 (table 12).

Arterial and venous events occurred more frequently in *JAK2* mutated patients (rates 2.74 and 1.13) compared to triple-negative patients (rates 1.77 and 0.72). MF patients with elevated white blood cell count (WBC, $>11 \times 10^9$) at diagnosis had higher rates of arterial events (2.88), venous events (1.24) and major bleeding (2.77) compared to MF patients with WBC$<11 \times 10^9$ (rates 1.96, 0.83 and 2.05, respectively).

During 12 years of follow up, the cumulative incidence of arterial and venous events, major bleeding and all-cause stroke was significantly higher in patients with MF. Regarding ACM, the MF patients had a significantly higher incidence compared to corresponding controls, and this significance persisted when patients were analyzed separately as PMF and SMF (figure 5).

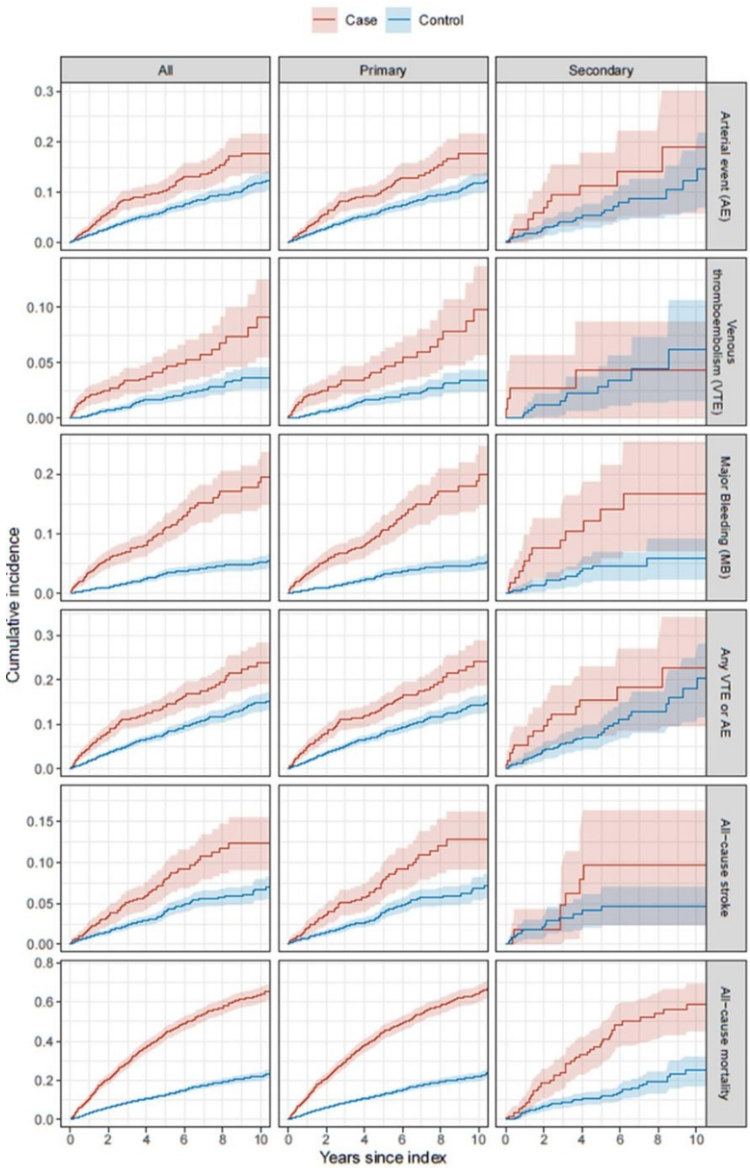

Figure 5 *Kaplan-Meier curves illustrating outcomes in myelofibrosis, primary myelofibrosis, and secondary myelofibrosis over a 12-year follow-up period. Cases are shown in red and controls in blue, with shaded areas indicating 95% confidence intervals (130)*

A multivariable analysis was conducted to determine risk factors for the outcomes investigated. JAKi therapy was associated with an increased risk of arterial or venous events (HR 2.16, 95% CI 1.27-3.68, p=0.0046),

as was treatment with LMWH (HR 3.16, 1.67-5.96), $p<0.001$). Additionally, a prior arterial or venous event and older age emerged as independent risk factors for thromboembolic events. In contrast, the *JAK2* mutation did not reach statistical significance as a risk factor for arterial or venous events.

The use of antithrombotic drugs (antiplatelet agents, anticoagulants, and LMWH) was not associated with an increased risk of major bleeding in our cohort of MF patients. Treatment with JAKi, a high WBC at diagnosis and a medical history including a previous venous thrombosis were all associated with an increased risk of major bleeding (HR 2.04, 1.15-3.63, $p=0.015$, HR 1.16, 1.08-1.24, $p<0.001$ and HR 2.01, 1.03-3.90, $p=0.04$, respectively, figure 6).

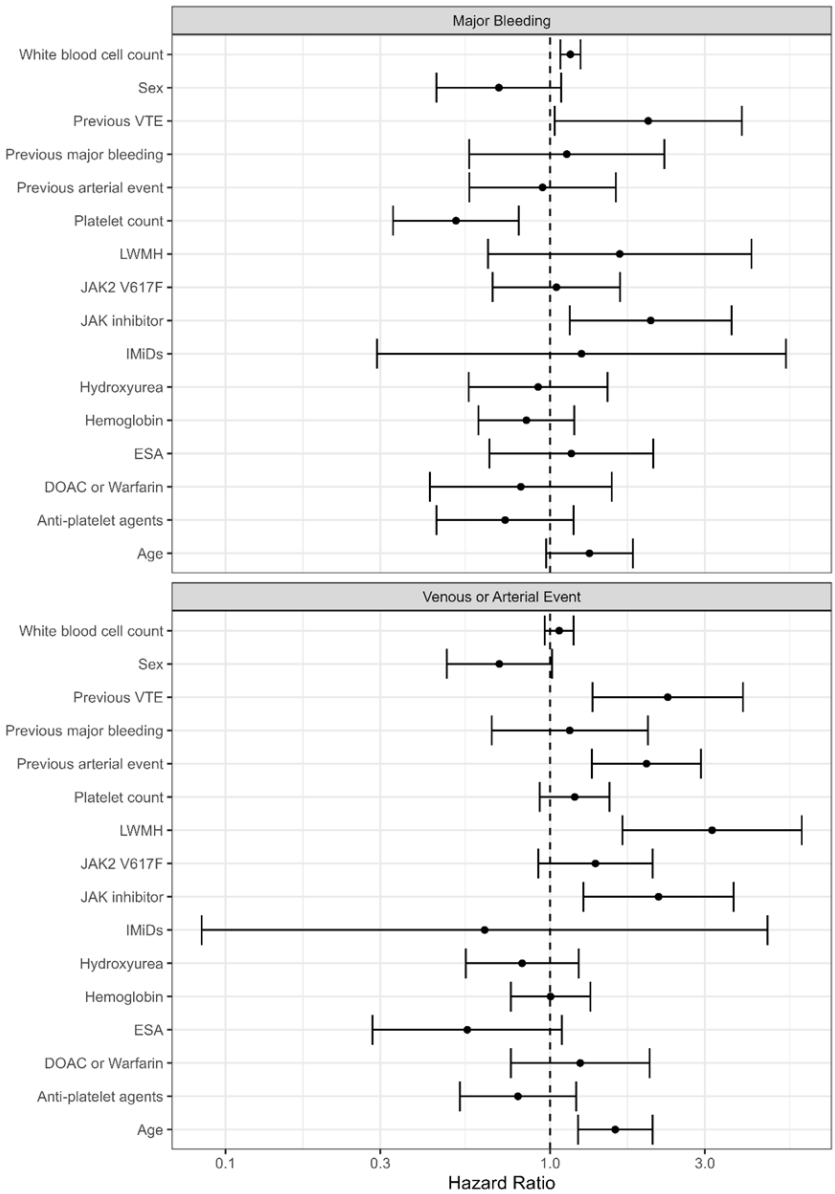

Figure 6 *Forest plot presenting risk factors for major bleeding and arterial or venous events in patients with myelofibrosis. The model was adjusted for age, sex, presence of the JAK2 V617F mutation, hemoglobin levels, white blood cell count, platelet count, history of thrombosis or bleeding, symptom-directed therapy, and use of antithrombotic treatment* (130). *AE arterial event, ESA erythropoiesis-stimulating agents, DOAC direct oral anticoagulants, IMiDs immunomodulatory drugs, LMWH low-molecular weight heparin, VTE venous thromboembolism*

Paper IV

The study cohort consisted of 2604 patients with PV and 3141 patients with ET. At diagnosis, 18.6% of PV patients were classified as low-risk, while 81.4% were considered high-risk. According to the r-IPSET thrombosis scoring system, the ET patients were classified as follows: very low (10.6%), low (14.5%), intermediate (16.5%), and high risk (58.5%).

HU was administered to 88.7% of high-risk PV patients, while IFN and JAK inhibitors were given to 5.6% and 3.6% of the high-risk PV patients, respectively. Among low-risk PV patients, 53.0% received HU and 20.3% IFN during follow-up. In ET patients, HU was used in most intermediate- and high-risk individuals (93.2% and 92.9%), compared with 38.9% of very low-risk and 15.3% of low-risk patients. The majority of PV patients, both low- and high-risk underwent phlebotomy to maintain stipulated hematocrit (90.9% and 84.1%, respectively).

During follow-up, the PV patients experienced 287 arterial and 143 venous events. The events primarily occurred in high-risk individuals, with the highest rates observed in patients treated with busulfan. High-risk PV patients receiving IFN had the lowest arterial event rates, while high-risk PV patients treated with HU had the lowest venous event rates (table 12).

Among ET patients, 228 arterial and 118 venous events occurred, mostly in higher-risk groups. Stratification by mutational status showed the highest arterial event rates in *MPL*-mutated patients, the lowest in *CALR*-mutated patients, and the highest venous event rates in *JAK2*-mutated patients.

Overall, 180 and 149 major bleeding events were reported in the PV and ET patients, respectively, with the highest rates in high-risk patients treated with P32. No major bleeding occurred in PV patients receiving JAK inhibitor therapy in our cohort (table 13 and 14).

The overall rate of ACM in PV and ET patients were 4.78 and 3.72 events per 100 patient-years. The highest rate of ACM was seen in high-risk PV and ET patients treated with busulfan and P32, in contrast to IFN treated patients who experienced the lowest rates of ACM in both PV and ET (table 13 and 14).

Table 13 *Rates of first arterial event, venous thromboembolism, major bleeding and all-cause mortality by treatment and risk stratification group in 2604 polycythemia vera patients in the Swedish MPN Register. The table gives rate (number of patients). Rate is events/patient time, where time is measured in 100-years units.*

	Event	Rate all patients n=2604	Rate low-risk PV n=485	Rate high-risk PV n=2119
All patients	AE	1.94 (2604)	0.64 (485)	2.32 (2119)
	VTE	0.94 (2604)	0.33 (485)	1.11 (2119)
	MB	1.18 (2604)	0.51 (485)	1.37 (2119)
	ACM	4.78 (2604)	0.56 (485)	5.94 (2119)
No cytoreductive treatment	AE	2.36 (1857)	0.69 (430)	3.34 (1427)
	VTE	1.11 (1857)	0.29 (430)	1.59 (1427)
	MB	1.53 (1857)	0.58 (430)	2.08 (1427)
	ACM	6.35 (1857)	0.74 (430)	9.54 (1427)
Phlebotomy	AE	1.90 (2224)	0.61 (441)	2.31 (1783)
	VTE	0.88 (2224)	0.28 (441)	1.07 (1783)
	MB	1.16 (2224)	0.46 (441)	1.37 (1783)
	ACM	4.21 (2224)	0.52 (441)	5.31 (1783)
Hydroxyurea	AE	1.89 (2136)	0.79 (257)	2.04 (1879)
	VTE	0.86 (2136)	0.52 (257)	0.91 (1879)
	MB	0.97 (2136)	0.26 (257)	1.07 (1879)
	ACM	4.01 (2136)	0.51 (257)	4.50 (1879)
Interferon	AE	0.96 (220)	0.45 (101)	1.54 (119)
	VTE	0.72 (220)	0.22 (101)	1.28 (119)
	MB	0.95 (220)	0.68 (101)	1.25 (119)
	ACM	1.29 (220)	0.00 (101)	2.73 (119)
JAK inhibitors	AE	2.17 (115)	0.00 (38)	2.90 (77)
	VTE	1.68 (115)	1.74 (38)	1.66 (77)
	MB	0.00 (115)	0.00 (38)	0.00 (77)
	ACM	4.54 (115)	1.72 (38)	5.43 (77)
Busulfan	AE	4.96 (118)	35.19 (2)	4.48 (116)
	VTE	2.69 (118)	0.00 (2)	2.74 (116)
	MB	2.14 (118)	0.00 (2)	2.18 (116)
	ACM	13.05 (118)	0.00 (2)	13.28 (116)
P32	AE	2.61 (32)	0.00 (1)	2.88 (31)
	VTE	0.88 (32)	0.00 (1)	0.97 (31)
	MB	3.47 (32)	0.00 (1)	3.83 (31)
	ACM	16.13 (32)	0.00 (1)	17.75 (31)

AE arterial event, *ACM* all-cause mortality, *MB* major bleeding, *PV* polycythemia vera, *P32* radioactive phosphorus, *VTE* venous thromboembolism

Table 14 *Rates of first arterial event, venous thromboembolism, major bleeding and all-cause mortality by treatment and risk stratification group according to revised International Prognostic Score for Essential Thrombocythemia in 3141 essential thrombocythemia patients in the Swedish MPN Register. The table gives rate (number of patients). Rate is events/patient time, where time is measured in 100-years units.*

	Event	Rate all ET patients n=3141	Rate very low-risk ET n=323	Rate low-risk ET n=444	Rate intermediate-risk ET n=502	Rate high-risk ET n=1790
All patients	AE	1.23 (3141)	0.44 (323)	0.16 (444)	0.95 (502)	1.84 (1790)
	VTE	0.63 (3141)	0.13 (323)	0.22 (444)	0.77 (502)	0.80 (1790)
	MB	0.79 (3141)	0.31 (323)	0.32 (444)	0.70 (502)	1.12 (1790)
	ACM	3.72 (3141)	0.56 (323)	0.32 (444)	4.30 (502)	5.20 (1790)
No cyto-reductive treatment	AE	0.90 (2117)	0.31 (277)	0.14 (405)	0.80 (301)	2.56 (1073)
	VTE	0.40 (2117)	0.00 (277)	0.23 (405)	0.40 (301)	0.96 (1073)
	MB	0.83 (2117)	0.31 (277)	0.24 (405)	1.61 (301)	1.91 (1073)
	ACM	3.46 (2117)	0.53 (277)	0.23 (405)	8.13 (301)	8.55 (1073)
Hydroxy-urea	AE	1.41 (2494)	0.49 (133)	0.15 (165)	0.96 (468)	1.71 (1663)
	VTE	0.76 (2494)	0.48 (133)	0.29 (165)	0.87 (468)	0.77 (1663)
	MB	0.78 (2494)	0.49 (133)	0.45 (165)	0.45 (468)	0.97 (1663)
	ACM	3.82 (2494)	0.64 (133)	0.72 (165)	3.28 (468)	4.43 (1663)
Interferon	AE	0.91 (237)	1.10 (67)	0.00 (50)	0.00 (12)	1.49 (104)
	VTE	0.20 (237)	0.00 (67)	0.00 (50)	0.00 (12)	0.49 (104)
	MB	0.51 (237)	0.00 (67)	0.80 (50)	0.00 (12)	0.75 (104)
	ACM	0.50 (237)	0.36 (67)	0.00 (50)	2.48 (12)	0.72 (104)
Anagrelide	AE	2.09 (246)	0.91 (32)	1.70 (21)	1.12 (43)	2.64 (140)
	VTE	0.34 (246)	0.00 (32)	0.00 (21)	0.00 (43)	0.64 (140)
	MB	1.38 (246)	0.91 (32)	1.69 (21)	2.35 (43)	1.27 (140)
	ACM	4.23 (246)	0.90 (32)	0.00 (21)	3.24 (43)	6.31 (140)
Busulfan	AE	2.30 (118)	0.00 (1)	0.00 (1)	0.00 (27)	3.44 (83)
	VTE	1.14 (118)	0.00 (1)	0.00 (1)	2.33 (27)	0.84 (83)
	MB	1.14 (118)	0.00 (1)	0.00 (1)	2.29 (27)	0.84 (83)
	ACM	12.42 (118)	0.00 (1)	0.00 (1)	11.27 (27)	12.57 (83)
P32	AE	2.02 (50)	– (0)	– (0)	4.05 (6)	1.98 (40)
	VTE	2.67 (50)	– (0)	– (0)	0.00 (6)	2.72 (40)
	MB	4.23 (50)	– (0)	– (0)	4.22 (6)	4.88 (40)
	ACM	14.47 (50)	– (0)	– (0)	8.01 (6)	16.21 (40)

AE arterial event, *ACM* all-cause mortality, *ET* essential thrombocythemia, *MB* major bleeding, *P32* radioactive phosphorus, *VTE* venous thromboembolism

We then assessed risk factors for arterial and venous events during treatment periods by performing multivariable analysis. PV patients with a medical history including a venous or arterial event had an increased risk of recurrent event, as did those patients presenting with leukocytosis at diagnosis and those of advanced age. Phlebotomy was also associated with an increased risk of venous or arterial event in PV patients. Among ET patients, elevated WBC at diagnosis, higher age, prior arterial or venous events and use of LMWH were identified as independent risk factors for thromboembolic events. In contrast, HU therapy and treatment with antiplatelet agents had a protective effect regarding arterial or venous events in both PV and ET (figure 7 and 8).

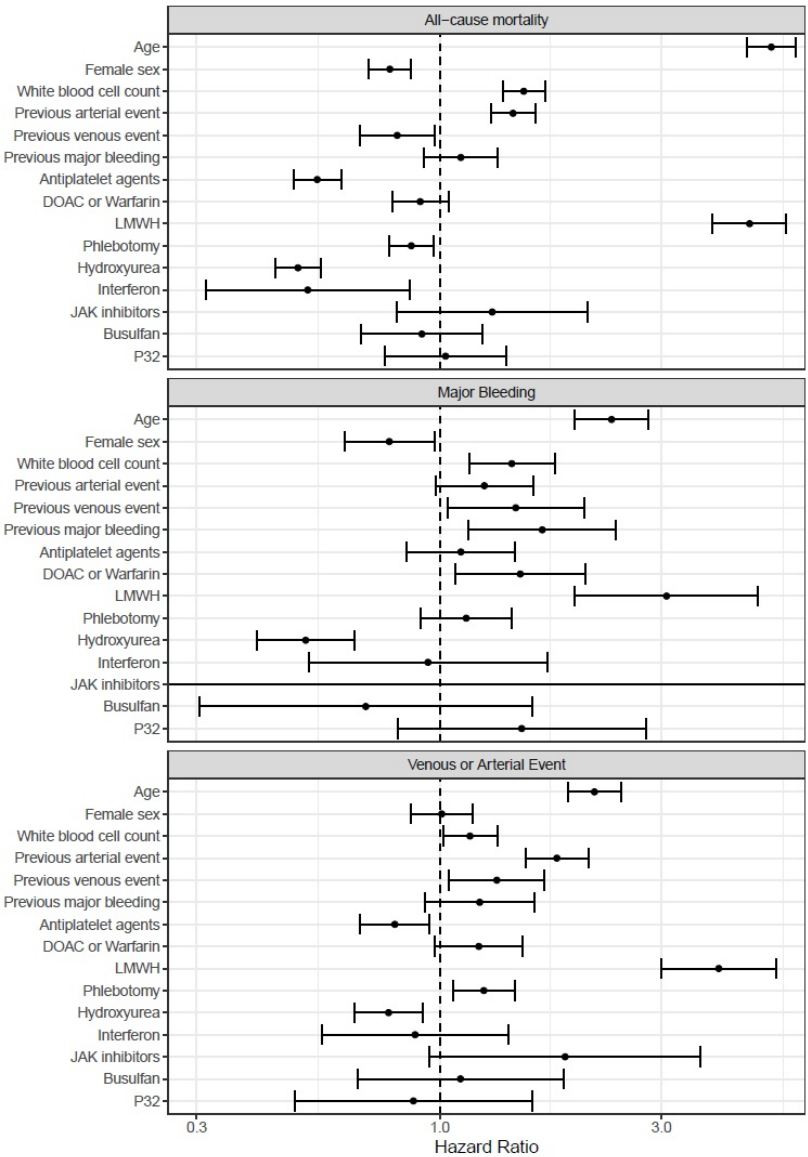

Figure 7 *Forest-plot illustrating risk factors of arterial or venous events, major bleeding, and all-cause mortality in 2604 patients with polycythemia vera. The model is adjusted for age, sex, white blood cell counts at diagnosis, previous thrombotic event or bleeding, phlebotomy, cytoreductive treatment and antithrombotic therapy. DOAC direct oral anticoagulants, LMWH low-molecular weight heparin, P32 radioactive phosphorus*

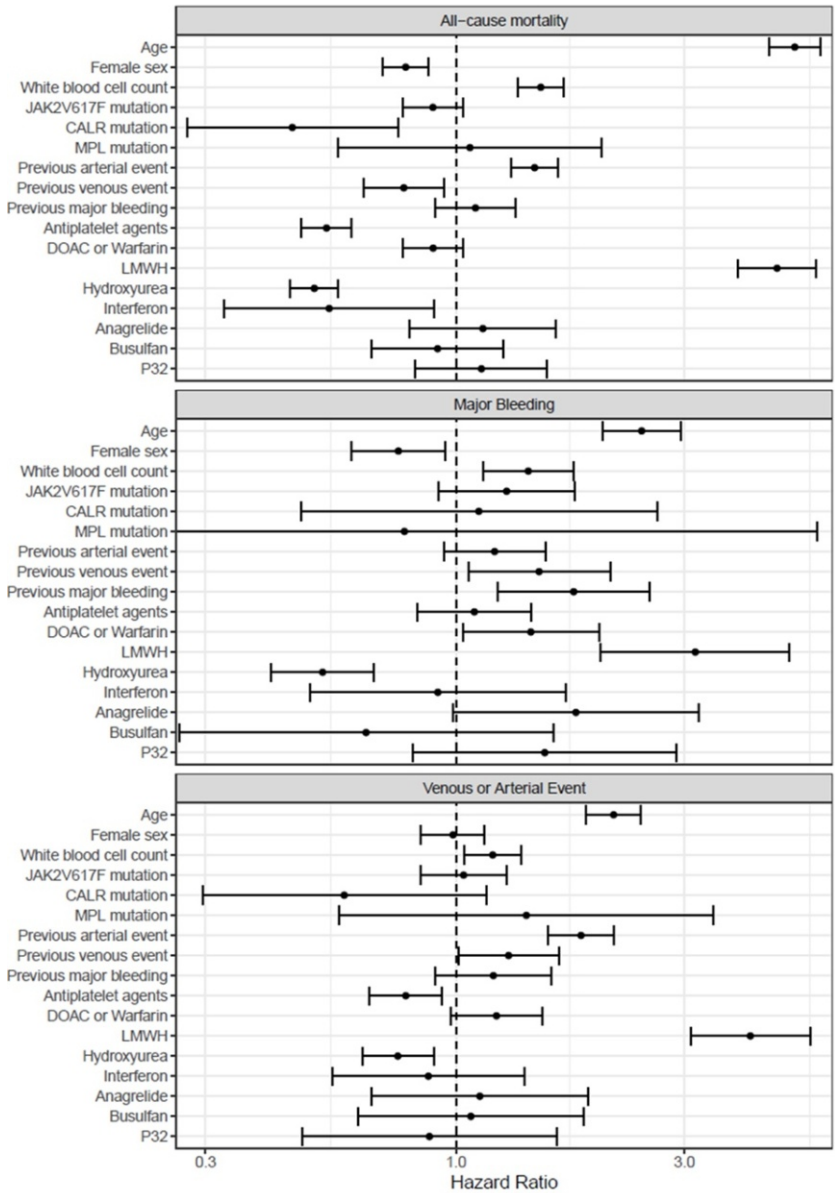

Figure 8 *Forest plot illustrating risk factors for arterial and venous events, major bleeding, and all-cause mortality among 3141 patients with essential thrombocythemia. The model was adjusted for age, sex, mutational status (JAK2 V617F, CALR, or MPL), white blood cell count at diagnosis, history of thrombosis or bleeding, cytoreductive therapy, and antithrombotic treatment. DOAC direct oral anticoagulants, LMWH low-molecular weight heparin, P32 radioactive phosphorus*

In both PV and ET several risk factors for major bleeding were identified in multivariable analysis: high WBC levels at diagnosis, a previous major bleeding, a previous venous event, advanced age, and treatment with anticoagulants (DOAC, Warfarin or LMWH). HU and female sex were associated with a reduced risk of hemorrhage in PV and ET patients (figure 7 and 8).

High age, elevated leukocytes at diagnosis, previous arterial event, and use of LMWH were, in multivariable analysis, all associated with an increased risk of ACM in both PV and ET patients. Conversely, female sex, prior venous events, and treatment with antiplatelet agents, HU, or IFN were related to reduced ACM risk in PV and ET, with the addition of phlebotomy also being associated with a lower risk of ACM among PV patients (figure 7 and 8).

Discussion

Paper I

How common is erythrocytosis?

In our first study we wanted to investigate how frequent elevated blood values are in a real-world patient cohort. Among all adult patients that were diagnosed with a venous or arterial event in the county of Norrbotten during 2017-2018 13.2% of the patients had elevated Hb/Hct at one time point or more. The frequency of erythrocytosis in the general population has been assessed in the large Lifelines cohort in the Netherlands. In total 3.4% (4995/147076) of the participants had a Hb/Hct that would fulfill the WHO criteria for PV (Hb >16.5 g/dL in men and >16.0 g/dL in women or Hct >49% in men and>48% in women) (131). In a smaller cohort of 609 patients diagnosed with ischemic or hemorrhagic stroke 116 (19.0%) had erythrocytosis (132). The higher frequency of erythrocytosis in our cohort and in the stroke cohort mentioned above cannot be fully explained, but it is possible that these groups included older patients with cardiopulmonary disease, resulting in secondary erythrocytosis and consequently higher Hb levels.

What about thrombocytosis?

A large number of the patients, 17.6%, in our selected cohort had thrombocytosis (platelets >450 × 10^9/L) at one or more time points. How common is thrombocytosis in the general population? The NHANES study reports prevalence of unexplained thrombocytosis (140.2 per 100 000 individuals), and this study is based on a survey made in the general population in the United States (133). The other studies are based on different patient cohorts, and the reported frequency of thrombocytosis in an elderly out-patient population were 1.9% (134), while a Turkish study reported 1.6% of all patients at inpatient and outpatient clinics having thrombocytosis (135). In patients admitted to internal medicine units, patients having acute VTE or patients admitted to intensive care units (ICU) the frequency of thrombocytosis were 7.8%, 20.2% and 2.1%, respectively (136-138). The frequency of thrombocytosis in the cohort with acute VTE (136) correlates with the frequency of thrombocytosis in our cohort. The lower frequency of thrombocytosis among patients in ICU can probably be explained by the fact that critically ill patients to a higher degree presents with thrombocytopenia rather than thrombocytosis.

Unexplained erythrocytosis

In a large proportion of the patients with erythrocytosis (71.6%) in our cohort we did not find an underlying condition explaining the elevated blood values, and these patients were therefore categorized as having unexplained erythrocytosis. In a study from Switzerland on *JAK2* unmutated erythrocytosis, 30.0% of the patients with erythrocytosis had no underlying condition explaining the elevated blood values. In this retrospective study, the lower proportion of unexplained erythrocytosis may be attributed to the availability of investigations such as p50 measurement, nuclear assessment of red blood cell mass, and detailed information on smoking habits and underlying cancers known to be associated with erythrocytosis (including renal, adrenal, genitourinary, respiratory, cerebral, or hepatocellular malignancies)(66), data not available in our study.

Access to NGS and other genetic analysis may help determine underlying causes in individuals presenting with erythrocytosis. A recent study from Spain evaluated 117 patients with idiopathic erythrocytosis using NGS and found that 5.1% had PV, 6.8% familial erythrocytosis, 36% secondary erythrocytosis, and 15% self-limited erythrocytosis, whereas 28.2% remained idiopathic (139).

When designing this study, our working hypothesis was that patients with unexplained erythrocytosis would have a higher rate of recurrent events, because this subgroup might include patients with undiagnosed PV. However, this hypothesis was proven incorrect. Patients classified as having unexplained erythrocytosis actually exhibited the lowest rates of recurrent thrombotic events, the most favorable event-free survival, and the longest restricted mean survival time. Thus, within this cohort, unexplained erythrocytosis appears to represent a more benign condition compared with the other subgroups.

Unexplained thrombocytosis

The majority of patients (90.9%) with thrombocytosis in our study were classified as reactive thrombocytosis and only 6.8% were categorized as unexplained thrombocytosis. In a large Turkish cohort comprising 2000 patients with thrombocytosis, 4.2% were found to have no identifiable cause for their elevated platelet counts (135), aligning with the findings of our study. Likewise, Griesshammer et al. reported thrombocytosis of uncertain etiology in 3.1% of 732 patients (140).

The use of NGS can clarify the etiology of elevated platelet counts. In the aforementioned Spanish study, 58 patients with thrombocytosis were evaluated using NGS; 13 were diagnosed with ET, two with familial thrombocytosis, seven experienced spontaneous resolution, nine were classified as having secondary thrombocytosis, and six remained without a clear explanation and were categorized as idiopathic (139).

Recurrent thrombotic events and event-free survival

Since the index event in study I was thrombotic in nature, the patients in our cohort had already demonstrated a predisposition to thrombosis. Among the various subgroups, the highest recurrence rate was observed in patients with unexplained thrombocytosis, where 9 out of 47 individuals (20%) experienced another thrombotic episode, including three AMI and three TIA. Conversely, patients with unexplained erythrocytosis exhibited the lowest recurrence rate, with only 7.7% developing a new arterial or venous thrombotic event.

In previously published studies patients with essential thrombocytosis have shown to have increased risk of thrombotic complications compared to reactive thrombocytosis (135, 140), in current study the patients categorized as having reactive thrombocytosis had a slightly higher rate of recurrent event compared to patients with a diagnosed MPN. The lower rates of recurrent event among the MPN patients may be attributed to a more stringent follow-up, cytoreductive treatment and use of antithrombotic agents. Why the patients with unexplained thrombocytosis in our cohort experienced a higher frequency of recurrent events is unclear. Time-limited anticoagulant therapy, presence of an undiagnosed malignancy, an unrecognized MPN, or another inflammatory condition associated with increased risk of thrombosis could provide possible explanations.

Thrombotic events at or prior to a diagnosis of SE have been shown to be almost equally common as in patients diagnosed with PV (141), and during follow-up the rates of thrombotic events is comparable to the rates in low-risk PV (142). An abstract from 2022 unexpectedly revealed that patients with secondary erythrocytosis experienced higher rates of perioperative thrombosis compared to PV patients, possibly due to underappreciated risk of thrombosis and lack of management strategies for patients with SE undergoing surgery (143). Among patients classified as having SE in our cohort, 10.4% experienced a recurrent thrombotic event, a frequency comparable to that observed in patients diagnosed with MPN (10.5%).

Patients with SE and unexplained thrombocytosis showed the highest rates of recurrent events in this study. Both groups also had the most inferior event-free survival, indicating an increased thrombotic risk and the need for comprehensive evaluation to identify underlying causes and prevent future thromboembolic events.

Inferior survival in patients with secondary erythrocytosis and unexplained thrombocytosis

Patients with SE and unexplained thrombocytosis also exhibited the most inferior restricted mean survival time. Patients with SE often have advanced pulmonary or cardiac disease, which can explain the inferior survival time. The underlying causes of the reduced survival observed in patients with unexplained thrombocytosis are not known. These patients may, however, have undiagnosed malignancies or other inflammatory conditions that could negatively affect life expectancy. Further investigation into the underlying conditions within this subgroup could provide valuable insight into the factors driving their reduced survival.

Paper II

Rates of thromboembolic events, major bleeding and all-cause mortality in PV and ET

The important studies by Hultcrantz et al have shown the increased risk of thrombotic events, reduced life expectancy and increased risk of overall mortality in patients with MPN (88, 112, 113). Our study confirms these earlier results, demonstrating an elevated risk of thrombotic events and all-cause mortality, despite the use of newer therapies. The PV patients included in our study experienced significantly higher rates of all arterial and venous events, with the exception of cerebral venous thrombosis, compared to corresponding controls, and among ET patients the rates of VTE and major bleeding were increased compared to controls. Excess ACM was observed in both PV and ET patients when compared with controls.

Comorbidities matter…

Previous studies have shown that presence of cardiovascular risk factors impact frequency of thrombotic events in patients with MPN (97-99, 144), and in line with these studies, we found that hypertension and ischemic heart disease were, in multivariable analysis, associated with an increased risk of arterial event in both PV and ET patients. Atrial fibrillation and previous ischemic stroke were identified as independent risk factors for arterial events among PV patients and a previous venous thrombosis was identified as a risk factor for recurrent venous events among PV and ET patients, results confirming previously published research (95, 99, 100).

In PV, a previous VTE or major bleeding as well as diabetes mellitus emerged as risk factors for major bleeding. Among the ET patients, a medical history including ischemic stroke or VTE was associated with an increased risk of major bleeding. The increased risk of bleeding associated with these conditions can be explained by use of antithrombotic treatment following the VTE and ischemic stroke. In a review by Nicol et al as many as 39 potential risk factors for bleeding in PV and ET were listed, and a previous bleeding episode was highlighted as one risk factor of interest (145), in line with the results from our cohort. Diabetes mellitus was associated with an increased risk of major bleeding among the PV patients in current study, an association that has not been previously described and needs to be confirmed in future studies.

… and so does age

When analyzing the impact of age on the various outcomes in the PV and ET patients in our cohort, we found that the risk of arterial or venous events and all-cause stroke was approximately twice as high in patients aged 70 compared with those aged 60. Furthermore, the risk of ACM was more than doubled in both ET and PV patients aged 70 compared with those aged 60. According to current guidelines, age above 60 is one of the criteria distinguishing low- from high-risk disease regarding thrombotic complications (51, 52), and our findings reinforce the validity of this threshold.

Paper III

In a review by Verstovsek et al the authors state that very few studies have investigated the frequency of thrombotic events and major bleeding in patients diagnosed with MF (146), and with our third study we try to fill this gap of knowledge.

In the aforementioned review, eight studies including between 72 and 707 patients reported the frequency of thrombotic events in MF (both overt MF and pre-PMF), and three studies with cohorts of 25 to 180 patients reported on bleeding events. Compared with these studies, our cohort is considerably larger, allowing for a more robust assessment of thrombotic and bleeding risks.

How common is thromboembolic events and major bleeding in MF?

Among the 1079 patients with MF included in our cohort, the rates of both thromboembolic events and major bleeding were significantly higher than in corresponding controls, and also exceeded the rates of the same events previously described in patients with PV and ET (129). The event rates in our cohort were marginally higher compared with those reported from the Spanish Registry of Myelofibrosis (102). The discrepancy may in part reflect differences in data sources, as our events were retrieved from multiple large healthcare registries rather than a single registry.

Diverging rates of thrombotic events in different treatment groups

While this study was not intended to provide head-to-head comparisons between treatments, the marked variation in arterial event rates, from 0.37 to 4.67 events per 100 patient-years across treatment groups, was noteworthy, and the highest rate of arterial events occurred in MF patients treated with JAKis. One possible explanation for the higher event rates in this treatment group is that in Sweden JAKis are only reimbursed for patients classified as high-risk or intermediate-2 according to the IPSS scoring system. This difference in thrombotic events is of interest and should be investigated further, since other JAKis used in rheumatoid arthritis and other inflammatory conditions have been associated with an increased risk of major adverse cardiovascular events (147).

Risk factors for arterial or venous events in MF

A previous VTE or arterial event as well as age were recognized as independent risk factors for arterial or venous events among the MF patients in our cohort, results coherent with previously published studies (148, 149). Use of JAKis and LMWH was also associated with an increased risk of thrombotic events. JAKis as a risk factor of thrombotic events has, to our knowledge, previously not been described and therefor warrants further investigation. The use of LMWH is probably concentrated among patients with a high thrombotic risk in whom other anticoagulants are contraindicated, such as in cases of thrombocytopenia or when LMWH are used as a bridging treatment in the peri- and postoperative setting. This potential selection bias may account for the higher risk of thrombotic events observed with LMWH therapy.

Risk factors for major bleeding in MF

In our cohort of MF patients, leukocytosis at diagnosis, a medical history including venous thrombosis and ongoing JAKi therapy were identified as independent risk factors for major bleeding. A previous thrombosis and elevated WBC have previously been identified as risk factors for bleeding in patients diagnosed with MF (89, 150) whereas ongoing JAKi therapy has, to our knowledge, not been described as a risk factor for major bleeding. Since JAKis are primarily used in high- and intermediate-2 risk MF patients in Sweden, it is reasonable to speculate that these individuals have more advanced disease, possibly including thrombocytopenia, which could in turn increase the risk of major bleeding.

Paper IV

Treatment patterns in PV and ET

In the fourth study we described treatment patterns in 5745 PV and ET patients during a total follow-up time of 43612 patient-years. We found that high-risk patients with both PV and ET were, to a large extent, managed in accordance with existing guidelines. Surprisingly, a relatively large number of patients categorized as low-risk at diagnosis received cytoreductive treatment during follow-up. Approximately half of the low-risk PV patients were treated with HU during the course of the study, a larger proportion than previously described (151-153), and among ET patients 38.9% of the very low- and low-risk patients received HU therapy during follow-up, results comparable to a previous study from Canada (154). Current guidelines only recommend cytoreductive treatment to high-risk PV and ET patients, and in certain circumstances in low-risk patients, such as frequent need of phlebotomy, pronounced symptoms, uncontrolled leukocytosis or thrombocytosis or progressive splenomegaly (155, 156). The exact indications for cytoreductive therapy among the low-risk patients in our cohort were not available due to registry-based data retrieval; however, a proportion of patients had likely exceeded the age threshold of 60 years, a criterion for high-risk disease, and therefore met the requirement for cytoreductive therapy.

Higher rates of thrombotic events in *MPL* mutated ET patients

When analyzing the rates of thrombotic complications in ET patients according to mutational status, we found higher rates of arterial events among *MPL* mutated patients while patients with *JAK2* mutations had the highest rates of venous events. The higher frequency of arterial events among *MPL* mutated patients in our cohort differs somewhat from studies conducted at the Mayo Clinic and in Florence, where arterial event rates were similar between *MPL* and *JAK2* mutated patients (99, 100). In multivariable analysis adjusted for age, sex, prior events, and ongoing treatment, the *MPL* mutation was not associated with an increased risk of thrombosis, suggesting that other factors may explain the higher incidence of arterial events in *MPL* mutated patients. The *MPL* mutated patients in our cohort had a higher mean age than the patients with other or no mutations, and this could possibly explain the higher rates of arterial events.

Risk factors for thrombotic events in PV and ET

In both PV and ET, the previously recognized risk factors age and a medical history of previous arterial or venous event (157, 158) also emerged as risk factors in our cohort. In addition, in PV phlebotomy was associated with increased risk of thrombosis. We interpret the need for phlebotomy as a marker of inadequate hematocrit control, which is a known risk factor for thrombosis (96). In both PV and ET, use of LMWH was identified as a risk factor for thromboembolic event. LMWH is frequently used as a prophylactic intervention in situations with an elevated risk of thrombotic complications, such as during immobilization or in the perioperative and postoperative periods, which may partly explain the observed association with an increased risk of thrombosis, although the exact indication for LMWH in our cohort is not known.

Protective effect of HU and antiplatelet agents

In both PV and ET patients, multivariable analysis showed that HU therapy as well as treatment with antiplatelet agents had a protective effect against thrombotic events. For the past twenty years, antiplatelet agents have been a cornerstone in the management of thrombotic complications in PV (94). The protective effect of HU against thrombotic events in both PV and ET in our cohort confirms previously published results (157, 159). The exact mechanism by which HU reduces the risk of thrombosis is not fully elucidated. Beyond lowering hematocrit, HU also decreases WBC, thereby potentially reducing pro-inflammatory cytokines and the cytokine-driven thrombus formation (160).

Leukocytosis at diagnosis - a risk factor for thrombotic events, major bleeding, and all-cause mortality

A recent prospective study has finally established leukocytosis as a risk factor in both low-risk and high-risk PV (161). In our cohort of patients with PV and ET, leukocytosis at diagnosis emerged as an independent predictor of arterial and venous thrombotic events during follow-up. Furthermore, elevated WBC counts at diagnosis were associated with an increased risk of major bleeding and ACM in both PV and ET, findings that are consistent with previous reports (162, 163).

Strengths and limitations

Paper I

Paper I is a retrospective cohort study including all adult patients diagnosed with a thrombotic event during 2017 and 2018 in the county of Norrbotten. It is foremost a descriptive study on frequency of elevated blood values in a cohort of common patients in an internal medicine unit. The study has several strengths, it is population-based, the data collected is reliable and the cohort size is large. Limitations of this study include lack of information on important risk factors for cardiovascular events such as smoking, diabetes, hypertension and hyperlipidemia. Curent study does not contain information of diagnostic tests (such as EPO levels, mutational status and results of spirometry) which could identify underlying condition causing the elevated blood values. When collecting the data from the medical charts it was not always possible to grade severity of underlying cardiopulmonary disease. In a subset of patients, the limited number of blood samples collected made it difficult to verify persistently elevated values. Another limitation of this study is that all included patients had a proven thrombotic predisposition, as the inclusion criterion was a previous thrombotic event. This may introduce selection bias. To better understand the effect of elevated blood values on thrombotic risk, it would be informative to include a control group without a prior thrombotic event in future studies.

Paper II-IV

Paper II-IV are all registry-based cohort studies with data retrieved from the MPN register as well as multiple Swedish health care registers. Given the very high coverage of the MPN register, the study data can be regarded as representing all MPN patients in Sweden. The data from the MPN register was cross-linked to the data from several national health registries allowing us to efficiently study the effect of the exposure (= the MPN diagnosis) on the different outcomes as well as treatment patterns in a large cohort of participants.

Paper II and III included five controls to each case, and these were matched by age at MPN diagnosis, geographic region, and gender. The controls were randomly selected by Statistics Sweden. To minimize potential bias from temporal changes in diagnostics and treatments, cases and controls were followed during the same calendar period. The use of matched controls ensure comparability between the two groups and reduce possible confounding factors such as age and gender. By using multiple controls, we sought to improve the estimation of what

was typical in the population, reduce the impact of outliers, maximize the information obtained from each patient in comparison with its matched controls and improve the reliability of the results.

One of the major strengths of these studies are the population-based study design, with the inclusion of a large number of MPN patients, which is rare in this field. The high level of coverage and validity of the included health care registers ensures high quality of the data retrieved. Since the entire population of MPN patients is included in these studies, the risk of selection bias is minimized. The long follow-up period is another strength of these studies. Since many patients with MPN have an expected life expectancy of many years, it is important to assess outcomes in the context of long-term treatment.

Like all registry-based studies, our studies have several limitations. Due to the registry-based data retrieval it is not possible to verify data on an individual level. While most of the registers included have automated data transfer, the MPN register and the stroke register rely on manual data entry, and therefore the possibility of reporting errors (information bias) cannot be completely excluded, although the MPN register were validated in 2022 with good consistency between the reported data and the source data (164). Our studies do not include data on transformation into MF and AML, which of course impact the analysis of survival and ACM. The absence of data on smoking and hyperlipidemia, both important cardiovascular risk factors, represents another limitation of these studies. The NPR provides data from inpatient care as well as specialized outpatient care but does not capture information from primary care. Since many patients, such as those with type II diabetes mellitus or hypertension, are primarily managed in the primary care setting, this represents a limitation of our studies.

In paper III and IV we use data on treatments retrieved from the PDR as well as the MPN register. Data on IFN, transfusions, phlebotomy and P32 were collected from the MPN register and all other therapies from the PDR. We calculated treatment periods based on strict standard dosing for each drug to avoid overestimating treatment and treatment-related complications, which could have resulted in a modest underestimation of the actual treatment time. When retrieving the information on the different therapies from the PDR one letter in the code for IFN was missing, which resulted in incomplete information on IFN therapy. By the time this error was discovered, the code key was no longer available; therefore, this information had to be obtained from the

MPN register. Although the PDR provides information on prescribed and dispensed drugs, it cannot confirm actual patient use or adherence.

Registry-based studies

Randomized controlled trials (RCTs) are regarded as the gold standard of study designs. Conducting an RCT in rare disorders such as MPN is extremely challenging, as recruiting a sufficient number of patients to achieve adequate statistical power is both difficult and time-consuming.

Registry-based studies are increasingly common and the Nordic countries have a leading role in registry-based research, owing to the availability of personal identification numbers and the possibility to link to the multiple registers that has been established. This approach offers several advantages: the registers provide clinical information from individuals across wide geographic areas with long-term follow-up, and the large sample sizes allow for the analysis of diseases and outcomes over time with high statistical power. The information from registries represent real-world data from routine clinical practice rather than the controlled conditions in an RCT and thereby strengthen the external validity (which means that the results can be generalized to a larger population). Furthermore, registry-based studies are less costly and easier to conduct than randomized trials, as the data are already collected. These registers also facilitate research on rare diseases, which includes many hematological disorders, by providing adequate sample sizes.

In contrast to the benefits of registry-based studies, there are a lot of limitations as well. Conducting reliable registry-based research requires high-quality data and detailed knowledge of the content in the registries when planning the study. Missing data, which represents a form of information bias, can be a problem in registry-based studies. Therefore, it is important to perform data validation and quality checks before analysis. If data are missing, it is also crucial to assess whether there is a pattern in the missing variables that could influence the study results (165). When variables necessary to answer the research question are missing, linking the registry data with other registers or data sources can be a useful approach. The accuracy of diagnoses recorded in different registries may vary, as certain ICD codes carry greater significance in discharge documentation. For instance, recording a diagnosis of AMI may be prioritized over the diagnosis of obesity in the discharge summary, and this discrepancy should be recognized when conducting registry-based studies.

Confounding occurs when the relationship between an exposure and an outcome is influenced by another third variable, and this can be an issue in registry-based research since the data already has been collected without control from the researcher (165). Confounding (both known and unknown) is automatically balanced by randomization, which is not possible in registry-based studies. In cohort studies (and registry-based studies), confounding can be partially handled through matching, but this method only accounts for known confounders. Multivariable analysis and stratification are other methods used to control confounding. Confounding by indication or indication bias occurs when the reason a patient receives a treatment is itself related to the outcome. An example of confounding by indication could be the MF patients prescribed JAKis in paper III who were observed to have higher rates of arterial events and major bleeding. These patients are probably high-risk or intermediate-2 risk patients, and by that also having an inferior survival, as well as an increased risk of thrombotic events (102). To evaluate the effect of JAKis and other therapies regarding thrombotic outcomes, studies of on MF patients stratified according to risk classification and ongoing therapy should be performed.

Internal validity is how well we are measuring the strength of association between exposure and outcome in the study population (165). Since registry-based studies are observational, they cannot fully eliminate confounding or selection bias and therefore have a limited internal validity compared to RCTs. By including the whole population, the selection bias can be minimized and by using matched controls and multivariable analysis we can, to some extent, control confounding. It is also important to interpret the results of registry-based studies as associative rather than casual.

In cohort studies, a key question for researchers is often: “Did we obtain a representative sample?” Various statistical methods, such as confidence intervals and hypothesis testing with p-values, are commonly used to assess sampling error. However, in registry-based studies that include the entire population, these methods may be less relevant (166).

When using registry-based data there are rigorous precautions to preserve the confidentiality of patient information. The information in the NPR, PDR, and CDR are protected by absolute confidentiality, representing the highest level of legal protection for sensitive information. Exceptions to this principle of confidentiality to use the data in research may be granted following ethical approval, provided it is clear that no data can be attributed to an individual and that no person will be harmed by the release of the data. Before the National Board of

Health and Welfare discloses any information, a confidentiality assessment is carried out in accordance with the Public Access to Information and Secrecy Act (167).

Conclusions

I. One third of the patients who experienced an arterial or venous event showed elevated Hb, Hct, or platelet levels on one or more occasions. Among these, one third had no apparent underlying condition explaining the abnormalities and should therefore be further evaluated to exclude an MPN.

II. The rate of VTE, major bleeding, and ACM was significantly higher among both ET and PV patients compared with corresponding controls. Additionally, the PV patients exhibited a significantly increased rate of arterial events and all-cause stroke compared to the controls. Across all age groups, PV patients had higher rates of arterial and venous events, while ACM was significantly increased in both PV and ET patients older than 50 years.

III. Among MF patients the rates of major bleeding, arterial and venous events as well as ACM were significantly increased compared to matched controls. Treatment with JAK inhibitors was associated with an increased risk of major bleeding, arterial and venous events, likely reflecting the more advanced disease in the patients receiving this therapy.

IV. A considerable proportion of PV and ET patients classified as low-risk at diagnosis received cytoreductive treatment during follow-up, which may partly be explained by patients reaching 60 years and thereby meeting the high-risk criterion. Treatment with HU or IFN was associated with a reduced risk of ACM in both PV and ET. Moreover, HU was associated with a reduced risk of major bleeding and thrombotic events in both patient groups.

Future considerations

While completing these four studies, several new questions have emerged, which could potentially serve as topics for future research.

In study I, the underlying causes of thrombocytosis in patients classified as having unexplained thrombocytosis remain unclear, as does the optimal management strategy to prevent recurrent events, including whether more intensive antithrombotic therapy is warranted. Further investigation of the causes of death in this patient group could also help identify treatable conditions and reduce mortality.

Among patients with unexplained erythtrocytosis we found the lowest rates of recurrent thrombotic events as well as the most favourable restricted mean survival time. It would be intriguing to do further analyses to find the underlying causes of the elevated Hb/Hct among these patients. And are these underlying conditions more benign when it comes to recurrent thrombotic events?

In MPN, elevated WBC is a risk factor for both thrombotic events and reduced survival. A similar association between leukocytosis and poorer prognosis has been observed in other conditions, including acute myocardial infarction (168), cancer (169) and covid-19 (170). Since study I includes WBC data, it would be of interest to analyze whether leukocyte count also affects the risk of recurrent events and survival across the different patient subgroups in our cohort.

In paper II, we found that diabetes mellitus was associated with major bleeding in PV patients. It would be valuable to explore this finding further and assess whether adjustments in antithrombotic therapy could lower the risk of major bleeding in these patients.

In the first research plan that we made in the beginning of this doctoral training we had an idea of investigating underlying causes of all elevated blood values that had been collected in adult patients in the county of Norrbotten during 2017 and 2018. We received ethical approval and thereafter a list of patients from the laboratory at Sunderby hospital. Using the diagnostic cut-offs for Hb levels defined in the 2016 revision of the WHO classification, we identified 13444 men and 3054 women who met these criteria, an overwhelming number of patients. It soon became clear that performing detailed investigations to determine the exact

underlying causes would not be possible, both because of time limitations and the high cost of genetic analyses. Still, if time and funding were no obstacles, this would have been a dream project to carry out.

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