From Department of Medicine, Division of Hematology, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden

CLINICAL AND EPIDEMIOLOGICAL STUDIES IN MYELOPROLIFERATIVE NEOPLASMS

Malin Hultcrantz

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ABSTRACT

Myeloproliferative neoplasms (MPN), including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are clonal hematopoietic disorders characterized by excessive terminally differentiated myeloid cells. MPNs can progress to secondary myelofibrosis or acute myeloid leukemia (AML)/myelodysplastic syndromes (MDS). Although progress has been made in the understanding of the pathogenesis and management of MPNs, there are still unresolved issues regarding prognosis, causes of death, and risk factors for leukemic transformation. In patients with hematological malignancies, the risk of suicide and suicide attempts is largely unknown.

We conducted a population-based study to establish patterns of survival in 9,384 MPN patients identified from the Swedish Cancer Registry between 1973 and 2008. Relative survival ratios were computed as measures of patient survival. Relative survival was significantly lower in all MPN subtypes compared to expected survival in the general population, reflected in10-year relative survival ratios of 0.64 (95% confidence interval (CI); 0.62-0.67) in PV, 0.68 (0.64-0.71) in ET, and 0.21 (0.18-0.25) in PMF, respectively. Excess mortality was observed in patients of all MPN subtypes during all four calendar periods (p <0.001). Nevertheless, survival improved significantly over time (p <0.001); however, the improvement was less pronounced after the year 2000 and was confined to patients with PV and ET. In conclusion, our findings underline the assertion that all MPNs should be considered serious diseases that reduce life expectancy and highlight the need to improve treatment strategies for these patients.

Through the Swedish Cancer Registry and our national MPN cohort we identified 9,563 MPN patients diagnosed between 1973 and 2005 and their 37,643 matched controls to assess excess mortality and causes of death. Cumulative incidence functions, calculated using a flexible parametric model, were used to estimate 10-year probabilities of death with 95% CIs for six categories of causes of death. The 10-year probability of dying from infections in male MPN patients aged 70-79 years at diagnosis were 4.5% (matched controls; 2.3%), from hematological malignancy 13.7% (0.2%), from cardiovascular disease 16.8% (15.0%), from cerebrovascular disease 5.5% (5.1%), from solid tumor 9.7% (11.5%), and from other disorders 24.9% (14.9%). The excess mortality in MPN patients declined due to a decrease in deaths from hematological malignancies during the first calendar period (1973-1982), infections, and in younger MPN patients, from cardiovascular disease. The overall improvement in 10-year mortality, observed in both patients and matched controls over time, was mainly explained by declines in cardiovascular death. In conclusion, the improved survival over time is multifactorial and can only partly be attributed to improved management of the underlying hematological malignancy.

We conducted a nested case-control study to assess the role of MPN treatment and subsequent AML/MDS risk. From a nationwide MPN cohort (n=11,039; diagnosed 1958-2005), we identified 162 patients (cases) with transformation (153 and nine with subsequent AML and MDS diagnosis, respectively) and their 242 matched controls (MPN patients without AML/MDS transformation). Using logistic regression, odds ratios (ORs) were calculated as measures of AML/MDS risk. Forty-one (25%) of the 162 MPN patients with AML/MDS transformation were never exposed to alkylating agents, radioactive phosphorous (P³²), or hydroxyurea (HU). The ORs for cases receiving 1 to 499 g, 500 to 999 g, more than 1,000 g of HU were 1.5 (95% CI; 0.6-2.4), 1.4 (0.6-3.4), and 1.3 (0.5-3.3), respectively, for AML/MDS development (not significant). Patients with MPNs who received P³² doses greater than 1,000 MBq and more than 1 g of alkylating agents had a 4.6-fold (2.1-9.8; p<0.002) and 3.4-fold (1.1-10.6; p<0.015) increased risk of AML/MDS, respectively. Thus, the risk of AML/MDS development after MPN diagnosis was not associated with HU treatment at any dosage. The fact that only 32% of patients with AML/MDS transformation received doses found here to be leukemogenic indicates a major role for non-treatment-related factors.

To define incidence and risk factors for suicide and suicide attempts in patients with hematological malignancies, we conducted a population-based study in 47,220 patients with hematological malignancies and their 235,868 matched controls. Using Cox regression, the hazard ratios (HRs) for suicide and suicide attempts (combined end-point) in patients with hematological malignancies was 1.9 (95% CI; 1.5-2.3) compared to matched controls during the first three years after diagnosis. When more than three years had elapsed, there was no excess risk of suicide/suicide attempts (HR 1.1; 0.9-1.4). Patients with multiple myeloma carried the highest risk, HR 3.4 (2.3-5.0), and a pre-existing psychiatric disorder was strongly associated with an increased risk of suicide and suicide attempts (HR 23.3; 16.6-32.6). Although suicides contributed marginally to mortality in patients with hematological malignancies, awareness of risk factors for suicide/suicide attempts can facilitate identification of high-risk patients and enable preventive interventions.
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Alk</td>
<td>Alkylating agents</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>Breakpoint cluster region-Abelson fusion (gene)</td>
</tr>
<tr>
<td>BU</td>
<td>Busulfan</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CLL</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td>DIPSS</td>
<td>Dynamic international prognostic scoring system</td>
</tr>
<tr>
<td>EBMT</td>
<td>European Group for Blood and Marrow Transplantation</td>
</tr>
<tr>
<td>EMRR</td>
<td>Excess mortality rate ratio</td>
</tr>
<tr>
<td>ET</td>
<td>Essential thrombocythemia</td>
</tr>
<tr>
<td>HL</td>
<td>Hodgkin lymphoma</td>
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<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>HU</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>ICD</td>
<td>International Statistical Classification of Diseases</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IPSET</td>
<td>International prognostic scoring system for essential thrombocythemia</td>
</tr>
<tr>
<td>IPSS</td>
<td>International prognostic scoring system</td>
</tr>
<tr>
<td>JAK2</td>
<td>Janus Kinase 2</td>
</tr>
<tr>
<td>MBq</td>
<td>Megabecquerel</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplastic syndromes</td>
</tr>
<tr>
<td>MF</td>
<td>Myelofibrosis</td>
</tr>
<tr>
<td>MM</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>MPL</td>
<td>Gene coding for thrombopoietin receptor</td>
</tr>
<tr>
<td>MPN</td>
<td>Myeloproliferative neoplasm</td>
</tr>
<tr>
<td>MPN-U</td>
<td>Myeloproliferative neoplasm unclassifiable</td>
</tr>
<tr>
<td>NHL</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>P(^{32})</td>
<td>Radioactive phosphorous</td>
</tr>
<tr>
<td>PET-MF</td>
<td>Post essential thrombocythemia myelofibrosis</td>
</tr>
<tr>
<td>PMF</td>
<td>Primary myelofibrosis</td>
</tr>
<tr>
<td>pPMF</td>
<td>Prefibrotic primary myelofibrosis</td>
</tr>
<tr>
<td>PPV-MF</td>
<td>Post polycythemia vera myelofibrosis</td>
</tr>
<tr>
<td>PV</td>
<td>Polycythemia vera</td>
</tr>
<tr>
<td>PVSG</td>
<td>Polycythemia Vera Study Group</td>
</tr>
<tr>
<td>RSR</td>
<td>Relative survival ratio SCT</td>
</tr>
<tr>
<td>SIR</td>
<td>Standardize incidence ratio</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
1 INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a group of clonal diseases characterized by excess hematopoiesis affecting one, two, or three cell lineages. The MPNs consists of three major subtypes: polycythemia vera (PV), essential thrombocytemia (ET), and primary myelofibrosis (PMF). Although the entities PV, ET, and PMF, had been described earlier, the interrelatedness of the MPNs was first proposed by Dr. William Dameshek in 1951 (Figure 1). He stated that these diagnoses were all characterized by excess bone marrow proliferation “perhaps due to a hitherto undiscovered stimulus.” Due to the similarities he found and the difficulties in distinguishing between PV, ET and PMF, he suggested that they should be considered as “closely related” and called them “myeloproliferative disorders”. This was long before the molecular background of the diseases was known.

In 2005, several research groups simultaneously discovered a mutation affecting the pseudokinase region of the Janus Kinase 2 (JAK2). This mutation is found in all MPN subtypes and due to the activating nature of the mutation, leads to constant stimulation of myeloid proliferation as already suggested by Dameshek. Since then, a number of additional disease-related mutations have been described. JAK2 mutation-status has been incorporated into the classification systems and also, the nomenclature has been changed from myeloproliferative disorders to myeloproliferative neoplasms to underline the neoplastic nature of these diseases.
MPNs are characterized by a relatively indolent course, which can be complicated by thromboembolic events, progression to secondary myelofibrosis (MF), and transformation to acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS). Advances have been made regarding the understanding of disease mechanisms and management of the MPNs. The importance of phlebotomy to a hematocrit <0.45 in PV patients suggested by Thomas Pearson in 1978 and the discovery of a leukemogenic effect of several cytoReducive therapies led to changes in the management of these diseases. There are however many unresolved clinical issues both regarding disease pathophysiology and optimal treatment. There are still uncertainties regarding survival among MPN patients due to the lack of large studies with long follow-up time. In the hitherto studies, patients with PMF have consistently been reported to have reduced life expectancy. Patients with PV have, in the majority of studies, been observed to have moderately reduced survival. In contrast, according to most reports, but not all, survival of patients with ET is not affected by the disease. In addition, causes of death in MPN patients are not well described. There is a need for better prevention of disease complications such as thromboembolism, progression to secondary MF and transformation to AML/MDS. The potential leukemogenic effect of certain cytoReducive treatments, primarily hydroxyurea (HU), is still a matter of debate. Even though great advances have been, the molecular background of the MPNs is not completely known. How the MPN-associated genetic mutations affect prognosis and if the recently introduced targeted treatments, such as JAK2 inhibitors, can affect disease progression remains to be elucidated.

MPNs and hematological malignancies can greatly affect the quality of life of patients. The diseases, their complications and treatments as well as the psychological strain of a cancer diagnosis can affect the emotional and physical well being of patients. Effects on quality of life can range from minor complaints to fatigue, pain, severe depression with suicidal ideation and even suicides. The incidence and risk factors for suicides in patients with hematological malignancies are not fully known.
1.1 MYELOPROLIFERATIVE NEOPLASMS (MPNS)

1.1.1 Polycythemia vera

Polycythemia vera (PV) was first described in 1892 by Dr. Louis Henri Vaquez in a patient with erythrocytosis and hepatomegaly. Later, Dr. William Osler described several similar patients with a new clinical entity he called Vaquez’s disease. Both Vaquez and Osler recognized a high incidence of stroke in these patients.

The incidence of PV is 1.5-2.0/100,000 persons/year. PV is characterized by an excess erythropoiesis with elevated hemoglobin and hematocrit. The bone marrow is hypercellular and dominated by erythropoiesis (Figure 2) but there is also a certain degree of panmyeloid proliferation leading to elevated white blood cell (WBC) and platelets counts in many PV patients. The JAK2 V617F mutation is found in >95% of patients with PV and an additional 3% of PV patients are positive for mutations in exon12 of the JAK2 gene. The exon 12 mutation results in a more isolated erythropoiesis without leukocytosis and thrombocytosis.

In Sweden, PV was earlier diagnosed according to the Polycythemia Vera Study Group (PVSG) diagnostic criteria and since 2001, patients are diagnosed according to the World Health Organization (WHO) classification system (Table 1). The diagnostic criteria have been fairly constant during the last decades with the addition of the JAK2 V617F mutation in the most recent WHO-classification in 2008.

![Figure 2](image)

Figure 2. Bone marrow of a patient with polycythemia vera showing dominance of erythropoiesis and enlarged megakaryocytes.

Patients with PV have a high risk of thromboembolic events while progression to secondary MF and transformation to AML/MDS are more rare events, see chapter 1.3. Other signs and symptoms of PV can include headaches, dizziness, pruritus which often is aquagenic, fatigue, splenomegaly, and microvascular disturbances such as erythromelalgia.

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Patients are defined as high risk if age is greater than 60 years or if there is a history of previous thrombosis; cytoreductive treatment is recommended if any one of these risk factors are present. A preliminary risk score in PV has been presented and a refined risk score is now under construction. In addition to age >60 years and a history of thrombosis, this risk score includes WBC counts >11 x10^9/L and cytogenetic abnormalities as risk factors for a worse overall survival.

In very early reports of untreated patients, median survival was around 18 months. With the introduction of modern treatments, life expectancy is now significantly longer but the reported survival in PV varies in different studies. Patients with PV are in the majority of studies observed to have a moderately reduced survival but survival has also been reported to be similar to that of the general population.

Table 1. Diagnostic criteria for PV according to the 2008 WHO classification system.

<table>
<thead>
<tr>
<th>Major criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hemoglobin &gt; 185 g/L in men, &gt;165 g/L in women, or hematocrit &gt; 0.52 in men and &gt; 0.48 in women, or other evidence of increased red cell volume*</td>
</tr>
<tr>
<td>2. Presence of JAK2 V617F or other functionally similar mutation such as JAK2 exon 12 mutation</td>
</tr>
<tr>
<td>Minor criteria</td>
</tr>
<tr>
<td>1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation</td>
</tr>
<tr>
<td>2. Serum erythropoietin level below the reference range for normal</td>
</tr>
<tr>
<td>3. Endogenous erythroid colony formation in vitro</td>
</tr>
</tbody>
</table>

Diagnosis requires the presence of both major criteria and 1 minor criterion or the presence of the first major criterion together with 2 minor criteria.

* Hemoglobin or hematocrit greater than 99th percentile of method-specific reference range for age, sex, altitude of residence or hemoglobin greater than 170 g/L in men, 150 g/L in women if associated with a documented and sustained increase of at least 20 g/L from an individual’s baseline value, or elevated red cell mass greater than 25% above mean normal predicted value.

1.1.2 Essential thrombocythemia

Essential thrombocythemia (ET) was the last of the classical MPN subtypes to be described, the first report is from 1934 by Dr. Emil Epstein and Dr. Alfred Goedel. The incidence of ET is similar to PV with 1.5-2.5 new cases per 100,000 inhabitants per year.

ET is characterized by a normocellular bone marrow with an excess of large megakaryocytes (Figure 3). A diagnosis of ET requires sustained thrombocytosis and bone marrow changes consistent with ET, and in the absence of clonal markers, the exclusion of secondary thrombocytosis. The clonal markers in clinical use in Sweden today are JAK2 V617F and MPL mutations, positive in around 60% and 1-3% of patients, respectively. During recent years, certain modifications of the diagnostic criteria have been made. Like PV, ET was initially diagnosed with PVSG criteria, and since 2001, according to the WHO criteria (Table 2). In the WHO classification, presence of significant reticulin or any collagen fibrosis excludes the diagnosis of ET. Other major changes are the differentiation of ET from
prefibrotic PMF (see chapter 1.1.3), the incorporation of JAK2 V617F mutation-status and the lowering of the platelet threshold for ET from ≥ 600 x10^9/L to ≥ 450 x10^9/L. 8,45

Patients with ET are often asymptomatic, clinical signs and symptoms if present are fatigue, headaches, and microvascular symptoms. 8,46,47 There is a significant risk of arterial and venous thrombosis but the risk of progression to secondary MF and transformation to AML/MDS is low in patients with ET, see chapter 1.3.

ET patients with age >60 years, a history of thrombosis, or platelet counts >1,500 x10^9/L have been considered as high risk patients and cytoreductive treatment is recommended if any one of these risk factors are present. 35,48 Recently, a prognostic scoring system, International Prognostic Scoring System for ET (IPSET), was introduced which incorporates age >60 years, history of thrombosis, and WBC count >11.0 x10^9/L as risk factors. 40 IPSET predicts overall survival but is not validated for predicting the risk of progression to post-ET myelofibrosis (PET-MF) or AML since there were too few events during follow-up time. 40 In addition, a thrombosis-risk score called IPSET-thrombosis was developed including the risk factors age >60 years, previous thrombosis, cardiovascular risk factors (hypertension, diabetes mellitus, tobacco use), and presence of JAK2 V617F. Patients were divided into three different risk groups low, intermediate, and high with annual thrombotic risks of 1.03%, 2.35%, and 3.56%, respectively. 47

Survival of patients with ET have in the majority of studies reported to be similar to that of the general population. 15,17,21,22,49 Most of these studies have however included a limited number of patients and/or a limited follow-up time. However, in a study with follow-up longer than 10 years, observed survival was shorter compared to expected survival in the general population. 25

Figure 3. Bone marrow of a patient with ET showing large mature megakaryocytes.
Table 2. Diagnostic criteria for ET according to the earlier PVSG classification\(^4^4\) and the 2008 WHO classification system.\(^8\)

<table>
<thead>
<tr>
<th>PVSG criteria for ET</th>
<th>WHO 2008 criteria for ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Platelet count greater than 600 x10(^9)/L</td>
<td>1. Sustained platelet count &gt;450x10(^9)/L</td>
</tr>
<tr>
<td>2. Hematocrit less than 0.40 or normal red blood cell mass</td>
<td>2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased number of enlarged, mature megakaryocytes. No significant increase of left-shift of neutrophil granulopoiesis or erythropoiesis.</td>
</tr>
<tr>
<td>3. Stainable iron in the marrow or normal red blood cell mean corpuscular volume.</td>
<td>3. Not meeting WHO criteria for PV, PMF, BCR-ABL positive chronic myeloid leukemia, MDS, or other myeloid neoplasms.</td>
</tr>
<tr>
<td>4. No Philadelphia chromosome or BCR-ABL gene rearrangement</td>
<td>4. Demonstration of JAK2 V617F or other clonal marker, or in the absence of JAK2 V617F, no evidence for reactive thrombosis. All four criteria must be met.</td>
</tr>
<tr>
<td>5. Collagen fibrosis of the bone marrow is absent or less than one third of the biopsy area without leukoerythroblastic blood film</td>
<td></td>
</tr>
<tr>
<td>6. No cytogenetic or morphologic evidence for MDS</td>
<td></td>
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<tr>
<td>7. No cause for a reactive thrombocytosis</td>
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</tbody>
</table>

1.1.3 Primary myelofibrosis

Primary myelofibrosis (PMF) was first described by Dr. Gustav Heuck in 1879, in two young patients with massive splenomegaly, and an increased number of abnormal leukocytes.\(^50\) PMF has been called agnogenic myeloid metaplasia, chronic idiopathic myelofibrosis, and myelofibrosis with myeloid metaplasia. In 2006, there was a consensus to exclusively use the term primary myelofibrosis.\(^51\)

The incidence of PMF is around 0.3-1.5/100,000 persons per year.\(^8,15,29\) Sixty percent of patients are JAK2 V617F positive\(^4^2\) and an additional 5-10% of patients harbor mutations in MPL.\(^4^2,4^3,5^3,5^4\) PMF is characterized by an excess production of reticulin and/or collagen fibrosis in the bone marrow. In the earlier stages of the disease, a hyperproliferative phase can be seen, characterized by elevated WBC and platelet counts and often normal hemoglobin levels. As the disease progresses, the bone marrow fibrosis eventually displaces the hematopoietic cells leading to cytopenias in one or more cell lineages. In this stage, PMF patients often have extramedullary hematopoiesis resulting in splenomegaly and the presence of circulating immature cells in the peripheral blood, termed leukoerythroblastic blood picture.\(^8\) Patients can experience early satiety and abdominal discomfort due to the splenomegaly.\(^4^7,5^4\) In addition, cytokine activation and inflammation lead to constitutional symptoms including fever, night sweats, cachexia and pruritus.\(^5^5,5^6\)

Early or prefibrotic PMF (pPMF) was introduced as a new entity in the 2001 WHO criteria.\(^3^3,5^7\) This entity may mimic ET with marked thrombocytosis but patients with pPMF often have higher levels of lactate dehydrogenase and there are significant differences in bone marrow histology compared to ET. Histologically, ET is characterized by large and abundant megakaryocytes while megakaryocytes in pPMF show atypia, dense chromatin structures, and form dense clusters (Figure 4), which is not seen in ET.\(^8,4^5\) Patients with pPMF often have more pronounced granulocytic proliferation and decreased erythropoiesis compared to ET.\(^8,4^5\) Most pathologists agree on this classification, however there is still some controversy concerning the diagnostic differentiation.\(^5^8\)
After the introduction of pPMF, a number of patients previously diagnosed as ET, for example with thrombocytosis and a limited degree of bone marrow fibrosis, are now reclassified as having pPMF. Patients with pPMF have a higher risk of progression to overt MF, transformation to AML, and a worse overall survival compared to patients with ET. Patients with pPMF also have a higher risk of major bleedings but the risk of thrombotic events appears similar in the two entities.

Three different risk scores for PMF patients have been developed during recent years: the International Prognostic Scoring System (IPSS), the dynamic IPSS (DIPSS) and DIPSS-plus. The IPSS assesses the prognosis at the time of PMF diagnosis while DIPSS and DIPSS-plus can be used anytime during follow-up. Based on the number of clinical risk factors, patients are divided into four risk groups; low, intermediate-1 intermediate-2, and high risk. The IPSS and DIPSS incorporate five risk factors; age ≥60 years, haemoglobin <100 g/L, constitutional symptoms, WBC count ≥25 x10⁹/L, and ≥1% circulating blasts. In the DIPS-plus, three additional risk factors have been included; cytogenetic abnormalities (+8, -7/7q-, I(17q), inv(2), -5/5q-, 12p-, or 11q23 rearrangements), platelet count <100x10⁹/L, and red blood cell transfusion dependency. In addition to these risk scores, elevated levels of cytokines (interleukin (IL)-8, IL-2R, IL-12, and IL-15) and free light chains have also been correlated with shortened survival.

PMF is the subtype associated with the worst survival, with overall survival reported to be approximately five years, ranging from 27 to 135 months depending on IPSS score.

Table 3. Diagnostic criteria for PMF according to the WHO 2008 criteria.

<table>
<thead>
<tr>
<th>Major criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Presence of megakaryocyte proliferation and atypia*, usually accompanied by either reticulin and/or collagen fibrosis, or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (i.e., prefibrotic cellular-phase disease)</td>
</tr>
<tr>
<td>2. Not meeting WHO criteria for PV, chronic myeloid leukemia, MDS, or other myeloid neoplasm</td>
</tr>
<tr>
<td>3. Demonstration of JAK2 V617F or other clonal marker (e.g., MPL W515L/K), or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minor criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Leukoerythroblastosis</td>
</tr>
<tr>
<td>2. Increase in serum lactate dehydrogenase level</td>
</tr>
<tr>
<td>3. Anemia</td>
</tr>
<tr>
<td>4. Palpable splenomegaly</td>
</tr>
</tbody>
</table>

**Diagnosis requires meeting all 3 major criteria and 2 minor criteria.**

*Small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering.
1.1.4 Myeloproliferative neoplasm unclassified

Myeloproliferative neoplasm unclassified (MPN-U) was introduced in the Swedish Cancer Register in 1993. This is not a strict entity but a group of patients with unclassified MPN who do not fulfill the criteria for PV, ET, or PMF. These might be patients early in the disease course but most often represent patients in later disease stages with fibrosis where the initial diagnosis cannot be determined.8

1.2 MOLECULAR AND CYTOGENETIC BACKGROUND

The spectrum of genetic abnormalities found in the MPNs has increased over the last eight years. As mentioned, in 2005 an activating mutation in the JAK2 gene was discovered by four different research groups simultaneously.3,6 The JAK2 V617F is a point mutation in the JAK2 gene on chromosome 9 where phenylalanine is substituted for valine in the 617 position. The mutation affects the pseudokinase region of the JAK2 which is situated in the intracellular part of the erythropoietin receptor and leads to constant stimulation of hematopoiesis.3,4 More than 95% of PV patients are JAK2 V617F positive and around 50-60% of ET and PMF patients harbor this mutation.5,40 In addition, mutations in the exon 12 region of the JAK2 gene have been described; these are found in approximately 3% of PV patients and are not seen together with the JAK2 V617F mutation.30 The allele burden of JAK2 V617F is highest in patients with PV and lower in ET and PMF patients.64 Overall, the JAK2 V617F allele burden has not been correlated to patient survival except in PMF where JAK2 V617F positivity with a low allele burden has been associated with a reduced survival.64,65

A number of additional mutations have been described in MPN patients since the original discovery of the JAK2 V617F mutation (Table 4). Many of these mutations affect the JAK-STAT signaling pathway, some direct and some through epigenetic mechanisms.66,67 The
The majority of MPN patients do not have cytogenetic abnormalities and cytogenetic
assessment is not part of routine work-up. Cytogenetic abnormalities are found in 33%, 11%,
and 7% of patients with PMF, PV and ET, respectively.\textsuperscript{74-76} The most common abnormalities are
del (20q), del (13q), +8, +9, and abnormalities in chromosomes 1, 5, and 7. Many of these are
seen in other hematological malignancies, most frequently in MDS, but abnormalities +9 and
13q are relatively specific to MPNs.\textsuperscript{77} Prognostic significance has been described for cytogenetic
abnormalities in PMF but not in PV or ET.\textsuperscript{62,74,76} As mentioned previously, in PV, a new
prognostic system is under construction where cytogenetic abnormalities are incorporated as one
of several prognostic factors.\textsuperscript{36}

In addition to the above mentioned genetic changes, familial aggregation of MPNs has been
described with a 5-7 fold increased risk in first-degree relatives.\textsuperscript{78} In this study by Landgren et
al, no anticipation was seen; however Rumi et al at observed a lower median age in second-
generation compared to first-generation MPN patients in their family studies.\textsuperscript{79,80} The germline
mutations are currently unknown but a predisposing haplotype (46/1) in the \textit{JAK2} locus has been
described.\textsuperscript{81,82}

### 1.3 CLINICAL COURSE

MPNs are associated with increased risks of thrombosis and bleeding. Disease progression can
occur; ET can phenotypically develop into PV\textsuperscript{49,83} and both PV and ET can progress to
secondary MF. PV, ET, and PMF can at any time transform to AML/MDS (Figure 5).
1.3.1 Thrombosis and bleeding

Patients with MPNs have a high risk of both arterial and venous thromboembolic complications. In PV, 11-39% of patients present with a major thrombosis and around 20% develop thrombosis during follow-up. A high hematocrit is associated with an elevated risk of major thrombosis and cardiovascular death, this finding was recently confirmed in a large randomized controlled trial. In patients with ET, the frequency of thromboembolic events in different studies ranges from 10% to 30% at diagnosis and between 8% and 31% during follow-up. The risk of arterial thrombosis is higher than the risk of venous thrombosis but patients of any subtype can develop large liver and splanchnic vein thrombosis. In addition to age >60 years and history of thrombosis, the presence of cardiovascular risk factors, a high WBC count, and JAK2 V617F positivity in ET have been observed as risk factors for thrombosis. Additional underlying mechanisms and risk factors that have been suggested are activation of neutrophil granulocytes and platelets, tissue-factor bearing microparticles, inflammation, endothelial activation, and gender differences where young women have a higher risk of abdominal venous thrombosis. No study has hitherto confirmed a correlation between high platelet counts and an elevated risk of thrombosis.

In both pPMF and overt PMF, the risk of thrombosis is similar to the risk observed in ET. The annual risk of thrombosis was approximately 2% in a large study of PMF patients. JAK2 V617F positivity has also been associated with an elevated risk of thrombosis in PMF.

The risk of major bleeding is elevated in MPN patients although bleeding complications are not as common as thromboembolic complications. The cumulative incidence of bleeding in PV and ET is around 5-6% and in pPMF, the cumulative incidence was 12% in a recent study. Risk factors for bleeding are history of previous bleeding event, thrombocytopenia, and the use of aspirin, and vitamin K antagonists. A higher risk of bleeding has also been noted in patients treated with a combination of aspirin and anagrelide. Platelet counts >1,500 x10^9/L is associated with a higher risk of bleeding, possibly due to acquired von Willebrand disease.
In addition, patients with splenic vein thrombosis and/or splenomegaly are at risk of upper gastrointestinal bleeding from gastric varices.\textsuperscript{91}

1.3.2 Transformation to myelofibrosis and acute leukemia

In PV, the 15-year risk of progression to the so called spent phase or post-PV myelofibrosis (PPV-MF) is 6-34\%.\textsuperscript{38,97,98} In ET, the risk of progression to secondary MF is lower compared to PV, the 15-year risk is around 4-9\%.\textsuperscript{38,41}

MPN patients who transform to AML/MDS have a dismal prognosis.\textsuperscript{99-102} In PV, the 10-year risk of transformation to AML or MDS is 5-10\% but the reported frequency ranges from 2\% to 20\%.\textsuperscript{20,103-106} The risk of transformation to AML/MDS increases with time after diagnosis.\textsuperscript{103} The risk of leukemic transformation in ET is reported to be between 0.65\% and 3.3\% which is lower than in the other subtypes.\textsuperscript{40,49,107} PMF carries the highest risk of transformation, around 20\%.\textsuperscript{11,54,101}

Older age and elevated leukocyte counts have been proposed as risk factors for leukemic transformation.\textsuperscript{49,103,108} In PMF, the DIPSS has apart from predicting survival also been shown to predict transformation to AML.\textsuperscript{109} In addition, several mutations have been associated with leukemic transformation, namely IDH1/2, IKZF1, TP53, NF1, RUNX1, NRAS, SRSF2, and DNMT3A.\textsuperscript{7,110-113} JAK2 V617F positivity in MPN patients has not been correlated to an increased risk of transformation to AML. As mentioned, patients with JAK2 V617F positive disease at MPN diagnosis can transform to JAK2 V617F negative AML.\textsuperscript{72} AML secondary to MPN is often characterized by a more complex karyotype compared to de novo AML indicative of a worse prognosis.\textsuperscript{112}

Treatment with alkylating agents and radioactive phosphorous (P\textsuperscript{32}) have been associated with a higher risk of transformation.\textsuperscript{22,89,106,114} The potential leukemogenic effect of HU is still a matter of debate.\textsuperscript{26} There are no conclusive randomized studies on treatment and AML transformation in patients with MPNs due the late-appearing and rare events in a long-term disease course, and reluctance to randomly assign patients to receive potentially leukemogenic therapies.

1.4 TREATMENT

Patients with MPNs have been treated with different cytoductive treatments and phlebotomy. The goals of treatment have been to prevent disease complications, give symptom relief, and palliation. There is currently no treatment that can prevent progression to myelofibrosis or transformation to AML/MDS and only recently, studies have shown that specific treatments can prolong survival in PMF patients.\textsuperscript{115} Apart from allogeneic stem cell transplantation there is no known cure for MPN.

Phlebotomy for PV has been used since the disease was first described.\textsuperscript{28,116} In the 1970s the importance of phlebotomy was emphasized by Pearson et al and the benefit of phlebotomy to a hematocrit level <0.45 was recently stressed in a pivotal trial.\textsuperscript{9,87}

Treatment of PV and the other MPNs have included skeletal radiation therapy (in 1917), acetylsalicylic acid (1918), potassium arsenite (1933), lead acetate (1942), nitrogen mustard (1950), triethylene melamine (1952), pyrimethamine (1954), 6-mercaptopurine (1962), uracil mustard (1964), chlorambucil (1965) and dapsone (1966). P\textsuperscript{32} was introduced in 1940, busulfan 1958, pipobroman 1962, hydroxyurea and melphalan in the 1970s and
interferon-α in the 1980s.\textsuperscript{116,117}

The PVSG was founded in 1967 and conducted trials for optimization of treatment in PV until 1997. One of their most important trials was PV01 in which a higher risk of transformation to AML was observed in patients treated with chlorambucil and P\textsuperscript{32} compared to patients treated with phlebotomy alone.\textsuperscript{106} Several studies have since then confirmed a leukemogenic effect and an elevated risk of secondary cancer associated with alkylating agents.\textsuperscript{20,114} Alkylating agents, primarily busulfan (BU), were previously one of the first line treatments in MPNs. However, their use has decreased after the leukemogenic effects were recognized.\textsuperscript{10}

HU is a non-alkylating agent which inhibits the enzyme ribonucleotide diphosphate reductase thereby inhibiting DNA-synthesis and cell growth.\textsuperscript{118} The PVSG recommended HU as first line therapy in PV since their prospective trials did not support a leukemogenic effect of HU.\textsuperscript{10} Some investigators have reported a pronounced leukemogenic effect while others have not.\textsuperscript{119-121}

In the large European Collaboration on Low-dose Aspirin trial, 518 patients with PV were randomized to low-dose aspirin or placebo. In the aspirin arm, there was a reduced risk of the combined end point of nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes (relative risk 0.40, \textit{p}=0.03). Since then, low-dose aspirin is standard treatment in PV and also many patients with ET and PMF.\textsuperscript{122,123}

In ET, Cortelazzo et al showed that patients treated with HU had a lower risk of thrombosis compared to patients on placebo.\textsuperscript{124} In the British PT1-trial, HU was associated with a lower risk of arterial thrombosis and progression to MF but a higher risk of venous thrombosis compared to anagrelide in patients with high risk ET.\textsuperscript{94} Recently, the Anahydet study was presented where HU was again compared to anagrelide but this time in high risk ET diagnosed according to the WHO criteria. This study showed no difference in the risk of arterial or venous thrombosis or bleeding.\textsuperscript{125}

In phase II studies, treatment with interferon-α has been shown to induce molecular remissions in PV and reduce bone marrow fibrosis in early stages of PMF.\textsuperscript{126,127} A lower than expected rate of thrombosis has been observed during interferon-α treatment and interferon-α is not considered to be leukemogenic.\textsuperscript{126} However, interferon-α is associated with considerable side effects often leading to treatment discontinuation.\textsuperscript{126}

The first two randomized controlled trials (Comfort I and II) on the effect of the JAK2 inhibitor ruxolitinib vs placebo and vs best available therapy in intermediate-2 and high risk PMF were recently published.\textsuperscript{115,128} There was a decrease in spleen size and symptom burden in the experimental arm of both studies and in Comfort I, a survival benefit was observed in the ruxolitinib arm compared to patients on placebo.\textsuperscript{115}

Therapies that are currently being tested primarily in PMF are additional JAK2 inhibitors, immunomodulators, histone deacetylase inhibitors, mTOR inhibitors interfering with epigenetic mechanisms and signaling pathways.\textsuperscript{20}

Allogeneic stem cell transplantation (SCT), introduced during the 1970s and 80s, is the only known cure for MPN but is associated with a substantial transplantation-related mortality.\textsuperscript{129,130}

Apart from the studies mentioned above, there has been few randomized controlled trials in MPNs which explains why treatment recommendations are to a large extent based on expert opinion. In 2011, a consensus committee consisting of European and American MPN experts
published guidelines for treatment of Philadelphia negative myeloproliferative neoplasms. The Swedish MPN Study Group was founded in 1994 and the first national guidelines were published in 1998. Later, the Nordic MPN Study Group was formed and the latest version of the Nordic MPN guidelines was recently been published.

1.4.1 Current Nordic guidelines

To summarize treatment strategies, patients with PV are treated with phlebotomy and low-dose aspirin, unless contraindicated. In ET, low dose aspirin is also recommended for high risk patients but should be avoided in patients with platelets >1.500x10^9/L due to the risk of acquired von Willebrand syndrome and thereby risk of bleeding. High risk PV and ET patients are treated with cytoreduction, the first line treatment in patients <60 years is pegylated interferon-α and in patients >60 years, HU is the drug of choice.

Treatment for PMF is aimed at relieving symptoms, i.e. anemia can be treated with erythropoietin stimulating agents, elevated blood counts are treated with cytoreduction and splenomegaly can be treated with cytoreductive agents, JAK2 inhibitors, or splenectomy. In younger patients with intermediate 2 or high risk PMF, allogeneic stem cell transplantation should be considered.

1.5 QUALITY OF LIFE AND SUICIDES IN CANCER PATIENTS

1.5.1 Quality of life

Until recently, the quality of life in MPN patients was not well studied. Generally, these diseases are not considered to have a large impact on the quality of life of patients, except in later stages of PMF. Recent studies have, however, shown that the quality of life in the majority of patients is impaired already at diagnosis. In 2007, one of the first quality of life studies was published where up to 80% of patients reported fatigue as a major problem, even in the absence of disease-related features and treatment. In recent years, scoring systems have been introduced and validated in many languages and quality of life is now included as end-point in most randomized controlled trials.

1.5.2 Suicide

Patients with solid tumors have an overall higher risk of committing suicides compared to individuals in the general population. The highest risk was observed in male patients and patients with cancer of the lung, the upper respiratory and gastrointestinal tracts due to the negative effect on quality of life and basic functions such as the ability to breathe, talk, and eat. Disease characteristics predicting a poor prognosis at diagnosis, rapid progression, advanced stage, and limited treatment options have also been correlated with an elevated risk of suicide in cancer patients. In non-cancer patients, the presence of a psychiatric disorder is a strong risk factor for suicide and suicide attempts and male sex is associated with a higher risk. A few studies have shown an association between cancer suicides and an underlying psychiatric diagnosis as well as depression that emerges after the cancer is diagnosed. In Sweden, around 1,200 persons commit suicide every year and the incidence
has decreased steadily since the 1970s. In studies on time trends in cancer suicides declines over time have been also observed. Suicide and suicide attempts have not been studied in detail in patients with hematological malignancies. In some studies, hematological malignancies have been included in subgroup analyses with diverging results. In Misono’s and Hem’s studies patients with any hematological malignancy had a higher suicide risk while no risk increase was seen in a study from California. Björkenstam et al showed a higher risk of suicides in patients with AML while in patients with PV and MF, the risk of suicide was lower than that of the general population. Males with multiple myeloma (MM) had an increased risk while males with leukemia had risks lower than average in a study from the American Surveillance, Epidemiology, and End Results data base.
2 AIMS

The overall aim of these studies is to improve the management of patients with MPNs by increasing our understanding of these disorders and more specifically to:

Define patterns of survival and causes of death in MPN patients and compare the findings to those of the general population.

Elucidate the potential leukemogenic effect of different cytoreductive treatments with a special focus on hydroxyurea.

Assess incidence and risk factors for suicide and suicide attempts in patients with hematological malignancies including MPNs.
3 SURVIVAL AND CAUSES OF DEATH IN MPN PATIENTS (I-II)

3.1 METHODS, PATIENTS, AND CONTROLS

3.1.1 Relative survival in MPN patients

Information regarding patients diagnosed with a malignant disease in Sweden is by law reported to the population-based nationwide Swedish Cancer Register which was established in 1958. It is mandatory for clinicians to prospectively report all incident cancers to the register. From 1984, a double reporting system was introduced for MPNs (for both clinicians and pathologists/cytologists) increasing the registries coverage. All dates and causes of death are reported to the Cause of Death Register. The cross-linkage of registries is facilitated by the unique national registration number which is given to all Swedish residents.

All patients diagnosed with an MPN reported to the Swedish Cancer Register January 1st 1973 to December 31st 2008 were included. By cross-linkage to the Cause of Death Register, information on date of deaths was obtained. Patients were followed until death, emigration or end of follow-up (December 31st 2009), whichever occurred first.

Information on the number of allogeneic stem cell transplantations was obtained from the European Group for Blood and Marrow Transplantation (EBMT) Registry which was founded in 1974.

Relative survival ratios (RSRs) were computed as measures of patient survival. Relative survival is defined as the observed survival in the patient group (where all deaths are considered events) divided by the expected survival of a comparable group from the general population, which is assumed to be free from the cancer under study. RSR provides a measure of total excess mortality associated with a diagnosis of MPN irrespective of whether the excess mortality was directly or indirectly associated with the MPN. Expected survival was estimated using the Ederer II method from the Swedish population life tables stratified by age, sex, and calendar period.

As described in paper I, the 1-, 5-, 10-, 15- and 20-year RSRs with 95% confidence intervals (CIs) were calculated for MPN patients during four calendar periods: 1973–1982, 1983-1992, 1993–2000, and 2001-2008. In the most recent calendar period, 1-, 5- and (due to the limited follow-up time) 8-year RSRs were calculated. Relative survival ratios were calculated separately in patients with different MPN subtypes: PV, ET, PMF, and MPN-U and in addition, RSRs were analyzed in patients diagnosed before versus after 1993 which was when the category MPN-U was introduced. Separate RSRs were calculated for patients in five age categories, <50, 50-59, 60-69, 70-79, and ≥80 years, and separately for men and women.

Excess mortality rate ratios (EMRRs) were calculated using Poisson regression in order to estimate the effects of the factors described above while controlling for potential confounding factors. All EMRRs were adjusted for age, sex and calendar period of diagnosis.
3.1.2 Causes of death in MPN patients and matched controls

We identified all patients diagnosed with an MPN reported to the Swedish Cancer Register from 1973 to 2005. In addition, we retrieved information on MPN patients through a national MPN network (the Swedish Myeloproliferative Neoplasm Study Group) comprising all hematology/oncology centers in Sweden, to include additional MPN patients.\(^{156}\)

For each MPN patient, four controls matched by sex, year of birth, and county of residence were selected randomly from the Swedish Register of Total Population. All controls had to be alive and free of hematological malignancy at the time of MPN diagnosis for the corresponding patients.

By linking the national registration number to the Causes of Death Register, data on cause and date of death was collected from January 1\(^{st}\) 1973 to December 31\(^{st}\) 2007 (end of follow-up). Cause of death was classified into six different categories: infection, solid tumor, hematological malignancy, cardiovascular disease, cerebrovascular disease and other disorders. The category hematological malignancy included patients that transformed to AML/MDS and non-transformed MPN patients where no underlying cause of death other than the MPN was specified. The category cardiovascular disease included deaths due to arterial thromboembolism whereas deaths from venous thromboembolism, congestive heart disease, and cardiac arrhythmias were included in other disorders.

A flexible parametric survival model was used to estimate the cause-specific mortality rates for the six different categories of causes of death in MPN patients compared to matched controls during the first 10 years after diagnosis.\(^{157,158}\) Results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs). The main analysis considered all subtypes combined, and results are presented for all MPN subtypes together, if not specified otherwise. Separate analyses were performed for men and women and for five age groups, 18-49, 50-59, 60-69, 70-79 and ≥80 years. Calendar period of diagnosis was categorized into four groups, 1973-1982, 1983-1992, 1993-2000, and 2001-2005. Likelihood ratio tests were used for model selection. The final model included the variables patient status (MPN patient or matched control), sex, age group, and period of diagnosis as well as an interaction term between patient status and age group. No time-dependent effects were found to be significant so the proportional hazards assumption was assumed to be reasonable for all six causes of death.

The different causes of death were treated as competing events and the probability of death from each of the six causes was estimated as a function of time. This is known as the cumulative incidence function and can be obtained through transformation of the cause-specific mortality rates obtained from the flexible parametric model.\(^{158}\)

An additional analysis was carried out to evaluate the probabilities of death for the subtypes PV, ET, and PMF. As MPN-U was not introduced until 1993 and therefore does not cover all four periods, it was not considered in the subtype analysis.
3.2 RESULTS

3.2.1 Relative survival in MPN patients

A total of 9,384 patients with MPN were identified (PV n=4,389, ET n=2,559, PMF n=1,048 and MPN-U n=1,388). Patient survival was considerably lower in all subtypes of MPN compared to expected survival in the general population. The RSRs for patients diagnosed between 1993 and 2008 are shown in Figure 6. Compared to PV, patients with PMF and MPN-U had higher overall excess mortality, EMRR in PMF was 4.38 (95% CI 3.90-4.91) and in MPN-U 4.57 (3.87-5.41). Patients diagnosed with ET before 1993 had an inferior survival compared to patients with PV. However, after 1993, the relationship was the opposite with 10-year RSRs of 0.72 (0.67-0.76) and 0.83 (0.79-0.88) for PV and ET, respectively. All MPN subtypes were associated with a significantly increased excess mortality during all calendar periods. During the most recent calendar period (2001-2008), the 8-year RSRs were 0.84 (0.77-0.90), 0.91 (0.84-0.97), 0.48 (0.39-0.57), and 0.54 (0.47-0.60) in patients with PV, ET, PMF, and MPN-U, respectively.

![Survival by subtype, 1993-2008](image)

<table>
<thead>
<tr>
<th>Subtype</th>
<th>1-year RSR</th>
<th>5-year RSR</th>
<th>10-year RSR</th>
<th>15-year RSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV</td>
<td>0.99 (0.98-1.00)</td>
<td>0.91 (0.88-0.94)</td>
<td>0.72 (0.67-0.76)</td>
<td>0.56 (0.49-0.63)</td>
</tr>
<tr>
<td>ET</td>
<td>1.00 (0.99-1.01)</td>
<td>0.95 (0.92-0.97)</td>
<td>0.83 (0.79-0.88)</td>
<td>0.66 (0.59-0.74)</td>
</tr>
<tr>
<td>PMF</td>
<td>0.81 (0.76-0.84)</td>
<td>0.42 (0.37-0.48)</td>
<td>0.19 (0.12-0.27)</td>
<td>0.13 (0.06-0.23)</td>
</tr>
<tr>
<td>MPN-U</td>
<td>0.87 (0.85-0.89)</td>
<td>0.63 (0.59-0.66)</td>
<td>0.49 (0.44-0.53)</td>
<td>0.39 (0.32-0.47)</td>
</tr>
</tbody>
</table>

*Figure 6. Cumulative relative survival among MPN patients diagnosed in Sweden between 1993 and 2008 stratified by subtype. 95% confidence intervals are shown within parenthesis.*
A significant improvement in RSRs was observed over time in an analysis of the whole MPN cohort (p<0.001, Figure 7). Compared to patients diagnosed in 1973-1982, the EMRR was 0.60 (0.53-0.67) for patients diagnosed 1983-1992, 0.29 (0.25-0.34) for patients diagnosed 1993-2000, and 0.23 (0.19-0.27) for patients diagnosed 2001-2008. The improvement between the two most recent calendar periods was of borderline significance (p=0.046). In a stratified analysis of patients diagnosed before and after 1993, a significant improvement in RSRs over time was seen in patients with PV and ET while no improvement was observed in PMF patients; the 10-year RSR was 0.21 (0.17-0.25) for PMF patients diagnosed before 1993 and 0.19 (0.12-0.27) after 1993.

Higher age at MPN diagnosis was associated with poorer survival. Improved survival rates were seen in all age groups, in all but the most recent calendar period (2001-2008). Women had a significantly superior survival with an EMRR of 0.72 (0.66-0.78), compared to men (reference 1.00).

Between 1973 and 2008, 71 allogeneic stem cell transplantations were reported in MPN patients to the EBMT Registry.

<table>
<thead>
<tr>
<th>Years since diagnosis</th>
<th>1-year RSR</th>
<th>5-year RSR</th>
<th>10-year RSR</th>
<th>15-year RSR</th>
<th>20-year RSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973-1982</td>
<td>0.82 (0.80-0.84)</td>
<td>0.61 (0.58-0.64)</td>
<td>0.45 (0.43-0.49)</td>
<td>0.32 (0.28-0.35)</td>
<td>0.21 (0.18-0.24)</td>
</tr>
<tr>
<td>1983-1992</td>
<td>0.88 (0.87-0.90)</td>
<td>0.70 (0.67-0.72)</td>
<td>0.54 (0.51-0.57)</td>
<td>0.39 (0.36-0.42)</td>
<td>0.28 (0.25-0.31)</td>
</tr>
<tr>
<td>1993-2000</td>
<td>0.94 (0.92-0.95)</td>
<td>0.80 (0.77-0.82)</td>
<td>0.64 (0.61-0.67)</td>
<td>0.50 (0.46-0.54)</td>
<td>-</td>
</tr>
<tr>
<td>2001-2008</td>
<td>0.95 (0.94-0.96)</td>
<td>0.82 (0.80-0.84)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 7.** Cumulative relative survival among MPN patients in Sweden between 1973 and 2008 stratified by calendar period of diagnosis. 95% confidence intervals are shown within parenthesis.
3.2.2 Causes of death in MPN patients and matched controls

A total of 9,563 patients and 37,643 matched controls were included in the study. Mortality was higher in MPN patients than in matched controls in all age groups, during all calendar periods, and for all causes of death. In male MPN patients aged 70-79 at diagnosis, the HR of dying from cardiovascular disease was 1.5 (95% CI 1.4-1.7), from cerebrovascular disease HR 1.5 (1.3-1.8), from solid tumor HR 1.2 (0.99-1.3), and from other disorders HR 2.3 (2.1-2.6; Table 5). The risk for MPN patients of dying from infection (HR 2.7; 2.4-3.1) and from hematological malignancy (HR 92.8; 70.0-123.1) are shown for all ages combined due to low number of controls dying from these causes.

Table 5. Hazard ratios and 95% confidence intervals (within parenthesis) of cause-specific deaths for MPN patients compared to controls.

<table>
<thead>
<tr>
<th>Patient age at diagnosis (years)</th>
<th>18-49</th>
<th>50-59</th>
<th>60-69</th>
<th>70-79</th>
<th>≥80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>2.7</td>
<td>(2.4-3.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid tumor</td>
<td>2.5</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>(1.3-4.7)</td>
<td>(0.9-1.9)</td>
<td>(0.9-1.4)</td>
<td>(0.99-1.3)</td>
<td>(0.8-1.2)</td>
</tr>
<tr>
<td>Hematological malignancy</td>
<td>92.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>8.9</td>
<td>2.2</td>
<td>(70.0-123.1)</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>(4.0-19.8)</td>
<td>(1.6-3.1)</td>
<td>(1.5-2.2)</td>
<td>(1.4-1.7)</td>
<td>(1.4-1.8)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>8.8</td>
<td>4.7</td>
<td>2.8</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(0.9-97.3)</td>
<td>(2.6-8.5)</td>
<td>(2.1-3.7)</td>
<td>(1.3-1.8)</td>
<td>(1.2-1.7)</td>
</tr>
<tr>
<td>Other disorders</td>
<td>5.2</td>
<td>4.3</td>
<td>3.7</td>
<td>2.3</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>(3.1-8.7)</td>
<td>(3.2-5.7)</td>
<td>(3.2-4.4)</td>
<td>(2.1-2.6)</td>
<td>(1.6-2.0)</td>
</tr>
</tbody>
</table>

In the cumulative incidence function analysis (competing risk model), male MPN patients diagnosed between the ages 70 and 79 years during the calendar period 1993-2000 and their matched controls were used as an example. These patients had an overall 10-year probability of death of 75.0% compared to 49.0% in matched controls (Figure 8). The excess mortality in MPN patients was mainly explained by death from infection, (MPN patients 4.5% vs. matched controls 2.3%), hematological malignancy (13.7% vs. 0.2%), and other disorders (24.9% vs. 14.9%), all differences being statistically significant. There were no significant differences regarding deaths due to cardiovascular disease (16.8% vs. 15.0%), cerebrovascular disease (5.5% vs. 5.1%), or solid tumor (9.7% vs. 11.5%) between this group of male MPN patients and their matched controls. In female patients (same age group and calendar period), the distribution of causes of death was similar but the overall probability of death was lower; 61.0% for MPN patients and 36.3% for matched controls 10 years after diagnosis (Figure 9). Figures 8 and 9 show 10-year probabilities with 95% confidence intervals for the six causes of death in males and females, respectively. In the younger age groups, MPN patients had a higher probability of dying from cardiovascular and cerebrovascular disease compared to matched controls (Figure 11).

The most common causes of death within the category other disorders were congestive heart failure, pulmonary diseases, arrhythmias, and dementia. Venous thrombosis was a less frequent cause of death in the category of other disorders.
In the analyses of MPN subtypes, patients with PMF had the highest total probability of dying during the first 10-years after diagnosis. In PV and ET, the most common cause of death was cardiovascular disease while patients with PMF had a higher probability of dying from hematological malignancy. The pattern of causes of death due to infection, solid tumor, cerebrovascular death and other disorders was similar in all MPN subtypes (Figure 10).

The excess mortality in MPN patients decreased over time primarily due to a decline in deaths from hematological malignancy. This decline in 10-year mortality was observed during the first calendar period (1973-1982), thereafter, the probability of dying from hematological malignancy remained relatively stable during the three most recent calendar periods (Figures 11 and 12). Decreased 10-year mortality from infections and in the younger age groups, also from cardiovascular diseases contributed to the reduction in excess mortality. The overall mortality decreased over time in both patients and matched controls due to reduced probabilities of deaths from cardiovascular disease (Figures 11 and 12).
3. DISCUSSION

In these two studies on survival and causes of death, patients with MPNs had an inferior relative survival as well as a higher probability of death from any cause compared to the general population. There was an excess mortality during all calendar periods and in all MPN subtypes, including ET. The major causes of death in MPN patients were cardiovascular events and hematological malignancy. Interestingly, the RSRs improved significantly over time. There was a decrease in deaths due to cardiovascular disease in both patients and controls while the decrease in excess mortality in MPN patients was mainly explained by reduced probability of death due to hematological malignancies, infections, and in young patients, also cardiovascular diseases.

Overall, PV was associated with an inferior relative survival similar to findings in earlier reports.\textsuperscript{13,16,18,20,38} PV was the subtype with the highest proportion of cardiovascular deaths and cerebrovascular deaths compared to the other subtypes. In previous studies, major causes of
death in PV were thromboembolic events and transformation to AML.\textsuperscript{18-20} Relative survival improved significantly over time. Several factors may have contributed to the improved survival observed in PV. Aspirin prophylaxis and stringent adherence to the hematocrit goals for phlebotomy have been shown to be beneficial in preventing thromboembolic complications.\textsuperscript{35,87,122} In addition, a more widespread blood count screening, introduced during the second and third calendar periods,\textsuperscript{159} most likely led to an earlier establishment of MPN diagnoses and thereby a better overall survival (lead time bias).\textsuperscript{153}

ET was associated with an excess mortality throughout the study period. This finding contrasts results from previous studies stating that survival is not affected by the disease.\textsuperscript{15,21,22,24,49} Possible explanations for this discrepancy may be limited patient samples, short follow-up, or patient selection in earlier studies. In a few studies with follow-up longer than 10 years, an inferior survival in ET was also observed.\textsuperscript{24,25} In ET, the most common cause of death was cardiovascular disease supporting reports on high risks of arterial thrombosis in ET.\textsuperscript{88,94} RSRs improved significantly over time in ET, reflecting better disease management but also improved diagnostics. Possible misclassification with inclusion of patients with early PMF in the ET group may have contributed to the low RSR in ET before 1993. An accurate diagnosis differentiating pPMF from ET is important not only for predicting overall survival but also for assessing the risk of disease complications.\textsuperscript{41,59,60} The reported number of ET patients in each calendar period increased over time, probably reflecting a better coverage of the register rather than a true increase in incidence. A recent report from the Swedish Cancer Register revealed a high coverage, >90%, of MPN diagnoses during recent years.\textsuperscript{123} There may also have been a certain selection in reporting of only the severe ET cases during the first calendar period, resulting in a better survival rate with increasing registration of patients with less aggressive disease during the latter part of the study period. These factors may have contributed to the low RSRs and higher probability of death from hematological malignancy in ET patients during the earlier calendar periods of this study. Future studies will elucidate whether or not ET classified according to WHO is associated with a reduced survival.

PMF was associated with the highest excess mortality, consistent with previous studies on survival in PMF.\textsuperscript{11-15,23} In addition, we found no improvement in survival over time. In a later detailed analysis stratified by age group, we observed an improvement over time in the younger age groups.\textsuperscript{160} Similar to the findings recently presented in an Italian study,\textsuperscript{12} the most common cause of death in PMF patients was hematological malignancy reflecting the higher risk of disease progression and transformation to AML/MDS compared with patients of other subtypes.\textsuperscript{161} Additionally, these patients experience a high risk of thromboembolic and hemorrhagic events, contributing to the excess mortality.\textsuperscript{31,52,61} Several novel therapies have been investigated in PMF, such as thalidomide, lenalidomide, and JAK2 inhibitors, but only a few patients during the study period were included in clinical trials of these therapies in Sweden.\textsuperscript{128,162} Promising data on symptom relief, reduction of spleen size, and also improved overall survival has been presented in patients treated with JAK2 inhibitors.\textsuperscript{115,128}

Deaths due to cardiovascular events decreased over time in both patients and controls. During the study period, there has been a general improvement in prevention and treatment of cardiovascular disease. Reduced smoking, treatment of hyperlipidemia, and better acute management of vascular events are factors contributing to the decreased mortality from vascular disease.\textsuperscript{163,164}
Death from hematological malignancy was one of the main contributing factors to the excess mortality in MPN patients compared to matched controls. The probability of dying from hematological malignancy decreased between the first and second calendar period, which may in part be explained by the decreasing use of leukemogenic treatments during the first calendar period. Thereafter, the probability of dying from hematological malignancy remained stable throughout the study period. Apart from the decreased use of P, alkylators, strategies for cytoreductive treatment did not undergo any major changes during the three most recent calendar periods. Allogeneic stem cell transplantation was introduced during the 1970s but only a small number of MPN patients were eligible for this treatment during the study period.
The risk of deaths from infectious diseases decreased over time reflecting improved diagnostics and treatment of infectious complications. The probabilities of deaths due to solid tumor were similar in MPN patients and in matched controls. The observed excess mortality due to other disorders in MPN patients may, at least to a certain degree, reflect a higher frequency of co-morbidities which may have contributed to the medical workup eventually leading to the detection of the MPN.

In the cause of death study (II), we analyzed both HRs, showing relative risks, and the cumulative incidence function, showing absolute risks in the presence of the competing risks. Due to the competing causes of death, the results from the two measures differ slightly. The cause-specific mortality rates of all of the six causes of death were higher in MPN patients than in the matched controls. However, in absolute terms, when we take into account that patients are at risk of dying from more than one cause, the proportion of deaths from each cause is not consistently higher in MPN patients than in the controls. Also, by preventing patients from dying of certain causes they will have a higher risk of dying from something else, hence the increases of deaths from other causes.

There was a higher mortality in men compared to women. Similar patterns have been observed in several hematological malignancies but the underlying causes for the better outcome in women are unknown. There may be variations in tumor characteristics, management, and/or life style factors; which need to be elucidated in future studies.

Older age at MPN diagnosis was associated with poorer survival. As survival is already adjusted for the mortality rates in older patients, the higher mortality in older patients may reflect more aggressive diseases and/or a higher rate of treatment related complications. Some authors report a less optimal stringency to treatment guidelines and a less restrictive use of leukemogenic therapies in older patients due to the expected shorter survival. Since life expectancy is increasing in all age groups, the importance of keeping to treatment recommendations also in older patients should be emphasized.

The highest relative risks of cardiovascular deaths were seen in the younger age groups, partly since the low absolute number of deaths in the younger age groups will result in greater relative change. Patients younger than 60 years are currently considered at low risk of thrombosis and cytoreductive therapy is not recommended. This age group showed the largest improvement over time however; further optimization of thromboprophylaxis in MPNs is of great importance and better risk stratification measures to identify and treat high-risk patients below the age of 60 years are needed.

Strengths of these studies are the population-based design, the large size and long follow-up. Relative survival is a reliable measure of excess mortality, whether it is directly or directly associated with the cancer under study, and estimates are independent of accurate classification of cause of death. In the second study on causes of death, one of the limitations is the uncertainty regarding classification in the Cause of Death Register. The reporting is user-dependent and the rate of autopsies has decreased during the last decades. Another limitation in these studies is the lack of detailed medical information such as clinical and laboratory data in the Swedish Cancer Register, therefore, we were not able to confirm individual diagnoses. In addition, as discussed, there were changes in the classification system during the study period which may have influenced the comparison of survival and risk of complications in different MPN subtypes over time.
Figure 11. Probability (%) of dying from the six categories of causes of death in relation to calendar period of diagnosis for male MPN patients aged 50-59 years at diagnoses and their matched controls.

Figure 12. Probability (%) of dying from the six categories of causes of death in relation to calendar period of diagnosis for male MPN patients aged 70-79 years at diagnoses and their matched controls.
In summary, in this large population-based study, we found all MPN subtypes to have a significantly reduced life expectancy compared to the general population, even in the most recent calendar period. The excess mortality was mainly explained by death from the underlying hematological malignancy, infections, and other disorders. Survival improved over time in all age groups and in patients with PV and ET. Overall, there was no improvement in survival of patients with PMF, and only a marginal improvement was observed between the two most recent calendar periods in the whole MPN cohort. The excess mortality in MPN patients decreased due to reduced probabilities of death from hematological malignancy and infections, and in younger patients also cardiovascular disease. The decline in deaths from cardiovascular disease over time in both MPN patients and controls most likely reflects a general improvement in the prevention and treatment of cardiovascular diseases. Taken together, the reason for the observed improvement in survival in MPN patients over time is multifactorial and can only marginally be attributed to improvements in the disease-specific treatment of the underlying MPN. The reduced life expectancy seen in this study in all MPN subtypes underlines the need to optimize our current treatment strategies and highlights the need for effective disease-modifying therapies to improve the outlook for MPN patients.
4 TRANSFORMATION TO ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES (III)

4.1 METHODS, PATIENTS, AND CONTROLS

All patients diagnosed with an MPN reported to the Swedish Cancer Register from 1958 to 2005 were identified. Additional cases not reported to cancer register were identified from a national MPN network, the Swedish Myeloproliferative Neoplasm Study Group, which includes all hematology/oncology centers in Sweden.

Information of a subsequent diagnosis of AML or MDS in patients within the MPN cohort was obtained through the Swedish Cancer Register. Detailed information on treatment (type of therapy, cumulative dose, duration of treatment) and laboratory variables at diagnosis, including full blood count, bone marrow examination (at MPN diagnosis and at transformation), and any other tumor preceding AML/MDS, was collected from medical records of both cases and controls. Patients and controls were excluded if the diagnosis of MPN or AML/MDS was incorrect, if there was lack of relevant medical information, or if they had received chemotherapy or radiotherapy for a non-MPN malignancy.

The risk of AML was analyzed in the MPN patients identified through the Swedish Cancer register. The absolute risks with respect to MPN subtype are presented by means of Kaplan-Meier curves (Figure 13). The risk in relation to the expected risk in the population is presented as standardized incidence ratio (SIR; i.e., ratio of observed to expected numbers of AML cases). The expected number of cases was estimated by multiplying the age-, sex-, and calendar year-specific person-years of follow-up for patients with MPNs who developed AML, with the corresponding AML rates in the general population obtained from the Swedish Cancer Register. Ninety-five percent CIs for the SIRs were determined based on the assumption of Poisson-distributed number of observed cases. All patients were observed from date of MPN diagnosis to date of death, emigration, diagnosis of AML, or end of follow-up, whichever occurred first. In this set of patients, the risk of AML transformation in relation to WBC count at diagnosis was calculated using χ² test. Patients who developed MDS were not included in these analyses because this diagnosis was not registered in the Swedish Cancer Register until 1993.

We then performed a nested case-control study where the risk of transformation to AML/MDS was analyzed in relation to exposure to MPN treatment. For each patient with MPN and a subsequent AML/MDS diagnosis (i.e. cases), up to two patients with MPNs without AML/MDS matched for MPN subtype, year of birth (±5 years), sex, and date of MPN diagnosis (±1 year) were identified (i.e. controls). A control patient could be used for more than one patient case but with adjusted follow-up time. In this analysis, 65 patient cases were excluded because there were no matched controls available in the database.

Conditional logistic regression was used to analyze the risk of transformation in relation to cumulative doses of HU, P³², and alkylating agents. The matching factors were adjusted for age, sex, calendar period, and MPN subtype. A subset analysis of patients with PV/ET and risk of AML were performed. Results are presented as odds ratios (ORs) with 95% CIs. Analysis of survival after AML in relation to MPN treatment was performed in all patients with relevant clinical information.
4.2 RESULTS AND DISCUSSION

From the Swedish Cancer Register, we identified a total of 292 MPN patients who transformed to AML (n=271) or MDS (n=21). Of these, 130 patients were excluded due to reasons mentioned above. A total of 162 patients (153 with AML and 9 with MDS) were included in the study of treatment exposure. Two hundred and forty-two control patients who had not transformed to AML/MDS were identified. Thirty-four percent of patients were diagnosed before the year 1980. The majority of MPN cases had PV (68%) followed by ET (16%), PMF (9%) and MPN-U (7%).

The SIR for developing AML was 35.1 (95% CI 30.6-39.9) in MPN patients. The highest risk of transformation was seen in PMF (SIR, 63.8; 42.7-91.6), followed by PV (SIR, 33.0; 27.8-38.9), ET was the subtype associated with the lowest risk (SIR, 24.7; 17.3-34.2). The calculated SIRs are probably conservative estimates due to underreporting of AML/MDS transformation to the cancer register. Overall, there was no significant difference in risk of transformation to AML between men and women. A WBC count ≥9.0 x10^9/L at MPN diagnosis was observed in 62.0% of patient cases and 66.4% of controls, respectively. The median WBC count of patient cases and controls with AML at diagnosis was 10.6 and 10.8 x10^9/L, respectively. Thus, earlier reports on the association between leukocytosis and elevated risk of transformation to AML could not be confirmed.169

![Graph](image.png)

**Figure 13.** Risk of AML transformation in relation to time since MPN diagnosis according to subtype. Analyses included all patients with MPNs with AML transformation who were identified from the Swedish Cancer Register

There was no significant association between HU exposure and risk of transformation to AML/MDS. The ORs for cases receiving 1 to 499 g, 500 to 999 g, and more than 1,000 g of HU were 1.5 (95% CI, 0.6 to 2.4), 1.4 (0.6 to 3.4), and 1.3 (0.5 to 3.3), respectively, for AML/MDS development (not significant). The portion of patients on HU alone was similar in MPN cases and controls (21%; Table 6). The potential leukemogenic effect of HU has
remained a controversial issue for several years.\textsuperscript{26,103,170} Many of the earlier studies reporting a leukemogenic effect of HU are retrospective and there may be a significant amount of patient selection in these studies. There may also be confounding by indication\textsuperscript{171} i.e. patients may be at higher risk of transformation not because of treatment-related factors but rather because of a longer disease course and/or more aggressive disease biology, causing exposure to higher doses and/or multiple drugs.\textsuperscript{172} We cannot rule out confounding by indication in our study, however, there was no significant difference in cumulative dosages of HU in transformed cases and controls (Table 7). Patients treated with HU during long time periods for benign diseases such as sickle cell anemia have not been reported to carry an increased risk of developing AML.\textsuperscript{173} Based on our findings, we cannot totally exclude a leukemogenic effect of HU but if any, the potential risk is limited compared with the risk associated with the disease itself.

Table 6. Risk of AML/MDS Transformation according to preceding treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Patient cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>None</td>
<td>41</td>
<td>25</td>
</tr>
<tr>
<td>Alk only</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>P\textsuperscript{32} only</td>
<td>39</td>
<td>24</td>
</tr>
<tr>
<td>HU only</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>Alk + P\textsuperscript{32}</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Alk + HU</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>HU + P\textsuperscript{32}</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Alk + P\textsuperscript{32} + HU</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

MPN cases who had received high doses of P\textsuperscript{32} or alkylators were at a higher risk of transformation to AML/MDS. When the cumulative doses were analyzed, the ORs were 4.6 (2.1-9.8) in patients treated with P\textsuperscript{32} doses of \( \geq 1000 \) MBq and 3.4 (1.1-10.6) in patients treated with \( \geq 1.0 \) g of alkylating agents (Table 7). Lower exposure to P\textsuperscript{32} and alkylating agents were not associated with significantly increased risks of transformation. In an analysis restricted to PV and ET only, results were essentially the same. Results were also unchanged when the analysis was restricted to AML and MDS was excluded. Although based on small patient numbers, this may indicate the existence of a threshold exposure of P\textsuperscript{32} and alkylators for AML/MDS transformation in MPNs.

Time to AML transformation differed between the treatment groups. In patients with no cytoreductive treatment and with HU only, 40% and 42% respectively, transformed more than five years after diagnosis. In contrast, a majority of patients treated with alkylating agents (76%), P\textsuperscript{32} (77%), or combinations of the two (91%) experienced transformation after more than 5 years. This may corroborate the notion that HU is nonleukemogenic because the majority of patients administered P\textsuperscript{32} and/or alkylators experienced transformation at a later time point.

A higher risk of transformation was observed in MPN cases treated with two or more types of cytoreductive treatments compared to patients receiving single-agent treatment at any dose level (Table 8). This may reflect a true risk elevation associated with sequential therapy but also, as mentioned, reflect a more aggressive underlying MPN contributing further to the risk of leukemic transformation (confounding by indication).\textsuperscript{22,120,174}
Importantly, 25% of transformed cases had never received cytoreductive therapy prior to transformation, indicating that MPNs have an inherent propensity of leukemic transformation. In addition, only 32% of patients with transformed disease were exposed to cumulative doses of P\textsuperscript{32} and/or alkylating agents shown here to be leukemogenic.

**Table 7.** Risk of AML/MDS in all patients and controls with MPNs in relation to cumulative dose of cytoreductive treatment. ORs are mutually adjusted for the other treatments. Figures in bold denote significant differences.

<table>
<thead>
<tr>
<th>Cumulative doses</th>
<th>Patient cases</th>
<th>Controls</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HU (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>111 (69)</td>
<td>179 (74)</td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>1-499</td>
<td>24 (15)</td>
<td>29 (12)</td>
<td>1.5</td>
<td>0.6-2.4</td>
</tr>
<tr>
<td>500-999</td>
<td>14 (9)</td>
<td>15 (6)</td>
<td>1.4</td>
<td>0.6-3.4</td>
</tr>
<tr>
<td>≥1,000</td>
<td>13 (8)</td>
<td>19 (8)</td>
<td>1.3</td>
<td>0.5-3.3</td>
</tr>
<tr>
<td>P\textsuperscript{32} (MBq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>92 (57)</td>
<td>161 (67)</td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>1-499</td>
<td>14 (9)</td>
<td>21 (9)</td>
<td>1.5</td>
<td>0.6-3.3</td>
</tr>
<tr>
<td>500-999</td>
<td>16 (10)</td>
<td>32 (13)</td>
<td>1.1</td>
<td>0.5-2.2</td>
</tr>
<tr>
<td>≥1,000</td>
<td>40 (25)</td>
<td>28 (12)</td>
<td><strong>4.6</strong></td>
<td><strong>2.1-9.8</strong></td>
</tr>
<tr>
<td>Alkylating agents (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1-0.49</td>
<td>15 (9)</td>
<td>26 (11)</td>
<td>1.1</td>
<td>0.5-2.3</td>
</tr>
<tr>
<td>0.5-0.99</td>
<td>11 (7)</td>
<td>12 (5)</td>
<td>1.7</td>
<td>0.6-5.0</td>
</tr>
<tr>
<td>≥1.000</td>
<td>12 (7)</td>
<td>8 (3)</td>
<td><strong>3.4</strong></td>
<td><strong>1.1-10.6</strong></td>
</tr>
</tbody>
</table>

Survival in transformed patients was poor, median survival was three months and survival was independent of previous therapy for the MPN (Figure 14). Patients with PV and PMF tended to have worse survival after AML transformation compared to transformed ET patients; however, the difference was not statistically significant. In the majority of earlier studies on AML secondary to MPN, observed median survival has been 3-6 months.\textsuperscript{100,175} The few long-term survivors in our cohort had all undergone allogeneic SCT, which has been reported to be the only effective treatment of post-MPN AML.\textsuperscript{99}

**Table 8.** Risk of AML/MDS transformation according to number of cytoreductive treatment types. Figures in bold denote significant differences.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>P\textsuperscript{32} only</td>
<td>1.5</td>
<td>0.8-2.8</td>
</tr>
<tr>
<td>Alkylating agent only</td>
<td>0.9</td>
<td>0.4-2.1</td>
</tr>
<tr>
<td>HU only</td>
<td>1.2</td>
<td>0.6-2.4</td>
</tr>
<tr>
<td>Mixed treatment (two or three)</td>
<td><strong>2.9</strong></td>
<td><strong>1.4-5.9</strong></td>
</tr>
</tbody>
</table>

Strengths of this study are its large size and the population-based design. The use of a nested case-control design based on information from nation-wide registries and medical records minimizes the risk of recall bias. One limitation is the underreporting to the registries both of MPN patients, particularly in the earlier years of this study, but most notably the underreporting of transformed cases. In total, 2.6% of patients transformed to AML/MDS which is low compared to other studies.\textsuperscript{54,103} The study was also limited by the relative lack of matched controls in spite of the large cohort of MPN patients. We therefore did a sensitivity
analysis with more relaxed matching criteria including 44 additional transformed AML/MDS patients and the corresponding matched controls. The results were similar to the main analysis which further corroborates the robustness of our data.

Figure 14. AML survival in relation to previous MPN therapy

In conclusion, the MPNs have an inherent propensity to transform to AML/MDS regardless of treatment as 25% of transformed patients had never been exposed to any cytoreductive medication. Higher doses of P32, alkylating agents, and sequential use of two or more cytoreductive agents are associated with an elevated risk of leukemic transformation. HU, even at high doses, was not associated with an increased risk of transformation to AML or MDS. Thus, we conclude that the risk of transformation is to a large extent associated with the disease itself and any potential leukemogenic effect of HU is of less importance.
5 SUICIDE AND SUICIDE ATTEMPTS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES (IV)

5.1 METHODS, PATIENTS, AND CONTROLS

All patients with a hematological malignancy reported to the Swedish Cancer Register between 1992 and 2006 were included in the study. For each patient, five controls matched for age, sex, and county of residence were chosen randomly from the Swedish Register of Total Population. All controls had to be alive and free of any preceding hematologic malignancy at the time of diagnosis for the corresponding patient.

Since 1964, the National Inpatient Register holds information on all somatic and psychiatric hospital discharges with a high level of coverage.\textsuperscript{176} From the Inpatient Register, information on admissions due to suicide attempts and due to psychiatric disorder was obtained. Pre-existing psychiatric disorder was defined as at least one admission with a psychiatric diagnosis prior to the cancer diagnosis. All psychiatric diagnoses, including substance abuse, were considered. Suicide and suicide attempts were defined as \textit{Intentional self-harm X60-X84} from ICD10 and \textit{Suicide and self-inflicted injury E950-E959} from ICD9. \textit{Events of undetermined intent Y10-Y34} (ICD10) and \textit{Injury Undetermined whether accidental or purposely inflicted E980-E989} (ICD9) were also included because a substantial portion of these deaths are considered to be suicides.\textsuperscript{134,177}

Information of suicides, suicide attempts and pre-existing psychiatric disorder were obtained by cross-linkage to the Cause of Death Register and the National Inpatient Register.

For patients with consummated suicides during the first three years following diagnosis, detailed information on patient characteristics, stage and progression of the disease as well as treatment was collected from patient medical records. The presence of substantial pain was defined as pain complaints being noted in the medical record and/or treatment with continuous pain medication.

Patients and controls were followed from the date of diagnosis or the corresponding time for the controls, until death, emigration, or end of follow-up. Suicide attempts were assessed until December 31\textsuperscript{st} 2006 and suicides until December 31\textsuperscript{st} 2005 due to delayed reporting to the Cause of Death Register. Cox-regression was used to analyze the risk of suicide and suicide attempts and results are presented as HRs with 95% CIs. In addition, incidence of suicide and suicide attempts were analyzed in relation to pre-existing psychiatric disorder in both patients and controls. Results are presented as events per thousand person-years of follow-up.

The HRs for suicide and suicide attempts were analyzed in relation to age, sex, and time after diagnosis. Since the increased risk was confined to the first three years after diagnosis and was of the same magnitude regarding both suicides and suicide attempts, the results represent the combined end-point suicide/suicide attempts appearing during the first three years after diagnosis unless not otherwise specified. Separate analyses were performed for the different subtypes of malignancies: non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), multiple myeloma (MM), acute leukemia (including both acute myeloid leukemia and acute lymphoblastic leukemia), and chronic lympho- and myeloproliferative disorders. The latter group consisted mainly of chronic lymphocytic leukemia (CLL), chronic myeloid leukemia...
(CML), and myeloproliferative neoplasms (MPNs) including the MPN subtypes PV, ET, and PMF.

5.2 RESULTS AND DISCUSSION

A total of 47,220 patients and 235,868 matched controls were identified. Fifty-five percent were men and median age at diagnosis was 70 years (range 18-102 years). Among patients, there were 54 suicides and 158 suicide attempts, of which 36 and 100, respectively, occurred during the first three years after diagnosis.

The risk of suicide and suicide attempt was twice as high in patients with hematological malignancies compared to the matched controls, HR 1.9 (95% CI 1.5-2.3). The risk was elevated during the first three years after diagnosis; there was no statistically significant excess risk when more than three years had elapsed. An analysis only including consummated suicides showed identical risks, HR 1.9 (1.3-2.8 p=0.0005) during the first three years after diagnosis, and HR 1.2 (0.8-1.8) after three or more years of follow-up (Table 9). Similar patterns, with highest risks shortly after diagnosis, have been reported for patients with solid tumors and in studies where hematological malignancies have been included in subgroup analyses.\textsuperscript{133-136,138,139,142,178} In a recent Swedish study, the risk of suicide and cardiovascular death was significantly elevated during the first year, even the first weeks, after a cancer diagnosis indicating that a cancer diagnosis is associated with both emotional and physical stress.\textsuperscript{178} A higher risk of suicide has also been associated with advanced disease stage, poor prognosis, and rapid progression.\textsuperscript{139} After more than three years following diagnosis, patients who are still alive may either be cured or may have adapted emotionally to the cancer diagnosis.

The overall excess risk associated with hematological malignancy was not significantly modified by age at diagnosis. The number of suicides/suicide attempts was higher among male patients men but when compared to controls, the HRs were similar in men and women. In general, men have a higher propensity of committing suicide but studies on gender differences in cancer patients have shown great variations.\textsuperscript{137,139-141} There was a trend towards a lower risk in patients diagnosed after 1999 (HR 1.6; 1.2-2.1) compared to patients diagnosed before 1999 (HR 2.2; 1.7-2.9) although this difference was not statistically significant (p=0.065; Table 10). Similar results with decreasing risk of cancer suicides have been previously reported.\textsuperscript{135,147}

### Table 9. Suicide and suicide attempts in relation to time after diagnosis in patients compared to matched controls.

<table>
<thead>
<tr>
<th>Event Type</th>
<th>No of events</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suicides/suicide attempts ≤3 years after diagnosis</td>
<td>136</td>
<td>1.9</td>
<td>1.5-2.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Suicides ≤3 years after diagnosis</td>
<td>36</td>
<td>1.9</td>
<td>1.3-2.8</td>
<td>0.0005</td>
</tr>
<tr>
<td>Suicides/suicide attempts &gt;3 years after diagnosis</td>
<td>76</td>
<td>1.1</td>
<td>0.9-1.5</td>
<td>0.2855</td>
</tr>
</tbody>
</table>

MM was associated with the highest risk of suicide and suicide attempts (HR 3.4; 2.3-5.0) during the first three years after diagnosis. MM is in most cases incurable and both the disease itself, leading to physical impairment and painful bone lesions, and its treatment can greatly impact the quality of life of patients.\textsuperscript{179} Treatment regimens, including chemotherapy, autologous stem cell transplantation, irradiation, and novel agents are associated with side effects such as neuropathy and fatigue.\textsuperscript{180} In addition, treatment with high doses of
corticosteroids, included in most treatment regimens, can induce depression and psychosis, both known risk factors for suicide.\textsuperscript{181,182} A high risk of suicide has been observed in patients with solid tumors that greatly impair the quality of life and physical functioning.\textsuperscript{136} In a study from the American Surveillance, Epidemiology, and End Results database, Kendal et al indicated an elevated risk of suicide in male MM patients compared to women; this study did however not include a control population.\textsuperscript{141} It is of great importance that disease-specific treatment as well as the psychological care in MM patients is optimized.

There was a significant increase in risk of suicide and suicide attempts in patients with NHL (HR 1.7; 1.2-2.3). In patients with HL, acute leukemia, and CLL/CML/MPN there was a tendency towards an increased risk, however this was not statistically significant (Table 10). Some investigators have reported that patients diagnosed with malignancies with a poor prognosis, including acute leukemias, are at a higher risk of suicide.\textsuperscript{135,147} New treatment options have emerged especially for patients with MM, NHL, and CML during the study period.\textsuperscript{183-186} Simultaneously, there was a trend towards a decrease in risk of suicide/suicide attempts in patients diagnosed during the second calendar period. Many of the recently introduced treatments have a favorable effect on the quality of life in these patients but their potential impact on risk of suicide needs to be elucidated.

There was a multiplicative interaction in patients with a pre-existing psychiatric disorder and a hematological malignancy (Table 10). Compared to controls without a pre-existing psychiatric disorder, the following risks were seen during the first three years of follow-up: patients with a pre-existing psychiatric disorder HR 23.3 (16.6-32.6), controls with a pre-existing psychiatric disorder HR 10.8 (8.8-13.2), and patients without pre-existing psychiatric disorder HR 1.8 (1.4-2.3; Table 10). It is well established that persons with psychiatric disorders are at an elevated risk of committing suicide. However, to our knowledge, the interaction between hematological malignancies and a pre-existing psychiatric disorder and risk of suicide/suicide attempts has not been studied in detail before. The elevated relative risks were reflected also in higher incidences of suicide/suicide attempts per 1,000 person years in patients and controls with pre-existing psychiatric disorders (Figure 15). In accordance with the relative risks, there was no difference in incidences between in patients and controls when three or more years had elapsed.

The most common psychiatric disorder in these patients was depression. Depression is often underdiagnosed and undertreated in cancer patients\textsuperscript{187,188} and several authors have previously shown that patients with cancer have an elevated risk of depression compared to the general population.\textsuperscript{142,144,189,190} In addition, depression has been shown to correlate with more rapid cancer progression.\textsuperscript{191} Hypotheses of a common immunological background of cancer and depression have been suggested. Neurobiological and immunological changes including the serotonin system, hyperactivity in the hypothalamo-pituitary-adrenal axis, and dysregulation of a number of different cytokines have been shown to correlate with both depression and cancer and may be a contributing underlying cause of both diseases.\textsuperscript{192-194} One of these cytokines was IL-6 which also has been observed in high levels in MM patients and has been associated with MM progression.\textsuperscript{195-197} In addition, IL-6 has been suggested as one of the cytokines associated with disease-related fatigue seen in MPN.\textsuperscript{132} The potential immunological relationship needs to be further clarified and if this association is shown to be truly causative, it may have great implications for the treatment of depression in cancer patients as well as the cancer itself.
Table 10. Risk of suicide/suicide attempts in relation to calendar period of diagnosis, sex, age, type of hematological malignancy, and history of psychiatric disorder during the first three years after diagnosis

<table>
<thead>
<tr>
<th>Calendar period of diagnosis</th>
<th>Patients ≤ 3 years of follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of events</td>
</tr>
<tr>
<td>1992 – 1998</td>
<td>80</td>
</tr>
<tr>
<td>1999 – 2006</td>
<td>56</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>72</td>
</tr>
<tr>
<td>Female</td>
<td>64</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td></td>
</tr>
<tr>
<td>≤69</td>
<td>63</td>
</tr>
<tr>
<td>≥70</td>
<td>73</td>
</tr>
<tr>
<td>Subtype</td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>46</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>11</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>37</td>
</tr>
<tr>
<td>Acute leukemia (AML/ALL)</td>
<td>9</td>
</tr>
<tr>
<td>Other (CLL/CML/MPN)</td>
<td>33</td>
</tr>
<tr>
<td>History of psychiatric disorder</td>
<td></td>
</tr>
<tr>
<td>Patients without a pre-existing psychiatric disorder (n=45,178)</td>
<td>92</td>
</tr>
<tr>
<td>Patients with a pre-existing psychiatric disorder (n=2,042)</td>
<td>44</td>
</tr>
<tr>
<td>Controls without a pre-existing psychiatric disorder (n=224,841)</td>
<td>325</td>
</tr>
<tr>
<td>Controls with a pre-existing psychiatric disorder (n=11,027)</td>
<td>147</td>
</tr>
</tbody>
</table>

* Test of homogeneity. This significance is mainly contributed by a higher risk for multiple myeloma. HR= hazard ratio, CI= confidence interval, AML= acute myeloid leukemia, ALL= acute lymphoblastic leukemia. CLL= chronic lymphocytic leukemia, CML= chronic myeloid leukemia and MPN= myeloproliferative neoplasms.

Thirty-six patients committed suicide during the first three years after diagnosis. Of these, 23 had NHL, nine had MM, two had AML, and two had PV. The majority of patients were in remission or on active therapy aiming at remission. Twelve patients (33%) were on active treatment which included corticosteroids in 10 patients. Fifteen patients were in remission with no ongoing treatment and six patients were in a palliative phase. Substantial pain was noted in the medical record of 14 patients. Sixteen patients (44%) had a pre-existing psychiatric disorder and nine patients had attempted to commit suicide prior to the suicide. In these 36 patients, depression was the most common psychiatric disorder followed by alcohol abuse. Thirteen patients did not have a pre-existing psychiatric disorder and were not in a palliative phase, five of these had MM. Only a minority of patients were in a palliative phase where the by others termed “rational suicides” may be found, reflecting the wish to put an end to suffering and pain from an advanced incurable cancer. The methods used for suicides were, in order of
frequency: hanging, intoxication (most often with sedatives), jumping/falling, drowning, use of firearms, and deaths due to burns/fire.

The first step of suicide prevention is to identify patients at an increased risk, which in this study indicated patients who were recently diagnosed, patients with MM, and a pre-existing psychiatric disorder. Additional risk factors observed in previous studies are male sex, poor social support, advanced disease, and emotional distress including a feeling of hopelessness.\textsuperscript{199,200} There are reports stating that patients who committed suicide visited their doctor shortly before the suicide, 25\% within the week before, and that suicides are often committed shortly after discharge from the hospital.\textsuperscript{145,177} Early identification of these patients is important since effective treatment in multidisciplinary teams, including psychological intervention and anti-depressive treatment, can lead to a better quality of life and reduce the risk of suicide and suicide attempts.\textsuperscript{187,191,200-202}

Strengths of this study are the population-based design including a large number of patients under a period of 16 years. The study includes information from Swedish registries, which have a high quality and a high level of coverage,\textsuperscript{150,176} as well as detailed information from individual medical records. One limitation is the possibility of underreporting of suicides to the Cause of Death Register. There may also be underestimation of suicide attempts and pre-existing psychiatric disorders since this information was obtained from the National Inpatient Register only including patients who had been admitted to hospital with these diagnoses. In addition, psychiatric complaints emerging after the cancer diagnosis are not analyzed in this study. In a preliminary report, we observed that patients with AML had a higher risk of suicide than MM patients.\textsuperscript{203} In this report a different time period and statistical method were used. We therefore find the results from the current study, where matched controls are used as comparison, more reliable and accurate.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure15.png}
\caption{Suicide and suicide attempts among patients and controls with and without a pre-existing psychiatric disorder per year, starting at diagnosis shown as incidence per 1,000 person-years.}
\end{figure}
In summary, in this large population-based study we found patients with hematological malignancies, especially MM, to have a higher risk of suicide and suicide attempts compared to matched controls during the first three years following diagnosis. The risk elevation was strongly associated with a pre-existing psychiatric disorder. Future studies are needed to elucidate the underlying neuropsychiatric and immunological causes, which may be shared in depression and cancer. Even though the absolute number of suicide/suicide attempts was small, awareness of risk factors such as male sex, a MM diagnosis, short time since diagnosis, and a pre-existing psychiatric disorder may facilitate identification of high-risk patients. Our results emphasize, in addition to optimal treatment of the malignant disease, the need for psychological assessment and early detection of cancer patients at high-risk of committing suicide in order to enable effective prevention of suicide/suicide attempts.
6 SUMMARY AND CONCLUSIONS

Survival and cause of death studies (I-II)
Patients with MPNs have an inferior relative survival compared to the general population. Cardiovascular disease was a major contributing factor for mortality in MPN patients. However, the excess mortality mainly consisted of a high risk of dying from the underlying hematological malignancy. Survival improved over time but there was still significant excess mortality during the most recent calendar period under study (2001-2008).

Transformation to acute myeloid leukemia and myelodysplastic syndromes in MPN patients (III)
There was a significantly increased risk of transformation to AML/MDS in patients treated with high doses of alkylating agents and Pt32, and in patients treated with two or more cytoreductive regimens. There was no correlation between treatment with HU at any cumulative dose and risk of transformation to AML or MDS. Twenty-five percent of cases with AML/MDS had never been exposed to cytoreductive agents, indicating a major role for non-treatment-related factors.

Suicide and suicide attempts in patients with hematological malignancies (IV)
Patients with hematological malignancies carry a two-fold elevated risk of suicide and suicide attempts. Risk factors are male sex, a diagnosis of MM, and a pre-existing psychiatric disorder. Although suicides contributed marginally to mortality in patients with hematological malignancies, awareness of risk factors can facilitate identification of high risk patients and enable preventive interventions.
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