

**IMPLEMENTATION OF THE JAK2V617F MUTATION ANALYSIS IN  
THE PATHWAY OF SUSPECTED MYELOPROLIFERATIVE  
NEOPLASMS IN GROOTE SCHUUR HOSPITAL**

---

**DR ERMA POULET**

Student number: PLTERM001

---

**SUBMITTED TO THE UNIVERSITY OF CAPE TOWN**

In partial fulfillment of the requirements for the degree of  
MMed (Master of Internal Medicine) Faculty of Health Sciences

---

**DATE OF SUBMISSION**

5 September 2016

---

**SUPERVISOR**

Dr Estelle Verburgh

Senior Consultant Haematology  
Department Internal Medicine  
Groote Schuur Hospital

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.



DEDICATION

To all those who have so believed in me.

*"Like a wild flower; she spent her days, allowing herself to grow, not many knew of her struggle, but eventually all; knew of her light."*

Nikki Rowe

## **TABLE OF CONTENTS**

**Acknowledgments**

**Declaration of Originality**

**Thesis Abstract**

### **Structured Literature Review**

Introduction

Discussion

Conclusion

References

### **Journal Ready Manuscript**

Introduction

Methods

Results

Discussion

Conclusion

Abbreviations

Author contributions

Disclosures

References

### **Appendices and Supporting Material**

Plagiarism Report

Ethics Approval

Data collection Sheet

## **ACKNOWLEDGMENTS**

I am eternally grateful to the following for their contributions to this dissertation:

The Groote Schuur Hospital Records department, especially Mrs. Imelda Booysen for obtaining the large number of folders I had requested.

The Haematology department clerks for their friendly assistance in obtaining records from within the departmental.

Dr Ania Köller and the Haem Pathology laboratory registrars who helped me down in the Fishbowl with the laboratory data collection.

Dr Katherine Antel and Mrs. Katherine Manning for their help with the statistical analysis.

Ms. Karen Shires in the molecular laboratory for her constructive critical analysis of my first protocol, and her kind intent.

Prof Peter Raubenheimer for an open-door policy when we experienced an obstruction.

Dr Estelle Verburgh for her mentorship, guidance, encouragement and shoulder to cry on during this project.

And finally, to John Gardner, my love, my partner and my stronghold throughout this process of intense growth.

## DECLARATION OF ORIGINALITY

This research report is my own original work and no part of it has ever been published in any format. The work is solely for the purpose of this degree and is to be submitted to the University of Cape Town only.

The results of this study will be discussed in the format of a publication submitted to a journal of our choice.

SIGNED

Signed by candidate

ON 5 SEPTEMBER 2016

Signature removed

**CHAPTER ONE**

Structured Literature review



# STRUCTURED LITERATURE REVIEW

## **1. Introduction**

## **2. The history of the Myeloproliferative Neoplasm**

1. Bird's eye view on developing guidelines for MPN
2. The Revelation of the JAK2 V617F mutation
3. JAK2V 617F mutation molecular mechanism
4. Brief summary of developments prompted by JAK2 V617F
5. Other mutations diagnostic in MPN

## **3. JAK2 V617F in Haematological Disorders other than Philadelphia negative MPN**

## **4. Exploring the groups within MPN**

1. Polycythaemia Vera (PRV)
  1. Hb/Hct debate
  2. WHO criteria
  3. WHO algorithm
  4. Serum Erythropoietin
  5. Red Cell Mass
  6. Bone Marrow Biopsy
  7. Endogenous Erythroid Colonies
  8. Utility of Venesection
  9. A Perplexing Entity: "Idiopathic Erythrocytosis"
2. Essential Thrombocytosis (ET)
  1. WHO criteria
  2. WHO algorithm
  3. Complications of ET
3. Primary Myelofibrosis (PMF)
  1. WHO criteria
  2. WHO algorithm

## **5. Spectrum of MPN - Overlap between PRV/ET/PMF**

## **6. JAK2 V617F mutation in the presence of thrombosis**

1. The holy grail – clutching at straws for a cause?
2. Splanchnic thrombosis
3. Other sites of thrombosis

## **7. Conclusion**

## Abbreviations

**aCML** for atypical chronic myeloid leukaemia;

**BMB** for Bone marrow biopsy;

**BCS** for Budd-Chiari Syndrome;

**BRVO** for Branch retinal vein occlusion;

**CALR** for calreticulin mutation;

**CMML** for chronic myelomonocytic leukaemia;

**CVRO** for central retinal vein occlusion;

**CVT** for cerebral vein thrombosis;

**DVT** for deep vein thrombosis;

**EEC** for endogenous erythroid colony;

**EPO** for erythropoietin;

**ET** for essential thrombocytosis;

**Hb** for hemoglobin;

**Hct** for hematocrit;

**IE** for idiopathic erythrocytosis;

**IMF** for idiopathic myelofibrosis;

**IVC** for inferior vena cava;

**JAK2** for JAK2V617F

**MDS/MPN** for myelodysplastic/myeloproliferative neoplasm;

**MPL** for myeloproliferative leukaemia virus oncogene;

**MPN** for myeloproliferative neoplasm;

**MMM** for myelofibrosis with myeloid metaplasia;

**PE** for pulmonary embolism;

**PMF** for primary myelofibrosis or primary chronic myelofibrosis;

**PRV** for polycythaemia Vera;

**RARS-T** for Refractory Anaemia with Ringed Sideroblasts associated with Thrombocytosis;

**RCM** for red cell mass;

**RVT** for retinal vein thrombosis;

**RVO** for retinal vein occlusion;

**VTE** for venous thromboembolism;

## List of Figures:

- Figure 1:** Symptoms of PRV
- Figure 2:** 2008 WHO PRV criteria
- Figure 3:** 2008 WHO PRV algorithm
- Figure 4:** Causes of an Erythrocytosis
- Figure 5:** 2008 WHO criteria for ET
- Figure 6:** Causes of a Thrombocytosis
- Figure 7:** 2008 WHO algorithm for ET
- Figure 8:** Complications of Essential Thrombocytosis
- Figure 9:** Causes of Bone Marrow Fibrosis
- Figure 10:** Complications of PMF
- Figure 11:** 2008 WHO criteria for PMF
- Figure 12:** 2008 WHO algorithm for PMF
- Figure 13:** Overlap between MPN subgroups
- Figure 14:** Risk factors for Thromboembolism
- Figure 15:** Risk factors for Upper Limb fibrosis

## 1. Introduction

The myeloproliferative neoplasms (MPN), previously known as myeloproliferative disorders (MPD), are a group of clonal hematopoietic stem cell malignancies in which there is an increased proliferation or accumulation of one or more subtypes of myeloid cells instead of the destruction of normal haematopoiesis as usually associated with malignancy (de Lacerda, *et al* 2014).

Excluding the acute myeloid malignancies, the chronic group is divided into the Philadelphia chromosome (BCR/ABL-1 gene) positive chronic myeloid leukemia (CML) and negative groups. The latter comprises Polycythaemia Vera (PRV), characterized by red cell proliferation, essential thrombocythemia (ET) with marked thrombocytosis, and primary myelofibrosis (PMF), where bone marrow reticulin and collagen deposition is dominant (de Lacerda, *et al* 2014). The suggestion has been made to view PRV, ET, and PMF not as separate diseases, but as different manifestations of the same disease, or a combination of both (Spivak and Silver 2008).

In this review of the literature, we will concentrate on the pivotal discovery of the JAK2 V617F mutation on exon 14 of chromosome 9p in 2005, in illustration of the revolution it brought about. Its discovery not only provided new insights into the molecular basis of the MPD/MPN but also new molecular approaches to their diagnosis (Spivak and Silver 2008).

## 2. The history of the Myeloproliferative Neoplasm

### 2.1. Bird's eye view on guideline development in MPN

Endeavours at diagnostic criteria for PRV, ET and PMF stem from as early as the 1940's (Michiels, *et al* 2015) with Dameshek as the first to propose speculative theories involving an unknown factor either stimulating bone marrow excessively, or the lack of inhibition of a mysterious factor (Dameshek 1950).

On the basis of CML, following the discovery of the disease-specific Philadelphia chromosome in 1982, the origin of ET/PRV/PMF was also expected to be involving constitutively active tyrosine kinase activity, (James, *et al* 2005b) but until 2005, this was yet to be proven.

The first diagnostic criteria for MPN was brought out in 1967. Debate persisted furiously between the experts over the years, but without consensus groups practiced according to their own convictions. Guideline after guideline was being published, because physicians continued to be confounded by the MPN's.

### 2.2. The Revelation of the JAK2 V617F mutation

In 2005, the identification of the JAK2 mutation and its significance to change myeloproliferative disorders to myeloproliferative neoplasms, was broadcasted to the medical world in no less than four publications by four independent research groups across the globe over a period of 2 months.

Baxter, *et al* (2005a), described the single point mutation (Val617Phe) in JAK2, in a population of known MPN patients. Short on their heels, followed James, *et al* (2005a) in their description of JAK2V617F in PRV patients. Levine, *et al* (2005b) and a Swiss group, Kralovics, *et al* (2005) published within the next month with similar methods and findings.

What the JAK2 mechanism unveiled is the common stem-cell clonal heritage of the MPNs, and that its diverse phenotypes were all the result of abnormal signal transduction from a series of mutations affecting the tyrosine kinases (Tefferi and Vardiman 2008b).

### **2.3. JAK2 V617F mutation molecular mechanism**

The acquired mutation within JAK2 exon 14 was identified on codon 617 of the JAK2 JH2 domain. There, valine (V), is replaced by phenylalanine (F). The ensuing JAK2V617F mutation interferes with the auto-inhibitory activity of the JH2 pseudokinase part, leading to enhanced activity and constitutive activation of the tyrosine kinase function. These mutated haematopoietic stem cells become hypersensitive to haematopoietic growth factors resulting in the lineage abnormalities characteristic of MPN (Bench, *et al* 2013, Michiels, *et al* 2015).

The JAK2 kinase activity is furthermore influenced by both the allele burden and various steps up- or downstream of the signalling pathways. (Michiels, *et al* 2015)

In summary, all of the driver mutations lead to JAK2 activation in MPNs, and the development of cytoses are related to an activation of cytokine receptor signalling. This hallmarks MPN, even where no mutation has been identified yet (Kiladjian 2012).

### **2.4. Brief summary of developments prompted by JAK2 V617F**

In the revised 2008 WHO classification for chronic myeloid neoplasms, the phrase “disease” is replaced by “neoplasm” for the first time. With the renewed interest in this group of diseases, terminology for thrombocythaemia and myelofibrosis also became standardised, where before there existed several different names for each.

JAK2 is nowadays considered *conditio sine qua non* for the diagnosis of PV and it simplified diagnosing ET and PMF in at least 50% of cases (Tefferi 2008).

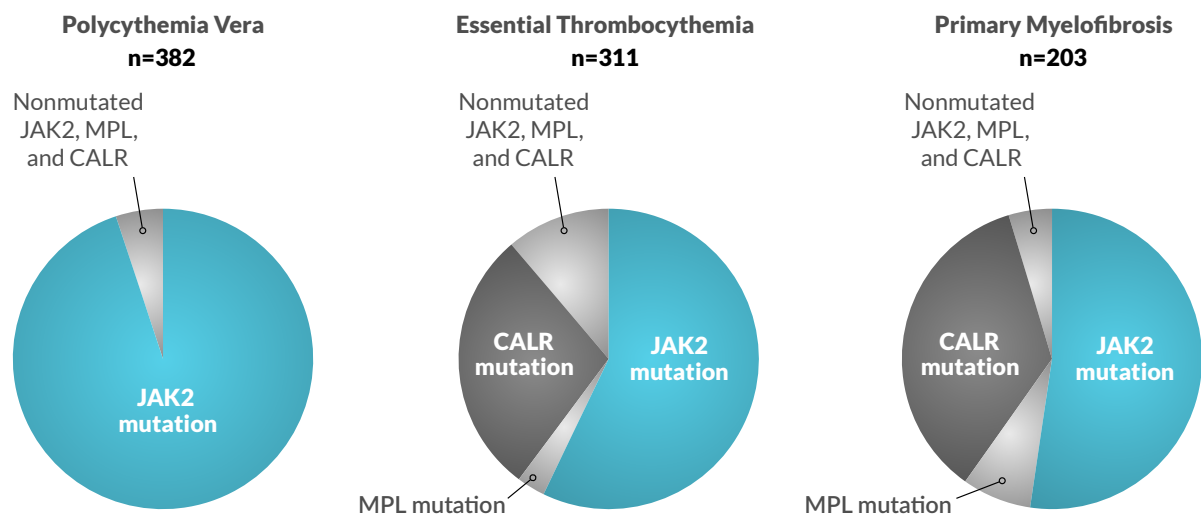
While not the focus of this review, the improved understanding of disease pathogenesis also resulted in new targeted therapies (Stein, *et al* 2011).

### **2.5. Other mutations diagnostic in MPN**

The most important mutations other than JAK2V617F include the JAK2 exon 12, MPL exon 10 and the most recently identified CALR mutation. The JAK-STAT pathway appears to be activated in all MPN, regardless of founding driver mutations. Increasingly, however, the difference is manifested with effects on the clinical course and outcomes (63). Even though they are not available in our setting at present, they may become relevant in the future as they are implemented in standard diagnostic algorithms.

A table borrowed from an NEJM publication(63), illustrates the distribution of these mutations across the spectrum of MPN eloquently:

Distribution of JAK2, MPL, and CALR Mutations in Philadelphia Chromosome-Negative Myeloproliferative Neoplasms  
FIGURE 0.



REFERENCE

Klampf T, Gisslinger H etc. Somatic mutations of calreticulin in myeloproliferative neoplasms. NEJM 2013; 369 (25): 2379-90

### 2.5.1. JAK2 V617F exon 12

In the small group of JAK2 exon 14 negative PV patients, 3% of patients do demonstrate the exon 12 mutation. This finding confirms the strong association between the JAK2 mutations and MPN. Patients with this mutation usually present with early-stage PV with a good outcome and normal life expectancy according to Michiels (3), but Tefferi holds their outcome to be prognostically similar to the exon 14 patients (36). Additional observations by the latter include presentation with predominantly erythroid myelopoiesis, subnormal EPO and a younger age at diagnosis. Many of these JAK2V617F exon 14 negative cases were up to identification of the exon 12 mutation regarded under the umbrella of “idiopathic erythrocytosis”. (64)

### 2.5.2. MPL

MPL was discovered prior to the JAK2V617F mutation, in 2004, based on a case of congenital ET. Henceforth, it was noted to exist in ET and MF but rarely in PV (3). It occurs in around 4% of ET patients and 8% of PMF patients (36).

The characteristics of patients with both JAK2 and MPL mutations are different from those with only the JAK2 in that there are no features of prodromal PV at diagnosis, they do not evolve into overt PV during follow-up, don't have deranged EPO and ferritin levels and have different bone marrow characteristics(65).

### 2.5.3. CALR

This mutation was only discovered in 2013. In frequency, it is the second most common JAK-STAT activating mutation after the JAK2V617F (63). CALR mutations are absent in PV, occur in up to 35% of PMF and 24% of ET patients (36) but are mutually exclusive with both JAK2 and MPL mutations.

The clinical significance of this mutation is that in both ET and PMF, patients with the CALR as opposed to JAK2 or MPL mutations, had longer overall survival, and a lower risk of death. CALR mutations are present in up to 73% of patients who

don't have JAK2 or MPL mutations and it is believed by some authors(63) to fill the current molecular diagnostic gap in MPN.

### 3. JAK2 V617F in Haematological Disorders other than Philadelphia negative MPN

Although the JAK2 mutation provides clear evidence for malignant, clonal haematopoiesis, it is non-specific to the Philadelphia negative MPN, being described in low frequencies in other myeloid, but not in lymphoid malignancies(Kesler, *et al* 2000, Kiladjian 2012, Levine, *et al* 2005a).

In the general population the prevalence was 0.2%(Nielsen, *et al* 2011).

Patients with the MDS/MPN overlap group present with both features of dysplasia and marrow proliferation. Hereby included is CMML, MDS/MPN-unclassifiable and RARS-T and atypical CML (aCML).

These disorders are rare – chronic myelomonocytic leukaemia (CMML) incidence is 0.3 per 100 000 in the US annually(Rollison, *et al* 2008). Only 8% of them in turn contain a JAK2 mutation(Kiladjian 2012). In RARS-T the finding of JAK2 (60%) and MPL mutations (10%) have been cause for debate whether it truly is a separate entity or a not simply a special form of ET due to the similarity of their phenotype(Gerds 2014, Malcovati, *et al* 2009). In CML, extraordinary case reports only exist where BCR-ABL and JAK2 occur concomitantly. It is also exceedingly rare in aCML(Wang, *et al* 2015, Zhou, *et al* 2015).

In AML it seems the JAK2 is most common in patients who transform from a pre-existing MPN although *de novo* events have been described(Levine, *et al* 2005a).

Defining patients on the basis of a shared JAK2 mutation seems impossible, with the mutation only being a piece of the puzzle(Kiladjian 2012).

## 4. Exploring the three major groups within MPN

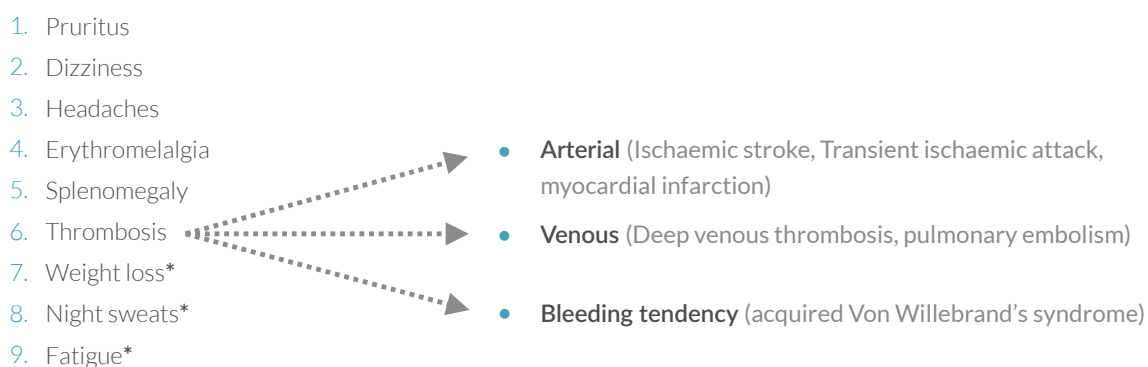
### 4.1. Polycythaemia Vera (PRV)

PRV is distinguished from other causes of erythrocytosis by its clonal mechanism. Up to 95% of PRV is JAK2 V617F positive, but another 4% of PRV cases have their mutation within exon 12 (Michiels, *et al* 2015, Passamonti 2012, Stein, *et al* 2014).

PRV patients have a reduced life expectancy compared to the normal population, mainly from cardiovascular complications or disease transformation(Stein, *et al* 2014). Two-thirds present from 50 years onwards(Passamonti 2012, Passamonti, *et al* 2003, Vannucchi 2014b). They are frequently symptomatic with a wide range of problems, as evidenced in figure 1 (de Lacerda, *et al* 2014, Passamonti 2012, Spivak 2003).

## Symptoms of PRV

FIGURE 1.



\*Once classical constitutional symptomatology like night sweats or weight loss appear, the concern of transformation develops

### REFERENCE

- De Lacerda JF, Oliveira SN, Ferro JM. Chronic myeloproliferative diseases. *Handb Clin Neurol.* 2014;120:1073-81  
Passamonti F. How I treat polycythemia vera. *Blood.* 2012;120(2):275-84  
Spivak JL. Diagnosis of the myeloproliferative disorders: resolving phenotypic mimicry. *Semin Hematol.* 2003;40(1 Suppl 1):1-5.

PRV is diagnosed according to the WHO criteria of 2008 (Passamonti 2012, Tefferi and Vardiman 2008b). An FBC is a relevant starting point and panmyelosis is more predictive of PRV than an isolated erythrocytosis (Passamonti 2012). An associated splenomegaly, usually not massive, associated with a panmyelosis also tips the scale more in favour of PRV. The patient's presentation, and clinical information collected at the time will influence the diagnostic approach to erythrocytosis (Vannucchi 2014b).

#### 4.1.1. Haemoglobin(Hb) and haematocrit(Hct) Debate

Hb, Hct and red cell mass (RCM) all act as surrogate markers of an elevated red cell mass in erythrocytosis (Johansson, *et al* 2005). A fierce debate still rages regarding which of the 3 red cell parameters should be used as a diagnostic hallmark of PRV.

The 2008 WHO criteria included Hb instead of Hct, and left a backdoor open with Hct more than 99<sup>th</sup> percentile acknowledged as equivalent (Tefferi and Vardiman 2008b). However, directly opposing opinions are reflected in the literature (Alvarez-Larran, *et al* 2012, Jacob, *et al* 2012, Johansson, *et al* 2005).

The only consensus currently exists where values are conspicuously elevated. The BCSH guidelines states that males presenting with sustained Hct values >60% (Hb >18.5g/dL) or female with Hct >56% (Hb >16.5g/dL) could be assumed to have an absolute erythrocytosis and therefore do not require confirmatory studies (Barbui, *et al* 2014c, McMullin, *et al* 2005, Vardiman, *et al* 2009).

4.1.2. **The 2008 WHO criteria** (Vardiman, *et al* 2009) is the current standard of diagnosis, (Passamonti 2012, Tefferi and Barbui 2015, Vannucchi 2014b) shortly to be revised (Barbui, *et al* 2014c, Silver, *et al* 2013).

## 2008 WHO PRV criteria

FIGURE 2.

### Polycythemia vera **major** criteria

- Hemoglobin >18.5 g/dL (men) >16.5 g/dL (women)\*
- Presence of JAK2 V617F or similar mutation

### Polycythemia vera **minor** criteria

- Bone marrow trilineage myeloproliferation
- Subnormal serum erythropoietin level
- Endogenous erythroid colony growth

\* Hemoglobin or hematocrit values above the 99th percentile of the reference range for age, sex, or altitude of residence; or red cell mass >22% above mean normal predicted or hemoglobin >17g/dL (men), or >15 g/dL (woman) if associated with sustained increase of  $\geq 2$ g/dL from baseline that cannot be attributed to correction of iron deficiency.

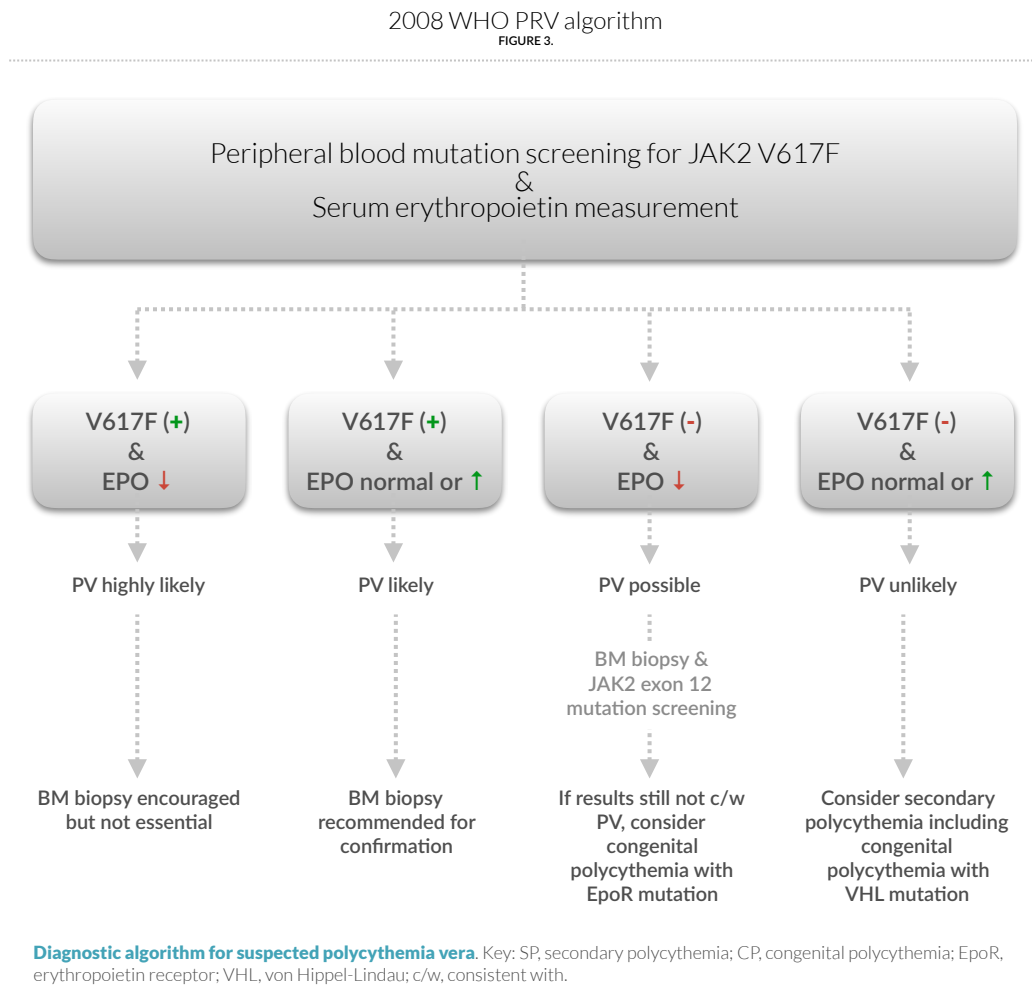
**Diagnosis of polycythemia vera requires meeting both major criteria and one minor criterion or the first major criterion and two minor criteria**

#### REFERENCE

Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114(5):937-51.

The JAK2 mutations simplified the diagnosis, necessitating investigation only in the mutation negative group (McMullin, *et al* 2007). Some authors still advocate ruling out acquired erythrocytosis prior to JAK2 when faced with an isolated erythrocytosis, based on the clinical history obtained (Passamonti 2012, Vannucchi 2014b), including abdominal imaging, lung functions, chest X-ray, polysomnography depending on factors like heart/lung co-morbidities, obesity, smoking, and suspicion of hepatic/renal tumours.

4.1.3. The 2008 WHO algorithm for PV (Tefferi and Vardiman 2008b) starts with JAK2 & s-EPO measurement:



REFERENCE

Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia* (08876924). 2008;22(1):14-22

#### 4.1.4. Serum erythropoietin levels (sEPO)

Erythropoietin is the principal hormone regulating erythropoiesis, secreted by the kidney in adults. The major stimulus for EPO is low O<sub>2</sub> saturation due to anaemia, or hypoxemia (Messinezy M 2002).

sEPO has been controversial because of conflicting results in the literature about the reliability and specificity of a low sEPO level. The big issues were a lack of standardisation of the test, and heterogeneity in methods by major studies, hindering comparison (Mossuz P 2004). On its own, it could be confusing because a low sEPO is suggestive, but not specific of PRV. A normal sEPO does not exclude PRV (Silver, *et al* 2013, Spivak and Silver 2008).

Most experts in the field today include sEPO in their erythrocytosis algorithms as a useful adjunct, done concomitantly with the JAK2. A low sEPO strongly eludes to an autonomous mechanism, and is expected where the JAK2 mutation is detected. Even in the absence of the JAK2, a low sEPO would be a motivating factor for requesting further genotyping in the form of exon 12 testing. However, with a negative JAK2 and a normal/increased sEPO, secondary causes would have to be ruled out first (Vannucchi 2014b).

#### **4.1.5. Red cell mass (RCM)**

Erythrocytosis can be either true or apparent. Mechanisms for true erythrocytosis includes primary (autonomous) or secondary factors. Using the nuclear medicine RCM and plasma volume determination helps differentiate between absolute and apparent erythrocytosis(Lorberboym, *et al* 2005).

The hallmark of true polycythaemia or erythrocytosis is an increased proportion of red blood cells(RBC) in the peripheral blood in relation to plasma volume. In apparent polycythaemia a lowered plasma volume with an increased or normal RCM skews the ratio. Surrogates of red cell volume, including Hb, Hct and red cell count may not always reflect the total RCM because of variations in plasma volume.

RCM limitations are well recognized, but it's biggest drawback is that not all centres have access to this valuable resource. Nevertheless, where available, it remains widely employed and is still accepted as the gold standard for determination of the red cell mass. Furthermore, as a test it is also cumbersome, and lacks precision in obese individuals, in states of dehydration and even in patients on commonly prescribed medication like diuretics (Barbui, *et al* 2014c, Spivak and Silver 2008).

Marked disagreements regarding the utility of RCM existed before the JAK2 discovery and continues to divide the haematological community. Supporters of RCM are adamant that it is to remain in the guidelines, provided the prescribed method is used and the indication is to detect suspected early cases. Opponents of the nuclear medicine RCM/plasma volume test, argue that its limitations exceed its benefits and that other tests incl. bone marrow histology, sEPO and biologic markers have made it redundant(Tefferi 2005).

#### **4.1.6. Bone marrow biopsy (BMB)**

BMB is not compulsory for the diagnosis of PRV.

The rationale for BMB in the investigation of erythrocytosis is dependent on the clinical scenario(Vannucchi 2014a). It is most useful in cases where the diagnosis is in doubt(Barbui, *et al* 2014c).

#### **4.1.7. Endogenous erythroid colonies (EEC)**

Mention has to be made of this investigative modality, however it was already in 2008 declared as "a research tool of historic interest"(Spivak and Silver 2008), only available in research laboratories(McMullin, *et al* 2005).

#### **4.1.8. Utility of phlebotomy (venesection)**

A procedure in use since antiquity, venesection has both therapeutic and preventative roles. Lowering the haematocrit reduces blood viscosity and may be useful in all forms of erythrocytosis, though sparse evidence exists. Apart from its role in erythrocytosis/PRV, it is also used in haemochromatosis and porphyria cutanea tarda(Cook 2010, Randi, *et al* 2015).

In PRV, phlebotomy is accepted as the mainstay of treatment with cytoreductive therapy as a supportive therapy. But who else should be venesected?

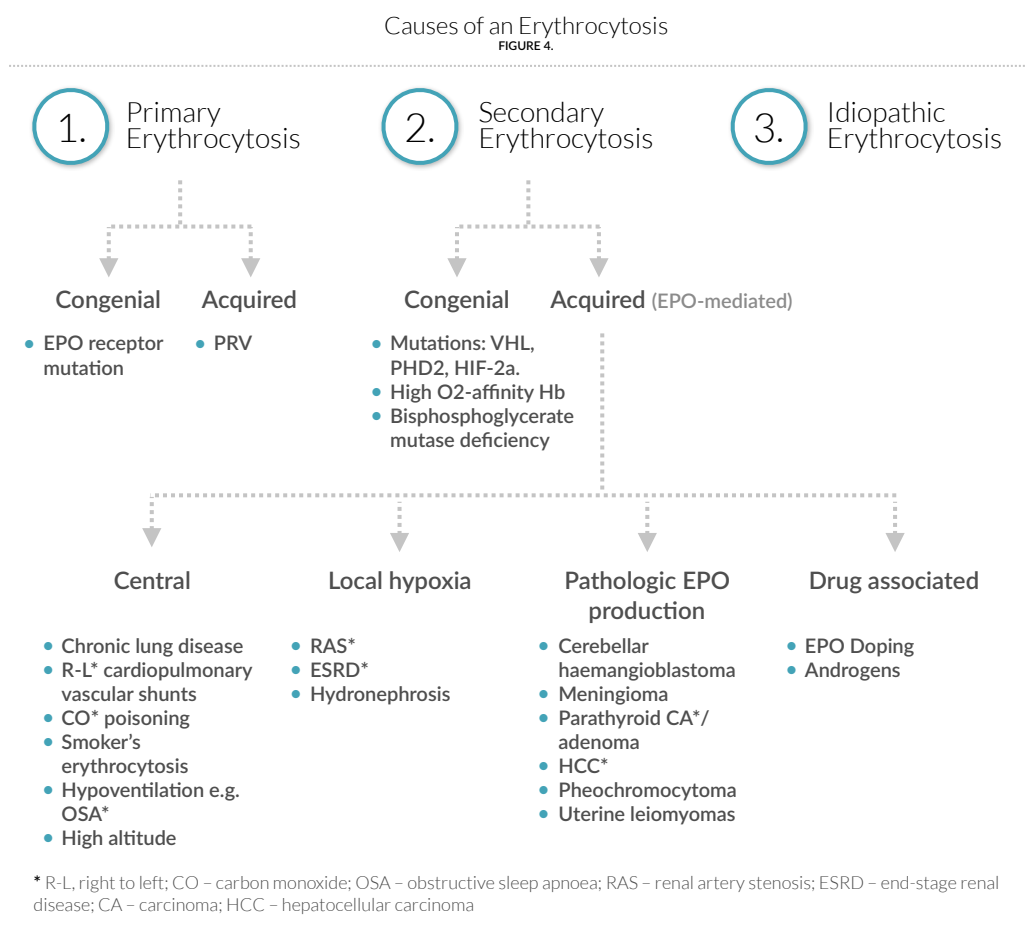
According to Assi *et al*(Assi and Baz 2014), relative polycythaemias incl. Gaisbock's syndrome, patients with tumours, living at altitude, Cushing's syndrome, on exogenous steroid replacement (iatrogenic or illegal!) and smokers are not eligible. Unfortunately, no sources for these statements are supplied.

Eligible patients according to this article are in hypoxic heart or lung conditions, including chronic obstructive pulmonary disease. The latter is, however, mostly related to smoking and smokers were included on the list of ineligible. No reasons are also given as to why one should be eligible or not. Apart from these disparities, renal transplant patients with erythrocytosis and poorly controllable blood pressures, as well patients with cyanotic heart disease are listed as eligible. Unfortunately, due to the inconsistencies mentioned, this article is not completely sound.

Other articles focus more on the use of phlebotomy in proven PV, but there is a paucity of information for those with secondary causes of erythrocytosis on the indications, benefits or disadvantages of phlebotomy.

#### 4.1.9. A Perplexing Entity: “Idiopathic Erythrocytosis”

Idiopathic erythrocytosis is an absolute erythrocytosis with no known cause, diagnosed by exclusion of primary and secondary erythrocytosis. Absolute erythrocytosis is defined as an increase in the RCM >125% of the predicted value for the body mass of the patient, or surrogate markers i.e. Hb and Hct with values as mentioned.



**REFERENCE**

Adapted from: McMullin MF. Idiopathic erythrocytosis: a disappearing entity. Hematology Am Soc Hematol Educ Program. 2009;629-35

Once the myriad known causes have been excluded, a large group of patients remain in whom no mechanism for the erythrocytosis has been identified, labelled idiopathic erythrocytosis (IE). Prior to the discovery of the JAK2 clone, many PRV patients were classified in this group. Ironically it still is the most common type of erythrocytosis in clinical practice, but

has been poorly studied in the literature compared to PRV and prominent authors such as Tefferi cautions against applying the term too eagerly (Finazzi, *et al* 2006, McMullin 2009, Pearson and Messinezy 2001).

It is thought that these patients could conceivably harbour an exceedingly rare form of congenital erythrocytosis or JAK2-wild type PRV form, which is beyond the scope of this article (Randi, *et al* 2015).

Clinical characteristics of this group is discordant and inconsistent. One third has low-, two-thirds normal EPO values. Management is on an individual basis, taking into account other cardiovascular and background risk factors for thrombosis. Phlebotomy may/may not be of value. An arbitrary cut-off of Hct 54% exist beyond which phlebotomy is considered, aiming for values between 45-50%, depending on practicalities. Cytoreductive treatment is unanimously rejected in those without evidence of a malignant clone (McMullin 2009).

## 4.2. Essential Thrombocytosis (ET)

The presence of a chronic state of thrombocytosis in which reactive causes have definitively been excluded, as well as the inability to ascribe the thrombocytosis to one of the other MPN subgroups (PMF, PRV) is what defines ET (Harrison and Green 2003). The WHO defines the cut-off as  $>450 \times 10^9/L$  with extreme thrombocytosis as platelets of  $>1000 \times 10^9$  (Bleeker and Hogan 2011).

4.2.1. The 2008 WHO criteria (Vardiman, *et al* 2009) for the diagnosis of ET, as follows:

2008 WHO criteria for ET  
FIGURE 5.

### Major criteria

1. Platelet count  $\geq 450 \times 10^9/L$
2. Megakaryocyte proliferation with large and mature morphology
3. Not meeting WHO criteria for PRV, PMF, CML, MDS or other myeloid neoplasm
4. Presence of JAK2 V617F or other clonal marker and no evidence of reactive thrombocytosis

Diagnosis of ET requires meeting all 4 major criteria = 1. + 2. + 3. + 4.

#### REFERENCE

Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, *et al*. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114(5):937-51.

Patients who present with an increased platelet count are a heterogeneous group, with multiple conditions which could manifest this way. Harrison *et al* stated the position clinicians and researchers alike were faced with, eloquently: "The major

difficulty in making a diagnosis in ET is the lack of a definitive position criterion analogous to the presence of BCR/ABL transcripts in CML(Harrison and Green 2003).

The first step is therefore to carefully evaluate and rule out the causes of a reactive thrombocytosis as well as to ascertain the persistence (chronicity) of the thrombocytosis. Comorbidities, history, other features of the FBC and past platelet counts have to be taken into consideration(Bleeker and Hogan 2011).

Causes can be broadly divided into spurious, reactive or clonal, with reactive by far the most common cause(Beer, *et al* 2011, Bleeker and Hogan 2011). Reactive and clonal conditions can co-exist, especially in persistent thrombocytosis(Bleeker and Hogan 2011). Measuring circulating acute phase reactants is not always reliable as they may have returned to normal by the time the thrombocytosis is detected(Folman, *et al* 2001). Iron-deficiency anaemia need to be ruled out, with s-ferritin and transferrin to be demonstrated normal before moving on to other causes. The following table illustrate the potential causes of reactive thrombocytosis:

Causes of a Thrombocytosis  
FIGURE 6.

Myeloid malignancy	Reactive (secondary) thrombocytosis	Familial thrombocytosis	Spurious thrombocytosis
<ul style="list-style-type: none"> <li>• ET</li> <li>• PRV</li> <li>• PMF</li> <li>• CML</li> <li>• RARS-T</li> <li>• MDS with isolated del(5q)</li> </ul>	<ul style="list-style-type: none"> <li>• Blood loss or iron deficiency</li> <li>• Infection or inflammation</li> <li>• Disseminated malignancy</li> <li>• Drug effect*</li> <li>• Hyposplenism or congenital asplenia</li> <li>• Hemolytic anaemia</li> </ul>	<ul style="list-style-type: none"> <li>• Mutations in MPL, TPO, or unknown genes</li> </ul>	<ul style="list-style-type: none"> <li>• Cryoglobulinaemia</li> <li>• Cytoplasmic fragmentation**</li> <li>• Red cell fragmentation</li> </ul>

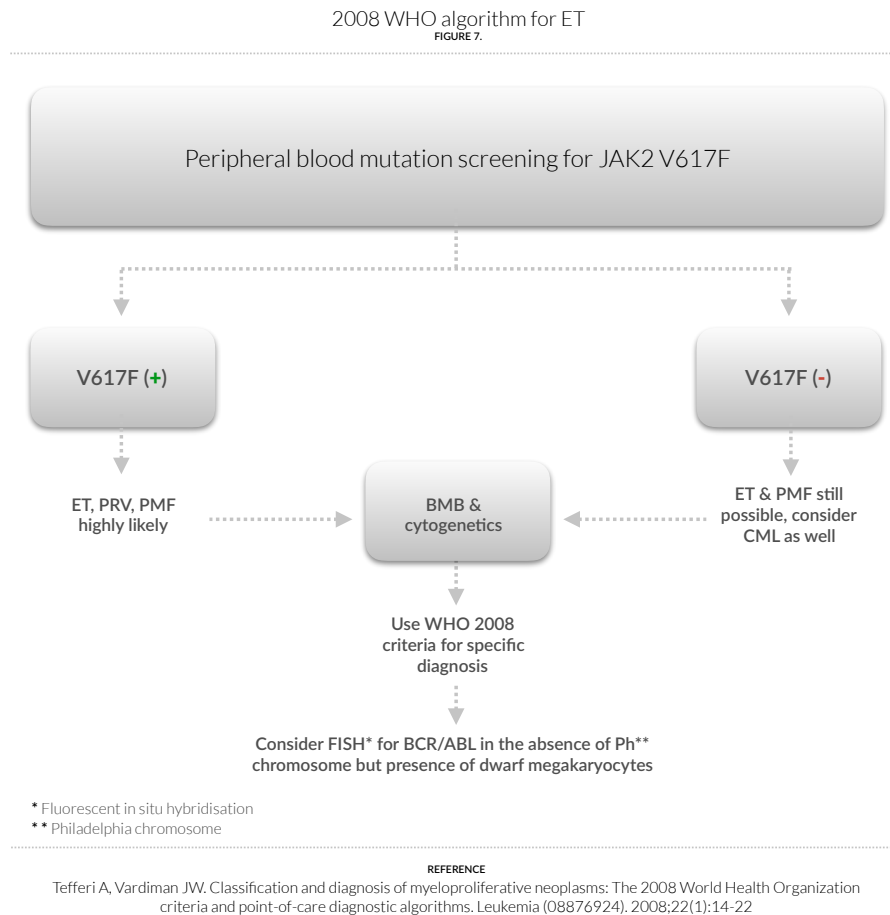
\* e.g. Vincristine, adrenaline, all-trans-retinoic-acid  
\*\* accompanying myeloid/lymphoid neoplasia

REFERENCE

Adapted from: Beer PA, Erber WN, Campbell PJ, Green AR. How I treat essential thrombocythemia. *Blood*. 2011;117(5): 1472-82.

Once reactive or spurious causes have been excluded, the investigator can move on to the next phase. Regretfully, this is where the WHO start their algorithm of investigation, which is a pity, since it skips a number of basic steps, including history and examination.

#### 4.2.2. 2008 WHO algorithm for ET (Tefferi and Vardiman 2008b)



In ET, a BMB is mandatory, even where JAK2 is positive. This provides confirmation of the diagnosis (vs. PMF), prognostic information and serves as a useful baseline if the patient later undergoes transformation (Beer, *et al* 2011).

In the molecular era following the JAK2 discovery pinning it down to a clonal mechanism has been promoted, as JAK2 is prevalent in 55% of ET cases (Tefferi and Barbui 2015). In an additional 8% of cases the MPL mutation (which interestingly was discovered antecedent to the JAK2) also tests positive (Michiels, *et al* 2015).

The majority of ET patients is female with diagnosis around 60 years. The true prevalence of ET is uncertain because patients are shown to have a normal life-expectancy (Fenaux, *et al* 1990).

Presentation is with a wide range of symptoms, from vasomotor-like (dizziness, headache, atypical chest pain, syncope, visual disturbances, paraesthesia and even erythromelalgia as in PRV), but also with thrombosis and bleeding, as well as pregnancy complications (Bleeker and Hogan 2011).

#### 4.2.3. Complications of ET

Compared to reactive thrombocytosis, where the risk of thrombotic complications are proven to be low, in PV & ET thrombosis is a cause of major morbidity and mortality and the main factor in determining treatment approach (Bleeker and Hogan 2011, Elliott and Tefferi 2005, Marchioli, *et al* 2005). Even patients on cytoreductive treatment continue to suffer

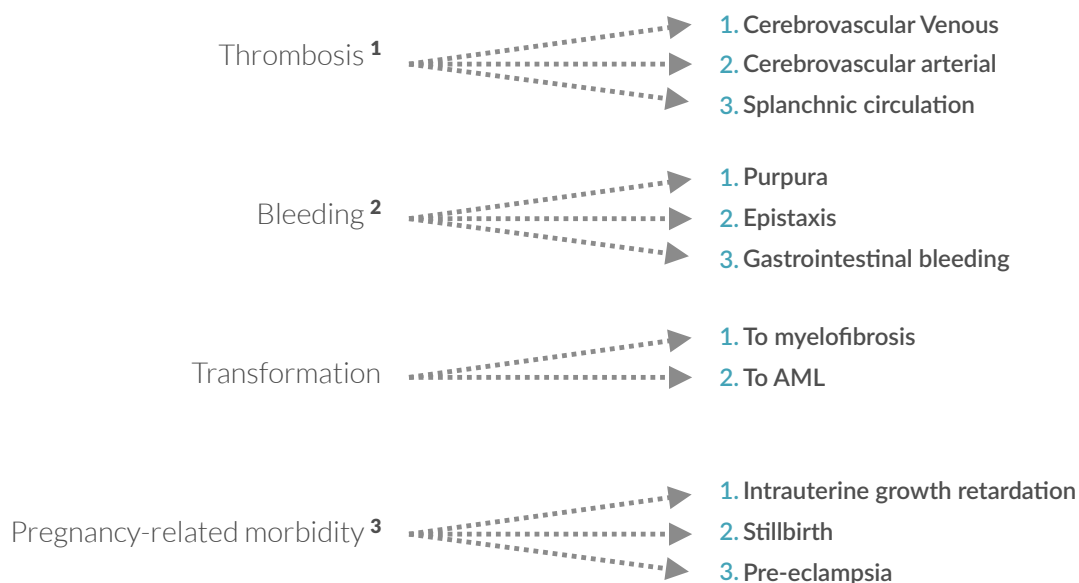
from thrombosis. Age is also a significant risk factor, as well as ET and PRV patients with a concomitant leukocytosis(Bleeker and Hogan 2011).

Other manifestations of ET include bleeding tendencies and transformation. Bleeding has especially been noted in extreme thrombocytosis (Beer, *et al* 2011, Ozer, *et al*).

ET patients also have more pregnancy-related morbidity, with intra-uterine growth retardation, stillbirth and pre-eclampsia in up to 25-50% of pregnancies. JAK2-positive ET patients have an even higher rate(Beer, *et al* 2011, Passamonti, *et al* 2007) than their ET JAK2 negative counterparts.

### Complications of essential thrombosis

FIGURE 8.



<sup>1</sup> Mostly arterial compared to PRV where mostly venous

<sup>2</sup> Mechanism related to either platelet dysfunction or acquired von Willebrand's disease

<sup>3</sup> In 20-25% of pregnancies in ET patients. JAK2 positive patients more affected than JAK2 negatives

#### REFERENCE

Beer PA, Erber WN, Campbell PJ, Green AR. How I treat essential thrombocythemia. *Blood*. 2011;117(5):1472-82.  
Passamonti F, Randi ML, Rumi E, Pungolino E, Elena C, Pietra D, et al. Increased risk of pregnancy complications in patients with essential thrombocythemia carrying the JAK2 (617V>F) mutation. *Blood*. 2007;110(2):485-9

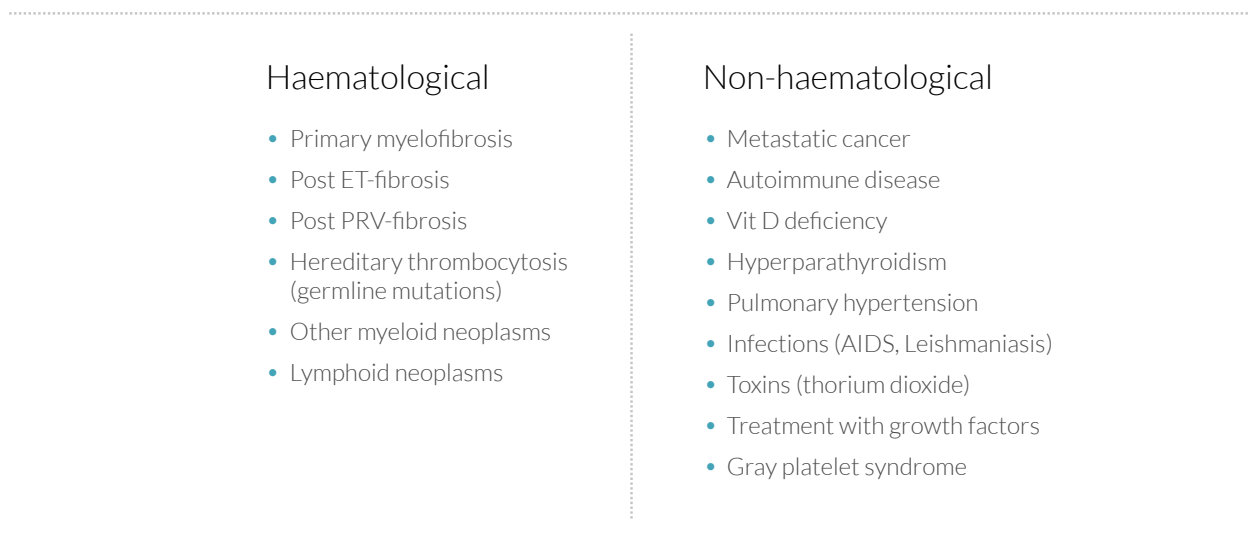
### 4.3. Primary Myelofibrosis(PMF)

PMF is the least common of the MPN's. It is difficult to define as an entity, because the disease process could be a result of multiple causes. Up to recently, not even the name could be agreed upon: primary chronic myelofibrosis (PMF), idiopathic myelofibrosis (IMF) or myelofibrosis with myeloid metaplasia (MMM) were all synonyms (Okamura 2003). Some authors even claim that "biologically, there is no such thing as 'primary' myelofibrosis"(Spivak and Silver 2008).

In PMF clonal myeloproliferation is accompanied by a secondary inflammatory state characterized by bone marrow stromal changes and abnormal cytokine expression(Tefferi 2011). Bone marrow fibrosis can, however, be due to both haematologic and non-haematologic conditions as summarised in figure 9. Even within MPN, a phenotypically similar entity exist with post-PRV or post-ET fibrosis(Tefferi 2011). However, a distinction is made by Tefferi in referring to "myelofibrosis" only in terms of PMF, and with every other cause the phrase "with bone marrow fibrosis", i.e. "MDS with bone marrow fibrosis"(Tefferi 2011).

Causes of Bone Marrow Fibrosis

FIGURE 9.



REFERENCE

Tefferi A. How I treat myelofibrosis. Blood. 2011;117(13):3494-504.

Average age at diagnosis is 66 years. Males are predominantly affected in 3:2 fashion (Tefferi, *et al* 2012). Typical symptoms and causes of morbidity are summarised below in figure 10 (Tefferi 2014). PMF is debilitating and chronic with a reduced life expectancy and an impaired quality of life (Vannucchi 2010).

### Symptoms of PMF

FIGURE 10.

- 
- |  |        |   |
|--|--------|---|
| 1. Severe anaemia                                      |        |   |
| 2. Hepatosplenomegaly                                  |        |   |
| 3. Constitutional                                      | .....▶ | ● <b>Fatigue, weight loss/ cachexia, night sweats</b>   |
| 4. Pruritus  |        |   |
| 5. Splenic infarcts                                    |        |   |
| 6. Bone Pain   |        |   |
| 7. Thrombosis  |        |   |
| 8. Bleeding  |        |   |
| 9. Portal hypertension sequelae                        | .....▶ | ● <b>Variceal bleeding</b>  |
| 10. Non-Hepatosplenic<br>extramedullary haematopoiesis | .....▶ | ● <b>Symptoms depends on site e.g. lungs, lymph nodes, spinal<br/>cord, retroperitoneum etc</b> |
- 

#### REFERENCE

Tefferi A. Primary myelofibrosis: 2014 update on diagnosis, risk-stratification, and management. *Am J Hematol.* 2014;89(9): 915-25.

Once PMF is suspected on the basis of the clinical presentation, a BMB becomes mandatory to elucidate the morphologic, cytogenetic and molecular characteristics. As illustrated with the wide differential diagnosis, simply illustrating marrow fibrosis does not confirm PMF (Tefferi 2011).

A number of mutations are also associated with PMF, none of them, however, are PMF-specific (Tefferi, *et al* 2012). JAK2 is detected in 50-60% of PMF cases, MPL in 8%, and in the mutually exclusive group, a further 35% are CALR-positive (Kralovics, *et al* 2005, Tefferi and Barbui 2015).

4.3.1. The diagnosis is finally made with the WHO criteria (Vardiman, *et al* 2009).

2008 WHO criteria for PMF

FIGURE 11.

Major criteria

- Megakaryocyte proliferation and atypia accompanied by reticulin  $\pm$  collagen fibrosis, or in the absence of overt fibrosis, megakaryocyte must be accompanied by increased marrow cellularity and granulocyte proliferation (i.e. prefibrotic myelofibrosis)
- Not meeting WHO criteria for PRV, CML, MDS or any other myeloid neoplasm
- Presence of JAK2 V617F or other clonal marker and no evidence of reactive marrow fibrosis

Minor criteria

- Leukoerythroblastosis
- Increased serum LDH
- Anaemia
- Palpable splenomegaly

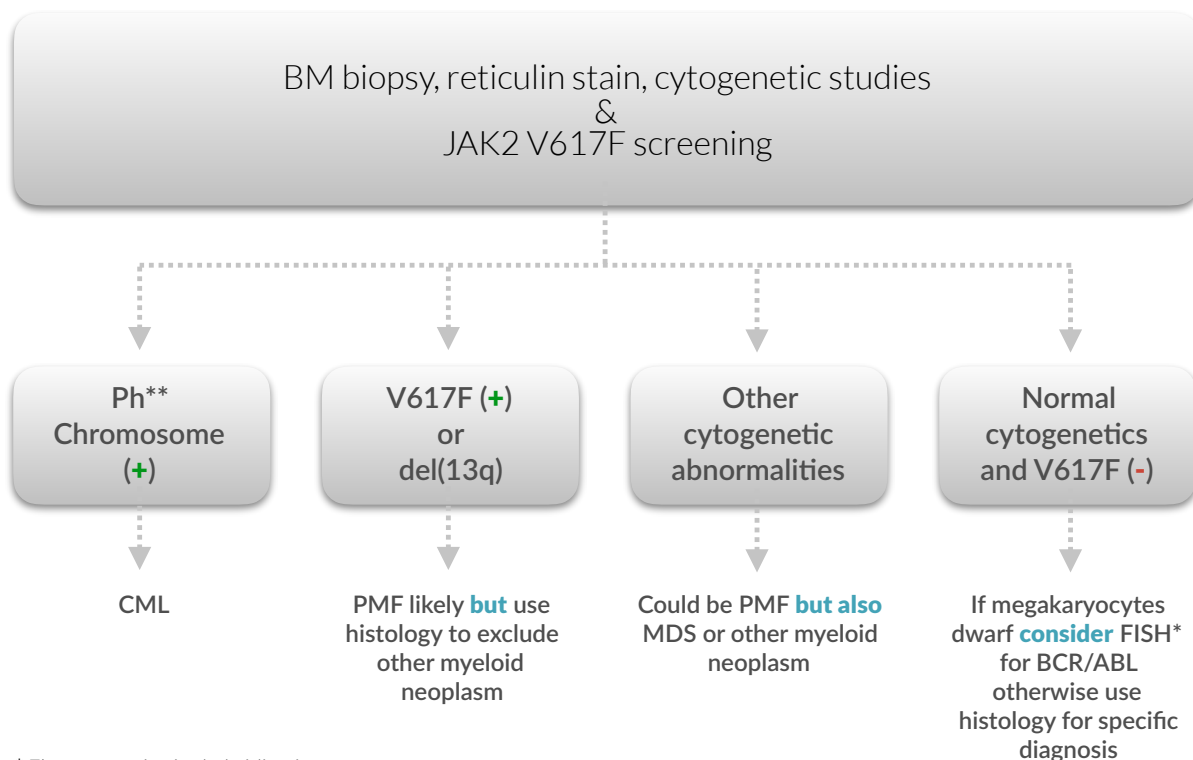
**Diagnosis of PMF requires all 3 major criteria and 2 minor criteria**

REFERENCE

Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, *et al*. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114(5):937-51.

4.3.2. The WHO algorithm for diagnosing PMF(Tefferi and Vardiman 2008b):

2008 WHO algorithm for PMF  
FIGURE 12.



\* Fluorescent in situ hybridisation

\*\* Philadelphia chromosome

REFERENCE

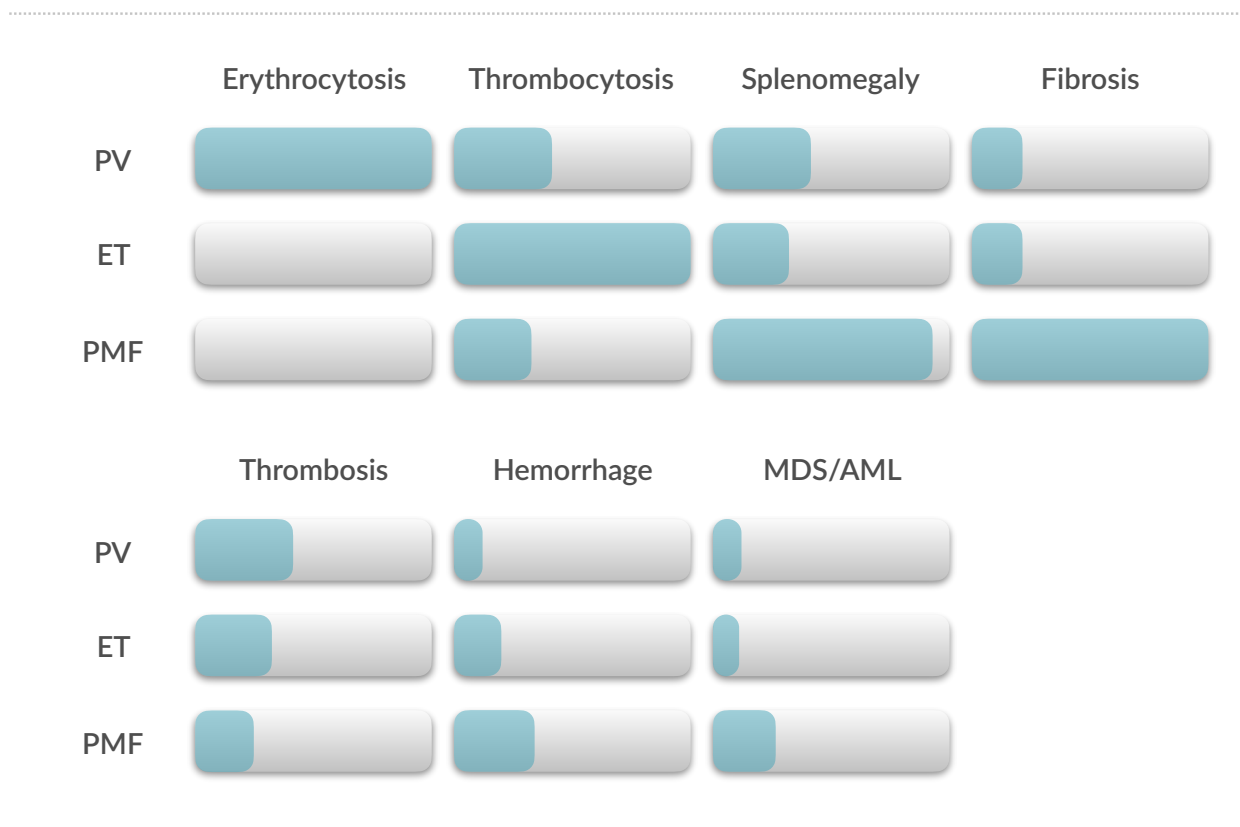
Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-51.

## 5. Spectrum of MPN - Overlap between PRV/ET/PMF

It is not always easy to distinguish the origin of the MPN proven by a JAK2 mutation. Multiple overlaps in clinical presentation and evolution exist, as these illustrations, borrowed from Kiladjian et al. (Kiladjian 2012) demonstrate. The suggestion has been made whether V617F positive ET/PMF/PRV did not simply evolve according to a continuum model in which the 3 entities were different stages of MPN(Kiladjian 2012).

Characteristics and Complications Overlap between MPN subgroups

FIGURE 13.



REFERENCE

Adapted from Kiladjian JJ. The spectrum of JAK2-positive myeloproliferative neoplasms. Hematology Am Soc Hematol Educ Program. 2012;2012:561-6.

Particular controversy often arises during the attempted diagnosis of ET because of its heterogeneity and the need to exclude other disorders. Distinguishing the border between ET and early “pre-fibrotic” PMF is complex, and some has even debated the traditional view of separate entities. The main arguments are: ET myelofibrosis and PMF are clinically indistinguishable, they share JAK2 and MPL mutations, and thrombocytosis often predates the PMF diagnosis by many years(Beer, *et al* 2011).

Furthermore, phenotypic overlap also exists between PRV and ET, and uncertainty reigns about where to draw the line between them. ET patients certainly could have a raised Hct, and PRV patients a thrombocytosis. For simplicity, Beer et al. recognised this problem but still classified patients with a positive JAK2 and a raised Hct as PRV(Beer, *et al* 2011). In the absence of specific diagnostic biologic markers, the evaluation and final diagnosis has to be based on expert judgement(Spivak 2003).

Taking into account the spectrum of disease represented by a JAK2 mutation, it is clear that JAK2 only unambiguously provides evidence of malignant, clonal and finally, myeloid haematopoiesis.

## 6. JAK2 V617F mutation in the presence of thrombosis

### Clutching at straws for a cause?

Thrombophilia denotes conditions that are associated with an increased risk of thromboembolism, hypercoagulable state or variations in the fibrinolytic system (Smalberg, *et al* 2011).

The understanding of the aetiology of thrombosis has improved over the years and finding a cause is a challenge the physician is frequently faced with. It is multifactorial, and seldom with only a single risk factor present (Smalberg, *et al* 2011). MPNs, especially PV and ET are now recognized as acquired risk factors in the aetiology of thrombosis (Klampfl, *et al* 2013, Smalberg, *et al* 2011). ET patients will have up to 50% incidence of thrombosis within a decade of diagnosis, predominantly arterial. Splanchnic thrombosis is characteristic of MPN, particularly in young women, and, to a lesser extent, the cerebral circulation (Martinelli, *et al* 2014, Schafer 2004, Whitlatch and Ortel 2008).

Despite the multifactorial aetiology of DVT or PE, thrombosis at unusual sites like the splanchnic veins more often raises questions about underlying causes (Martinelli, *et al* 2014).

### Splanchnic Vein Thrombosis

The Budd Chiari syndrome (BCS) and portal vein thrombosis (PVT) are grouped under splanchnic thrombosis (Janssen and Leebeek 2006, Smalberg, *et al* 2012).

MPNs are the most common underlying cause and can be identified in nearly 30-50% of BCS and 15-30% of PVT patients. (Colaizzo, *et al* 2007, De Stefano, *et al* 2007, Dentali, *et al* 2009a, Kiladjian, *et al* 2008, Smalberg, *et al* 2011) In both conditions the peripheral blood count changes may be masked, owing to portal hypertension and consequences thereof i.e. hypersplenism, iron deficiency and haemodilution, therefore the conventional criteria of MPN are of limited value (Janssen and Leebeek 2006, Smalberg, *et al* 2011). "Occult MPN" refers to them, and previously the clinician only had limited means to rely on for making the diagnosis (Smalberg, *et al* 2012).

Screening for JAK2 rendered a new diagnostic tool and has since been indispensable in the routine diagnostic workup of these conditions. In contrast, the JAK2 exon 12 and MPL mutations have no specific association with splanchnic vein thrombosis and should not be routinely screened for. Why the MPNs and JAK2 are so strongly analogous to splanchnic vein thrombosis remains a mystery (Smalberg, *et al* 2012).

### Other sites of thrombosis

It remains puzzling why patients with similar prothrombotic factors develop diverse forms of thrombosis. Why does one person develop a DVT/PE and another a splanchnic thrombosis? Local factors are potentially involved in the pathogenesis of thrombosis at specific sites, but the underlying mechanisms elude us (Smalberg, *et al* 2011). In 2009, a meta-analysis appraised the role of JAK2 in various forms of thrombosis, incl. SVT, DVT of lower limbs or PE, cerebral vein thrombosis, and

retinal vein thrombosis. A strong association between JAK2 and splanchnic vein thrombosis was found, but its prevalence in the other forms of idiopathic VTE was no different to that of the general population(Dentali, *et al* 2009a, Shetty, *et al* 2010).

### Cerebral sinus venous thrombosis (CVT)

The incidence of CVT is uncertain because epidemiological studies are lacking. Young female adults are mostly affected. The superior sagittal and transverse sinus are mostly involved, but in two-thirds of case more than one sinus is involved(Martinelli and De Stefano 2010).

Martinelli *et al.* (Martinelli, *et al* 2014) looked particularly at the characteristics and course of CVT in MPN patients. In their study, CVT complicated MPN in  $\pm$  1% of cases. Also relevant is that ET patients with the JAK2 mutation were especially at risk. Nearly half of their patients with CVT were diagnosed with the two conditions concomitantly, but regrettably, no mention is made of the FBC characteristics at the time of diagnosis (i.e. was there a clue in the counts eluding to an MPN?).

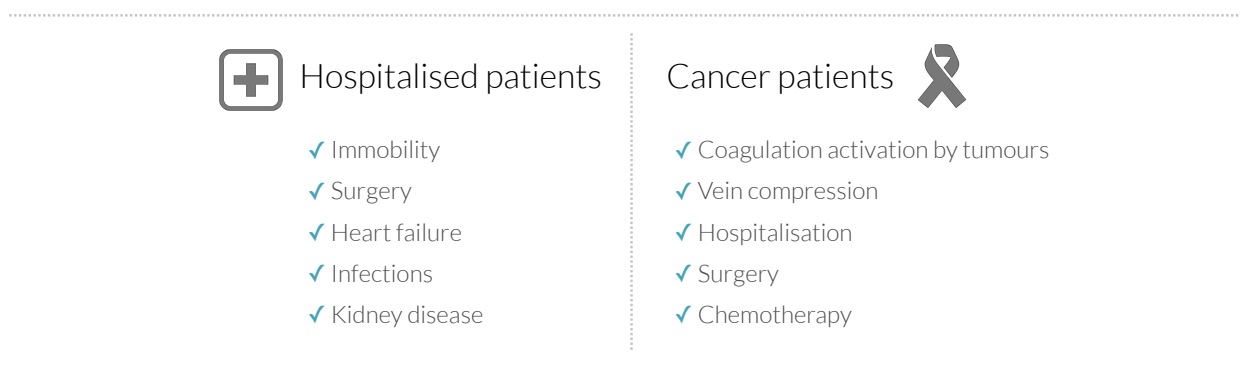
In a meta-analysis by Dentali *et al.*, JAK2 had a prevalence of 2.57% in CVT. This is slightly increased from the presence of the mutation in the general population. Compared to the corresponding 32% in SVT, it is clear that blindly screening for JAK2 is less justified(Dentali, *et al* 2009a).

### Venous PE & lower extremity DVT

Risk factors for common venous thromboembolism (VTE) are eloquently summarized by Smalberg *et al* (Smalberg, *et al* 2011). Herein it shows that stronger risk factors (Protein C-, antithrombin deficiency, antiphospholipid antibodies, malignancy, immobilisation, surgery and obesity) carry more weight than weaker risk factors like MPN, hormonal factors or autoimmune disease. Investigations should therefore focus on stronger factors first.

#### Risk factors for Thromboembolism

FIGURE 14.



#### REFERENCE

Smalberg JH, Kruij MJ, Janssen HL, Rijken DC, Leebeek FW, de Maat MP. Hypercoagulability and hypofibrinolysis and risk of deep vein thrombosis and splanchnic vein thrombosis: similarities and differences. *Arterioscler Thromb Vasc Biol.* 2011;31(3): 485-93.

Hospitalised and cancer patients are particularly known to have multiple risk factors for common VTE – see figure 14 (Smalberg, *et al* 2011). It follows naturally that age-appropriate screening is undertaken in patients who present with idiopathic VTE, especially if recurrent(Whitlatch and Ortel 2008). In contrast to the risk factors summarised in figure 14, the

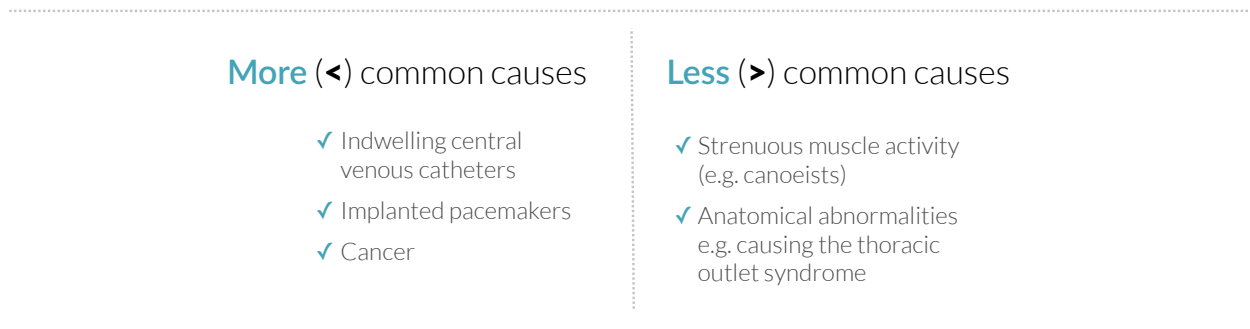
mean prevalence of MPN in VTE is only 0.88%. Dentali et al. conclude that patients with VTE should not be screened for the presence of the JAK2 mutation, as the yield is nearly negligible(Dentali, *et al* 2009a).

### Upper Extremity Thrombosis

A high degree of mobility in the upper limbs is often quoted as a reason why thrombosis here is relatively rare. The subclavian vein is most often involved. Causes are elaborated on in figure 15 (Martinelli and De Stefano 2010). No specific association between MPN and upper extremity thrombosis has been elucidated.

Risk factors for Upper Limb thrombosis

FIGURE 15.



REFERENCE

Martinelli I, De Stefano V. Rare thromboses of cerebral, splanchnic and upper-extremity veins. A narrative review. *Thromb Haemost.* 2010;103(6):1136-44.

### Retinal Vein Thrombosis (RVT)

RVT refers to occlusion in the central retinal vein(CRVO) or in a branch retinal vein (BRVO). RVT occurs similar in both sexes, commonly in advanced age, and usually unilateral. It shares traditional systemic risk factors for thrombosis, i.e. hypertension, diabetes and arteriosclerosis. Screening for thrombophilia in RVT produced conflicting results, where factor V Leiden and prothrombinG20210A are not associated, but antiphospholipid antibodies and hyperhomocysteinaemia are proven as risk factors(Martinelli and De Stefano 2012).

The JAK2 prevalence in RVT was only 0.99% in the Dentali's meta-analysis(Dentali, *et al* 2009b). Hermans unambiguously concluded that JAK2 is not implicated in this scenario with his article "The JAK2 V617F mutation is not a cause of of central retinal vein occlusion"(Hermans, *et al* 2008).

## 7. Conclusion

The discovery of the JAK2 V617F mutation came as an exciting development in Haematology already in 2005, and even though several years have gone by wherein this test had been available in our hospital there had been no data available on utility in our unique South African setting. Instead, we still have to rely on European and American information. We reviewed the literature on the various subgroups within MPN (i.e. PRV, ET and PMF) and the guidelines that sprung forth since 2005 and in our study, aimed to apply them retrospectively to the patients that were already under our care. Our objectives were confined to the diagnostic aspects of MPN and the investigative approach to an elevated haemoglobin and platelet count.

With regards to erythrocytosis we studied the debates raging within the Haematological community on the use of surrogate markers for absolute erythrocytosis and the use of ancillary measures such as the nuclear RCM and sEPO. We identified a significant group of patients during our study where the aetiology of the erythrocytosis seemed unclear, and reviewed the available literature on the entity of idiopathic erythrocytosis.

The presence of the JAK2 V617F mutation and the prevalence of thrombosis and its respective aetiology were also reviewed, in order to determine whether the widespread use of this mutation in thrombosis was justified and apply these findings in the future to the patients under our care.

Gaps identified in the literature were the striking paucity of information available on the effects of androgen therapy on the Hb/Hct, as well as the influence on these markers following a splenectomy. It became very apparent that “smoker’s polycythaemia” remains a poorly delineated concept where the overlap with early chronic lung disease is difficult to define. Even less consensus exist regarding the application of venesection in erythrocytosis groups where a clonal mechanism could not be identified.

In conclusion, we conducted a broad overview of literature surrounding the clonal marker of JAK2 V617F and attempted with the knowledge gained, to apply this to our retrospectively identified population.

## 8. References

1. de Lacerda JF, Oliveira SN, Ferro JM. Chronic myeloproliferative diseases. *Handb Clin Neurol*. 2014;120:1073-81.
2. Spivak JL, Silver RT. The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis, and primary myelofibrosis: an alternative proposal. *Blood*. 2008;112(2):231-9.
3. Michiels JJ, Berneman Z, Schroyens W, De Raeve H. Changing concepts of diagnostic criteria of myeloproliferative disorders and the molecular etiology and classification of myeloproliferative neoplasms: from Dameshek 1950 to Vainchenker 2005 and beyond. *Acta Haematol*. 2015;133(1):36-51.
4. Dameshek W. Physiopathology and course of polycythemia vera as related to therapy. *J Am Med Assoc*. 1950;142(11):790-7.
5. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling.pdf. *Nature*. 2005.
6. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *The Lancet*. 2005;365(9464):1054-61.
7. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005;434(7037):1144-8.
8. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005;7(4):387-97.
9. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005;352(17):1779-90.
10. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia (08876924)*. 2008;22(1):14-22.
11. Bench AJ, White HE, Foroni L, Godfrey AL, Gerrard G, Akiki S, et al. Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant mutations. *Br J Haematol*. 2013;160(1):25-34.
12. Kiladjian JJ. The spectrum of JAK2-positive myeloproliferative neoplasms. *Hematology Am Soc Hematol Educ Program*. 2012;2012:561-6.
13. Tefferi A. Essential thrombocythemia, polycythemia vera, and myelofibrosis: Current management and the prospect of targeted therapy. *Am J Hematol*. 2008;83(6):491-7.
14. Stein BL, Crispino JD, Moliterno AR. Janus kinase inhibitors: an update on the progress and promise of targeted therapy in the myeloproliferative neoplasms. *Curr Opin Oncol*. 2011;23(6):609-16.
15. Kesler A, Ellis MH, Manor Y, Gadoth N, Lishner M. Neurological complications of essential thrombocytosis (ET). *Acta Neurol Scand*. 2000;102(5):299-302.
16. Levine RL, Loriaux M, Huntly BJP, Loh ML, Beran M, Stoffregen E, et al. The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood*. 2005;106(10):3377-9.
17. Nielsen C, Birgens HS, Nordestgaard BG, Kjær L, Bojesen SE. The JAK2 V617F somatic mutation, mortality and cancer risk in the general population. *Haematologica*. 2011;96(3):450-3.
18. Rollison DE, Howlader N, Smith MT, Strom SS, Merritt WD, Ries LA, et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. *Blood*. 2008;112(1):45-52.
19. Gerdts AT. I Walk the Other Line: Myelodysplastic/Myeloproliferative Neoplasm Overlap Syndromes. *Curr Hematol Malig Rep*. 2014;9(4):400-6.

20. Malcovati L, Della Porta MG, Pietra D, Boveri E, Pellagatti A, Galli A, et al. Molecular and clinical features of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. *Blood*. 2009;114(17):3538-45.
21. Wang Y, Xia J, Wu X, Hu Y. A Patient With BCR-ABL and JAK2 V617F Double-Positive Myeloproliferative Neoplasm With Overlapping Clinical Phenotypes. *Am J Med Sci*. 2015;350(1):68-9.
22. Zhou A, Knoche EM, Engle EK, Fisher DA, Oh ST. Concomitant JAK2 V617F-positive polycythemia vera and BCR-ABL-positive chronic myelogenous leukemia treated with ruxolitinib and dasatinib. *Blood cancer journal*. 2015;5:e351.
23. Passamonti F. How I treat polycythemia vera. *Blood*. 2012;120(2):275-84.
24. Stein BL, Moliterno AR, Tiu RV. Polycythemia vera disease burden: contributing factors, impact on quality of life, and emerging treatment options. *Ann Hematol*. 2014;93(12):1965-76.
25. Vannucchi AM. How I treat polycythemia vera. *Blood*. 2014;124(22):3212-20.
26. Passamonti F, Malabarba L, Orlandi E, Barate C, Canevari A, Brusamolino E, et al. Polycythemia vera in young patients: a study on the long-term risk of thrombosis, myelofibrosis and leukemia. *Haematologica*. 2003;88(1):13-8.
27. Spivak JL. Diagnosis of the myeloproliferative disorders: resolving phenotypic mimicry. *Semin Hematol*. 2003;40(1 Suppl 1):1-5.
28. Johansson PL, Safai-Kutti S, Kutti J. An elevated venous haemoglobin concentration cannot be used as a surrogate marker for absolute erythrocytosis: a study of patients with polycythaemia vera and apparent polycythaemia. *Br J Haematol*. 2005;129(5):701-5.
29. Alvarez-Larran A, Ancochea A, Angona A, Pedro C, Garcia-Pallarols F, Martinez-Aviles L, et al. Red cell mass measurement in patients with clinically suspected diagnosis of polycythemia vera or essential thrombocythemia. *Haematologica*. 2012;97(11):1704-7.
30. Jacob M, Annaheim S, Boutellier U, Hinske C, Rehm M, Breymann C, et al. Haematocrit is invalid for estimating red cell volume: a prospective study in male volunteers. *Blood Transfus*. 2012;10(4):471-9.
31. Barbui T, Thiele J, Vannucchi AM, Tefferi A. Rethinking the diagnostic criteria of polycythemia vera. *Leukemia*. 2014;28(6):1191-5.
32. McMullin MF, Bareford D, Campbell P, Green AR, Harrison C, Hunt B, et al. Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. *Br J Haematol*. 2005;130(2):174-95.
33. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114(5):937-51.
34. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2015 update on diagnosis, risk-stratification and management. *Am J Hematol*. 2015;90(2):162-73.
35. Silver RT, Chow W, Orazi A, Arles SP, Goldsmith SJ. Evaluation of WHO criteria for diagnosis of polycythemia vera: a prospective analysis. *Blood*. 2013;122(11):1881-6.
36. McMullin MF, Reilly JT, Campbell P, Bareford D, Green AR, Harrison CN, et al. Amendment to the guideline for diagnosis and investigation of polycythaemia/erythrocytosis. *Br J Haematol*. 2007;138(6):821-2.
37. Messinezy M WN, El-Hemaidi I, Marsden JT, Sherwood RS, Pearson TC. <Serum erythropoietin values in erythrocytoses and in primary thrombocythaemia.pdf>. *Br J Haematol*. 2002;
38. Mossuz P GF, Magali D. Diagnostic value of serum EPO.pdf. *Haematologica*. 2004(89):1194-8.
39. Vannucchi A. how I treat PV.pdf. *Blood*. 2014;124(22):3212.
40. Lorberboym M, Rahimi-Levene N, Lipszyc H, Kim CK. Analysis of red cell mass and plasma volume in patients with polycythemia. *Arch Pathol Lab Med*. 2005;129(1):89-91.

41. Tefferi A. The rise and fall of red cell mass measurement in polycythemia vera. *Curr Hematol Rep.* 2005;4(3):213-7.
42. Cook LS. Therapeutic phlebotomy: a review of diagnoses and treatment considerations. *J Infus Nurs.* 2010;33(2):81-8.
43. Randi ML, Bertozzi I, Cosi E, Santarossa C, Peroni E, Fabris F. Idiopathic erythrocytosis: a study of a large cohort with a long follow-up. *Ann Hematol.* 2015.
44. Assi TB, Baz E. Current applications of therapeutic phlebotomy. *Blood Transfus.* 2014;12 Suppl 1:s75-83.
45. Finazzi G, Gregg XT, Barbui T, Prchal JT. Idiopathic erythrocytosis and other non-clonal polycythemia. *Best Practice & Research Clinical Haematology.* 2006;19(3):471-82.
46. McMullin MF. Idiopathic erythrocytosis: a disappearing entity. *Hematology Am Soc Hematol Educ Program.* 2009:629-35.
47. Pearson TC, Messinezy M. Idiopathic erythrocytosis, diagnosis and clinical management. *Pathol Biol (Paris).* 2001;49(2):170-7.
48. Harrison CN, Green AR. Essential thrombocythemia. *Hematol Oncol Clin North Am.* 2003;17(5):1175-90, vii.
49. Bleeker JS, Hogan WJ. Thrombocytosis: diagnostic evaluation, thrombotic risk stratification, and risk-based management strategies. *Thrombosis.* 2011;2011:536062.
50. Beer PA, Erber WN, Campbell PJ, Green AR. How I treat essential thrombocythemia. *Blood.* 2011;117(5):1472-82.
51. Folman CC, Ooms M, Kuenen BB, de Jong SM, Vet RJ, de Haas M, et al. The role of thrombopoietin in post-operative thrombocytosis. *Br J Haematol.* 2001;114(1):126-33.
52. Fenaux P, Simon M, Caulier MT, Lai JL, Goudemand J, Bauters F. Clinical course of essential thrombocythemia in 147 cases. *Cancer.* 1990;66(3):549-56.
53. Elliott MA, Tefferi A. Thrombosis and haemorrhage in polycythaemia vera and essential thrombocythaemia. *Br J Haematol.* 2005;128(3):275-90.
54. Marchioli R, Finazzi G, Landolfi R, Kutti J, Gisslinger H, Patrono C, et al. Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. *J Clin Oncol.* 2005;23(10):2224-32.
55. Ozer FL, Truax WE, Miesch DC, Levin WG. Primary hemorrhagic thrombocythemia. *The American Journal of Medicine.* 28(5):807-23.
56. Passamonti F, Randi ML, Rumi E, Pungolino E, Elena C, Pietra D, et al. Increased risk of pregnancy complications in patients with essential thrombocythemia carrying the JAK2 (617V>F) mutation. *Blood.* 2007;110(2):485-9.
57. Okamura T. Medical progress in idiopathic myelofibrosis. *Intern Med.* 2003;42(6):461-2.
58. Tefferi A. How I treat myelofibrosis. *Blood.* 2011;117(13):3494-504.
59. Tefferi A, Lasho TL, Jimma T, Finke CM, Gangat N, Vaidya R, et al. One Thousand Patients With Primary Myelofibrosis: The Mayo Clinic Experience. *Mayo Clin Proc.* 2012;87(1):25-33.
60. Tefferi A. Primary myelofibrosis: 2014 update on diagnosis, risk-stratification, and management. *Am J Hematol.* 2014;89(9):915-25.
61. Vannucchi A. MF from palliation to targeted therapy. *N Engl J Med.* 2010;363(12):1180-2.
62. Smalberg JH, Kruip MJ, Janssen HL, Rijken DC, Leebeek FW, de Maat MP. Hypercoagulability and hypofibrinolysis and risk of deep vein thrombosis and splanchnic vein thrombosis: similarities and differences. *Arterioscler Thromb Vasc Biol.* 2011;31(3):485-93.
63. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med.* 2013;369(25):2379-90.

64. Whitlatch NL, Ortel TL. Thrombophilias: when should we test and how does it help? *Semin Respir Crit Care Med*. 2008;29(1):25-39.
65. Martinelli I, De Stefano V, Carobbio A, Randi ML, Santarossa C, Rambaldi A, et al. Cerebral vein thrombosis in patients with Philadelphia-negative myeloproliferative neoplasms. An European Leukemia Net study. *Am J Hematol*. 2014;89(11):E200-5.
66. Schafer AI. Thrombocytosis. *N Engl J Med*. 2004;350(12):1211-9.
67. Janssen HL, Leebeek FW. JAK2 mutation: The best diagnostic tool for myeloproliferative disease in splanchnic vein thrombosis? *Hepatology*. 2006;44(6):1391-3.
68. Smalberg JH, Arends LR, Valla DC, Kiladjian JJ, Janssen HL, Leebeek FW. Myeloproliferative neoplasms in Budd-Chiari syndrome and portal vein thrombosis: a meta-analysis. *Blood*. 2012;120(25):4921-8.
69. Colaizzo D, Amitrano L, Tiscia GL, Scenna G, Grandone E, Guardascione MA, et al. The JAK2 V617F mutation frequently occurs in patients with portal and mesenteric venous thrombosis. *J Thromb Haemost*. 2007;5(1):55-61.
70. De Stefano V, Fiorini A, Rossi E, Za T, Farina G, Chiusolo P, et al. Incidence of the JAK2 V617F mutation among patients with splanchnic or cerebral venous thrombosis and without overt chronic myeloproliferative disorders. *J Thromb Haemost*. 2007;5(4):708-14.
71. Dentali F, Squizzato A, Brivio L, Appio L, Campiotti L, Crowther M, et al. JAK2V617F mutation for the early diagnosis of Ph- myeloproliferative neoplasms in patients with venous thromboembolism: a meta-analysis. *Blood*. 2009;113(22):5617-23.
72. Kiladjian JJ, Cervantes F, Leebeek FW, Marzac C, Cassinat B, Chevret S, et al. The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. *Blood*. 2008;111(10):4922-9.
73. Shetty S, Kulkarni B, Pai N, Mukundan P, Kasatkar P, Ghosh K. JAK2 mutations across a spectrum of venous thrombosis cases. *Am J Clin Pathol*. 2010;134(1):82-5.
74. Martinelli I, De Stefano V. Rare thromboses of cerebral, splanchnic and upper-extremity veins. A narrative review. *Thromb Haemost*. 2010;103(6):1136-44.
75. Martinelli I, De Stefano V. Extra-abdominal venous thromboses at unusual sites. *Best Practice & Research Clinical Haematology*. 2012;25(3):265-74.
76. Dentali F, Squizzato A, Brivio L, Appio L, Campiotti L, Crowther M, et al. JAK2V617F mutation for the early diagnosis of Ph- myeloproliferative neoplasms in patients with venous thromboembolism: a meta-analysis. *Blood*. 2009;113(22):5617-23.
77. Hermans C, Slachmuylder V, Gala JL, Lambert C, Detrait M. The JAK2 V617F mutation is not a cause of central retinal vein occlusion. *Thromb Haemost*. 2008;100(3):515-6.

CHAPTER TWO

# Journal Ready Manuscript



# ROLE OF THE JAK2V617F MUTATION ANALYSIS IN THE DIAGNOSTIC EVALUATION OF SUSPECTED MYELOPROLIFERATIVE NEOPLASMS IN GROOTE SCHUUR HOSPITAL

## Abstract

We studied the implementation of JAK2 mutation analysis in conjunction with the World Health Organisation (WHO) guidelines in the pathway to MPN diagnosis in 279 patients presenting with one of three clinical scenarios: erythrocytosis, OR leukocytosis and/or thrombocytosis and/or splenomegaly; OR patients with thrombosis without cytoses. Patients were investigated for MPN and managed in the haematology clinic of Groote Schuur Hospital. We studied the association of clinical and laboratory variables with clonal vs non-clonal diagnoses. In 120/297 patients MPN was confirmed: Polycythemia vera (PV), (n=51, 100% JAK2 mutated); essential thrombocytosis, (n=41, 42% JAK2 mutated); primary myelofibrosis (n=28, 57% JAK2 mutated). The 2016 WHO haemoglobin/haematocrit thresholds in PV were validated. Idiopathic erythrocytosis (IE) found in 44 patients. Bone marrow histology, but not serum EPO level, was essential to differentiate between clonal and non-clonal erythrocytosis. Both PV and IE patients complied with the criteria of absolute erythrocytosis on peripheral blood, yet nuclear red cell mass identified critical differences between clonal and non-clonal erythrocytosis. No patient venesected for non-clonal erythropoiesis developed thrombocytosis. JAK2 mutation analysis applied with the WHO diagnostic algorithm efficiently differentiated true clonal myeloproliferation from reactive cytoses. Lifestyle and metabolic factors such as smoking and thrombosis were not associated with either clonal or non-clonal erythrocytosis, and were equally present in mutated and unmutated essential thrombocytosis.

## Introduction

The Philadelphia-negative classic myeloproliferative diseases mainly comprise essential thrombocytosis (ET), primary myelofibrosis (PMF) and polycythaemia vera (PV)(Michiels, *et al* 2006). The molecular findings of the past decade have confirmed the clonal nature of the disorder – hence the name change to myeloproliferative neoplasms (MPN) (Baxter, *et al* 2005b, James, *et al* 2005a, Kralovics, *et al* 2005, Levine, *et al* 2005b) The pivotal discovery of the JAK2 V617 mutation in 2005(Spivak and Silver 2008) brought about a major advance in our ability to diagnose these three disorders. Previously, in many cases, it had been problematic to define the diagnostic distinction between a myeloproliferative neoplastic disease and a reactive proliferative disorder, but we can now confidently define the majority of these disorders based on the presence of this mutation.

Regarding the application of the JAK2 mutation analysis in patients with suspected MPN, it is expected that up to half of patients with ET and PMF will be JAK2 negative(Tefferi and Barbui 2015). Newly emerging markers such as CALR and MPL provide further evidence of clonality and have implications for prognosis(Arber, *et al* 2016).Expert interpretation of the histology on bone marrow examination (BME) in close adherence to the World Health Organisation (WHO) diagnostic algorithm will confirm JAK2 negative ET and PMF and thereby rule out other causes of leukocytosis, thrombocytosis and splenomegaly(Tefferi and Vardiman 2008a).

In patients investigated for erythrocytosis, one could argue that ascertaining a clonal diagnosis of PV should be fairly straightforward because more than 95% JAK2 positivity has been reported(Michiels, *et al* 2015, Passamonti 2012). Nevertheless, after confirming JAK2-positive PV and ruling out secondary causes of erythrocytosis, the clinician is confounded by a sizeable patient population with likely non-clonal, yet sustained erythrocytosis(McMullin 2012, Randi, *et al*

2016). The designation 'idiopathic erythrocytosis' is used for this patient population regarded to have an absolute erythrocytosis – defined as an increased red cell mass more than 125% of the predicted value – as expressed in either raised haemoglobin (hb) or haematocrit (hct), or nuclear red cell mass (RCM)(Pearson, *et al* 1995) (Johansson, *et al* 2005, McMullin, *et al* 2005). To outrule JAK2 negative PV, there is the WHO criterium for trilineage proliferation on BME. A low serum erythropoietin level (sEPO) is an important part of the diagnostic algorithm for PV, but it is unclear if it has additional value to substantiate true clonal erythropoiesis(Barbui, *et al* 2015, Mossuz, *et al* 2004).

We studied the implementation of the JAK2 mutation analysis, in conjunction with the diagnostic algorithm for respectively PMF, ET and PV, in the patient population of our tertiary referral center. We wanted to see firstly how the JAK2 test would be distributed in our three Philadelphia-negative MPN populations. However, we were equally interested in the rest of the population who were tested and an MPN ruled out. If this population can be better defined, we reasoned that it could inform a more targeted utilization of the JAK2 test in our institution. In this regard we examined if smoking and a new malignancy were more closely associated with the reactive or non-clonal conditions. Likewise the relationship of leukocytosis or thrombocytosis with clonal versus non-clonal erythrocytosis was of interest(Randi, *et al* 2016).

Therefore, our second aim was to analyse patients within the subgroups based on the clinical reasoning behind requesting the JAK2 test. JAK2 mutation analysis was requested based on three specific indications, firstly in patients investigated for a myeloproliferative neoplasm due to the presence of erythrocytosis, secondly based on leukocytosis and/or thrombocytosis variably associated with splenomegaly; and thirdly, in patients where the occurrence of a thrombotic episode prompted investigation. Although the occurrence of a thrombotic episode is often the first step leading toward a diagnosis of MPN, nevertheless it is only in splanchnic vein thrombosis that there is consensus that JAK2 mutation screening is indicated(Dentali, *et al* 2009a, Yonal, *et al* 2012). Our third aim was to determine the incidence of thrombosis in all patients at diagnosis and to correlate it with their underlying disorder. This would additionally determine if thrombotic episodes in absence of cytososes ever led to a diagnosis of MPN in our population.

## Methods

### **Patient cohort and clinical assessment criteria:**

We retrospectively analysed the clinical records of 279 patients who had been tested for the JAK2V617F mutation, as part of diagnostic investigation for MPN by the Division of Haematology (Department of Medicine) Groote Schuur Hospital, Cape Town during the period 2007 to 2014. Ethical consent was obtained by the University of Cape Town Health Faculty Research Ethics Committee (number 091/15). All clinical tests analysed were previously performed by the National Health Laboratory Service at Groote Schuur hospital, using accredited, standard methodology. Specifically, the JAK2 V617F mutation was detected by PCR amplification and melting curve analysis of the amplicons, using highly specific hybridization probes as described previously. The sensitivity in PV and ET was respectively 78% and 40 %(McClure, *et al* 2006). The RCM was measured using chromium 51-labeled red blood cells and iodine (<sup>125</sup>I)-labeled albumin, respectively, following the guidelines proposed by the international committee on standards in Haematology (ICSH)(Lorberboym, *et al* 2005).

### Clinical assessment was carried out within 3 groups based on clinical reasoning behind JAK2 mutation analysis test:

- 1) **Patients investigated for erythrocytosis (n=138):** These patients had erythrocytosis with or without other cytos. This included all patients with hb and hct values above local normal threshold values (>17g/dL and/or >48% for males and >16g/dL and/or >46% for females). We also recorded the higher World Health Organisation (WHO) thresholds (Vardiman, *et al* 2009) (hb in males > 18.5g/dL; females >16.5g/dL) as well as the two British Committee for Standards in Haematology (BCSH) thresholds (hct in males >52% and >60%; females > 48% and >56%)(McMullin, *et al* 2005).
- 2) **Patients investigated for cytosis (leukocytosis and/or thrombocytosis) or for splenomegaly (n=103):** These patients presented with neutrophil leukocytosis and/or thrombocytosis and/or splenomegaly suspicious for MPN. The thresholds as given by our NHLS laboratory were as follows: platelets 178-400 x 10<sup>9</sup>/L, white cell count 4.00 – 10.00 x 10<sup>9</sup>/L.
- 3) **Patients investigated primarily for thrombotic events (n=37):** These patients were distinguished from the erythrocytosis and other cytos groups in that blood counts were in the normal range.

### Clinical assessment was based on:

- **General and demographic data:** age at date of JAK2 mutation analysis, gender, previous disease history, smoking history, clinical scenario leading to JAK2 test including new diagnosis of malignancy within six months of MPN diagnosis.
- **Review of MPN diagnosis:** A diagnostic review for PV, ET or MPN or other MPN-related disorder was made on all patients using the diagnostic algorithm of the 2008 WHO based on history and clinical examination, full blood count (FBC) values at presentation and/or at date of JAK2 mutation testing as well as during follow-up, BME, spleen size, sEPO, and RCM result(Tefferi and Vardiman 2008a).
- **Thrombotic events:** The prevalence of thrombotic events in the year before and at diagnosis was recorded in all patients. Thrombotic events included venous or arterial thromboembolism such as cerebrovascular, peripheral vascular and acute coronary events as well as venous thromboembolism.
- **Occurrence of cytos on follow-up in erythrocytosis patients:** We recorded platelets/leukocytes above normal laboratory values at diagnosis and again at follow-up during venesection we recorded the occurrence of thrombocytosis and leukocytosis and the need for cytoreductive therapy for leukocytosis/thrombocytosis.

### Statistical analysis:

Data was collected on a password-protect excel spreadsheet and analysed using Stata (Version 13.1; Stata Corp, College Station, Texas, USA). Descriptive statistics was used to characterise the patient cohort, where continuous variables are expressed as mean ( $\pm$  standard deviation) when variables are normally distributed or median (interquartile range) for data that were skewed. Categorical variables were represented using frequency distribution. The two idiopathic erythrocytosis groups were combined in order to conduct a two-sample test of proportions for the difference in frequencies in smoking and thrombosis between polycythemia vera and idiopathic erythrocytosis. P-values of <0.05 were considered statistically significant.

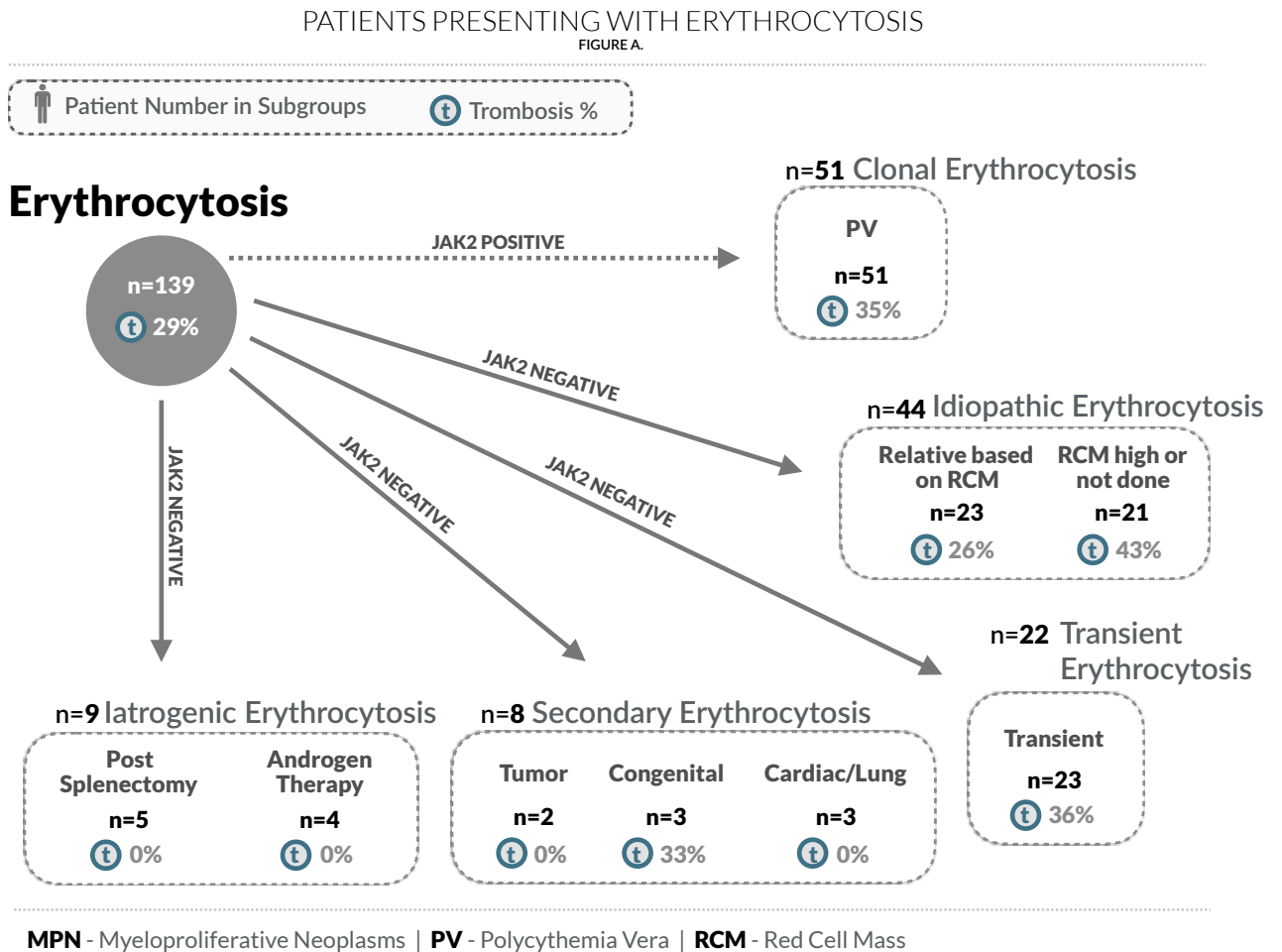
## Results

Our cohort consisted of 158 (57%) male and 120 (43%) female patients; mean age at testing 51.0 years (female=52.7 years, male=50.3 years). Patients were analysed in the three modes of presentation as described under methods (Figure 1). Eighty-six (31%) patients tested positive for the JAK2 mutation of whom 2 with myelodysplastic/myeloproliferative disorder

(MDS/MPN). An additional 36 patients were diagnosed with JAK2 negative MPN – in total 120 (43%) patients with MPN and 2 patients with JAK2 positive MDS/MPN.

**Patients Investigated For Erythrocytosis With or Without Other Cytoses (n=139):**

We subdivided patients presenting with erythrocytosis into 5 groups with regards to shared diagnostic characteristics as laid out below and in Figure 1. The five subgroups were clonal, idiopathic, transient, secondary and iatrogenic erythrocytosis. We then described shared characteristics between the groups in table 1, including: diagnostic sEPO, measures used for establishing absolute erythrocytosis (hb/hct and RCM) and prevalence of thrombosis and smoking.



- Clonal erythrocytosis: JAK 2 positive Polycythaemia Vera (PV) (n=51).** Thrombocytosis was present in 40 patients (78%) at diagnosis and leukocytosis in 30 patients (59%). All were venesected to keep hct<45, placed on aspirin if not contra-indicated, and 43 patients (84%) needed cytoreductive therapy to normalise platelet count.
- Idiopathic erythrocytosis (IE) (n=46).** These patients had a sustained erythrocytosis, but none of them was JAK2 positive. They had variable levels of sEPO as seen in Table 1. However, even in cases with low sEPO, the BME was not indicative of MPN and JAK2 negative PV was ruled out. Hb/hct values indicated sustained absolute erythrocytosis; 37 patients (80%) were venesected during follow-up. Unless contra-indicated, patients were placed on aspirin but none received cytoreductive therapy. Based on RCM result patients were subdivided:

- 2.1. **Idiopathic yet RCM proven apparent erythrocytosis (n=25).** Patients in this group had a RCM study indicating a apparent or spurious erythrocytosis. 7 had thromboses at or prior to diagnosis. Thrombocytosis or leukocytosis was seen at diagnosis in only 2 patients. Thrombocytosis or leukocytosis developed in none of 18 patients venesected during follow-up.
- 2.2. **Idiopathic erythrocytosis (n=21).** RCM was either not carried out in these patients, or confirmed an absolute erythrocytosis in 4 patients. No thrombocytosis was seen at diagnosis, and only 3 patients had leukocytosis. Thrombocytosis or leukocytosis did not develop in 19 patients venesected during follow-up.
3. **Transient erythrocytosis (n=20).** Raised Hb/Hct values were non-sustained (hence, transient). Venesections (<3) were carried out during the first few months of follow-up in 5 patients but could be terminated with no rebound erythrocytosis. In 2 patients RCM confirmed relative erythrocytosis. At diagnosis 4 patients had transient leukocytosis and 1 patient had transient thrombocytosis.
4. **Secondary erythrocytosis (n=8).** One patient had cardiac cyanotic disease. Two patients were referred for JAK2 analysis after the respiratory physicians had ruled out lung disease, yet after a few years of follow-up early cor pulmonale became evident. Three patients were judged to have congenital secondary polycythemia – one with high oxygen affinity hemoglobin and two young patients with suspected mutations in the oxygen sensing pathway. Leukocytosis/thrombocytosis was not present at diagnosis or follow-up with venesection in the congenital cases. All 6 these patients had very high sEPO. The remaining 2 patients with subnormal sEPO values presented with a new cancer – cervix carcinoma and nasal neuroblastoma – in both cases erythrocytosis resolved after tumor resection so was clearly associated with the tumor.
5. **Iatrogenic erythrocytosis (n=9).** Five patients underwent splenectomies for diverse reasons (e.g. traumatic, ITP, spherocytosis) and 4 patients received androgen replacement therapy (e.g. post total orchidectomy, hypogonadism, macroprolactinoma and gender reassignment).

Within the erythrocytosis group, we also described malignancies associated with MPN, as well as whether patients had splenomegaly at diagnosis and noted the presence of a raised white cell count or thrombocytosis at initial diagnosis and the effects on the counts following venesection.

**Malignancies diagnosed at or within 6 months of MPN evaluation:** In 2 patients with secondary erythrocytosis a malignancy was resected at diagnosis. No malignancies in IE and transient erythrocytosis; 7 new malignancies in 51 PV patients (14%).

**Splenomegaly at diagnosis:** Recorded in 23/51 (45%) PV patients and in 1 patient with IE.

**Leukocytosis and/or thrombocytosis at diagnosis and after venesection:** As described below under subgroups.

**Table 1. The five subgroups of patients investigated for erythrocytosis (n=139): Distribution of serum Erythropoietin level (sEPO); Hb/Hct level according to the WHO and BCSH guidelines; Red Cell Mass (RCM); thrombosis at diagnosis and smoking status**

	Clonal erythrocytosis- JAK2 positive polycythemia vera n=51	Idiopathic erythrocytosis n = 44		Transient erythrocytosis n=22	Secondary erythrocytosis n=8	Iatrogenic erythrocytosis n= 9
		Apparent erythrocytosis based on RCM n=23	RCM either not evaluated n= 17 or high n=4			
<b>sEPO level</b>						
Low (<2.6)	71% (6/21)	5% (1/20)	38% (8/21)		25% (2/8)	
Normal (2.6 – 18.5)	29% (15/21)	80% (16/20)	43% (9/21)	83% (10/12)		50% (2/4)
High (>18.5)		15% (3/20)	19% (4/21)	17% (2/12)	75% (6/8)	50% (2/4)
<b>Measures for establishing absolute erythrocytosis:</b>						
Hb/Hct consistent with increased red cell mass*	100% (51/51)	100% (23/23)	100% (21/21)	64% (14/22)	100% (8/8)	33% (3/9)
Hct >60 in males, >56 in females **	39% (20/51)	57% (13/23)	76% (16/21)	32% (7/22)	100% (8/8)	33% (3/9)
RCM not raised (relative erythrocytosis)		100% (23/23)		100% (2/2)		100% (5/5)
RCM raised (absolute erythrocytosis)	100% (9/9)		100% (4/4)		100% (4/4)	
<b>Thrombosis at diagnosis***</b>	35% (18/51)	26% (6/23)	43% (9/21)	36% (8/22)	0% (0/8)	0% (0/9)
<b>Smokers †</b>	63% (32/51)	78% (18/23)	81% (17/21)	81% (13/16)	50% (4/8)	100% (4/4)

\* Threshold values for absolute increase in erythrocytosis as set for Hb and Hct by the 2008 WHO and the BSCH respectively (McMullin, *et al* 2005, Vardiman, *et al* 2009).

\*\* A1 criterion used in BCSH algorithm for diagnosing JAK2 negative PV (McMullin, *et al* 2005, McMullin, *et al* 2007)

\*\*\* There was no significant difference in the proportion of thrombosis between PV and IE (35% in PV vs 34% in IE) (p=0.92).

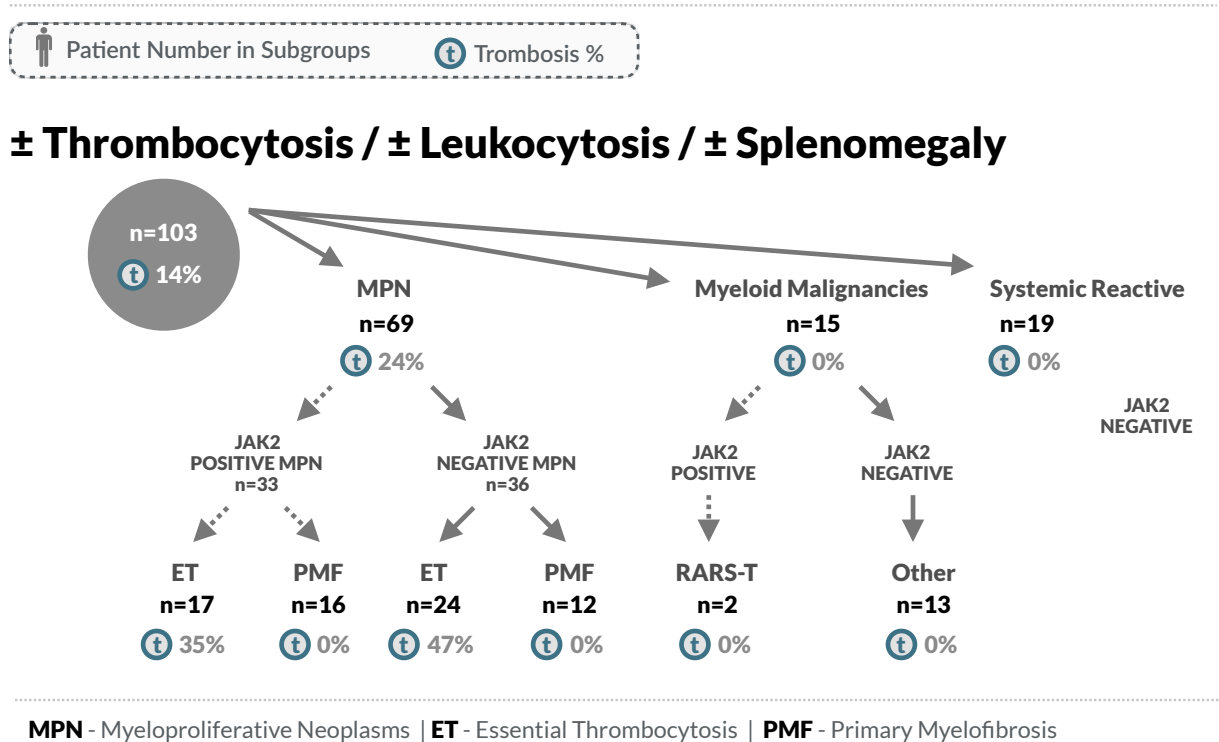
† There was no significant difference in the proportion of smokers between PV and IE (63% in PV versus 80% in IE) (p=0.07).

Abbreviations: Haemoglobin (Hb), haematocrit (Hct), red cell mass (RCM)

## Patients Investigated For Leuco- or Thrombocytosis or for Splenomegaly: n=103

In patients who primarily presented with cytosés *other than* erythrocytosis or with splenomegaly, our observations were as follows. In addition, we noted the prevalence of thrombosis and their smoking histories in this group.

PATIENTS PRESENTING WITH OTHER CYTOSÉS OR SPLENOMEGALY  
FIGURE B.



Sixty-nine of these patients were proven with an MPN (whether JAK2 positive or negative), 15 patients had a myeloid malignancy and 19 patients were systemically ill and had other explanations for the cytosés.

1. Within the MPN proved group:

ET was diagnosed in 41 cases, with JAK2 positive in 17 (42%). Twenty-eight PMF cases were identified, of whom 16 (57%) JAK2 mutated.

2. Within the myeloid malignancy group:

In 15 cases the JAK2 analysis was done in the presence of other known myeloid disorders (aplastic anaemia n=2, CML n=4, myelodysplastic-myeloproliferative neoplasms n=5, acute myeloid leukaemia n=4) to explore fibrotic or proliferative features. The JAK2 mutation was detected in the 2 patients (13%) diagnosed with MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)(Arber, *et al* 2016).

3. Nineteen systemically ill patients who had cytosés were investigated and none were JAK2 mutated – deemed reactive because cytosés were explained by the clinical syndromes (figure 1).

**Thrombosis prevalence and smoking history at diagnosis:** No thromboses recorded in PMF patients or in the 19 patients with reactive cytosés. Thromboses were recorded in 42% of ET (see figure 1). JAK2 was not significantly associated with thrombosis in ET (p=0.7). There were 24 smokers (59%) among ET patients and 16 (57%) among PMF patients.

**Malignancies diagnosed at or within 6 months of MPN evaluation:** One ET patient (2%), 2 PMF patients (7%) and 7 patients with reactive cytosés (41%) had newly diagnosed malignancies.

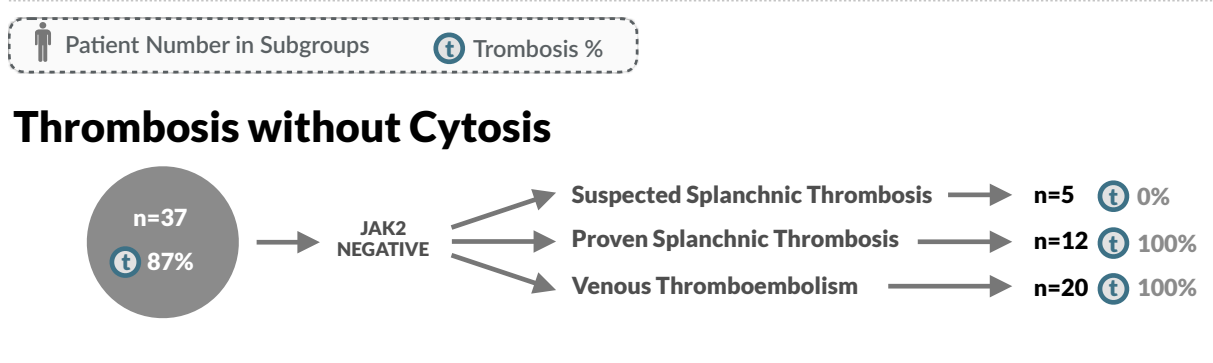
**Splenomegaly at diagnosis:** Spleen clinically or radiologically enlarged in 5 ET patients. All PMF patients had splenomegaly. Splenic mass present in one patient with reactive cytosés.

## Patients Investigated Solely For Thrombotic Events: n=37

In this group of patients with thromboses, the JAK2 mutation was requested in the absence of any form of cytosis, whether it be erythro-, thrombo- or leukocytosis. We looked at the presumed reasoning behind the requesting of a JAK2 in this setting. All 37 patients were JAK2 negative.

### PATIENTS PRESENTING WITH THROMBOSIS BUT NO CYTOSIS

FIGURE C.



1. In 20 patients (stroke n=5; VTE n=15) the prompt for JAK2 testing was a thrombotic event, unaccompanied by cytosis or splenomegaly.
2. In 17 patients without cytosises investigation was performed for intra-abdominal thrombotic events, of whom 12 with proven splanchnic vein thrombosis: (Budd-Chiari syndrome n=6, portal vein thrombosis n=4, inferior vena cava thrombosis n=2)
3. Five patients had no thrombotic event, yet were investigated for possible thrombosis in the diagnostic work-up of liver cirrhosis.

## Overall Occurrence of Thrombotic Events in All Patients:

Figure 1 gives a schematic representation of thrombotic events as they occurred in all subgroups. Apart from the 37 patients solely investigated for thrombosis, there were thrombotic events in 14/138 patients investigated for erythrocytosis (table 1), and in 17 patients (with ET) of 103 patients investigated for cytosises. JAK2 positive splanchnic vein thrombosis were exclusively found in the cytosises groups: ET (n=2; PV (n=1). Patients with splanchnic vein thrombosis investigated in the subgroup without cytosises were JAK2 negative.

## Discussion

A myeloproliferative neoplasm could be detected in 43% of the study population investigated with JAK2 mutation analysis. The distribution of the JAK2 mutation in the PV (100%), ET (42%) and PMF (57%) groups was in the region of previously reported values.(Kralovics, *et al* 2005, Tefferi and Barbui 2015) In the small group of patients with other myeloid disorders, the JAK2 mutation was found in 13%, namely two patients with MDS/MPN-RS-T.

Using bone marrow histology in conjunction with the WHO diagnostic criteria, it was not difficult to distinguish ET and PMF patients from those with non-clonal reactive and inflammatory cytoses. PMF was perfectly correlated with splenomegaly, but the same could not be said of ET cases. Therefore, absence of splenomegaly was not a good indicator of reactive cytoses. Moreover, malignancies occurred as frequently at diagnosis among patients with clonal cytoses than with non-clonal cytoses. Nothing in our analysis suggested that the baseline clinical scenario of ET and PMF patients could be easily distinguished from that of non-clonal cytoses cases. Given the low numbers of non-clonal cases tested over a seven-year period in our institution, it is clear that the JAK2 mutation analysis was not indiscriminately employed to explore reactive thrombocytosis and leukocytosis.

In cases investigated for erythrocytosis, we expected to find some clinical scenarios that should have directed the clinician away from the JAK2 test. Indeed, in a few patients the erythrocytosis was clearly the result of a medical intervention. In a cost-restrained environment JAK2 testing in these individuals may better be avoided. The fact that erythrocytosis post-splenectomy is due to redistribution of blood volume was beautifully illustrated in the normal RCM recorded in these patients. The link between androgen replacement therapy and a rising hb/hct should also be well known, but it is intriguing that RCM study in these individuals revealed spurious erythrocytosis, suggesting androgens may induce a reduced plasma volume rather than raised red cell mass(Dickerman, *et al* 1999, Messinezy and Pearson 1993, Spivak and Silver 2008).

In a minority (14%) of cases, erythrocytosis was not sustained beyond the initial episode and JAK2 mutation analyses were consistently negative. Also in these cases the JAK2 mutation analysis may have been requested prematurely and might better have been re-considered if erythrocytosis persisted at subsequent follow-up.

There was only one secondary erythrocytosis case – with congenital cardiac cyanosis – where JAK2 testing was clearly inappropriate. Ruling out the other patients with secondary erythrocytosis was only possible with the clarity afforded by retrospective analysis and in these cases JAK2 testing was essential. All these secondary cases had high sEPO. In contrast, in two patients with JAK2 negative erythrocytosis and underlying malignancy, extremely low sEPO levels erroneously pointed towards MPN instead of a secondary cause. After resection of tumors in both patients the erythrocytosis – presumably driven by an EPO-like substance - resolved completely. This was an unanticipated finding as tumor-related erythrocytosis is supposed to be caused by high EPO levels.(Samyn, *et al* 2004) We were unable to find similar cases in the literature.

JAK2 mutation analysis made it possible to unequivocally diagnose PV in 37% of erythrocytosis cases. Even in PV cases with thrombocytosis, we had no difficulty distinguishing PV from ET – a hb/hct that was not in the normal range for our laboratory consigned a patient to PV as opposed to ET. This dispensed with the spectre of 'masked polycythemia vera' and accords with the lower hb/hct threshold in JAK2 positive PV that is now suggested in the revised WHO classification(Arber, *et al* 2016, Barbui, *et al* 2014b, Barbui, *et al* 2015)

In JAK2 negative erythrocytosis cases with low sEPO, we investigated if low sEPO was associated with true clonal PV cases. However, in none of these cases the BME findings were consistent with MPN. Therefore, we could not confirm even a single JAK2 negative PV in our series. In agreement with others, we found low sEPO to have no added diagnostic value (Ancochea, *et al* 2014). Diagnostic accuracy in our patients was in accordance with the literature with a low sEPO in only 71% of JAK2 positive PV cases (Ancochea, *et al* 2014).

We expected the remaining third of erythrocytosis cases without clear primary or secondary causes to be a diagnostic challenge. British and Italian investigators have done the bulk of work trying to make sense of idiopathic erythrocytosis (IE) (McMullin 2009, Randi, *et al* 2016). By definition patients in their registries have an absolute erythrocytosis of idiopathic origin, manifested either by a RCM of 125%, or a raised hct above 52 in males and 48 in females. The 44 patients classified as IE all had hb/hct values consistent with an absolute increase in red cell mass according to the WHO and BCSH criteria (see Table 1). By definition FBC findings alone are sufficient to classify patients with IE (and therefore absolute erythrocytosis), however, in the majority (23/27) of our IE patients, RCM revealed an apparent and not an absolute erythrocytosis (Johansson, *et al* 2005, McMullin 2012). No further clarification was gained from sEPO, as patients with IE have variable low and high levels unrelated to RCM, as was also confirmed in our cohort (McMullin 2012).

Thus, with few exceptions, an apparent increase in RCM was associated with IE patients. And without exception, an absolute increase in RCM was observed in JAK2 positive PV patients. This suggests an innate difference in the actual red cell mass between clonal and non-clonal erythropoiesis, regardless of the appearance on peripheral blood. In spite of the similar laboratory indices, PV patients are more polycythaemic than IE patients by this measure. Note furthermore that the higher BCSH A1 criterium for absolute erythrocytosis (hct >60 in males and >56 in females) was present at diagnosis in only 41% of PV and in 44% of IE patients. This percentage is at once much lower than expected for patients with supposed absolute erythrocytosis, as well as startlingly similar for two patient groups with completely disparate RCM results. Expert opinion maintains that a hct >60, is always correlated with an absolute increase and needs no confirmation with RCM to prove absolute erythrocytosis (McMullin 2012). Our results do not confirm this, and on the contrary indicate that RCM is a more stringent measurement of raised whole body red cell volume and is more closely aligned with clonal erythropoiesis than hb/hct levels.

Another good indicator of the lack of clonality underlying IE, is that, consistent with the findings of Randi *et al.*, not a single one of our IE patients developed thrombocytosis following repeated venesection (Randi, *et al* 2016). This in contrast to the majority of JAK2 positive PV cases in our series needing cytoreductive therapy for thrombocytosis in addition to venesection. Evidence is lacking that IE patients should be managed in the same way as PV patients but venesection to keep the hematocrit at least below 50% is widely practised (Randi, *et al* 2016). Even through questions of etiology and management remain, these patients should be clearly labelled 'idiopathic erythrocytosis' so that they can be expertly followed and studied for as yet obscure clonal or non-clonal mechanisms of disease.

Based on an empiric impression of the high incidence of smoking, it was at first tempting to explain away idiopathic erythrocytosis as 'smoker's polycythemia'. The latter remains a poorly delineated concept where the significance of a raised carboxyhemoglobin or raised red cell mass and the overlap with early chronic lung disease is difficult to define in the clinical setting. However, the similarly high prevalence of smoking in both our PV and IE population, did not point to an additional contribution of smoking to idiopathic erythrocytosis. Indeed, the prevalence of lifestyle (smoking) and metabolic (thrombosis) determinants between clonal and non-clonal erythrocytosis were strikingly similar in our analysis (Table 1).

Thrombosis and smoking were equally prevalent at diagnosis in JAK2 mutated vs unmutated ET, and thrombosis at 42%, was higher than the 14% incidence in a recent analysis from the Mayo clinic(Haider, *et al* 2016). Perhaps in our population this is related to poor access to health care resulting in sustained thrombocytosis with late diagnoses and more thrombotic events. In our patient population with PV, thrombosis was seen in 29%, not dissimilar to the recently reported 21% thrombosis at diagnosis in 1545 PV patients(Barbui, *et al* 2014a). Not a single patient presenting with thrombosis without cytos tested positive for MPN. Cytoses at diagnosis was also a hallmark of the 3 MPN patients with splanchnic vein thrombosis in our series, although splanchnic vein thrombosis cases with masked MPN have been described in the literature. Our results confirm that, with the possible exclusion of splanchnic vein thrombosis, the JAK2 mutation analysis should not be requested in patients with thrombosis without cytos(Yonal, *et al* 2012). It is noteworthy that in our cohort, fewer patients with splanchnic thromboses were proven to have a myeloproliferative neoplasm, than comparatively described in the literature.

Chronic inflammation and metabolic dysregulation have been shown to influence pathogenesis of MPN(Lindholm Sorensen and Hasselbalch 2015, Sorensen and Hasselbalch 2016). Our limited analysis, however, show the features of smoking and thrombosis to be equally present in clonal and non-clonal cytos. This is a novel finding and, in future we aim to further explore lifestyle and cardiovascular risk factors in patients investigated for MPN.

We conclude that the JAK2 mutation used in conjunction with the BCSH and 2008 WHO algorithms was an excellent clinical tool for differentiating between true clonal myeloproliferation and reactive cytos. Our analysis confirms that the lower hb/hct thresholds in PV are a logical step in the JAK2 era, as now also formalised in the 2016 WHO algorithm(Arber, *et al* 2016). The WHO guidelines for PV brought a new emphasis on trilineage marrow proliferation to the exclusion of other minor criteria and our results confirm that BME is indispensable to differentiate between clonal and non-clonal JAK2 negative erythrocytosis. RCM, which has become redundant in JAK2 positive PV, remains an important tool to further characterise JAK2 negative erythrocytosis(Alvarez-Larran, *et al* 2012), however, due to limited availability its use is restricted. Our finding regarding the lack of agreement between peripheral blood erythrocytosis and RCM measurements in patients with IE has clinical and pathogenetic implications for further study.

## References

- Alvarez-Larran, A., Ancochea, A., Angona, A., Pedro, C., Garcia-Pallarols, F., Martinez-Aviles, L., Bellosillo, B. & Besses, C. (2012) Red cell mass measurement in patients with clinically suspected diagnosis of polycythemia vera or essential thrombocythemia. *Haematologica*, 97, 1704-1707.
- Ancochea, A., Alvarez-Larran, A., Morales-Indiano, C., Garcia-Pallarols, F., Martinez-Aviles, L., Angona, A., Senin, A., Bellosillo, B. & Besses, C. (2014) The role of serum erythropoietin level and jak2 v617f allele burden in the diagnosis of polycythaemia vera. *British Journal of Haematology*.
- Arber, D.A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M.J., Le Beau, M.M., Bloomfield, C.D., Cazzola, M. & Vardiman, J.W. (2016) The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*, 127, 2391-2405.
- Assi, T.B. & Baz, E. (2014) Current applications of therapeutic phlebotomy. *Blood Transfus*, 12 Suppl 1, s75-83.
- Barbui, T., Carobbio, A., Rumi, E., Finazzi, G., Gisslinger, H., Rodeghiero, F., Randi, M.L., Rambaldi, A., Gisslinger, B., Pieri, L., Bertozzi, I., Casetti, I., Pardanani, A., Passamonti, F., Vannucchi, A.M. & Tefferi, A. (2014a) In contemporary patients with polycythemia vera, rates of thrombosis and risk factors delineate a new clinical epidemiology. *Blood*, 124, 3021-3023.
- Barbui, T., Thiele, J., Carobbio, A., Guglielmelli, P., Rambaldi, A., Vannucchi, A.M. & Tefferi, A. (2014b) Discriminating between essential thrombocythemia and masked polycythemia vera in JAK2 mutated patients. *Am J Hematol*, 89, 588-590.
- Barbui, T., Thiele, J., Vannucchi, A.M. & Tefferi, A. (2014c) Rethinking the diagnostic criteria of polycythemia vera. *Leukemia*, 28, 1191-1195.
- Barbui, T., Thiele, J., Vannucchi, A.M. & Tefferi, A. (2015) Rationale for revision and proposed changes of the WHO diagnostic criteria for polycythemia vera, essential thrombocythemia and primary myelofibrosis. *Blood Cancer J*, 5, e337.
- Baxter, E.J., Scott, L.M., Campbell, P.J., East, C., Fourouclas, N., Swanton, S., Vassiliou, G.S., Bench, A.J., Boyd, E.M., Curtin, N., Scott, M.A., Erber, W.N. & Green, A.R. (2005a) Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *The Lancet*, 365, 1054-1061.
- Beer, P.A., Erber, W.N., Campbell, P.J. & Green, A.R. (2011) How I treat essential thrombocythemia. *Blood*, 117, 1472-1482.
- Bench, A.J., White, H.E., Foroni, L., Godfrey, A.L., Gerrard, G., Akiki, S., Awan, A., Carter, I., Goday-Fernandez, A., Langabeer, S.E., Clench, T., Clark, J., Evans, P.A., Grimwade, D., Schuh, A., McMullin, M.F., Green, A.R., Harrison, C.N., Cross, N.C. & British Committee for Standards in, H. (2013) Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant mutations. *Br J Haematol*, 160, 25-34.
- Bleeker, J.S. & Hogan, W.J. (2011) Thrombocytosis: diagnostic evaluation, thrombotic risk stratification, and risk-based management strategies. *Thrombosis*, 2011, 536062.
- Colaizzo, D., Amitrano, L., Tiscia, G.L., Scenna, G., Grandone, E., Guardascione, M.A., Brancaccio, V. & Margaglione, M. (2007) The JAK2 V617F mutation frequently occurs in patients with portal and mesenteric venous thrombosis. *J Thromb Haemost*, 5, 55-61.
- Cook, L.S. (2010) Therapeutic phlebotomy: a review of diagnoses and treatment considerations. *J Infus Nurs*, 33, 81-88.
- Dameshek, W. (1950) Physiopathology and course of polycythemia vera as related to therapy. *J Am Med Assoc*, 142, 790-797.
- de Lacerda, J.F., Oliveira, S.N. & Ferro, J.M. (2014) Chronic myeloproliferative diseases. *Handb Clin Neurol*, 120, 1073-1081.
- De Stefano, V., Fiorini, A., Rossi, E., Za, T., Farina, G., Chiusolo, P., Sica, S. & Leone, G. (2007) Incidence of the JAK2 V617F mutation among patients with splanchnic or cerebral venous thrombosis and without overt chronic myeloproliferative disorders. *J Thromb Haemost*, 5, 708-714.

- Dentali, F., Squizzato, A., Brivio, L., Appio, L., Campiotti, L., Crowther, M., Grandi, A.M. & Ageno, W. (2009a) JAK2V617F mutation for the early diagnosis of Ph- myeloproliferative neoplasms in patients with venous thromboembolism: a meta-analysis. *Blood*, 113, 5617-5623.
- Dickerman, R.D., Pertusi, R., Miller, J. & Zachariah, N.Y. (1999) Androgen-induced erythrocytosis: is it erythropoietin? *American Journal of Hematology*, 61, 154-155.
- Elliott, M.A. & Tefferi, A. (2005) Thrombosis and haemorrhage in polycythaemia vera and essential thrombocythaemia. *Br J Haematol*, 128, 275-290.
- Fenaux, P., Simon, M., Caulier, M.T., Lai, J.L., Goudemand, J. & Bauters, F. (1990) Clinical course of essential thrombocythemia in 147 cases. *Cancer*, 66, 549-556.
- Finazzi, G., Gregg, X.T., Barbui, T. & Prchal, J.T. (2006) Idiopathic erythrocytosis and other non-clonal polycythemia. *Best Practice & Research Clinical Haematology*, 19, 471-482.
- Folman, C.C., Ooms, M., Kuenen, B.B., de Jong, S.M., Vet, R.J., de Haas, M. & von dem Borne, A.E. (2001) The role of thrombopoietin in post-operative thrombocytosis. *Br J Haematol*, 114, 126-133.
- Gerds, A.T. (2014) I Walk the Other Line: Myelodysplastic/Myeloproliferative Neoplasm Overlap Syndromes. *Current Hematologic Malignancy Reports*, 9, 400-406.
- Haider, M., Gangat, N., Lasho, T., Abou Hussein, A.K., Elala, Y.C., Hanson, C. & Tefferi, A. (2016) Validation of the revised International Prognostic Score of Thrombosis for Essential Thrombocythemia (IPSET-thrombosis) in 585 Mayo Clinic patients. *American Journal of Hematology*.
- Harrison, C.N. & Green, A.R. (2003) Essential thrombocythemia. *Hematol Oncol Clin North Am*, 17, 1175-1190, vii.
- Hermans, C., Slachmuylder, V., Gala, J.L., Lambert, C. & Detrait, M. (2008) The JAK2 V617F mutation is not a cause of central retinal vein occlusion. *Thromb Haemost*, 100, 515-516.
- Jacob, M., Annaheim, S., Boutellier, U., Hinske, C., Rehm, M., Breymann, C. & Krafft, A. (2012) Haematocrit is invalid for estimating red cell volume: a prospective study in male volunteers. *Blood Transfus*, 10, 471-479.
- James, C., Ugo, V., Le Couedic, J.P., Staerk, J., Delhommeau, F., Lacout, C., Garcon, L., Raslova, H., Berger, R., Bennaceur-Griscelli, A., Villeval, J.L., Constantinescu, S.N., Casadevall, N. & Vainchenker, W. (2005a) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*, 434, 1144-1148.
- Janssen, H.L. & Leebeek, F.W. (2006) JAK2 mutation: The best diagnostic tool for myeloproliferative disease in splanchnic vein thrombosis? *Hepatology*, 44, 1391-1393.
- Johansson, P.L., Safai-Kutti, S. & Kutti, J. (2005) An elevated venous haemoglobin concentration cannot be used as a surrogate marker for absolute erythrocytosis: a study of patients with polycythaemia vera and apparent polycythaemia. *Br J Haematol*, 129, 701-705.
- Kesler, A., Ellis, M.H., Manor, Y., Gadoth, N. & Lishner, M. (2000) Neurological complications of essential thrombocytosis (ET). *Acta Neurol Scand*, 102, 299-302.
- Kiladjian, J.J. (2012) The spectrum of JAK2-positive myeloproliferative neoplasms. *Hematology Am Soc Hematol Educ Program*, 2012, 561-566.
- Kiladjian, J.J., Cervantes, F., Leebeek, F.W., Marzac, C., Cassinat, B., Chevret, S., Cazals-Hatem, D., Plessier, A., Garcia-Pagan, J.C., Darwish Murad, S., Raffia, S., Janssen, H.L., Gardin, C., Cereja, S., Tonetti, C., Giraudier, S., Condat, B., Casadevall, N., Fenaux, P. & Valla, D.C. (2008) The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. *Blood*, 111, 4922-4929.
- Klampfl, T., Gisslinger, H., Harutyunyan, A.S., Nivarthi, H., Rumi, E., Milosevic, J.D., Them, N.C., Berg, T., Gisslinger, B., Pietra, D., Chen, D., Vladimer, G.I., Bagienski, K., Milanese, C., Casetti, I.C., Sant'Antonio, E., Ferretti, V., Elena, C., Schischlik, F., Cleary, C., Six, M., Schalling, M., Schonegger, A., Bock, C., Malcovati, L., Pascutto, C., Superti-Furga, G., Cazzola, M. & Kralovics, R. (2013) Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med*, 369, 2379-2390.

Kralovics, R., Passamonti, F., Buser, A.S., Teo, S.S., Tiedt, R., Passweg, J.R., Tichelli, A., Cazzola, M. & Skoda, R.C. (2005) A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*, 352, 1779-1790.

Levine, R.L., Loriaux, M., Huntly, B.J.P., Loh, M.L., Beran, M., Stoffregen, E., Berger, R., Clark, J.J., Willis, S.G., Nguyen, K.T., Flores, N.J., Estey, E., Gattermann, N., Armstrong, S., Look, A.T., Griffin, J.D., Bernard, O.A., Heinrich, M.C., Gilliland, D.G., Druker, B. & Deininger, M.W.N. (2005a) The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood*, 106, 3377-3379.

Levine, R.L., Wadleigh, M., Cools, J., Ebert, B.L., Wernig, G., Huntly, B.J., Boggon, T.J., Wlodarska, I., Clark, J.J., Moore, S., Adelsperger, J., Koo, S., Lee, J.C., Gabriel, S., Mercher, T., D'Andrea, A., Frohling, S., Dohner, K., Marynen, P., Vandenberghe, P., Mesa, R.A., Tefferi, A., Griffin, J.D., Eck, M.J., Sellers, W.R., Meyerson, M., Golub, T.R., Lee, S.J. & Gilliland, D.G. (2005b) Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*, 7, 387-397.

Lindholm Sorensen, A. & Hasselbalch, H.C. (2015) Smoking and philadelphia-negative chronic myeloproliferative neoplasms. *European Journal of Haematology*.

Lorberboym, M., Rahimi-Levene, N., Lipszyc, H. & Kim, C.K. (2005) Analysis of red cell mass and plasma volume in patients with polycythemia. *Arch Pathol Lab Med*, 129, 89-91.

Malcovati, L., Della Porta, M.G., Pietra, D., Boveri, E., Pellagatti, A., Galli, A., Travaglio, E., Brisci, A., Rumi, E., Passamonti, F., Invernizzi, R., Cremonesi, L., Boulwood, J., Wainscoat, J.S., Hellstrom-Lindberg, E. & Cazzola, M. (2009) Molecular and clinical features of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. *Blood*, 114, 3538-3545.

Marchioli, R., Finazzi, G., Landolfi, R., Kutti, J., Gisslinger, H., Patrono, C., Marilus, R., Villegas, A., Tognoni, G. & Barbui, T. (2005) Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. *J Clin Oncol*, 23, 2224-2232.

Martinelli, I. & De Stefano, V. (2010) Rare thromboses of cerebral, splanchnic and upper-extremity veins. A narrative review. *Thromb Haemost*, 103, 1136-1144.

Martinelli, I. & De Stefano, V. (2012) Extra-abdominal venous thromboses at unusual sites. *Best Practice & Research Clinical Haematology*, 25, 265-274.

Martinelli, I., De Stefano, V., Carobbio, A., Randi, M.L., Santarossa, C., Rambaldi, A., Finazzi, M.C., Cervantes, F., Arellano-Rodrigo, E., Rupoli, S., Canafoglia, L., Tieghi, A., Facchini, L., Betti, S., Vannucchi, A.M., Pieri, L., Cacciola, R., Cacciola, E., Cortelezzi, A., Iurlo, A., Pogliani, E.M., Elli, E.M., Spadea, A. & Barbui, T. (2014) Cerebral vein thrombosis in patients with Philadelphia-negative myeloproliferative neoplasms. An European Leukemia Net study. *Am J Hematol*, 89, E200-205.

McClure, R., Mai, M. & Lasho, T. (2006) Validation of two clinically useful assays for evaluation of JAK2 V617F mutation in chronic myeloproliferative disorders. *Leukemia*, 20, 168-171.

McMullin, M.F. (2009) Idiopathic erythrocytosis: a disappearing entity. *Hematology Am Soc Hematol Educ Program*, 629-635.

McMullin, M.F. (2012) Diagnosis and management of congenital and idiopathic erythrocytosis. *Ther Adv Hematol*, 3, 391-398.

McMullin, M.F., Bareford, D., Campbell, P., Green, A.R., Harrison, C., Hunt, B., Oscier, D., Polkey, M.I., Reilly, J.T., Rosenthal, E., Ryan, K., Pearson, T.C., Wilkins, B. & General Haematology Task Force of the British Committee for Standards in, H. (2005) Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. *Br J Haematol*, 130, 174-195.

McMullin, M.F., Reilly, J.T., Campbell, P., Bareford, D., Green, A.R., Harrison, C.N., Conneally, E. & Ryan, K. (2007) Amendment to the guideline for diagnosis and investigation of polycythaemia/erythrocytosis. *Br J Haematol*, 138, 821-822.

Messinezy, M. & Pearson, T.C. (1993) Apparent polycythaemia: diagnosis, pathogenesis and management. *European Journal of Haematology*, 51, 125-131.

Messinezy M, W.N., El-Hemaidi I, Marsden JT, Sherwood RS, Pearson TC (2002) Serum erythropoietin values in erythrocytoses and in primary thrombocythaemia.pdf. *Br J Haematol*.

- Michiels, J.J., Berneman, Z., Schroyens, W. & De Raeve, H. (2015) Changing concepts of diagnostic criteria of myeloproliferative disorders and the molecular etiology and classification of myeloproliferative neoplasms: from Dameshek 1950 to Vainchenker 2005 and beyond. *Acta Haematologica*, 133, 36-51.
- Michiels, J.J., De Raeve, H., Berneman, Z., Van Bockstaele, D., Hebeda, K., Lam, K. & Schroyens, W. (2006) The 2001 World Health Organization and updated European clinical and pathological criteria for the diagnosis, classification, and staging of the Philadelphia chromosome-negative chronic myeloproliferative disorders. *Semin Thromb Hemost*, 32, 307-340.
- Mossuz P, G.F., Magali D (2004) Diagnostic value of serum EPO.pdf. *Haematologica*, 1194-1198.
- Mossuz, P., Girodon, F., Donnard, M., Latger-Cannard, V., Dobo, I., Boiret, N., Lecron, J.C., Binquet, C., Barro, C., Hermouet, S. & Praloran, V. (2004) Diagnostic value of serum erythropoietin level in patients with absolute erythrocytosis. *Haematologica*, 89, 1194-1198.
- Nielsen, C., Birgens, H.S., Nordestgaard, B.G., Kjær, L. & Bojesen, S.E. (2011) The JAK2 V617F somatic mutation, mortality and cancer risk in the general population. *Haematologica*, 96, 450-453.
- Okamura, T. (2003) Medical progress in idiopathic myelofibrosis. *Intern Med*, 42, 461-462.
- Ozer, F.L., Truax, W.E., Miesch, D.C. & Levin, W.G. Primary hemorrhagic thrombocythemia. *The American Journal of Medicine*, 28, 807-823.
- Passamonti, F. (2012) How I treat polycythemia vera. *Blood*, 120, 275-284.
- Passamonti, F., Malabarba, L., Orlandi, E., Barate, C., Canevari, A., Brusamolino, E., Bonfichi, M., Arcaini, L., Caberlon, S., Pascutto, C. & Lazzarino, M. (2003) Polycythemia vera in young patients: a study on the long-term risk of thrombosis, myelofibrosis and leukemia. *Haematologica*, 88, 13-18.
- Passamonti, F., Randi, M.L., Rumi, E., Pungolino, E., Elena, C., Pietra, D., Scapin, M., Arcaini, L., Tezza, F., Moratti, R., Pascutto, C., Fabris, F., Morra, E., Cazzola, M. & Lazzarino, M. (2007) Increased risk of pregnancy complications in patients with essential thrombocythemia carrying the JAK2 (V617F) mutation. *Blood*, 110, 485-489.
- Pearson, T.C., Guthrie, D.L., Simpson, J., Chinn, S., Barosi, G., Ferrant, A., Lewis, S.M. & Najean, Y. (1995) Interpretation of measured red cell mass and plasma volume in adults: Expert Panel on Radionuclides of the International Council for Standardization in Haematology. *British Journal of Haematology*, 89, 748-756.
- Pearson, T.C. & Messinezy, M. (2001) Idiopathic erythrocytosis, diagnosis and clinical management. *Pathologie Biologie*, 49, 170-177.
- Randi, M.L., Bertozzi, I., Cosi, E., Santarossa, C., Peroni, E. & Fabris, F. (2015) Idiopathic erythrocytosis: a study of a large cohort with a long follow-up. *Ann Hematol*.
- Randi, M.L., Bertozzi, I., Cosi, E., Santarossa, C., Peroni, E. & Fabris, F. (2016) Idiopathic erythrocytosis: a study of a large cohort with a long follow-up. *Annals of Hematology*, 95, 233-237.
- Rollison, D.E., Howlader, N., Smith, M.T., Strom, S.S., Merritt, W.D., Ries, L.A., Edwards, B.K. & List, A.F. (2008) Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. *Blood*, 112, 45-52.
- Samyn, I., Fontaine, C., Van Tussenbroek, F., Pipeleers-Marichal, M. & De Greve, J. (2004) Paraneoplastic syndromes in cancer: Case 1. Polycythemia as a result of ectopic erythropoietin production in metastatic pancreatic carcinoid tumor. *Journal of Clinical Oncology*, 22, 2240-2242.
- Schafer, A.I. (2004) Thrombocytosis. *New England Journal of Medicine*, 350, 1211-1219.
- Shetty, S., Kulkarni, B., Pai, N., Mukundan, P., Kasatkar, P. & Ghosh, K. (2010) JAK2 mutations across a spectrum of venous thrombosis cases. *Am J Clin Pathol*, 134, 82-85.
- Silver, R.T., Chow, W., Orazi, A., Arles, S.P. & Goldsmith, S.J. (2013) Evaluation of WHO criteria for diagnosis of polycythemia vera: a prospective analysis. *Blood*, 122, 1881-1886.

- Smalberg, J.H., Arends, L.R., Valla, D.C., Kiladjian, J.J., Janssen, H.L. & Leebeek, F.W. (2012) Myeloproliferative neoplasms in Budd-Chiari syndrome and portal vein thrombosis: a meta-analysis. *Blood*, 120, 4921-4928.
- Smalberg, J.H., Kruip, M.J., Janssen, H.L., Rijken, D.C., Leebeek, F.W. & de Maat, M.P. (2011) Hypercoagulability and hypofibrinolysis and risk of deep vein thrombosis and splanchnic vein thrombosis: similarities and differences. *Arterioscler Thromb Vasc Biol*, 31, 485-493.
- Sorensen, A.L. & Hasselbalch, H.C. (2016) Antecedent cardiovascular disease and autoimmunity in Philadelphia-negative chronic myeloproliferative neoplasms. *Leukemia Research*, 41, 27-35.
- Spivak, J.L. (2003) Diagnosis of the myeloproliferative disorders: resolving phenotypic mimicry. *Semin Hematol*, 40, 1-5.
- Spivak, J.L. & Silver, R.T. (2008) The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis, and primary myelofibrosis: an alternative proposal. *Blood*, 112, 231-239.
- Stein, B.L., Crispino, J.D. & Moliterno, A.R. (2011) Janus kinase inhibitors: an update on the progress and promise of targeted therapy in the myeloproliferative neoplasms. *Current Opinion in Oncology*, 23, 609-616.
- Stein, B.L., Moliterno, A.R. & Tiu, R.V. (2014) Polycythemia vera disease burden: contributing factors, impact on quality of life, and emerging treatment options. *Ann Hematol*, 93, 1965-1976.
- Tefferi, A. (2005) The rise and fall of red cell mass measurement in polycythemia vera. *Curr Hematol Rep*, 4, 213-217.
- Tefferi, A. (2008) Essential thrombocythemia, polycythemia vera, and myelofibrosis: Current management and the prospect of targeted therapy. *American Journal of Hematology*, 83, 491-497.
- Tefferi, A. (2011) How I treat myelofibrosis. *Blood*, 117, 3494-3504.
- Tefferi, A. (2014) Primary myelofibrosis: 2014 update on diagnosis, risk-stratification, and management. *Am J Hematol*, 89, 915-925.
- Tefferi, A. & Barbui, T. (2015) Polycythemia vera and essential thrombocythemia: 2015 update on diagnosis, risk-stratification and management. *Am J Hematol*, 90, 162-173.
- Tefferi, A., Lasho, T.L., Jimma, T., Finke, C.M., Gangat, N., Vaidya, R., Begna, K.H., Al-Kali, A., Ketterling, R.P., Hanson, C.A. & Pardanani, A. (2012) One Thousand Patients With Primary Myelofibrosis: The Mayo Clinic Experience. *Mayo Clinic Proceedings*, 87, 25-33.
- Tefferi, A. & Vardiman, J.W. (2008a) Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia*, 22, 14-22.
- Tefferi, A. & Vardiman, J.W. (2008b) Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia (08876924)*, 22, 14-22.
- Vannucchi, A. (2010) MF from palliation to targeted therapy. *N Engl J Med*, 363, 1180-1182.
- Vannucchi, A. (2014a) how I treat PV.pdf. *Blood*, 124, 3212.
- Vannucchi, A.M. (2014b) How I treat polycythemia vera. *Blood*, 124, 3212-3220.
- Vardiman, J.W., Thiele, J., Arber, D.A., Brunning, R.D., Borowitz, M.J., Porwit, A., Harris, N.L., Le Beau, M.M., Hellstrom-Lindberg, E., Tefferi, A. & Bloomfield, C.D. (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*, 114, 937-951.
- Wang, Y., Xia, J., Wu, X. & Hu, Y. (2015) A Patient With BCR-ABL and JAK2 V617F Double-Positive Myeloproliferative Neoplasm With Overlapping Clinical Phenotypes. *Am J Med Sci*, 350, 68-69.
- Whitlatch, N.L. & Ortel, T.L. (2008) Thrombophilias: when should we test and how does it help? *Semin Respir Crit Care Med*, 29, 25-39.

Yonal, I., Pinarbasi, B., Hindilerden, F., Hancer, V.S., Nalcaci, M., Kaymakoglu, S. & Diz-Kucukkaya, R. (2012) The clinical significance of JAK2V617F mutation for Philadelphia-negative chronic myeloproliferative neoplasms in patients with splanchnic vein thrombosis. *Journal of Thrombosis and Thrombolysis*, 34, 388-396.

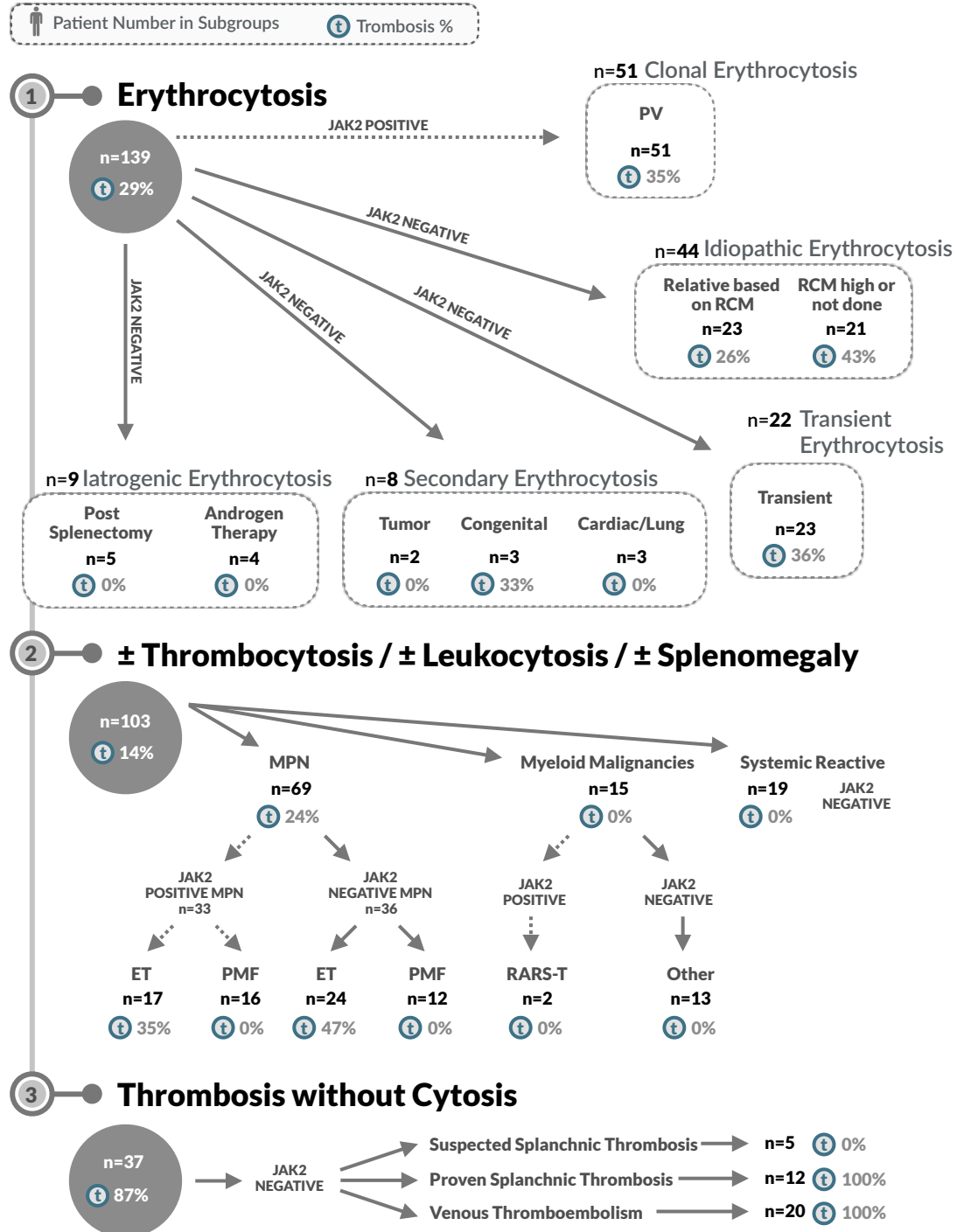
Zhou, A., Knoche, E.M., Engle, E.K., Fisher, D.A. & Oh, S.T. (2015) Concomitant JAK2 V617F-positive polycythemia vera and BCR-ABL-positive chronic myelogenous leukemia treated with ruxolitinib and dasatinib. *Blood Cancer J*, 5, e351.

CHAPTER THREE

# Addendum



Figure 1. Three Clinical Scenarios Prompting JAK V617F analysis in 279 patients: erythrocytosis; thrombocytosis ± leukocytosis ± splenomegaly; and thrombosis without cytos. Subgroups created by application of the JAK2 mutation analysis in conjunction with WHO guidelines in the MPN diagnostic pathway. The incidence of thrombosis at diagnosis in all patient groups is shown.



MPN - Myeloproliferative Neoplasms | PV - Polycythemia Vera |  
 ET - Essential Thrombocytosis | PMF - Primary Myelofibrosis | RCM - Red Cell Mass

## GROOTE SCHUUR HOSPITAL

Enquiries: Dr Bernadette Eick

E-mail : [Bernadette.Schmitz@westerncape.gov.za](mailto:Bernadette.Schmitz@westerncape.gov.za)

Dr E. Verburgh  
Haematology  
5<sup>th</sup> Floor – Chris Barnard Building  
**FACULTY HEALTH SCIENCES**

E-mail: [Estelle.Verburgh@uct.ac.za](mailto:Estelle.Verburgh@uct.ac.za)

Dear Dr Verburgh

**RESEARCH PROJECT: An Audit Of The Implementation Of The JAK2 V617F Mutation Analysis In The Pathway Of Suspected Myeloproliferative Disorders In Groote Schuur Hospital (FCP Candidate: E. Poulet)**

Your recent letter to the hospital refers.

You are hereby granted permission to proceed with your research.

Please note the following:

- a) Your research may not interfere with normal patient care.
- b) Hospital staff may not be asked to assist with the research.
- c) No hospital consumables and stationary may be used.
- d) **No patient folders may be removed from the premises or be inaccessible.**
- e) Please introduce yourself to the person in charge of an area before commencing.
- f) Please discuss the study with HOD before commencing.
- g) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
- h) Confidentiality must be maintained at all times.

I would like to wish you every success with the project.

Yours sincerely



**DR BERNADETTE EICK**  
**CHIEF OPERATIONAL OFFICER**

**Date:** 11<sup>th</sup> March 2015

C.C. Mr. L. Naidoo  
Professor E. Weimann  
Professor B. Mayosi  
Professor N. Novitzky

G46 Management Suite, Old Main Building,  
Observatory 7925

Tel: +27 21 404 6288 fax: +27 21 404 6125

Private Bag X,  
Observatory, 7935

[www.capegateway.gov.za](http://www.capegateway.gov.za)



**UNIVERSITY OF CAPE TOWN**  
**Faculty of Health Sciences**  
**Human Research Ethics Committee**



**Room E52-24 Old Main Building**  
**Groote Schuur Hospital**  
**Observatory 7925**  
**Telephone [021] 406 6492 • Facsimile [021] 406 6411**  
**Email: [Sumayah.ariefdien@uct.ac.za](mailto:Sumayah.ariefdien@uct.ac.za)**  
**Website: [www.health.uct.ac.za/fhs/research/humanethics/forms](http://www.health.uct.ac.za/fhs/research/humanethics/forms)**

**03 March 2015**

**REC/REF: 091/2015**

**Dr E Verburgh**  
Haematology  
5<sup>th</sup> floor  
Chris Barnard Building  
FHS

**Dear Dr Verburgh**

**Project Title: AN ADULT OF THE IMPLEMENTATION OF THE JAK2 V617F MUTATION ANALYSIS IN THE PATHWAY OF SUSPECTED MYELOPROLIFERATIVE DISORDERS IN GROOTE SCHUUR HOSPITAL (FCP candidate E Poulet)**

Thank you for your response letter, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

**Approval is granted for one year until the 28 March 2016.**

Please submit a copy of institutional approval to the HREC.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

***We acknowledge that the following student:- Dr Erma Poulet is also involved in this project.***

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

**Please quote the HREC REF in all your correspondence.**

Yours sincerely

**Signed**

**PROFESSOR M BLOCKMAN**  
**CHAIRPERSON, HSF HUMAN ETHICS**

**Hrec/ref:091/2015**

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

F. NAME

**S. NAME + INITIAL**

ADDRESS

AREA + POSTCODE

**GSH NUMBER**

DOB: ##/##/##

**SEX: M/F (H2)**

TEL #:

### Ward where JAK2 done

This field can contract and expand as required

### If E5 - reason for referral

This field is expandable, enabling the user to enter data directly on the computer

X  
X  
X

### Co-morbidities

This field is expandable, enabling the user to enter data directly on the computer

X  
X  
X

### Radiology

This field can contract and expand as required

X  
X

### Investigations incl. blood & BMB

This field is expandable, enabling the user to enter data directly on the computer

X  
X  
X

### Medication

This field is expandable, enabling the user to enter data directly on the computer

X  
X  
X

### Final diagnosis

This field is expandable, enabling the user to

enter data directly on the computer

X  
X  
X

### JAK2

Appropriate	
Probably appropriate	
Inappropriate	
Probably appropriate	

### APPROVED

Name	Date	Signature

[preferences](#)

Processed on: 01-Sep-2016 21:36 SAST  
 ID: 700273162  
 Originality Report Word Count: 9362  
 Submitted: 1

Document Viewer

Similarity Index	Similarity by Source	
5%	Internet Sources:	3%
	Publications:	3%
	Student Papers:	1%

[include quoted](#) [include bibliography](#) [excluding matches < 12 words](#) ▼

mode: [show highest matches together](#) ▼

## STRUCTURED LITERATURE REVIEW 1.

Introduction 2. The history of the Myeloproliferative Neoplasm 1. Bird's eye view on developing guidelines for MPN 2. The Revelation of the JAK2 V617F mutation 3. JAK2V 617F mutation molecular mechanism 4. Brief summary of developments prompted by JAK2 V617F 3. JAK2 V617F in Haematological Disorders other than Philadelphia negative MPN 4. Exploring the groups within MPN 1. Polycythaemia Vera (PRV) 1. Hb/Hct debate 2. WHO criteria 3. WHO algorithm 4. Serum Erythropoietin 5. Red Cell Mass 6. Bone Marrow Biopsy 7. Endogenous Erythroid Colonies 8. Utility of Venesection 9. A Perplexing Entity: "Idiopathic Erythrocytosis" 2. Essential Thrombocythosis (ET) 1. WHO criteria 2. WHO algorithm 3. Complications of ET 3. Primary Myelofibrosis (PMF) 1. WHO criteria 2. WHO algorithm 5. Spectrum of MPN - Overlap between PRV/ET/PMF 6. JAK2 V617F mutation in the presence of thrombosis 1. The holy grail - clutching at straws for a cause? 2. Splanchnic thrombosis 3. Other sites of thrombosis 7. Conclusion 1 Abbreviations aCML for atypical chronic myeloid leukaemia; BMB for Bone marrow biopsy; BCS for Budd-Chiari Syndrome; BRVO for Branch retinal vein occlusion; CALR for calreticulin mutation; CMML for chronic myelomonocytic leukaemia; CVRO for

1 1% match (Internet from 06-Apr-2016)  
<http://atvb.ahajournals.org>

2 1% match (publications)  
 Ayalew Tefferi. "Myeloproliferative Neoplasms: Essential Thrombocythemia, Polycythemia Vera, and Primary Myelofibrosis", Practical Hemostasis and Thrombosis Key/Practical Hemostasis and Thrombosis, 05/05/2010

3 1% match (Internet from 19-Mar-2010)  
<http://www.nature.com>

4 < 1% match (publications)  
 Martinelli, Ida, and Valerio De Stefano. "Extra-abdominal venous thromboses at unusual sites", Best Practice & Research Clinical Haematology, 2012.

5 < 1% match (publications)  
 Jan Michiels. "The 2001 World Health Organization and Updated European Clinical and Pathological Criteria for the Diagnosis, Classification, and Staging of the Philadelphia Chromosome-Negative Chronic Myeloproliferative Disorders". Seminars in Thrombosis and Hemostasis, 06/2006

6 < 1% match (publications)  
 Randi, Maria Luigia, Irene Bertozzi, Elisabetta Cosi, Claudia Santarossa, Edoardo Peroni, and Fabrizio Fabris. "Idiopathic



Signed



Signed

Estelle Verburgh

## ***The British Journal of Haematology* Instructions to Authors**

All papers should be submitted on the world wide web at <http://mc.manuscriptcentral.com/bjh>. Authors will need their entire manuscript in electronic format. Full instructions, a user ID and password are available at the site. Microsoft Word, Powerpoint and Excel files are automatically converted to PDF format for compatibility. Other recommended files types are RTF, GIF and JPEG. Users may have difficulties viewing other file types. Where possible, files should be combined and artwork embedded in the main manuscript file to aid users in downloading and viewing papers. Technical support for submission can be obtained from the ScholarOne Support website at <http://mcv3support.custhelp.com> or by telephoning +1 434 817 2040 ext 167.

Editorial correspondence should be sent to *BJH Office, 11 Auchinbaird, Sauchie, Alloa FK10 3HB, UK, Telephone and Fax +44 (0) 1259 220869, e-mail [lorna@bjhaem.co.uk](mailto:lorna@bjhaem.co.uk)*. Authors unable to submit their manuscript online should contact the BJH Office.

### **Submission of papers**

*The British Journal of Haematology* invites papers on original research in clinical, laboratory and experimental haematology. All papers should include only new data which have not been published elsewhere. All authors are expected to disclose any commercial affiliations as well as consultancies, stock or equity interests and patent-licensing arrangements that could be considered to pose a conflict of interest regarding the submitted article. Specifics of such disclosures will remain confidential. If appropriate, general statements in the acknowledgements regarding such disclosures may be recommended by the editors.

Wiley Blackwell will dispose of all hardcopy or electronic material submitted two issues after publication.

### **NEW: Pre-submission English-language editing**

Authors for whom English is a second language may choose to have their manuscript professionally edited before submission to improve the English. A list of independent suppliers of editing services can be found at [http://authorservices.wiley.com/bauthor/english\\_language.asp](http://authorservices.wiley.com/bauthor/english_language.asp). All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

### **Ethical policy and guidelines**

*BJH* encourages its contributors and reviewers to adopt the standards of the [International Committee of Medical Journal Editors](#). *BJH* will not consider papers that have been accepted for publication or published elsewhere. Copies of existing manuscripts with potentially overlapping or duplicative material should be submitted together with the manuscript, so that the Editors can judge suitability for publication. The Editors reserve the right to reject a paper on ethical grounds.

**Please read the Ethical Policies of BJH by clicking [here](#).**

### **Disclosure and competing interests statement**

Authors are required to disclose financial interests in any company or institution that might benefit from their publication. A competing interest exists when a primary interest (such as patients' welfare or the validity of research) might be influenced by a secondary interest (such as financial gain or personal rivalry).

It may arise for the authors of a British Journal of Haematology article when they have a financial interest that may influence their interpretation of their results or those of others. Financial interests are

paper, E contributed the knockout mice for the study and G designed the research study and wrote the paper’.

### **Role of professional medical writers in peer-reviewed publications**

Please ensure that you follow the guidelines by the [European Medical Writers Association](#) on the role of medical writers. The guidelines emphasise the importance of respecting widely recognised authorship criteria, and in particular of ensuring that all people listed as named authors have full control of the content of papers. The role of professional medical writers must be transparent. Please name any professional medical writer among the list of contributors to any article for *British Journal of Haematology* (not only original research papers), and specify in the acknowledgements and statement of competing interests for the article who paid the writer. Writers and authors must have access to relevant data while writing papers.

### **Copyright**

If your paper is accepted, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services; where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper.

### **For authors signing the copyright transfer agreement**

If the OnlineOpen option is not selected the corresponding author will be presented with the copyright transfer agreement (CTA) to sign. The terms and conditions of the CTA can be previewed in the samples associated with the Copyright FAQs below:

CTA Terms and Conditions [http://exchanges.wiley.com/authors/faqs---copyright-\\_301.html](http://exchanges.wiley.com/authors/faqs---copyright-_301.html)

### **For authors choosing [OnlineOpen](#)**

If the OnlineOpen option is selected the corresponding author will have a choice of the following Creative Commons License Open Access Agreements (OAA):

Creative Commons Attribution Non-Commercial License OAA

Creative Commons Attribution Non-Commercial -NoDerivs License OAA

To preview the terms and conditions of these open access agreements please visit the Copyright FAQs hosted on Wiley Author Services [http://exchanges.wiley.com/authors/faqs---copyright-\\_301.html](http://exchanges.wiley.com/authors/faqs---copyright-_301.html) and visit <http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html>.

If you select the OnlineOpen option and your research is funded by certain funders [e.g. The Wellcome Trust and members of the Research Councils UK (RCUK) or the Austrian Science Fund (FWF)], you will be given the opportunity to publish your article under a CC-BY license supporting you in complying with your Funder requirements. For more information on this policy and the Journal’s compliant self-archiving policy please visit: <http://www.wiley.com/go/funderstatement>.

For RCUK, Wellcome Trust, FWF authors click on the link below to preview the terms and conditions of this license:

Creative Commons Attribution License OAA

To preview the terms and conditions of these open access agreements please visit the Copyright FAQs hosted on Wiley Author Services [http://exchanges.wiley.com/authors/faqs---copyright-\\_301.html](http://exchanges.wiley.com/authors/faqs---copyright-_301.html) and visit <http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html>.

## **Research papers**

The majority of papers published in the Journal report original research into scientific and clinical haematology. All papers are subject to review and authors are urged to be brief; long papers with many tables and figures may require shortening if they are to be accepted for publication.

## **Short reports**

Short reports which offer significant insight into scientific and clinical haematological processes may be published. They may include up to 1500 words of text, two figures or tables or one of each, and up to 15 references. A summary of up to 100 words should be followed by continuous text, subdivided if appropriate. Short reports could include important preliminary observations, short methods papers, therapeutic advances, and any significant scientific or clinical observations which are best published in this format. Publication of initial results which will lead to more substantial papers will generally be discouraged. Although submission of case reports is not encouraged, these will be considered if the report includes novel scientific material or is of especial clinical interest. Authors will receive proofs.

## **Annotations and reviews**

These are normally invited contributions but suitable papers may be submitted to the Editor for consideration for this purpose. Previous issues of the Journal should be consulted for style of contribution and length.

## **Letters to the Editor**

Correspondence which relates to papers which have recently appeared in the Journal may be published. The Editor reserves the right to invite response from the original authors for publication alongside. In addition, letters dealing with more general scientific matters of interest to haematologists will be considered. Letters should be as short as possible (but no more than 1000 words of text, two figures or tables or one of each, and up to 10 references).

Correspondence to the journal is accepted on the understanding that the contributing author licences the publisher to publish the letter as part of the journal or separately from it, in the exercise of any subsidiary rights relating to the journal and its contents.

## **Images in Haematology**

Authors can submit for consideration an illustration (or, where appropriate, two or more related images) which is interesting, instructive and visually attractive, with a few lines of explanatory text and a maximum of six contributors. If there are more than three authors then the corresponding author should outline the contribution of each author that justifies their inclusion. The images (e.g. a clinical photograph, radiology, cytology, histology, a laboratory test) should be submitted in a digital format online at <http://bjh.manuscriptcentral.com>. High-quality glossy prints, transparencies, or digital files (see <http://authorservices.wiley.com/bauthor/illustration.asp>) should be sent to the BJH Office on acceptance. Because of space constraints, there are usually no references in the 'Images' section. However, it is at the discretion of the Images Editor to accept one reference if it appears essential to the contribution in question.

## **Announcements**

Information about scientific meetings that are likely to be of general interest to readers of the Journal may be published at the discretion of the Editor. These should be sent to the Editor as early as possible prior to the event. Text should be as concise as possible, with a maximum of 150 words.

## **Preparation of manuscripts**

Manuscripts should be formatted with wide margins and bear the title of the paper with the name and address of the author(s), together with the name of the hospital, laboratory or institution where the work

has been carried out. Authorship should be restricted to individuals who have made a significant contribution to the study. The name, full postal address and e-mail address of the author to whom readers should address correspondence and reprint requests should be given on the first page; this will appear as a footnote in the journal and the publishers will send proofs to this author at the given address unless contrary instructions are written on the manuscript. Correspondence during the peer-review process will be with the author indicated during submission. A running short title of not more than 60 characters and spaces should be included. An informative summary of not more than 200 words must be included at the beginning of the paper and supplied when prompted during the online submission process. Papers should normally be divided into summary, introduction, methods (and/or materials), results, discussion, acknowledgements and references. SI units should be used throughout. Human DNA, gene, protein, and DNA restriction and modification enzyme nomenclature should be standardized as follows:

- 1) Human genes and alleles should be italicized capitals;
- 2) Human protein designations are the same as the gene symbol (i.e., written in upper case), but not italicized;
- 3) When distinguishing between mRNA, genomic DNA and cDNA, the relevant term should be given after the gene symbol, e.g. BCL2 cDNA;
- 4) D numbers should be as described in the Guidelines for Human Gene Nomenclature (Wain *et al*, 2002, *Genomics*, **79**, 464-470; <http://www.gene.ucl.ac.uk/nomenclature/guidelines.html>)
- 5) Nomenclature for DNA restriction and modification enzymes and their genes should follow Roberts *et al* (2003, *Nucleic Acids Research*, **31**, 1805-1812; <http://nar.oupjournal.org/cgi/content/full/31/7/1805>).

The Editor reserves the right to make textual changes.

### **Keywords**

Five keywords must be supplied after the summary.

### **Headings**

The main categories of headings are side capitals, side italics and shoulder italics. If necessary, small capitals may be used for subsidiary main headings. For examples see articles in a recent issue of the Journal.

### **Illustrations**

Illustrations should be referred to in text as, e.g., Fig 2, Figs 2, 4–7, using Arabic numbers. Individual figure files should bear a reference number corresponding to a similar number in the text, prints should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is doubt as to the orientation of an illustration the top should be marked with an arrow. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched.

Where printed, diagrams should be on separate sheets. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it. In the full-text online edition of the journal, figure legends may be truncated in abbreviated links to the full-screen version. Therefore the first 100 characters of any legend should inform the reader of key aspects of the figure.

It is the policy of the Journal for authors to pay the full cost for the reproduction of their colour artwork. Therefore, please note that if there is colour artwork in your manuscript when it is accepted for publication, Wiley-Blackwell require you to complete and return a colour work agreement form before your paper can be published. This form can be downloaded as a PDF from the internet [here](#).

Once completed, the original hard copies of the form should be posted to the following address:

Customer Services (OPI)  
John Wiley & Sons Ltd, European Distribution Centre  
New Era Estate  
Oldlands Way  
Bognor Regis  
West Sussex  
PO22 9NQ  
United Kingdom

If you are unable to access the internet, or are unable to download the form, please contact the Editorial Office and they will be able to email or fax a form to you. **Any article received by Wiley Blackwell with colour work will not be published until the form has been returned.**

### **Electronic artwork**

We would like to receive the artwork accompanying accepted manuscripts in electronic form. Please save vector graphics (e.g. line artwork) in encapsulated Postscript format (EPS), and bitmap files (e.g. half-tones) in tagged image file format (TIFF). Detailed information on our digital illustration standards is available at <http://authorservices.wiley.com/bauthor/illustration.asp>.

### **Tables**

Tables should be as few as possible and should include only essential data; they should be printed on separate sheets and should be given Roman numerals.

### **References**

We recommend the use of a tool such as Reference Manager for reference management and formatting. Reference Manager reference styles can be searched for at: <http://www.refman.com/support/rmstyles.asp>. Only papers closely related to the author's work should be cited. References should be made by giving the author's surname with the year of publication in parentheses. Where the reference contains more than two authors it should be given at each mention in the text with only the first surname plus et al, e.g. Jones et al (1948). If several papers by the same author (s) and from the same year, or by the same author but different subsequent authors in the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Jones et al (1948a, b). All references should be brought together at the end of the paper in alphabetical order, with all authors, titles of journals spelt out in full, and with both first and last page numbers given. The style to be used is that of any recent issue of the Journal.

### **Supporting information**

Supporting Information can be a useful way for an author to include important but ancillary information with the online version of an article. Examples of Supporting Information include additional tables, data sets, figures, movie files, audio clips, 3D structures, and other related nonessential multimedia files. Supporting Information should be cited within the article text, and a descriptive legend should be included. It is published as supplied by the author, and a proof is not made

available prior to publication; for these reasons, authors should provide any Supporting Information in the desired final format.

For further information on recommended file types and requirements for submission, please visit: <http://authorservices.wiley.com/bauthor/suppinfo.asp>

### **Production office**

Wiley, 1 Fusionopolis Walk, #07-01 Solaris South Tower, Singapore 138628

Tel.: +65 6643 8465 / Fax: +65 6643 8008

### **Proofs**

Proofs (except for Correspondence) The corresponding author will receive an email alert containing a link to a web site. A working e-mail address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from the site. Acrobat reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site: <http://www.adobe.com/products/acrobat/readstep2.html>. This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available. Excessive changes made by the author in the proofs, excluding typesetting errors will be charged separately.

### **NEW: Online production tracking is now available for your article through Wiley Blackwell's Author Services**

Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The author will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript. Visit <http://authorservices.wiley.com/bauthor> for more details on online production tracking and for a wealth of resources including FAQs and tips on article preparation, submission and more.

### **Early View**

*The British Journal of Haematology* is covered by Wiley Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Articles are, therefore, available for publication as soon as they are ready, rather than having to wait for the next scheduled print issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in a final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are, therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid, and can be continued to be used to cite and access the article.

### **Offprints**

Free access to the final PDF offprint of your article will be available via [Author Services](#) only. Please therefore sign up for author services if you would like to access your article PDF offprint and enjoy the many other benefits the service offers. Paper offprints of the printed published article may be purchased if ordered via the method stipulated on the instructions that will accompany the proofs.

*The British Journal of Haematology* is covered by *Current Contents*, *Chemical Abstracts*, *Current Clinical Cancer*, *CABS*, *ISI/BIOMED*, *Science Citation Index* and *ASCA*.

**Internet**

Information on this journal and other Wiley Blackwell publications is on the Wiley Online Library homepage at: <http://wileyonlinelibrary.com/journal/bjh>