POPULATION-BASED LONG-TERM FOLLOW-UP OF PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS: Complications and Prognosis

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Dr. William Dameshek
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Perhaps it is possible to resolve all of these dilemmas, conflicts, antagonisms and confusions by considering, not that the various conditions listed are different but that they are closely inter-related. It is possible that these various conditions –"myeloproliferative disorders" — are all somewhat variable manifestations of proliferative activity of the bone marrow cells, perhaps due to a hitherto undiscovered stimulus. This may affect the marrow cells differently or irregularly with the result that various syndromes, either clear-cut or transitional, result.

—William Dameshek "Some Speculations on the Myeloproliferative Syndromes," *Blood*, 1951

Review: *Physiopathology, Etiologic Factors, Diagnosis, and Course of Polycythemia Vera as Related to Therapy According to William Dameshek, 1940-1950,* by Jan Jacques Michiels.

To *Fethi, Nina, Sara and Frida*

ABSTRACT

Philadelphia chromosome negative myeloproliferative neoplasms (Ph-MPNs) are rare clonal hematological malignancies, mainly including polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF). Patients with these diseases run a high risk of vascular complications and may transform to acute myeloid leukemia (AML). Population-based studies relating to these issues are few in number.

The aims were *(i)* to investigate the rate of AML development in subtypes of Ph-MPNs, *(ii)* to determine whether clinical and bone marrow findings at diagnosis have an impact on survival and vascular complications in PV and ET, *(iii)* to find prognostic tools based on clinical findings in newly diagnosed PV and *(iv)* to describe the "real"-life data from newly diagnosed PV and ET. We have investigated these issues in population-based material; study (*i*) and (*iii*) were based on patients from both Gothenburg, Sweden, and the Côte d`Or area, France. Study (*ii*) was based only from Gothenburg and study (*iv*) comprised PV and ET patients in the National MPN Registry.

In the median observation period of 15 years, 7% (56 of 795) of patients with Ph-MPN transformed to AML. The yearly rate of AML transformation was significantly higher in MF (1.09%) compared with that of ET (0.37%) and PV (0.38%); ($p = 0.02$ and $p = 0.002$ respectively). Patients with PV had a significantly shorter survival compared with the general Swedish population (HR 1.66; CI: (1.38-1.99); $p < 0.001$). For ET, however, the corresponding survival differences did not reach statistical significance (HR 1.23; CI: (0.97-1.51); $p = 0.089$). Low hemoglobin at the time of diagnosis predicted poor survival in ET ($p = 0.0281$) and splenomegaly predicted poor survival in PV ($p = 0.037$). Using multivariate analysis, independent risk factors at diagnosis for survival in PV patients were identified as age > 70 years, WBC > 13×10^9/L and thrombotic events. Patients with none of these risk factors had a 10-year relative survival (RS) of 84%, compared with 59% and 26% in patients with one and two or three risk factors respectively.

In the fourth study, we showed that vascular complications preceded an MPN diagnosis in 35% of ET and 37% of PV and multivariate analysis identified low hemoglobin as a risk factor for thromboembolic complications in PV ($p = 0.012$), while in ET age > 65 years, WBC > 12×10^9 /L and the presence of the JAK2 V617F mutation were independent risk factors ($p = 0.0004$, $p = 0.0038$ and $p = 0.0016$ respectively).

Keywords: essential thrombocythemia, polycythemia vera, myelofibrosis.
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Sammanfattning (Swedish)

Philadelphia kromosomnegativa (Ph-) myeloproliferativa neoplasier (Ph-MPN) omfattar huvudsakligen följande sjukdomar: Polycytemia vera (PV), Essentiell trombocytemi (ET) och Myelofibros (MF). MF kan vara primär, utan föregående PV/ET, eller sekundär, dvs. föregåtts av PV/ET, som också kallas post ET/PV MF.

Ph-MPN är sällsynta klonala blodsjukdomar med en sammanlagd incidens på mindre än 6 per 100,000 invånare och år. Sjukdomarna karaktäriseras av proliferation av stamceller i benmärgen. Vid PV ses, så kallad, panmyeloisk proliferation, dvs. omfattande alla tre cellinjer. Vid ET ses proliferation huvudsakligen av megakaryocyter, som är moderceller för blodplättar (trombocyter). Vid MF ses proliferation av avvikande megakaryocyter, dessutom förekomst av bindväv (fibros) och så kallad extramedullär (utanför benmärgen) blodbildning, vilket i sin tur kan leda till mjältförstoring. Diagnosen av dessa sjukdomar är numera baserad på WHO-kriterier som innefattar blodvärden, benmärgsmorfologi och molekylära tester. Dessa sjukdomar har flera gemensamma karakteristika: benägenhet för tromboemboliska händelser och blödningar, samt en ökad risk för transformation till akut myeloisk leukemi (AML).

Under våren 2005 gjordes ett stort genombrott avseende förståelsen av uppkomsten av Ph-MPN. Fyra oberoende forskargrupper upptäckte då mutation i Janus kinas 2 genen, JAK2 V617F. Sedan dess har ytterligare mutationer upptäckts: JAK-2 exon 12, MPL mutationer och Calreticulin (CalR) mutationer. Analys av dessa mutationer ingår numera i utredning av misstänkt Ph-MPN, härmed har diagnostiken förenklats och förbättrats. Vi vet nu att JAK2 V617F mutation förekommer hos drygt 95% av PV patienter och hos 60-70% av ET/MF patienter. De PV patienter som inte har JAK2 V617F mutation, dvs. ca. 5%, beräknas hälften ha JAK 2 exon 12 mutation. Detta betyder att närmare 100% av PV patienter har mutationer som kan upptäckas via blodprov och detta kan användas vid screening av patienter med högt hemoglobin/EVF och man kan undvika onödiga benmärgsprov hos en del patienter. CalR är den näst vanligaste mutationen i Ph-MPN och förekommer hos 25% av ET och 30% av MF patienterna. MPL-mutation förekommer hos 3-5% av ET patienter och ca 5-8% av MF patienter. Således förekommer någon av ovanstående mutationer hos cirka 90% av ET och MF patienter.

Syfte

Det huvudsakliga syftet med inkluderade arbeten har varit att utföra populationsbaserade studier och genom detta: *(i)*, Studera andelen patienter som utvecklar AML i varje diagnosgrupp (delarbete I). *(ii)*, Studera om blodvärden, mjältstorlek och benmärgsfynd vid diagnos av ET och PV har inverkan på överlevnad, propp-blödningsbenägenhet och/eller transformation till MF eller AML (delarbete II). *(III),* Skapa en prognostisk modell för PV utifrån kliniska fynd vid diagnos (delarbete III) och *(iv)*, Undersöka om ålder, blodvärden, JAK2 V617F mutation vid diagnos är riskfaktorer för vaskulära händelser (delarbete IV)

Metoder och resultat

Delarbete I

Alla patienter som fick diagnosen PV, ET och MF vid Göteborgs-sjukhusen och alla patienter med motsvarande diagnos i Côte d`Or området, Frankrike mellan 1980-2004 ingick. Median observationstiden var 15 år. Under den här tiden hade 16% av MF, 5,7% av PV och 5,6% av ET patienter utvecklat AML. Den årliga transformationsfrekvensen till AML var 1,09% (MF), 0,38% (PV) och 0,37%, (ET). Skillnaden mellan ET och MF, PV och MF var statistisk signifikanta. Ett oväntat resultat var att 17 av 18 de PV patienter som utvecklade AML var kvinnor.

Delarbete II

Alla patienter som fick diagnosen PV och ET vid Göteborgs-sjukhusen ingick, observationstiden var i median 15 år. Studien visar minskad överlevnad, i jämförelse med generella populationen, när det gäller PV, däremot sågs ingen signifikant skillnad när det gäller ET. Lågt hemoglobin (Hb), vid diagnos, predikterade kortare överlevnad hos patienter med ET och mjältförstoring, vid diagnos, predikterade kortare överlevnad hos patienter med PV. Lågt Hb, höga vita blodkroppar (LPK), ökad cellhalt och ökad retikulinhalt i benmärgen, vid diagnos, predikterade högre risk för transformation till MF och/eller AML hos patienter med ET. Ökad retikulinhalt och mjältförstoring, vid diagnos, predikterade högre risk för transformation hos PV patienter.

Under uppföljningstiden hade 45% av patienter med ET och 47% med PV utvecklat åtminstone en vaskulär-händelse. De faktorer, vid diagnos, som predikterade vaskulära händelser var högt LPK hos patienter med PV. Låg retikulinhalt och låg cellhalt i benmärgen hos patienter med ET föreföll vara riskfaktorer för arteriell tromboembolism.

Delarbete III

Alla patienter med PV vid Göteborgs sjukhusen och motsvarande patienter vid Côte d`Or område, Frankrike ingick, median observationstiden var 11 år. Relativ överlevnad (RS) var 93%, 83% och 46% efter 5,10 respektive 20 år. Multivariatanalys visade att ålder > 70 år, LPK >13x10^9/L och tidigare vaskulär-händelse, vid diagnos, var oberoende riskfaktorer för överlevnad, baserat på dessa riskfaktorer har en prognostisk modell skapats: Lågrisk PV patienter, utan någon av dessa riskfaktorer, hade en beräknad 10-års RS på 84%. Intermediär risk PV, med en riskfaktor, hade en beräknad 10-års RS på 59%. Hög risk PV, med två eller tre riskfaktorer, hade en beräknad 10-års RS på 26%.

Delarbete IV

Det sista delarbetet är baserat på data från det Nationella MPN-registret där registrering varit obligatoriskt sedan 2008 och som, i det närmaste, är heltäckande. Detta gör att registret ger en mer sann bild av Ph-MPN än studier från sjukhusregister. Med dagens täckningsgrad på cirka 95% kan man genomföra högkvalitativa populationsbaserade studier avseende incidens, diagnostik och komplikationer. Alla patienter med ET och PV som registrerats fr.o.m. januari 2008 t.o.m. oktober 2015 i det Nationella MPN-registret ingick. Totalt innefattar studien 1105 patienter med PV och 1284 med ET. Trettiosju procent av patienter med PV och 35% patienter med ET hade redan haft åtminstone en vaskulär-händelse vid diagnos, majoriteten var tromboemboliska. 98% av PV och 64% ET patienter var bärare av JAK2 V617F mutationen.

Frekvensen av vaskulära händelser, innan diagnos, var signifikant högre hos ET patienter med JAK2 V617F mutation jämfört med de utan denna mutation. Multivariatanalys visade att ålder > 65 år, förekomst av JAK2 V617F mutation och höga LPK, vid diagnos, oberoende riskfaktorer. Hos patienter med PV var bara lågt Hb vid diagnos oberoende riskfaktor för vaskulära händelser. Det förelåg en signifikant korrelation mellan lågt Hb och högt LPK hos både PV och ET patienter.

Sammanfattning

Vaskulära komplikationer inträffar i ansenlig andel av patienter innan eller vid Ph-MPN diagnos. JAK2 V617F mutation är en riskfaktor för tromboemboliska händelser hos patienter med ET. Överlevnad hos patienter med Ph-MPN varierar beroende på diagnosgrupp. MF har sämre prognos och ET har bättre prognos. Sjukdomsduration och ålder vid diagnos har inverkan på överlevnad både hos ET och PV patienter likaså lågt Hb vid ET och höga LPK vid PV.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Abdulkarim K,** Girodon F, Johansson P, Maynadié M, Kutti J, Carli P-M, Bovet E, Andréasson B. AML transformation in 56 patients with Ph- MPD in two well defined populations. Eur J Haematol, 2009. **82**(2): p. 106-11.

II. **Abdulkarim K,** Ridell B, Johansson P, Kutti J, Safai-Kutti S, Andréasson B. The impact of peripheral blood values and bone marrow findings on prognosis for patients with essential thrombocythemia and polycythemia vera. Eur J Haematol, 2011. **86**(2): p. 148-55.

III. Bonicelli G, **Abdulkarim K,** Mounier M, Johansson P, Rossi C, Jooste V, Andréasson B. Maynadié M, Girodon F. Leucocytosis and thrombosis at diagnosis are associated with poor survival in polycythaemia vera: a population-based study of 327 patients. Br J Haematol, 2013. **160**(2): p. 251-254.

IV. **Abdulkarim K,** Samuelsson J, Johansson P, Andréasson B. Risk factors for vascular complications and treatment patterns at diagnosis of 2389 PV and ET patients: Real-world data from Swedish MPN registry; Eur J Haematol, 2017.98(6) p.577-583.

CONTENT

ABBREVIATIONS

ABL	Abelson murine leukemia virus oncogene
AML	Acute myelogenous leukemia
BCR	Breakpoint cluster region
CalR	Calreticulin
CI	Confidence interval
CML	Chronic myelogenous leukemia
EPO	Erythropoietin
ET	Essential thrombocythemia
IPSET	International prognostic score for ET
RS	Relative survival
Ph-	Philadelphia negative
MDS	Myelodysplastic syndrome
MF	Myelofibrosis
MPD	Myeloproliferative disorder
MPL	Myeloproliferative leukemia virus oncogene.
MPN	Myeloproliferative neoplasm
PV	Polycythemia vera
PVSG	Polycythemia vera study group
WBC	White blood cell
WHO	World Health Organization

1 INTRODUCTION

The classical myeloproliferative neoplasms (MPNs), also called Philadelphia chromosome negative (Ph-) MPNs or BCR/ABL-MPNs, are rare clonal hematological malignancies mainly including; polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF). Primary myelofibrosis is diagnosed in patients without any known preceding PV or ET, whereas post-PV MF and post-ET MF are transformations preceding earlier PV and ET.

Patients with these neoplasms have similar characteristics such as running an increased risk of thromboembolic and hemorrhagic complications. Furthermore, they run the risk of transformation into acute myeloid leukemia (AML). In PV, the reported incidence of AML ranges between 1-4% after 10 years of observation. The risk is higher in patients with MF (8-23%) and lower in ET (0.5-1%) (1-5).

1.1 History

Louis Henry Vaquez, a French physician, was the first to describe an unknown entity in a patient with persistent erythrocytosis and cyanosis in 1892, and it is likely to be the first description of PV.

In the early 1900s, Osler presented the first series of patients with the new syndrome and he established the original diagnostic criteria for PV with the triad of cyanosis, erythrocytosis and splenomegaly (6).

The term “myeloproliferative syndromes” was first used by Dameshek in the early 1950s, when he described these conditions as being characterized by the excessive production of mature blood cells (7) and he is considered to be the father of the MPNs.

In 1960, two American scientists (Peter Nowell and David Hungerford) discovered the Philadelphia (Ph) chromosome in patients with chronic myeloid leukemia (CML) (8) and, with this discovery, CML was distinguished from other MPNs; hereby Ph-MPNs.

The diagnosis of these disease entities was historically based on clinical criteria. Among the most important sets of criteria, before the modern ones, Berlin in 1975 (9), and Murphy et al. in 1986 (10) should be mentioned.

The WHO criteria were established in 2001 and bone marrow histology has finally been included in the diagnostic criteria for PV, as well as for ET (11). The WHO criteria have been revised several times since this first version.

The term "neoplasm" was first introduced in 2008 by the authors of the WHO classification of tumors of hematopoietic and lymphoid tissues. The previous name, MPD (**M**yelo **P**roliferative **D**isorder), was thus replaced by MPN (**M**yelo **P**roliferative **N**eoplasm) (12).

1.2 Definitions and diagnostic criteria

PV is characterized not only by the proliferation of the erythroid lineage but also by the megakaryocytic and granulocytic lineages, so-called panmyeloid (panmyelosis) proliferation. ET is characterized by megakaryocytic proliferation, resulting in an increase in platelets. MF is more heterogeneous, with collagen fibrosis, megakaryocytic proliferation, with atypical megakaryocytes, and extramedullary hematopoiesis.

Prior to 2005, the molecular pathogenesis of the Ph-MPN was unknown. In the spring of 2005, a major breakthrough occurred, with respect to the understanding of Ph- MPN, owing to the discovery of point mutation in the Janus Kinase 2 gene, JAK2 V617F mutation, by four independent research groups (13-16). Since then, additional mutations have been identified: myeloproliferative leukemia virus oncogene (MPL W515L) (17), point mutation in JAK2 exon 12 (18) and calreticulin (CalR) (19, 20), to name just a few, which also thought to play a role in the pathogenesis of Ph-MPNs. The mutations are considered to be driving factors, leading to the proliferation and maturation of hematopoietic stem cells, but there is doubt about whether they cause the disease by themselves.

With the discovery of the JAK2 V617F mutation, the diagnosis of Ph-MPNs has improved considerably and been simplified. Close to 95% of PV patients have this mutation, while about 60% of ET and MF carry this mutation (21, 22). JAK2 exon 12 is present in about half of PV patients

who lack JAK2 V617F mutations (23). The analysis of JAK2 mutations can be used in the work-up of patients with high hemoglobin and hematocrit. The need for painful and, in some cases, unnecessary bone marrow examinations has been reduced especially in patients with only high hemoglobin and/or hematocrit.

The diagnosis of Ph-MPNs nowadays is based on clinical, morphological, i.e. bone marrow biopsy, and molecular criteria.

Bone marrow from PV patients with the JAK2 exon 12 mutation displays erythrocytic hyperplasia, without the morphological abnormalities in the megakaryocytic or granulocytic cell lineage (24).

The MPL W515L mutation is present in 3-5% of patients with ET and 5-8% of patients with MF.

A mutation in the CalR gene is present in 25% and 35% patients with ET and MF respectively. CalR mutation is mostly mutually exclusive with both JAK2 and MPL W515L mutations. More than fifty mutations in CalR genes, all in exon 9, are described in the literature and the majority of them are non-pathogenic. The two most frequent pathogenic CalR mutations correspond to a 52-bp nucleotide deletion (Type 1) and a 5bp nucleotide insertion (Type II) (25). These mutations occur at different frequencies in ET and MF: in ET, Type 1 and Type 2 mutations are closely distributed (55%/35%), while, in MF, Type 1 is predominant (75%/15%) (26). Some clinical observations have been made with respect to the types of CalR mutation. In MF, the presence of the CalR Type 1 mutation is associated with superior survival, as compared to JAK2 V617F mutated patients, whereas the CalR Type 2 mutation is found to be unfavorable and it is associated with a higher blast percentage and leukocyte count (27). In ET, the Type 2 CalR mutation is associated with a significantly higher platelet count compared with the Type 1 variant. Blood counts in both patients with CalR Type 1 and Type 2 variants are associated with higher platelet and lower hemoglobin and leukocyte counts compared with the levels in patients with JAK2 V617F mutations (28).

Next generation sequencing (NGS) has enabled the identification of several other acquired mutations in Ph-MPNs; ASXL1, TET2 and DNMT3A, to mention a few. These mutations are not restricted to only Ph-MPNs, but they are more common in other myeloid malignancies, such as MDS and AML. ASXL1 mutation is the second most common

among epigenetic regulators, after TET2, in Ph-MPNs (29), and is associated with a poor prognosis in MF patients and a higher risk of AML transformation (29, 30).

In conclusion, it is possible to claim that we find detectable molecular mutations in almost 100% of patients with PV, and in about 90% of patients with MF and ET.

The WHO criteria were established in 2001 and have been revised several times (11, 12, 31). The latest WHO criteria, established in 2016, are shown in ***Table 1***.

Table 1. 2016 WHO diagnostic criteria for PV, ET, prefibrotic PMF, and overt PMF.

Arber et al. Blood 2016;127:2391-2405

	Polycythemia vera (PV)	Essential thrombocythemia (ET)
Major criteria	1. Hemoglobin >16.5 g/dL in men, >16.0 g/dL in women *or* Hematocrit >49% in men, >48% in women *or* increased red cell mass >25% above mean normal predicted value. 2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (diffrences in size) 3. Presence of *JAK2* (V617F) or *JAK2* exon 12 mutation	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 criteria	Subnormal serum erythropoietin level	Presence of a clonal marker or absence of evidence for reactive thrombocytosis
	Diagnosis requires meeting all *three* major criteria or the first *two* major criteria and the minor criterion.	Diagnosis requires meeting all *four* major criteria or the first *three* major criteria and the minor criterion.

	Prefibrotic PMF	Overt PMF
Major criteria	1. Megakaryocytic profileration and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis 2. Not meeting WHO criteria for BCR-ABL1*, CML, PV, PMF, 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 BM reticulin fibrosis (minor reticulin fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic myelopathies)	1. Presence of megakaryoctic proliferation and atypia, accompanied by either reticulin and/or colloagen fibrosis grades 2 or 3 2. Not meeting WHO criteria for BCR-ABL1*, CML, PV, PMF, 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 BM reticulin fibrosis (minor reticulin fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic myelopathies)
Minor criteria	Presence of at least *one* of the following confirmed in 2 consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis (WBC count ≥11 × 10^9/L) • Palpable splenomegaly • LDH level increased to above upper normal limit of institutional reference range	Presence of at least 1 of the following confirmed in 2 consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis (WBC count ≥11 × 10^9/L) • Palpable splenomegaly • LDH level increased to above upper normal limit of institutional reference range • Leukoerythroblastosis
	Diagnosis requires meeting all *three* major criteria, and at least *one* minor criterion. Note: 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.	

1.3 Incidence and prognosis

Ph-MPNs are regarded as a rare neoplasm with a cumulative yearly incidence of less than 6 per 100,000 individuals.

The reported annual incidence of PV is between 0.4-2.8 per 100,000 persons and the corresponding figures are ET is 0.38-1.7 per year for ET and 0.1-1 per 100,000 persons per year for MF (32-34).

These neoplasms generally occur in the middle-aged or advanced-aged adults, with a median age at the time of diagnosis of 69 years for PV, 68 years for ET and 70 years for MF (33-35).

The prognosis for these disease entities varies depending on the subtypes of MPN. Generally, the relative survival rate (RS) is lower in MF compared with PV, and in PV compared with ET (36). The Survival of patients with WHO-defined ET does not differ significantly from that of an age- and gender-standardized European population (37).

Ph-MPN patients run a higher risk of vascular complications, both thrombotic and hemorrhagic complications, compared with the general population (38-41)). Thrombotic complications may occur in both arteries and veins. In a substantial portion of Ph-MPN, vascular complications precede the diagnosis (42). This is the main cause of morbidity and mortality in this patient population.

Patients with ET who harbor JAK2 V617F run a high risk of thrombosis and may progress to PV in contrast to ET patients with the CalR mutation, who run a lower risk of thrombosis but a higher risk of transformation to myelofibrosis (43). Patients with a CalR-mutated ET have a better survival than those with MPL-mutated ET (29).

Patients with MF who harbor the CalR mutation have a relatively indolent clinical course compared with those with the JAK2 V617F or MPL mutation, while those with triple negativity have the poorest prognosis (44, 45).

Age > 60 years, a previous history of vascular events and a platelet count of > 1500 x10^9/L are three risk factors for vascular complications in ET. The presence of one of the above-mentioned risk factors is regarded as an indication for cytoreductive treatment (46) by the Nordic MPN Care Program. The International Prognostic Score for Essential Thrombocythemia (IPSET) thrombosis also regards the JAK2 V617F mutation as an additional risk factor (47).

The IPSET prognostic model was developed on the basis of an international study of 867 patients with the WHO-defined ET (48).
Age > 60 years, prior thrombosis and leukocytosis (WBC > 11x10^9/L) were the factors that significantly affected survival.

In PV, age > 60 years and previous vascular events are also regarded as risk factors for further vascular complications, thereby necessitating cytoreductive treatment (49).

In MF, there are currently three prognostic models; the IPSS (International Prognostic Scoring System), DIPSS (Dynamic International Prognostic Scoring System) and DIPSS-plus.

The IPSS comprises five independent inferior prognostic variables: age > 65 years, hemoglobin < 10g/dl, circulating blasts > 1%, WBC count > 25x10^9/L and the presence of constituitional symptoms, each with one point (50). The DIPSS also uses the same prognostic variables, but it assigns two points to low hemoglobin (51).

The DIPSS-plus incorporates three additional prognostic variables on top of the above mentioned ones. They are a platelet count of < 100 x10^9/L, an unfavorable karyotype and a transfusion requirement (52).

The IPSS estimates survival at the time of diagnosis, whereas the DIPSS and DIPSS–plus can be applied at any time during the clinical course.

These models are based on clinical and hematological parameters, but recent studies indicate the involvement of driver mutation as an independent factor.

The prognostic scoring system with the IPSS, DIPSS and DIPSS-plus is illustrated in ***Table 2***.

Risk factors for vascular complications, myelofibrotic/AML transformations and survival are summarized in ***Table 3*****.**

Table 2 Prognostic scoring system for the IPSS, DIPSS and DIPPS-plus.

Risk factors and corresponding score contribution to prognostic models

Risk factors:	score contribution **IPSS**	score contribution **DIPSS**	score contribution **DIPSS-plus**
• Age >65 year	1	1	-
• Constitutional symptoms	1	1	-
• Hemoglobin < 10 g/dL	1	2	-
• WBC count >25 × 10^9/L	1	1	-
• Circulating blasts ≥ 1%	1	1	-
• RBC transfusion need	-	-	1
• PLT count <100 × 10^9/L	-	-	1
• Unfavorable karyotype	-	-	1
• DIPSS score	-	-	see note
note: DIPSS score (DIPPS low =0, DIPPS int-1 = 1, DIPPS int-2 = 2, DIPSS high = 3)			

Risk groups and clinical relevance

total sum score of risk factors	Risk group	(median survival, years) **IPSS**	(median survival, years) **DIPSS**	(median survival, years) **DIPSS-plus**
0	Low risk	11.3 y	20 y	15 y
1	Intermediate-1 risk	7.9 y	14.2 y	6.6 y
2	Intermediate-2 risk	4.0 y	4.0 y	2.9 y
≥ 3	High risk	2.3 y	1.5 y	1.3 y

IPSS estimates survival at the time of diagnosis

DIPSS and DIPSS-plus estimates can be applied anytime during clinical course

Table 3. Conventional and molecular risk factors for patients with MPNs.

Diagnosis	Vascular complications	Myelofibrotic & AML transformations	Survival
ET	Age ≥60 years Previous thrombosis. JAK2 V617F mutation. High platelet count ($\geq 1500x10^9$//L	CalR mutation (MF only) *Other mutations in myeloid genes(MF/AML)	Age≥ 60 years. Previous thrombosis. Leukocytosis. *Other mutation in myeloid genes.
PV	Age≥ 60 years Previous thrombosis	JAK2 V617F- mutant allele burden>50 (MF) *Other mutations in myeloid genes (AML).	Leukocytosis. Previous thrombosis. *Other mutations in myeloid genes.
MF		age≥65 years Anemia (Hb<10g/L) Leukocytosis (WBC count>25x109/L). Thrombocytopenia (<100x109/L). Circulating blasts≥1% Unfavorable karyotype Triple negativity. *Other mutations in myeloid genes.	age≥65 years Anemia (Hb<10g/L) Leukocytosis (WBC count>25x109/L). Thrombocytopenia (<100x109/L). Circulating blasts≥1% Unfavorable karyotype Triple negativity. *Other mutations in myeloid genes.

1.4 Treatment

The treatment of PV includes phlebotomy with a target hematocrit of < 45% (49, 53), low-dose-aspirin (54) and, in those with a high risk profile, treatment with cytoreductive agents. The main aim of treatment is to minimize the risk of vascular complications.

The main aim of the treatment of ET is to prevent thrombotic and bleeding complications. In ET patients with an extremely high platelet count (> 1500 $x10^9$/L), low-dose aspirin should not be given, because of an increased risk of bleeding caused by acquired von Willebrand syndrome (55). Low-dose aspirin is given in low-risk ET patients with peripheral vascular symptoms, such as erythromelagia, or in the presence of cardiovascular risk factors. Low-risk ET patients with the CalR mutation are not found to benefit from therapy with low-dose aspirin, which does not appear to affect the risk of thrombosis but is associated with a higher bleeding tendency (56). On the other hand, ET patients with a high-risk profile require low-dose aspirin and cytoreductive treatment with a target platelet count of < 400 x 10^9/L.

In MF, the treatment focuses on the management of anemia and splenomegaly and the improvement of constitutional symptoms. MF is associated with an enormous symptom burden with a reduction in quality of life. The other treatment option is allogenic stem-cell transplantation (SCT) and this is the only potentially curative treatment, but it is reserved for younger patients with an intermediate II/ high risk IPSS score. It can be applied to younger low/intermediate-1 patients who progress to higher DPSSS/DIPSS-plus during follow-up (57-59).

Treatment with erythropoietin (EPO) has been shown to increase the hemoglobin concentration in 20-60% of MF patients (60, 61), but the limitation is that all patients may not respond and it could facilitate spleen enlargement.

Ruxolitinib is the only JAK1/JAK2-inhibitor approved in Europe and the USA and its approval was based on two randomized studies; COMFORT 1 and COMFORT 2 (62, 63). Ruxolitinib has been shown to reduce spleen size and improve constitutional symptoms. A significant positive effect on survival has also been seen in patients who have been treated with Ruxolitinib, despite no clear effect on the elimination of the mutant cell clone. Interferon-α is a treatment option, especially in younger patients with the hyperproliferative stage of the disease (64, 65).

2 AIMS OF THE STUDIES

The overall aim was to conduct population-based studies in patients with Ph-MPNs, with particular emphasis on prognosis and complications.

Paper I

To investigate the rate of AML development in patients with Ph-MPN in the City of Gothenburg, Sweden, and the Côte d'Or area, France

Paper II

To review all the available bone marrow biopsy specimens from patients with PV and ET, as established according to PVSG criteria, and to correlate the findings at the time of diagnosis with clinical outcome, survival and vascular events and transformation into MF and AML

Paper III

To find the prognostic tools based on the clinical and laboratory findings at the time of diagnosis of patients with PV

Paper IV

To describe "real-world" data from a cohort of patients with newly diagnosed PV and ET and to investigate the possibility of any correlations between peripheral blood counts, EPO, JAK2 V617F mutational status and vascular complications prior to diagnosis

3 METHODS

Papers I, II and III

Gothenburg is the second largest city in Sweden. All patients with suspected hematological disorders are usually examined at either of the two main university hospitals (Sahlgrenska and Östra).

Since 1980, the population of the Côte d`Or area, France, has been covered by a special registry of hematological malignancies. The Swedish and the French regions are similar with respect to population, each with close to 500,000 inhabitants.

In almost all patients with suspected Ph-MPN, a bone marrow biopsy was performed as part of the diagnostic work-up; all these biopsies were initially thoroughly examined and, within the studies, re-examined by experienced hematopathologists.

Clinical files were reviewed; data such as, age, gender, peripheral blood counts, EPO, vascular complications and the AML/MF transformation of all patients were retrieved and analyzed retrospectively.

The Polycythemia Vera Study Group (PVSG) criteria were used to establish the PV and ET diagnosis until 2001, when the WHO criteria were introduced (9, 10). MF was considered to be present in patients with bone marrow fibrosis of unexplained origin, together with splenomegaly and a leukoerythroblastic cell reaction in peripheral blood, and who did not fulfil the criteria for PV, ET and myelodysplastic syndrome (MDS).

Paper IV

All the information for the study was extracted from the National MPN registry, to which it has been mandatory to report since 2008. In this registry, age, gender, blood counts, EPO and JAK2 V617F mutation status at diagnosis were recorded and, in later years, MPL status and CalR mutation status were also added. Vascular events, prior to diagnosis, were also documented. Plans for initiating or continuing therapy, including antiplatelets/ anticoagulants, phlebotomy and myelosuppressive treatment, were registered. The diagnoses of the included patients were based on the WHO criteria from 2008 (12).

3.1 Statistical methods

Standard statistical methods were used for the calculation of means, median values and standard deviations. Differences in time from Ph-MPN diagnosis to the onset of AML development were compared using the Mann-Whitney U test (Paper I).

Differences between groups were tested with Fisher's exact test (Paper I). Survival was determined by the Kaplan-Meier method (Papers I &III).

The probability of survival was calculated with the Kaplan-Meier log-rank test (Paper II). A multivariate analysis with Poisson regression was used and the hazard ratio is given (Paper II, Paper III). Pitman's test was used to calculate correlations (Paper II).

Significant factors were identified by a Cox regression. To estimate the excess mortality directly or indirectly due to the disease, RS was calculated (Paper III).

When applicable, $p < 0.05$ was considered statistically significant. Differences in the distribution of variables among categories were calculated using the Mann-Whitney test. The Chi-square test was used for comparisons between groups. Correlations were analyzed with Pearson's product correlation test (Paper IV).

4 RESULTS

Paper I

A total of 795 Ph-MPN patients (392 from the Gothenburg area and 403 from the Côte d`Or region) were included in the study.

At the median follow-up period of 15 years, 56 subjects (7%) had developed AML. Patients with PV and MF who developed AML were a bit younger at the time of diagnosis as compared to the total PV and MF population. Their mean ages were 66 years and 70 years for PV and 68 and 70 years for MF respectively. There was, however, no difference between the patients with ET who developed AML and those who did not; the mean ages were 68 years in both groups.

Most of the patients were treated with hydroxyurea (HU) as a single agent (32⁄56), eight patients were treated with radiophosphorous alone (P^{32}), eight patients were treated with combinations of myelosuppressive agents, two patients were treated with interferon-α and six patients had never received any cytoreductive therapy.

The average time from diagnosis until the initiation of myelosuppressive therapy for the patients from Gothenburg was significantly longer compared with those from the Côte d'Or area, 14 ± 18 months and 3 ± 6 months respectively (p=0.001).

The yearly incidence of AML transformation for the total of 795 patients investigated was 0.38% in PV, 0.37% in ET and 1.09% in MF. The incidence of AML development was significantly higher in MF as compared to both PV and ET (p = 0.002 and p = 0.02 respectively). The difference between the incidence of AML development between the PV and ET patients was not statistically significant (p = 0.41).

Seventeen of the 18 PV patients who transformed to AML were females.

The mean time from Ph-MPN diagnosis to AML development was 71 ± 55 months (range 2–237). The mean time from MF diagnosis to AML transformation was 42 ± 33 months, which was significantly shorter than

that for both PV and ET (88 ± 56 and 76 ± 57 months [p = 0.0075 and p = 0.027 respectively]). The rate of yearly AML transformation appears to be relatively continuous over time in all three MPN entities, as illustrated in **Figure 1**.

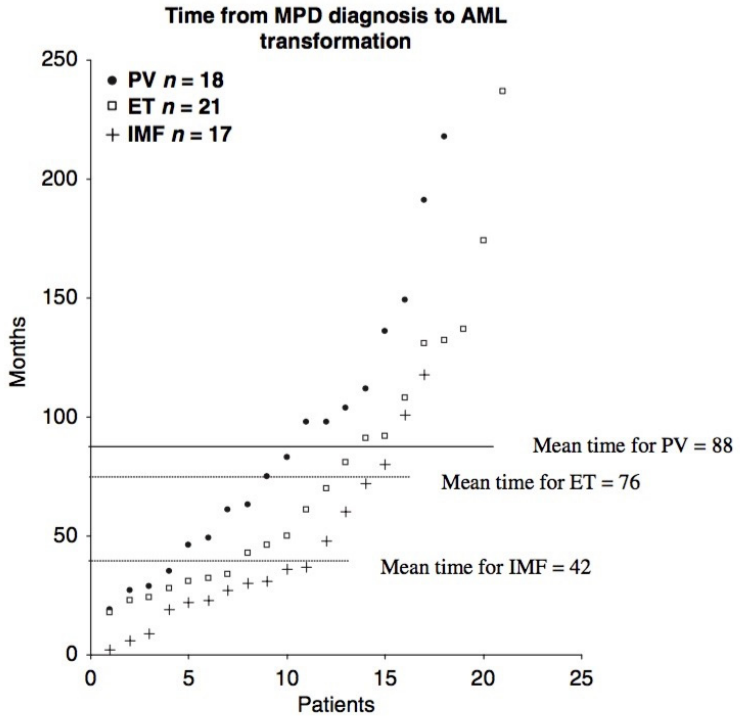

Figure 1 The mean time from MPD diagnosis to AML transformation for 18 patients with PV, 21 with ET and 17 with IMF were 88, 76 and 42 months, respectively. The patients of the three subtypes were sequentially arranged along the x-axis with respect to their individual durations from diagnosis to AML transformation.

The average survival after the development of AML was short (4.6±5.5 months) and there was no significant difference in survival after AML transformation between Ph-MPN entities.

Paper II

A total of 280 Ph-MPN patients (150 PV and 130 ET), diagnosed in Gothenburg during the time-period 1983-1999, were compared with the normal Swedish population with respect to expected survival. Only PV or ET patients verified with a bone marrow biopsy were included. The effects of age, gender, calendrical time, and time of observation were adjusted for.

Patients with PV had a significantly poorer survival compared with individuals in the general Swedish population (hazard ratio 1.66; 95% CI: (1.38-1.99); $p < 0.001$). In contrast, there was no significant difference regarding ET patients and the general population (hazard ratio 1.23; 95% CI: (0.97-1.51); $p = 0.089$). The hazard ratio of death for ET versus PV was 0.65 (95% CI: (0.49-0.87); $p = 0.004$). The probability of overall survival 10 years from diagnosis was 58% for the patients with ET compared with 40% for the patients with PV ($p < 0.001$).

Multivariate analysis was performed separately for patients with ET and PV. A low hemoglobin concentration was the only independent parameter significantly affecting survival in ET ($p = 0.028$). A high white blood cell (WBC) count did not quite reach statistical significance ($p = 0.07$).

In PV, the only parameter independently reaching the level of significance affecting survival was the presence of splenomegaly ($p = 0.0037$).

Of the 130 patients with ET, 11 developed AML and seven MF (13%).

Thirteen of the 150 patients with PV developed AML and 13 experienced a transformation into MF (17%). Even if these patients were excluded from the analysis, the risk of death was increased in the PV group (risk

ratio 1.46, 95% CI: (1.18-1.79), $p < 0.001$), whereas the patients with ET did not differ significantly from the general population (risk ratio 1.01, 95% CI: (0.78-1.29), $p = 0.957$).

The bone marrow biopsies from the 44 patients who experienced AML and MF transformation were re-evaluated with respect to the WHO criteria for Ph-MPN diagnosis. Six of 11 patients with ET who developed AML and all seven patients with MF transformation had MF features according to the WHO classification criteria. The corresponding figures for the patients with PV were four of 13 with AML development and five of 13 with MF transformation.

A low hemoglobin concentration and high WBC counts have been shown to be independent risk factors for transformation in patients with ET ($p = 0.0037$ and $p = 0.0306$ respectively), whereas, in PV, none of the tested blood counts predicted transformation.

Reticulin content in the bone marrow was found to be a risk factor for transformation for both PV and ET patients ($p = 0.0164$ and $p = 0.0359$ respectively). Bone marrow cellularity also appeared to be a predictor of AML or MF development in ET ($p = 0.0103$), but, in PV, significance was not quite reached ($p = 0.0536$).

Splenomegaly at diagnosis was found to be a risk factor for transformation in PV ($p = 0.0028$) but not in ET ($p = 0.0616$).

During the follow-up period and one year prior to diagnosis, 59 of the 130 (45%) patients with ET and 70 of the 150 (47%) patients with PV experienced at least one vascular event.

Patients with PV who had an elevated WBC count were found to have a significantly higher frequency of venous thrombosis compared with patients with normal values ($p = 0.045$).

In patients with ET, a low reticulin content and low cellularity in the bone marrow was significantly related to arterial thrombosis ($p = 0.011$ and $p = 0.0002$ respectively).

Arterial thrombotic events occurring from the time of diagnosis had a frequency of 3.4% / patients / year for the patients with ET and 4.0% /

patients/year for the patients with PV. The corresponding frequencies for venous thrombotic events were 0.7 and 2.2%/patients/year and 1.6 and 1.7%/patients/year, for bleeding complications, for ET and patients with PV respectively.

Paper III

A total of 327 PV patients (188 from Gothenburg and 139 from the Côte d'Or area, 175 females and 152 males) with a median age at diagnosis of 71 years (range: 21– 95) were analyzed. The median follow-up was 11 years. The incidence of PV was similar, irrespective of which set of diagnostic criteria were used (PVSG or WHO) (1.97 vs. $1.8/10^5$ inhabitants), in contrast to ET (1.55 vs. $2.0/10^5$ inhabitants).

Two hundred and forty-four patients (137 females and 107 males) died at a median age of 81 years. The cause of death was reported in 174 cases and was related to thrombotic events in 21%, secondary AML in 17%, solid tumors in 17% and chronic heart failure in 15% of the patients.

Patients who had had a thrombotic complication at the time of diagnosis ran a higher risk of dying from a thrombotic event ($p < 0.01$).

Relative survival (RS) was 93%, 72% and 46% after five, 10 and 20 years of disease duration respectively. No difference in RS was seen between Gothenburg and the Côte d'Or area.

When univariate RS analysis was performed, risk factors for poor survival were identified as age > 60 or > 70 years ($p = 0.011$ and $p < 0.001$ respectively), leukocytosis > mean WBC (11×10^9/L), ($p = 0.04$) or median WBC (13×10^9/L), ($p = 0.004$) and thrombosis at the time of diagnosis ($p = 0.007$).

In contrast, neither the hemoglobin concentration or hematocrit nor the platelet count was significantly associated with poor survival.

Multivariate analysis for RS revealed age > 70 years, leukocytosis $>13\times10^9$/L and thrombosis at diagnosis as significant predictors of survival.

Among the patients younger than 60 years of age, only a thrombotic event at diagnosis was an independent risk factor for poor RS.

Based on these three prognostic factors, a model for RS was constructed to define three groups at risk: a high risk for patients with two or three risk factors, an intermediate risk with one risk factor and a low risk with no risk factors.

The ten-year RS was 26%, 59% and 84% in the high, intermediate and low-risk groups respectively (p =0.001), **(Figure 2).**

Transformation to AML occurred in 30 (9.2%) PV patients (22 females and 8 males) during the observation period.

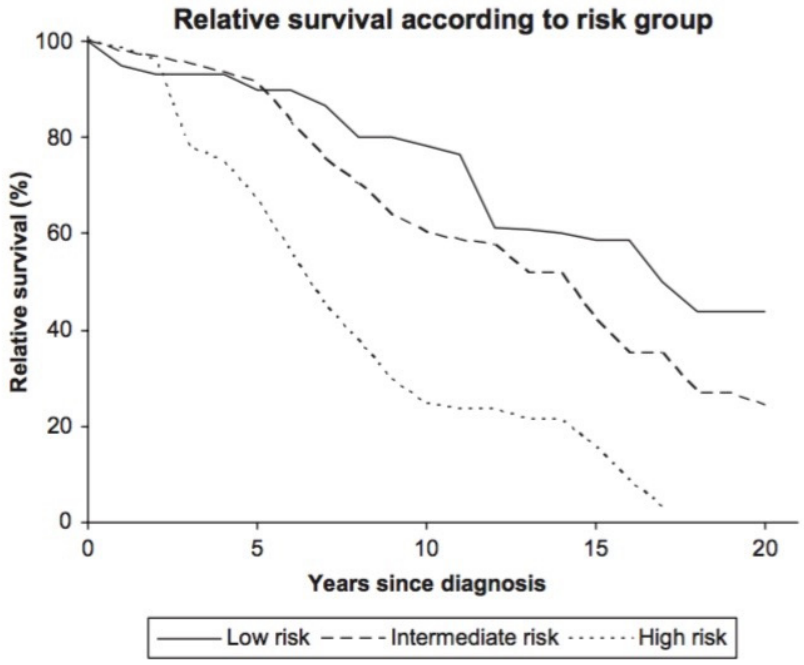

Figure 2. Relative survival curves for polycythemia vera patients stratified according to the three risk factors of age > 70 years, leukocytosis > 13 x 10^9 /L and thrombosis at diagnosis.

Paper IV

A total of 1,284 newly diagnosed ET patients and 1,105 PV patients was reported. The annual incidence for ET was 2.0 per 100,000 inhabitants and the corresponding figure for PV was 1.8 per 100,000 inhabitants.

The median age at diagnosis for ET was 68 years (range 13-94) and, for PV, 69 years (range 17-98) ($p = 0.003$).

Sixty-four percent of the ET patients carried the JAK2 V617F mutation and the corresponding frequency in PV was 98%.

Among the ET patients, 35% had suffered from at least one vascular complication prior to diagnosis. Only 12.5% of these complications were bleedings and the rest were thromboembolic.

Univariate analyses revealed that ET patients with vascular complications had a significantly lower mean hemoglobin concentration and mean hematocrit compared with the patients without vascular events ($p = 0.002$ and $p = 0.005$ respectively). The mean WBC was significantly higher in the complication group ($p = 0.031$). No difference was seen regarding platelet concentrations in the two groups.

The frequency of ET patients harboring the JAK2 V617F mutation was 71% in the group with vascular complications compared with 60% in the group without ($p < 0.001$).

No significant difference was seen regarding EPO concentrations when ET patients with or without vascular complications were compared.

A multivariate analysis was performed with respect to thromboembolic events in ET, including age higher or lower than 65 years, gender, WBC higher or lower than 12×10^9/L, the presence of the JAK2 V617F mutation, hemoglobin, hematocrit and platelets higher or lower than the median concentration. The odds ratio was significantly elevated for age > 65 years, WBC > 12×10^9/L and the presence of the JAK2 V617F mutation ($p = 0.0004$, $p = 0.0038$ and $p = 0.0016$ respectively).

Thirty-seven percent of patients with PV had a vascular event before diagnosis. The difference in frequencies of vascular complications

between PV and ET patients was not significant ($p = 0.4$). Thirteen percent of these events were bleedings.

In both ET and PV patients, the four major types of thromboembolic events were cerebrovascular, cardiovascular, deep vein thrombosis/pulmonary embolism and peripheral embolism.

Univariate analyses revealed that, at diagnosis, the PV patients with earlier vascular complications had a significantly higher mean age, lower hemoglobin concentration, and lower hematocrit compared with the PV patients without vascular complications ($p < 0.001$ for each parameter).

Despite lower hemoglobin concentrations, the mean EPO levels were significantly lower in the complication group ($p = 0.028$). No significant differences between means in the two groups were seen regarding WBC or platelets.

In the multivariate analysis, with the same parameters as in ET, only hemoglobin below the median concentration significantly influenced the odds ratio for thromboembolic complications prior to diagnosis in PV ($p = 0.021$).

In both PV and ET, a significant correlation was found between a higher WBC count and a lower hemoglobin concentration ($p < 0.001$ and $p = 0.0015$ respectively).

Seven of 509 (1.4%) patients with ET and 19 of 438 (4.3%) patients with PV had palpable splenomegaly ($p = 0.01$). Constitutional symptoms, in the same cohort, were significantly more common among the PV patients (19.9%) compared with the ET patients (6.9%) ($p < 0.001$).

Low-dose aspirin treatment (ASA) was prescribed for 59% of the PV patients, while 4% of the patients received other anticoagulant therapies, mainly warfarin or clopidogrel. The frequency of ASA treatment was identical in the ET patient group (59%), whereas 2% of the patients were treated with other anticoagulants.

Cytoreductive therapy was initiated in 48% of the PV patients and in 68% of the ET patients ($p < 0.001$).

Sixty-three percent of the PV patients with prior vascular complications were started on myelosuppressive therapy, compared with 46% in the

group of PV patients without vascular events prior to diagnosis ($p < 0.001$). The corresponding frequencies for ET patients were 88% and 59% respectively ($p < 0.001$). Hydroxyurea was used in more than 90% of the patients in need of treatment.

5 DISCUSSION

Patients with Ph-MPN display similarities and divergences as relating to peripheral blood counts, bone marrow morphology, the presence of driver mutations and survival.

Among the Ph-MPN entities, it is indisputable that MF has the poorest prognosis (36). ET is generally considered to have the best prognosis, but there is controversy about whether patients with ET have the same life expectancy as the general population (1, 36, 66-68). In our study (Paper II), we have shown that patients with PV had a significantly poorer survival compared with individuals from the normal Swedish population and also compared with the ET cohort. On the other hand, no significant survival difference was found when the patients with ET were compared with the general population.

The risk of AML transformation in patients with Ph-MPN is a well-established phenomenon and a wide range of transformation frequencies have been reported (1, 2, 4, 5, 66, 69). It is true that the transformation frequencies are dependent on subtypes of Ph-MPN and MF patients run a greater risk of transformation to AML compared with patients with PV and ET.

It is well known that the use of P^{32} and combinations of alkylating agents is associated with a higher incidence of AML development (2, 66, 69-72), while other treatments like IFN have no documented leukemogenic effect (73) and large retrospective and population-based studies have repeatedly shown the absence of an association between hydroxyurea and leukemia development (2, 74). Hydroxyurea is by far the most frequently used agent in our studies.

In our population-based study, which included all the Ph-MPN patients in two comparable regions in Sweden and France (Paper I), a total of 795 patients were studied. During the median follow-up period of 15 years, 56 (7%) subjects developed AML. The frequency of AML development in MF (16%) was significantly higher compared with both PV (5.7%) and ET (5.6%). These results are almost identical to those in the study conducted by Tefferi et al. (66). We also showed that the transformation to AML in MF patients could not possibly be explained by pre-transformation therapy, since most of MF patients did not receive any cytoreductive treatment. The finding that 14% of patients who developed

AML had not been treated with myelosuppressive agents, apart from interferon-α, highlights the fact that factors other than treatment-related factors might play a role. Our findings are consistent with those of a population-based study performed by Björkhoim et al., which showed that a quarter of MPN patients who developed AML/MDS were never exposed to cytoreductive therapy (69). We also showed that AML transformation could occur at any time in the disease course, by demonstrating a relatively steady and continuous risk of transformation over time. The yearly rate of AML transformation appeared to be fairly consistent in all MPN entities. Our observation does not support the hypothesis that AML transformation only occurs late in the course of Ph-MPNs after years of treatment with myelosuppressive agents. Our results indicate that MPNs run an intrinsic risk of transformation into AML and this supports the hypothesis that the occurrence of unfavorable mutations in the course of MPNs plays a major role in AML transformation.

One striking result in our studies was that 17 of 18 PV patients who developed AML were female, in spite of the fact that the male/female ratio for the whole PV population was 0.85. The fact that the mean age for the diagnosis of female PV subjects was 72 years, while it was 69 years for males, cannot be the only explanation. This finding has also been high-lighted in the work by Finazzi et al. (2), who concluded that women with PV ran a significantly higher risk of AML development compared with men ($p < 0.002$). On the other hand, a study conducted by Björkholm et al. (69) did not reveal any significant gender differences, but the risk was higher in females without attaining statistical significance. We have no explanation for our finding. We believe that the use of the NGS-method will be helpful in identifying risk patients in the future. A recent study with NGS-method revealed that adverse variant mutations, which are associated with inferior survival, such as ASXL1, SRSF2 and SH2B3, had a prevalence of 15% in both ET and PV (75).

It has been illustrated in several retrospective cohort studies that a number of factors may play a role in survival. Long disease duration and higher age at diagnosis are independent risk factors for both for ET and PV (1, 76-78). In accordance with the aforementioned studies, our study (Paper II) showed that disease duration and age in both ET and PV were highly significant risk factors ($p = 0.0006$ and $p=0.0001$ respectively for ET and $p = 0.0038$ and $p=0.0001$ respectively) for PV.

In **Paper III**, we have shown that younger patients with PV, i.e. those aged < 65 years at the time of diagnosis, have a median OS of 17.5 years, similar to what has been described by Kiladjian et al. (79).

According to our study (**Paper II**), the clinical finding at diagnosis that affected survival significantly in ET was low hemoglobin and this is in accordance with other studies (80, 81). Like those in other studies, our results confirm that a low hemoglobin concentration is an independent risk factor for transformation to MF and AML. At the time of diagnosis, splenomegaly in PV resulted in a significant impairment of survival. Splenomegaly has previously been shown to be a risk factor for the development of MF (82). Leukocytosis is a risk factor for thromboembolic events in both PV and ET and this finding likely explains the negative impact of leukocytosis on survival (Papers II-III), which is consistent with other studies (83-85).

Using multivariate analysis for relative survival (RS), we have shown (**Paper III**) that age > 70 years, leukocytosis with WBC > 13 x 10^{97}/L and thrombotic event prior to diagnosis were independent factors for poor RS. For younger (age < 60 years) patients, only earlier thrombotic events at diagnosis predicted poor survival. A model for RS in PV was constructed. We defined three risk groups: low with none of the risk factors mentioned above, intermediate with one and high with two or three risk factors. The ten-year RS was 84%, 59% and 26% in the low, intermediate and high risk groups respectively ($p < 0.001$). A multi-center study performed by Tefferi et al. identified advanced age, leukocytosis, a history of venous thrombosis and an abnormal karyotype as risk factors for OS (86).

To the best of our knowledge, our registry-based study (**Paper IV**) is the largest truly population-based study of PV and ET patients. Registration in the MPN registry is mandatory for both pathology departments and clinicians from hematology departments and this ensures good coverage of newly diagnosed Ph-MPNs, in our case 95%.

The incidence of PV appears to be consistent with previous reports from western Sweden, i.e. with patients diagnosed during the last decades of the 20th century (33). On the other hand, a clear increase in the ET incidence has occurred during our inclusion period. This could have different explanations; the change in the criteria by the WHO in 2008 (12), with a lowering of the threshold for platelets from 600 x 10^9 /L to 450 x $10^{9/}$/L, the discovery of driver mutations which are very important

tools in the work-up of patients with thrombocytosis and the use of automated blood sample analysis that brings more patients into diagnostic consideration.

The fact that 35% of patients with ET and 37% of patients with PV had already experienced at least one vascular event prior to diagnosis highlights the fact that vascular complications before diagnosis are a common phenomenon and this is confirmed in other studies (39, 42). A rapid diagnosis, when Ph-MPN is suspected, and the early start of treatment in those with high-risk disease are important and could possibly prevent many vascular complications.

It has been shown in several studies that the presence of the JAK2 V617F mutation in ET appears to be a risk factor for vascular complications (47, 87). In our study (**Paper IV**), we were also able to show that the frequency of this mutation was significantly higher (71%) in those with vascular complications, prior to diagnosis, compared with those without (60%) ($p=0.016$). Our results do not prove that treatment lowering the JAK2 V617F allele burden is able to decrease the risk of vascular events, but it could be hypothesized that therapy affecting this driver mutation also produces a favorable prognosis in terms of minimizing thromboembolism and hemorrhages. Follow-up data are reported to the National MPN Registry every three years and it will hopefully be possible to answer this question in up-coming reports.

The role of leukocytosis as a risk factor for thromboembolism has been discussed for many years, despite the fact that several studies have shown an association between elevated WBC and vascular events (88, 89). It is included in the IPSET prognostic model of survival and it is currently unclear how this information should be applied in clinical treatment decision-making (88, 90). In our studies, we have found a significant association between an elevated WBC count and thromboembolism, both in the retrospective cohort study **(Paper II**) and in the prospective registry study (**Paper IV**). Elevated leukocytes in PV and a WBC of $> 12 \times 10^9$/L in ET were thus found to be independently associated with the presence of vascular complications prior to diagnosis, when multivariate analysis was performed.

The goal of treatment in ET, as recommended by the Nordic care program, is lowering the platelet count ($< 400 \times 10^9$/L), even though no studies have demonstrated a significant correlation between platelet concentration and thrombosis. Nor did we find any relationship between

the platelet level and vascular events in our two studies addressing this question (**Papers II** and **IV**). Results from the prospective primary thrombocythemia (PT-1) trial indicate a significant association between thrombosis and leukocytosis but not thrombocytosis (55). This suggests that the effort of keeping the platelet count below 400 x 10^9/L can be seen as a surrogate goal and treatments lowering platelets probably also affect other blood parameters, such as WBC, and thereby have a positive effect on the risk of vascular events. Future treatments should not primarily be focused on lowering the platelet count for thrombosis prevention.

The fact that patients with low hemoglobin at diagnosis in PV ran a significantly higher risk (p=0.021) of thromboembolic events prior to diagnosis was unexpected. It could be hypothesized that the low hemoglobin is not the cause of vascular events, but the higher leukocytes that we found are instead associated with the low hemoglobin concentration. Another possible explanation might be that some of these PV patients with low hemoglobin had masked PV (mPV) and may have had the disease undiagnosed for a long time and thereby been at risk of thromboembolic complications for a long time. A study performed by Barbui et al. compared patients with overt PV and mPV. Patients with mPV displayed a more frequent history of arterial thrombosis (91). The term mPV was re-introduced for JAK-mutated patients who revealed PV-characteristics in their bone marrow morphology but displayed lower levels of hemoglobin, i.e. between 16 and 18.5g/dL for men and 15-16.5g/dL for women. This was one of the rationales for revising the WHO classification for MPNs in 2016.

We also showed that patients with PV, at diagnosis, had significantly more constitutional symptoms compared with ET patients, which is consistent with the findings described by Abelsson et al., who reported the most impaired quality of life in newly diagnosed PV patients compared with other MPN entities (92).

In conclusion, our studies indicate that the subtype of Ph-MPN, age, duration of MPN, low hemoglobin and/or leukocytosis at diagnosis have an impact on survival. The JAK2 V617F mutation in ET is a risk factor for thromboembolic events and these events may occur prior to diagnosis in more than one-third of patients.

6 SUMMARY AND CONCLUSIONS

Paper I

In the retrospective study of 795 Ph-MPN patients with a median observation time of 15 years, 56 patients developed AML. The frequency of AML development was significantly higher in patients with MF compared with patients with ET and PV. The average time from MPN diagnosis to AML was significantly shorter in patients with an initial diagnosis of MF compared with PV and ET.

Patients with MF therefore have inferior survival compared with both PV and ET patients and are more prone to transform into AML. Average survival after a post-MPN AML diagnosis was short and that was independent of the MPN subtypes.

Paper II

We investigated a total of 280 PV and ET patients retrospectively. Over the median observation time of 15 years, patients with PV had significantly shorter survival compared with a comparable general population. ET patients did not demonstrate any significant difference in survival compared with the general population. Low hemoglobin at diagnosis in ET and splenomegaly in PV were risk factors for poor survival. Increased reticulin content and splenomegaly at diagnosis in PV predicted transformation to MF, while the corresponding risk factors in ET were increased reticulin, low hemoglobin and a high WBC.

PV patients thus have inferior survival compared with patients with ET. In ET, low hemoglobin at diagnosis predicted not only inferior survival but also transformation to MF, while in PV the same was true for splenomegaly at diagnosis.

Paper II

This retrospective study of 327 patients with PV addresses factors affecting survival. Multivariate analysis identified three independent risk factors; age > 70 years, WBC > 13 x 10^9 /L and thromboembolism prior to diagnosis. The 10-year RS for patients with no risk factors was 84%, with one risk factor 59% and with two or three risk factors 26%.

We are therefore able to classify PV patients into three risk groups: high risk with two or three risk factors, intermediate risk with one risk factor and low risk with no risk factors.

Paper IV

A prospective observational registry study comprising a total of 1,105 patients with PV and 1,284 patients with ET showed the presence of vascular complications, prior to diagnosis, in 35% of the ET and 37% of the PV population. Multivariate analysis identified three independent risk factors for vascular complications in ET; age > 65years, WBC > $12x10^9$/L and the presence of the JAK2 V617F mutation, while in PV low hemoglobin was the only significant risk factor.

The fact that vascular complications among newly diagnosed PV and ET had affected more than one-third of these patients therefore underlines the importance of early diagnosis and treatment to prevent further complications. Myelosuppressive treatment should be considered in JAK2-V617F-mutated ET, irrespective of age.

7 FUTURE PERSPECTIVES

Our future- plan in this field is to analyze the presence of other mutations by using NGS (next generation sequencing). Special emphasis will be placed on those patients with triple negativity, i.e. who do not harbor JAK 2 V617, MPL or CalR mutations. Other patient categories that will be of interest in this respect are patients with transformation.

We are also planning a further analysis of the MPN registry cohort in upcoming years i.e. from the follow-up data which will be reported to the National MPN Registry every three years. We believe this will provide us with more information about the outcome for the MPN cohort in a "real-world" setting.

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