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MORBIDITY AND CHILDBIRTH IN MYELOPROLIFERATIVE NEOPLASMS

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Karolinska Institutet

Stockholm 2023

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2023

ISBN 978-91-8016-953-0
Cover illustration: Pegasus by Carl Milles, photo Yanan Li

Morbidity and Childbirth in myeloproliferative neoplasms

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at Gene, Neo, Flemingsberg, April 21st 2023 9.00 AM

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To Agnes, Albert and Kristian, my beloved family

POPULÄRVETENSKAPLIG SAMMANFATTNING

Myeloproliferativa neoplasier (MPN) är en grupp av kroniska blodcancersjukdomar, där man har för mycket röda blodkroppar (polycytemia vera), blodblättar (essentiell trombocytemi) eller fiberbildning i benmärgen (myelofibros). De flesta människor med MPN lever länge med sin sjukdom, även om sjukdomen kan medföra komplikationer. Patienter med MPN har tex en högre risk att drabbas av blodproppar. Sjukdomen kan också utvecklas, fiberbildning kan tillkomma, och för en del patienter med MPN övergår sjukdomen i en akut leukemi.

Den här avhandlingen fokuserar på annan sjuklighet hos MPN-patienter. I den första studien visas att patienter med MPN har en högre risk för att drabbas av andra sekundära cancersjukdomar jämfört med människor utan MPN, framför allt hudcancrar.

I den andra studien visas att patienter med MPN har en ökad risk för att drabbas av allvarliga infektioner än individer utan MPN. Det tycks inte spela någon roll vilken typ av läkemedel man behandlas med, däremot så är risken större hos de som har myelofibros, dvs fiberbildning i benmärgen.

Majoriteten av de som drabbas av MPN är i övre medelåldern, men 10-20% är i fertil ålder vid diagnos. I den tredje studien undersöks hur det går för MPN-patienter i samband med graviditet. Generellt går det bra, men det finns en ökad risk för att barnen föds för tidigt. Det visas också att graviditet vid MPN är vanligare än man tidigare trott.

I den fjärde studien visas att patienter med MPN i genomsnitt har lägre födslotal jämfört med individer utan MPN. Personer med essentiell trombocytemi, den vanligast typen av MPN hos unga kvinnor, har ungefär samma födslotal som friska kvinnor, däremot sågs att kvinnor med andra typer av MPN har lägre födslotal. Missfall var inte statistiskt säkerställt ökat, dödfödsel var för ovanligt för att någon skillnad skulle kunna påvisas.

ABSTRACT

Myeloproliferative neoplasms (MPN) are a family of chronic hematologic cancers, characterized by excess proliferation of myeloid cell lineages, or fibrosis of the bone marrow. Patients with MPN generally have a long expected survival. To elucidate morbidities during the disease course, e.g. second malignancies and infections, and outcome and prognosis of pregnancy and childbirth, we performed four large population-based cohort studies based on data from Swedish health registers, and compared outcomes to those of matched controls.

We found that patients with MPN are at increased risk of second cancers, both solid and hematologic. The hazard ratio (HR) of developing a solid cancer was 1.6 (I.5-1.7), where skin cancers had the largest risk increase, but cancers of the brain, lung, pancreas, kidney and endocrine organs were also significantly increased.

Patients with MPN also had a twofold risk of infections, HR 2.0 (1.9-2.0), leading to hospitalization or death compared to controls. An increase was evident in all subtypes of MPN, but significantly higher in patients with primary myelofibrosis.

Among women with MPN, there were 342 pregnancies beyond gestational week 22/28 in women with MPN in Sweden 1973-2018. Preterm birth, in particular iatrogenic preterm birth, was significantly increased, but not thrombosis, bleeding or other obstetric complications. Low birthweight was similarly increased to preterm birth, but there was no increase in low birthweight babies in pregnancies with term delivery. The incidence of childbirth during the last decade was 12.2 per 100,000 childbirths.

In women with MPN birthrates were reduced by 22%, HR 0.78 (0.67-0.90) compared to matched controls. In a subgroup analysis, the HR of childbirth was not reduced in patients with essential thrombocythemia. The rate of miscarriage was not statistically significantly increased, HR 1.25 (0.89-1.76.) Stillbirth was significantly more common in MPN patients prior to the MPN diagnosis, (p=0.013).

In conclusion, there is significant morbidity in the MPN population, with increased risk of second cancers and infections. Pregnancy outcomes are generally better than previously anticipated, however there is an increased risk of preterm birth, and birthrates in MPN are lower than in the general population.

LIST OF SCIENTIFIC PAPERS

I. **Landtblom AR,** Bower H, Andersson TM, Dickman PW, Samuelsson J, Bjorkholm M, et al. Second malignancies in patients with myeloproliferative neoplasms: a population-based cohort study of 9379 patients. Leukemia. 2018;32(10):2203-10.

II. **Landtblom AR**, Andersson TM, Dickman PW, Smedby KE, Eloranta S, Batyrbekova N, et al. Risk of infections in patients with myeloproliferative neoplasms-a population-based cohort study of 8363 patients. Leukemia. 2021;35(2):476-84.

III. **Landtblom AR**, Andersson TM, Johansson ALV, Wendel SB, Lundberg FE, Samuelsson J, et al. Pregnancy and childbirth outcomes in women with myeloproliferative neoplasms-a nationwide population-based study of 342 pregnancies in Sweden. Leukemia. 2022;36(10):2461-7.

IV. **Landtblom AR**, Andersson TM, Johansson ALV, Lundberg FE, Samuelsson J, Björkholm M, et al. Childbirth Rates in Women with Myeloproliferative Neoplasms – a population based matched cohort study - *Manuscript*

These articles are referred to by their roman numerals throughout, and are presented in full at the end of this thesis

RELATED SCIENTIFIC PAPERS NOT INCLUDED IN THESIS

Hultcrantz M, **Ravn Landtblom A**, Andreasson B, Samuelsson J, Dickman PW, Kristinsson SY, et al. Incidence of myeloproliferative neoplasms - trends by subgroup and age in a population-based study in Sweden. J Intern Med. 2020;287(4):448-54.

Ahlstrand E, Samuelsson J, Lindgren M, Pettersson H, Liljeholm M, **Ravn-Landtblom A**, et al. Highly reduced survival in essential thrombocythemia and polycythemia vera patients with vascular complications during follow-up. European journal of haematology. 2020;104(3):271-8.

Lindgren M, Andreasson B, Samuelsson J, Pettersson H, Enblom-Larsson A, **Ravn-Landtblom A**, et al. Survival and risk of vascular complications in myelofibrosis-A population-based study from the Swedish MPN group. European journal of haematology. 2022;109(4):336-42.

Batyrbekova N, Bower H, Dickman PW, **Ravn Landtblom A**, Hultcrantz M, Szulkin R, et al. Modelling multiple time-scales with flexible parametric survival models. BMC Med Res Methodol. 2022;22(1):290.

Landtblom AR, Ungerstedt J, Hedlund A, Tobiasson M, Deneberg S, Jadersten M. Omalizumab alleviates pruritus in myeloproliferative neoplasms. Haematologica. 2023. *Accepted*

CONTENT

LIST OF ABBREVIATIONS

AML	Acute myeloid leukemia
HSCT	Hematopoietic stem cell transplantation
BAT	Best available therapy
CI	Confidence Interval
DIPSS	Dynamic International Prognostic Score system
ET	Essential thrombocythemia
EBMT	European Society for Blood and Marrow Transplantation
ELN	European Leukemia Net
HELLP	Hemolysis, elevated liver enzymes, low platelet counts
HR	Hazard ratio
HU	Hydroxyurea
IFN	Interferon
IPSS	International Prognostic Score System
IPSET	International Prognostic Score for Essential Thrombocythemia
LMWH	Low molecular weight heparin
MF	Myelofibrosis
MIPSS	Mutation Enhanced International Prognostic Score System
MPN	Myeloproliferative Neoplasms
MPN-U	MPN-Unclassifiable
PMF	Primary Myelofibrosis
Pre-PMF	Prefibrotic primary myelofibrosis
PV	Polycythemia Vera
RCT	Randomized Clinical Trial
SIR	Standardized Incidence Ratio
VAF	Variant allele frequency

1 INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a family of closely related chronic hematologic malignancies, characterized by an excess proliferation in myeloid cell lineages and/or bone marrow fibrosis. The family consists of Polycythemia Vera (PV) characterized by high hemoglobin and hematocrit, Essential Thrombocythemia (ET) characterized by increased platelet levels, and primary myelofibrosis (PMF) characterized by increased fibrosis in the bone marrow. PMF can be further subdivided into prefibrotic myelofibrosis (pre-PMF), and overt PMF with more reticulin fibrosis in the bone marrow. MPN-Unclassifiable is MPN that cannot be further subclassified into PV, ET, or PMF. These diseases share clinical, morphological, and molecular features with each other but constitute distinct disease entities.(1)

MPNs are indolent in nature, in particular ET, followed by PV, with PMF being associated with a more prominent shortening of life expectancy. PV and ET may progress to secondary myelofibrosis, and all MPN may transform to blast phase MPN, with dismal prognosis. Complications associated with MPN with high impact on morbidity and mortality are thrombotic and hemorrhagic events. MPNs are mainly diagnosed in middle-aged or elderly individuals, but 10-20% of patients are of fertile age at diagnosis.(2)

In this thesis, additional complications were investigated; second cancers in paper I and infections in paper II. Prognosis of childbearing and pregnancy are explored in paper III and IV.

The purpose of assessing complications and childbearing is to improve management of patients with MPN. Accurate knowledge on risks and prognosis are essential for optimizing patient care.

2 LITERATURE REVIEW

2.1 EPIDEMIOLOGY

The incidence of MPNs is 1.5-2 per 100 000 person/year for PV and ET respectively, and 0.6 per 100 000 person/year each in PMF and MPN-U respectively. There are thus 400-500 new diagnoses of MPN in Sweden each year. Since MPN patients have an expected long survival, the prevalence of MPN is higher than indicated by incidence. The median age at MPN diagnosis is 65-70 years but may occur in all ages, including childhood and young adults, Table 1. There are some gender differences in incidence of MPN, with ET having a slightly higher incidence in women, and PV, PMF, and MPN-U a higher incidence in men.(2, 3) In Sweden, the number of patients diagnosed with MPN-U has decreased over time, which is likely attributable to more accurate classification of subtypes.(2)

Age (years)	Number MPN diagnosed 2000–2014	Crude incidence rate/ 100 000 person-years (95% CI)
<18	11	0.04 (0.02–0.07)
18–39	274	0.70 (0.62–0.79)
40–49	431	2.32 (2.11–2.55)
50–59	869	4.83 (4.52–5.17)
60–69	1451	9.53 (9.05–10.03)
70–79	1926	18.61 (17.79–19.45)
≥80	1319	18.26 (17.30–19.28)

CI, confidence interval; MPN, myeloproliferative neoplasm.

Tabel 1. Incidence of MPN in Sweden by age category.(2)

2.2 HEREDITY AND ETIOLOGY

Hereditary factors can affect the risk of developing MPN. The risk is increased by 5-7-fold in first-degree relatives of MPN patients.(4) Familial clustering occurs, 8% of MPNs are reported to be familial.(5) There are two known heredity mechanism; one is a commonly occurring haplotype in *JAK2* 46/1 that increases the risk of developing MPN with a low penetrance,(6) and the second a variant in the *TERT* gene with a stronger association to MPN.(7)

The risk of developing MPN is higher in patients with autoimmune diseases.(8) Agricultural and petrochemical occupational exposure including benzene, are shown in some studies to increase the risk of MPN. An association is described between smoking and PV, and with obesity and ET.(9-12) However, associations of external exposures are week, and not consistent through different studies.

2.3 A HISTORICAL PERSPECTIVE

Gustav Heuck described PMF in 1879 in two young patients with massive splenomegaly and characteristic findings in blood and bone marrow.(13) PV was first described by Louis Henri Vaquez in 1892, with speculations of hematopoietic hyperactivity.(14) The clinical picture was confirmed and more extensively described by William Osler in 1903. The term Vaquez-Osler disease is an old eponym for PV.(14, 15) ET was first described as hemorrhagic thrombocythemia by Emil Epstein and Alfred Goedel in 1934, several others contributed to describing the clinical syndrome, as well as the morphologic features of blood and bone marrow.(16)

In an editorial in 1951 in Blood, William Dameshek concluded that these conditions were closely related to one another, driven by an hitherto undiscovered stimulus and called them Chronic Myeloproliferative Disorders.(17) The Philadelphia chromosome was discovered in 1960, separating chronic myeloid leukemia from the other MPNs.(18) A common stem cell origin of clonal myeloproliferation was shown by Fialkow and colleagues in PV, ET, and PMF 1976-1981 based on G6PD isoenzymes studies.(19-21)

The Polycythemia Vera Study Group (PVSG) was started in 1967 by an American hematologist, Louis Wasserman, in the era of war on cancer and National Cancer Act. Diagnostic criteria were set up, and randomized clinical trials performed. The first was PSVG01 were venesectio alone was compared to venesectio in combination with chlorambucil or P32, favoring venesectio alone due to lower incidence of acute myeloid leukemia (AML). In another trial, venesectio alone was compared to hydroxyurea (HU), were HU was favored due to lower incidence of thrombosis, without an increase in AML. The PSVG was active until 1997.(22)

Venesectio is medical treatment with ancient roots, it has been used since Hippocrates days for a wide variety of conditions, but in modern medicine still has a place PV and hemochromatosis. HU and busulphan entered the scene in the 1970s. Historical treatments includes skeletal radiation therapy, lead compounds, nitrogen mustard, pipobroman, chlorambucil, uracil mustard, 6-mercaptopurin, and dapsone amongst others.(22) In a Danish

study from 1962, survival in PV patients was described, from first symptom or accidental finding of PV, half of the untreated patients died within 18 months, suggesting severity of natural disease course of symptomatic PV, Figure 1.(23)

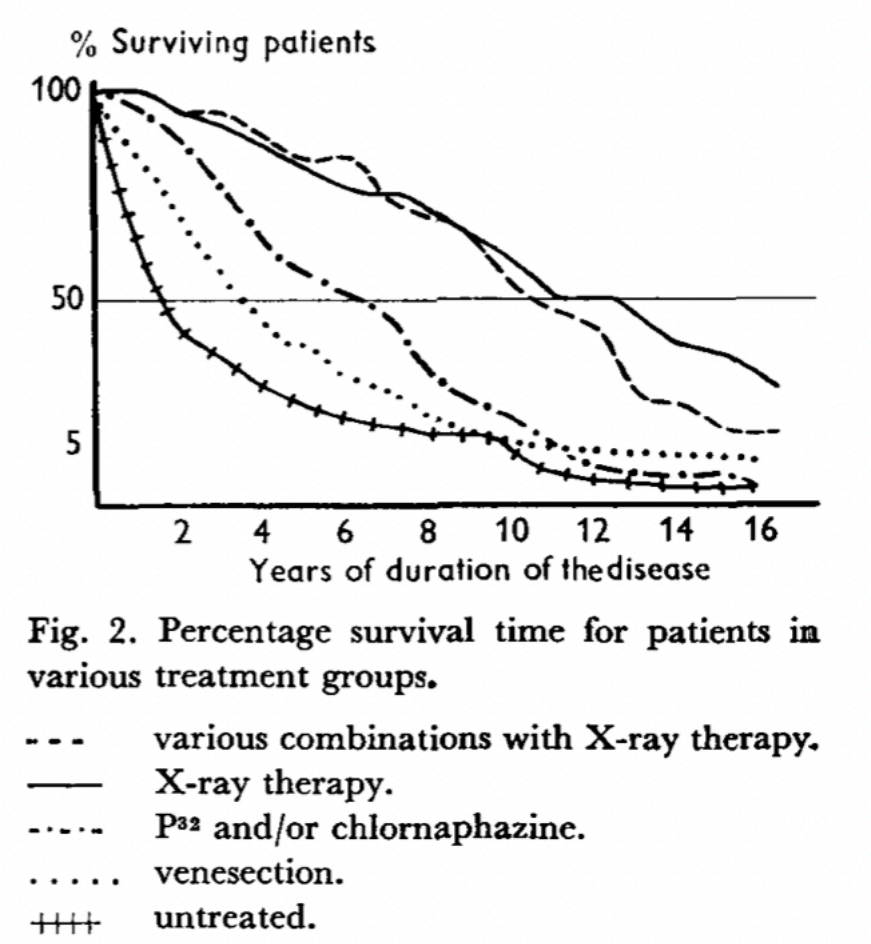


Figure 1. Survival in Danish patients with PV per treatment type.(23)

The MPNs close internal relationship and perhaps Dameshek's hitherto undiscovered stimulus was confirmed in the molecular era. First, the *JAK2* V617F mutation was described in four simultaneous publications in 2005 (24-27), followed by the *MPL*-mutation in 2006 (28) and the *CALR*-mutation in 2013.(29) They are considered driver mutations, being found in the majority of MPN patients. During recent years, there has been a rapid expansion of knowledge on genetic and molecular background in MPNs and other cancers. It has been demonstrated by extensive sequencing, that the MPN driver mutations emerge early in life, in utero or childhood, and the mutated clones expand slowly, to yield a phenotypic disease much later in life.(30)

2.4 DIAGNOSIS

2.4.1 Diagnostic criteria

An MPN diagnosis is established based on blood counts, clinical parameters, bone marrow morphology, and identification of driver mutations. The current diagnostic criteria used in Sweden are WHO 2016, Table 2 and 3.(31) In comparison with WHO 2008, the main changes include lowered thresholds for hemoglobin, (from 185 g/L in men and 165 g/L in women, to 165 g/L and 160 g/L) in PV and thrombocytosis (from 600 x 10^9/L to 450 x 10^9/L) in ET. Another important difference is that pre-PMF was recognized as a separate entity. Two new competing sets of diagnostic criteria for myeloid neoplasms were presented in 2022, WHO 2022(32) with minor adjustments regarding MPNs compared to WHO 2016, and ICC, the International Consensus Classification of myeloid neoplasms and acute leukemias.(33) There are no major differences in these three classifications regarding diagnosis of chronic phase MPN.

In Sweden, the PVSG diagnostic criteria was used until the turn of the millennium, when they were gradually replaced by WHO diagnostic criteria.(31, 34, 35) The Nordic MPN Study Group has presented management guidelines, and are very similar to European guidelines issued by European Leukemia Network (ELN).(36) Since 2021, there are national Swedish guidelines.(37)

	Polycythemia vera (PV) [a]	***Essential thrombocythemia (ET)*** [b]
Major criteria		
1	Hemoglobin > 16.5 g/dL(men) Hemoglobin > 16.0 g/dL (women) or Hematocrit > 49% (men) Hematocrit > 48% (women) or increased red cell mass (RCM)[c]	Platelet count ≥ 450 × 10^9/L
2	BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)	BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant left-shift of neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers[d]
3	Presence of *JAK2* or *JAK2* exon 12 mutation	Not meeting WHO criteria for *BCR-ABL1* + CML, PV, PMF, MDS, or other myeloid neoplasms
4		Presence of *JAK2*, *CALR* or *MPL* mutation
Minor criteria		
1	Subnormal serum erythropoietin level	Presence of a clonal marker (e.g., abnormal karyotype) or absence of evidence for reactive thrombocytosis

Table 2. Diagnostic criteria for PV and ET according to WHO 2016. PV requires all three major criteria or the two first major and the minor criteria. ET requires all four major criteria or the three first and minor criteria. (1, 31, 38)

	Primary myelofibrosis (PMF) [a]	
	Prefibrotic/early PMF (pre-PMF)	**Overt PMF**
Major criteria		
1	Megakaryocytic proliferation and atypia[b], without reticulin fibrosis > grade 1[c], accompanied by increased age-adjusted BM cellularity, granulocytic proliferation and often decreased erythropoiesis	Megakaryocyte proliferation and atypia[b] accompanied by either reticulin and/or collagen fibrosis (grade 2 or 3)
2	Not meeting WHO criteria for *BCR-ABL1* + CML, PV, ET, MDS, or other myeloid neoplasm	Not meeting WHO criteria for *BCR-ABL1* + CML, PV, ET, MDS or other myeloid neoplasm
3	Presence of *JAK2, CALR, or MPL* mutation or in the absence of these mutations, presence of another clonal marker[d] or absence of minor reactive BM reticulin fibrosis[e]	Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence, the presence of another clonal marker[d] or absence of evidence for reactive BM fibrosis[f]
Minor criteria		
1	Presence of one or more of the following, confirmed in two consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11×10^9/L • Palpable splenomegaly • LDH level above the upper limit of the institutional reference range	Presence of one or more of the following confirmed in two consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11×10^9/L • Palpable splenomegaly • LDH level above the upper limit of the institutional reference range • Leukoerythroblastosis

Table 3. Diagnostic criteria PMF, prefibrotic and overt PMF according to WHO 2016. (1, 31, 38) Diagnosis of both pre-PMF and overt PMF requires all three major criteria and one minor criterion.

2.4.2 Morphology

Bone marrow morphology is assessed on trephine biopsies. In PV, panmyelosis is present with trilineage proliferation and hypercellularity, often 80-100%. Maturation is complete, and low or depleted iron stores is a common finding. Megakaryocytes may be hyperlobulated. Peripheral smears may show erythrocytosis, microcytosis if iron deficiency is present, leukocytosis, thrombocytosis, and slight basophilia. In ET, the bone marrow cellularity is normal or mildly increased. Megakaryocytes are increased in numbers and atypical with large hyperlobulated nuclei, "staghorn" appearance, Figure 2. They may be located in loose clusters. Erythroid and granulocytic proliferation is usually normal. Fibrosis can be grade 1 or absent. Peripheral smears may show thrombocytosis with platelet anisocytosis.(39)

In pre-PMF there is usually hypercellularity and large dysplatic megakaryocytes, with hyperlobulated and hyperchromatic nuclei, often with aberrant ratio between nuclei and cytoplasm, and may be described as "cloud-like". They appear in loose or dense clusters. In over PMF, the marrow can by hypocellular, and interspersed with fibers, arbitrary graded 1-3. Bone trabeculae may be broad and bony, osteosclerosis. Peripheral smears in later stages of PMF shows leukoerythroblastosis and teardrop erythrocytes.(38, 40)

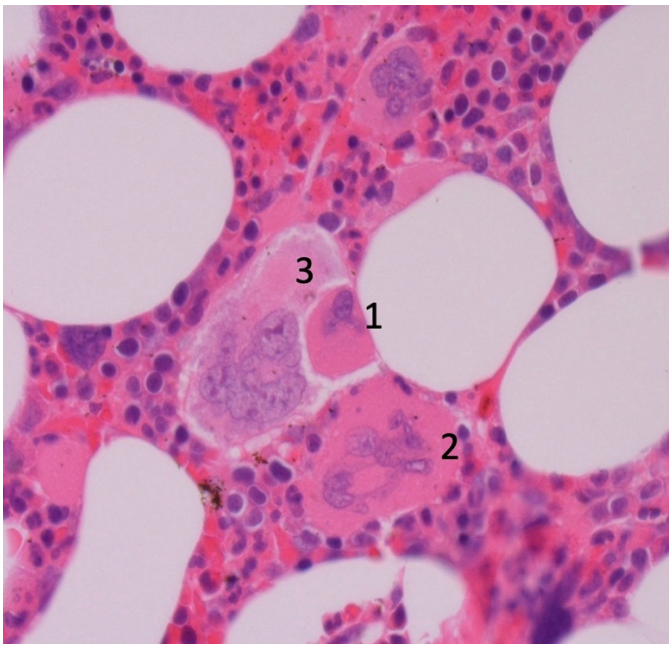


Figure 2. Megakaryocytes cluster. 1. Normal sized megakaryocyte 2. Enlarged megakaryocyte with hyperlobulated nucleus, as in ET. 3. Enlarged megakaryocyte with hypolobulated cloud-like nucleus as in PMF. Photo Birgitta Sander

2.4.3 Symptoms and quality of life

Symptoms that may occur in PV and ET are headaches, fatigue, dizziness, visual disturbances, and erythromelalgia, due to microcirculatory disturbances. Aquagenic pruritus, or pruritus related to external heat, for example in saunas, is most commonly found in PV, but may occur in all MPN subtypes.(41) Patients with PMF may in addition experience symptoms related to cytopenias, splenomegaly; abdominal discomfort and early satiety, or constitutional symptoms; recurring fever, night sweats, and cachexia. Patients can also experience pruritus, bone pain, and muscle pain.(42)

In a multinational online questionnaire-based study, symptoms were quantified in MPN patients. More than 90% of the respondents reported symptoms, the most frequent symptom being fatigue, Figure 3. The total symptom burden affects quality of life and work capacity negatively, and was not correlated to disease risk group classification. (42) This is supported

by data from the US and Sweden. (43, 44)

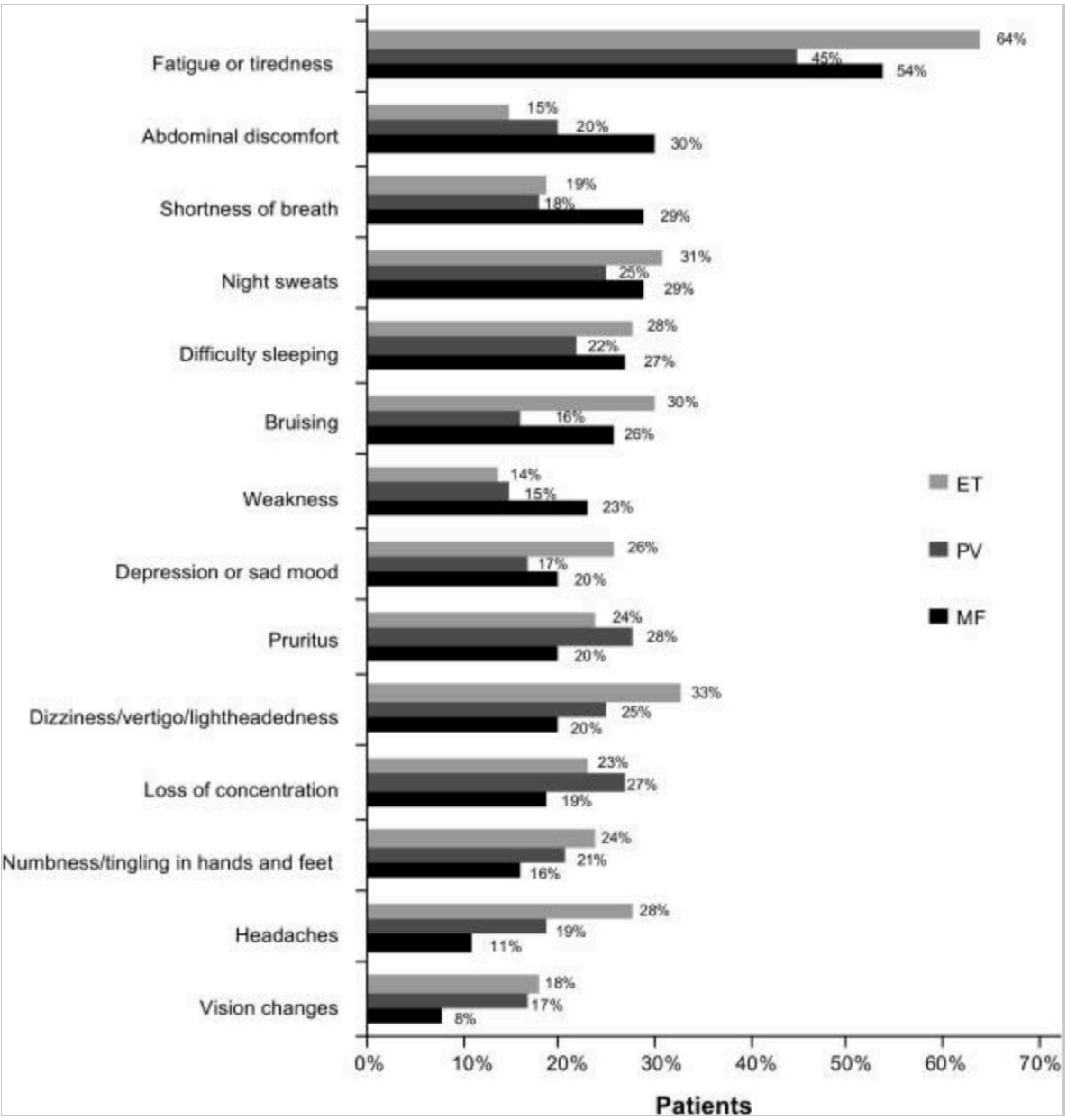


Figure 3. Symptoms described by 699 MPN patients in an online survey study.(42)

MPN-Symptom assessment form total symptom score (MPN-SAF TSS) is a validated instrument of grading symptoms, and commonly used in clinical studies.(45)

2.4.4 Molecular landscape

2.4.4.1 Driver mutations

There are three known driver genes; *JAK2* V617F or exon 12 (24-27, 46), *MPL* W515L mutation(28), and Calreticulin (*CALR*).(29, 47) The driver mutations are usually mutually

exclusive and all mutations results in activation of the JAK-STAT intracellular signaling pathway, Figure 4. The *JAK2* V617F mutation leads to a constitutive activation of the erythropoietin-receptor, and to a lesser extent the thrombopoietin-receptor and the granulocyte colony stimulating factor-receptor, while *MPL* and *CALR* mutations mainly activates to two latter receptors.(48) Studies using murine models have confirmed that *MPL* and *CALR*-mutations results in an ET or PMF phenotype, (28, 49, 50) while *JAK2* V617F in murine models may produce all MPN phenotypes.(51, 52) *CALR* mutations are heterogenous; two main variants exist, type 1 (52 base pair deletion) or type 1-like, and type 2 (5 base pair insertion) or type 2-like, with type 1 being more associated with myelofibosis, but also with thrombosis.(53) In general, *CALR*-mutated patients are generally younger at diagnosis and *CALR*-mutated ET patients have higher platelet counts and lower risk of thrombosis compared to those with *JAK2*-mutations. On the other hand, PMF patients with *CALR*-mutations have a better prognosis than *JAK2* or *MPL*-mutated cases.(54-56)

JAK2 mutations and to a lesser extent *CALR* mutations can also be present in individuals who do not fulfil the criteria for an MPN-diagnosis, in clonal hematopoiesis of indeterminate potential, and is found in increasing levels in older individuals. Also in non-MPN individuals, *JAK2* mutations are associated with an increased risk of thromboembolism and abnormal blood counts, and may precede a diagnosis of MPN.(57)

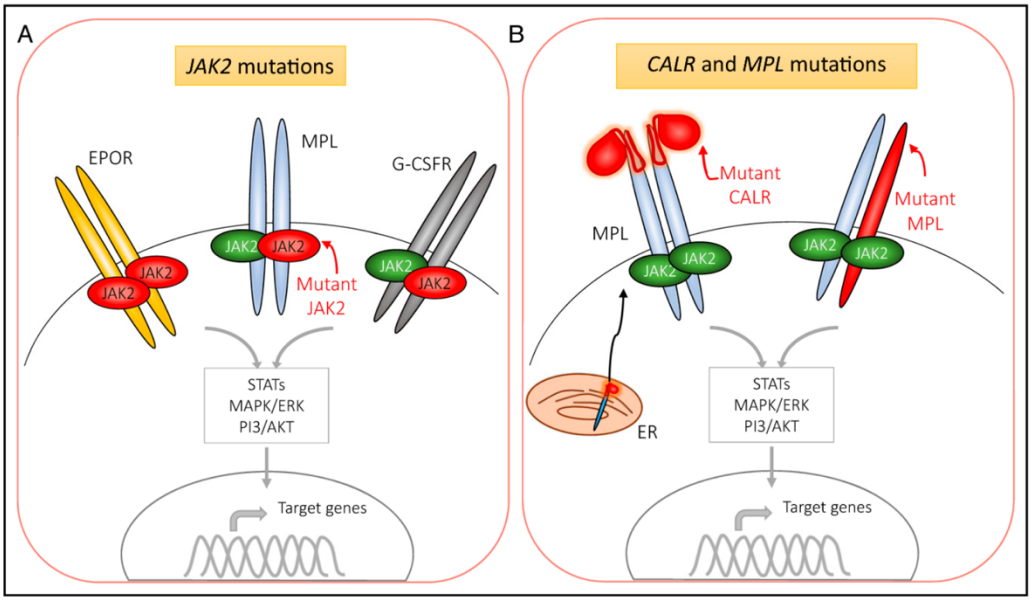


Figure 4. Mutant JAK2 (A) and mutant CALR and MPL (B) and their effects on the EPO-receptor, TPO-receptor, and G-CSF receptor.(48)

In PV, 95-98 % of patients harbor the *JAK2* V617F mutation. In *JAK2* V617F-negative PV cases, mutations in *JAK2* exon 12 may occur, and is associated with isolated erythrocytosis, Figure 5.(58) About 50 %-60 % of ET and PMF-patients harbor the *JAK2* V617F mutations, 20-40 % have *CALR* mutations and 4 % *MPL* mutations.(59) In 10-15 % of patients with ET and PMF, no known driver (*JAK2*, *MPL* or *CALR*) mutation is detected. These patients are heterogenous, and may carry other mutations of clonal hematopoiesis, or non-canonical mutations in *MPL* and *JAK2*.(60)

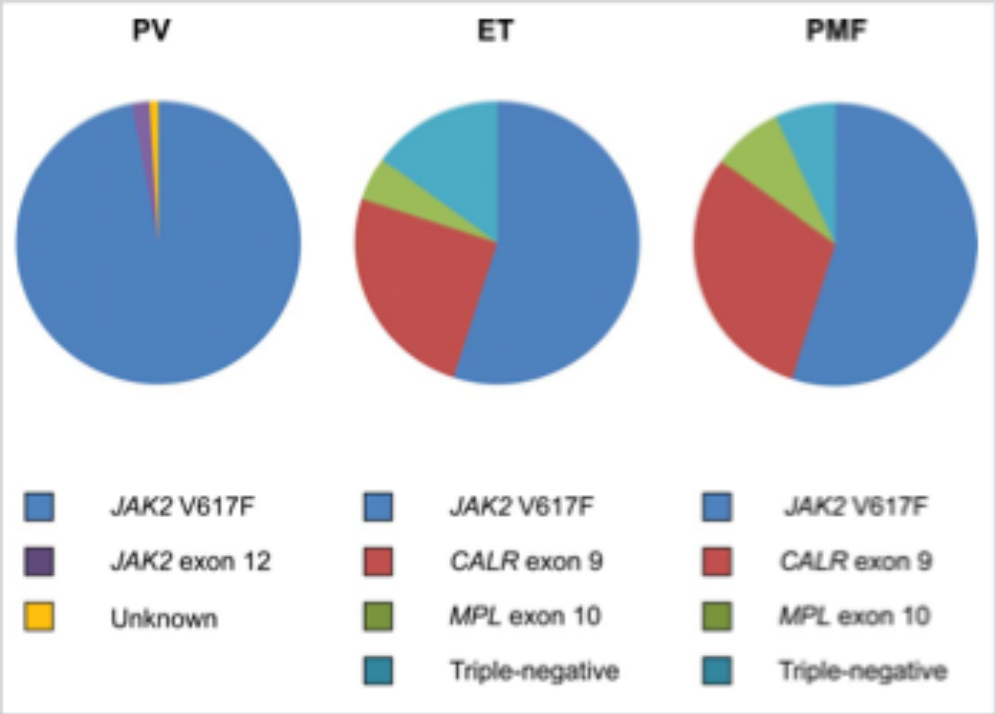


Figure 5. Proportion of driver mutations in PV, ET, and PMF. (61)

It is not fully understood why the same driver mutations can cause different MPN subtypes, and which other factors affects disease phenotype. The sequential order of acquisition of mutations have been shown to influence disease phenotype, which is most studied in *JAK2, TET2,* and *DNMT3A*. The order of mutations may affect the microenvironment and the competitive advantages of the clone.(62, 63) Clones with homozygosity for *JAK2*-mutations are commonly found in PV, while in ET most *JAK2* mutated clones are heterozygotic.(64) Additional mutations in *NFE2*, 9p-Loss of heterozygosity, or *JAK 46/1* haplotype favors erythroid proliferation and a diagnosis of PV, whereas germline polymorphisms associated with thrombocytosis favored a diagnosis of ET.(65)

2.4.4.2 Additional mutations

Additional mutations occur in one third of MPN patients, including *TP53* as well as mutations in genes involved in chromatin regulation and RNA splicing such as *EZH2,*

IDH1/2, ASXL1, SRSF2, KRAS, NRAS and *RUNX1* amongst others.(65) The number of additional mutations is higher in PMF than in ET and PV, and increases with advancing patient age.(29)

2.4.4.3 Cytogenetics

In chronic phase PV, 20%, have cytogenetic abnormalities, most commonly del 20q, +8, +9, -Y and is associated with inferior survival. Abnormalities increase in frequency in accelerated and blast phase, and complex karyotype is associated with blast phase.(66, 67) In chronic phase ET 7 % of patients are reported to have cytogenetic abnormalities, and is similarly associated with inferior survival.(68) In PMF, 30-55% are reported to have cytogenetic abnormalities at diagnosis, and cytogenetic abnormalities including complex karyotype predicts risk of leukemic transformation.(69-71)

2.5 RISK ASSESSMENT

In PV, conventional risk stratification is based on 1) previous thrombosis and 2) age above 60-65 years, and if one or both criteria are present, the patient is considered to have a high risk of thrombosis. This risk stratification is supported by the ECLAP-study and endorsed by the ELN guidelines.(72, 73) There are also reports suggesting that leukocytosis and cardiovascular risk factors affect risk of thrombosis and survival, although results are conflicting.(74, 75)

The International Prognostic Score for Essential Thrombocythemia (IPSET) has been demonstrated to be more precise in predicting risk of thrombosis in ET and the IPSET-thrombosis is currently recommended by the ELN.(73, 76-78)

There is also a Mutation enhanced International Prognostic Score System (MIPSS) for overall survival; MIPSS PV includes *SRSF2* mutation, age >67 years, leukocyte >15 $x10^9$/L, and history of thrombosis, while MIPPS ET includes mutations in *SRSF2, SF3B1, U2AF1,* or *TP53*, age >60 years, leukocytes >$11x10^9$/L, and male sex.(79)

In PMF, several risk scores for survival are available, based on clinical parameters alone or in combination cytogenetic and molecular parameters. The first was the Lille score, that included hemoglobin <100 and leukocytosis either <4 or >30 $x10^9$/L.(80) Based on clinical parameters are International Prognostic Scoring System (IPSS) was validated for use at diagnosis, and Dynamic International Prognostic Scoring System (DIPSS) was validated for

stratification along the disease course. They include the same parameters; age >65 years, presence of constitutional symptoms, hemoglobin <100 g/L, leukocytes >25 x10^9/dL, and circulating blasts ≥ 1%, but DIPSS doubles the prognostic weight of anemia.(81, 82) In DIPSS-plus, transfusions, thrombocytopenia <100 x10^9/L, and unfavorable karyotype was added, further increasing the stratification accuracy.(83)

The MIPPS70 score and later MIPPS70 plus version 2.0 improved the discrimination between risk groups, and includes bone marrow fibrosis grade, absence of *CALR* type 1 mutation, and presence of high molecular risk mutations; *ASXL1, EZH2, SRSF2, IDH1/2* and *U2AF1* (only in v 2.0), Figure 6. Severity of karyotype and anemia was included. The circulating blasts level was adjusted to ≥2%. Age was omitted in this risk score, as it was originally developed for allogenic stem cell transplantation candidates. (84, 85)

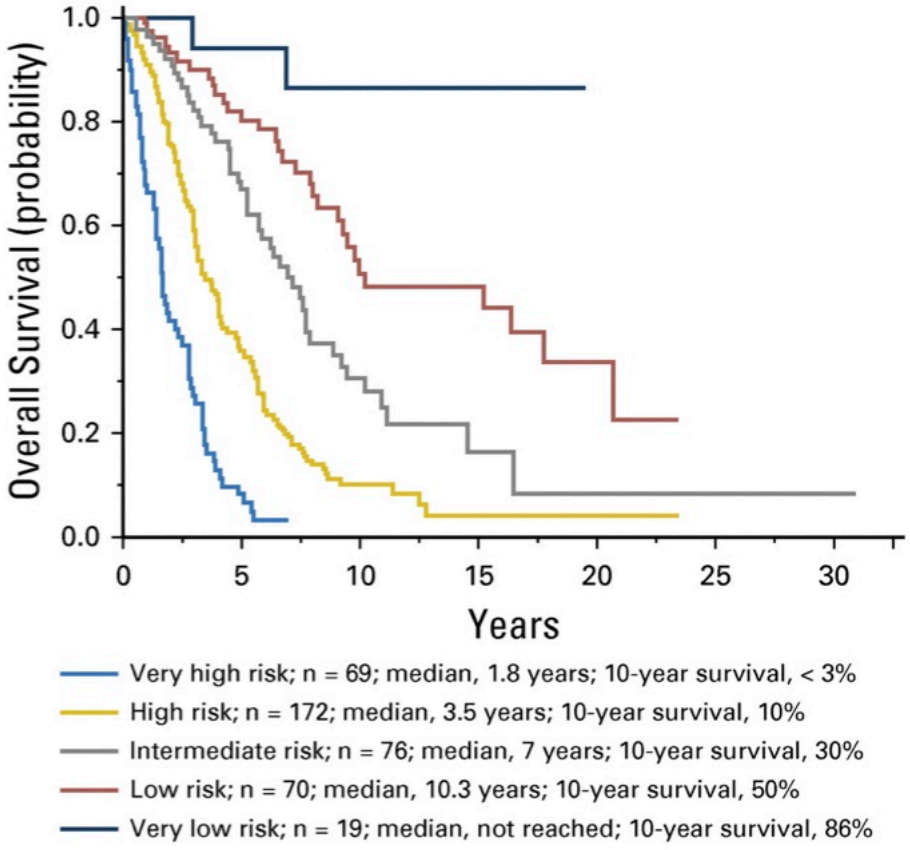


Figure 6. Risk stratification by MIPSS70+ version 2.0 in patients of all ages with PMF.(85)

Acknowledging that patients with secondary MF are different from those with PMF, Myelofibrosis Secondary to PV and ET Prognostic Model (MYSEC-PM) was developed specifically for post-ET and post-PV MF, mainly based on clinical parameters, but also

includes absence of *CALR* type 1 mutation.(86, 87) How to optimally integrate molecular data into risk stratification of secondary MF is not fully demonstrated.(88)

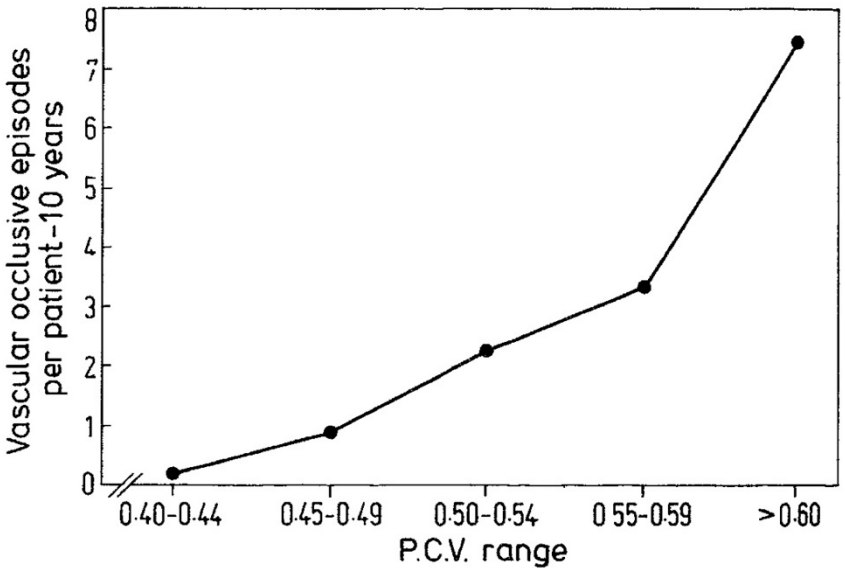
2.5.1 Treatment considerations

The goal of treatment varies depending on disease stage. In PV, ET, and early proliferative phases of myelofibrosis treatment goal is freedom from thrombohemorrhagic events, and treatment is aimed at normalizing blood counts.(89, 90) Indication of cytoreductive treatment is based on risk assessment, where it is indicated in high risk patients, patients with very high platelet counts, microvasculatory symptoms not otherwise manageable, or if venesectio is insufficient to keep target hematocrit <0.45.

Pre-PMF treatment considerations are extrapolated from ET and PMF.(91) In overt PMF, treatment is guided by risk assessment, and whether the patient has symptoms requiring specific management, such as anemia, splenomegaly, or constitutional symptoms.

2.5.2 Venesectio

An important determinant of blood viscosity is hematocrit.(92) Target hematocrit in PV have been historically debated, and threshold successively lowered, Figure 7.(93) In the CYTO-PV trial it was clearly demonstrated that keeping hematocrit by venesectio and/or HU below 0.45 compared to 0.45-0.50 significantly reduced the risk of major thrombosis and cardiovascular death, the rate was 1.1 vs 4.4 events per 100 person-years.(94)



Relation of P.C.V. range to number of vascular occlusive episodes per 10 patient-years in patients with primary proliferative polycythæmia.

Figure 7. Relation of vascular occlusive events and hematocrit in 69 PV patients 1960-1975.(93)

2.5.3 Aspirin

Aspirin was first shown to increase the risk of bleeding in patients with MPN by the PSVG, when investigated in doses of 900 mg per day in combination with dipyridamole.(95) Aspirin was later reintroduced in lower doses and was shown to be safe and efficacious in reducing vascular events in PV patients in the ECLAP trial.(96) In ET, there is no randomized controlled trial (RCT) to assess the value of aspirin, however in analogy with the ECLAP trial for PV and supported by retrospective data, aspirin is considered beneficial in ET.(97, 98) A protective effect from aspirin was however not evident in *CALR*-mutated patients without other risk factors.(99) Platelet turnover may be increased in ET patients, resulting in aspirin resistance, which can be overcome by twice a day dosing.(100) Aspirin may also reduce microvascular symptoms including erythromelalgia.(101) In pre-PMF and PMF the role of aspirin is less clear and requires individual assessment.(91)

2.5.4 Hydroxyurea

The anti-tumorous properties of HU has been known for long, at least since the 1960s.(102) It has been used in a wide variety of conditions, alone or in combination; MPN, viral infections, sickle-cell anemia, brain tumors, and is currently investigated for other conditions such as AML and Alzheimer disease.(103-106) HU acts by reversibly inhibiting ribonucleotide reductase and inhibits DNA synthesis and causes arrest in the S-phase of the cell cycle. In high doses or prolonged exposure DNA damages and oxidative stress occurs.(107)

HU has a well-documented efficacy in PV and ET in protecting against vascular events and is recommended as first-line cytoreduction by the ELN.(108-111) In the Cyto-PV trial keeping the hematocrit <0.45 by venesectio and HU was superior to a treatment goal of <0.50. (94) Propensity score matching of ECLAP data supports HU over venesectio alone in high-risk individuals, due to lower mortality and fewer cardiovascular events. Disease progression also tended to be lower in the HU-treated patients, although not significant.(112) Real-world retrospective data, albeit with short follow-up, supports protective effects against thrombosis and improved survival in older PV and ET-patients compared to non HU-treated patients.(113, 114)

In MF patients HU may reduce symptoms, splenomegaly, and hyperproliferation, but responses are generally not durable.(115)

HU is generally well tolerated, with discontinuation rates of 5-10%. Adverse events include gastrointestinal symptoms, pneumonitis, drug-fever, and mucocutaneous adverse events including cutaneous vasculitis, painful ulceration, premalignant and malignant skin conditions.(116, 117)

Cytoreductive therapy in relation to leukemic transformation have been a long-standing controversy, in particular regarding HU. A Swedish nested case-control study investigated treatment in relation to leukemic transformation and found that HU alone was not associated to increased risk of transformation. One fourth of patients experiencing transformation in that study had never received any cytoreductive therapy, suggesting the risk of transformation is inherent to the MPN itself.(118) However, there were indications of associations between pipobroman, busulphan and radioactive phosphorus (P32) and increased risk of transformation to AML, in particular when serially used.(118, 119) No association between IFN and leukemic transformation was shown.(120, 121)

2.5.5 Interferons

Recombinant interferon (IFN) was first reported to have effect in MPN in the 1980s, and has since been investigated in numerous trials.(122, 123) In a phase II trial of 79 patients, peg-IFNα 2a was shown to be efficient in normalizing blood counts and reducing symptoms, but also to reduce the variant allele frequecngy (VAF) of the *JAK2* mutation as well as normalize the histopathologic morphology.(124) The effects remain, in some patients several years after discontinuation. Most studies include recombinant IFN-α, during recent years most commonly pegylated, either α2a (Pegasys) or α2b (Pegintron), IFN-β is less studied. (125)

There has been a longstanding absence of randomized studies directly comparing the two main options of cytoreductive treatment, HU and IFN. In 2022 a study of 168 PV and ET patients randomized to HU or pegylated IFN-α2a, with similar effects on clinicohematologic response and thrombotic events. HU was superior in histopathologic response and IFN in molecular response. Adverse advents were more common in the IFN arm.(126) In the Danish DALIAH-trial, newly diagnosed MPN patients were randomized to HU or IFN, *JAK2* mutated patients were more likely to have a clinicohematologic response with reduced *JAK2* VAFs in comparison to *CALR*-mutated patients. *DNMT3A* mutations tended to occur increasingly during IFN treatment in comparison with during HU.(127)

Monopegylated ropeginterferon α2b, was studied in early PV in comparison with HU in PROUD-PV and CONTINUATION-PV, the complete hematologic response was similar at 12 months, and significantly higher at 36 months with ropeginterferon.(121) The VAFs of *JAK2* mutations were continuously decreasing in the ropeginterferon arm, but not in the HU arm.(128)

Adverse events associated to IFN treatment includes autoimmune, neuropsychiatric and flu-like symptoms. Discontinuation rates during long-term treatment were as high as 40-50% in the DALIAH trial, compared to 17% on ropeginterferon in PROUD-PV and 8% in CONTINUATION-PV.(121, 127) Direct comparisons between ropeginterferon and other pegylated IFN are lacking, there is however real-world descriptions of 5 patients with suboptimal response or intolerance on pegylated IFN, with improved outcomes on ropeginterferon.(129)

Evidence supports the use IFNs in early, low, or intermediate -1 disease MF, but not in advanced MF, however this may change with ropeginterferon or combinations.(130, 131)

2.5.6 JAK-inhibitors

The first JAK-inhibitor, ruxolitinib, was approved in 2011 after the COMFORT trials, and was shown to improve constitutional symptoms and reduces spleen size, in comparison with placebo and BAT.(132, 133) Adverse events included anemia, thrombocytopenia and opportunistic infections. Follow-up of the initial trial imply improved survival in the ruxolitinib arm despite cross-over.(134) In 2019 the second JAK2-inhibitor, fedratinib was approved in the US, with a warning about Wernicke encephalopathy, after the JAKARTA trials in Ruxolitinib-exposed and ruxolitinib-naïve patients.(135-137) Momelotinib was shown to have similar effects on spleen size, better effect on transfusion dependency but inferior effect on symptoms in the SIMPLIFY I trial, compared to ruxolitinib.(138) In a post-ruxolitinib setting, it failed to demonstrate improved spleen reduction compared to BAT.(138, 139) Pacritinib was assessed in the PERSIST trials, with superior spleen responses compared BAT excluding JAK inhibitors, and in thrombocytopenic MF patients, spleen responses were superior compared to BAT including ruxolitinib. Besides JAK-inhibition, it also exerts effect on *FLT3*.(140, 141) Momelotinib and pacritinib are thought to be more suitable options in cytopenic MF. Although JAK inhibitors are revolutionary for many MF-patients, responses are generally not durable, and anemia and thrombocytopenia may be severe dose-limiting toxicities. The prognosis in MF post ruxolitinib is poor and constitutes an unmet need.(142)

2.5.7 Other treatment options

Anagrelide reduces platelet levels, and was shown to reduce risk of thrombotic events in the PT1 and ANAHYDRET trials in ET patients.(110, 143) Other cytoreductive agents are intermittent busulphan and radioactive phosphorus, P32, where fear or increased leukemic transformations limits its use to elderly patients with intolerance to other options.(37, 144) Other agents used in PMF includes steroids, lenalidomide, danazol, and erythropoietin. Regular transfusions are an alternative in MF-related anemia.

2.5.8 Allogenic transplantation

Hematopoietic stem cell transplantation (HSCT) is a curative treatment option in myelofibrosis. Candidates for HSCT are selected by a high risk assessment, for example by DIPSS, DIPSS+ or MIPSS70+ v2 in PMF or in case of post-PV or post-ET myelofibrosis on MYSEC-PM.(82, 83, 85, 145, 146) Patients with intermediate -2 or high-risk disease, aged <70 years are considered for HSCT. ELN/EBMT 2015 consensus also recommends considering HSCT in intermediate-1 patients <65 years of age with transfusion dependence, circulating blasts in peripheral blood >2%, or adverse cytogenetics.(147)

Non-relapse mortality limits the use of HSCT in MF.(148) A myelofibrosis-specific Transplant Scoring System has been developed to predict transplantation outcomes, MTSS.(149) Age was previously shown to be a strong factor for inferior outcome, and survival was reduced from those over 55 years of age.(150) The negative impact of high risk-mutations may be overcome by HSCT.(151) Splenomegaly negatively affected both non-relapse mortality and time to engraftment, and requires management prior to transplantation, either by JAK inhibition or splenectomy.(152) Outcomes of transplantation were significantly better in chronic phase MPN than in accelerated (10-19% blasts) or blast phase (>20% blasts).(153, 154)

2.5.9 Novel agents in clinical trials

Several new treatment options are being investigated, particularly for MF, many but not all in combination with ruxolitinib. Phase II trials were recently presented for numerous promising agents. Navitoclax is a BCL-X_L/BCL-2 inhibitor where improvements were seen in anemia, fibrosis, and constitutional symptoms in patients resistant to ruxolitinib .(155) The BET

inhibitor pelabresib yielded splenic, morphologic, and symptomatic responses in combination with ruxolitinib in ruxolitinib-resistant patients.(156) Imetelstat is a telomerase inhibitor, where improvements in morphology, molecular response, and clinical benefits were demonstrated in a recent phase II trial.(157) In PV, rusferitide, a hepcidin mimetic, have been shown to reduce the need of venesectio and maintain target hematocrit.(158) The LSD-1 inhibitor bomedemstat was well tolerated and found to reduce platelet counts, constitutional symptoms, and allelic burden in ET, with manageable toxicities.(159) Many of these agents are currently being tested in phase III trials, with potential to change current guidelines.

2.6 DISEASE PROGRESSION

2.6.1 Secondary Myelofibrosis

Patients with PV and ET can progress to secondary MF and are then referred to as post-PV/ET MF, Figure 8. Secondary MF bears many similarities to PMF, but are not identical.(87) The cumulative incidence of progression to MF is quantified to 6-14 % in 15 years in PV.(160) In ET, the 10 year cumulative incidence of myelofibrotic progression is 0.8-4.5 %, and depends on accurate distinction of ET from pre-PMF.(66, 161)

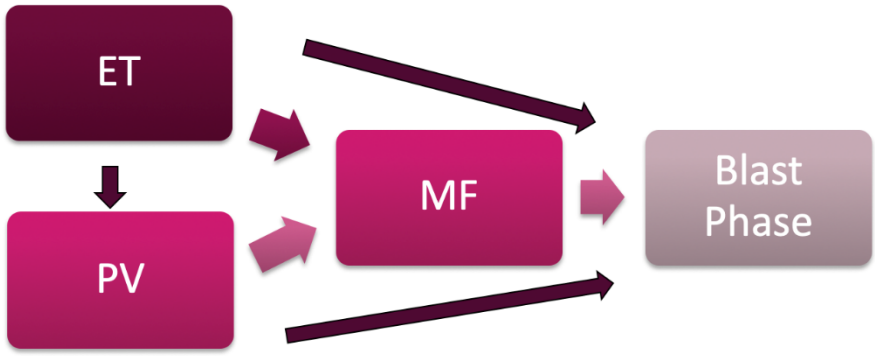


Figure 8. Patterns of MPN progression.

2.6.2 Accelerated phase and blast phase

MPN with 10-19% bone marrow blasts is defined as accelerated phase and >20% blasts is defined as blast phase.(31) An increased rate of leukemic transformation was noted also in patients with blasts >5%.(162) The risk of blast phase disease (leukemic transformation) is significantly higher in PMF than in PV and ET, it is 8-20 % in 10 years in PMF, 2.3-10 % in 10 years in PV, and 0.7-5 % in ET, where the lower end of the spectrum may be represented by “true” ET where a clear distinction from pre-PMF has been made.(66, 161, 163-165) MPN

blast phase carries a dismal prognosis, with a median survival of 3-6 months.(163, 166, 167) Treatment options in accelerated and blast phase include chemotherapy, hypomethylating agents alone or in combination with ruxolitinib, HSCT, targeted therapy if actionable mutation is present, or inclusion in clinical trials.(168)

2.7 SURVIVAL AND CAUSE OF DEATH

The median survival is around 18-20 years in ET, 13-15 years in PV, and 4-6 years in PMF.(164, 169) The median survival was reported to be longer in individuals that are younger at diagnosis.(170) The relative survival was inferior to general population in all MPN subtypes and all age groups.(171) This was true even in the low-risk subset of patients with ET and PV.(169, 172) The survival was significantly better in "true" ET than in pre-PMF, and this should be kept in mind when interpreting data prior to the WHO 2016 revision.(173) In post-ET and post-PV MF, median survival was reported to be 9.3 years.(145) The survival in PMF was highly variable, and depending on clinical, cytogenetic and molecular data, the 5-year survival ranged from 7% to 91 %, corresponding to a median survival ranging from 2 to 28 years.(174)

The causes of death in MPN patients were investigated in a Swedish population-based study, showing a HR of dying from infections of 2.7 (95% CI 2.4-3.1) in all MPN and all age-groups. The HR of dying from solid tumors was significantly increased in the youngest age-category, 18-49 years, HR 2.5 (95% CI 1.3-4.7). Death from cardiovascular disease was significantly increased in all age categories as were death from cerebrovascular disease in all but the youngest age category (18-49 years of age).(175)

2.8 COMPLICATIONS

2.8.1 Thrombosis

One of the major clinical problems in MPN is the increased risk of thromboembolic events, both on the arterial side, with myocardial infarction and stroke, and on the venous side, with pulmonary embolism, deep vein thrombosis, and thrombosis in more unusual locations such as portal veins, splanchnic veins, or cerebral sinus veins.(97, 176-178) In a population-based study from Sweden, the rate of arterial and venous thrombosis was compared to matched controls. The HR of arterial thrombosis was 3.0 (95% CI 2.7-3.4) the HR of venous

thrombosis was 9.7 (95 % CI 7.8-12.0) at 3 months after MPN diagnosis. The HR of thrombosis compared to controls decreased within the first years of diagnosis but remained significantly elevated also at 1 and 5 years. There were no significant differences in the HR of venous and arterial thrombosis between MPN subtypes.(179) These findings highlight the need for prompt diagnostic work-up and initiation of treatment in patients with suspected MPN, and support that active treatment significantly reduces risk of thrombotic events. *JAK2*-mutated patients with ET and PMF were at a higher risk of thrombotic events in than *CALR*- and *MPL*-mutated cases.(54, 55)

2.8.2 Bleeding

MPN patients have an increased risk of bleeding.(180) There are many pathophysiologic causes for this including functional changes in platelet and endothelium function. Thrombocytopenia may occur in PMF. Thrombocytosis, in particular extreme thrombocytosis, paradoxically causes increased risk of bleeding similar to acquired von Willebrand disease with reduction of large von Willebrand multimers.(181) Anticoagulant and platelet inhibitory treatment further add to the bleeding diathesis, as does pre-PMF or MF subtype.(182-184)

2.8.3 Second malignancy

Transformation to acute myeloid leukemia and myelodysplastic syndrome is well-known in MPNs. An association to lymphoproliferative neoplasms has been described,(185-187) as well as to selected solid tumors, such as skin, lung, and kidney cancer in patients with PV and ET in register-based studies.(188, 189) An increased risk of solid cancers prior to MPN diagnosis has also been reported.(189, 190) Solid malignancy was shown to be a significant cause of death in MPN patients.(175, 191) Concomitant MPN decreased the 5-year survival from the second cancers.(192) Large population-based studies of all MPN subtypes, and risk of second malignancies in relation to controls were however lacking.

2.8.4 Infections

Infections are a common and serious complication in patients with hematologic malignancies. Elevated infection risks are seen in AML, myelodysplastic syndrome, myeloma, chronic lymphatic leukemia, and infections are associated with significant morbidity and mortality.(193-196) The risk of infections in patients with hematologic malignancies were related to underlying pathologies in hematopoietic cell lines and immune system, cytotoxic

treatments, neutropenia, hypogammaglobinemia, but were also associated with modern targeted therapies.(197)

The risk of infections in MPN patients has been of emerging interest since ruxolitinib was introduced.(198) An increased risk of infections in patients on ruxolitinib has been described in numerous case reports; common bacterial infections such as respiratory tract infections, urinary tract infections, viral infections such as herpes zoster, and opportunistic infections, pneumocystis jiroveci, and reactivation of latent tuberculosis or hepatitis B have been reported.(199-202) These associations did however not meet statistical significance in a meta-analysis and systematic reviews.(203, 204) Whether there is an underlying excess risk of infections in MPN or related to MPN treatment has not been studied in a population-based manner. There were thus several questions related to infections in MPN patients that remained to be answered.

2.9 CHILDBIRTH IN MPN

2.9.1 Pregnancy

At diagnosis, 10-20 % of patients with MPN are of fertile age.(2) Pregnancy as well as the MPN itself are complicated situations from a hemostatic point of view, with increased risk of both thrombosis and bleeding. In previous literature, the live childbirth rates, i.e. proportion of pregnancies ending with a live birth, are reported to be around 70 % in women with ET and 65% in PV, respectively.(205-208) For PMF, only case reports and smaller case series exist, live birth rate have thus not been calculated.(209, 210) Pregnancy complications that have been described in numerous case series include spontaneous abortion, sometimes repeated, stillbirth, preterm birth, low birth weight, preeclampsia as well as placental infarctions.(211, 212) Maternal complications include thrombotic and hemorrhagic events.(213)

Recently, a British prospective population-based study reported higher live birthrates in a study of 58 women with MPN, there were 58 live children including 2 twin pregnancies, 1 stillbirth, and 1 miscarriage. Fifteen percent of neonates had a low birthweight, and 13% required admission to neonatal care.(214) Passamonti et al found a 3.4-fold increase in fetal loss in comparison with age-matched Italian statistical data.(215) Few other studies have described fetal outcomes and the majority of studies to date on pregnancy outcomes in MPN

have been cases series, thus population-based assessment of maternal and fetal outcomes, miscarriage, and stillbirths are lacking.(206, 207, 216)

2.9.2 Pregnancy management

The management of pregnancies in MPN patients are mainly based on expert opinion, retrospective case series and metanalysis the published case series. Aspirin and interferon were suggested to improve live birth rate in a meta-analysis by Maze et al, while no benefit of adding low molecular weight heparin (LMWH) was shown.(205) There are no RCTs or prospective studies addressing the choice of treatment during pregnancy. Whether aspirin protects against fetal loss is not completely clarified, however most but not all evidence support a protective roll.(205, 215, 217) Historically, the use of aspirin in pregnancies in MPN started around the turn of the millennium, and has gradually increased. Current recommendations include aspirin for all pregnant women with MPN and addition of LMWH and IFN if high-risk markers of poor pregnancy outcome are present (see below).(37, 42, 206, 218) In PV, venesection may be used to keep the hematocrit at mid-gestation appropriate range.(218)

The criteria for defining pregnancy risks were suggested by Griesshammer and classify the patients as high risk based on the following:

- Previous thrombosis or severe bleeding
- Previous inferior pregnancy outcomes
- Platelet count rising to 1,500 $x10^9$/L.(206)

Inferior pregnancy outcome was defined as >3 first trimester pregnancy loss, or >1 second or third trimester loss, birthweight below 5^{th} percentile for gestation, stillbirth, or preeclampsia necessitating preterm delivery.(206) Similar criteria for inferior pregnancy outcomes are practised by Harrison et al, adapted from antiphospholipid syndrome.(219, 220) In a report by Randi et al, no diffference in pregnancy outcome was observed between low and high risk pregnancies.(221)

There are contradictory results on whether a *JAK2* mutation is associated with inferior outcomes.(215, 221) Interestingly, in women without a diagnosis of MPN and normal blood counts, harboring a *JAK2* V617F mutation was more common among those experiencing fetal loss compared to those who had live births.(222)

Blood values in MPN patients often improve during pregnancy. In a study by How et al on pregnancies in women with ET, platelets decreased by 43%, and a larger decrease in platelets was associated with improved pregnancy outcomes, Figure 9.(208) In the general population, a platelet decrease of 17 % is expected in pregnancy.(223)

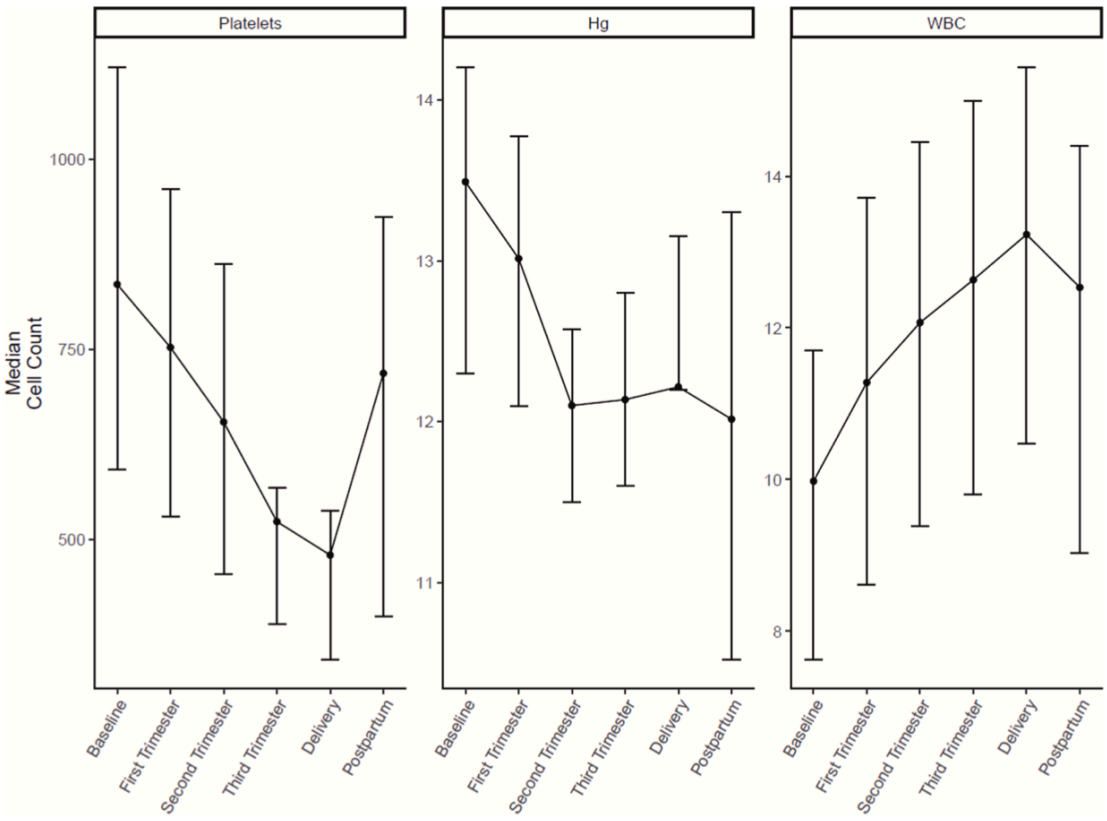


Fig. 1. GESTATIONAL CELL COUNT TRENDS.

Figure 9. Blood values in 49 pregnancies in women with ET. (208)

2.9.3 Fertility and reproductive patterns in MPN

Investigating pregnancy outcomes in women with MPN raise questions on childbirth rates and patterns of childbirth. There are to our knowledge no studies on female fertility or childbearing patterns in women with MPN.

3 RESEARCH AIMS

The overall aim of this thesis is to advance the knowledge on complications in patients with MPN and on childbirth in women with MPN. The long-term purpose of this added knowledge is to improve management of patients with MPN. The specific aims of this thesis are:

To thoroughly determine the risk of developing second malignancies in MPN patients compared to population controls.

To thoroughly determine excess morbidity due to infections in MPN patients compared to population controls, and to assess effect of cytoreductive treatments on risk of infections.

To assess and quantify pregnancy outcomes, including maternal and fetal outcomes including fetal loss, as well as and childbearing patterns in women with MPN, and compare those to non-MPN population controls.

4 MATERIALS AND METHODS

4.1 CENTRAL REGISTERS AND SOURCE POPULATION

Sweden is a country with 10.5 million residents. All resident individuals have a personal identification number that is used in all contacts with health care and authorities, which allows for crosslinking between health registers.(224) Health care is tax-funded and provided publicly. Hematology/Oncology is mainly a hospital-based specialty. Maternal and pediatric care is completely free of charge.

The data for paper I-IV is sourced from national registers with high quality and prospectively collected data. The National Board of Health and Welfare holds several health care registers, one of the oldest being the Swedish Cancer Register, where reporting of all incident cases of malignancy is mandatory by law since 1958. Since 1984 there is a double reporting routine, where both clinicians and pathologists are obliged to report incident cancers. Quality assessment of the Cancer Register have found an overall high degree of completeness, however somewhat lower in indolent cancers (225-227), while correct differentiation of new primary malignancies and metastasis is high.(228)

The Patient Register was started in 1964 and consists of the Inpatient Register that records all diagnoses from hospital discharges with nationwide coverage since 1987.(229) The Outpatient Register was introduced nationwide in 2001 and captures the diagnoses of all outpatient visits to specialty clinics, not including primary care. Emergency department visits are recorded since 2016 in the Outpatient Register.

The Cause of Death register holds information on causes of death for all deaths in Sweden since 1952.(230) The Medical Birth Register was started in 1973 and records data from pregnancies from gestational week 22, prior to 2008 from week 28. It includes antenatal maternal information as well as data on delivery method, diagnoses, and neonatal status at birth, with a high degree of completeness, 95-97%. The quality of the register is validated by National Board of Health and Welfare.(231) In the Register of Prescribed Drugs all prescribed and dispensed drugs at pharmacies are automatically recorded since July 2005, also held by National Board of Health and Welfare.

Statistics Sweden, the official authority for population statistics in Sweden, holds the Register of Total Population with information on all Swedish residents, including migration data. The Multi-Generation Register includes all individuals that are born 1932 or later, and who

resided in Sweden at any time-point after 1961, and holds information on links to biological parents.(232)

These registers have been used in one or more of the included studies for defining the cohorts, exposure, inclusion and exclusion criteria, censoring, and outcomes. The source population for paper I and II is the entire Swedish population, in paper III and IV the source population is individuals in Multi-Generation Register.

4.2 STUDY POPULATIONS AND OUTCOMES

4.2.1 Paper I

MPN patients aged 18 or older, diagnosed between January 1st 1973 and December 31st 2009, were identified from either Cancer Register or Inpatient Register. They were followed until December 31st 2010. Four controls for each MPN patient were randomly selected from the Total Population Register, matched by age, sex, and region of residency. Exclusion criteria were previous cancer, both solid and hematologic, for MPN patients and controls. Controls had to be alive at the date of their corresponding patient's MPN diagnosis. Censoring was made at death, emigration, or end of study. Outcome was a diagnosis of solid or hematologic cancers, defined by ICD codes in Cancer Register.

4.2.2 Paper II

We included all MPN patients aged 18 or older, with a diagnosis of MPN in Cancer Register between January 1st 1992 and December 31st 2013, with follow up until December 31st 2015. Four controls were matched for each MPN patient from Register of Total Population, matched by birth year and sex. Exclusion criteria for patients and controls were previous hematologic malignancy. Censoring was done at death, emigration, end of study or diagnosis of another hematologic malignancy, as the research question was to assess infections during chronic phase MPN. Outcomes, a wide range of serious infections leading to hospitalization or death, were identified from the Inpatient Register and the Cause of Death Register.

4.2.3 Paper III and IV

In paper III, the Multi-Generation Register was linked to the Medical Birth Register to identify pregnancies between January 1st 1973 and December 31st 2017. From these pregnancies, we selected all pregnancies where the mother-to-be had received a diagnosis of

MPN in the Cancer Register, Outpatient Register, or Inpatient Register, and aged 16 or older at diagnosis. We also included women where the diagnosis was made during pregnancy or in the first two months postpartum. The pregnancies in women with MPN were matched 1:1 to another pregnancy, by maternal age at pregnancy, year of pregnancy, previous parity and whether the present pregnancy was singleton or duplex. If more than one pregnancy occurred in the same woman with MPN, separate controls were matched for each pregnancy. Pregnancy outcomes were identified from the Medical Birth Register, outcomes of maternal bleeding or thrombosis from the Medical Birth Register, Inpatient Register, and Outpatient Register.

In paper IV, women aged 15-44 at MPN diagnosis, and diagnosed between January 1st 1973 and December 31st 2018 were included. Population controls from Multi-Generation Register were matched 1:4, by age and year of birth. Both patients and controls needed to be free of other hematologic malignancy, alive and residing in Sweden at diagnosis/matching date. Censoring was made at death, emigration, turning 45, end of study, or receiving a diagnosis of another hematologic malignancy. Primary outcome was time to first live childbirth during follow-up. Secondary outcomes were time to first miscarriage and stillbirth during follow-up, as well as the total number of children in women turning 45 years during the study. We also compared baseline data; history of stillbirth, miscarriage, repeated miscarriage (three or more), recent miscarriage (within 2 years), mean number of children, and proportion of women with previous childbirth.

A diagnosis of MPN in paper III and IV was identified from either Cancer Register, Inpatient Register, or Outpatient Register. If Outpatient Register was used, two separate occasions with a diagnosis of MPN were required.

4.3 STATISTICAL METHODS

Paper I and II are similar in study design and methodology, they are both cohort studies with an MPN cohort and a matched control cohort, with inclusion and matching at MPN diagnosis, and a longitudinal follow-up until outcome of interest, end of study, or censoring. Both paper I and II were analyzed statistically with methods of survival analysis. In paper I Cox regression assuming proportionality were used to calculate HR with 95% CIs, and non-proportional flexible parametric models to assess changes in HRs (with 95% Cis) over time. In paper I, standard incidence ratios (SIRs) were also calculated to facilitate comparison to other studies in the field, as well as cumulative incidences.

In paper II, a flexible parametric model was used to calculate HRs with 95% CIs. All hospitalization or death due to infection, as well as more specific outcomes were studied and presented separately, including type of infection (bacterial, viral, fungal), location (pulmonary, urinary tract etc) and specific microbial pathogens (Streptococci, E. coli etc.)

An additional analysis of the rate of infections in relation to cytoreductive treatment was performed in a subgroup of patients where this information was available, i.e. those diagnosed 2006-2013, with follow-up until 2015. Patients without cytoreductive treatments were used as reference. Patients were considered to be on treatment with a drug if they had been prescribed it during the last 18 months, if two or more different cytoreductive treatment prescriptions were made within a 6-month period they were considered to be on combined treatments, and if no prescriptions were made during 18 months period they were considered untreated. Patients could thus contribute with time at risk in different treatment categories. HRs with 95% CIs for hospitalization due to infection were calculated for the different treatment categories.

In paper I and paper II, separate analyses were performed by MPN subtype, age-groups, sex, and calendar period of MPN diagnosis. In the survival analysis, patients were followed until outcome of interest while all other outcomes were ignored in each analysis. In paper I and II, in order to reduce the risk of reverse causality affecting the results, a separate analysis was performed where follow-up started one year after MPN diagnosis.

In paper III, the outcomes were compared between patients and controls, both in absolute numbers and percentages in all identified pregnancies. Matching variables were ignored in the analysis.(233) In the first pregnancy after MPN diagnosis in each patient, p-values were calculated using two-sided Fisher exact test, $p>0.05$ was considered significant. Restricting to one pregnancy for each individual was done in order to avoid a mix of dependent and non-dependent data.

In paper IV a flexible parametric model was used to analyze childbirths and miscarriages after diagnoses, and proportional hazards models were used to estimate HRs with 95% CIs and non-proportional hazards were introduced to compare HRs in relation to time, cumulative incidence and to produce graphs. In cumulative incidence, competing risks were not considered. Proportions in baseline data were compared using two-sided Fisher exact test, means were calculated using the Students' t-test and $p<0.05$ was considered significant.

In paper III and IV, as sensitivity analysis, we performed separate analysis by source of MPN diagnosis; the Cancer Register, Inpatient Register, or Outpatient Register to assess the

diagnoses from the Patient Registers as a diagnosis of MPN from the Cancer Register is considered more valid. In paper IV, as a sensitivity analysis, we also started follow-up at nine months after diagnoses, in order to exclude women and controls that were pregnant at the time of MPN diagnosis.

4.4 ETHICAL CONSIDERATIONS

All four sub-studies in this thesis are epidemiological and solely based on register data. The registers used are pre-existing and include all residing individuals nationwide. When extracting datasets from the National Board of Health and Welfare and Statistics Sweden, we received de-identified (paper I and II) or pseudo-anonymized data (paper III and IV). In case of pseudo-anonymized data, the code key is kept at National Board of Health and Welfare for a restricted time, hence we have no access to the code key or identity of the participants. Regardless of anonymization, the datasets contain large amounts of sensitive personal data, and identification of individuals may yet be possible. The data is treated as sensitive personal data, with rigorous safe keeping. It is kept at the Department of Medical Epidemiology and Biostatistics at Karolinska Institutet, where there is significant experience in keeping of data, and only involved researchers have access. However, keeping sensitive personal data is associated to potential risks of loss of integrity, for example in digital intrusion attacks.

Informed consent was waived for the studies in the thesis, as specified in the ethic permissions and approvals. We had no contact with the study subjects and many of the study subjects, particularly in paper I and II, are not alive today, hence informed consent would have led to a more selected inclusion, and a risk of negative emotions for families that would have been contacted for consent.

Despite this, the risk of a negative impact of participants can be considered small. The positive effect of what we can learn when being able to access and cross-link large amount of population-based data, outweighs the risks.

Ethical approval was granted for all preformed studies by the regional ethical review board and or after 2019 by Swedish Ethical Review Authority, with reference numbers 2005/206-31/3 with amendments 2013/1353-32 and 2014/1610-32, 2017/73-31, 2020-05539, and 2022-02992-02.

5 RESULTS

5.1 PAPER I: SECOND MALIGNANCIES

We identified 9,739 MPN patients and 35,682 matched population controls. The median age was 67.5 years and 48% were men. The MPN subtype was PV in 45%, ET in 28%, PMF in 15%, and MPN-U in 12%, respectively. In total, 1,192 patients were diagnosed with a second non-hematologic malignancy after a median follow-up time of 6.1 years. A significantly increased rate of any non-hematologic malignancy was observed, HR 1.6 (I.5-1.7), Table 4. Of non-hematologic malignancies, the highest increases in rate were observed for skin cancers, both melanoma and non-melanoma, and for cancers of the brain, kidneys, endocrine organs, pancreas, lung, and head-neck. There were no significant differences in the HRs of solid tumors across MPN subtypes, calendar periods, or between men and women.

As expected, the rate of developing AML was significantly increased, HR 46 (32.6-64.9), with higher HRs in patients with PMF and MPN-U. The rate of lymphoma was also increased, with a higher HR in PMF patients, Table 5.

Type of cancer	HR	95% CI	Number of events in MPN patients
All non-hematologic	*1.6*	1.5 - 1.7	1185
Non-melanoma skin cancer	2.8	2.4 - 3.3	252
Brain	2.8	1.9 - 4.2	37
Kidney	2.8	2.0 - 4.0	49
Endocrine organs	2.5	1.6 - 3.8	33
Malignant melanoma	1.9	1.4 - 2.7	53
Pancreas	1.8	1.2 - 2.6	32
Lung	1.7	1.4 - 2.2	92
Head and neck	1.7	1.2 - 2.7	32
Esophagus and stomach	1.6	1.2 - 2.2	53
Breast	1.4	1.1 - 1.7	106
Prostate	1.2	1.1 - 1.4	190
Liver and gallbladder	1.3	0.9 - 1.9	31
Colon, rectum, and anus	1.1	0.9 - 1.3	129
Female genital organs	1.2	0.9 - 1.5	73
Urinary tract excluding kidney	1	0.7 - 1.3	46

Table 4. HRs with 95% CIs of solid tumors. (234)

MPN subtype	AML HR (95% CI) n = 278	Lymphoma HR (95% CI) n = 90	Multiple myeloma HR (95% CI) n = 16
All MPN	46.0 (32.6 - 64.9)	2.6 (2.0 – 3.3)	1.7 (1.0 - 3.0)
PV	38.1 (23.5 - 62.0)	1.9 (1.3 - 2.8)	1.6 (0.7 - 3.5)
ET	26.1 (13.4 - 50.9)	2.3 (1.3 - 3.9)	1.4 (0.5 - 3.6)
PMF	99.2 (41.0 - 240.1)	6.0 (3.4 - 10.8)	9.0 (1.8 - 44.0)

Table 5. HRs with 95% CIs of solid tumors and hematologic malignancies by MPN subtype. (234)

The HR of any solid tumor and of skin cancers, both melanoma and non-melanoma, showed a tendency to increase over time from MPN diagnosis, Figure 10. The HR of AML was stable over time after MPN diagnosis. SIRs with 95% CIs were calculated to facilitate comparability to other studies in the field, and were similar or slightly lower than the HRs. Cumulative incidence with 95% Cis, accounting for death as a competing event, for MPN patients and matched controls were calculated separately by age group and sex, during the last calendar period, 2002-2009, Figure 11. Cumulative incidence curves diverged the most for middle-aged patients and were overlapping for patients above 80 years.

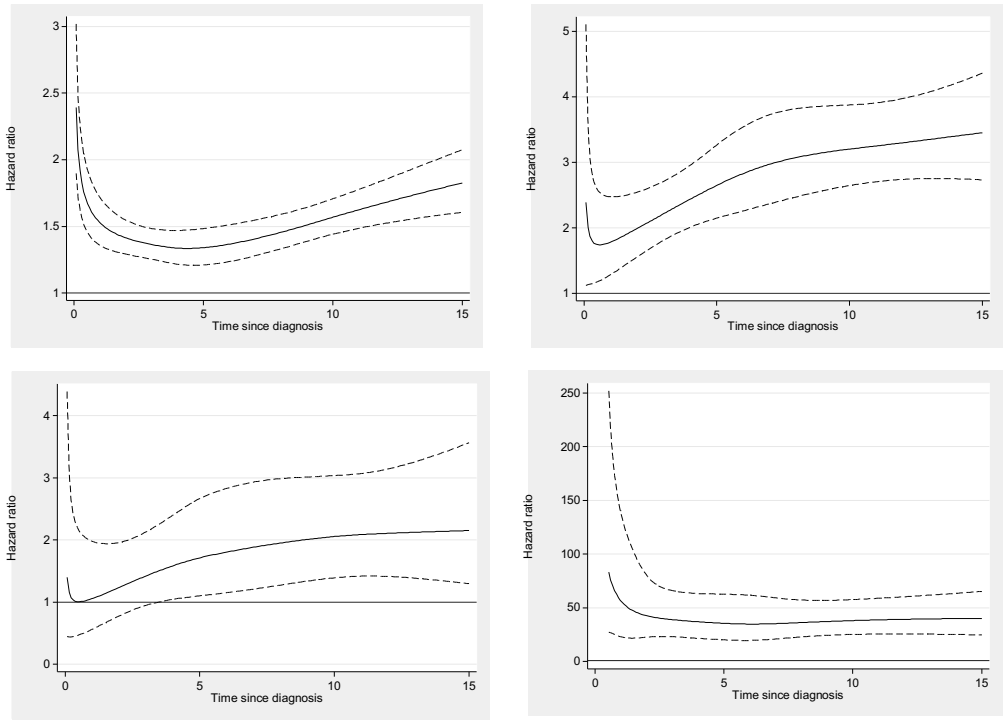


Figure 10. HRs with 95% CIs in relation to time from MPN diagnosis; top left all non-hematologic malignancy, top right non-melanoma skin cancer, bottom left melanoma skin cancer, and bottom right AML

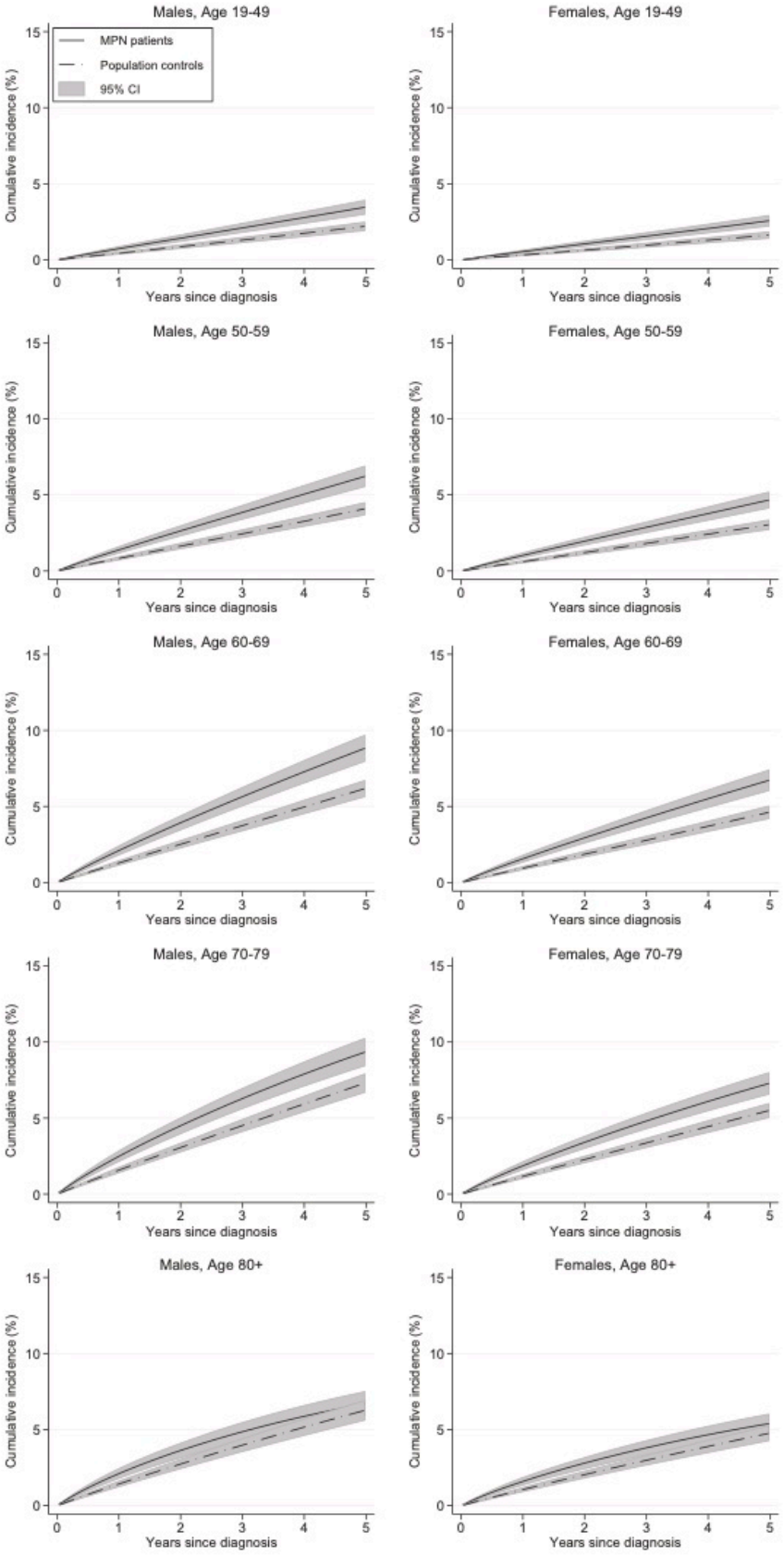


Figure 11. Cumulative incidence of second malignancies for MPN patients and controls, diagnosed 2002-2009, and shown separately by age group and sex.

5.2 PAPER II: INFECTIONS

We identified 8,363 MPN patients and 32,405 controls identified between the years 1992 and 2013. Median age was 71 years, 53% were women. The HR of serious infection, requiring hospitalization or leading to death, was 2.0 (1.9-2.0), based on 3,095 events in MPN and 8,615 in controls, Table 6. The HRs of more serious infections such as sepsis, meningitis and encephalitis, as well as osteomyelitis more pronouncedly increased. There were no significant differences between men and women, or between calendar periods of MPN diagnosis. There was a trend towards a higher HR of infection in the younger age categories. The rate of infection was significantly elevated in all MPN subtypes, and the elevation was more pronounced in PMF. The HR of any infection in MPN-U was 2.4 (2.2-2.6). This pattern was consistent in most infectious outcomes investigated, Table 6.

Outcome	MPN (all) HR (95% CI)	PV HR (95% CI)	ET HR (95% CI)	PMF HR (95% CI)
Combined outcomes				
Any infections	2.0 (1.9–2.0)	1.7 (1.6–1.8)	1.7 (1.5–1.8)	3.7 (3.2–4.1)
Bacterial infections	1.9 (1.8–2.0)	1.7 (1.6–1.8)	1.6 (1.5–1.8)	3.4 (3.0–3.9)
Viral infections	2.1 (1.9–2.3)	1.8 (1.5–2.1)	1.7 (1.4–2.1)	5.2 (3.8–7.1)
Fungal infections	2.9 (2.5–3.5)	2.6 (1.9–3.4)	2.2 (1.6–3.0)	8.0 (4.9–13.3)
Infection type				
Sepsis	2.6 (2.4–2.9)	2.1 (1.8–2.5)	2.0 (1.6–4.7)	6.2 (4.8–8.0)
Pneumonia	2.0 (1.9–2.1)	1.8 (1.6–2.0)	1.6 (1.4–1.8)	3.8 (3.2–4.6)
Urinary tract infections	1.6 (1.5–1.7)	1.5 (1.4–1.7)	1.5 (1.3–1.6)	2.2 (1.8–2.8)
Skin and soft tissue infections	2.1 (1.8–2.4)	1.8 (1.4–2.3)	1.6 (1.2–2.0)	4.9 (3.2–7.4)
Gastrointestinal infections	2.3 (2.1–2.6)	2.3 (1.9–2.8)	1.7 (1.3–2.0)	4.8 (3.4–6.8)
Infectious agent				
Streptococci excl. pneumococci	2.3 (1.7–3.1)	2.4 (1.5–3.8)	1.1 (0.6–2.0)	8.7 (4.0–19.1)
Staphylococci	2.4 (2.0–2.8)	1.6 (1.2–2.1)	1.8 (1.3–2.4)	5.5 (3.6–8.6)
Escherichia coli	1.9 (1.6–2.2)	1.8 (1.4–2.4)	1.7 (1.2–2.2)	3.7 (2.1–6.5)
Varicella zoster	2.0 (1.4–2.8)	0.98 (0.5–2.0)	2.5 (1.4–4.5)	7.3 (2.9–18.3)

Table 6. HRs of infection in total and per MPN subtype.(235)

The cumulative incidence of infection or death due to infection is shown in Figure 12. The HR of infection was consistently increased from MPN diagnosis, with small changes over time, Figure 13.

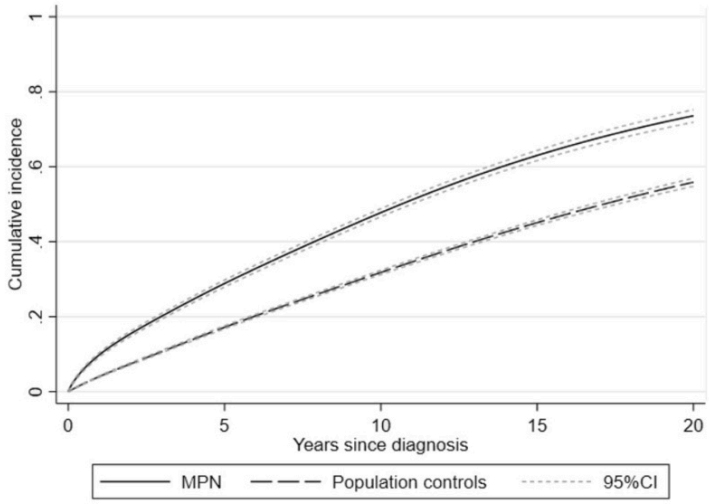


Figure 12. Cumulative Incidence of serious infection in MPN and controls, with 95% confidence intervals.(235)

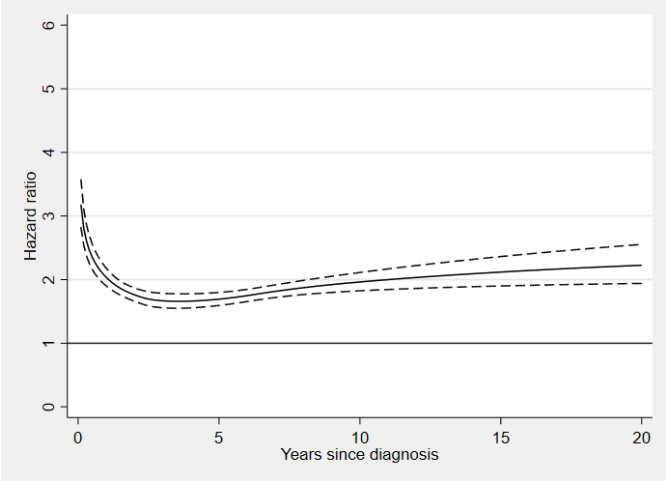


Figure 13. HR of any infection with 95% CI in relation to time from diagnosis for MPN patients in comparison with controls.(235)

The subset of patients where treatment data was available during the latter part of the study period, 2006-2013, included 3,659 patients, together collecting 15,211 person-years in different treatment categories. MPN patients with no cytoreductive treatment was used as a reference. There was no significant difference in the rate of any infection between patients without cytoreductive treatment, or on treatment with HU, IFN, or anagrelide. The rate of infection was higher in patients treated with ruxolitinib, other MPN-related drugs including busulphan, danazol, erythropoietin, thalidomide or lenalidomide, or combination treatments, compared to patients without cytoreductive treatment, Table 7. Of note, there were few patients with relatively short time at risk in the ruxolitinib group.

Table 4 Effect of treatment on risk of any infection in the MPN cohort during the last calendar period, patients diagnosed 2006–2013 with follow up until 2015.

Treatment	All MPN HR (95% CI)	Number of patients, MPN	Time at risk, years, MPN	PV HR (95% CI)	ET HR (95% CI)	PMF HR (95% CI)
No cytoreductive treatment	1.0 reference	3501	4 994	1.0 reference	1.0 reference	1.0 reference
Hydroxyurea	1.0 (0.9–1.2)	2432	8 276	1.1 (0.8–1.4)	1.0 (0.7–1.4)	1.1 (0.7–1.7)
Interferon	1.1 (0.7–1.8)	301	778	1.0 (0.4–2.4)	0.6 (0.1–2.5)	1.1 (0.4–3.1)
Anagrelid	0.9 (0.5–1.7)	148	337	1.3 (0.4–2.0)	0.6 (0.2–1.6)	2.2 (0.5–9.4)
Ruxolitinib	4.1 (1.3–13.0)	35	23	–	–	4.6 (1.4–15.8)
Other MPN related drugs	2.8 (2.2–3.6)	302	436	0.9 (0.3–3.0)	2.2 (1.1–4.3)	2.6 (1.7–3.9)
Combinations of any of the above	1.9 (1.3–2.7)	492	368	1.4 (0.5–3.4)	1.1 (0.5–2.6)	1.8 (1.0–3.3)
Total number of patients, MPN		3659		1182	1369	461
Total time at risk, years			15,211	5140	6174	1378

HR hazard ratio, *CI* confidence interval, *MPN* myeloproliferative neoplasms, *PV* polycythemia vera, *ET* essential thrombocythemia, *PMF* primary myelofibrosis.

Analyses are adjusted for age category, sex, and year of MPN diagnosis. The category assigned other MPN-related drugs include busulphan, danazol, erythropoietin, thalidomide, lenalidomide. No treatment is used as reference.

Table 7. HRs of infection in relation to treatment in patients diagnosed with MPN 2006-2013, follow-up until 2015.(235)

5.3 PAPER III: PREGNANCY OUTCOMES

We identified 342 pregnancies in women with MPN during the years 1973-2017, and the same numbers in controls. Of these, 229 pregnancies were the first pregnancy after the MPN diagnosis. The majority, 70%, occurred in patients with ET, 13% occurred in PV, 10% in PMF, and 8 % in MPN-U, respectively. Five pregnancies in both patients and controls were duplex. Median year of pregnancy was 2007 and the median maternal age was 32 years, Figure 14. The MPN diagnosis was established prior to the pregnancy in 80% of the MPN pregnancies, while 20% of patients received their diagnosis during pregnancy or during the immediate postpartum period. The median time from MPN diagnosis to delivery was 3.7 years (IQR 1.3-7.7 years). Self-reported data regarding miscarriages prior to the index pregnancy revealed no significant differences between MPN patients and controls. The incidence of pregnancy 2007-2017 was 12.2 per 100,000 childbirths, only including those diagnosed prior to conception.

Women with MPN were significantly more likely to give birth preterm, Table 8. Low birthweight and very low birthweight were more common among women with MPN, while small for gestational age was not, Table 8 and Figure 15. Subanalysis of preterm birth revealed that iatrogenic preterm birth, defined as pre-labor cesarean section or induction prior to gestational week 37+0, was significantly increased in women with MPN, but not spontaneous preterm birth. No risk factors of preterm labor could be identified, however there

was a tendency towards a higher proportion of preterm deliveries during earlier years, 16% between 1973-2006, and 9% between 2007-2017. There was no statistically significant difference in neonates with chromosomal abnormalities or malformations.

There were no statistically significant differences in the proportion of pregnancies complicated by bleeding or thrombosis. Among all MPN pregnancies, 1% of patients had a venous thrombosis and there were no arterial thromboses. A higher proportion of women with MPN had bleedings or transfusions, for example 24 (9%) of MPN patients bled >1000 ml during delivery or postpartum compared to 14 (5%) controls, however these differences were not statistically significant. Furthermore, there were no significant differences in cases of preeclampsia, HELLP (hemolysis, elevated liver enzymes and low platelets), and gestational hypertension. However, having a Cesarean section was significantly more common in MPN, 31% compared to 16% (p<0.001).

	All identified pregnancies		***First pregnancy after MPN diagnosis***		
	MPN patients n=342 (%)	Controls n=342 (%)	MPN patients n=229 (%)	Controls n=229 (%)	p-value
Low birthweight <2500 g	29 (8)	11 (3)	22 (10)	10 (4)	**0.042**
Very low birthweight <1500 g	7 (2)	0 (0)	7 (3)	0 (0)	**0.015**
Small for gestational age	15 (4)	7 (2)	11 (5)	7 (3)	0.472
Preterm < week 37	42 (12)	14 (4)	33 (14)	10 (4)	**<0.001**
Moderate preterm, week 32-37	31 (9)	12 (4)	22 (10)	9 (4)	**0.024**
Very preterm, week 28-31	6 (2)	2 (0.6)	6 (3)	1 (0.4)	0.122
Extremely preterm, <week 28	5 (1)	0 (0)	5 (2)	0 (0)	0.061
*Iatrogen preterm**	19 (6)	2 (0.6)	16 (8)	2 (1)	**0.001**
*Spontaneous preterm**	18 (6)	12 (4)	13 (6)	8 (4)	0.371
Low birthweight, term	6 (2)	4 (1)	3 (2)	4 (2)	1.000
Fetal malformations and chromosomal abnormalities	15 (4)	10 (3)	9 (4)	7 (3)	0.800
Stillbirth	2 (0.6)	0 (0)	1 (0.4)	0 (0)	1.000
Neonatal death	1 (0.3)	1 (0.3)	1 (0.4)	1 (0.4)	1.000

** Variables where data is only available from 1990 and onwards, total number of patients and controls from 1990 are 322, when restricted to first pregnancy 212.*

Table 8. Outcomes of the newborn child: gestational age, birthweight, and mortality. P-value is calculated only in first pregnancy after MPN diagnosis, Fisher exact test was used and

p<0.05 considered significant (bold). Neonatal death is defined as a live birth, with death occuring between day 0 and 28.(236)

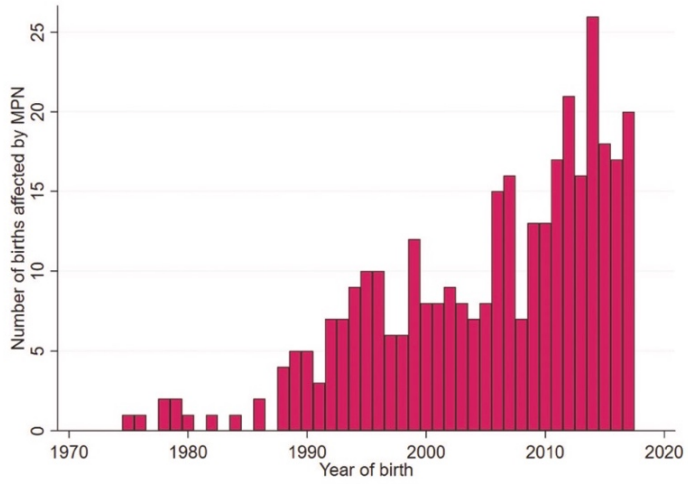


Figure 14. Frequency of childbirth in women with MPN per year.(236) Note that the Outpatient Register was started in 2001.(236)

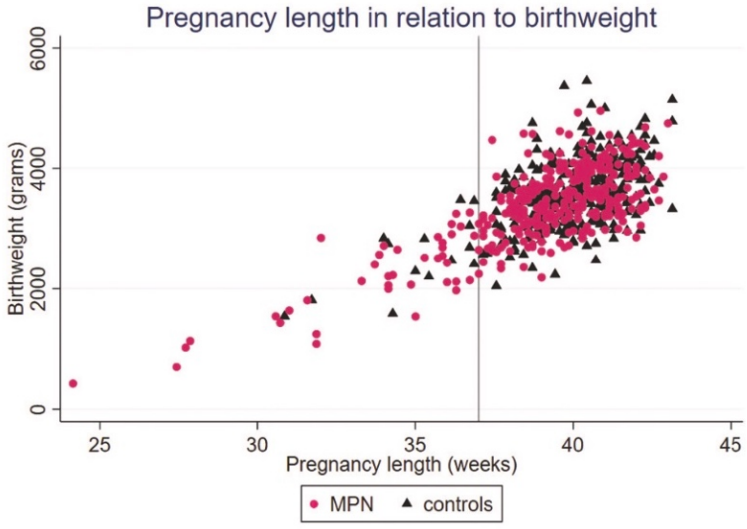


Figure 15. Pregnancy length in relation to birthweight in MPN and controls. The vertical line marks gestational week 37+0, before which births are considered preterm.(236)

5.4 PAPER IV: CHILDBIRTH PATTERN

We identified 1,141 women with MPN aged 15-44 years and 4,564 controls, with a median age of 36 years. At diagnosis, a lower proportion of women with MPN compared to their age-

matched counterparts had children, 61.0% vs 67.3% (p<0.001). The mean number of children at MPN diagnosis was lower, 1.29 in women with MPN compared to 1.43 in controls (p=0.001), Table 9. Among the women with an MPN diagnosis (or matching date) after 2003, the proportion of with previous miscarriage (≥1), repeated miscarriage (≥3), or recent miscarriage (within the 2 years prior to diagnosis) was similar among patients and controls. Only miscarriages after 2001 were included, as the Outpatient Register was used to obtain information of miscarriages. Women with MPN were more likely to have a history of stillbirth than controls, (p= 0.013).

	MPN Total 1141 n (%)	*Controls Total 4564 n (%)*	*p-value*
Mean number of children	1.29	1.42	**0.001**
Parous at diagnosis	696 (61.0)	3073 (67.3)	**<0.001**
*Miscarriage**	79 (12.4)	318 (12.5)	1.000
*Recent miscarriage (within 2 years)**	16 (2.5)	59 (2.3)	0.770
*Repeated miscarriage (≥3)**	14 (2.2)	54 (2.1)	0.879
Stillbirth	11 (1)	16 (0.4)	**0.013**

*Table 9. History of fetal loss at diagnosis. *Data on miscarriages is available from 2001, and is only analyzed in subjects from 2003 and onwards, total number is 636 MPN patients and 2,544 controls.*

After the MPN diagnosis, the rate of live childbirths was reduced, HR 0.78 (0.68-0.90) in women with MPN compared to controls, Table 10. In the subgroup analysis, the HR was not reduced in patients with ET, but statistically significantly reduced in patients with PV, PMF, and MPN-U. The HR of childbirth tended to be lower in women aged 15-25 at diagnosis. The birthrates and HR of birthrates in relation to time from diagnosis/matching date initially declined and then stabilized at a lower level, Figure 16 and 17.

The HR of miscarriage during follow up was 1.25 (0.89-1.76) in women with MPN compared to controls. During follow-up, there was one stillbirth among a woman with MPN and six among controls, which were too few for comparative statistical analysis.

In a sensitivity analysis where analysis started 9 months after MPN diagnosis/matching date, the HR of childbirth was 0.72 (0.61-0.89) and the HR of miscarriage was 1.06 (0.72-1.55).

Women with MPN who turned 45 during the study period had on average 1.81 children, the corresponding mean among controls was 2.01. In this group 82.2% of the MPN patients had ever given birth to a child, compared to 87.5% of controls.

In this study, we explored the reasons for censoring and as expected, the reason for censoring for the majority of participants were end of study or turning 45 years of age. However, 57 (5%) women with MPN were censored due to death, compared to 19 (0.4%) of controls.

	Childbirths in MPN	*Childbirths in controls*	*Hazard Ratio*	*95% Confidence interval*
All	221	1131	0.78	0.68-0.90
Age: 15-25	69	424	0.64	0.49-0.82
26-35	128	612	0.83	0.69-1.01
36-44	24	95	1.06	0.68-1.65
Subtype: PV	26	177	0.50	0.33-0.76
ET	156	676	1.02	0.86-1.22
PMF	18	150	0.45	0.28-0.74
MPN-U	21	128	0.52	0.33-0.82

Table 10. Number of events, defined as first childbirth during follow up, and HRs of live childbirth during follow-up, per age group and MPN subtype.

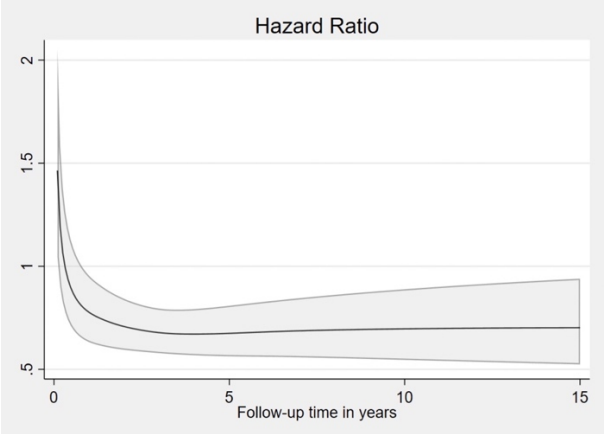


Figure 16. HR with 95% CI in relation to childbirth in women with MPN compared to controls.

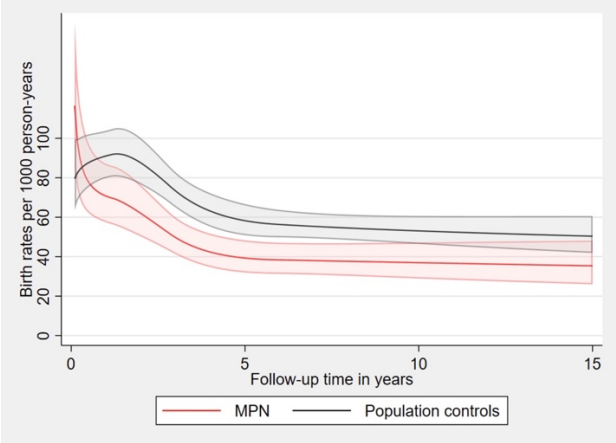


Figure 17. Birth rate per 1000 person-years with 95% CI for women aged 26-35 and MPN diagnosis/matching during the years 1989-2003, for MPN patients and controls, based on a flexible parametric model without assuming proportionality.

6 DISCUSSION

6.1 SECOND MALIGNANCIES

6.1.1 Interpretation of findings

In this large population-based study, patients with MPN are at increased risk of second malignancies, in particular of the skin, kidneys, lung, pancreas, brain, head-neck, esophagus and endocrine organs. Patients with MPN were also at increased risk of hematologic malignancies, AML is well recognized, but in paper I, an increased risk of lymphoma and multiple myeloma was also demonstrated. The increased cancer risk may be of multifactorial etiology, including genetic susceptibility, immune dysfunction and cytoreductive treatment.

6.1.2 Context

Paper I was one of the first large population-based studies of second malignancy in MPN, and during recent years, significant progress has been made in the field of second malignancies. The increased cancer risk is now well-documented and summarized in a review by Brabrand and all with over 65,000 MPN patients, including the 9,379 patients from paper I, with increased risk of cancers in the skin, lung, kidney, and thyroid, but not in colon, breast and prostate. Hematologic cancers in the lymphoid and plasma cell lines were also increased across most studies. The absolute number of second malignancy was higher in older age group, but in comparison to a control population, the relative risk was higher in the younger age groups.(237) This was comparable to our results, although Swedish patients may be overrepresented in the review.(189) In a Danish study of PV and ET patients with a second malignancy, these patients were found to have an inferior prognosis compared to matched non-MPN patients with the same type of cancer; risk of death was between 1.2 - 2.3 times higher than in controls. The difference was evident irrespective of whether the second cancer was localized, spread regionally, or with distant metastasis.(192) Patients with MPN are also shown to have an increased risk of a malignancies prior to MPN diagnosis.(190, 238)

6.1.3 Association to cytoreductive treatment

An effort to clarify the relationship to cytoreductive treatment was made by Barbui et al, in a nested case-control study where the authors demonstrated a significantly increased risk of non-melanoma skin cancer in patients exposed to HU, pipobroman, ruxolitinib and treatment combinations. The risk of other solid cancers or hematologic cancer was not found to be related to treatment.(119) In a Danish retrospective study of risk of second malignancies in

patients exposed to HU, HU + IFN or IFN alone, odds ratio of second malignancy was increased for both HU and HU + IFN.(239) A smaller Czech study reported an increased risk of second malignancies, both skin and solid, associated with HU. (240) A study from the Surveillance, Epidemiology, and End Results (SEER) database could not demonstrate an increased risk of second malignancies in an elderly MPN population treated with HU compared to non-treated, however follow-up time was only three years.(241) In the COMFORT-2 study of ruxolitinib, a significant increase of non-melanoma skin cancer was noted.(242) This was further demonstrated by Lin et al in a retrospective study with a 10-year follow up, where ruxolitinib-exposed patients had a more than doubled risk of non-melanotic skin cancer, and in particular in *JAK2*-unmutated patients.(243) An increased risk of skin cancers in ruxolitinib treated PV and MF patients was confirmed in real-world studies, but not of other second malignancies.(244, 245) Rampotas et al presented a case series of 71 patients on ruxolitinib treatment and whos' non-melanoma skin cancers had a more aggressive disease course with more metastatic spread, and higher recurrence rates and mortality than anticipated.(246) In an ecologic study, where risk of second malignancy in PMF patients where compared pre and post ruxolitinib introduction in 2011, an increased risk of second malignancies were demonstrated after 2011, but not an increased survival.(247) There is thus a clear association with HU and ruxolitinib and non-melanoma skin cancers.

The association with lymphoproliferative neoplasms in MPN patients has also sparked interest.(185, 186) The *JAK2* mutation has been identified in lymphoma cells, and a high proportion of patients with MPN and concurrent lymphomas are *JAK2*-mutated.(185, 248) In a Danish study of 97 patients with MPN and concomitant or subsequent lymphomas, the relative risk increase of peripheral T-cell lymphomas was noted, as was inferior prognosis in diffuse large B-cell lymphoma.(249) Proteomic studies has revealed molecular differences between lymphomas from MPN patients and non-MPN patients, suggesting a different underlying tumor biology.(250) The association of lymphomas and ruxolitinib treatment has been investigated with conflicting results.(251, 252)

The potential mechanism of the increased risk related to HU and ruxolitinib may differ. While HU affects DNA synthesis, the increased risk in ruxolitinib may be associated to immunosuppression with reduced oncosurveillance, and T-lymphocyte and dendritic cell inhibition which are important in skin cancer control.(253) This may be related to the JAK inhibition itself, and therefore a possible effect of other JAK-inhibitors too. To date, an increased risk of skin cancers has not been demonstrated with other JAK-inhibitors but

ruxolitinib, but they have been available for a shorter time period, and have been used by fewer patients.

6.1.4 Genetic predisposition

An increased risk of developing MPN following thyroid cancer, kidney cancer, melanoma and non-melanoma skin cancer has been demonstrated.(190, 238) This bidirectionality may suggest a shared susceptibility. The contributing role of genetics in this susceptibility is gradually becoming clearer.

Clonal hematopoiesis may constitute a link between solid and hematologic cancers. It is an increasing concern in patients with solid cancers, and will become a more common clinical consideration as more sequencing including liquid biopsies is being performed.(254) In a targeted next generation sequencing study of tumor material and matched blood samples from 8,810 patients, 25 % of cancer patients had evidence of clonal hematopoiesis, *DNMT3A* being the most common mutation. Presence of clonal hematopoiesis was associated with shortened survival, although only statistically significant only in patients with presumptive driver mutations.(255) Cancer therapy, including chemotherapy was shown to favor expansion of certain mutations, e.g. *TP53* and *PPM1D.*(255) Clonal hematopoiesis is associated with increasing age, smoking and inflammation, in which a positive feedback loop may favor the mutated clone.(256) In patients with *JAK2* V617F clonal hematopoiesis an increased risk of lymphoma has been demonstrated.(257)

Regarding specific genetic predisposition, variants in the *TERT* gene may predispose to both hematologic malignancies including MPN and sold tumors, such as glioma, bladder cancer, thyroid cancer. Other genes with mutations or polymorphisms identified by GWAS studies in MPN patients are associated with a predisposition to solid cancers, such as *MECOM*, *SH2B2*, *TET2*, *ATM*, *CHEK2*, *ASXL1*, *DNMT3A*, *IDH2*, *NF1*.(258) Attempts at clarifying the genetic character of MPN with and without second cancers have been made by Hsu et al, demonstrating enrichment in genes involved in inflammatory pathways confirmed by excess levels of plasma cytokines.(259)

6.1.5 Clinical implications

There are several challenges related to second malignancies in MPN in regards to prevention and management. The optimal choice of treatment in an MPN patient with a high risk of second malignancy is not clear, especially if IFN is not appropriate. Non-melanoma skin cancers are common in an elderly population, and to generalize the Swedish elderly population; it is to a large degree of Caucasian descent, fair-skinned and sun-loving, making

this is a common clinical dilemma. Patients may be advised to use adequate sun protection, and seek medical attention if skin changes or other symptoms of a new malignancy occur. Dermatologic surveillance may be indicated in patients with higher risk, for example those with previous skin cancers. General surveillance is likely not indicated, but vigilance in case of emerging symptoms is essential. How to improve outcomes in second malignancies with MPN is another important question. It is clear that with new treatment options and as more patients have been treated with JAK inhibitors, second malignancies will need to be considered and thoroughly investigated.

6.2 INFECTIONS

6.2.1 Interpretation of findings

We found a two-fold increased risk of infections leading to hospitalization or death. The risk increase was significant in all MPN subtypes but highest in PMF. When comparing patients without cytoreductive treatment to patients treated with HU and IFN, the risk of infection was similar.(235) The large majority of patients included in paper II were not treated with ruxolitinib, our results can thus be interpreted as a baseline risk of infection prior to the introduction of JAK-inhibitors. To our knowledge, there is no other population-based study where risk of infection is assessed across the entire MPN population.

The risk of infections was not different between patients with HU, IFN, and untreated patients, which implies that the increased risk of infection is intrinsic to the MPN itself. Retrospective studies of treatments are always associated a risk of confounding by indication, but as we did not see an increased risk with HU or IFN, any major impact of these treatments was unlikely. However, for ruxolitinib, confounding by indication was likely since it was mainly used in intermediate 2 and high-risk MF. Similarly, the increased risk in patients on treatment combinations may be affected by confounding by indication as only the more complex MPN patients are usually on combination treatment. The result of paper II does not discourage use of common cytoreductive agents such as HU and IFN, due to fear of infections. Emerging treatment options needs to be evaluated regarding risk of infection, keeping the increased baseline risk in mind. In patients with the highest risk of infections, prophylactic measures may be warranted, in particular against herpes zoster, and all MPN patients should be prompted to follow general vaccination guidelines.

6.2.2 Context

Whether risk of infections was increased in patients with MPN was a question that was raised first by the introduction of JAK-inhibitors and further emphasized by the Covid-19 pandemic. Paper II focuses on serious infections requiring hospitalization or leading to death. Simultaneously a questionnaire-based study of 948 MPN patients was published by Crodel et al that included non-serious infections. Around half of the respondents had experienced at least one infection during the last year, of which 73.8% required outpatient contact, and 12.1% hospitalization. The risk of infection was higher in patients treated with IFN, ruxolitinib or combinations, and higher in MF patients.(260) During the Covid -19 pandemic, inferior outcomes in MPN patients were presented, but population-based comparisons to controls were not performed.(261, 262)

6.2.3 JAK-inhibitors and infections

There are several studies focusing on risk of infections in ruxolitinib-treated patients, including the original COMFORT trials, propensity score matching studies, systematic reviews, metanalysis, and real-world studies. Controversy still exists on whether ruxolitinib increases the risk of infections in general, however data supports that the risk of herpes zoster infections is increased.(132, 133, 203, 204, 263, 264) Additionally, the underlying indications for ruxolitinib treatment are also associated with an increased the risk for infections. Polverelli et al demonstrated in a retrospective study of 507 MF patients, with or without ruxolitinib, that high IPSS score and splenomegaly were risk factors for infections.(265) The same authors later reported on 446 ruxolitinib-treated MF patients, risk factors for infection in this population was high age, high IPSS score, previous infections and splenomegaly, while duration of ruxolitinib treatment and spleen response was associated with a reduced risk of infections.(266)

Information on the risk of infection in other JAK inhibitors is limited due to shorter time on the market. In the SIMPLIFY trial there were more infections in the momelotinib arm than the ruxolitinib arm.(138) In a trial of fedratinib, there were more infections in the fedratinib arm compared to placebo.(136) Infections were also a significant adverse event in the PERSIST trials of pacrintinib.(267) In PV, ruxolitinib treatment was associated with increased risk of herpes zoster in the RESPONSE trials.(268)

The JAK-STAT system is essential for hematopoiesis and immune cell regulation, proliferation, differentiation and cytokine signaling.(269) Due to the observed immunosuppressive properties, JAK inhibitors are being investigated and clinically used in

rheumatologic diseases and graft-versus-host disease.(270, 271) The degree of effects on immune function vary between ruxolitinib, fedratinib, pacritinib, and momelotinib depending on the selectivity of the JAK inhibition. A risk of infections is thus implied in all JAK-inhibitors, as may be expected from their mechanism of action.(272, 273)

6.2.4 Immune function in MPN

The immune system is dysregulated in patients with MPN. Hyperinflammation is present, monocytes and macrophages are increased, and the cytokine milieu is altered. There are data on dysfunctions in T-cells, NK cells and dendritic cells, which is further enhanced by ruxolitinib.(199, 269, 274-277) There are increasing evidence of an inflammatory state in MPN with increased inflammatory cytokine signaling, decreased inhibition of the same, and dysregulation of the immune system. The activation of the immune system may be related to both MPN pathogenesis, disease progression, and symptom burden.(278, 279) This dysregulation may also affect risk of infections.

6.3 PREGNANCY AND CHILDBIRTH

6.3.1 Interpretation of findings

Pregnancy outcomes were overall better in paper III than previously reported, however we did find a significantly increased risk of preterm birth, in particular iatrogen preterm birth, in women with MPN. Bleeding, thrombosis, preeclampsia, HELLP and gestational hypertension were not significantly increased. The incidence of pregnancy in MPN was considerably higher than demonstrated by Alimam et al.(214) In paper III, Sweden, a country with 10.5 million inhabitants, had during the last decade 15-20 pregnancies per year in women with MPN. As we found that pregnancies in MPN are less rare than expected, studies aiming to improve management of pregnancy in MPN international collaboration should be feasible.

In paper IV, childbirth rates were statistically significantly lower in women with MPN, but interestingly not evident in ET. Total number of children was lower in MPN already at diagnosis, suggesting a biologic effect of MPN on childbirth rate. Stillbirth was statistically significantly increased prior to MPN diagnosis. Misscarriage was not statistically significantly increased neither prior to diagnosis nor after. Paper IV is the first in which birthrates are estimated in women with MPN.

To conclude the positive findings of pregnancy and childbirth in women with MPN, it is clear childbearing should not be dissuaded. It is of outmost importance that women have access to adequate information on prognosis of pregnancy outcomes, in order to make informed choices, and not let life-changing decisions be influenced by excessive fear. Paper III and IV are the first population-based studies of pregnancy and childbearing in MPN in comparison with matched controls, and together provides a good knowledge basis to have conversations with young female patients on childbearing.

6.3.2 Context

Previous studies on pregnancy in MPN are hampered by low numbers of described pregnancies and significant heterogeneity in reported pregnancy outcomes. Interestingly, the only other population-based study by Alimam et al also has better outcomes than most published case series.(214) This suggests that selection of patients matters, that small case series may tend to favor inferior outcomes, and demonstrates the importance of reflecting over possible selection bias, in particular when cases are non-consecutive. Selection of patients may have been biased towards patients with inferior outcomes, and conclusions on optimal management are limited by risk of confounding by indication. As an example, patients treated with IFN had improved rates of live birth, and IFN treated patients can be assumed to be have been classified as a higher risk warranting the treatment. This poses the question of a potentially stronger protective roll of IFN than indicated, and whether IFN should be recommended in patient groups that today are classified as low risk. Additional questions include whether aspirin is indicated in *CALR* mutated patients, and if adding LMWH to aspirin and IFN further improves outcomes in patients without previous thrombosis.

6.3.3 Maternal complications

The proportion of MPN women affected by thrombosis during pregnancy in paper III was 1% including antepartum and postpartum period, thus lower than previously reported. We also assessed bleeding during pregnancy, delivery or postpartum bleedings >1000 ml, affecting 14% and 9% of pregnancies in MPN, compared to 8% and 5% in controls. In a meta-analysis by Skeith et al on ET pregnancies, the total proportion with antepartum venous thromboses was 1.1%, and 1.4% during the postpartum period, all events of venous thrombosis occurred in patients without LMWH prophylaxis.(213) Skeith et al also reported 1.6% antenatal bleedings, and 1.9% postpartum bleedings. Comparison between studies requires careful interpretation, since definitions differed, and we included all MPN subtypes, while Skeith

only reported on ET. A lower proportion with thrombosis and higher proportions of bleedings may also suggest that a higher proportion of our patients were treated with aspirin or LMWH.

Preterm birth and fetal loss are outcomes of special interest in paper III and IV, and will thus be outlined further below.

6.3.4 Preterm birth

Preterm birth was significantly increased in women with MPN compared to controls in paper III. Interestingly, when subdividing between iatrogen preterm and spontaneous preterm birth, iatrogen was significantly increased compared to controls, but not spontaneous. Patients with MPN are recommended to be followed by obstetrician-led maternal care and, may have been examined more thoroughly during pregnancy. Preterm birth is reported to occur in 15 % of pregnancies by Aliman et al(214), similar to our findings. Low birthweight and very low birthweight occurred in 8% and 2% in our study, as compare to 15% and 6% in the study by Alimam et al. In a review by Gangat et al, the proportion with preterm birth varied considerably between included studies, 2 – 20% in ET, and 0 – 25% in PV. When results are combined from the largest included case series in ET, 8.1% preterm birth is reported, however with significant heterogeneity, which is lower than 12% in MPN in paper III.(280)

6.3.5 Fetal loss

The question of fetal loss is approached in paper III and paper IV through different aspects. In paper III, where all participants were included at pregnancy, self-reported history of previous miscarriage did not differ between MPN patients and controls. In paper IV, HR of miscarriage was 1.25 (0.89-1.76). When delaying start of the underlying timescale to 9 months after diagnosis, the HR was 1.06 (0.72-1.55). Similar to paper III, there were no differences in history of miscarriages at diagnosis, when information as obtained from the Patient Registers.

In paper III, there were few events of stillbirth, 2 in women with MPN and 1 in a control. In paper IV, history of stillbirth, prior to MPN diagnosis was significantly increased. After MPN diagnosis, there were too few events of stillbirth for comparative statistical analysis, 1 in MPN and 6 in controls. Optimistically but not certainly, this may be interpreted as that the treatment offered to the patients add some protection against stillbirth. We did not have access to treatment data, which is likely relevant since both IFN and aspirin are suggested to increase live birthrates.(205)

The rate of miscarriages and stillbirth is dependent on rate of pregnancies. Theoretically, since there was no statistically significant difference in rate of miscarriage, but there was a significantly lower HR of live childbirth, a larger proportion of pregnancies in women with MPN may be affected by miscarriages.

In the review by Gangat et al of larger ET studies, the miscarriage rate was 28.7% and stillbirth 1.8%.(216) In PV and PMF, data is more limited; the largest MF cohort is 24 pregnancies, 29% miscarriage, and the largest PV cohort is of 121 patients with miscarriage in 24% and stillbirth in 10%.(217, 281) In the 43 pregnancies in PV in paper III, there was one stillbirth. The rarity of stillbirth and heterogeneity among reported incidences add uncertainty. The study by Alimam et al was performed by outreaching to consultant-led maternity centers through United Kingdom Obstetric Surveillance System in the UK, first trimester miscarriages may thus be underestimated.

Miscarriage during pregnancy has been shown to predict future thrombotic risk in patients with ET.(221) Potentially, other obstetric complications may also predict vascular events, and this effect is likely transferrable to all MPN subtypes.

6.3.6 Childbirth pattern

Paper IV is the first in which childbearing patterns in women with MPN are described, hence there is nothing to compare our results to. We found that the rate of childbirth was lower in MPN than in controls, this was however not evident in ET. It remains unclear whether the childbirth rates are lower due to a biologic effect of the MPN or an effect of choices and attitudes toward childbearing that may differ between women with MPN and controls. Attitudes and desire to have children may in MPN be affected by fear of pregnancy complications, heredity factors, future health concerns, or potential negative effect on offspring by medications. The literature on pregnancy in MPN portrays pessimistic outcomes in pregnancy, which may also have affected patients and clinicians' attitudes.

Although MPNs are neoplastic diseases, they are indolent in nature and may in the context of childbearing be more comparable to chronic non-neoplastic diseases. In an Australian study in patients with chronic physical non-communicable diseases, such as diabetes and hypertension, it was showed that the women ideally wanted the same number of children as their peers but expected to have fewer children than healthy subjects, and were less likely to access reproductive health care.(282) To some cancer patients, fear of decreased fertility or infertility is equally distressing as the cancer itself, which stresses the importance of questions regarding childbearing. It has been shown that women with cancer may be reluctant to voice

fertility concerns if not asked specifically while clinicians prefer to have adequate information prior to discussing the subject.(283)

6.4 METHODOLOGICAL CONSIDERATIONS

6.4.1 General

All of the included studies are cohort studies, with MPN being the exposure, and outcomes of interest varying between paper I-IV. A common feature of the outcomes is that they are frequent events in the general population; cancers, infections, and childbirth. The exposure on the other hand, MPN, is rarer, which makes the cohort design appropriate and efficient. Although RCT are considered the gold standard of study designs, all questions are not feasible or ethical to answer by RCTs. RCTs are also associated with other limitations such as cost-effectiveness, and are most appropriate for interventions that require a reasonably short follow up. Cohort studies are the observational equivalent of an RCT, were the function of the matching corresponds to randomization.

The included studies are all based on cross-linking of prospectively collected data from national health registers, for both inclusions of patients and for outcomes. This methodology has great advantages when it comes to power, it allows us to efficiently study large numbers of participants. The population-based inclusion reduces risk of selection bias, and the data is captured equally for patients and controls.

All included studies used matched controls for comparison, which makes the results more understandable, relatable, and interpretable, and allows for estimation of the effect of MPN. The same inclusion and exclusion criteria can be applied for controls and patients studied, in contrast to general statistical comparison, eg SIR.

A widely recognized weakness with register-based methodology is the lack of individual data on lab values, mutational status or given treatment for example. It is however embedded in the methodology to only use data existing in registers, as it would not be feasible to gather medical chart data in this large number of patients, and doing so would affect the unselected inclusion, due to geographically or temporally varying availability. One solution to improve access to individual information would be combining different registers. For example, molecular data and initial lab values are captured in the MPN Blood Cancer Quality Register since 2008. Treatment data on prescribed drugs can be found in the Register of Prescribed

Drugs, since July 2005, and were used in paper II. The reason for not including data from these registers in paper I, III and IV was the limited overlapping time frame with our study periods.

Identification of MPN in registers was complicated by the difference in numbers of patients registered in Cancer Register, Inpatient Register and Outpatient Register. Ideally, all MPN patients would be in the Cancer Register, almost all would exist in Outpatient Register, and only those having required inpatient care would be found in Inpatient Register. Vice versa, all patients found in Outpatient Register or Inpatient Register should be registered in Cancer Register. In paper III and IV register completeness was a concern, as only 26 % of patients were identified through Cancer Register. Diagnoses found in Cancer Register are considered to be more valid. Although the completeness of Cancer Register is generally high, one might suspect that it is lower in diagnoses that do not rely on morphology alone. To overcome the insecurity of a lower completeness of Cancer Register, we required two occasions with diagnoses in Outpatient Register. Additionally, we performed as a sensitivity analysis separately by source of inclusion where the patient populations identified through Outpatient Register and Cancer Register were found to have similar outcomes.

The studies included had long follow-up times, which is an important strength as expected survival for MPN patients is long, and allows for inclusion of larger numbers of patients. However, historical truncation of data occurs, that affects both exposure and outcome, and needs to be considered in analysis and interpretation. Diagnostic criteria and preferred treatments have changed during the study period, for example the lowered thresholds of platelets and hemoglobin in ET and PV respectively. The distinction of pre-PMF also requires consideration, as pre-PMF patients may formerly have been classified as ET. We have thus been careful to compare estimates over calendar periods.

The level of individual detail is lower in register-based studies, leading to reduced possibilities to answer several additional questions, such as identification of individual risk factors. In contrast, the large number of patients and population-based inclusion allows for robustness in estimates and other types of details, such as HR in relation to time and estimation of rate of uncommon events. To conclude, this of type of register-based cohort studies adds important knowledge and provides an excellent helicopter view.

6.4.2 Specific methodological considerations

6.4.2.1 Paper I

A diagnosis of MPN may lead to increased surveillance as well as patient awareness and attention to new symptoms. Being connected to a care institution may lead to increased discovery of second malignancies, and potentially constitute differential misclassification bias of outcomes, with the direction away from the null. To counteract this, HR over time was calculated, as most cancers at some timepoint become obvious, and a sensitivity analysis was performed.

6.4.2.2 Paper II

A difficulty worth mentioning associated to paper II, is how to define exposure to treatment in relation to register of prescribed drugs. In MPN doses and dose intervals of commonly used drugs, such as HU and IFN are highly variable, and adjusted from blood counts. As prescriptions are usually made to last for a year, a period of 18 months was chosen to consider a patient untreated. We assumed that current or recent exposure was more important for risk of infection than cumulative exposure.

6.4.2.3 Paper III

In paper III, a specific limitation was the lower proportion than expected of patients included from Cancer Register, suggesting underreporting in this young patient population. This insecurity in exposure, the risk of differential misclassification, would lead towards the null. It was handled by sensitivity analysis, with separate analysis per source of MPN inclusion, were the estimates were similar between the different sources. We thus expect a limited effect of this uncertainty. Although paper III with 342 pregnancies is one of the largest studies to date on pregnancy in MPN, power to estimate rare outcomes was an issue.

6.4.2.4 Paper IV

Many studies of childbearing in relation to cancer diagnosis, for example in Hodgkin lymphoma, have an underlying time-scale that starts at 9 months post diagnosis, in order to not include patients that were pregnant at diagnosis.(284) We chose not to do this due to several reasons. MPN is a neoplasm with a slow onset, where the diagnosis is commonly established with some latency. The biologic effect of MPN is thus likely to have been present at conception in those that were pregnant at diagnosis. This is supported by the fact that women with MPN had a lower number of children already at diagnosis in MPN. The

psychologic effect of the MPN-diagnosis on choice of having children is however not present, neither is the effect of MPN treatments. In our data, a higher proportion of women with MPN were pregnant at diagnosis/matching date than controls, pregnancy may increase the chances of disease detection since it is associated with health care contacts and attention to symptoms. On the other hand, starting follow-up at diagnosis is in line with the matching. As a sensitivity analysis, we delayed underlying time-scale by nine months, and in most outcomes the estimates were similar. The largest difference was seen in HR of miscarriage in sensitivity analysis, HR 1.06 (0.72-1.55).

6.4.3 Validity and generalizability

6.4.3.1 Internal validity

Internal validity refers to the correctness of the results for the studied participants, and may be reduced by confounding or bias. The perfect internal validity is defined as the counterfactual ideal, and is more realistically imitated in an RCT.

Confounding is controlled for through the matching and statistical analyses. Residual confounding, not possible to control for, in this study would be if MPN patients were more likely to seek medical attention at hospitals for symptoms of malignancy or infection, and controls in primary care, where diagnoses are not captured in national registers. It would also occur if awareness was different among patients and controls. However, an increased risk of infections and second malignancy in MPN, apart from the warning of skin cancer in HU, was not particularly well-known for the majority of the study period, making differences in both patient and clinician awareness less likely.

Missing data could through the designs of the studies be a potential problem in paper III. Degree of missing data was however low. We handled missing data as absence of the event in question and in numerical outcomes it was ignored.

The coherence with what is previously known seems reasonable, as in paper I, were comparison to similar studies are possible. For paper II and III there are few studies with less similar methodology to use for reference, and for paper IV there are no results by others to relate our results to. Taking all possible bias into account, and what impact they may have on the estimates, we consider our results to have a high degree of internal validity.

6.4.3.2 External validity

External validity refers to the ability from the results to draw conclusions and make inferences to the population from which the sample is chosen, to generalize the findings, and cannot exist without internal validity. The study sample in this methodology was all MPN patients in Sweden rather than selected a sample, controls were sampled. We do consider the samples representative. Sample sizes are large enough to produce robust estimates with narrow confidence intervals for main outcomes, in particular in paper I and II. There were secondary outcomes and subgroup analysis that are limited by small numbers, and in paper I and II, power was a limitation. What may differ between countries is access to MPN treatment, e.g. IFN. Ethnicity may affect risk of for example skin cancers. Access to maternal care also varies widely between countries, as does incidence of preterm birth and cesarean section. To conclude, the population-based inclusion ensures a high degree of external validity, and the results may be generalized to patients in countries with similar living conditions and with similar health care access and quality.

7 CONCLUSIONS

In these large population-based studies, it was demonstrated that:

- Patients with MPN have an excess risk of second malignancies, in particular in the skin, but also lung, brain, kidney, pancreas, head neck, esophagus and stomch, and endocrine organs. The excess risk seems to increase with time from diagnosis. Hematologic malignancies of other cell lines, such as lymphoid was also increased.
- Patients with MPN have a two-fold rate of being hospitalized or dying from infection. The excess risk is higher in PMF patients. The risk of infections is not affected by commonly used cytoreductive treatments such as HU or IFN.
- Pregancy in MPN is more common and associated with fewer maternal complications than anticipated. Preterm birth, in particular iatrogen preterm birth is a concern, and sgnificantly increased in MPN patients compared to controls.
- Childbirth rate was lower in MPN than controls, however not in ET. Stillbirth was increased prior to diagnosis. Miscarriage was not increased before diagnosis, and not statistically significantly increased after diagnosis.

8 POINTS OF PERSPECTIVE

8.1 METHODOLOGY

Population-based epidemiological studies are a unique tool to inform us about disease course of patients with MPN. The large amount of prospectively collected data constitutes an important complement to laboratory, clinical and interventional studies. Further technical improvements with automated data entry including laboratory, molecular and treatment data can significantly improve the quality and detail of the health registers going forward.

8.2 MYELOPROLIFERATIVE NEOPLASMS

There are several unmet needs in MPN today, and numerous questions remain to be answered in order to improve prognosis and quality of life.

In chronic phase MPN there is a need of risk assessment tools and biomarkers that allows accurate and highly specific prognostication, for early identification of individuals with a higher risk of both thrombosis and disease progression to myelofibrosis and blast phase. It would be useful for selection of patients for transplantation and personalize surveillance strategies depending on risk of progression. Distinguishing patients with an excellent prognosis are also important, to ease the psychologic burden in living with a chronic malignancy. Risk assessment and new biomarkers would also be needed to select individuals for studies on new treatments that may prevent or protect from progression. Surrogate markers for response and disease modification are highly wanted.

The longevity of most MPN patients makes several important research questions difficult to answer, due to the long follow-up time that would be required to observe effects. Especially questions regarding disease modification and long-term safety of cytoreduction are difficult to answer by conventional scientific methods of high standards. RCTs with follow-up that span over decades are non-existent and even if they would be performed, the outcomes would likely be blurred by cross-over and loss-to-follow-up. This highlights the importance of identifying appropriate surrogate markers and subgroups with higher progression risks. Illustrating the difficulties in studying disease modification in MPN, we still do not know with certainty whether the treatments we have had access to since at least the 1980s; HU and IFN offers protection against disease progression and leukemic transformation.

We need to understand which patient benefits from which treatment, what is the optimal timing of initiating treatment, and whether sequencing of treatments matters. Next generation

sequencing and cytogenetics are a part of prognostication, currently mainly for prognostication in MF and as a diagnostic aid in triple-negative ET. The interplay of genetic alterations, epigenetics or other factors may be complex and of importance. The future will tell what role artificial intelligence or machine learning will play in aiding understanding and interpretation of different risk factors.

Obviously, there is an unmet need of more and better treatment options. Blast phase MPN still carries a dismal prognosis. Patients with MF may have significant symptomatology, poor prognosis, and suboptimal response to JAK-inhibitors. Less obvious but also important is the need cytoreductive options for patients with PV, ET, and pre-PMF, where treatment is ongoing for many years, and thus must be safe, highly tolerable, have minimal effect on quality of life, and preferably reduce the risk of clonal evolution.

Irrespective of subtype and disease stage, the goal of treatment needs to shift. This is underscored by the fact that relative survival is inferior in all MPN subtypes. Freedom of thrombotic and hemorrhagic events and symptom reduction are obvious targets, but the goal needs to be widened to include disease modification, suppression of the malignant clone, avoiding evolution, monitoring for arising and more aggressive subclones. Reduction of mortality from blast phase MPN is likely achieved by preventing it, rather than trying to treat it once it has occurred.

The chronic nature of MPN, and the good prognosis for most patients has likely led to a priority of research resources to more emergent malignancies, and diseases associated with acute mortality. Although most patients with MPN have a good prognosis, MPNs cause significant impact on patients' lives, with increased morbidity and mortality.

9 ACKNOWLEDGEMENTS

There are so many people I wish to express my gratitude towards for enabling this work. Firstly, all the patients with MPN in Sweden. Together you have provided this treasure of data, that we have tried to do the most of, and I hope it will be of use for you, and future patients.

Malin Hultcrantz, my main supervisor, for always showing me the way forward, for sharing your commitment, ambition, fearlessness and joy. From across the Atlantic Ocean, you have managed to always be a present supervisor, offering me support through hard times, and providing me with confidence and eternal inspiration.

Therese Andersson, my co-supervisor, for your endless support, patience, time and thorough methodologic discussions. Without you, statistics would have been so much more difficult and less fun.

Jan Samuelsson, my co-supervisor, who has always had time to offer for a conversation, help with patient care, sharing your expertise, and for your dedication to promoting younger colleagues in various contexts.

Magnus Björkholm, my co-supervisor, for your strategic advice and very meticulous readings. I wish I one day can read as thoroughly as you.

All the other collaborators in my studies, for generously sharing your expertise, and taking your time to improve our work.

The Swedish MPN-group for interesting discussions, and great cooperation with national guidelines and cancer patient pathways

All of my brilliant clinical colleagues, both at Internal Medicine at Södersjukhuset and at Hematology Department at Karolinska. I learn so many new things from all of you, every day, and I am deeply impressed and inspired by your efforts and capability to improve the lives of your patients. I can't tell you enough how much I appreciate this! Amongst many others, I would like to thank Olga Stromberg for being such a great colleague, Johanna Ungerstedt for helping me out with everything, and Martin Jädersten for including me in your omalizumab project.

My clinical supervisors during residency for your guidance, Marlene Mozart, Göran Wållinder, Jan Samuelsson, Eva Ottosson, Patrik Andersson, and mostly Birgitta Wiking Goine.

The wonderful hematology nurses at Karolinska and Södersjukhuset

All of my patients

Birgitta Sander for morphology photos in this book

The teachers and students at KI/Region Stockholm Research School in Epidemiology for all the challenging methodologic discussions.

Everyone else that has supported me along the way, taking your time to offer me pieces of knowledge or strategic advice. They say science is a hard and competitive, I have only met warmth, support and a willingness to bring others forward.

To all of my friends outside of work for bringing joy to my life, amongst others, Karin, Rafaela, Anna, Katharina, Anna, Linnea, Jonna… …

Kristina and Stefan, for helping out with everything in our family. Agnes and Albert will forever remember hot chocolate in cold weather after preschool. You are wonderful grandparents and parents-in-law.

Solveig and Pär, my parents, for letting me grow up surrounded by support, love and encouragement, and my brother Anders for always being there. I wish my father was still here, the way he was.

Last, but certainly not least, thank you to my wonderful family; Kristian for always being there for me, my partner in life. Agnes and Albert, for showing me what is important in life. I love you all!

This thesis was supported by Blodcancerfonden and Radiumhemmets forskningsfonder

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