PHILADELPHIA CHROMOSOME NEGATIVE MYELOPROLIFERATIVE NEOPLASMS AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirement for the degree of Master of Medicine (MMed)

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DECLARATION

I, Yusuf Mayet declare that this report is my own unaided work. It is being submitted for the degree of Master of Medicine in the Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand. It has not been presented for any other degree or examination at this or any other University.

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Dr Yusuf Mayet MBBCh (Wits) FCP (SA) Date

DEDICATION

To my wife, Zahraa

for her love, patience, and encouragement

and to my family

for their undivided support

ETHICS COMMITTEE APPROVAL

This research was approved by the Ethics Committee for Research on Human Subjects, University of the Witwatersrand (Clearance Certificate Number: M121175).

ABSTRACT

BACKGROUND

Myeloproliferative Neoplasms (MPN) are a heterogeneous group of diseases that are characterized by clonal proliferation of the erythroid, myeloid, or megakaryocytic lineages. The discovery of the JAK2 V617F mutation has provided new insights into the disease and has broadened the therapeutic landscape. The management of MPN is aimed at relieving symptoms of disease, managing and modifying the signs of disease, and preventing complications. There is a lack of data with regard to MPN in South Africa and this study attempts to understand this disease entity as represented by patients at a large public sector hospital.

PATIENTS AND METHODS

This study is a retrospective review of adult patients seen at the Clinical Haematology Unit, Department of Internal Medicine at Chris Hani Baragwanath Academic Hospital during the period 01/01/1987 to 31/12/2011. The aim of the study was to determine the profile of patients diagnosed with Polycythaemia Vera (PV), Essential Thrombocythaemia (ET), and Primary Myelofibrosis (PMF), and in particular to describe the demographics, clinical presentation, management, and outcomes of patients with MPN.

RESULTS AND DISCUSSION

A total of 94 patients were analyzed of which 37% were diagnosed with ET, 18% were PV, and 45% PMF. The median age of presentation was 63, 54, and 64 for ET, PV, and PMF respectively with a female to male ratio of 1.24:1 for all patients. Fatigue, weight loss, and pruritus were the most common presenting symptoms, while splenomegaly was found in 80% of patients and was the predominant clinical manifestation. It was noted that hydroxyurea was the most important form of cytoreductive therapy used, especially in ET where 91% received it. Venesection was performed in 100% of PV patients. JAK2 V617F mutation testing became available in 2006 and of all the patients tested, 100% (ET), and 89 %(PV), and 89 %(PMF) were positive. Only 5% of patients achieved a

complete response whilst on the treatment available. Transformation to acute myeloid leukaemia was shown in 9% of the patients, whilst 22% ultimately resulting in death.

CONCLUSION

Ph1 chromosome negative MPN in South Africa is an uncommon disease which most commonly occurs in the 55 to 65 year old age group. Overall, it is slightly more common in females compared to males, with particular reference to ET. PMF is the most common Ph1 chromosome negative MPN, followed by ET and PV. Patients with Ph1 chromosome negative MPN present typically with fatigue, constitutional symptoms and splenomegaly. Presentation to hospital with thrombotic events and bleeding was also significant. The mainstay of treatment is variable, depending on the nature of the MPN i.e. venesection for PV, and hydroxyurea for ET and PMF. Complications such as arterial and venous thrombosis, bleeding and leukaemic transformation occurred at a similar rate to that described in the literature. JAK2 V617F mutation investigation was available since 2005, and 93% of all patients that were tested, proved to have the mutation. More frequent and routine testing of this mutation is needed in the future. Other treatment options such as anagrelide, interferon alpha, and JAK inhibitors were used at a very low frequency in our population of MPN patients. The prognosis of patients correlates with other studies of MPN, with PMF showing the worst prognosis and lowest median overall survival rate, and the highest transformation rate to acute leukaemia. More widespread use of JAK2 inhibitors in the future may impact favourably on the adverse prognosis and poor survival.

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ABBREVIATIONS

Allo-SCT	Allogenic Stem Cell Transplant
BU	Busulphan
CAL-R	Calreticulin
CML	Chronic Myeloid Leukaemia
DIPSS	Dynamic International Prognostic Scoring System
EPO	Erythropoietin
ET	Essential Thrombocythaemia
ELN	European Leukaemia Network
FDA	Food and Drug Administration
Hct	Haematocrit
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HU	Hydroxyurea
IFN	Interferon alpha
IPSET	International Prognostic Score for ET
IPSS	International Prognostic Scoring System
IWG-MRT	International Working Group Myeloproliferative Neoplasms Research and Treatment
JAK2	Janus Kinase 2
JAK-STAT Pathway	Janus Kinase and Signal Transducer and Activator of Transcription Pathway
LDH	Lactate Dehydrogenase
MPD	Myeloproliferative Disorders
MPL	Myeloproliferative Leukaemia Virus
MPN	Myeloproliferative Neoplasms
MPN-SAF	Myeloproliferative Neoplasm Symptom Assessment Form
Ph1	Philadelphia Chromosome
PI3	Phosphoinositide 3-kinase
32P	Phosphorus32
PV	Polycythaemia Vera
PCR	Polymerase Chain Reaction
PMF	Primary Myelofibrosis
TPO-R	Thrombopoeitin Receptor
UA	Uric Acid
WHO	World Health Organization

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

In 1951, William Dameshek coined the term “Myeloproliferative Disorders (MPD)” as a clinico-pathological entity which included chronic myelogenous leukaemia (CML), polycythaemia vera (PV), essential thrombocythaemia (ET), and primary myelofibrosis (PMF) (1). The word disorders in “myeloproliferative disorders” has now been replaced with the word “neoplasms”, according to the World Health Organization (WHO) classification of myeloproliferative neoplasms (MPN) published in 2008 (2).

The discovery of myeloid cells occurred as early as the 17th century when van Leeuwenhoek and Lieutaud described red cells and white cells respectively (3). The description of platelets was attributed to Alfred Donne in 1842 while Bizzozero was credited with their role in homeostasis (1). Before Dameshek, leukaemias were classified into myeloid and lymphoid subtypes by Paul Ehrlich in 1880, and the notion that a common stem cell gave rise to distinct cell lineages was shown (4).“Splenic Medullary Leukaemia” was a term used by Heuck in 1879 for 2 patients with an increased number of abnormal leucocytes and the presence of marrow fibrosis, which is now known as PMF (5). In 1892 a man presented to Dr Louis Henri Vaquez, with features of chronic “cyanosis”, hepatosplenomegaly, and marked erythrocytosis which he considered to be congenital heart disease (6). William Osler reviewed 18 similar cases in 1908 and referred to these as “Vaquez disease” or “polycythaemia with cyanosis, thus PV was first recognized (7). ET was the last of the MPN to be described in 1934 by Epstein and Goedel (1). The interrelationship between the MPN was supported by the discovery of post PV myelofibrosis in 1935 and this suggested that trilineage myeloproliferation arose from a common primitive reticulum cell (8). Substantial progress has been made since these early descriptions of the MPN and more exciting developments were to follow.

Molecular and cytogenetic mechanisms of the MPN have been studied for many years. The identification of the fusion gene BCR/ABL and the t(9;22) translocation which generates the Philadelphia chromosome (Ph1) as the critical pathogenetic event in CML has led to a better understanding of this entity, as well as the development of specific therapy targeting the molecular defect (9). Hence the development of the tyrosine kinase inhibitor, imatinib , led to one of the most successful therapeutic agents in the history of haematological malignancies (10). Furthermore, in 2005, the discovery of a point mutation in the Janus Kinase 2 (JAK2) gene in the majority of patients with Ph1 negative MPN were reported (11). The mutation was called the JAK2 V617F and further revolutionized our knowledge of the disease pathogenesis and led to redefining of diagnostic criteria.

The MPN are a heterogeneous group of diseases that are characterized by increased proliferation of the erythroid, megakaryocytic or myeloid lineages. They are thought to arise from transformation of a haemopoetic stem cell. They are classified into 9 different entities, which includes the 4 common MPN i.e. CML, PV, ET, and PMF, as well as uncommon entities such as Chronic Neutrophilic Leukaemia, Chronic Eosinophilic Leukaemia, Hypereosinophilic Syndrome, Systemic Mastocytosis, and MPN Unclassifiable (12). The three major non-leukaemic, Ph1 negative disorders in this classification are PV, ET, and PMF.

1.2 PATHOGENESIS

The origins of MPN from a clone of haematopoietic stem cells was confirmed by Phillip Fialkow and colleagues in the 1960's and 1970's (13). Techniques demonstrating inactivation of the X-chromosome were performed on women with PV or ET, who carried a polymorphic variant of the X-linked glucose-6-phosphate dehydrogenase gene (14). This suggested that some myeloid cells were clonally derived. The defining phenotype of these disorders thus includes an increased red cell mass in PV, an increased platelet count in ET, and fibrosis of the bone marrow in PMF. The subsequent discovery of the somatic mutation in the JAK2 gene further facilitated how a molecular defect was accountable for these disorders (15).

Normal haematopoiesis is dependent on the JAK-STAT (Janus Kinase and Signal Transducer and Activator of Transcription) pathway. The Janus Kinase (JAK) group consists of JAK1, JAK2, JAK3, and TYK2. Cytokine receptors, upon binding of cytokines, recruit and activate JAK kinases (16). This results in phosphorylation of downstream signalling pathways such as phosphoinositide 3-kinase (PI3), RAS, and transcription factors termed STATs (signal transducers and activators of transcription) (16). JAK2 is particularly involved in transducing intracellular signalling by the receptors for erythropoietin, thrombopoietin, interleukin 5, interleukin 3, and granulocyte-macrophage colony stimulating factor (17). STAT 3 and STAT 5 phosphorylation mediated by JAK2 leads to nuclear translocation of STAT heterodimers and homodimers, which bind to DNA sequences on genes that regulate cell proliferation, differentiation, and apoptosis (18). Aberrations in these pathways have been shown to be the underlying cause for leukaemias and MPN (19). Since these MPN are closely related, transitional forms may exist, as well as evolution from one form to another (20).

1.2.1 JAK2 V617F Mutation

An acquired point mutation in the JAK2 gene was discovered in 2005, in the majority of patients with Ph1 negative MPN (21). This was a milestone in our understanding of Ph1 negative MPN. This mutation at codon 617 of the JAK2 protein is a guanine to thymidine transversion, which substitutes a phenylalanine for a valine (22). Studies have demonstrated that the mutation occurs in more than 95% of patients with PV, and in 50 to 60% of patients with ET or PMF (23). Most patients with MPN are heterozygous for JAK2 V617F, but there is a subset of patients, particularly with PV, that are homozygous for the JAK2 V617F allele (24). Homozygosity of JAK2 V617F results from acquired uniparental disomy at chromosomal locus 9p24. Also demonstrated in studies is the fact that homozygous subclones produce increased signalling and thus a more robust clinical and haematological picture, than the heterozygous subtype, particularly in patients with PV (24). It is also hypothesised that the level of JAK2 V617F kinase activity could determine the phenotype of the MPN (25). Therefore according to this hypotheses, low kinase activity would lead to thrombocythemia, while high levels of activity would to polycythaemia or myelofibrosis (26).

JAK2 is a cytoplasmic tyrosine kinase receptor involved in transducing intracellular signalling, as mentioned previously. The effects of the JAK2 V617F mutation are activation of STAT5, MAPK/ERK, and PI-3K/AKT signalling pathways (27). This thus causes further changes in the cell cycle, cell differentiation and apoptosis in a cytokine independent or hypersensitive manner. Promotion of the G1/S transition in haematopoietic cell lines occurs with upregulation of cyclin D2 and down regulation of p27 (28). The anti-apoptotic effect of the mutation is due to the overexpression of BCL-XI, as a

consequence of STAT5 activation (29). The net result is the production of a diverse range of proteins that promote cell survival and proliferation.

Associations between the JAK2 V167F mutation and the clinical presentation of MPN have been documented. Patients with high mutational loads were shown to present more commonly with advancing age and were also more likely to suffer with life threatening complications (30). An increased thrombotic as well as bleeding risk were demonstrated (31). The incidence of progression to large splenomegaly, higher leucocyte counts, as well as transformation of the MPN to the fatal acute leukaemia was also increased in patients with the JAK2 V617F mutation, especially in patients with PMF (32). Conflicting results have been seen with regard to the impact of the mutation with overall survival (33). Some studies showed that the mutation had no impact, while others showed that patients with PMF and a positive JAK2 V617F mutation had poorer survival, but no consensus has been reached (33).

The JAK2 V617F mutation has definitely led to a better understanding of the mechanism of the MPN process, but has also given us further knowledge on risk stratification, provided us with new tools of diagnosis of MPN, and ultimately has led to the trials and production of therapeutic options. Since 2005, many JAK2 inhibitors have been developed, majority of which are being evaluated under clinical trials (34).

Almost all patients with PV have been shown to be JAK2 V617F positive and about 50 to 60% of patients with ET and PMF, thus the question has been proposed as to how patients, who are JAK2 V617F negative, still display similar clinical features to those who have the mutation. This may suggest that they result from the deregulation of the same JAK/STAT pathway, activated by the occurrence of other gain of function mutations (35).

1.2.2 Other Mutations

The JAK2 V617F mutation is a gain of function mutation located in exon 14. However several gain of function JAK2 mutations have been discovered since, on exon 12 (36). They have been shown to cause hypersensitivity and activation of the erythropoietin receptor signalling pathways. These mutations on exon 12 were shown in studies to be characterized by isolated erythrocytosis at clinical onset in patients who are JAK2 V617F negative, and also to have a similar disease progression to patients who are JAK2 V617F positive (37). A prospective study on 338 patients diagnosed with PV revealed that 4.1% of these patients carried a JAK2 mutation on exon 12.

The MPL (Myeloproliferative Leukaemia Virus) oncogene encodes the Thrombopoeitin Receptor (TPO-R) protein. MPL is key in growth and survival of megakaryocytes. Gain of function MPL mutations have also been described that is associated with an MPN phenotype, and includes splenomegaly, thrombocytosis, myelofibrosis, and an increased risk of thrombosis (38). Subsequently, other MPL mutations including MPLW151L and MPLW151K were described in patients with ET and PMF at frequencies ranging from 3% to 15% (39).

Another mutation that has been recently described is Calreticulin (CAL-R). CAL-R is an oncogene present in the endoplasmic reticulum, involved in ensuring proper glycoprotein folding, contributing to calcium homeostasis, and also influences processes such proliferation, apoptosis and immune responses (40). A CAL-R mutation was found in 70% to 84% of patients diagnosed with ET and PMF without JAK2 or MPL mutations (41). The significant number of patients with this mutation definitely warrants further research into a possible new diagnostic approach to MPN, as well as provides new insight into further therapeutic options.

1.3 CLINICAL PRESENTATION

Many patients have been diagnosed with a MPN after an incidental abnormal finding on full blood counts, having presented to their general practitioner with non-specific symptoms. However, there are patients that have presented with much more debilitating features such as thrombosis or haemorrhage. These features lead to significant morbidity and mortality amongst the afflicted patients. The symptomatic burden may be severe and also present in most patients with the disease. The signs and symptoms are a result of the disease process such as hyperviscosity in PV, thrombocytosis in ET, or extramedullary haemopoeisis in PMF.

Non-specific symptoms such as fatigue, headaches, dyspnoea, blurred vision, night sweats, and abdominal discomfort have been reported in patients with a MPN. Hypermetabolic symptoms such as a low grade fever, weight loss, and night sweats have also been reported, especially in patients with PMF (42). A more common complaint in those with PV has been pruritus. Aquagenic pruritus which is severe epidermal itching evoked by contact of water, has been reported in 43% of patients in one study (43). Another symptom that causes distress to patients particularly with PV and ET is erythromelalgia. This is the burning sensation of hands and feet with associated erythema or cyanosis, and is deemed as pathognomonic of microvascular thrombotic complications (44). Both these symptoms seem to be fairly responsive to aspirin treatment. Altered blood viscosity leads to symptoms of epigastric distress as well as transient visual disturbances due to alterations in the gastric

and retinal blood flow. Patients with PMF also tend to complain of bone pain, as a result of osteosclerosis or periostitis. A prospective study on patients with MPN was able to formulate a Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF), which is able to gauge MPN severity and debilitation (45).

Organomegaly in the form of splenomegaly and hepatomegaly are common in MPN due to the process of increased cell turnover as well as extramedullary haemopoeisis. Massive splenomegaly is the hallmark of PMF, and also carries the risk of splenic infarction (46). Pallor is the result of anaemia and other features such as petechiae, or purpura is secondary to bleeding from abnormal platelet function. The more serious and fatal complications are associated with thrombotic and haemorrhagic manifestations of disease. Thrombotic arterial and venous disease is a common presenting feature of MPNs, with increased rates seen in PV and ET. Risk factors for arterial thrombosis includes advanced age, previous thrombosis, a history of cardiovascular risk factors and JAK2 V617F positive disease (47). Studies also revealed that 23% of patients diagnosed with splanchnic vein thrombosis were further diagnosed with a MPN (48). The fact that all Ph1 negative MPN have the propensity to transform into each other, may make it difficult to classify initially, however they may also transform into an acute leukaemia. This could be part of the evolution of disease, however in a prospective study of 1683 patients, it was noted that the use of cytoreductive agents in patients with PV showed an increased risk for transformation to acute myeloid leukaemia (49).

The diagnosis of MPN is first suspected when abnormalities of the full blood count accompanies the clinical features mentioned. Laboratory features include an increase in haemoglobin (Hb), haematocrit (Hct), uric acid (UA) and lactate dehydrogenase (LDH) in patients with PV, however decreased levels of erythropoietin (EPO) are seen. A thrombocytosis in ET, and anaemia with a leucoerythroblastic picture on the blood film, is commonly reported in PMF. However, the diagnosis is always not obvious, and may require further investigations such as a bone marrow aspirate and trephine to assess for features such as fibrosis, other bone marrow infiltration, and to rule out possible chronic myeloid leukaemia (CML). Of importance is to rule out other reactive causes of thrombocytosis, or secondary causes of polycythaemia, before a diagnosis is made. The investigation of JAK2 V617F mutation by Polymerase Chain Reaction has been available to South Africa since 2005, and proves valuable in the diagnosis of MPN.

1.4 CLASSIFICATION

Table 1: The 2008 World Health Organization diagnostic criteria for Polycythaemia Vera, Essential Thrombocythaemia, and Primary Myelofibrosis (2)

	PV*	ET§	PMF#
Major criteria	1 Hgb >18.5 g/dL (men)>16.5g/dl (women) or Hgb >17g/dL (men), or >15g/dL (women) if associated with a sustained increase of ≥ 2g/dL from baseline that cannot be attributed to correction of iron deficiency or± 2 Presence of JAK2V617F or similar mutation	1 Platelet count ≥450 × 10^9/L 2 Megakaryocyte proliferation with large and mature morphology. No or little granulocyte or erythroid proliferation. 3 Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm. 4 Demonstration of JAK2V67F or other clonal marker or no evidence of reactive thrombocytosis.	1 Megakaryocyte proliferation and atypia¦ accompanied by either reticulin and/or collagen fibrosis, or in the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased bone marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (ie prefibrotic PMF). 2 Not meeting WHO criteria for CML, PV, MDS or other myeloid neoplasm. 3 Demonstration of JAK2V617F or other marker or no evidence of reactive bone marrow fibrosis.
Minor criteria	1 BM trilineage myeloproliferation 2 Subnormal serum Epo level 3 EEC growth		1 Leukoerythroblastosis 2 Increased serum LDH 3 Anemia 4 Palpable splenomegaly

WHO indicates World Health Organization; PV, polycythaemia vera; ET, essential thrombocythaemia; PMF, primary myelofibrosis; Hgb, haemoglobin; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; BM, bone marrow; Epo, erythropoietin; LDH, lactate dehydrogenase; EEC, endogenous erythroid colony.

*The diagnosis of PV requires meeting either both major criteria and 1 minor criterion or the first major criterion and 2 minor criteria.

§The diagnosis of ET requires meeting all 4 major criteria.

#The diagnosis of PMF requires meeting all 3 major criteria and 2 minor criteria.

±Or Hgb or hematocrit greater than the 99th percentile of reference range for age, sex or altitude of residence or red cell mass > 25% above the mean normal predicted.

¦small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering.

In 1967 Louis Wasserman assembled a group of investigators and founded the Polycythaemia Vera Study Group (50). They were grouped in order to study PV, establish diagnostic criteria, and also to conduct further clinical trials. In 2001 the WHO published new criteria for MPN, which addressed certain deficiencies in previous ones, such as the emphasis on bone marrow findings (51). Since the discovery of JAK2 and MPL mutations, a further revision has been made to the criteria for MPN in the 2008 WHO publication (2).

The diagnosis for PV includes 2 major criteria i.e. an increased haemoglobin level and the presence of JAK2 V617F or similar mutation, and 3 minor criteria i.e. trilineage myeloproliferation on bone marrow investigation, a subnormal serum EPO, and the growth of endogenous erythroid colonies in the absence of EPO. Either both major criteria or 2 minor criteria is sufficient to make a diagnosis of PV, or even 1 minor criteria and the first major criteria (2).

An ET diagnosis requires 4 major criteria which consists of a platelet count of ≥ 450 x 10^9/L, a megakaryocyte proliferation with large and mature morphology, a JAK2 V617F or similar mutation, and not meeting criteria for PV, PMF, CML, or other myeloid neoplasm (2). Bone marrow features of fibrosis or increased cellularity, the presence of clonal markers, as well as features of leukoerythroblastosis, anaemia, palpable splenomegaly and a raised LDH, are all criteria required for a diagnosis of PMF (2).

With an ever growing knowledge of the Ph1 negative MPNs with regard to future cytogenetic and molecular discoveries, there will certainly be a new revision of the WHO 2008 criteria. Currently though it seems that bone marrow histology plays an integral part in making a distinction between reactive and clonal myeloproliferation (52).

1.5 EPIDEMIOLOGY

There is a paucity of epidemiological data on MPN. A recent study in the United States of America utilized two major United States health insurance claims databases to estimate the prevalence of MPN from 2008 to 2010. The study findings concluded that the prevalence of PMF ranged from 3,6 to 5,7 per 100 000 patients, PV prevalence ranged from 45 to 57 per 100 000 patients, and ET prevalence was 39 to 57 cases per 100 000 patients (53). Another retrospective review of 2 112 patients in South East England showed incidences of 1,08 per 100 000 patients, 0,37 per 100 000 patients, and 1,65 per 100 000 patients, for PV, PMF and ET respectively (54). Other studies done comparing data across

Europe revealed a wide variation in the prevalence and incidence of MPN and again confirmed the importance of better epidemiological data needed to assess the burden of illness (55).

The number cases as well as the age specific incidence is also shown to increase with age, and incidences were in all ages seen to be higher for females than for males (56). Of interest is a study published in 1992, where epidemiological parameters were noted in a group of people in northern Israel. This study revealed a 10 to 20 fold higher incidence of MPN in Ashkenazi Jews compared to the Arab population, and emphasises the importance of genetic predisposition possibly interacting with acquired factors in the pathogenesis of the disease (57). Demographic data for Africa is severely lacking and further research needs to be carried out to compare the burden of MPN with the rest of the world.

1.6 MANAGEMENT OF MPN

The treatment of MPN has evolved over the past years, especially since the discovery of the JAK2 V617F mutation. Newer and hopefully more effective treatment is being researched and many trials are still ongoing. Due to the heterogeneous nature of the disease, the timing of treatment and which agents to use, has been difficult in the management process. As stated before, MPN have a significant burden of disease symptoms and a heightened risk for thrombotic and haemorrhagic complications. There has also been no therapy discovered that has led to cure of the disease, except for allogeneic stem cell transplantation. Therefore, it is seen historically that the aim of treatment was concentrated on alleviating symptoms of disease and preventing debilitating complications.

In 2011, a panel of experts were convened by the European Leukemia Network (ELN) to produce an evidence based algorithm for the treatment of MPN (58). Factors addressed by this group included the correct time to initiate treatment as well as a recommendation of agents to be used. Life expectancy of ET and PV may not be diminished but patients diagnosed with PMF unfortunately do not fare as well. It is for this reason that the goals of treatment for PV and ET were similar and those for PMF would be more individualized. The primary goals of therapy for PV and ET are to prevent the complications of thrombosis and bleeding, as well as halting the progression to myelofibrosis and acute myeloid leukaemia, and finally to control disease related symptoms (59). In contrast, the goals for PMF is to alleviate symptoms caused by factors such as cytopaenias and splenomegaly, as well as workup for possible allogenic stem cell transplant (Allo-SCT).

According to the treatment recommendations proposed by the ELN, before initiating therapy in a patient who has been diagnosed with a MPN, the prognosis as well the symptomatic burden should be assessed (60). The Myeloproliferative Neoplasm Symptom Assessment Form total symptom score (MPN-SAF TSS) is a scoring system that utilizes 10 symptoms of disease and is shown to accurately assess symptom burden (60). This can also be used to assess symptomatic response after treatment has been initiated. The prognostic assessment of MPN can be achieved by commonly used scoring systems. These scores have been validated in predicting survival and risk of thrombosis. Tefferi et al developed a model for assessment of prognosis for PV, which is still commonly used (61). The International Prognostic Score for ET (IPSET) is a model developed to assess prognosis of WHO defined ET (62). PMF prognostic assessment can be estimated using the International Prognostic Scoring System (IPSS) at the time of diagnosis, while the Dynamic International Prognostic Scoring System (DIPSS) is used at any time during the disease process (63). Patients are thus put into a high risk or low risk category for prognosis and a plan of therapy can then take place.

After stratification of risk and MPN symptom burden, the treatment for PV and ET follow a similar course. Conventional therapy such as allopurinol to prevent secondary gout, as well as aspirin to reduce the risk of vascular events, may be initiated. Phlebotomy in patients with PV has been shown to also to reduce risks of thrombotic and cardiovascular events, when the haematocrit has been reduced to below 45% (64). Cytoreductive therapy would have to be initiated for patients with high risk profiles. Agents such as hydroxyurea (HU), anagrelide, interferon alpha (IFN), or busulphan (BU) may be used as single therapy or as combination therapy, although some may use these with caution due to their leukaemogenic risk. Clinical trials involving JAK2 inhibitors are also underway and results regarding their efficacy in PV and ET patients are eagerly awaited. Management of PMF is guided by symptomatology, and treatment such as corticosteroids, androgens, immunomodulatory agents (e.g. thalidomide), and splenectomy have been used for symptomatic anaemia (65). Blood and platelet transfusions should be given where necessary as well, but ideally high risk patients should be worked up for Allo-SCT and also be initiated on Ruxolitinib which has shown success on its effects on symptoms and splenomegaly (66).

JAK inhibitors have shown promise for PMF and the other trials for alternative therapy is also underway. These will try to ascertain whether targeting other pathways by using agents such as histone deacetylase (HDAC) inhibitors will help us further in the future. Unfortunately, agents such as the JAK inhibitors are not freely available in the state hospitals in South Africa and thus effective and more modern approaches to management is limited.

1.6.1 ASPIRIN

Aspirin is a salicylate drug that is also referred to as acetylsalicylic acid. Its main use is for its analgesic, antipyretic, and anti-inflammatory properties. Due to the fact that it inhibits the production of thromboxane, thus has antiplatelet effects, it is used in low doses for prevention of cardiovascular disease. It has thus also been assessed for the prevention of vascular events in PV and ET. The ECLAP study demonstrated that low dose aspirin can safely prevent thrombotic complications in patients with PV, who don't have contraindications to it (67). The role of aspirin in ET is less clear. It remains one the main frontline therapies for PV and ET patients (65). Side effects include gastrointestinal ulcers, risk of bleeding in patients who are predisposed, and Reyes syndrome in children.

1.6.2 PHLEBOTOMY

Phlebotomy, also known as bloodletting or venesection, has been part of medical therapy for around 3000 years. In the time of Hippocrates, around 460 BC, the removal of blood when one was ill was common practice. It was practiced in the past by using lancets, cupping, or even the use of leeches (68). The use of therapeutic phlebotomy is currently indicated for 3 disease processes: haemochromatosis, porphyria cutanea tada, and PV. One of the characteristics of PV is an increased red cell mass and thus a raised calculated haematocrit (Hct). Many studies were done over the years that proved that thrombotic events were related to the increased Hct level and therefore reducing the Hct would lead to preventing this complication (68). No consensus has been reached regarding the target Hct level, but a large trial in Italy called the CYTO-PV trial, revealed that Hct levels in patients with PV that were below 45% had a particularly lower rate of thrombosis and cardiovascular death (69). Other trials showed using combination therapy including HU, 32 Phosphorus (32P), and aspirin, also helped cement the importance of therapeutic phlebotomy in PV (68).

Agents such as HU or anagrelide may be used as an alternative, if the patient is intolerant to venesection. Complications of venesection may include tiredness or vasovagal responses, and iron deficiency may develop which should be supplemented for, when the patient becomes symptomatic (70). Of importance also, is to optimize other cardiovascular risk factors such as diabetes mellitus and hypertension, so as to further decrease the risk of thrombosis. The frequency of venesection is determined by the level of the Hct and therefore adequate monitoring is imperative.

1.6.3 HYDROXYUREA

Hydroxyurea is an antineoplastic agent that is also known as hydroxycarbamide. It acts by inhibiting the enzyme ribonucleotide reductase, therefore reducing the production of deoxyribonucleotides, which are important in DNA synthesis. This drug is used for its cytoreductive action, and therefore its use has been indicated for MPN such as PV, ET, and CML. Fetal haemoglobin concentration is also increased and thus hydroxyurea has been effective in the prevention of painful crises in sickle cell anaemia (71). It is administered orally and reaches peak plasma levels within 4 hours.

Many clinical trials over the years have proven the effective nature of hydroxyurea in MPN, due to its myelosuppressive actions. Cortelazzo et al demonstrated in their study of 114 patients with ET that hydroxyurea is effective in the prevention of thrombotic episodes (72). ELN has also now recommended that hydroxyurea be the first line cytoreductive agent for patients with high risk MPN (65). Other uses in MPN has been the reduction in spleen size in patients with PMF presenting with symptomatic splenomegaly (73).

All drugs have their pros and cons, and hydroxyurea is no different. Therefore, careful monitoring of the full blood count is necessary due to the suppressive nature of hydroxyurea on the bone marrow. Another concern for haematologists and oncologists has been the possible risk of leukaemogenesis. There is no definite evidence of this claim when hydroxyurea is administered as a single agent, however its use may increase the leukaemic potential of other cytoreductive drugs (74).

1.6.4 ANAGRELIDE

Anagrelide is a platelet lowering agent belonging to the class of phosphodiesterase inhibitor drugs. It is used in the treatment of thrombocytosis in patients with ET and is also recommended as second line therapy by ELN, in patients who are intolerant to hydroxyurea (65). Several studies have demonstrated its efficacy in lowering platelet counts in patients with ET, however there was also an increased incidence of marrow fibrosis associated with its use (75). The incidence of arterial and venous thrombosis was higher than hydroxyurea in clinical trials of patients with ET, where anagrelide was compared to hydroxyurea (75). Therefore, one can conclude that hydroxyurea may be superior to anagrelide in treatment of ET. The ANAHYDRET study however revealed that there was no significant difference between the two drugs with regard to thrombotic complications of ET (76). It still remains in the context of ET treatment, as a 2nd line therapy.

1.6.5 BUSULPHAN

Busulphan is an alkylating agent that has been utilised for many years in the treatment of patients with MPN. It acts by inducing cross linkages between DNA bases thereby preventing DNA replication. Other indications include bone marrow conditioning for patients undergoing bone marrow transplantation. Metabolism of the drug occurs in the liver. Therefore many drug interactions may result which have an effect on its metabolism. There has been caution regarding its use due to complications of severe cytopenias, pulmonary fibrosis, and possible leukaemogenicity, as reported in previous trials (49). When patients with PV become intolerant to phlebotomy or hydroxyurea, the options of further treatment is fairly limited. A study by Alvarez-Larran et al assessed busulphan in 36 patients with PV and ET who were refractory or intolerant to hydroxyurea (77). This study revealed that it is effective as a 2nd line option in elderly patients with ET or PV intolerant to hydroxyurea, but there was still a concerning risk of disease transformation (78).

1.6.6 INTERFERON

Interferons are a group of glycoproteins that are produced in cells in response to various stimuli, including viral, protozoal, and bacterial infections or by tumour cells. It was discovered in 1957 by Lindeman and Isaacs, who described it as a substance that could cause viral interference (78). Over the next few years research has been ongoing regarding its therapeutic effects in malignant disease. It has been recently classified into 2 groups by virtue of their ability to bind to common receptor types i.e. Type 1 and Type 2 (79). Interferon alpha, beta, omega, and tao belong to the Type 1 group, while interferon gamma belongs to the type 2 group. Functions include immunomodulatory, antiviral, and antitumor activity. The production of interferon by recombinant rDNA technology has allowed for research to be done regarding its efficacy against tumour cells. Interferon alpha has been used in the treatment of MPN, particularly CML, because of its ability to inhibit myeloproliferation and thus cause clinical and haematological reduction in disease. In a study of 279 patients revealed that interferon alpha was an effective alternative to the 1st line treatment in PV (80). Newer preparations manufactured by adding polyethylene glycol to the interferon, prolongs the action of the agent, and is called pegylated interferon. A phase 2 trial of 79 patients with either ET or PV also demonstrated complete response rates of 76% and 70% respectively, with the use of pegylated interferon alpha 2a (81). A further trial assessing the efficacy and safety of pegylated interferon alpha 2a against hydroxyurea in PV and ET patients is underway (65). The intolerability of the drug may be its downfall, with hepatic and neurological side effects, as well as ''flu-like'' symptoms such as fever and malaise.

1.6.7 PHOSPHORUS-32

Phosphorus-32 (32P) is a synthetic radionuclide that has been used in the treatment of haematological malignancies for many years. The localization of the radioisotope in leukaemic cells, as well as the liver, bone, and spleen was demonstrated by John Lawrence in 1939, and this was enough to suggest that it would have a future therapeutic role (82). It then became the agent used to treat chronic leukaemia as well as PV and ET. The use of 32P has been studied extensively, especially for its outcomes in PV and ET. In a prospective study of 431 patients in 1981, where many treatment modalities were used in patients with PV and ET, it was recommended that 32P be used in patients greater than 70 years of age (83). Currently, its use has been less favoured and newer treatments are used. The leukaemogenic potential of this agent and the difficulty in obtaining it has led to the reduction in its use and it currently is not a recommended treatment option (82).

1.6.8 DANAZOL

Danazol is a derivative of the synthetic steroid ethisterone. The luteinizing hormone increase in the middle of the menstrual cycle is suppressed by this agent, and thus it prevents ovulation. It is used in the treatment of endometriosis. Masculinization of patients, as well as hepatotoxicity, are some of the well- known side effects of extended use of this agent. Studies over the years have demonstrated its advantageous role in the treatment of patients with autoimmune haemolytic anaemia and immune thrombocytopenia (84) (85). It has also been used in the treatment of MPNs, particularly in the treatment of anaemia in patients with PMF (86). In a study of 30 patients diagnosed with PMF by Francisco Cervantes et al, danazol was used for the treatment of anaemia in these patients, with 37% showing a complete response (87). Another study of 18 elderly patients refractory to conventional therapy, demonstrated that the concurrent use of danazol with chemotherapy improved the quality of life in these patients (88). The beneficial role of danazol in patients with MPN with refractory pruritus was shown in a small study of 22 patients (89).

1.6.9 JAK INHIBITORS AND OTHER NEW THERAPIES

JAK inhibitors are newly discovered agents that modulate immune response in MPN and other disease states, by inhibiting one of the Janus Kinases and thus depressing the effect of the JAK-STAT pathway. Following the success of the tyrosine kinase inhibitor Imatinib for the treatment of CML, the discovery

of the JAK inhibitors in 2011 was hopefully the answer for Ph1 negative MPN. This has led to multiple trials in the recent past as well as currently, in the hope that a possible cure could be imminent for this disease process. Its uses may prove beneficial not only for MPN, but for other diseases such as rheumatoid arthritis and psoriasis (90) (91).

The improvement of symptoms, reduction in splenomegaly, and prolongation of life, are major factors that a new agent should aim to achieve in the treatment of MPN. The approval of use of Ruxolitinib by the U.S. Food and Drug Administration (FDA) for its use in PMF, was due to two important trials displaying successful administration of this JAK2 inhibitor (92). Ruxolitinib is an inhibitor of both JAK1 and JAK2 and has thus been studied extensively. The COMFORT 1 trial, where patients with intermediate or high risk myelofibrosis were given twice daily doses of ruxolitinib or placebo, revealed that the agent was effective in treatment of myelofibrosis (93). Reductions in the size of splenomegaly as well as improved survival and symptomatology were successfully demonstrated in this trial (93). The COMFORT 2 trial randomised 219 patients with myelofibrosis to Ruxolitinib or the Best Available Treatment (BAT), and this also demonstrated upto a 50% reduction in spleen size, as well as a probability of survival of 71% of patients after a 3,5 year follow up analysis (94). There are ongoing trials studying the effects of Ruxolitinib in patients with PV and ET (95). Side effects of Ruxolitinib include cytopenias, gastrointestinal upset, as well as bruising.

Other JAK inhibitors still undergoing trials include agents such as Momelotinib, Fedratinib, and Pacritinib, and we await their approval in the near future (96) (97). The potential for symptom reduction and overall better survival is promising but to date there is no data showing molecular or cytogenetic reversals (92). The fact that MPNs are a result of abnormal JAK-STAT pathway signalling, and that no obvious cure is currently available, this has led to other treatment targets being explored. Other pathways or molecules that influence cellular growth, differentiation, and apoptosis are being targeted in newer trials that are now underway. PI3 pathway inhibitors as well as mTOR inhibitors are being studied in combination with JAK inhibitors, hoping that another alternate approach can be demonstrated (98) (99). Another agent being studied is Ginovostat, a Histone Deacytelase Inhibitor, and in combination with Ruxolitinib, will hopefully show successful results in the treatment of MPN (100).

The discovery of the JAK inhibitors has certainly created excitement in the therapeutic approach to Ph1 negative MPN. First world countries are leading the way in the discovery of many new agents and thus new evolving treatment strategies are being studied. Although the private hospital institutions in

South Africa can acquire these new agents, the state sector is almost always reliant on conventional therapies.

1.6.10 ALLOGENEIC STEM CELL TRANSPLANTATION (Allo SCT)

Allo SCT is the process where haematopoietic stem cells are transplanted from a donor to a recipient. Donors must have a tissue Human Leucocyte Antigen (HLA) type that matches the recipient, and donors may be related to the recipient e.g. siblings, or they may be unrelated. The introduction of stem cell transplantation to the medical world was by E. Donnall Thomas in the 1950's (101). This led the way to future stem cell transplantations, especially in the treatment of leukaemias, lymphomas, as well as non-malignant haematological disease such as thalassemia and aplastic anaemia. Another type of stem transplantation is autologous stem cell transplantation (Auto SCT), which involves transplantation of stem cells, into a recipient, that has been extracted and harvested from the same person. The benefit of Auto SCT involve reductions in the post-transplant complications such as infections as well as graft-versus-host-disease, although an increase rate of disease relapse, especially in diseases like leukaemia have led to Allo SCT being favoured in certain circumstances.

The process also includes the conditioning of the bone marrow, and a myeloablative or a non-myeloablative approach can be used (102). Complications include infections, graft-versus-host-disease, veno-occlusive disease, and mucositis, which leads to increased incidence of morbidity and mortality. The use of Allo SCT may be the only cure for the Ph1 chromosome negative MPN, PMF. Allo SCT should be considered for intermediate to high risk patients diagnosed with PMF, where a survival of less than 5 years is expected (58). It would therefore be imperative, that after diagnosis and risk stratification of PMF, the physician should assess the candidacy for Allo SCT. Few studies have been done to demonstrate the success of Allo SCT in patients with PMF or post PV or Post ET myelofibrosis, and one of these studies showed a longer term relapse free survival (103). Other studies compared the use of reduced intensity conditioning before Allo SCT in patients with myelofibrosis, and this proved to be superior to the old myelo-ablative approach, with regard to increased survival (104) (105). More trials of stem cell transplantation use in the treatment of MPN is needed to further understand and perfect the process, and better the chances for a definite cure.

The emerging importance of stem cell transplantation worldwide has also led to multiple willing donors globally, and the formation of bone marrow registries. Locally, the South African Bone Marrow Registry (SAMBR) was established in Cape Town in 1991 (106). This offers South Africa the option of

Allo SCT for a variety of conditions. There is no doubt that Allo SCT has become an important mode of treatment for haematological disease, locally as well as internationally (107).

1.7 OUTCOMES

Several studies were conducted worldwide assessing patterns of survival in Ph1 negative MPN. The results of these demonstrate that patients with PV and ET have a good prognosis, with a slight reduction in overall survival compared to the general population (108). Also revealed was the fact that PMF had an inferior survival compared to ET and PV (108). The major causes of mortality and morbidity include thrombosis, haemorrhage and clonal evolution. In a large study called the European Collaboration on Low Dose Aspirin in PV (ECLAP), 41% of deaths were due to cardiovascular mortality (109). Leukaemic transformation carries a very poor prognosis for patients with MPN, even though they occur at low rates (110). Treatments of MPN are thus targeted at reducing these sometimes fatal complications of the disease.

Different scoring systems have been developed to assess prognosis in patients with MPN, such as the IPSET score for ET and the IPSS and DIPSS scores for PMF (111). This assists in risk stratifying patients and therefore creating an appropriate management plan. Factors that were universally shown to have caused significant morbidity and mortality were an increasing age of greater than 65 years, increased red cell and platelet counts, and a previous history of thrombosis (111). Criteria to define the outcomes or response to therapy for MPN have been revised and standardised by ELN and the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) (112) (113). No cure is yet available and thus a high index of suspicion and risk stratification is needed to try and alleviate some of the fatal complications of the disease and further improve survival rates.

1.8 CONCLUSION

Philadelphia chromosome negative myeloproliferative neoplasms are a rare haematological disease that has the ability to cause major morbidity and mortality. The better understanding of its pathogenesis especially in regard to the several mutations it exhibits, will hopefully lead to more effective therapy, and create better outcomes in the future.

1.9 OBJECTIVES OF THE STUDY

- To assess the demographics; clinical presentation; complications; and management of MPN
- To describe the prevalence of JAK2 V617F mutation in MPN
- To describe the treatment modalities that were available and used to treat patients with MPN
- To assess the outcome of different treatment modalities used to treat patients with MPN

CHAPTER 2: PATIENTS AND METHODS

2.1 STUDY DESIGN

A retrospective review of adult patients seen at the Clinical Haematology Unit, Department of Internal Medicine, Chris Hani Baragwanath Academic Hospital from the period 01/01/1987 to 31/12/2011.

2.2 SAMPLE POPULATION

There were a total of 116 patients identified in the sample population at the Clinical Haematology Unit, however, only 94 patients had records with sufficient information that were used in the study.

2.2.1 INCLUSION CRITERIA

- All patients ≥ 18 years of age
- All patients diagnosed specifically with PV, ET, and PMF

2.2.2 EXCLUSION CRITERIA

- Patients diagnosed with CML
- Patients diagnosed with other MPN (e.g. systemic mastocytosis, chronic neutrophillic leukaemia, chronic eosinophilic leukaemia, hypereosinophilic syndrome, and MPN unclassifiable)
- Patients whose records were inadequate and insufficient for data analysis

2.3 COLLECTION OF DATA

Data was collected retrospectively from patients diagnosed with MPN, excluding CML, for the period 01/01/1987 to 31/12/2011 (25 years). Permission was obtained from the Head of the Clinical Haematology Unit, as well as the Head of the Department of Internal Medicine, to perform the study.

A standardised data sheet which included a tick box list for documentation of the data was used (Appendix A and B).

The following information was obtained:

- Demographics: age and gender
- Clinical presentation based on documented history and examination
- Laboratory investigations including full blood counts, urea and electrolytes, haematinics, uric acid, and bone marrow characteristics
- Blood results were recorded for different periods in time i.e. at time of presentation, at time of best response to treatment, and at time of the patients last follow up visit or at the time of death
- JAK2 V617F mutation was detected by polymerase chain reaction testing and was obtained from the patient records
- Management including venesection, blood transfusions, medication, number of treatments, and duration of treatment, was obtained from the haematology files
- Information regarding the complications of MPN including arterial and venous thrombosis, bleeding and leukaemic transformation were obtained from the haematology files
- Outcomes for ET were defined as follows:
    - Complete response is defined as a platelet count ≤ 400 x 10^9/L, and no disease related symptoms, and a normal spleen size on imaging, and white blood cell count ≤ 10 x 10^9/L (114).
    - Partial response is defined as patients who do not fulfil the criteria for complete response, platelet count ≤ 600 x 10^9/L or a decrease > 50% from baseline (114).
    - Stable disease is defined as a response that does not satisfy complete or partial response
    - Death as a consequence of ET
- Outcomes for PV were defined as follows:
    - Complete response is defined as a haematocrit < 45% without phlebotomy, and platelet count ≤ 400 x 10^9/L, and white cell count ≤ 10 x 10^9/L, and normal spleen size on imaging, and no disease related symptoms (114)
    - Partial response is defined as patients who do not fulfil the criteria for complete response, haematocrit < 45% without phlebotomy, or response in 3 or more of the other criteria (114)
    - Stable disease is defined as a response that does not satisfy complete or partial response

- Death as a consequence of PV

- Outcome of PMF is defined as follows:
  - Complete response is defined as haemoglobin ≥ 10 g/dl and < the upper limit of normal limit, neutrophil count ≥ 1 x 10^9/L and < upper normal limit, and a platelet count ≥ 100 x 10^9/L, and a resolution of disease symptoms with no palpable liver and spleen, and bone marrow normocellularity with < 5% blasts and ≤ grade 1 myelofibrosis (113)
  - Partial response is defined as resolution of disease symptoms with no palpable liver or spleen, and either bone marrow features as above, or peripheral blood features as above (113)
  - Stable disease is defined as a response that does not satisfy criteria for complete or partial response
  - Death as a consequence of PMF

Patients' names and details were recorded and kept confidentially. A numerical coding system was used to ensure confidentiality. The REDCap research database was used to capture the information from the data collection sheet. REDCap is a research database that allows for the electronic capture of data by utilising a created electronic data collection sheet. It then also allows for the exportation of the information to a statistical program. It is a secure web based program that is accessed via the internet, and it is protected by a password for which the researcher uses to gain access.

2.4 DATA ANALYSIS

Once the data was collected, it was verified using the software provided by the REDCap program. The verified data was then exported to Microsoft Excel for further analysis. Microsoft Excel was then used for statistical analysis. Descriptive analysis in the form of mean and median was used for demographic statistics as well as blood results, using standard deviations or range for continuous variables. Mean and standard deviations were used for parametric data while median and interquartile ranges were used for non-parametric data. Categorical data was illustrated using frequency distribution tables, pie charts and bar charts. Further statistical analysis was shown by using graph pad instat.

Bivariate and multivariate analysis of categorical data was done by using contingency tables. Paired, parametric data was analysed using the paired t-test, while unpaired, non-parametric data was analysed using the Kruskal-Wallis test. Survival curves for non-parametric data was created using the

log-rank test (Mantel-Cox test). A p value of < 0.05 determined statistical significance. A statistician was consulted to assist and verify the methods used.

2.5 ETHICS

Ethics approval was granted unconditionally by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (Clearance Certificate Number: M121175). Informed consent was not needed from the patients due to the analysis being retrospective in nature.

CHAPTER 3: RESULTS

3.1 DEMOGRAPHICS OF PATIENTS WITH MPN

Table 2: Demographics of patients with MPN

	All Patients	ET	PV	PMF
NUMBER	94	35	17	42
AGE (years) (mean ± SD)	59,5 ± 14.4	58,4±16,5	52,8±12,2	63,2±12,2
AGE (years) (median ± IGR)	61,5 (21 -87)	63 (21 – 80)	54 (32 -78)	63,5 (39 – 87)
GENDER - MALE	42 (45%)	11 (31%)	10 (59%)	21 (50%)
FEMALE	52 (55%)	24 (69%)	7 (41%)	21 (50%)
M:F RATIO	1: 1.24	1: 2.2	1: 0.7	1: 1

A total of 94 records were obtained and reviewed from the Clinical Haematology Unit, Department of Internal Medicine, at Chris Hani Baragwanath Academic Hospital. These patient records were analysed in the 25 year period, from 1 January 1987 to the 31 December 2011.

Of the 94 records analysed 42 (45%) were diagnosed with PMF, 35 (37%) were diagnosed with ET, and the least amount 17 (18%) of patients were diagnosed with PV. The median age of patients diagnosed with MPN was 61.5 years with a mean age of 59.5. A younger median age of 54 years was identified in the PV group, while median ages of 63 and 63.5 were noted in the ET and PMF groups respectively.

As illustrated in figure 1, there is a slight female predominance (1.24:1) of all patients diagnosed with a MPN, with 55% of patients identified as female and 45% identified as male. A female predominance was shown in ET, while a male predominance was noted in PV and an equal gender ratio in PMF (see figures 2, 3, and 4).

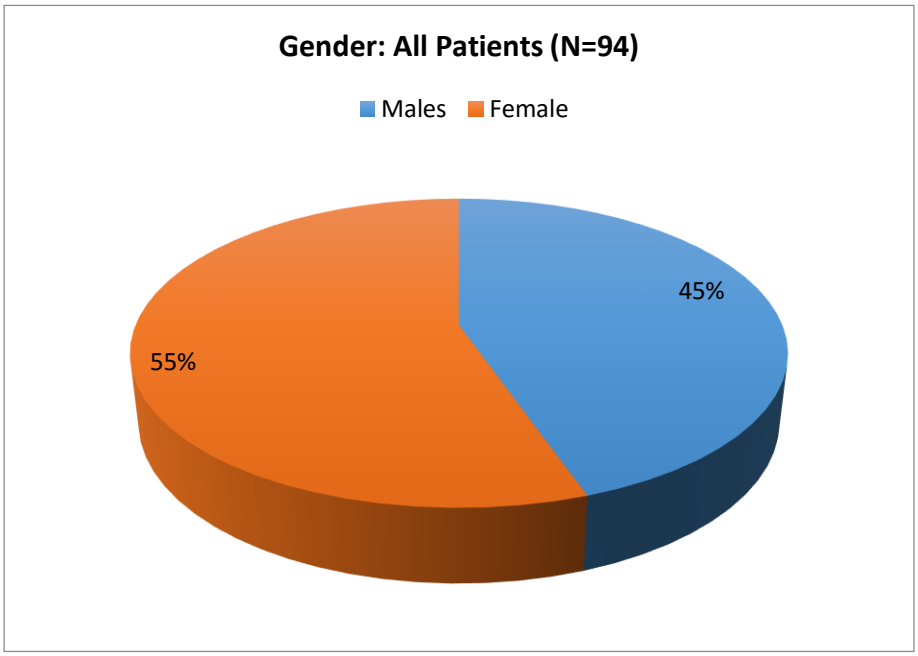

Figure 1: Gender distribution in MPN

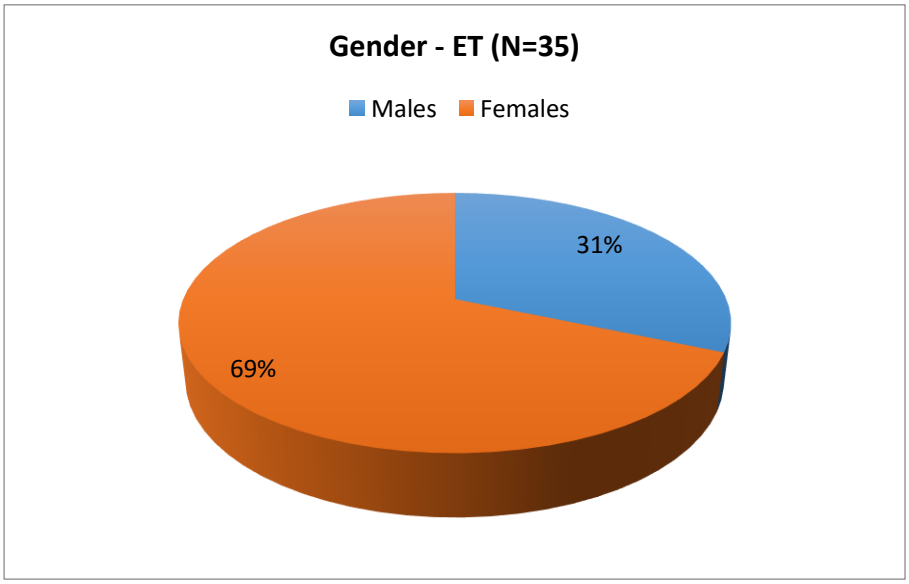

Figure 2: Gender distribution in ET

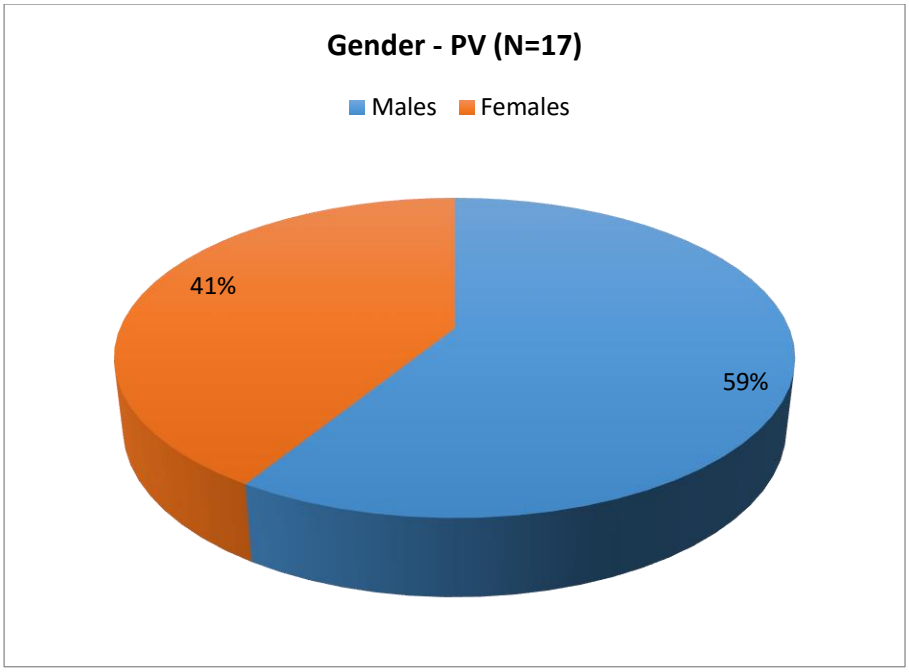

Figure 3: Gender distribution in PV

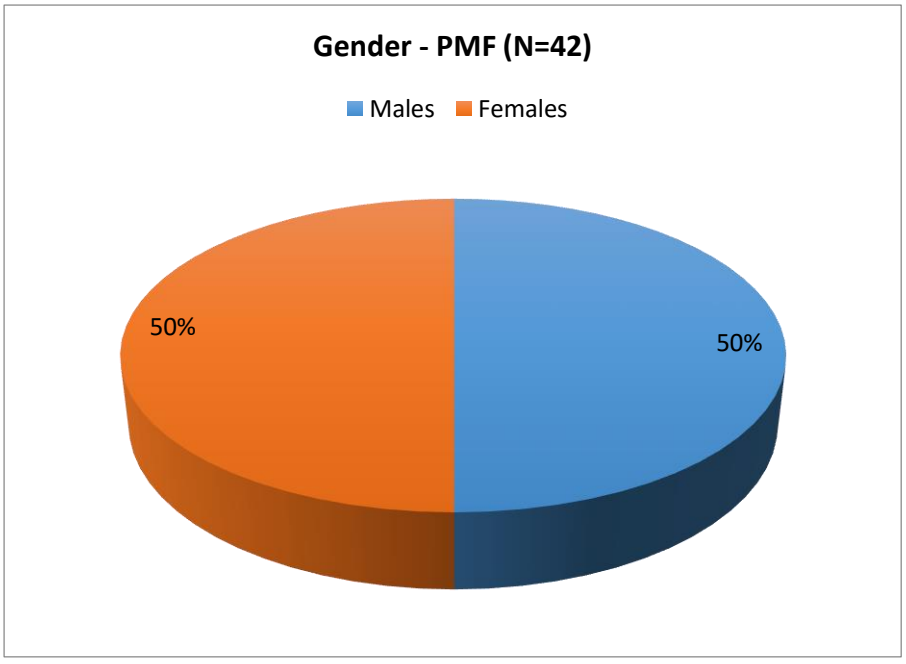

Figure 4: Gender distribution in PMF

3.2 PRESENTING SYMPTOMS IN PATIENTS DIAGNOSED WITH MPN

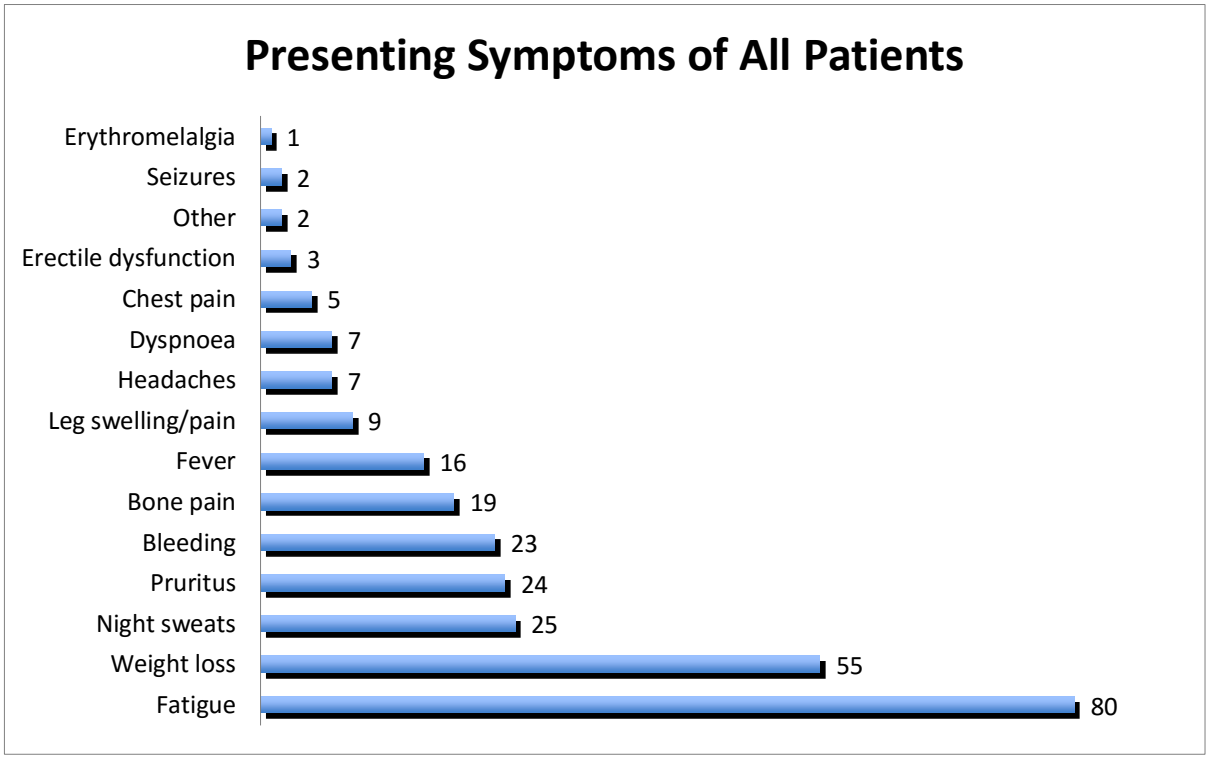

Figure 5: Frequency of symptoms of all patients at presentation

(Symptoms experienced in the ''Other'' category were abdominal pain and deafness)

Figure 5 illustrates multiple presenting symptoms of all 94 patients that were diagnosed with MPN. It is appreciated that the most common complaint in these patients was fatigue (85% i.e. 80 out of 94 patients), and as demonstrated in figures 6, 7 and 8, fatigue was also shown to be the most common complaint in ET (80%), PV (88%), and PMF (82%) respectively. Other common symptoms experienced in all patients were weight loss (58%), night sweats (26%), and pruritus (25%). Bleeding (epistaxis and gum bleeding) was much more common in patients diagnosed with ET and PMF than in patients with PV (see figures 6, 7, 8). Of interest is that patients diagnosed with PV had significantly more complaints of pruritus (59%). Figure 8 demonstrates that a significant amount of patients with PMF presented with weight loss (82%). Other symptoms that were shown during analysis included bone pain (20%), fever (17%), swelling or pain of the legs (9%), headaches (7%), dyspnoea (7%), and chest pain (5%), as seen in figure 5. Only 1 patient diagnosed with a MPN complained of erythromelalgia, while 3 patients with PMF experienced erectile dysfunction.

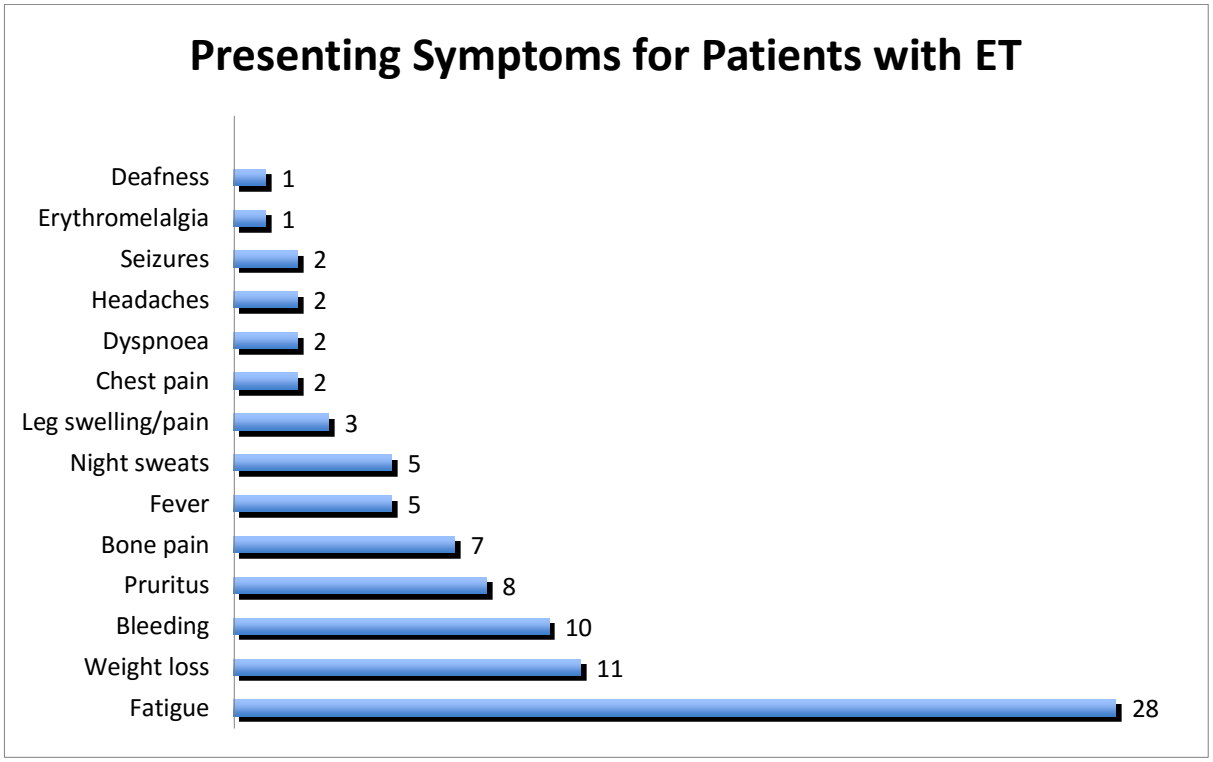

Figure 6: Frequency of symptoms in patients with ET

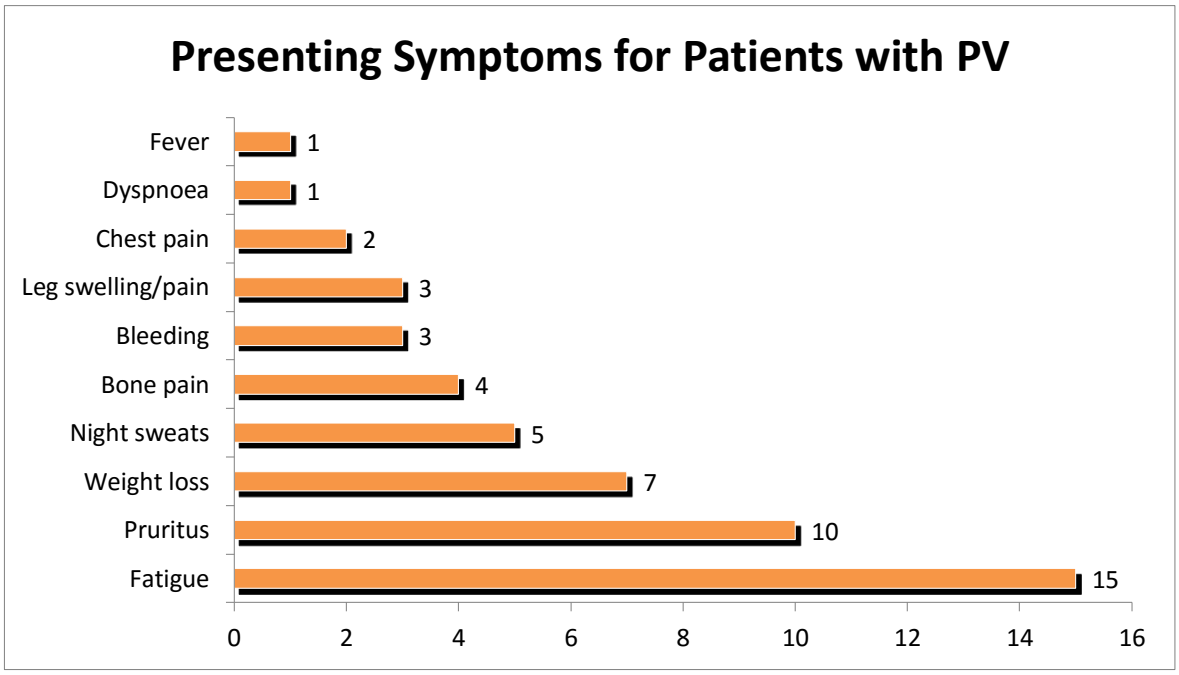

Figure7: Frequency of symptoms in patients with PV

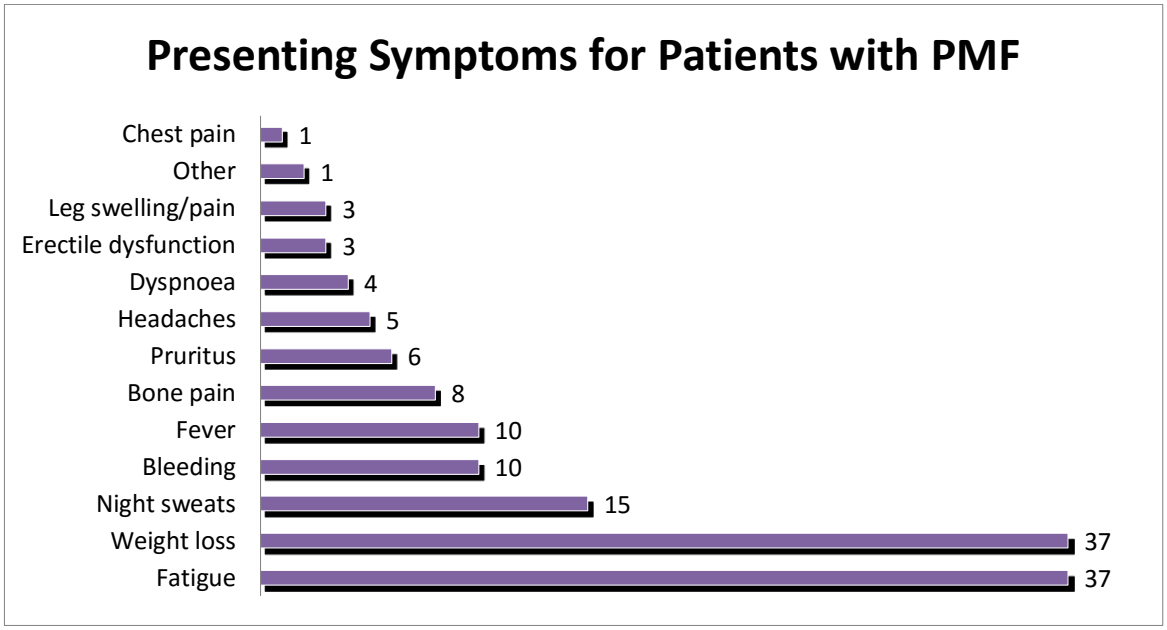

Figure 8: Frequency of symptoms in patients with PMF

3.3 CLINICAL PRESENTATION

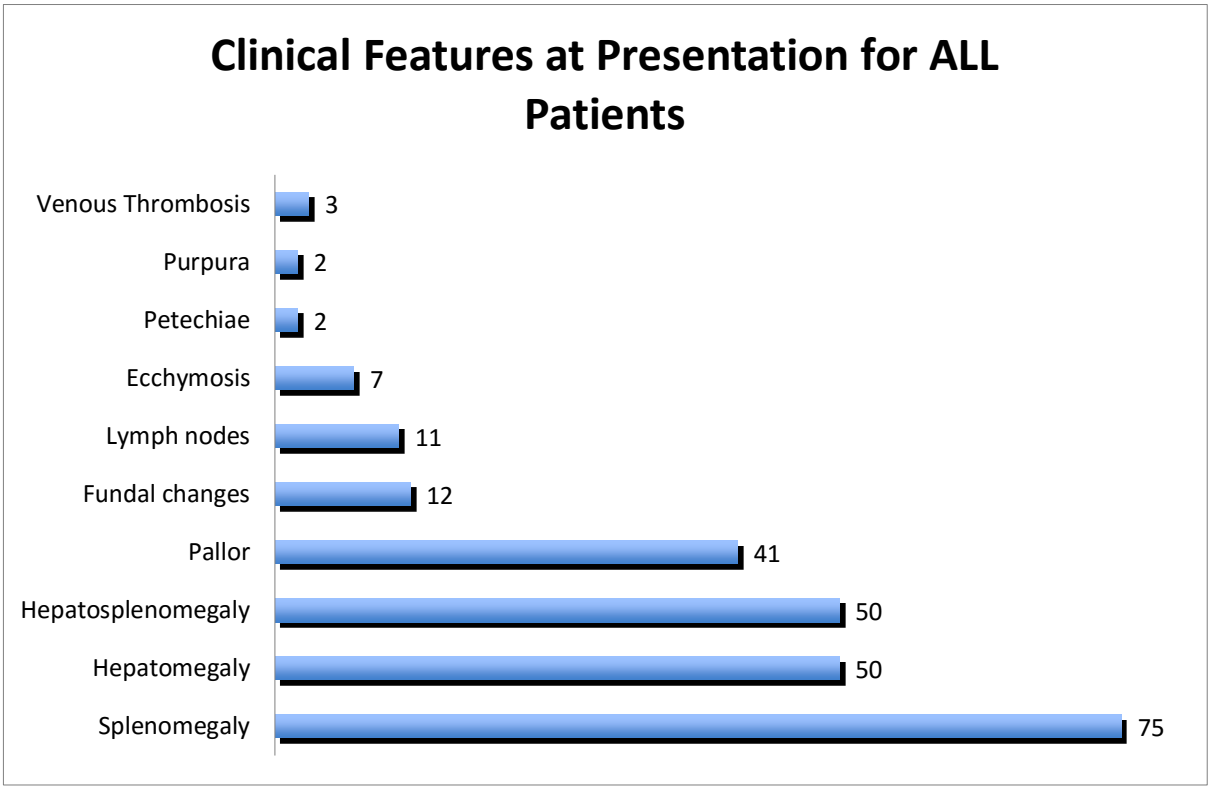

Figure 9: Frequency of clinical features found on examination

Figure 9 illustrates the clinical features that were present on examination of these patients when they were referred to the Clinical Haematology Unit. Organomegaly on abdominal examination was shown to be the most common clinical feature, with splenomegaly occurring in 80% of all patients examined whilst hepatomegaly occurred in 53% of patients. The simultaneous occurrence of hepatomegaly and splenomegaly was demonstrated in 53% of the patients at presentation. Other features seen on examination include pallor (43%), fundal changes (13%), lymphadenopathy (12%), venous thrombosis (3%), and evidence of bleeding into the skin (12%).

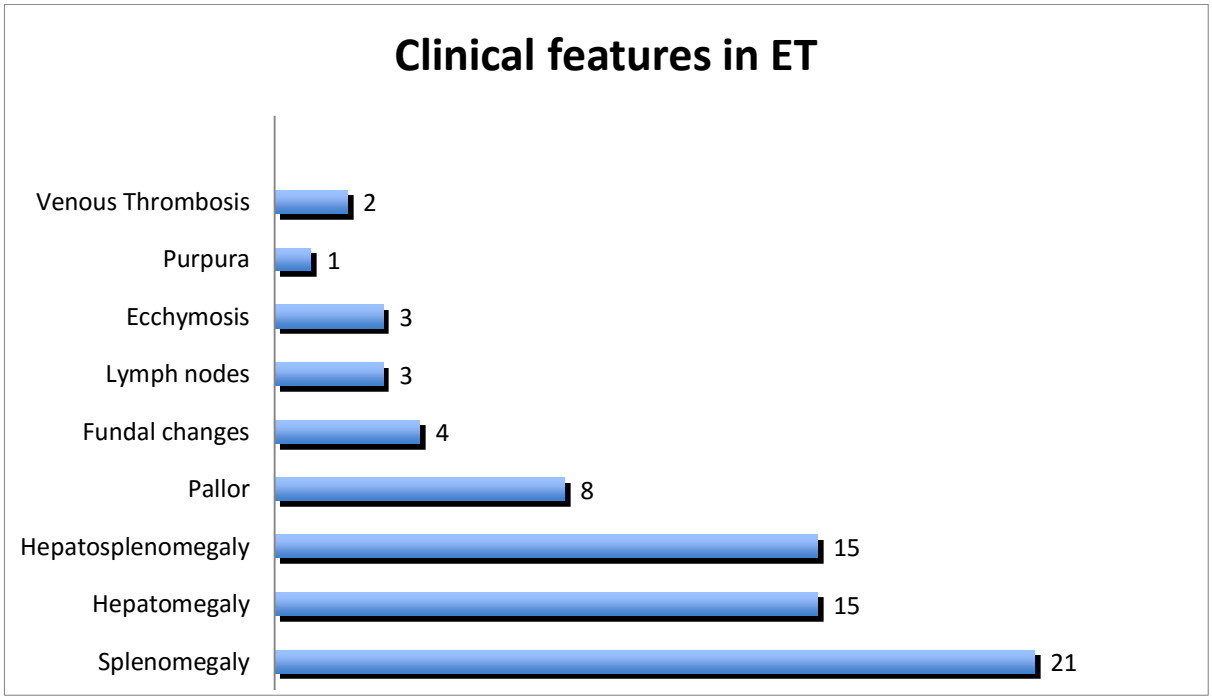

Figure 10: Frequency of clinical features on examination in patients with ET

Splenomegaly was the most common clinical feature in ET (60%). Other significant features were hepatomegaly (43%) and pallor (23%) (see figure 10). Patients with PV also had a splenomegaly in 76% of the records analysed, as seen in figure 11. The frequency of clinical features in PMF is illustrated in figure 12, with some striking data observed. Ninety seven percent of patients diagnosed with PMF had a splenomegaly while 71% had hepatomegaly on examination. Of note was that pallor was visible in 78% of PMF patients on presentation. Only 6 patients were recorded as having any form of bleeding into the skin. Two patients diagnosed with ET presented with splanchnic vein thrombosis, and only one patient with PV presented with deep vein thrombosis.

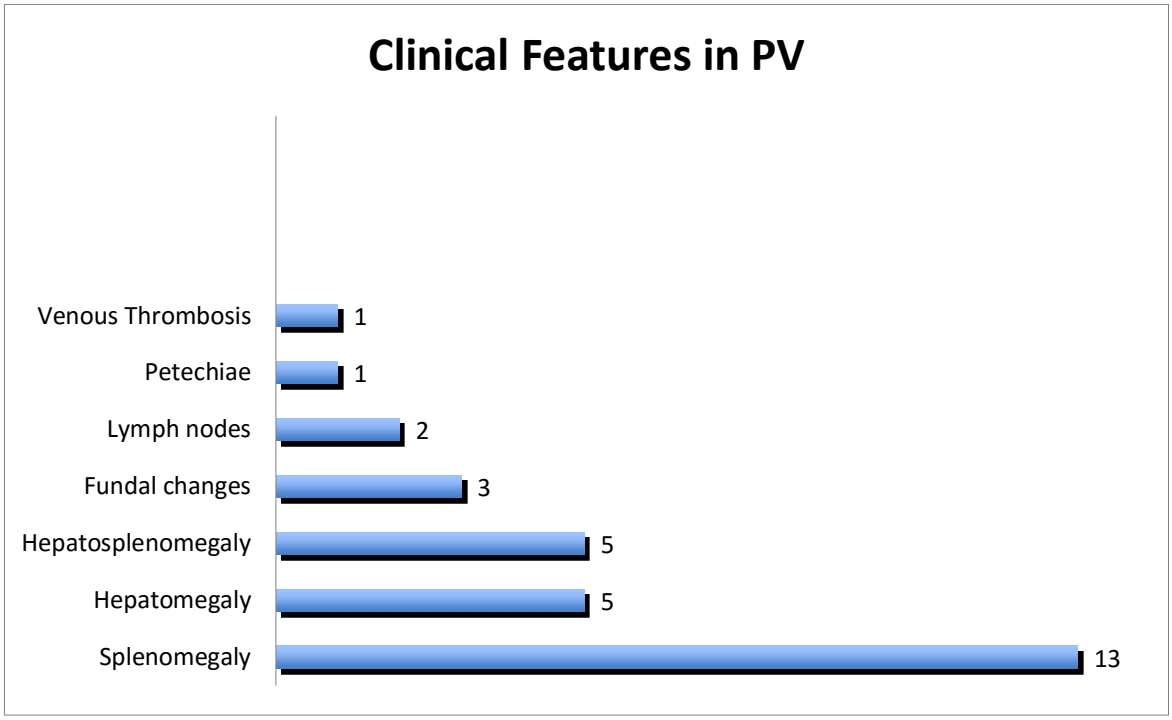

Figure 11: Frequency of clinical features at presentation in patients with PV

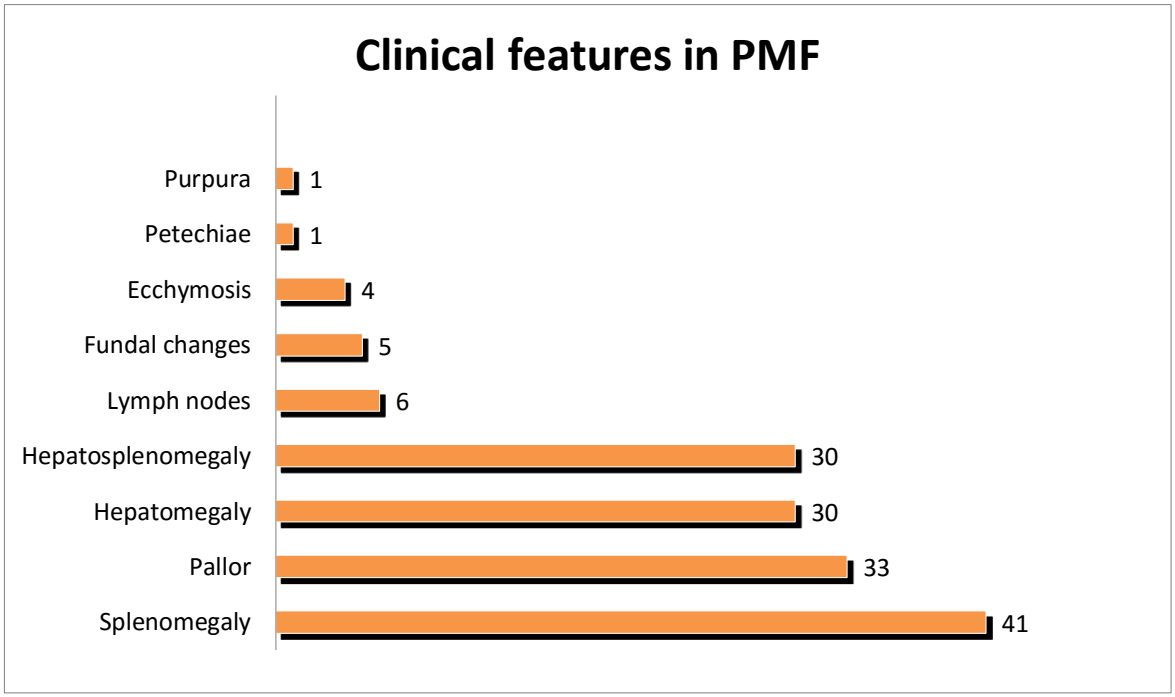

Figure 12: Frequency of clinical features at presentation in patients with PMF

3.3.1 HEPATOMEGALY IN PATIENTS WITH Ph1 NEGATIVE MPN

Table 3: Comparison of Hepatomegaly in Ph1 Negative MPN

Hepatomegaly	**All patients (n = 94)**	**ET (n = 35)**	**PV (n = 17)**	**PMF (n = 42)**
Number	50 (53%)	15 (42%)	5 (29%)	30(71%)
Size below costal margin (in cm) (median±IQR) *	4(2-13)	3(2-10)	3(2-4)	5(2-13)

*p value = 0,0381 (using Kruskal-Wallis test)

Table 3 demonstrates a comparison between the 3 diseases (ET, PV, and PMF) regarding the number of patients presenting with hepatomegaly, as well as the size of the liver palpable below the right costal margin in the midclavicular line. Fifty three percent of all patients presented with a hepatomegaly at presentation. It is noted that patients with PMF were most likely to present with hepatomegaly (71%), and patients with PV were least likely to have a hepatomegaly (29%). The findings with regard to hepatomegaly were statistically significantly different in the 3 different MPN.

3.3.2 SPLENOMEGALY IN PATIENTS WITH Ph1 NEGATIVE MPN

Table 4: Comparison of Splenomegaly in Ph1 Negative MPN

Splenomegaly	**All patients (n = 94)**	**ET (n = 35)**	**PV (n = 17)**	**PMF (n = 42)**
Number	75 (80%)	21(60%)	13 (76%)	41(98%)
Size below costal margin (in cm) (median±IQR) *	4(1-28)	3(1-16)	2(1-8)	7(2-28)

*p value = 0,001 (ANOVA using Kruskal-Wallis)

ET vs PV p value > 0,05; ET vs PMF p value < 0,01; PV vs PMF p value < 0,01

A comparison of the median size of splenomegaly as well the number of patients presenting with splenomegaly is shown in Table 4. Eighty percent of all patients presented with a splenomegaly. Ninety eight percent of patients diagnosed with PMF had a splenomegaly. In the other 2 diseases, 21 of the 35 patients diagnosed with ET (60%) had splenomegaly and 76% of PV patients had splenomegaly. Statistical significance was seen when comparing the median sizes in all 3 diseases but no difference was seen between the ET and PV group of patients. Furthermore the p values also show that the median size of the spleen in PMF was significantly larger than ET or PV.

3.3.3 ANALYSIS OF SIZE OF SPLENOMEGALY IN PMF

Table 5: Frequency of different sizes of splenomegaly in PMF

SPLENOMEGALY IN PMF	Number (%)
PMF Patients	41 (98%)
< 4 cm below costal margin	11(27%)
4-8 cm below costal margin	14 (34%)
> 8 cm below costal margin	16 (39%)

It is clearly demonstrated that splenomegaly is almost always present in patients with PMF in this cohort (98%). Table 5 also shows that the majority of PMF patients present with moderate to massive splenomegaly (73%), while 27% of patients present with mild splenomegaly.

3.4 ANALYSIS OF BLOOD RESULTS ON PRESENTATION

Most patients presenting to the hospital for investigation would have a battery of tests done to aid the physician in diagnosis. The pertinent blood tests assessed in patients with MPN include a Full Blood Count (with examination of the peripheral blood smear), haematinic analysis (including iron studies, red cell folate and vitamin B12), serum uric acid, as well as serum erythropoietin (in patients suspected of PV) prior to the era of the JAK2 V617F mutation analysis. Tables 6, 7, and 8 show blood results obtained at presentation for patients diagnosed with ET, PV, and PMF respectively. Where the distribution of values were skewed, the median was utilised, while the mean was utilised for values with a normal pattern of distribution.

The median White Cell Count for ET was elevated at 12,23 x 10^9/l, however the mean counts for PV and PMF were in the normal reference range. Mean Haematocrit (Hct) values were predictably high in patients with PV at 0,63 l/l, and normal reference values were seen in ET and PMF. The mean

Haemoglobin value in ET was 12,26 g/dl, but the mean value in PMF was 8,3 g/dl. Thrombocytosis was again predictably seen in patients presenting with ET, with a mean platelet count of 1250 x 10^9/l. Examination of the peripheral blood smear showed that teardrops were present in 73% of patients with PMF, while a leucoerythroblastic picture was seen in 21% of these patients. On analysing the values of the iron studies there were 9 patients that were iron deficient (5 with PMF and 2 with PV and ET respectively), but median ferritin values for all 3 groups were within the normal reference range. Folate deficiency was recorded in 4 patients with ET and 5 patients with PMF, but not in any patient with PV. Only 1 patient was diagnosed as having Vitamin B12 deficiency in the PMF group.

Human Immunodeficiency Virus (HIV) Elisa testing was undertaken in 76% of all patients diagnosed with MPN. A very low proportion of patients were infected with the virus, with only 2 patients with ET and 1 patient with PMF being diagnosed as HIV positive. Serum Erythropoietin (EPO) levels were measured in 70% of patients with PV with a median of 3,2 mU/ml, which is decreased according to the reference range used.

Table 6: Analysis of Blood Results of patients diagnosed with ET

ET	Number	Values (n = normal)
WCC (x 10^9/l) (mean ± SD)	N=35	12,23 ± 4,66 (n= 4 – 10)
RCC (x 10^{12}/l) (mean ± SD)	N=13	4,74 ± 1,23 (n= 3,9 – 5,8)
Hct (l/l) (mean ± SD)	N=33	0,39 ± 0,09 (n= 0,43 – 0,55)
Hb (g/dl) (mean ± SD)	N=35	12,26 ± 2,93 (n= 11,6 – 17,5)
MCV (fl) (mean ± SD)	N=35	81,92 ± 8,12 (n= 79 – 101)
Platelets (x10^9/l) (mean ± SD)	N=35	1250 ± 462 (n= 170 – 450)
Fe (µmol/l) (median ± IGR)	N=32	7,9 (1-33) (n= 10 – 30)
Sat Fe (%) (mean ± SD)	N=30	13,86 ± 8,1 (n= 25 – 50)
Transferrin (g/l) (mean ± SD)	N=33	2,53 ± 0,70 (n= 2 – 3,6)
Ferritin (µg/ml) (median ± IGR)	N=33	54 (14-1901) (n= 30 – 400)
HIV positive		2
HIV negative		22
HIV unknown		11
RC folate (nmol/l) (median ± IGR)	N=23	541 (82-4706) (n= 418 – 1158)
Vitamin B12 (pmol/l) (mean ± SD)	N=24	471 ± 189 (n= 145 – 637)
Uric acid (mmol/l) (median ± IGR)	N=26	0,37 (0,2-1,18) (n= 0,22 – 0,45)

(WCC = White Cell Count, RCC = Red Cell Count, Hct = Haematocrit, Hb = Haemoglobin, MCV = Mean Cell Volume, Fe = Iron, Sat Fe = % Iron Saturation, RC folate = Red Cell folate)

Table 7: Analysis of Blood Results of patients diagnosed with PV

PV	Number	Values (n = normal)
WCC (x 10^9/l) (median ± IGR)	N=17	8,01 (4,6-42,5) (n= 4 – 10)
RCC (x 10^{12}/l) (mean ± SD)	N=14	7,46 ± 1,13 (n= 3,9 – 5,8)
Hct (l/l) (mean ± SD)	N=17	0,63 ± 0,05 (n= 0,43 – 0,55)
Hb (g/dl) (mean ± SD)	N=17	20,6 ± 2,4 (n= 11,6 – 17,5)
MCV (fl) (mean ± SD)	N=17	83,9 ± 9,9 (n= 79 – 101)
Platelets (x 10^9/l) (mean ± SD)	N=17	371 ± 240 (n= 170 – 450)
Fe (µmol/l) (median ± IGR)	N=12	8,95 (3,2-30,9) (n= 10 – 30)
Sat Fe (%) (median ± IGR)	N=10	11 (4-40) (n= 25 – 50)
Transferrin (g/l) (mean ± SD)	N=12	2,96 ± 0,76 (n= 2 – 3,6)
Ferritin (µg/ml) (median ± IGR)	N=12	45,5 (15-1667) (n= 30 – 400)
HIV positive		0
HIV negative		13
HIV unknown		4
RC folate (nmol/l) (median ± IGR)	N=9	785 (336-8408) (n= 418 – 1158)
Vitamin B12 (pmol/l) (median ± IGR)	N=11	499 (323-1462) (n= 145 – 637)
Uric acid (mmol/l) (mean ± SD)	N=13	0,52 ± 0,17 (n= 0,22 – 0,45)
PaO_2 (mmHg) (mean ± SD)	N=6	80 ± 8 (n= 69 – 84)
Sats O_2 (%) (mean ± SD)	N=6	94 ± 2 (n= 90 – 98)
Serum EPO (mU/ml) (median ± IGR)	N=12	3,2 (1,2-11,6) (n= 4,1 – 19,5)

(WCC = White Cell Count, RCC = Red Cell Count, Hct = haematocrit, Hb = Haemoglobin, MCV = Mean Cell Volume, Fe=Iron, Sat Fe =% Iron Saturation, RC folate = Red Cell folate, EPO = serum erythropoietin)

Table 8: Analysis of Blood Results of patients diagnosed with PMF

PMF	Number	Values (n = normal)
WCC (x 10^9/l) (median ± IGR)	N=42	9,3 (1,5-90,5) (n= 4 – 10)
RCC (x 10^{12}/l) (mean ± SD)	N=24	3,01 ± 0,9 (n= 3,9 – 5,8)
Hct (l/l) (mean ± SD)	N=35	0,26 ± 0,07 (n= 0,43 – 0,55)
Hb (g/dl) (mean ± SD)	N=42	8,3 ± 3 (n= 11,6 – 17,5)
MCV (fl) (mean ± SD)	N=42	84,8 ± 8 (n= 79 – 101)
Platelets (x 10^9/l) (mean ± SD)	N=42	231 ± 203 (n= 170 – 450)
Fe (µmol/l) (median ± IGR)	N=32	9,1 (1,3-46) (n= 10 – 30)
Sat Fe (%) (median ± IGR)	N=23	20 (3-94) (n= 25 – 50)
Transferrin (g/l) (median ± IGR)	N=31	2 (1-20) (n= 2 – 3,6)
Ferritin (µg/ml) (median ± IGR)	N=32	276 (18-2247) (n= 30 – 400)
HIV positive		1
HIV negative		34
HIV unknown		7
RC folate (nmol/l) (median ± IGR)	N=25	590 (142-4103) (n= 418 – 1158)
Vitamin B12 (pmol/l) (median ± IGR)	N=27	741 (54-2000) (n= 145 – 637)
Uric acid (mmol/l) (median ± IGR)	N=27	0,45 (0,24-1,21) (n= 0,22 – 0,45)
Leucoerythroblastic picture on smear	N=33	7 patients
Teardrops on smear	N=33	24 patients
Blasts on smear	N=33	30 patients

(WCC = White Cell Count, RCC = Red Cell Count, Hct = Haematocrit, Hb = Haemoglobin, MCV = Mean Cell Volume, Fe = Iron, Sat Fe = % Iron Saturation, RC folate = Red Cell folate)

Table 9: Prevalence of hyperuricaemia in patients with Ph1 negative MPN

HYPERURICAEMIA	ET	PV	PMF
NUMBER TESTED	26	13	27
INCREASED URIC ACID LEVEL	12 (46%)	10 (77%)	10 (37%)
GOUT	1 (3%)	2 (12%)	2(5%)

(normal serum uric acid 0,22 – 0,45 mmol/l)

(for patients with gout – ET (N = 35), PV (N = 17), PMF (N = 42))

In MPN, uric acid production is increased. Serum uric acids levels were tested in 70% of patients. Seventy seven percent of patients with PV had hyperuricaemia while 46% and 37% of serum uric acid levels were raised in ET and PMF respectively. Hyperuricaemia seems to be more commonly seen in PV than in ET or PMF. However, the development of gout was uncommon, with only 5% of all patients having been diagnosed with this condition.

3.5 PREVALENCE OF THE JAK2 V617F MUTATION

Table 10: Analysis of the Prevalence of JAK2 V617F Mutation in patients with Ph1 negative MPN

JAK2 V617F MUTATION	ET	PV	PMF	ALL PATIENTS
NUMBER OF PATIENTS	35	17	42	94
POSITIVE	11	8	8	27
NEGATIVE	0	1	1	2
UNKNOWN	24	8	33	65

JAK2 V617F mutation is measured by Polymerase Chain Reaction (PCR) analysis. The test became available to the NHLS in 2005. A total of 29 patients (31%) were tested in this cohort. 11 patients with ET that were tested had the mutation while 8 patients with PV and PMF were to shown to be JAK2 V617F mutation positive (as illustrated in table 10). 4 patients that were tested did not have the mutation. Unfortunately a large proportion of patients were not tested for the mutation, probably due to the fact that the test was not discovered and not available prior to 2005.

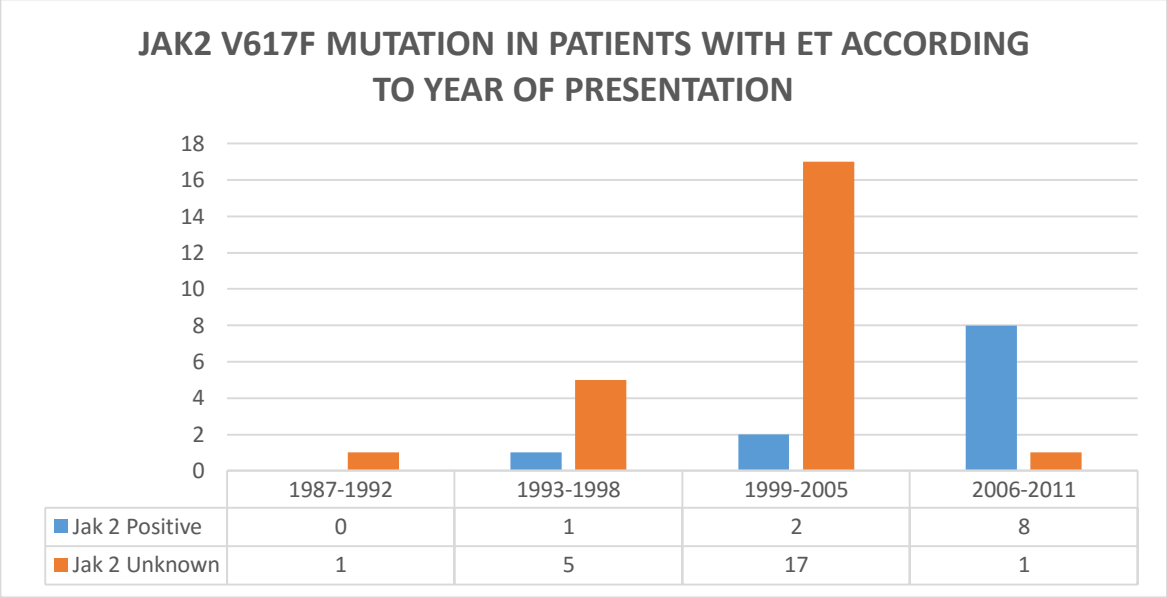

Figure 13: JAK2 V617F Mutation in ET according to year of presentation

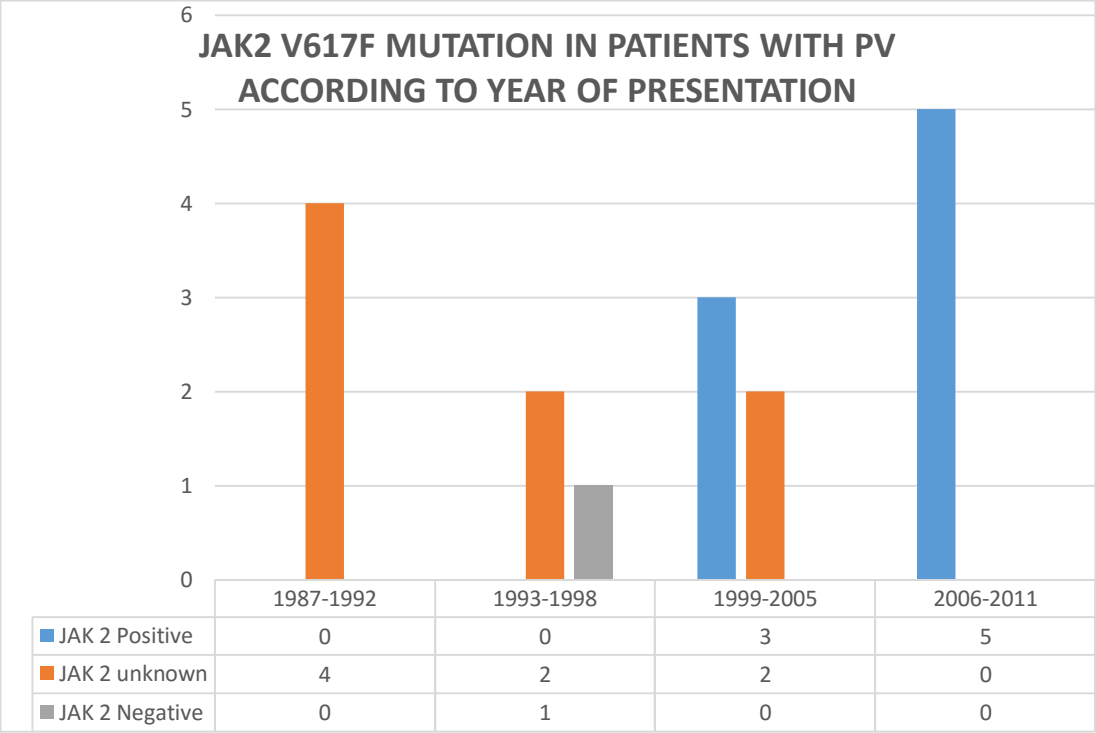

Figure 14: JAK2 V617F Mutation in PV according to year of presentation

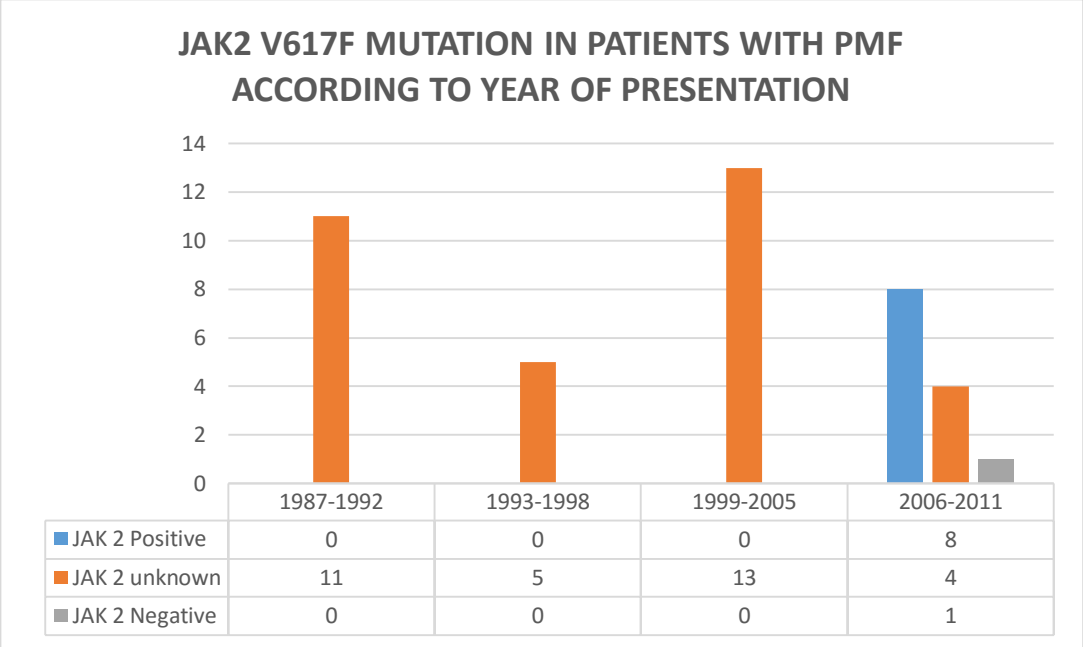

Figure 15: JAK2 V617F Mutation in PMF according to year of presentation

Figures 13, 14, and 15 illustrate the results of the JAK2 V617F mutation analysis of patients in the year of presentation. As demonstrated, most tests were done between the years 2006 – 2011. In the ET group, 8 patients tested as positive between these years while 5 PV patients and 8 PMF patients also tested positive during this time period. It is now routine practice to test all patients suspected of having MPN by doing the JAK2 V617F mutation analysis.

3.6 SUPPORTIVE MANAGEMENT

Table 11: Analysis of Supportive Treatment used in MPN

	All Patients (N=94)	ET (N=35)	PV (N=17)	PMF (N=42)
Folate	56 (60%)	21 (60%)	1 (6%)	34 (81%)
Iron	30 (32%)	5 (14%)	1 (6%)	24 (57%)
Allopurinol	66 (70%)	31 (89%)	13 (76%)	22 (52%)
Anticoagulation	10 (11%)	4 (11%)	5 (29%)	1 (2%)
Aspirin	16 (17%)	13 (37%)	3 (18%)	0 (0%)
Venesection	17 (18%)	0 (0%)	17 (100%)	0 (0%)
Blood Transfusion	27 (29%)	6 (17%)	0 (0%)	21 (50%)

A summary of the supportive treatment used is illustrated in Table 11. Folate tablets were given to 60% of all patients, with a large number of the 42 PMF patients receiving the drug. Iron supplementation was also most commonly given to patients with PMF with 57% of these patients having received either oral or intravenous replacement. Allopurinol was administered to 70% of patients with MPN. Anticoagulation in the form of warfarin or low molecular weight heparin was prescribed to 11% of patients. Aspirin was prescribed to 37% of ET patients, and 18% of patients with PV. Other supportive treatment included phlebotomy or venesection, as well as blood transfusions. Venesection is therapeutic in patients with PV and thus 100% of patients with PV were venesected. A total of 50% of PMF patients needed a blood transfusion. A small proportion of ET patients (17%) were also recorded as requiring a blood transfusion.

3.7 SPECIFIC MANAGEMENT

Table 12: Analysis of Drugs used to treat MPN

DRUGS USED	ET (N=35)	PV (N=17)	PMF (N=42)	ALL (N=94)
Hydroxyurea	32 (91%)	9 (53%)	24 (57%)	65 (69%)
Anagrelide	0 (0%)	0 (0%)	0 (0%)	0 (0%)
32P	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Busulphan	9 (26%)	1 (6%)	2 (5%)	12 (13%)
Corticosteroids	1 (3%)	0 (0%)	7 (17%)	8 (9%)
Danazol	0 (0%)	0 (0%)	5 (12%)	5 (5%)
Interferon alpha	2 (6%)	0 (0%)	0 (0%)	2 (2%)
Other*	1 (3%)	0 (0%)	0 (0%)	1 (1%)

(*Ruxolitinib)

The analysis of specific drugs used to treat MPN is demonstrated in Table 12. The most common specific treatment used in this cohort was hydroxyurea, with 91%, 53% and 69% of patients with ET, PV, and PMF receiving it respectively. In the earlier period of this study, busulphan was used as an alternative to hydroxyurea, especially in ET, with 26% of patients receiving this drug. Only 1 PV patient and 2 PMF patients in this cohort were given busulphan. 17% of PMF patients were prescribed corticosteroids in the form of oral prednisone, and only 1 patient with ET received it. Other drugs used either as an alternative or as an additional drug were danazol and interferon alpha. These were also among the least used drugs with only 2 patients receiving interferon alpha, while danazol was used in 5 patients (12%) of patients with PMF. 1 patient from the ET group was put on a trial using the JAK inhibitor called ruxolitinib. Anagrelide and 32P were not used in this cohort.

3.7.1 NUMBER OF DRUGS USED

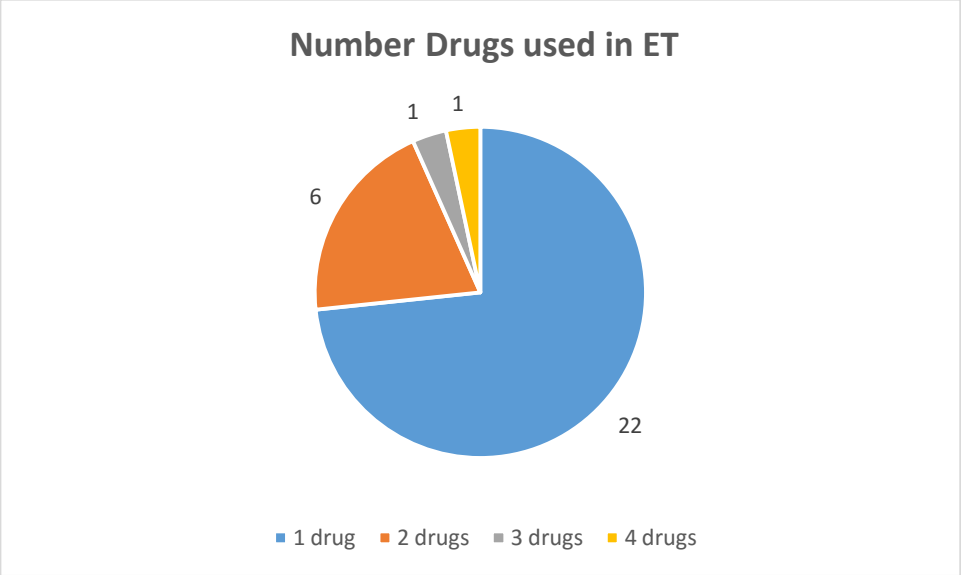

Figure 16: Number of Drugs used to treat ET

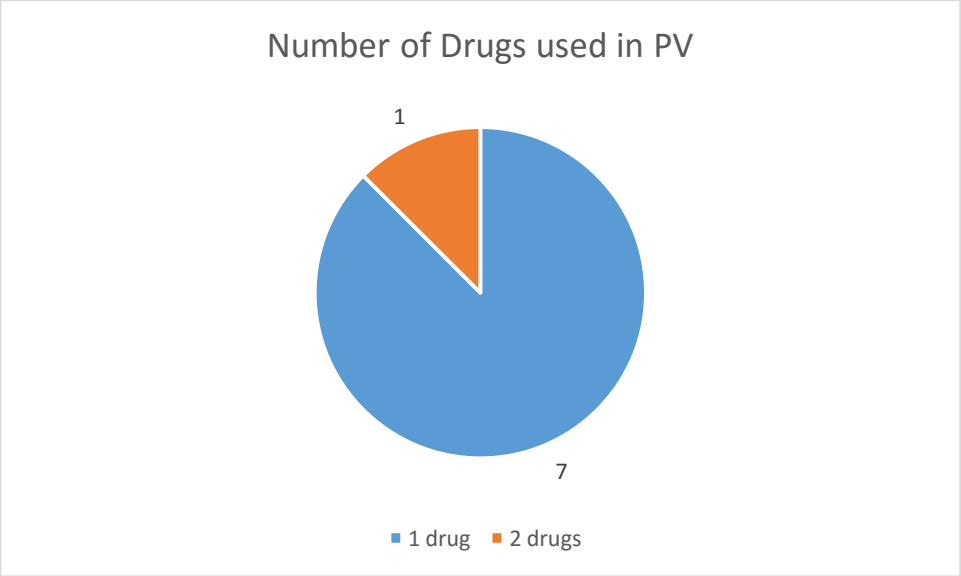

Figure 17: Number of Drugs used to treat PV

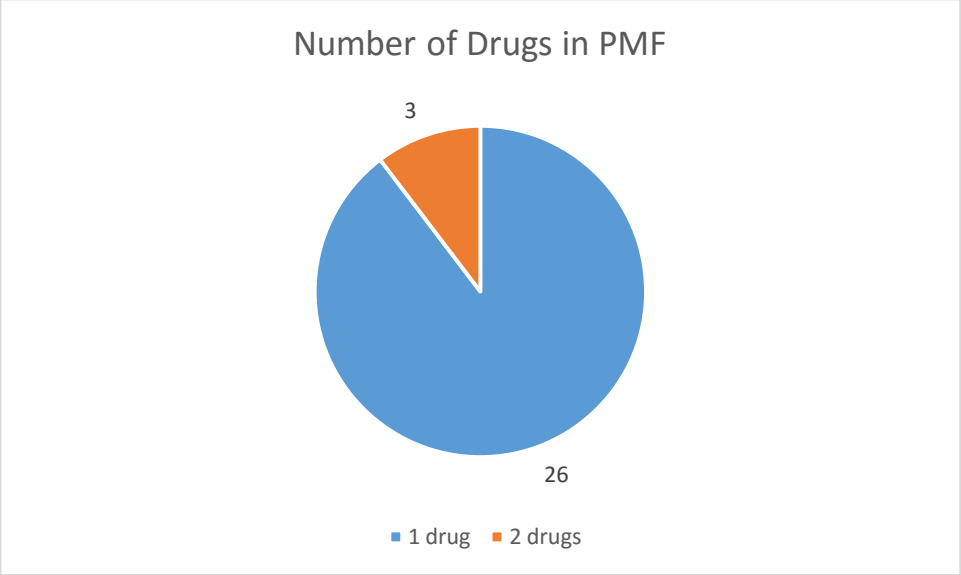

Figure 18: Number of drugs used to treat PMF

Figures 16, 17, and 18 illustrate the number of drugs used by patients with MPN in this cohort. The majority of patients with ET (63%) were treated with only drug i.e. hydroxyurea, while 6 patients were given 2 drugs simultaneously, and only 1 patient received 3 or 4 drugs at one time. Similar findings were observed in the PV and PMF groups, as it was common practise to use 1 drug (7 PV patients and 26 PMF patients). Danazol was utilised as a single drug in 3 PMF patients and another 4 PMF patients were prescribed corticosteroids as a single drug.

3.7.2 DURATION OF SPECIFIC TREATMENT USED

Tables 13 and 14 depict the duration of specific drugs used during the course of disease in patients with ET and PMF. A hundred percent of patients with PV were venesected, and 41% of PV patients were treated with hydroxyurea alone with a median duration of 207 days. 22 patients with ET were treated with hydroxyurea alone with a median duration of 226,5 days. Patients refractory to hydroxyurea were then initiated on busulphan, as was observed in 6 patients. Only 1 patient with ET received 3 drugs, with interferon alpha being used after the patient did not respond to hydroxyurea and busulphan. Hydroxyurea was used alone to treat patients with PMF in 45% of cases. The median duration of treatment in this group was 99 days. Danazol was also used in patients with PMF in 7% of cases with a median duration of 43 days. Other drugs used were corticosteroids in 4 patients with PMF, with the median duration of treatment being two and half months. Hydroxyurea was the most common specific drug treatment used in patients with MPN.

Table 13: Duration (in days) of specific treatment in patients with ET (N=30)

	Only Hydroxyurea	Hydroxyurea + Busulphan	Hydroxyurea + Busulphan + Interferon alpha	Hydroxyurea + Busulphan + Interferon alpha + Ruxolitinub
Number	22	6	1	1
Duration of hydroxyurea	226,5(22-2630)*	1252,5(57-5142)*	3358	2401
Duration of Busulphan		31(4-771)*	259	273
Duration of Interferon alpha			1134	519
Duration of Ruxolitinib				126

*(median ± IGR utilised when patient number >2)

Table 14: Duration (in days) of specific treatment in patients with PMF (N=29)

	Only Hydroyxurea	Hydroxyurea + busulphan	Hydroxyurea + Corticosteroids	Only Corticosteroids	Only Danazol
Number	19	1	2	4	3
Duration of Hydroxyurea	99(7-1366)*	1177	400		
Duration of Busulphan		372			
Duration of Corticosteroids			529,5	81(43-239)*	
Duration of Danazol					43(40-211)*

*(median ± IGR utilised when patient number > 2)

3.8 RESPONSE TO TREATMENT

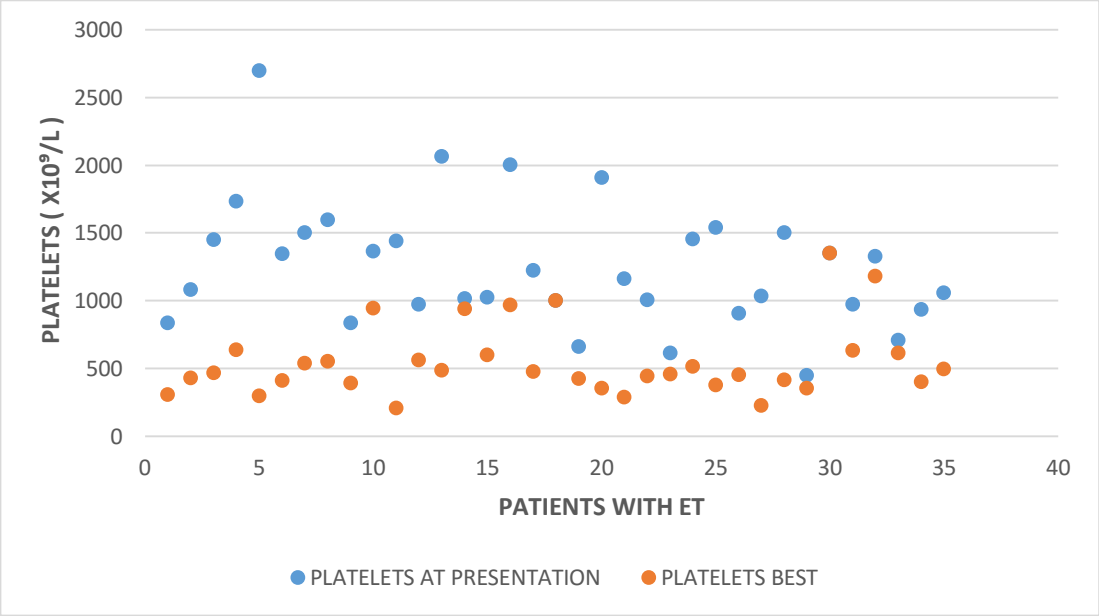

Figure 19: Platelets at Presentation vs Platelets Best recorded in ET

(P value = < 0,0001 using paired t-test)

Figure 19 (above) depicts the response of treatment in ET patients, by comparing the presenting platelet counts to the best recorded platelet counts. On viewing the line graph, it is seen that the best platelet count recorded are definitely lower than the presenting counts. This suggests that the treatment used is effective in lowering platelet counts in ET patients. The p value is also statistically significant and supports this notion.

Figure 20 (below) illustrates the response to treatment in patients with PV by comparing the presenting Hct levels against the best recorded Hct levels. Once again the line graph is indicative of lower Hct values recorded post treatment. The p value demonstrates a statistical significance and therefore also reveals that treatment used in PV was effective in decreasing the Hct in patients diagnosed with PV.

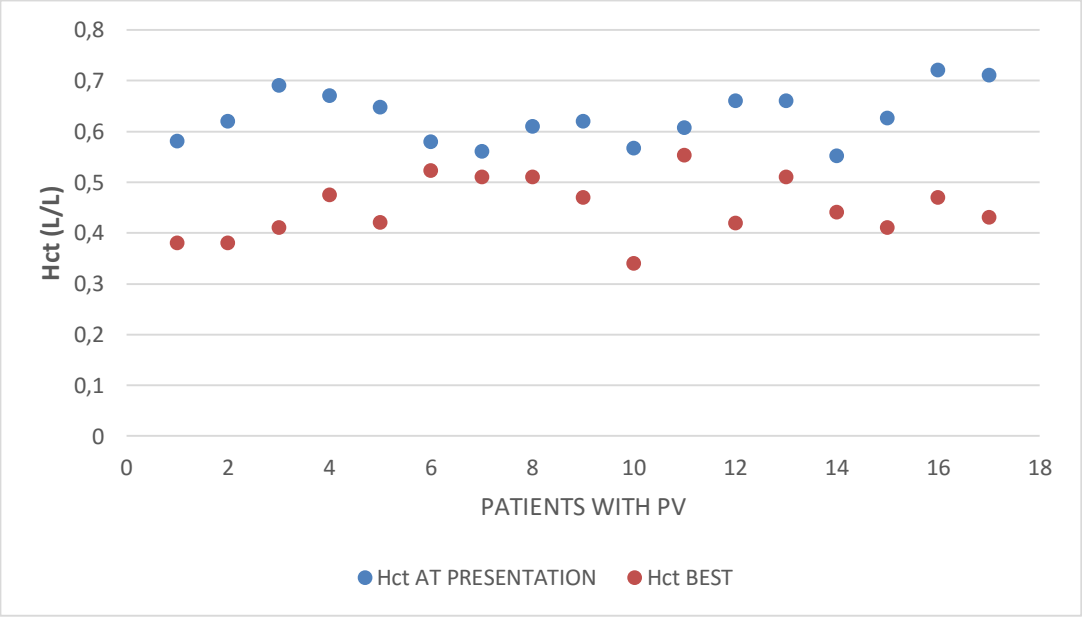

Figure 20: Haematocrit at Presentation vs Haematocrit Best recorded in PV

(Hct = Haematocrit)

(p value = 0,0001 using paired t test)

Table 15: Analysis of Venesections in patients with PV

Venesections in Patients with PV	
Number of patients venesected (%)	17 (100%)
Average number of venesections per patient	9 ±6
Median (± IGR) days per venesection	117 (15-1216)

As previously demonstrated, venesection was carried out in 100% of patients with PV, with the aim of lowering the haemoglobin, red cell count, and Hct, thereby lowering the blood viscosity and thus reducing the incidence of complications. The average number of venesections performed per patient in this cohort was 9 times. It was also noted that there was a median duration of approximately 4 months between each venesection. From these records it is suggestive that venesection therefore plays a pivotal role in the treatment of PV.

3.9 COMPLICATIONS OF MPN

Figure 21 illustrates the complications of MPN that occurred in patients in this cohort. Thrombotic complications include strokes, the occurrence of deep vein thrombosis and pulmonary thrombo – embolic disease, as well as intra-abdominal thrombus formation. The most common complication was bleeding (which included gingival bleeds, epistaxis and conjunctival haemorrhages) and this was demonstrated in 19% of all patients. 24% and 17% of PMF and ET patients suffered from a bleed respectively, while only 12% of PV patients were recorded as having a bleeding episode. Stroke occurred in 4 ET and PV patients, while no patients diagnosed with PMF had this debility. Only 3% of all patients developed deep vein thrombosis (2 PV patients) or a pulmonary embolus (1 patient in the PV, ET, and PMF group). Intra-abdominal thrombosis in the form of splanchnic vein thrombosis was diagnosed in only 2 (6%) of all ET patients.

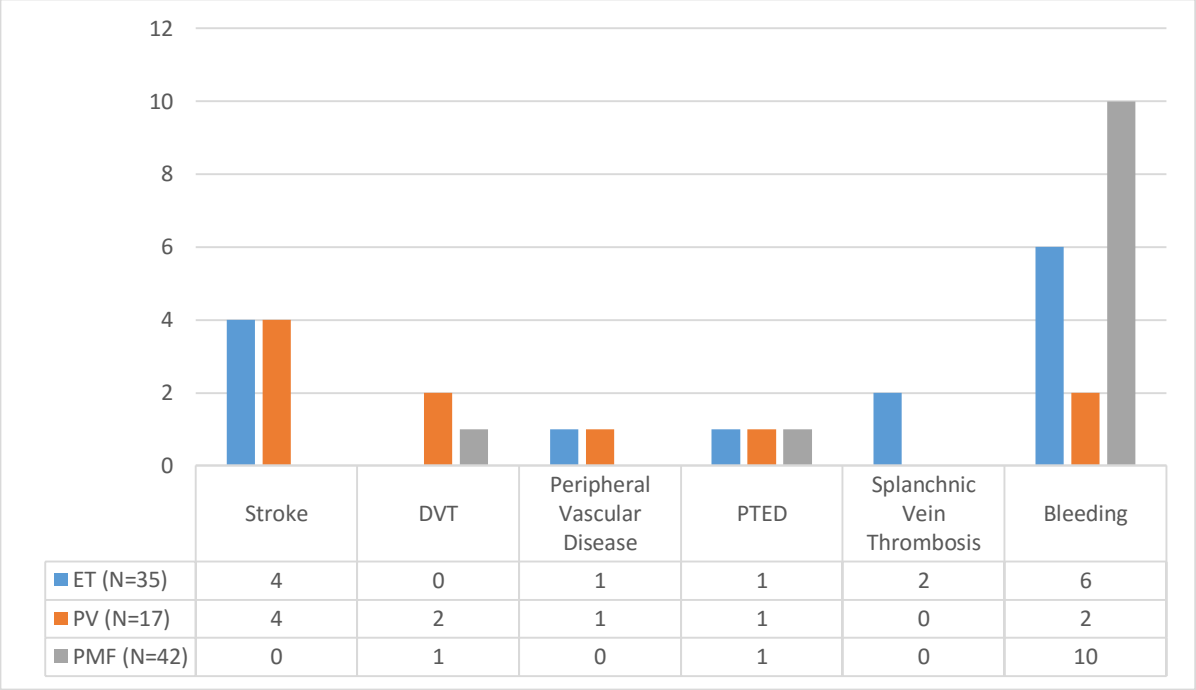

Figure 21: Frequency of Complications occurring in MPN

(DVT = Deep Vein Thrombosis, PTED = Pulmonary Thrombo-embolic Disease)

3.9.1 TRANFORMATION ANALYSIS

Table 16: Frequency of Transformation in Ph1 Negative MPN

	ET	PV	PMF
To ET	--	0	0
To PV	0	--	0
To MF	2 (6%)	2 (12%)	--
To AML	2 (6%)	0	6 (14%)

Another complication of MPN is the ability to transform into another form of MPN, as well as the fatal leukaemic transformation. Table 16 illustrates the frequency of transformation in this cohort. Disease transformation is shown to be an uncommon occurrence in this cohort, however, when it did occur, it was seen from ET and PV to myelofibrosis in 6% and 12% of patients respectively. Transformation to acute myeloid leukaemia was documented in 14% of patients with PMF and 6% of patients with ET.

3.10 OUTCOMES

The final clinico-haematologic responses of patients diagnosed with MPN were analysed using criteria suggested by the ELN working group. The major outcomes included complete response, partial response, and no response which was further subdivided into stable disease and death.

3.10.1 OUTCOMES OF PATIENTS TREATED

Table 17: Final Outcomes of ET, PV, and PMF

	ALL (N=94)	ET (N=35)	PV (N=17)	PMF (N=42)
Complete Response	5 (5%)	1 (3%)	2 (12%)	2 (5%)
Partial Response	7 (8%)	6 (17%)	1 (6%)	0 (0%)
Relapse	1 (1%)	0 (0%)	0 (0%)	1 (2%)
Stable Disease	52 (55%)	21 (60%)	12 (70%)	19 (45%)
Death	29 (31%)	7 (20%)	2 (12%)	20 (48%)

Table 17 depicts the final outcomes based on the best documented outcome at the last visit in patients with MPN. Overall the results documented reveal that the outcomes of treatment available to patients in this cohort was dismal. A total of 5% of patients showed a complete response in this cohort and only 7% of all patients had a partial response. The most promising results may have come from the ET group with 17% of patients being recorded as having a partial response. 55% of all patients had stable disease on treatment, with 60% of ET patients,

70% of PV patients, and 45% of PMF patients belonging to this group. A total of 31% of MPN patients in this cohort died, and the worst outcomes were seen in the PMF group as 48% of these patients lives ended in death. Death came about to only 2 patients with PV, however 20% of patients in the ET group also had a final outcome of death. The criteria recommended by the ELN seem to be quite stringent and therefore there was difficulty in maintaining a persistent complete response. However most of the patients were clinically stable during the course of the disease, even though many fluctuations of laboratory parameters were seen.

3.10.2 SURVIVAL CURVES

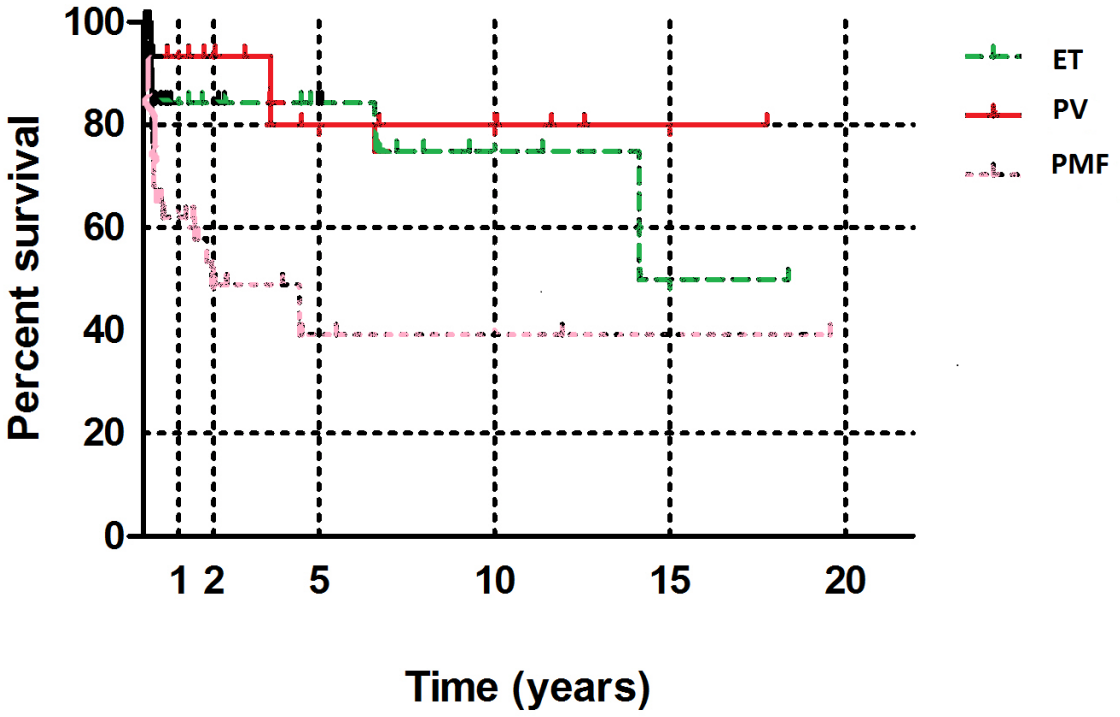

Figure 22: Survival Curves for ET, PV, and PMF

p value = 0,0079 (Chi^2 using log ranks (Mantel-Cox) analysis)

The survival curves of patients diagnosed with ET, PV, and PMF is depicted in Figure 22. On analyses of the survival curves, it is safe to say that PV and ET have a better chance of survival than PMF as the

disease progresses. The 5 year survival of PV was 80%, while the worst 5 year survival belongs to the PMF group with a 40% survival being illustrated. ET has the best 5 year survival in this cohort with 85%, however as time progresses it is shown that the percentage of survival decreases substantially to around 52% (15 year survival).

CHAPTER 4: DISCUSSION

4.1 INTRODUCTION

Myeloproliferative Neoplasms collectively represent a disease entity that was initially described as early as the 1950's (1). MPN are clonal proliferations of the 3 main blood cells lines and manifest with features of thrombosis and bleeding. Essential Thrombocythaemia, Polycythaemia Rubra Vera, and Primary Myelofibrosis differ from Chronic Myeloid Leukaemia in that they do not exhibit the Philadelphia Chromosome. Compared to CML, the other MPN mentioned above have been less well characterized. A retrospective analysis of the 3 Philadelphia Chromosome Negative Myeloproliferative Neoplasms was thus undertaken to better understand the patterns of the disease in this group, including the efficacy of treatment based on our management. The study reviewed 94 patients diagnosed with Ph1 negative MPN at Chris Hani Baragwanath Academic Hospital between 1987 and 2011 (25 years).

4.2 EPIDEMIOLOGY

During the 25 year period, 94 patients were diagnosed with a MPN, using criteria recommended by the Polycythaemia Study Group in the early years, and revised WHO criteria in 2001 and 2008 (115) (116). Of the patients studied, 45% were diagnosed with PMF, while 37% of the patients were diagnosed with ET, and 18% with PV. These figures differ from studies carried out in Europe and the United States where PV was shown to be the most common diagnosis and PMF the least common (53) (117). The reason for this difference is not entirely clear but could be due to PV being relatively benign and being managed by general physicians, while most of the PMF patients are referred to a specialised centre for further management. The ethnic group may also play a role. Many of the studies undertaken in Europe involve mostly Caucasian patients, while the vast majority of patients in this cohort are of African descent.

The median age at diagnosis of MPN was 61,5 years. Similar median ages to the MPN group were seen in the ET and PMF groups with 63 and 63,5 years respectively. However the median age of diagnosis at presentation was 54 years, almost a decade younger, in patients with PV. Our findings are similar to other cohorts where MPN has been shown to occur between the ages of 50 to 70 years, with a

median age of presentation of 61 years in patients with PV noted in one study, while median ages of 66 years for PMF and 54 years for ET were found in studies carried out at the Mayo Clinic (118) (119) (61). Thus, overall one can conclude that MPN is a disease most commonly of middle aged individuals.

The study also revealed that there is no significant gender disparity in the whole MPN group with a male to female ratio of 1: 1.24. However, twice as many females compared to males were seen in the ET group, while an equal number of males and females were diagnosed with PMF. A review of patients with MPN carried out in Sweden revealed a slight male predominance of 53% while studies at the Mayo Clinic showed a female predominance in the PMF group.

4.3 SYMPTOMATOLOGY

Symptoms associated with MPN are due to the features of the disease process. The most common symptom recorded in an online survey of 1179 patients is fatigue with 81% having complained of this symptom respectively (45). Other symptoms that patients complained about were pruritus and night sweats (52% and 49%), as well as bone pain, fever and weight loss (44%, 14% and 13% respectively) (45). Fatigue was also the most common symptom in our cohort with 85% of patients presenting with this symptom. Other common symptoms included weight loss and night sweats, which are constitutional symptoms. In our setting, other causes of constitutional symptoms such as HIV, tuberculosis and lymphomas must be excluded. Pruritus is another well-known symptom of MPNs, especially PV, and our cohort shows no difference, with 59% of PV patients presenting with this complaint. Erythromelalgia is an uncommon symptom as indicated in studies in the United States and in Norway, and this is also seen in our cohort with only 1 patient presenting with this complaint (120) (121). Thus, the presenting symptoms of patients with MPN in our cohort is similar to that published in the literature.

4.4 CLINICAL FEATURES

The occurrence of increased erythropoiesis, megakaryopoiesis, and fibrosis in the bone marrow leads to a variety of clinical features that may lead to increased morbidity and mortality in patients with MPN. These clinical features include organomegaly secondary to extramedullary haemopoiesis, as well as pallor and clinical evidence of bleeding due to progressive cytopenias. It is well demonstrated

in the literature that splenomegaly is a common clinical feature of MPN, especially in PMF. This is well illustrated in this cohort, as splenomegaly was the most common presenting clinical feature in 80% of the patients. All but 1 patient with PMF (98%) were recorded as having a splenomegaly on clinical examination. Splenomegaly leads to increased patient distress, caused by abdominal discomfort and pain, as well as compounding the effects of cytopenias due to splenic sequestration (122). The median size of the spleen palpated below the costal margin in PMF was 7cm and 73% of all PMF patients were shown to have a moderate to massive splenomegaly. This is in contrast to PV and ET patients who demonstrated median spleen sizes reflecting a small splenomegaly (< 4cm). This is also in keeping with findings in the literature. Symptomatic splenomegaly has been managed with different modalities of treatment such as hydroxyurea, radiotherapy, splenectomy and more recently the JAK2 inhibitors (122) (123). This will aim to reduce the size of the spleen and alleviate distressing pain as well as reduce the effects of hypersplenism. Hepatomegaly may be due to extramedullary haemopoeisis. Hepatomegaly is a common finding especially in PMF patients. More prominent hepatomegaly was found in this cohort in the PMF group compared to ET and PV groups.

Excessive bleeding due to platelet hypoactivity and dysfunction as well as thrombocytopenia may lead to anaemia, especially in patients with PMF. As the PMF progresses, excessive bone marrow fibrosis may occur, leading to cytopaenias. This manifests clinically as pallor, bleeding, petechiae, purpura, and ecchymosis. Pallor was evident in 78% of PMF patients in our study, which signifies that it is a common feature at presentation. Only 12% of the whole cohort presented with clinical evidence of petechiae, purpura or ecchymosis, which is in keeping with a study of cutaneous manifestations of ET in the United States, where 10% of patients presented with these features (124). Twelve patients in our study were recorded as having changes such as tortuous and dilated retinal vessels seen in PV from increased blood volume, and also haemorrhages of the retinal vessels which may also be observed (125). Lymphadenopathy was palpated in 12% of patients in our cohort and could be linked to other diseases such as HIV, tuberculosis, or even other viral infections. Lymphoproliferative disorders have been reported occasionally to be associated with MPN as shown in a study by Vannucchi et al (126). Lymphadenopathy is not a typical feature of MPN, and its presence should generally suggest another cause.

4.5 ANALYSIS OF BLOOD RESULTS

Haematological diseases are never assessed without the analysis of blood results in the patients affected. A diagnosis of MPN cannot be made unless relevant abnormalities are demonstrated and this is also the reason why many patients are referred to a specialised Haematology unit for further investigation. The occurrence of clonal proliferation of the erythropoietic and megakaryopoietic cell lines can be demonstrated on full blood counts and other important features can be shown on bone marrow investigation. Criteria for diagnosis of the Ph1 negative MPN have been revised in 2008 by the WHO (127). A platelet count of > 450 x 10^9/l is one of the criteria for ET and this is evident in our study with a mean platelet count of 1250 x 10^9/l at presentation. A similar study in Europe showed an average platelet count of 897 x 10^9/l at presentation (128). Leucocytosis was shown in the majority of the ET patients in this cohort with a mean of 12,23 x 10^9/L. The importance of this was demonstrated in a study by Carobbio et al, where leucocytosis was shown to be a risk factor for future thrombotic events in patients diagnosed with ET (129). The number of platelets though were not associated with any thrombotic risk, however a platelet count > 1500 x 10^9/l is associated with a form of acquired von Willebrand's disease, and thus an increased risk of bleeding (130).

Criteria for PV includes erythrocytosis with raised haemoglobin levels associated with low serum erythropoietin levels. Our cohort had a mean haemoglobin of 20,6 g/dl and a mean haematocrit of 0,63 l/l. Treatment recommendations are clear about reducing the haematocrit level to < 0,45 l/l as levels higher than this pose an increased risk for cardiovascular complications (131). Other factors of note were that neither the mean platelet or leucocyte counts were raised in PV patients. Very few patients in our study had oxygen saturation values recorded. Serum EPO levels are important in differentiating primary from secondary polycythaemia. Low serum EPO levels in patients suspected of PV helped considerably with the diagnosis in the past (this is largely replaced by JAK2 617F mutation analysis) while if the serum EPO levels are raised, then one has to suspect secondary causes of erythrocytosis such as chronic obstructive pulmonary disease. The median serum EPO levels in our cohort was 3,2 mU/ml, which is decreased according to our reference ranges (4,1-19,5 mU/ml) and correlates with the diagnosis of PV.

Features of decreased erythropoiesis and reticulin or collagen fibrosis in the bone marrow is a major criteria for the diagnosis of PMF. However, other features on the full blood count include an anaemia as well as a leucoerythroblastic picture on examination of the peripheral blood smear. Due to possible

poor or incomplete recording of patient results in our cohort by the attending physicians, only 7/42 patients (16,7%) had a leucoerythroblastic picture, while studies elsewhere do reveal that it is a common occurrence in PMF patients (132). Another common finding in the peripheral blood smear is of teardrop shaped red blood cells, and this correlates with our study as 72% of patients with PMF had this finding (133). Anaemia was found in the majority of patients with PMF (90,4%), likely due to multifactorial causes including reduced erythropoiesis, with a mean value of 8,3 g/dl. The IPSS is used to assess the prognosis of PMF patients at presentation while the DIPSS is used at any point in the disease process. These prognostic indices are predictors of inferior survival and include age > 65 years, haemoglobin < 10 g/dl, constitutional symptoms, leucocytosis of > 25 x 10^9/l, evidence of blasts > 1% and other factors such as transfusion requirements, unfavourable karyotype and platelets < 100 x 10^9/l (134). On further assessment of our cohort, patients referred to the Haematology unit that are diagnosed with PMF have an IPSS score of at least 3, which puts them in the high risk category and thus suggests poor prognosis (135).

Five patients in the study were iron deficient from factors other than bleeding related to MPNs. Three patients were HIV seropositive. This is likely to be coincidental rather than causal. The majority of patients with PV (77%) presented with hyperuricaemia. However, only two patients in this group and only 5 patients overall (with Ph1 negative MPN) developed secondary gout. This could be due to early and routine initiation of xanthine oxidase inhibitors like allopurinol in our patients (136).

4.6 JAK2 V617F MUTATION PREVALENCE

The discovery of the V617F mutation of Janus Kinase 2 in 2005 has changed the diagnostic landscape of MPN and is also impacting on therapy of the MPN. The PCR test for this mutation has been available in South Africa since 2006 and thus new patients and old patients were able to undergo this investigation and the diagnosis of MPN has been made easier and clearer. The presence of this mutation has also been introduced into the revised WHO criteria, thereby improving the diagnostic criteria for these diseases (2). Other mutations have also been discovered which alter the way the disease has been assessed in patients with ET and PMF especially. These mutations include the MPL and CALR mutations, and are thought to explain why some patients who do not have the JAK2 V617F mutation still present with a MPN (137). The frequencies of the JAK2 V617F mutation in the Ph1 negative MPN are estimated at approximately 98% of all PV patients, while at least 50 - 60% of patients with ET and PMF will have the mutation (137). The JAK2 V617F mutation has other implications

including an increased risk of leukaemic transformation as well as increased risk of thrombotic events (138) (139). In our study 29 patients were tested for the mutation, with 27/29 patients (93%) having a positive result and 2 patients a negative result. This leaves a significant proportion of patients who have an unknown JAK2 V617F mutation status (these are patients who may have died or were lost to follow up) and thus it was difficult to compare and analyse the significance of the mutation between the patients.

The investigation for the JAK2 V617F mutation in patients suspected of a MPN is now readily available and thus should be done in all patients at presentation. This will give us a better idea of the frequency of this mutation in MPN in our setting and help with diagnostic difficulties. Newer treatment aimed at JAK2 inhibition are still underway, however JAK2 inhibitors like Ruxolitinib has already been approved for myelofibrosis (140).

4.7 MANAGEMENT OF MPN

Therapy for MPN includes both supportive care and specific modalities such as cytoreductive therapy, targeted therapy and allogeneic stem cell transplantation. There has been no cure reported for MPN, and treatment is aimed at alleviation of symptoms and lessening disease burden, as well as reducing the risk of fatal complications.

Red cell folate deficiency was noted in 9/94 patients (9,6%) in our cohort, 5/42 (12%) of them belonging to the PMF group. Folate deficiency has been associated with MPN, likely secondary to ineffective haemopoeisis or due to drugs that antagonize folate, and due to increased cell turnover, which results in an increased utilization of folate. It is more common in PMF (141). Folate supplementation was prescribed to 81% of PMF patients and 60% of ET patients, in our study.

The occurrence of vascular events are most common in PV and ET and thus therapy aimed at reducing this includes aspirin. The ECLAP trial demonstrated that patients with PV benefitted from the use of aspirin, by reducing thrombotic events and with no significant risk of fatal bleeding (67). Only 16% of all patients were given aspirin (this was exclusively in the ET and PV patients). Patients with MPN may also have other comorbities such as hypertension or diabetes mellitus, which could also contribute to

thrombotic risk. Iron deficiency is not commonly seen in MPN, except for patients with PV receiving regular venesection, however, patients with evidence of bleeding may develop iron deficiency. Only 5 patients in our cohort had iron deficiency. However, oral iron supplementation was given to 57% of PMF patients as one of the measures to improve their anaemia.

Blood transfusions are most commonly required in patients with PMF as observed in our study. Transfusions are required for symptomatic anaemia. The need for blood transfusions were seen in 50% of patients with PMF during the course of their disease, and according to the DIPSS indicates a worse prognosis (134). Only 6 patients from the ET group required a blood transfusion, either due an acute bleed or secondary to complications of treatment. Phlebotomy or venesection was performed in all patients diagnosed with PV, with a mean requirement of 9 units venesected per patient. The time between each venesection was calculated to be on average at approximately 4 months. Conflicting opinions appear in the literature regarding venesection. Some authors are of the view that venesection alone increases thrombotic risk in the first 3 months and suggest combination therapy to avoid this, while others have demonstrated safety in venesecting small volumes at 2 monthly intervals (68). Venesection remains the mainstay of treatment in patients with PV.

Specific treatment includes cytoreductive therapy with hydroxyurea as the first line agent. It helps in suppressing the bone marrow and increasing fetal haemoglobin. In a study of a 100 patients with PV who were treated with phlebotomy alone or combined with hydroxyurea, the results indicate that hydroxyurea was effective at decreasing phlebotomy requirements without increasing toxicity after a mean duration of 69 months of use (142). Hydroxyurea is also capable of alleviating symptoms and reducing splenic size as demonstrated in another study where 52% of patients with PMF had a reduction in splenomegaly (143). Cortelazzo et al studied the benefits of hydroxyurea in ET patients in decreasing the thrombotic events by maintaining a platelet count below 600 x 10^9/l, and proved that it is beneficial in reducing thrombotic complications (72). The majority of the patients in our study were initiated on hydroxyurea (69%). It is most commonly used for patients with ET (91% of patients). Drugs such as anagrelide were not used in our setting. The ANHYDRET study revealed that anagrelide is not inferior to hydroxyurea with regard to thrombotic and bleeding manifestations but conversely another study showed that hydroxyurea had better outcomes in reducing complications (72) (76).

Busulphan was used in a study of 36 patients with ET and PV who were refractory or intolerant of hydroxyurea. The study showed that it is as effective as hydroxyurea, but recommended it as a 2nd line agent for patients refractory to hydroxyurea, in view of the toxicity as seen in 30% of the patients (77). Interferon alpha or pegylated interferon has also been recommended as a 2nd line therapy in MPN, especially in PMF (80). Limited use of both of these agents have been seen in our cohort with only 2 refractory ET patients having received interferon alpha, while 12 refractory patients with ET having received busulphan. This can be attributed to most patients having responded to hydroxyurea and only 14 patients showing treatment failure or intolerance to hydroxyurea. Ruxolitinib was prescribed to one patient with ET, who was refractory to 3 prior lines of treatment, and was thus put onto a clinical trial. Androgen therapy in the form of danazol was prescribed to 5 patients with PMF with refractory anaemia. This correlates with the literature where Cervantes et al demonstrated that danazol can be useful at an appropriate dose in improving haemoglobin levels (86).

In some patients, combination therapy was used. Examples include corticosteroids and hydroxyurea in PMF, and hydroxyurea and busulphan in refractory ET. The disadvantage of some of the combination therapies are an increased risk of leukaemogenesis as shown in a randomized clinical trial of ET patients, where busulphan combined with hydroxyurea showed an increased risk of second malignancies of 13% (144). The median duration of use of hydroxyurea was 226,5 days for ET, and 99 days for PMF. The reduced amount of days in the PMF group indicates that patients were either deemed refractory to hydroxyurea and another agent was added, or due to the late presentations in our population with advanced disease, which has a direct impact on poorer survival.

More recently, patients with MPN (in particular PMF) have been afforded access to JAK2 inhibitors (initially fedratinib and now ruxolitinib). The discovery of JAK inhibitors has revolutionized the way MPNs will be treated in the future. Drugs such as ruxolitinib and pacritinib are being evaluated in trials to determine whether Ph1 negative MPN can be treated more efficiently, just as tyrosine kinase inhibitors in the treatment of CML. Unfortunately due to the lack of funds in the state hospital sector in South Africa, these drugs are only available if patients are enrolled in a clinical trial. Therefore, the mainstay of treatment in this study remained cytotoxic therapy such as hydroxyurea.

The ultimate aim of therapy is to reduce debilitating symptoms, prevent complications of the disease, and thus improve quality of life. Analysis of platelet counts were carried out in all ET patients to assess the best response to treatment used in our patients with ET. Higher platelet counts increase the risk of bleeding and thrombosis and thus treatment aims to reduce this. There is a definite reduction in platelet counts in our patients with cytoreductive therapy, with a best recorded median platelet count of 466 x 10^9/l for all the patients with ET. This may not be a complete clinico-haematological response of < 400 x 10^9/l according to criteria by the ELN working group, however it will aid in reducing complications (114). The CYTO-PV trial showed evidence that patients with PV with haematocrit levels above 0,45 l/l developed more thrombotic and cardiovascular complications (69). More controlled haematocrit levels are thus recommended in PV patients. The response to phlebotomy by analysing the best haematocrit levels in our patients demonstrated a significant difference. Haematocrit levels were lowered with a median best haematocrit level of 0,44 l/l. This correlates with the level recommended to reduce thrombotic and cardiovascular complications.

4.8 COMPLICATIONS OF MPN

Thrombotic complications of MPN are a frequent problem and it has a multifactorial pathogenesis (145). Due to the age group that MPN normally present in, these patients generally have other comorbidities that also increase the risk of thrombotic and cardiovascular disease. These complications generally preclude the diagnosis of MPN, and the search for MPN occurs after they have occurred. An increased blood viscosity, altered von Willebrand factor function, dysfunctional platelets, are a few abnormalities that lead to thrombosis and bleeding. Thrombotic complications occur in about 50% of ET and PV patients (146). Twenty four percent of PV patients and 11% of ET patients developed an ischaemic stroke which could be partly due to other risk factors like smoking, diabetes mellitus, and hypertension. Venous thrombosis occurred in the form of deep vein thrombosis in 3 (3,2%) of the patients in our cohort, and pulmonary embolism in another 3 patients (3,2%). In a review of 891 patients with ET, 12% of patients developed either arterial of venous thrombosis, these findings are similar to our cohort (147).

Bleeding was predominantly mucocutaneous (epistaxis; into the skin such as purpura and ecchymosis). This was observed in 24% of patients with PMF and 17% of ET patients. This is also similar to the literature where a study by Finazzi et al of 1104 patients revealed that twice the number of PMF patients compared to ET patients developed bleeding during the course of their disease (148). A total

of 6% of patients with ET were diagnosed with portal vein thrombosis, which correlates with a study in Europe where 4% of patients developed abdominal vein thrombosis (149). This is a rare occurrence but one must suspect MPN if the thrombosis is unexplained.

Another fatal complication of MPN is their propensity to transform into acute leukaemia. Incidences of between 5% to 10% are seen in PV and ET, but the leukaemic transformation of PMF occurs at a higher rate of approximately 20% (150). Our study correlates with the literature where only 6% of ET patients developed acute myeloid leukaemia (AML), while 14% of PMF patients developed AML. Controversy exists regarding the leukaemogenic potential of hydroxyurea. More definite evidence is required in this regard, as a predisposition to the development of a malignancy maybe part of the natural history of the disease and other factors such the use of alkylating agents, radiotherapy, radioactive phosphorus etc. may also be contributory. Two patients (5,7%) developed post ET myelofibrosis and 2 patients (11,8%) developed post PV myelofibrosis, which proves it is a rare occurrence.

4.9 OUTCOMES OF THERAPY

The outcomes of therapy in this cohort was assessed by using the revised criteria for myelofibrosis, polycythaemia vera, and essential thrombocythaemia, recommended by the ELN and IWG-MRT consensus project (112) (113). In our cohort, therapy for MPN is dismal with regard to a ''cure''. Using stringent criteria by the ELN, only 5% of all patients achieved a complete response, while 7% of all patients achieved a partial response. This may be an indication that these diseases are ''chronic'' and rarely curable. Other factors in our patients that portend a poorer outcome include late presentations with more advanced stage disease, less stringent adherence to treatment and poor compliance with regard to long term follow up. Stable disease is defined as disease that neither improves to a complete or partial response, and also does not progress to a more severe disease. A high proportion of patients were observed in this group. Fatal disease occurred in 31% of all patients diagnosed with MPN in our study, with almost half of all PMF patients' having demised. Further, no patients received an allogeneic stem cell transplantation.

4.10 SURVIVAL

Survival in patients diagnosed with a MPN has been shown to be moderately reduced for patients with PV and ET, but significantly reduced in patients with PMF (151). Factors such as age > 65 years and a

haemoglobin level < 10 g/dl are seen as poor prognostic factors in patients with PMF (152). This correlates with our study as mean values for age and haemoglobin fall within this range and thus PMF patients would be expected to have an unfavourable course of disease. The median survival of PMF ranges from 4 to 5,5 years (42). ET has a median survival of 18,9 years in a study at the Mayo Clinic, but life expectancy worsens after the first 10 years after diagnosis (118). This could be attributed to development of thrombotic complications or transformation to leukaemia. Another study by Tefferi et al demonstrated a median survival of 14,1 years for PV, which is less than expected for the normal population (61).

The survival of patients in our study also correlates with the literature. PMF was shown to have a significantly decreased survival as the disease progresses with a 2 year survival of 50% and 5 year survival of 40%. ET and PV had much more improved 2 year and 5 year survival times compared to PMF. However, similar to the study at the Mayo Clinic, survival worsens as the time progresses with a 15 year survival of 52% (119). Our findings correlate with the findings in the Western world and prognostic factors are similar to that already described. Other comorbid conditions such as hypertension, diabetes mellitus, and chronic infections could also impact on survival in our patient population.

4.11 CONCLUSION

Philadelphia chromosome negative MPN in South Africa are generally uncommon diseases which typically occur in the 55 to 65 year old age group. It generally has no significant gender predilection. PMF is the most common subtype, followed by ET and PV. The common clinical presentation includes fatigue, constitutional symptoms and splenomegaly. Presentation to hospital may also be for thrombotic events and bleeding manifestations. The mainstay of treatment includes phlebotomy for PV, and hydroxyurea for ET and PMF. This therapy was able to produce stable disease in many patients but a complete therapeutic response was seen in only a very small number of patients. Complications such as arterial and venous thrombosis, bleeding and leukaemic transformation occurred at a similar rate to that described in the literature. JAK2 V617F mutation assays became available in 2006, and 93% of all patients that were tested, proved to have the mutation. Routine testing of this mutation is needed in all patients suspected of having a Ph1 negative MPN. JAK2 inhibitors are an emerging therapy with potential for use in more of our patients in the future. The prognosis of patients

correlates with studies of MPN, as described in the literature. PMF has been shown to have the worst survival of the Ph1 negative MPN.

4.12 LIMITATIONS OF THE STUDY

Due the retrospective nature of the study, aggregated data that was documented in patients' records were relied upon. Not all records were adequately completed and thus information was limited in some circumstances. The study was carried out at Chris Hani Baragwanath Academic Hospital, Clinical Haematology unit, Department of Medicine, and thus a selection bias may be present. Patients also may have been treated by general physicians and thus not referred to the Clinical Haematology unit, resulting in an underestimation of the number of possible patients with MPN. Non-compliance as well as patients lost to follow up is to be expected at a large institution such as Chris Hani Baragwanath Academic Hospital. Even though these limitations were present, the current study provides a fairly accurate representation and overview of the Ph1 negative MPN as seen at our public sector hospital over a 25 year period.

CHAPTER 5: REFERENCES

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APPENDIX A: DATA SHEET

STUDY NUMBER:

AGE:
GENDER:
DATE OF INITIAL PRESENTATION:

CLINICAL PRESENTATION

HISTORY

	NO	YES	Details (if yes)
SYMPTOMS: PRURITUS:			
WEIGHT LOSS:			
BONE PAIN:			
CHEST PAIN:			
FEVER:			
FATIGUE:			
NIGHT SWEATS:			
ERYTHROMELALGIA:			
BLEEDING:			
OTHER:			

DRUG HISTORY: .....

FAMILY HISTORY: .....

CLINICAL EXAMINATION

	NO	YES	Details (if yes)
PALLOR:			
LYMPHADENOPATHY:			
JAUNDICE:			
PETECHIAE:			
PURPURA:			
ECCHYMOSIS:			
HEPATOMEGALY:			
SPLENOMEGALY:			
FUNDAL CHANGES:			

NEUROLOGICAL FALLOUT:

TIA	CVA	OTHER	DETAILS

ANY NEW SIGNS OR SYMPTOMS DURING COURSE OF DISEASE:
.....

DIAGNOSIS:

	N0	YES
ET:		
PV:		
PMF:		

TREATMENT:

	NO	YES	DETAILS
FOLATE:			
IRON:			
ALLOPURINOL:			
ANTICOAGULANTS:			
ASPIRIN:			
PHLEBOTOMY:			
BLOOD TRANSFUSION:			
OTHER:			

PLATELET TRANSFUSION:

NO	YES	DURATION (IF YES)	NO. OF UNITS (IF YES)	FREQUENCY (IF YES)	INDICATIONS (IF YES)

SPECIFIC TREATMENT INCLUDING DATE OF INITIATION AND TERMINATION:

	NO	YES	INITIATION	TERMINATION
HYDROXYUREA:				
ANAGRELIDE:				
32P:				
BUSULPHAN:				
CORTICOSTERIODS:				
DANAZOL:				
INTERFERON ALPHA:				
OTHER:				

SPLENECTOMY:

NO	YES	DATE (IF YES)	INDICATION (IF YES)

TIME TO PARTIAL RESPONSE FROM INITIAL PRESENTATION DATE: .....

TIME TO COMPLETE RESPONSE FROM INITIAL PRESENTATION DATE: .....

RELAPSE:

NO	YES	DETAILS

TRANSFORMATION:

	NO	YES	DATE (IF YES)	OUTCOME (IF YES)	TREATMENT (IF YES)
TO ET:					
TO PV:					
TO PMF:					
TO AML:					

CURRENT STATUS:

	NO	YES	DETAILS
COMPLETE RESPONSE:			
PARTIAL RESPONSE OFF TREATMENT:			
PARTIAL RESPONSE ON TREATMENT:			

DEATH:

DATE OF DEATH: .....

CAUSE OF DEATH: .....

SURVIVAL (DATE OF HISTOLOGICAL DIAGNOSIS UNTIL DATE OF DEATH): .....

LOSS TO FOLLOW UP:

LAST DATE PATIENT SEEN: .....

STATUS OF PATIENT AT THAT DATE: .....

OTHER:

APPENDIX B: FLOW CHART OF INVESTIGATIONS

DATE										
WCC										
RCC										
HCT										
HB										
MCV										
PLATELETS										
NEUTROPHILS										
LYMPHOCYTES										
EOSINOPHILS										
BASOPHILS										
MONOCYTES										
BLASTS										
SMEAR COMMENTS										
NA										
K										
UREA										
CREATININE										
JAK2 V617F										
SERUM FE										
% SATS.										
TRANSF.										
FERRITIN										
MAGNESIUM										
PHOSPHATE										
CORR. CALCIUM										
HIV STATUS										
CD4 COUNT (IF +)										
VIRAL LOAD (IF +)										
RCF										
VIT B12										
URIC ACID										
PaO2										
O2 SATURATION										
EPO LEVEL										
TOTAL BILI.										

DIRECT BILI.										
TOTAL PROT.										
ALBUMIN										
ALP										
GGT										
ALT										
AST										

BONE MARROW ASPIRATE AND TREPHINE:

CELLULARITY: .....
ABNORMAL CELLS: .....
FOREIGN INFILTRATE: .....
MEGAKARYOPOIESIS: .....
ERYTHROPOIESIS: .....
GRANULOPOIESIS: .....
FIBROSIS: .....

CHEST X-RAY: .....
ABDOMINAL ULTRA SOUND: .....
OTHER IMAGING: .....

APPENDIX C: ETHICS APPROVAL LETTER

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Yusuf Mayet

CLEARANCE CERTIFICATE	**M121175**
PROJECT	Phildelphia Chromosome Negative Myelo-Proliferative Neoplasms at Chris Hani Baragwanath Hospital
INVESTIGATORS	Dr Yusuf Mayet.
DEPARTMENT	Department Internal Medicine
DATE CONSIDERED	30/11/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 30/11/2012 **CHAIRPERSON** ..... (Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof M Patel

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX D: TURNITIN LETTER FROM SUPERVISOR

Division of Haematology, Department of Medicine, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg

Chris Hani Road, Diepkloof, Soweto. Tel: +27 11 9339377, Fax: +27 11 9339449, email: moosa.patel@wits.ac.za

23 October 2015

The Chair

Postgraduate Studies Committee

Faculty of Health Sciences

University of the Witwatersrand

Re: Turn-it-in report: Dr Yusuf Mayet – MMed: 'Philadelphia Chromosome Negative Myeloproliferative Neoplasms at Chris Hani Baragwanath Academic Hospital'

I have reviewed the Turn-it-in report of Dr Mayet's MMed dissertation. The report identifies a similarity index of 8%. Much of this similarity relates to definitions which are standardized. The other information which bears a similarity has been appropriately referenced.

Thank you

Yours sincerely

Moosa Patel MBChB, FCP(SA), MMed(Wits), FRCP(Lond.), PhD(Wits)

Supervisor of the above study

Professor and Head of Clinical Haematology, Department of Medicine, Chris Hani Baragwanath Academic Hospital and the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa