**Sudan University of Science and Technology**

**College of Graduate Studies**

# Detection of JAK2-V617F Gene Mutation among Sudanese Patients with Chronic Myeloproliferative Neoplasms

# تحديد الطفره الجينيّه (JAK2-V617F) لدى مرضى أورام التكاثر النقوي المزمن بين السودانيين

**A thesis Submitted in fulfillment for requirement of the Ph.D in Hematology and Immunohematology**

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**2016**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (١) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (٢)
اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤) عَلَّمَ الْإِنْسَانَ مَا
لَمْ يَعْلَمْ (٥)

سورة العلق

آيه ١-٥

# Dedication

**To my father**

**To my mother**

**My brothers and sisters**

**To my wife**

**My daughter**

**To all members of my family and my friends**

# Acknowledgments

My best praise and thanks to Allah, the almighty most gracious and most merciful.

I would like to express my sincere gratitude to my supervisor Professor/ Babiker Ahmed Mohamed for sharing his great knowledge and experience with me. It has been a great experience for me to work with him.

I would like to acknowledge the tremendous support, advice and patience from my supervisor Assoc. Professor/ Fathelrahman Mahdi Hassan. He gave me best of suggestions and kind encouragement in all the time of research.

I owe a debt of gratitude to Dr. Ihsan M. Osman for great support and valuable assistance in the work.

My special thanks go to Dr. Modthir Abdelraheem Mohamed in department of hematology Sudan University for support me and gave me advice and suggestions in my work.

I would like to thank Dr. Mujtaba Elsamani Ahmed in Almukhtabar Company for helping me in bringing the reagent kits for analysis.

In addition, my gratitude goes to staff of Molecular Biology department at Radioisotopes centre Khartoum.

I cannot end without thanking Dr. Khalid Rahmtalah Kheder, for analysis of raw data.

My thanking goes to all people who support me in this work.

*Hashim AB*

**Abstract**

Myeloproliferative neoplasms (MPNs) are a clonal hematopoietic stem cell disorders, characterized by an overproduction of terminally differentiated myeloid cells such as erythrocytes, platelets, or granulocytes. The somatic mutation V617F in Janus associated kinase-2 (JAK2) gene is frequent in the majority patients with MPNs. The presence of the JAK2-V617F mutation is now part of clinical diagnostic algorithms, and JAK2 status is routinely assessed when Bcr/Abl-negative chronic myeloproliferative neoplasms (MPNs) are suspected.

This cross sectional study included 159 patients with Polycythemia Vera (PV), 55 with Essential thrombocythemia (ET) and 45 patients with Primary Myelofibrosis (PMF), was conducted from 2013 to 2016.

The aims of study were to detect the JAK2-V617F mutational status and allele burden, to fine out the differences in hematologic characteristics between JAK2-V617F positive and JAK2 wild type MPNs, and to examine the association of quantitative JAK2-V617F allele burden with hematological characteristics.

Genomic DNA extraction from peripheral blood leukocytes was carried out following the protocol of innuPREP kit (Analytik Jena/Germany). Allele specific PCR was used to detect the JAK2-V617F mutation, and quantitative real-time polymerase chain reaction (qRT-PCR) technology(Ipsogen JAK2 MutaQuant Kit, Germany) was used to determinate the percentage of mutated alleles in genomic DNA among JAK2-V617F positive MPNs. hematologic parameters were obtained from patient's medical records. Hematological features were compared between patients who were V617F-positiveand V617F-negative patients.

The JAK2-V617F mutation was detected in 71% of all patients with MPNs. The prevalence of JAK2-V617F mutation was 81.7% in PV, 56.4% in ET, and 51.1% in PMF patients. At diagnosis, the presence of JAK2-V617F was found to be significantly associated with high hemoglobin (P=.039) and Hemtocrit (P=.04) in PMF patients. The JAK2-V617F-positive ET patients were older than ET patients without mutation (P=.033). A JAK2-V617F allele burden was measured for 184 patients whom positive for the mutation; 130 PV, 31 ET and 23 PMF. Patient with PMF showed highest burden, then PV patients and ET patient had the lowest burden.

The mean allele burden of JAK2 V617F was 69.3% in PMF, 60.2% in PV and 31.5% in ET. These differences between the groups studied were statistically significant (P=0.00). The majority of JAK2V617F-positive ET patients were heterozygous, while homozygous genotype was frequently detected in PV patients. The prevalence of JAK2V617F mutation with high allele burden was (53.4 %) of PV patients, 12.7% of ET patients and 31.1% of PMF patients. These frequencies were different significantly among three subgroup (*P*=0.00). There was significant difference in leukocyte count and platelet count between heterozygous and homozygous groups for PV patients (*P*=.006) (P=000). Homozygous PMF patients associated with low platelet count (P=.015).

In conclusion, the JAK2-V617F mutation could be frequently detected in the Sudanese patients with MPNs, vast majority of polycythemia patients and around half of essential thrombocythemia and primary myelofibrosis have the mutation.

# المستخلص

مرض التكاثر النقوي المزمن هو مجموعة اضطرابات نقويه تتميز بالافراط في انتاج الخلايا الدمويهمثل كريات الدم الحمراء والصفائح الدموية و كريات الدم البيضاء.

الطفره الوراثيه(JAK2-V617F) التي تم اكتشافها في سنه ٢٠٠٥ تعتبر من اهم الطفرات الوراثيه التي تحدث عند غالبية مرضي اضطرابات التكاثر النقوي. ووجود هذه الطفره هو الآن جزء من خوارزميات التشخيص السريري، ويتم تقييم وضع(JAK2-V617F) بشكل روتيني عندما يشتبه بمرض التكاثر النقوي المزمن.

أجريت هذه الدراسة المقطعية من ٢٠١٣ الي ٢٠١٦ وشملت ثلاث مجموعات، الاولي كثره الحمر(PV) وتضم ١٥٩ مريض، الثانيه كثرة الصفيحات (ET) وتضم ٥٥ مريض، الثالثه التليف النقوي الاولي (PMF) وتضم ٤٥ مريضتم تشخيصها وفقا لمعايير منظمة الصحة العالمية.

هدفت الدراسة لمعرفة الطفره (JAK2-V617F) لدي الاشخاص المصابين باضطراب التكاثر النقوي المزمن، و قياس كمية الطفره بالنسبه المؤيه للحالات الايجابيه، ومقارنه الخصائص الدموية و السريرية بين المرضى الايجابين للطفره(JAK2-V617F) و المرضى السلبيين. وعلاوة على ذلك، دراسة علاقة كمية الطفره مع الخصائص الدموية و السريرية.

تم استخراج الحمض النووي الجيني من الكريات البيضاء في الدم الطرفي لكل عينه. وباستخدام تفاعل البلمرة المتسلسل (PCR) تم تحديد الطفره الجينيه في العينات. وتم قياس نسبة الطفره باستخدام تفاعل البلمره المتسلسل الكمي(qPCR). وقد تم الحصول على المعلمات المخبرية والسريرية من السجلات الطبية المرضي.

اوضحت الدراسه ان ٧١% من مجمل الحالات تحمل الطفره الجينييه، وكانت نسبه وجود الطفره بالتفصيل كلاتي : ٨١.٧% من مرضى كثرة الحمر، و ٥٦.٤% من مرضى كثرة الصفيحات، و ٥١.١ % من مرضي التليف النقوي الاولي يحملون الطفره الجينيه (JAK2-V617F).

هنالك علاقه ذات دلاله معنويه بين وجود الطفره و ارتفاع نسبة الهيمجلوبين والهيماتوكريت لدى المصابين بالتليف النقوي الاولي وكانت القيمه الاحصائيه (P=.039) (P=.033) علي التوالي. وان مرضى كثرة الصفيحات الحاملين للطفره الجينيه هم اكبر سنا مقارنة بمرضى كثرة الصفيحات غير الحاملين للطفره بقيمه احصائيه(P=.04).

اوضحت الدراسه ايضا ان متوسط نسبة الطفره الجينيه في الحالات الايجابيه لدى مرضى التليف النقوي الاولي كانت ٦٩.٣ %، بينما كانت النسبه ٦٠.٢ % لدى كثرة الحمره، اما مرضى كثرة الصفيحات كانت النسبه لديهم ٣١.٥%.

المرضي الذين لديهم نسبه الطفره اعلي من خمسين بالمائه في الخليه(تم اعتبارهم ذوي تركيب وراثي متماثل للطفره الجينه) ٥٣.٤ %، ٣١.١ %، و١٢.٧ % لدى من مرضى كثرة الحمر،مرضى التليف النقوي الاولي، و مرضىكثرة الصفيحات علي التوالي.

هنالك فرق ذات دلاله احصائيه في عدد الكريات البيض والصفيحات الدمويه بين المرضي كثرة الحمر ذوي التركيب الوراثي المتماثل للطفره الجينيه والمرضى ذوي التركيب الوراثي المتخالف بقيم احصائيه ($P$=.006)و (P=000) تواليا. هنالك ارتباط بين التركيب الوراثي المتماثل للطفره الجينيه و انخفاض عدد الصفيحات الدمويه عند مرضى التليف النقوي الاولي و القيمه الاحصائيه هي P=.015.

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| | |
|---|---|
| **AML** | Acute myeloid leukemia |
| **ATP** | Adenosine Triphosphate |
| **AS** | Allele- Specific |
| **ARMS** | Amplification refractory mutation system |
| **AGM** | Aorta-gonad-mesonephros |
| **ASXL1** | Additional Sex Combs-like 1 |
| **aCML** | atypical chronic myeloid leukemia |
| **BCR/ABL** | Breakpoint cluster region / Abelson (gene) |
| **BFU-E** | Blast Forming Unit-Erythroid |
| **bp** | base pair |
| **BM** | Bone Marrow |
| **cDNA** | ComplementaryDNA |
| **CALR** | Calreticulin |
| **CBL** | Casitas B.cell lymphoma |
| **MCD** | Mast cell disease |
| **CEL-NOS** | chronic eosinophilic leukemia not otherwise specified |
| **CEL** | Chronic eosinophilic leukemia |
| **HES** | hypereosinophilic syndrome |
| **CML** | Chronic myelogenous leukemia |
| **CMML** | Chronic myelomonocytic leukemia |
| **CNL** | Chronic neutrophilic leukemia |
| **CFC** | Committed colony- forming cell |
| **CIMF** | Chronic idiopathic myelofibrosis |
| **DIC** | Disseminated intravascular coagulopathy |
| **DNA** | Deoxyribonucleic acid |
| **del-** | deletion (chromosomal) |
| **der-** | derivative chromosome (chromosomal) |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **EEC** | Endogenous erythroid colonies |
| **EMPs** | Erythroid-myeloid progenitors |
| **E po** | Erythropoietin |
| **ET** | Essential thrombocythemia |

| | |
|---|---|
| **FERM** | 4.1, ezrin, radixin, moesin |
| **FGFR** | Fibroblast growth factor receptor |
| **FLT3** | Fms-like tyrosine kinase |
| **G-CSF** | Granulocyte colony-stimulating factor |
| **GM-CSF** | Granulocyte-macrophage colony-stimulating factor |
| **GTP** | Guanosine triphosphate |
| **Hb** | Hemoblobin |
| **Hct** | Hematocrit |
| **HGFs** | Hematopoietic growth factors |
| **HSC** | Hematopoietic stem cell |
| **HSCT** | Hematopoietic stem cells transplantation |
| **HRM** | High resolution melting |
| **IFNγ** | Interferon gamma |
| **IL** | Interleukin |
| **IGF-1** | Insulin-like growth factor-1 |
| **JAK** | Janus-Associated Kinase |
| **JAK-STAT** | Janus associated kinase-signal transducer and activator of transcription |
| **JMML** | Juvenile myelomonocytic leukemia |
| **JH2** | Janus Homology domain2 |
| **LOH** | Loss of heterozygosity |
| **M-CSF** | Macrophage colony-stimulating factor |
| **MAP** | Mitogen activated protein |
| **MDS** | Myelodysplastic syndromes |
| **MPL** | Thrombopoietin receptor |
| **mTOR** | mammalian target of rapamycin |
| **CMPDs** | Myeloproliferative disorders |
| **MPNs** | Myeloproliferative neoplasms |
| **NK cells** | Natural Killer cells |
| **NRTKs** | Non tyrosine receptor kinases |
| **PDGFR** | Platelet Derived Growth Factor Receptor |
| **PCM1** | Pericentriolar material1 |
| **BP** | Peripheral blood |

| | |
|---|---|
| **$Ph^-$** | Philadelphia chromosome- negative |
| **PTB** | Phosphor-tyrosine binding |
| **PI3K** | Phosphotidylinositol 3-kinase |
| **PV** | Polycythemia vera |
| **PVSG** | Polycythemia Vera Study Group |
| **PCR** | Polymerase chain reaction |
| **PMF** | Primary myelofibrosis |
| **PTKs** | Protein Tyrosine kinases |
| **PRV-1** | Polycythemia Vera Rubra gene 1 |
| **qRT-PCR** | Quantitative real-time polymerase chain reaction |
| **RARS-T** | Refractory anemia with ring sideroblasts and thrombocytosis |
| **SNPs** | Single nucleotide polymorphisms |
| **SOCS** | Suppressor of cytokine signaling |
| **SPSS** | Statistical Package for the Social Sciences |
| **SH2** | Src homology2 |
| **STAT** | Signal Transducers and Activators of Transcription |
| **SCT** | Stem cell transplantation |
| **t-** | Translocation |
| **TEL** | Translocation ETS leukemia |
| **TET2** | Ten eleven translocation |
| **Tpo** | Thrombopoietin |
| **TKI** | Tyrosine kinase inhibitor |
| **TNFα** | Tumor necrosis factor alpha |
| **TYK** | Tyrosine kinase |
| **PTPs** | Protein Tyrosine phosphatases |
| **WBCs** | White Blood Cells |
| **WHO** | World Health Organization |

# CHAPTER ONE

# 1. Introduction and Literature Review

## 1.1 Introduction

Myeloproliferative neoplasms (MPNs), are a clonal hematopoietic stem cell disorders, characterized by proliferation of one or more myeloid cell lineages in the bone marrow, and an overproduction of terminally differentiated myeloid cells such as erythrocytes, platelets, or granulocytes *(*Amy and Nicholas, 2013; Jones D, 2010; Bang S *et al*., 2006*)*. Chronic myeloproliferative disorders (CMPDs) were first described by William Dameshek In 1951 as a group of closely related disease entities *(*Dameshek, 1951*)*; the upcoming World Health Organization (WHO) classification system for hematopoietic tumors included these entities in a broader category of myeloproliferative neoplasms (MPNs) *(*Tefferi and Gilliland, 2007*)*. According to 2008 World Health Organization (WHO) classification of hematological neoplasms *(*Tefferi, 2010; Mascarenhas, *et al.,* 2012; Kim, *et al*., 2013*)*, MPNs are subclassified into eight separate entities: chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia, chronic eosinophilic leukemia-not otherwise specified, mastocytosis and MPN unclassifiable. Chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, and primary myelofibrosis are considered classic MPNs *(*Dameshek, 1951*)*. CML was separated as a distinct clonal disorder defined by a single chromosomal and gene rearrangement (BCR/ABL) *(*Mohamad, *et al*., 2013*)*. The term 'BCR-ABL1-negative' MPN, used interchangeably with the term 'Philadelphia chromosome- negative' MPN refers to PV, ET, and PMF *(*Mascarenhas, *et al*., 2012*)*. One of the most important discoveries associated with classical BCR-ABL1-negative MPNs was the identification of JAK2-V617F mutation in 2005*(*Baxter, *et al*., 2005; Kralovics, *et al*., 2005*)*. The JAK2V617F results from a somatic G to T mutation involving JAK2 exon 14, which leads to nucleotide change at position 1849 and the substitution of valine to phenylalanine at codon 617 *(*Tefferi, 2010*)*. This mutation causes growth factor independent autonomous proliferation of hematopoietic precursors *(*Thomas, 2007*)*. JAK2 is a cytoplasmic non receptor tyrosine kinase, that via its association with cytokine receptors serves as a signaling mediator for hematopoietic cytokines such as erythropoietin (E po), and

thrombopoietin (Tpo) to regulate cell proliferation and growth (Ross, 2012). The mutation affects the noncatalytic 'pseudo-kinase' domain (JH2) of JAK2, and disrupts its autoinhibitory function, resulting in constitutive activation of JAK2 mediated signaling pathways through the STAT5 and STAT3, phosphotidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase (Donal, *et al*., 2006; Ross, 2012). The Janus kinase 2 (JAK2) V617F mutation is the most common molecular abnormality in Ph−MPN and is found in more than 97% of patients with PV and approximately 50% of patients with either ET or PMF, and in smaller extent (~5%) of chronic myelomonocytic leukemia, myelodysplastic syndrome, systemic mastocytosis, and chronic neutrophilic leukemia (Angela, *et al*., 2007; Sang, *et al*., 2013). Therefore, the presence of the JAK2V617F mutation is now considered as a major diagnostic criterion in the 2008 World Health Organization diagnostic criteria for PV, whereas it is considered as a clonal marker for the diagnosis in ET and PMF (Barbara, *et al*., 2010). Despite this important discovery, many questions remain regarding the molecular basis of PV, ET, and PMF, and the role of the JAK2V617F allele in MPN pathogenesis, most importantly, the mechanism underlying the ability of the identical somatic mutation in JAK2 to contribute to three related, but clinically distinct, disorders remains unknown (Ross, 2008). But clinical, biological and pathological data have led to three potential hypotheses, One of them called the gene-dosage hypothesis, postulates a correlation between disease phenotype and the proportion of JAK2 (V617F) mutant alleles introducing the concept of allele burden, that is the ratio between mutant and wild type JAK2 in hematopoietic cells (Francesco and Elisa, 2008). The aims of this study were to determine the frequency of JAK2 V617F mutation in Sudanese patients with myeloproliferative disorders referred to RadioIsotope Center Khartuom (RICK), to quantify the JAK2 V617Fmutation among positive cases, and to examine the association of quantitative JAK2-V617F allele burden with laboratory characteristics and clinical phenotype, and to investigate the differences of laboratory parameters between patients with JAK2-V617F positive myeloproliferative neoplasms (MPNs) and JAK2 wild type MPNs. Numerous techniques have been published for detection of the JAK2 1849G > T mutation including, direct sequencing, allele-specific polymerase chain reaction (PCR), PCR-restriction fragment length polymorphism, pyrosequencing, and amplification refractory mutation system (ARMS)-PCR, and melt curve analysis or high resolution melting (HRM) (Huijsmans, *et al*., 2011). Allele-specific PCR detects the mutation

with a much higher sensitivity at 1 to 3%, and modified (ARMS)-PCR technique detects mutation with a highly reproducible analytic sensitivity of 0.05 to 0.1%, making it suitable for the detection of low levels of JAK2 mutation *(*Qiaofang, *et al*., 2007*)*. For quantitatively determine the proportion of single nucleotide polymorphisms (SNPs) in DNA samples, methods based on real-time quantitative polymerase chain reaction (qPCR) are preferred because of their higher sensitivity allowing monitoring of allele burden in a longitudinal fashion. In this study allele specific PCR based on (ARMS)-PCR technique was used to detect the mutation, and The quantitative real-time polymerase chain reaction (qRT-PCR) technology(Ipsogen JAK2 MutaQuant Kit, Germany) was used to determinate the percentage of mutated alleles in genomic DNA among JAK2-V617F positive MPNs.

In numerous published studies, the JAK2 V617F mutations was detected in (80% - 97.0%) cases of PVand in approximately 50–60% cases of essential ET or PMF *(*Baxter, *et al*., 2005; Kralovics, *et al*., 2005; Jones, 2005*)*

In this study The JAK2-V617F mutation was detected in 71% of patients with MPNs. The prevalence of the mutation in our study was 81.7% in PV, 56.4% in ET and 51.1% in PMF. The mean allele burden of *JAK2* V617F for positive MPNs was 69.3% in MF, 60.2% in PV and 31.5% in ET. The prevalence of JAK2V617F mutation with high allele burden (i.e. allele burden exceeded 50%) was 53.4% of PV patients, 12.7% of ET patients and 31.1% of PMF patients.

# 1.2 Literature Review

### 1.2.1 Normal hematopoiesis

Hematopoiesis is the ongoing process by which blood cells are produced from the hematopoietic stem cell (HSC) whose progeny can be either another HSC or a cell with more limited developmental potential *(*Amittha and Barbara, 2009*)*. The process of hematopoiesis regulates the production of myeloid, erythroid and lymphoid cells from bone marrow progenitors and maintains the correct balance of different blood cell lineages *(*Alister, 2002*)*. This well-regulated process also allows for replacement of cells lost through daily physiologic activities *(*Manula, *et al*., 2010*)*. The control of hematopoiesis depends on signals delivered through surface receptors of the hematopoietic superfamily present on progenitor cells in response to a complex cocktail of cytokines in the bone marrow microenvironment and peripheral blood *(*Alister C, 2002*)*.

#### 1.2.1.1 Hematopoietic stem cells (HSCs)

Hematopoietic stem cells (HSCs) are the multipotent stem cells, which are able to give rise to all types of blood cells including myeloid (monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, and dendritic cells) and lymphoid lineages (T cells, B cells, NK cells) (Figure 1.1 ). The multipotent capability of HSCs was first described as clonogenic bone marrow (BM) cells that can reconstitute the hematopoietic system in primary and secondary recipients *(*Aggarwal, *et al*., 2012*)*. Indeed, HSC have the ability to replenish their own compartment (i.e., they demonstrate self renewal) and to differentiate into progenitor cells and mature blood cells of all hematopoietic lineages. In adult mammals, blood cells are generated primarily within the medullary cavity of some bones. It is generally considered that HSC encountered in the adult bone marrow arise by replication and amplification of a stock of HSC that emerged early in ontogenesis, when the bone marrow had not yet formed *(*Manula, *et al*., 2010*)*. Approximately 0.1% of nucleated cell in the BM are pluripotent stem cells, and approximately 5% of these cells may be actively cycling at any one time. The stem cell pool maintains itself through a process of asymmetrical cell division: when stem cell divides, one daughter cell remains a stem cell and the other becomes committed colony- forming cell

(CFC). The proliferation and differentiation of CFC are controlled by hematopoietic growth factors (Stefan and Water, 2008). During the past several decades, hematopoietic stem cells transplantation (HSCT) has been used as standard treatment for various hematological disorders such as severe combined immunodeficiency, congenital neutropenia and malignancies (Aggarwal. *et al*., 2012).

### 1.2.1.2 Sites of hematopoiesis

In humans, hematopoiesis begins in the yolk sac and transitions into the liver temporarily before finally establishing definitive hematopoiesis in the bone marrow and thymus (Table 1.1). Blood development in vertebrates involves two waves of hematopoiesis: the primitive wave and the definitive wave. The primitive wave, which involves an erythroid progenitor, gives rise to erythrocytes and macrophages during early embryonic development (Galloway and Zon, 2003; Palis and Yoder, 2001). The primary purpose of the primitive wave is to produce red blood cells that can facilitate tissue oxygenation as the embryo undergoes rapid growth (Orkin S and Zon, 2008). In mammals, these erythroid progenitor cells first appear in blood islands in the extra-embryonic yolk sac early in development (Paik and Zon, 2010). The primitive wave is transitory, and these erythroid progenitors are not pluripotent and do not have renewal capability. Definitive hematopoiesis, by contrast, occurs later in development, notably at different time points in different species. In most organisms, there is a transient wave of definitive hematopoiesis that occurs in the blood islands and produces progenitors called erythroid-myeloid progenitors (EMPs) (McGrath, *et al*., 2011). Definitive hematopoiesis later involves HSCs, which are multipotent can give rise to all blood lineages of the adult organism. In vertebrates, definitive HSCs are born in the aorta-gonad-mesonephros (AGM) region of the developing embryo. They migrate to the fetal liver and then to the bone marrow, which is the location for HSCs in adults (Cumano and Godin, 2007).

Table 1.1 Sites of hematopoiesis (hoffbrand, 2006)

| **Fetus** | **Infants** | **Adults** |
|---|---|---|
| 0–2 months (yolk sac)<br>2-7 months (liver, spleen)<br>5-9 months (bone marrow) | Bone marrow (practically all bones) | Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur |

### 1.2.1.3 Hematopoietic growth factors

Hematopoietic growth factors (HGFs) constitute a family of glycoproteins that individually and in various combinations involving complex feedback mechanism regulate the self-renewal, cycle activation, proliferation, maturation, adhesion, functional integrity, and activation of mature blood cells, as well as hemostasis (Table 1.2) (Figure 1.1). The number of positive and negative regulatory factors that influence hematopoietic cell survival and development has grown to encompass not only colony-stimulating factors, but also interleukins, interferons, chemokines, and other growth factors *(*Janice, *et al*., 2003*)*. Regulation occurs at the level of the structured microenvironment (stroma), via cell-cell interactions and by way of the generation of specific hormones and cytokines: erythropoietin, interleukin 3, granulocyte-monocyte colony-stimulating factor (GM-CSF), monocyte-macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin 5, interleukin 4, and other less well-defined factors, including the megakaryocyte growth factors *(*Smith, 1990*)*. Most of HGF are produced by macrophages, fibroblast, and endothelial cells that comprise BM microenvironment *(*Gary and David, 2011*)*. HGFs appear to exert their effects primarily via binding to specific high-affinity receptors found on the cell membrane surface of appropriate cells *(*Stefan and Walter, 2008*)*. Many receptors (e.g. erythropoietin (epo) recptor, GM-CSF-R) are from the hematopoietin receptor super-family which dimerize after binding their ligand. Dimerization of the receptor leads to activation of a complex series of intracellular signal transduction pathways of three major ones are JAK/STAT, the mitogen activated protein (MAP) kinase and the phosphatidylinositol 3(PI3) kinase pathways *(*hoffbrand, 2006*)*.

Early progenitor-stimulating cytokines include SCF, IL-6, IL-3 and FLT3 ligand. These cytokines stimulate HSC to begin the proliferation process.

G-CSF and GM-CSF provide stimulation for myelopoiesis. G-CSF stimulates granulocytes proliferation. M-CSF stimulates macrophages proliferation.these hematopoietic growth factors are made by lymphocytes.

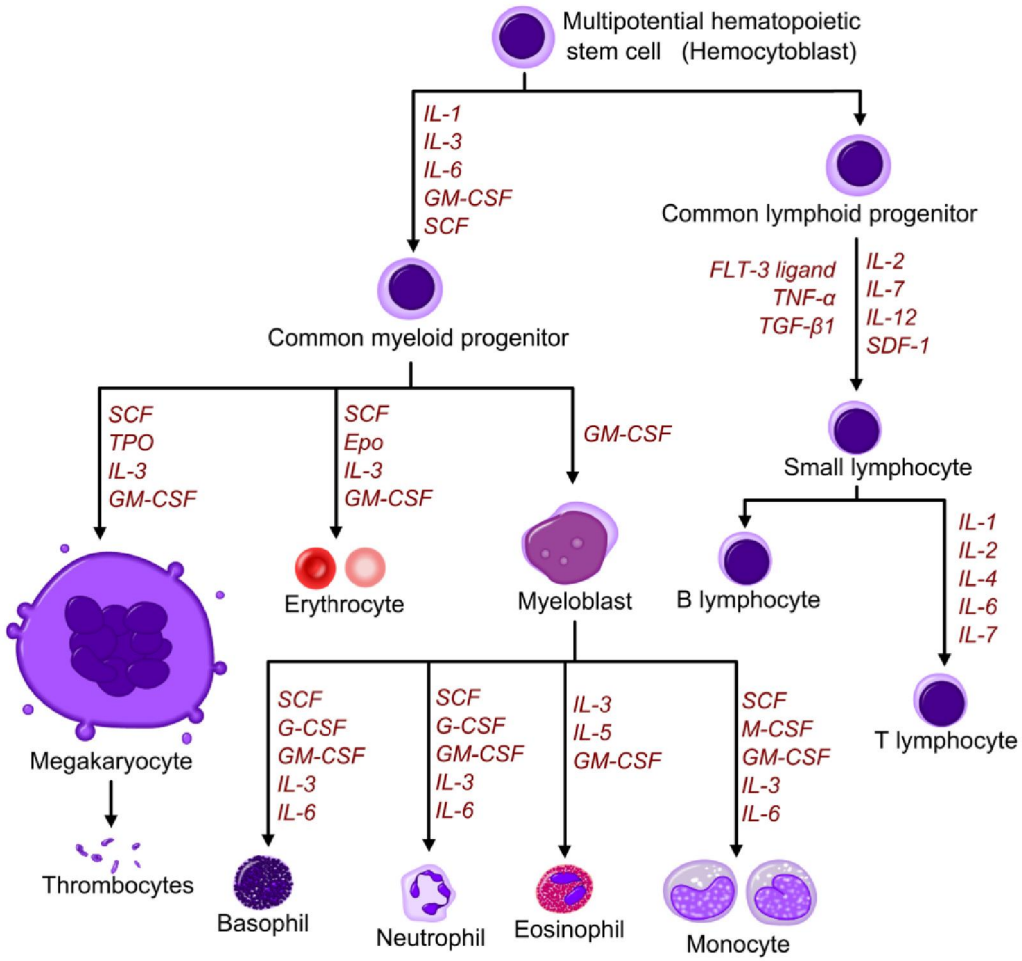
Erythropoietin stimulates erythroid differentiation resulting in the production of mature RBCs. Erythropoietin is made in the kidney as result of sensing hypoxemia by the periglomerular cells *(*Alvin and Hillard, 2012*)*.

Thrombopoietin (TPO), known as megakaryocyte growth and development factor, specifically regulates the production and differentiation of megakaryocytes. Beside the primary action in megakaryocytopoiesis, TPO also plays an important role in the growth and survival of hematopoietic stem cells *(*Bociek and Armitage, 1996*)*.

**Tabe 1.2** Hematopoietic growth factors *(*hoffbrand, 2006*)*.

| |
|---|
| Act on stomal cells |
| IL-1, TNF |
| Act on pluripotential stem cells |
| SCF, Flt-L |
| Act on multipotential progenitor cells |
| IL-3, IL-6 |
| GM-CSF, G-CSF |
| Thrombopoietin |
| Act on committed progenitor cells |
| G-CSF, M-CS, |
| IL-5(eosinophil-CSF) |
| Erythropoietin |
| Thrombopoietin |

IL interleukin, TNF tumor necrosis factor, SCF Stem Cell Factor




From http://www.slideshare.net/aspchnrt/hemopoisis

**Figure 1.1** schema of hematopoiesis including some important hemopoietic growth factors that influence differentiation and proliferation. SCF Stem Cell Factor, Tpo Thrombopoietin , IL interleukin, GM-CSF Granulocyte Macrophage Colony Stimulating Factor, Epo Erythropoietin, M-CSF Macrophage Colony Stimulating Factor G-CSF Granulocyte Colony Stimulating Factor, SDF-1 Stromal cell-Derived Factor 1, FLT-3ligand FMS like trrosine kinase3 ligand, TNF-a tumor necrosis factor alpha, TGF-B transforming growth factor beta

### 1.2.2 Myeloproliferative neoplasms (MPNs)

Chronic myeloproliferative neoplasms are group of hematologic disorders distinguish by clonal expansion of abnormal hematopoietic stem cells at different levels leading to hypercellular marrow with excessive terminal proliferation of the hematopoitic cell and peripheral blood granulocytosis, erythrocytosis, and/or thrombocytosis. This hyper proliferation process in certain conditions associated with BM fibrosis and extra-medullary hematopoiesis *(*Faramarz N*et al*., 2008*)*. Although there are spectrum of MPNs involving all of the different myeloid compartments, the most common MPNs are polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML) *(*Edward, *et al*., 2014*)*, are currently considered as classic MPNs *(*Tefferi, *et al*., 2011*)*.

The clonal hematopoietic stem cell origin of MPDs has been demonstrated by X-linked glucose-6-phosphate dehydrogenase (G-6PD) locus analysis *(*Fialkow, *et al*., 1967*)*. During development, somatic cells of females randomly inactivate one of the two X chromosomes. As a result, women heterozygous for a polymorphic X-linked allele have a mixture of cells expressing one or the other allele in normal tissues. If cells are clonal, i.e. they come from the same ancestor; all of the cells inactivate the same X chromosome, thus showing a homogeneous type of X-inactivation pattern. Based on this theory, Adamson et al showed that female PV patients had the same form of glucose-6-phosphate dehydrogenase (G6PD) in their erythrocytes, granulocytes, and platelets, demonstrating the clonal origin of PV *(*Adamson *et al*.,1976*)*. EI Kassar N and Tsukamoto N studied the polymorphism of the human androgen receptor gene (HUMARA), as well as the restriction fragment length polymorphisms (RFLP) of the X-chromosome phosphoglycerate kinase (PGK) and hypoxanthine phosphoribosyltransferase (HPRT) genes in female ET and PMF patients and found out that most of patients also had clonal hematopoiesis *(*EI Kassar, *et al*., 1995; Tsukamoto, *et al*., 1994*)*. All these data suggest that PV, ET and PMF originated from a multipotent hematopoietic progenitor or a stem cell, which acquired proliferative and/or survival advantage in the course of the disease.

#### 1.2.2.1 General features

In general, MPNS share several features, namely, involvement of a multipotent hematopoietic progenitor cell with dominance of the transformed clone over normal

hematopoiesis, autonomous proliferation of at least one cell line in the absence definable stimulus *(*Greil, *et al*., 2010*)*, furthermore, evolution into secondary myelofibrosis (post-PV and post-ET MF) for PV and ET, and subsequently to acute myeloid leukemia (AML) *(*Francesco and Elisa, 2008*)*. AML may occur directly from ET and PV without the intermediate step of MF, and Evolution to post-PV and post-ET myelofibrosis occurs at a rate of 10% to 20% after 15 to 20 years of follow-up. Progression to AML is less frequent in PV and ET (2-7%) than in PMF (8-30%), and ET patients may slowly progress to PV, especially those carrying the JAK2 (V617F) mutation *(*Francesco, *et al*., 2011*)*. Cytogenetic abnormalities of chromosome 1, 8, 9, 13 and 20 as well as genetic mutations in the JAK2 or MPL locus, epigenetic abnormalities as well as increased PRV-1 mRNA and impaired meqakaryocyte and platelet MPL expression are also shared features of classic MPNs *(*Greil, *et al*., 2010*)*.

#### 1.2.2.2 Clinical characteristics

Signs and symptoms of MPNs include arterial and venous thrombosis and hemorrhages especially in patients with essential thrombocythemia (ET) and polycythemia Vera (PV), microcirculatory problems including erythromelalgia(red, painful region on distal extremities due to ischemia from reduced blood flow from polycythemic red blood cell and/or thrombosis), and neurologic and visual disturbances *(*Alvin and Hillard, 2012*)*. Lower extremity deep vein thrombosis and pulmonary embolism are major thrombotic complications reported with increasing frequency in MPNs patients *(*Mohamad, *et al*., 2013*)*. Bleeding complications in the setting of high platelets count and platelet dysfunction can also occur. The excessive myeloid proliferation may also cause sequestration of myeloid cells in various organs (myeloid metaplasia), most often causing splenomegaly *(*Thomas, 2007*)*. Constitutional symptoms including weight loss, fatigue, and pruritus, fever, night sweats and bone pain can accompany these disorders. Patients with MPNs are at risk of transformation to acute myelogenous leukemia (AML). Without treatment, the risk of transformation is highest in chronic myelogenous leukemia (CML) (up to 90%) and lowest in ET *(*Greil, *et al*., 2010*)*.

### 1.2.2.3 Classifications of MPNs

The history of MPNs dates back to 1951, when William Dameshek coined the term ‘‘myeloproliferative disorders (MPD)’’ as a clinicopathologic category that included chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and erythroleukemia (Di Guglielmo syndrome) *(*Ayalew, *et al*., 2009*)*. Over the years, erythroleukemia and its variants has been recognized as acute erythroid leukemia, whereas the other four entities have continued to be referred to as MPDs *(*Martha and Ayalew, 2010*)*. In 2001, the World Health Organization (WHO) classification of myeloid neoplasms included CML, PV, ET, and PMF under the category of chronic myeloproliferative diseases (CMPDs). The CMPDs category also included other “nonclassic” MPD-like disorders such as chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia/hypereosinophilic syndrome (CEL/HES), and unclassified CMPD *(*Alessandro, *et al*., 2009*)*. And CMPDs grouped with MDS, MDS/MPD overlap, and mast cell disease (MCD) under the category chronic myeloid neoplasms *(*Martha and Ayalew, 2010*)*. In the revised 2008 WHO classification system, some important changes have been incorporated. The word disease in both CMPD and MDS/MPD was replaced by neoplasm (ie, CMPD was replaced by myeloproliferative neoplasm [MPN] and MDS/MPD was replaced by MDS/MPN) *(*Munjal, *et al*., 2012*)*. In addition, MCD was formally included in the MPN category. Another major change was the reorganization of CEL/HES into CEL not otherwise specified (CEL-NOS) and myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB, and FGFR1. The latter group was assigned a new category of myeloid neoplasms whereas CEL-NOS remained an entity within the MPN category *(*Ayalew, *et al*., 2009*)*. Finally the authors of the WHO classification system effectively incorporated molecular/cytogenetic disease markers and biologically relevant minor criteria to confirm histological impressions *(*Tefferi, *et al*., 2007).

#### 1.2.2.3.1 The 2008 WHO classification

The revisions in the criteria for the WHO classification of MPN have been influenced by two important factors: (1) the discovery of genetic abnormalities that can be used as diagnostic markers in *BCR-ABL1*–negative MPN and (2) better characterization of histologic features that aid in the identification of MPN subtypes *(*James, *et al*., 2009*)*.

Based on diverse clinical, morphologic, and molecular findings, the revised World Health Organization (WHO) classification system for hematopoietic malignancies recognizes MPN as a separate category which includes subcategories *(*Mascarenhas, *et al*., 2012; Edward, *et al*., 2014*)*, including chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), systemic mastocytosis, chronic eosinophilic leukemia not otherwise specified, chronic neutrophilic leukemia and MPN unclassifiable *(*Tefferi, 2010*)*. The first four subcategories described by William Dameshek in 1951 as related disorder, referred to as classic MPN *(*Tefferi and Gilliland, 2007*)*. CML is distinct among the MPNs in that it is defined by a specific cytogenetic abnormality (Philadelphia chromosome) *(*Francesca, *et al*., 2014*)*. Philadelphia chromosome- negative chronic Myeloproliferative neoplasms include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), subsumed as the classic MPNs as well as the following disorders: chronic neutrophilic leukemia (CNL), Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS), systemic mastocytosis and unclassifiable MPNs *(*Greil, *et al*., 2010)(Table 1.3).

Table 1.3 Classification of Myeloid Neoplasms According to the 2008 World Health Organization Classification Scheme *(*WHO, 2008*)*

1. Myeloproliferative neoplasms (MPN)
   - 1.1. Chronic myelogenous leukemia, BCR-ABL1–positive (CML)
   - 1.2. Polycythemia vera (PV)
   - 1.3. Essential thrombocythemia (ET)
   - 1.4. Primary myelofibrosis (PMF)
   - 1.5. Chronic neutrophilic leukemia (CNL)
   - 1.6. Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
   - 1.7. Mast cell disease (MCD)
   - 1.8. MPN, unclassifiable
2. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB, and FGFR1
3. MDS/MPN
   - 3.1. Chronic myelomonocytic leukemia (CMML)
   - 3.2. Juvenile myelomonocytic leukemia (JMML)
   - 3.3. Atypical chronic myeloid leukemia, BCR-ABL–negative (aCML)
   - 3.4. MDS/MPN, unclassifiable
4. Myelodysplastic syndromes (MDS)
5. Acute myeloid leukemia (AML)

### 1.2.2.4 Epidemiology of MPNs

The reported incidence of MPNs worldwide is ~0.1–3 cases per 100,000 individuals, with no significant differences observed between regions and countries. Classic MPNs are the most common MPNs, usually affecting the adult elderly individuals; however they can also be found in children *(*Alessandro, *et al*., 2009*)*. The average age is 60 years *(*Munjal, *et al*., 2012*)*. In a comprehensive report on the incidence of MPNs across the globe, in 2012, reported annual incidence rates ranged from 0.01 to 2.61, 0.21 to 2.27, and 0.22 to 0.99 per 100,000 for PV, ET, and PMF, respectively *(*Titmarsh, *et al*., 2014*)*. In a study described the incidence of MF and prevalence of MF, ET and PV in the United States between 2008 and 2010 based on data from two large health plans, The incidence of primary MF was about 1 per 100 000 per year and did not vary over the study years. The prevalence of PV (44-57 per 100 000) and ET (38-57 per 100 000) was much higher than that of MF (4-6 per 100 000) or subgroups containing MF (post-PV MF = 0.3-0.7 per 100 000; post-ET MF = 0.5-1.1 per 100 000) *(*Mehta, *et al*., 2014*)*. Others estimated incidence among the various registries in European population for PV of PV ranged from 0.4 per 100,000 per year to 2.8 per 100,000 per year, incidence of ET was between 0.38 per 100,000 per year and 1.7 per 100,000 per year, and the incidence rate of MF ranged from 0.1 per 100,000 per year to 1 per 100,000 per year. Furthermore, because of their relatively smooth clinical course, it is likely that many classic MPN cases actually go undetected or are not reported to registries. Among individuals aged 80 years or older, the rate was as high as 13.3 per 100,000 *(*Alessandro, *et al*., 2009*)*. As a group, PV, ET and PMF occur with about three times the frequency of CML *(*Domnita, 2010*)*; CML being not uncommon in children and young adults, there is slight male preponderance for MPNs except for ET *(*Munjal, *et al*., 2012*)*. ET is a disorder of middle age and is rare in childhood (one case per one million children). It is predominant in females (2:1). There is possibly family involvement. PMF was most commonly in the sixth or seventh decades of life and affecting both genders. Exposure to benzene or ionizing radiation is present in some cases and rare familial cases have been documented *(*Rosane, *et al*., 2012*)*.

### 1.2.3 Protein Tyrosine kinases (PTKs)

Tyrosine kinases are enzymes that catalyse the transfer of γ-phosphate from a purine nucleotide triphosphate, such as adenosine triphosphate (ATP) or guanosine triphosphate (GTP) to hydroxyl groups of specific amino acid residues of their protein substrates *(*Amy, 2010*)*. Tyrosine kinases play a critical role in the regulation of fundamental cellular processes including cell development, differentiation, proliferation, survival, growth, apoptosis, cell shape, adhesion, migration, cell cycle control, T-cell and B-cell activation, angiogenesis, responses to extracellular stimuli, neuro-transmitter signaling, platelets activation, transcription and glucose uptake *(*Groner, 2007*)*. Human genome sequence analysis has identified at least 90 tyrosine kinases [58 receptor tyrosine kinases (RTKs) and 32 non receptor tyrosine kinases (NRTKs)]. Based on their extracellular and non-catalytic domain sequences, both RTKs and NRTKs have been further grouped into 20 and 10 subfamilies, respectively *(*Groner, 2007*)*. The RTKs bind extracellular ligand, and span the cell membrane, and non-receptor tyrosine kinases NRTKs are located within the cell. RTKs contain an amino-terminal extracellular ligand binding domain (usually glycosylated), a hydrophobic transmembrane helix and cytoplasmic domain, which contain conservative tyrosine kinase core and additional regulatory sequences (that contain crucial carboxy-terminal tyrosine residues and receptor regulatory motifs). Ligand binding to the extracellular domain results in receptor dimerisation/oligomerisation, leading to activation of cytoplasmic tyrosine kinase activity and phosphorylation of tyrosine residues. Autophosphorylated tyrosine residues serve as a platform for the recognition and recruitment of specific set of signal-transdusing protein [such as proteins containing Src homology2 (SH2), and phosphor-tyrosine binding (PTB) domain], that modulate signaling responses. NRTKs have a common conserved catalytic domain (similar as RTKs) with modular amino-terminal, which have different adaptor protein motifs *(*Groner, 2007*)*. Both RTKs and NRTKs and their ligands are important mediators of normal haematopoiesis, and as a consequence deregulation of these signalling pathways by intergenic or intragenic mutation can result in leukaemogenesis *(*Amy, 2010*)*. The following sections briefly discuss tyrosine kinases involved in myeloid haematopoiesis.

### 1.2.3.1 Receptor tyrosine kinases (RTKs)

The PDGFR family includes the receptors KIT, FLT3R, PDGFRα, PDGFRβ and FMS. All of these except PDGFRα are believed to play important roles in maintaining a steady state of haematopoiesis, and are subject to tight regulation. Induction of signalling pathways JAK/STAT, PLCγ, PI3K and Ras-Raf-MEK/ERK are all observed in activation class. And the fibroblast growth factor receptor (FGFR) family members FGFR1 and FGFR3 are also targets of chromosomal rearrangement. Translocations that disrupt FGFR1 on chromosome 8p11 are closely associated with a defined 8p11 syndrome that present as an MPN with eosinophilia and other features such as T-lymphoblastic lymphoma, granulocyte sarcoma and rapid progression to acute leukaemia, usually of myeloid phenotype *(*Amy, 2010*)*.

The PDGF receptors, PDGFRα and PDGFRβ, are two highly related proteins 100, that bind a total of four ligands (PDGF A, B, C, and D), which exist in dimeric isoforms linked by disulphide bonds. Both receptors have a role in mesenchymal cell migration and proliferation, but their involvement in the pathogenesis of MPN is better understood. The most common PDGFRA fusion gene is now known to be FIP1L1-PDGFRA was described in association with an MPN phenotype characterized by eosinophilia and mastocytosis *(*Cools, *et al*., 2003*)*. Additional PDGFRA fusion genes have also been reported, for example BCR-PDGFRA was isolated in very rare cases with aCML. PDGFRB is also targeted by balanced chromosomal translocation, and more than 20 different fusions involving this gene have been recorded in patients with various MPNs. The best characterised abnormality is the t(5;12)(q33;p13) resulting in a ETV6-PDGFRB fusion gene. In an analogous manner to the BCR-ABL oncoprotein, pathogenicity is dependent on both the ETV6 dimerisation motif and the tyrosine kinase activity of PDGFRβ. However fusions involving PDGFRβ remain extremely rare *(*Golub, *et al*., 1994*)*.

The RTK KIT and its ligand stem cell factor (SCF) are essential for the development and differentiation of mast cells (*KIT*D816V and*KIT*V560G), plus other cells of the haematopoietic system *(*Furitsu, *et al*., 1993*)*.

FMS (also known as CSF1R) is the receptor for the macrophage colony-stimulating factor (M-CSF), and is important for the growth and differentiation of the monocyte, macrophage, osteoclast lineage *(*Coussens, *et al*., 1986*)*.

FLT3 (Fms-like tyrosine kinase) and FLT3 ligand play an important role in the development and expansion of multipotent haematopoietic stem cells and progenitors. Constitutive activation of FLT3 occurs either by internal tandem duplication (FLT3-ITD) of the juxtamembrane domain region or by point mutations usually involving the kinase domain. Both types of mutation constitutively activate FLT3. ITD mutations are a common finding in 25% of younger adults with AML, and approximately 7% of AML cases have point mutations in the activation loop of the kinase *(*Amy, 2010*)*.

#### 1.2.3.2 Non RTKs (NRTKs)

Lack of transmembrane domains situates NRTKs either in the cytosol or nucleus. The JAK (Janus-Associated Kinase) family is the most important NRTKs class with relevance for MPNs pathogenesis.

##### 1.2.3.2.1 Janus Kinase (JAK) family proteins

The JAK kinases are 116 to 140 kDs, and comprise approximately 1150 amino acid, involved in a variety of biological, process including haematopoiesis and regulation of immune system *(*Mughal, *et al*., 2014; William, 2013*)*. There are four mammalian JAK kinases, JAK1, JAK2, JAK3, and TYK2 *(*Ghoreschi, *et al*., 2009*)*. Each cell type expresses either three (JAK1, JAK2, TYK2), or in some cases all four JAK kinases (eg, lymphoid cells that also express JAK3) *(*William, 2013*)*. The genes of the four JAK family members in mammals are located on three different chromosomes. The first JAK family member originally identified as a novel class of PTK in human, Tyk2, is located on chromosome 19p13.2 clustered together with the Jak3 gene at 19p13.1, the genes coding for JAK1and JAK2 are located at chromosome 1p31.3 and 9p24 *(*Ghoreschi, *et al*., 2009*)*. In accord with their ubiquitous expression JAK1, JAK2, and TYK2 are activated by a variety of different sets of cytokines. For example, JAK2 is activated not only by IFNγ but also by growth hormone, Epo, prolactin, and hematopoietic cytokines, IL3, IL5, and GM-CSF *(*William, 2013*)*. Each JAK kinases have seven homologous domains (JH1-7)( Figure 1.2) , which includes the catalytic kinase domain (JH1) and catalytically inactive psedokinase domain

(JH2), it is hypothesized that the JH2 domain serves autoinhibitory function, and that the valine-to-phenylalanine substitution at codon 617 might abrogate autoinhibitory and result in constitutive tyrosine kinase activity (Hoffbrand, *et al*., 2011). The amino-terminus of JAK contains a SH2-like domain (JH3-JH4), and a band 4.1, ezrin, radixin, moesin (FERM) homology domain (JH6 and JH7). Mutations within the FERM domain of Jak3 have been reported to impair kinase activity (Ghoreschi, *et al*., 2009). In contrast with its role on the kinase activity, the FERM domain seems to be essential for binding of JAK to its receptor (Angus and Michael, 2003). Each member of the JAK family has a primary role in mediating a signaling process with some overlap between them. JAK1 plays a crucial role in the signaling of many pro-inflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor alpha (TNFα) (Mohamad, *et al*., 2013). JAK3 is important in lymphoid and JAK2 in myeloid cell proliferation and differentiation (Dino, *et al*., 2009), and TYK2 functions in association with JAK2 or JAK3 to transduce signaling of cytokines, such as IL-12 (Srdan, *et al*., 2011). JAK2 is located on chromosome 9p24 and includes 25 exons and its protein 1132 amino acids (Tefferi, 2010). It is a non receptor tyrosine kinase that is critical for normal hematopoiesis and orchestrates the intracellular signaling downstream of a numerous of hematopoietic cytokines including erythropoietin, thrombopoietin, interleukin-3, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Peter, *et al*., 2013). JAK2 kinase activity is regulated by homology domains, in particular, the JH1 (catalytic kinase domain) and the JH2 (the psedokinase domain) which serve an auto-inhibitory function (Ross, 2012). Since the identification of JAK2-V617F in MPN, additional mutations in JAK2 and other genes have been identified that also lead to constitutive activation of JAK/STAT signaling (Amy and Nicholas, 2013).

### 1.2.4 The JAK-STAT signaling pathway

JAK-STAT (Janus associated kinase-signal transducer and activator of transcription) pathway is one of the critical intracellular signaling cascades, by which signals can be transduced from the membrane to the nucleus (Ralph and Edward, 2011). Type I cytokine receptors, such as receptors for erythropoietin (Epo), thrombopoietin (Tpo), granulocyte colony stimulating factor (G-CSF) and most interleukins (IL) receptors, and type II interferons (IFN) lack intrinsic catalytic activity, and JAK-STAT signal transduction is firmly established as the major route from all class 1 and class 2

cytokine receptors to nucleus (Thomas and Mathias, 2012; Judith and Stefan, 2012). JAK-STAT signaling is important for a wide spectrum of cellular processes, including proliferation, survival or normal functioning of hematopoietic, immune, cardiac and other cells (Tefferi, 2010). Each of JAK family (JAK1, JAK2, JAK3 and TYK2) contains a conserved kinase domain and a related, but catalytically inactivate, pseudo-kinase domain at the carboxyl terminus. The pseudo-kinase domain might regulate the kinase activity of JAKs (Shuai and Bin, 2003). On the other hand, there are seven mammalian STATs: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6, which are highly homologous in several regions (Figure 1.2), including a SRC homology 2 (SH2) domain, which is involved in the activation and dimerization of STATs, a DNA-binding domain, and a transacivation domain which is located at the carboxyl terminus. The amino terminal region of STATs is involved in regulating STAT activity, such as the formation of STAT tetramers and tyrosine dephosphorylation (Shuai and Bin, 2003). STATs are normally present in the cytoplasm as inactive monomers before being recruited to receptor/JAKs complex (Rakesh, 2008). However, it has also been shown that STATs constitutively shuttle between the cytoplasm and nucleus before being retained in the nucleus following activation. The family of signal transducers and activators of transcription (STAT) consists of seven transcription factors that respond to a variety of cytokines, hormones, and growth factors (Dino, *et al*., 2009). Activation of The JAK-STAT pathway requires binding of an extracellular ligand to the transmembrane receptor, result in activation of receptor-associated JAKs. The interactions of JAK kinases with cytokines receptors is mediated by N-Terminal region, including JH6 and JH7 domains of the JAK kinases, and activated JAKs then autophosphorylate and phosphorylate their associated receptors to generate docking sites for SH2 domains of STATs (Angus and Michael, 2003). Once bound to the receptor/JAKs complex, STATs proteins are cross- phosphorylated and form either hetero- or homo-dimer, stabilized by the interaction between the SH2 domain of one molecule and the phosphor-tyr of the other molecule. Activated STAT-STAT dimer then translocate to nucleus where it bind to palindromic DNA sequence at the promoter of target genes to promote target gene transcription (Dino, *et al*., 2009). Although the STAT-STAT dimer formation can occur prior to pathway stimulation, only complexes activated by tyr-phosphorylation appear to include target gene expression. Recent studies have shown that JAK–STAT signaling can be regulated at many steps through distinct

mechanisms. Key regulators include the suppressor of cytokine signaling (SOCS) proteins and the recently discovered protein inhibitor of activated STAT (PIAS) family, as well as various protein tyrosine phosphatases (PTPs) *(*Shuai and Bin, 2003*)* (Figure 1.3).

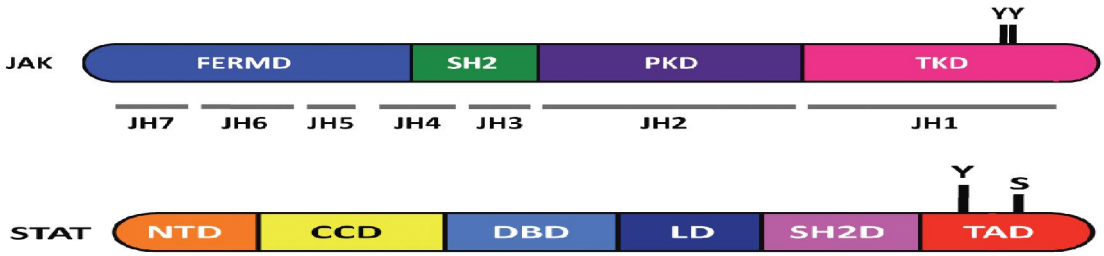
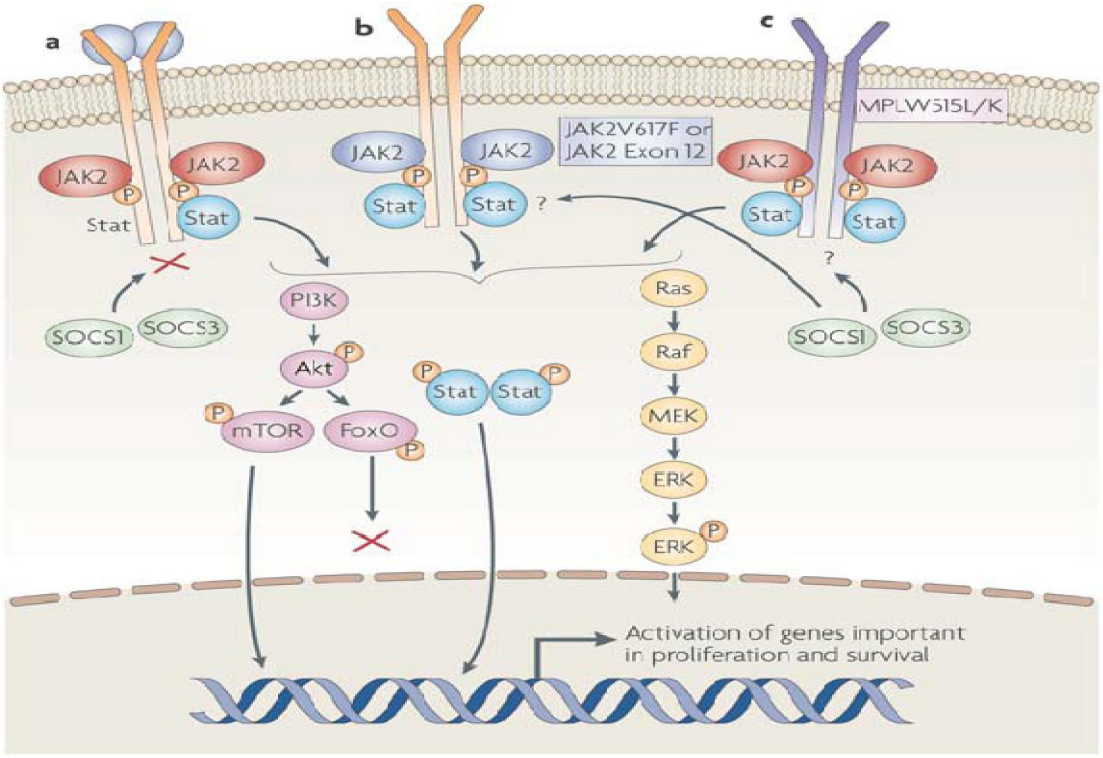



**Figure 1.2** Structures of JAKs and STATs

JAKs have 7 JAK-homology domains, named JH1-JH7. These form four main regions: the four-point-one, ezrin, radixin and moesin (FERM) domain (blue), the potential SRC homology 2 (SH2) domain (green), and the pseudokinase (purple) and tyrosine kinase domains (magenta). STATs have 6 domains, named N-terminal domain (orange), coil-coiled domain (yellow), DNA binding domain (turquoise), linker domain (navy), SH2 domain (pink) and transcriptional activation domain (red). Key phosphorylation tyrosine (Y) and serine (S) C-terminus residues are also depicted *(*Ana, *et al*., 2011*)*.




Levine RL, Nature Reviews Cancer 2007

**Figure 1.3** Signaling via the JAK-STAT pathway *(*Levine, 2007*)*

a) Binding of cytokine receptors with ligands results in Jak2 phosphorylation, recruitment and phosphorylation of Stats and activation of downstream signaling pathways including Stat transcription factors, PI3K pathway, and MAP kinase pathway. b) The JAK2-V617F and *JAK2* exon 12 mutant kinases bind receptors and get phosphorylated in the absence of cytokines, leading to ligand independent activation of downstream signaling pathways. c) *MPL*W515L/K mutants are able to phosphorylate wild-type JAK2 in the absence of TPO, which results in the activation of signaling pathways downstream of JAK2. SOCS proteins, most notably SOCS-1 and SOCS-3, normally mediate negative regulation of JAK2 signaling.

#### 1.2.4.1 Negative regulation of Jak-Stat signaling pathway

The magnitude and the duration of JAK2 signaling are tightly regulated by several mechanisms in order to prevent unnecessary signalization resulting in deregulated cell proliferation and malignant transformation. Multiple proteins including phosphotyrosine phosphatase (PTP), suppressor of cytokine signaling (SOCS), LNK, CBL and protein inhibitors of activated STAT (PIAS) are involved in the negative regulation of JAK2 mediated signaling.

#### 1.2.4.1.1 Protein tyrosine phosphatases (PTPs)

Three types of PTPs have been shown to negatively regulate JakStat signaling pathway. SH2-containing phosphatases (SHPs) have two family members SHP1 and SHP2. SHP1is mainly expressed in hematopoietic cells, while SHP2 is more ubiquitously expressed. They are characterized by the presence of two SH2 domains and a phosphatase catalytic domain *(*Basel, 2008*)*. SH2 domains of SHP-1 can bind to either phosphorylated JAKs or phosphorylated receptors to facilitate dephosphorylation of these activated signaling molecules *(*Jason, *et al*., 2004*)*. Mice deficient for Shp1 showed hematopoietic dysfunctions and displayed hyperphosphorylation of Jak1 and Jak2 following cytokine treatment. Silencing of Shp-1gene was detected in various hematologic malignancies, such as leukemia, lymphomas and myeloma *(*Oka, *et al* 2002*)*.

The receptor-like tyrosine phosphatase, CD45, a hematopoietic-specific phosphatase, is the second types of tyrosine phosphatase appear to have a role in regulating JAK/STAT signaling through a subset of receptors *(*Jason S., *et al* 2004*)*. Absence of CD45 leads to augmented Jak and Stat phosphorylation in hematopoietic cells. The third group of phosphotyrosine phosphatases includes phosphotyrosine phosphatase 1B (PTP1B) and T cell protein tyrosine phosphatase (TC-PTP), which have high similarities in their catalytic domains. They specifically recognize tyrosine residues in the Jak activation loop. PTP1B interacts with Jak2 and Tyk2 while TC-PTP dephosphorylates Jak1 and Jak3.In contrast to PTP1B, which is expressed in many tissues, TC-PTP is mainly expressed in hematopoietic cells and comprised of two isoforms created by alternative splicing, the nuclear form TC45 and the cytoplasmic form TC48. The nuclear form of TC-PTP is also responsible for the deactivation of nuclear Stat1 and Stat3 *(*Basel, 2008*)*.

#### 1.2.4.1.2 Suppressors of cytokine signaling (SOCS) proteins

SOCS proteins are small proteins that possess SH2 domains and conserved C-terminal SOCS/CIS boxes. The SOCS family of proteins has eight members: CIS (cytokine-inducible SH2 domain protein) and SOCS1–SOCS7 *(*Kile., *at al* 2002*)*. CIS, a cytokine inducible SH2 containing protein, is a target of JAK2-STAT pathway. Moreover, it contains an SH2 domain and binds to tyrosine-phosphorylated EPO and IL-3 receptors. Thus, CIS is a feedback modulator of STAT5; its expression is

induced by STAT5 and it negatively modulates STAT5 activation resulting in attenuation of JAK-STAT pathway *(*Yoshimura., *et al* 1995*)*. SOCS such as SOCS-1 associate with the activation loop of JAKs directly via its SH2 domain to inhibit the function of JAKs. Similarly the kinase inhibitory region of SOCS-1 and SOCS-3 can bind to the JAK catalytic pocket and block its catalytic activity. The third way is to target signaling proteins to the ubiquitin proteasome pathway through the SOCS box

**1.2.4.1.3 Protein inhibitor of activated STAT proteins (PIAS)**

PIAS are a family of genes involved in inhibiting the JAK-STAT signaling pathway. PIAS1 and PIAS3 interact with STAT1 and STAT3, respectively *(*Shuai., *et al* 200*)*. This PIAS-STAT interaction inhibits STAT binding and STAT-mediated gene activation. PIAS family is comprised of 5 members: PIAS1, PIASx, PIAS3-α, PIAS3-β and PIASy. They consist of a N-terminal LXXLL co-regulator domain, a zinc finger domain and a Cterminal acidic domain *(*Rakesh and Agrawal, 2005*)*.

LNK, also known as SH2B3, is a member of SH2B family of adopter proteins that is involved in negative regulation of cytokine receptor signaling. The LNK Src homology 2 (SH2) domain is essential for its inhibitory function. LNK is shown to inhibit TpoR and EpoR mediated signaling inhibited by attenuating JAK2 activation resulting in blunting of AKT, STAT and MAPK pathways *(*Tong., *et al* 2005*)*.

Ubiquitination is another way to control JAK2 signalization. The CBL are E3 ubiquitin ligases that attach an ubiquitin molecule, to growth receptors, tyrosine kinases and other molecules and may targets them for recycling or degradation (Schmidt M and Dikic I, 2005).

**1.2.5 JAK2 Mutations**

A number of chromosomal translocations involving JAK2 have been found in the leukemias and rarely in MPNs. The first described JAK2 somatic alteration indentified in human malignancy was a fusion of JAK2 to TEL (translocation ETS leukemia or ETV6). All described TEL- JAK2 fusions proteins include the JH1 kinase domain of JAK2, and occasional translocations have also been found to include the JH2 pseudokinase domain. The TEL- JAK2 fusions proteins results in constitutive kinase activation of JAK2 *(*Verstovsek and Tefferi, 2011*)*. ETV6-JAK2 fusion was isolated in a T-cell ALL patient, and later in a child with early B-precursor ALL and in adult aCML *(*Amy, 2010*)*. The second most frequently reported JAK2

translocations fuses JAK2 to PCM. The PCM1 gene encodes a centrosomal protein, pericentriolar material1. PCM-JAK2 fusion protein includes both JH1 kinase domain and JH2 pseudokinase domain *(*Verstovsek and Tefferi, 2011*)*. PCM1-JAK2 was identified in several cases of chronic and acute leukaemia, such as aCML, acuteerythroid leukaemia and T-cell lymphoma. A single case of aCML was also found to carry a BCR-JAK2 fusion gene *(*Amy, 2010*)*. Nevertheless, JAK2 translocations remain rare in MPN, especially when compared to the incidence of JAK2 V617F.

#### 1.2.5.1 The JAK2 V617F Mutation in MPNs

In 2005, several groups independently reported a somatic mutation in the Janus family kinase JAK2 gene (JAK-2V617F) in a maioraty of myeloproliferative neoplasms (MPNs) patients *(*Baxter, *et al* 2005; Levine, *et al*., 2005*)*. JAK2-V617F results from a somatic G to T transversion involving JAK2 exon 14, which leads to nucleotide change at position 1849 and the substitution of valine to phenylalanine at codon 617 (JAK-2V617F), affects the auto-inhibitory domain (JH2, pseudokinase) of JAK2 leading to constitutive activation of JAK2 and JAK/STAT signaling, and a consequent loss of control in cell proliferation and growth *(*Tefferi. 2010; Francesco, *et al*., 2011*)*. JAK is a non receptor tyrosine kinase that is critical for normal hematopoiesis and coordinates the intracellular signaling downstream *(*Peter, *et al*., 2013*)*. The affected codon is located in the JH2 pseudokinase domain of JAK2, and the mutation is generally considered to negatively affect the JH2-mediated auto-inhibitory functionality of the enzyme, resulting in constitutive activation of the tyrosine kinase function. This in turn results in dysregulation of JAK-dependent signal transduction and activation of multiple downstream effectors, including STAT3 and STAT5 *(*Mughal, *et al*., 2014*)*.

More than 95% of patients with PV and approximitly 60% of patients with ET and PMF were found to have the mutation *(*Baxter, *et al*., 2005; Kralovics, *et al*., 2005; Levine, *et al*., 2005*)*. It is also present in about 5-15% of patients with atypical CML or MDS/MPD and in approximately 50% of patients with ‘refractory anemia with ring sideroblasts and thrombocytosis’ (RARS-T). It is much less common in patients with other myeloid disorders such as MDS, CMML, CEL or AML *(*Christoph, *et al*., 2008*)*. The JAK2 V617F mutation can be detected in hematopoietic progenitors as

well as mature hematopoietic cells. Individual cells can either be heterozygous or homozygous for the JAK2 V617F mutation and the homozygous state is a result of mitotic recombination, which by microsatelite mapping was first demonstrated as loss of heterozygosity of chromosome 9p (9pLOH) *(*Thomas, 2007*)*. Although a majority of MPN patients carry heterozygous JAK2V617F mutation, loss of heterozygosity (LOH) on chromosome 9p involving the JAK2 locus has been observed in ~30% of MPN patients *(*Akada , *et al*., 2014*)*. Cell numbers homozygous for the JAK2 V617F mutation increases with time, suggesting a proliferative and survival advantage of mutant progenitor cells. Homozygosity in hematopoietic precursors is often found in PV, whereas it is a rare event in ET *(*Thomas, 2007*)*. The 9pLOH results in the loss of JAK2 WT allele and concomitant duplication of the JAK2V617F mutant allele via mitotic recombination. Interestingly, 9pLOH is more common in PMF and PV than in ET, JAK2V617F homozygosity via 9pLOH has been associated with more severe MPN phenotype. However, the contribution of 9pLOH in the pathogenesis of MPNs remains unclear *(*Akada , *et al*., 201*)*.

The JAK2-V617F mutation confers cytokine hypersensitivity, constitutive activation of the JAK-STAT pathway, and cytokine-independent growth of so-called endogenous erythroid colonies (EEC) in the majority of patients with PV *(*Alexandra, *et al*., 2013*)*. Currently, only limited knowledge about the underlying molecular mechanisms as well as the resulting dysregulations associated with JAK2-V617F expression exists. Data derived both from patients and mouse models suggest misbalanced signaling of the mutated kinase. In addition, there is strong evidence for deregulated signaling to be dependent upon the expression level of JAK2-V617F *(*Alexandra, *et al*., 2013*)*. The JAK2 V617F mutant induces several downstream signaling pathways; recapitulating in a persistent manner the pathways transiently activated by cytokines, i.e., STAT5/3, MAP-kinase, PI-3′-kinase/Akt and mTOR pathways. Interestingly, there are differences in STAT signaling between different MPNs. PV patients exhibit high STAT5 and STAT3 phosphorylation, while ET patients exhibit high STAT3 but low STAT5 phosphorylation. In contrast, myelofibrosis patients exhibit low STAT5 and low STAT3 phosphorylation *(*Judith and Stefan, 2012*)*. Several lines of evidence indicate that constitutive activation of JAK2-STAT5 and STAT3 signaling is the basis of PV, ET and PMF *(*Judith and Stefan, 2012*)*. Recently, it was shown that knockout of STAT5A/STAT5B prevents

induction of the myeloproliferative phenotype by JAK2-V617F, thereby establishing an obligatory role of STAT5 in MPNs *(*Judith and Stefan, 2012*)*. Furthermore, the choice and relative levels of activated STATs can have profound consequences on the lineage that is amplified. For example, down-modulation of STAT5 in $CD34^{+}$ cells promotes megakaryocyte differentiation, while activation of STAT5 promotes erythropoiesis. More recently, STAT1 was shown to be specifically associated with megakaryopoiesis *(*Judith and Stefan, 2012*)*.

#### 1.2.5.1.1 Biological consequences of JAK2-V617F mutation- Murine system

The Biological consequences of JAK2-V617F mutation have been clearly demonstrated by murine experiments using either retrovirally transfected hematopoietic stem cells in bone marrow transplantation settings or transgenic animals. Retroviral mouse models, using mouse JAK2 cDNA in which the mutation had been introduced, have demonstrated a PV phenotype which often progress to post PV-MF, but thrombocytosis is absent in these mice *(*Lacout, *et al*., 2006; Wernig, *et al*., 2006*)*. These models usually display polycythemia with erythrocytosis, leukocytosis and transformation to myelofibrosis. However the degree of leukocytosis and development to myelofibrosis varies, and is probably depend on the genetic background and gene dosage among other factors *(*Marty, *et al*., 2010*)*.

Very recently a JAK2-V617F mutant transgenic mouse model using human JAK2-V617F has been generated. These mice developed a phenotype resembling ET, with moderate neutrophilia and marked thrombocytosis that has never been observed with retroviral models. Through induction of the mutated transgene expression, and thus control over ratio of expression levels of mutated transgene to endogenus mouse JAK2, a variable phenotype ranging from thrombocytosis only to bilineage disease involving thrombopoiesis and granulopoiesis(ET-like) to the full trilineage PV-like phenotype was observed. The phenotype (a) and (b) were induced by JAK2-V617F/JAK2-wt ratios <1 or = 1respectively. High levels of JAK2 with V617F/JAK2-wt > 1 appear to inhibitory to megakaryopoiesis. This demonstrated by normal or decreased platelets count in retroviral murine CMPD-models as well as the lower on average platelet counts in observed PV patients as compared to ET patients *(*Tiedt, *et al*., 2008*)*. One possible explanation for this phenomenon is that JAK2-V617F downregulates MPL expression *(*Moliterno, *et al*., 2006*)*.

Ex vivo studies have provided some interesting results recently. The study of individual erythroid colonies derived from patients with PV and ET showed that JAK2V617F homozygosity apparently arises with similar frequencies in PV and ET, but expansion of a dominant homozygous subclone was found only in PV, likely due to acquisition of additional molecular lesions *(*Godfrey, et al., 2012*)*. In this model, development of a dominant JAK2V617F-homozygous subclone drives erythrocytosis in many PV patients, with alternative mechanisms operating in those with small or undetectable homozygous-mutant clones. Another study found that the thrombopoietin receptor MPL can induce antiproliferative effects in cells with high JAK activation levels, such as cells with the JAK2V617F mutation *(*Pecquet, *et al*., 2012*)*. These findings could explain why many PV and PMF patients with homozygous JAK2V617F show down-modulation of MPL levels in platelets, a mechanism allowing cells to escape this antiproliferative effect of MPL.

#### 1.2.5.1.2 JAK2-V617F allele burden

The most important question arose after the discovery of JAK-V617F mutation is how a single mutation might give rise to at least three different diseases. This question remains unanswered, but clinical, biological and pathological data have led to three potential hypotheses. First, the gene-dosage hypothesis, postulates a correlation between disease phenotype and the proportion of JAK2-V617F mutant alleles introducing the concept of allele burden, which is the ratio between mutant and wild type JAK2 in hematopoietic cells. The potential impact of the burden of JAK2 mutation on disease phenotype through the level of activation of downstream JAK/STAT signaling pathways is supported by several experimental and clinical observations. First, homozygous JAK2-V617F mutant erythroid colonies are observed in almost all patients with PV, but only rarely in those with ET. These data demonstrate the differences in JAK2-V617F gene dosage between PV and ET, and suggest that the higher levels of JAK2 activation associated with JAK2-V617F homozygosity might result in a PV phenotype, whereas a lesser degree of activation of JAK2 signaling might result in an ET phenotype *(*Ross, 2008*)*. There are substantial differences in the median burden of V617F allele in peripheral blood granulocytes among individual MPNs; the highest levels are found in PV and the lowest in ET patients, with most PMF patients displaying intermediate to high levels *(*Vannucchi, *et al*., 2008a; Passamonti, *et al*., 2006*)*. Most patients with MPN-MF

have high levels of the JAK2 V617F allele. Furthermore, erythroid progenitors harbouring the V617F mutation are more sensitive to erythropoietin than normal erythroid progenitors, and most erythropoietin-independent erythroid colonies are made up of homozygous progenitors *(*Delhommeau, *et al*., 2007*)*. Conceivably, duplication of the mutant allele is expected to result in a level of JAK2/STAT activation higher than in cells harbouring one mutant and one wild-type allele and in a greater activation of downstream genes *(*Vannucchi, *et al*., 2006; Kralovics, *et al*., 2005a*)*. This could be due to the loss of competition between normal and mutated allele and/or impaired interaction of mutant JAK2 with cellular regulators such as the suppressor of cytokine signalling-3 (SOCS3) *(*Hookham, *et al*., 2007, Alessandro, *et al*., 2011*)*. A second hypothesis advocates the existence of a pre-JAK2 phase in which additional somatic mutations or inherited predisposing alleles establish clonal hematopoiesis before the acquisition of JAK2-V617F. Thus, mutations other than JAK2 may determine disease phenotype directly or by co-operating with JAK2 mutations. Finally, host genetic factors may contribute to phenotypic diversity among myeloproliferative neoplasms *(*Francesco and Elisa, 2008*)*.

#### 1.2.5.1.3 Clinical correlations of JAK2-V617F mutation

Soon after identification of JAK2-V617F mutation in a vast majority of PV and half of ET and PMF patients, multiple studies attempted to fine out the correlations between *JAK2*-V617F mutational status or allele burden and clinicohematologic characteristics of these disorders. Parameters such as age, white blood cell (WBC) count, Hb concentration, Hct, spleen size, disease duration, pruritus, and fibrosis in subjects with MPN were associated with JAK2-V617F mutation status *(*Campbell, *et al*., 2005*)*. In numerous studies, the JAK2-V617F allele has been variably associated with higher indices of erythropoiesis, unchanged or decreased platelet counts, greater occurrence of thrombosis, increased BM fibrosis or cytoreductive treatments, older age, longer disease duration, or poorer survival in MPN *(*Kralovics, *et al*., 2005; Rudzki, *et al*., 2007*)*. There is now a growing interest in JAK2-V617F allele burden and its potential influence on disease phenotype, disease complications and evolution *(*Francesco and Elisa, 2008*)*. The effect of JAK2-V617F allele burden in MPNs has been demonstrated in several studies. It has been found that the higher JAK2-V617F allele burden in PV patients correlates with an increased risk of MF transformation, more advanced myelofibrosis, greater splenomegaly, higher white blood counts,

increased frequency of thrombosis including major cardiovascular events, and increased need for chemotherapy treatment. Interestingly, PMF patients with low JAK2-V617F allele burden had a worse overall and leukemia-free survival when compared with patients with either a high allele burden or wild-type status *(*Mohamad, *et al*., 2013*)*.

#### 1.2.5.1.4 JAK2-V617F detection methods

Several laboratory methods of *JAK2*V617F mutation detection are currently available, for the detection of minimal residual disease; highly sensitive quantitative assays are required *(*Huijsmans, *et al*., 2011*)*. In general, quantitative PCR methods are preferred because of their potential value in measuring mutant allele burden and monitoring treatment response.

**Conventional DNA sequencing:** A PCR amplified DNA sequence of interest (in this instance the mutated DNA region of JAK2) is processed through an automated sequencer that is based on the Sanger DNA sequencing principle. The method is semi quantitative, can distinguish a homozygous from a heterozygous pattern of mutation in the absence of mixed clonality, and the reported sensitivity is above 5% *(*James, *et al* 2006*)*.

**Pyrosequencing (sequencing-by-synthesis):** The technique of pyrosequencing is based on the detection of light signal (bioluminescence) from a luciferase reaction using pyrophosphates that are released by the incorporation of nucleotides by polymerase during *in vitro* DNA synthesis as source of ATP. Specific nucleotides are added stepwise to the reaction in order to determine the sequence of the DNA strand of interest that is used as a template for DNA synthesis. The method is suitable for quantitative measurements and can distinguish homozygous from heterozygous mutation pattern, and assay sensitivity is estimated at 5% *(*Jones, *et al*., 2005*)*.

**Melting curve assay:** A fluorescence-tagged primer that is complementary to either wild-type (WT) or mutated sequence is incorporated in a standard PCR reaction and differences in the dissociation temperature of the primer from either the WT or mutant sequence is used to differentiate between the two. In other words, mismatched binding dissociates at a lower temperature. The method is semiquantitative with 1% to 10% reported assay sensitivity *(*McClure, *et al*., 2006*)*.

**Allele-specific PCR:** There are several different methodologies that utilize allele-specific (AS) primers, in the context of both qualitative and quantitative platforms. One such assay is very sensitive (0.01%-0.1%) and involves the use of a mutation specific forward primer that results in the amplification of only the mutated sequence that is detected by capillary electrophoresis *(*McClure, *et al*., 2006*)*. Another AS-PCR method, referred to as amplification refractory mutation system (ARMS; reported sensitivity of 1%-2%), uses two primer pairs to specifically amplify both WT and mutant DNA sequence in a single reaction. In general, AS-PCR methods are suitable for quantitative measurements on a real-time PCR platform, using either genomic DNA or cDNA *(*Levine, *et al*., 2006; Lippert, *et al*., 2006*)*.

**BsaXI restriction analysis:** The JAK2-V617F mutation interferes with a *Bsa*XI restriction enzyme recognition site and therefore results in restriction digests with altered size following site-specific restriction analysis *(*Baxter, *et al*., 2005*)*. The method can discriminate homozygous from heterozygous mutation patterns. Using restriction enzyme *Bsa*XI has the ability to show both mutant and normal allele in a sample. However, the assay may cause misinterpretation because incomplete *Bsa*XI digestion would generate a mutant plus normal pattern in patients with only wild-type alleles *(*Qiaofang, *et al*., 2007*)*.

### 1.2.5.2 JAK2 exon 12 Mutations

Approximately 2% of well-characterized PV patients do not carry a JAK2-V617F mutation. In 2007, other JAK2 mutations (exon 12 JAK2) were described in many *JAK2*V617F-negative patients PV. These mutations cluster within small region of spanning codons 537-547, most of which affect Leu539 or Glu543. The region lies just upstream of SH2 pseudokinase domain and thought to affect interaction between SH1 and SH2 domains *(*Drew and John, 2010*)*. Mutations result in the gain-of function and activation of JAK2 similar to the V617F mutation *(*Scott, *et al*., 2007*)*. The JAK2 exon 12 mutations lead to high degree of cytokine independence and phosphorylatin of JAK2 substrates than does JAK-V617F. In murine models, JAK2K539L lead to elevated hematocrit, leukocytosis, and thrombocytosis but leukocytosis and thrombocytosis are less dramatic than with the V617F mutation. Similarly, patient carry JAK2 exon 12 mutations tend to display isolated erythrocytosis within the bone marrow, in contrast, to JAK-V617F-positive patients

who characteristically show hyperplasia of all myeloid three lineages (Drew and John, 2010). The JAK2 exon 12 mutations have not been detected in JAKV617F-negative ET or PMF. Hence mutations within JAK2 exon 12 lead to increase kinse activity that drives hematopoietic stem cell to ward erythropoiesis but not other hematopoietic cell lineage (Scott, *et al*., 2007).

### 1.2.5.4 JAK2 inhibitors

The discovery of the BCR-ABL1 inhibitor imatinib and its unprecedented success in the therapy of CML ushered hematology-oncology into the era of kinase inhibitors, where small molecules target kinases aberrantly activated in cancer cells, with a greater therapeutic index and safety compared to traditional chemotherapeutic agents (Fabio and Srdan , 2010; Srdan, *et al*., 2011). Activating JAK2 mutations in MPNs have been suggested to be an ideal target for inhibition due to their prevalence and apparent role in the disease phenotype (Mamatha, *et al*., 2012). As soon as 2 years after the publication of the first reports of the JAK2 V617F mutation in MPNs, several JAK2 inhibitors were entering clinical trials for patients with MF and later PV and ET, with differences in potency and kinase specificity (Fabio and Srdan , 2010). Initial clinical trials have been focusing specifically on MF due to a limited number of efficient targeted approaches and immediate therapeutic need (Mamatha, *et al*., 2012). Whoever clinical trials with JAK2 inhibitors showed significant improvements in splenomegaly and constitutional symptoms in patients with myelofibrosis but meaningful molecular responses was not documented (Bogani, *et al*., 2013). In ET and PV JAK2 inhibitor therapy may efficiently control blood cell count, as well as improve splenomegaly and control disease related symptoms. Most TKI in current clinical development are small molecules that act by competing with ATP (adenosine tri-phosphate) for the ATP-binding catalytic site in the TK domain, since ATP is the source of phosphate groups utilized by TK for phosphorylating protein targets. The V617F mutation locates outside the TK domain of JAK2, and current JAK2 inhibitors target both wild-type and mutated JAK2 indiscriminately. This could explain why these drugs are active in patients with both wild-type and mutated JAK2 (Fabio and Srdan, 2010). High affinity interactions with amino acids in this hydrophobic groove determine the specificity for each drug, currently there are many JAK2 kinase inhibitors in clinical trials (Mamatha, *et al*., 2012).

In 2011, the dual JAK1/JAK2 inhibitor INCB18424 (ruxolitinib; Jakafi) was approved for the treatment of myelofibrosis (MF) based on the ability of ruxolitinib to markedly reduce splenomegaly and ameliorate MF-associated symptoms. Despite the important clinical benefits of ruxolitinib therapy, JAK inhibition with ruxolitinib and with other JAK inhibitors does not substantively reduce mutant allele burden as is observed with BCR-ABL kinase inhibition in CML *(*Neha, *et al*., 2014*)*.

### 1.2.6 Molecular pathogenesis of MPNs

#### 1.2.6.1 Cytogenetic

The spectrum of cytogenetic aberrations in MPNs is heterogeneous, ranging from numerical gains and losses to structural changes, including unbalanced translocations *(*Faramarz, *et al*., 2013*)*. Chromosomal gains and losses rather than unbalanced translocations appear to be common in MPNs (Table 1.4). In fact at least 27 different chromosomal anomalies have been associated with MPNs. Unlike the Philadelphia chromosome in CML there is no pathognomonic chromosomal abnormality associated with MPNs *(*Reilly, 2008*)*. Chromosomal abnormalities are seen in 30 – 40% of patients with PV and MF and seen to indicate a poor prognosis. On the other hand, chromosomal abnormalities are rare in patients with ET. In cases suspicious for ET, cytogenetic studies are used for exclusion of other hematological malignancies associated with increased megakaryopoiesis such as 5q-syndrome or AML with inversion chromosome 3q *(*Faramarz, *et al*., 2008*)*. Chromosomal anomalies are found most frequently in PMF (up to 50%), followed by PV, whereas anomalies in ET and CEL are so infrequent (Table 1.5). The most common structural chromosomal anomalies of MPNs in order of frequency are t(9;22)(q34;q11.2), del(20)(q11q13), del(13)(q12q14), del(5)(q13q33), and del(12)(p12). Only the t(9;22) is diagnostic for CML *(*Tefferi,*et al*., 2009).

**Table1.4** Chromosomal aberrations in Myeloproliferative Neoplasms *(*Faramarz, *et al*., 2008*)*

| Chromosomal abnormality | Frequency % | Prognosis |
|---|---|---|
| Trisomy 1q | 8 | |
| 4q12 deletion (CHIC2) | | Response to imatinib |
| 5q32 aberrations (PDGFB) | | Response to imatinib |
| Monosomy 7 | 5 | |
| Trisomy 8 | 16 | Good prognosis |
| 8q11 rearrangement (PDGFR1) | | Poor prognosis |
| Trisomy 9 | 10 | Nuclear |
| 12p aberrations | 3 | |
| 13q deletion | 7 | Nuclear to good if sole aberration |
| 20q deletion | 9 | Good |

**Table1.5** Incidence of Chromosomal abnormalities in subtypes of MPNs *(*Faramarz, *et al*., 2008*)*

| Subtype | Frequency % |
|---|---|
| CML | 95 |
| PV | 34 |
| CIMF | 40 |
| CEL | 7-12 |
| ET | < 3 |

CIMF chronic idiopathic myelofibrosis

### 1.2.6.2 Pathogenetic contribution of mutations in MPNs

MPNs are clonal stem cell diseases characterized by an excessive production of blood cells because of hypersensitivity or independence from normal cytokine regulation. In CML, the bcr-abl fusion protein plays a key role in pathogenesis of CML, by constitutive tyrosine kinase (TYK) activity *(*Cherie and Philip, 2010*)*. Their cellular effect is exerted through activation of multiple signal transduction pathways that transduce oncogenic signals *(*Najia, *et al*., 2014*)*. Inhibition of the ABL kinase activity in patients results in disease regression, demonstrating that BCR-ABL is the oncogenic event responsible for the disease *(*William, *et al*., 2011*)*.

In contrast to our detailed understanding of chronic myeloid leukemia pathogenesis, we only have some essential clues to the molecular pathogenetic mechanisms for PV, ET, and PMF. A major clue was the recognition of increased signaling through the JAK-signal transducer and activator of transcription (STAT) pathway, comprised of JAKs and STATs, as well as through the phosphatidylinositol 3-kinase (PI3K)-AKT (also known as protein kinase B) pathway in erythroid and myeloid cells *(*Delhommeau, *et al*., 2006*)*. The most significant clue to date came in 2005 with the identification of the somatic mutation in JAK2 gene (JAK2-V617F) *(*Tariq, *et al*., 2014*)*. The mutation in JAK2 exon 14, which occurs in at least 95% of patients with PV and about 60% of those with PMF and ET, results in a valine (V) to phenylalanine (F) substitution at codon 617 *(*Zhe, *et al*., 2007; Alvin and Hillard, 2012; Mohamad, *et al*., 2013*)*. The mutation occurs within the JAK2 pseudokinase domain (JH2) and disrupts its autoinhibitory function, resulting in constitutive activation of JAK2-mediated signaling pathways through the STAT5 and STAT3, PI3K and extracellular signal-regulated kinase *(*Ross, 2012*)*. Generally an increasing number of mutations that directly or indirectly affect JAK-STAT signaling are being implicated in the pathobiology of MPNs *(*Mughal, *et al*., 2014*)*.

Several other mutations which lead to constitutive activation of the JAK2-STAT5 pathway and manifest in MPNs phenotypes, and are therefore seen as functionally similar, if not equivalent to the JAK2-V617F mutation have been reported *(*Greil, *et al*., 2010*)*. The first identification of somatic mutation which activates JAK2 in JAK2V617F-negative MPN was the identification of somatic point mutations at codon 515 of MPL in 10% of JAK2V617F- negative PMF in 2006. MPLW515L results from a G to T transition at nucleotide 1544 (exon 10), resulting in a tryptophan to leucine substitution at codon 515. Different mutations at codon 505 and 515 have subsequently been identified in ET and PMF patients *(*Tefferi, 2010*)*. In aggregate, MPL mutations are seen in 4% of ET patients, and 10% of PMF patients (absent in PV). Functional studies showed that MPLW515 directly activates JAK2 and downstream signaling pathways, and when expressed in vivo results in distinct MPN phenotype notable for thrombosis, splenomegaly, and bone marrow fibrosis. These observations provided the first evidence of an alternate mutational pathway to activate JAK2 in JAK2V617F- negative MPN patients, and provided the first genetically accurate in vivo model of PMF *(*Pikman, *et al*., 2006*)*.

In 2007, other JAK2 mutations (exon 12 *JAK2*) were described in JAK2-V617F-negative patients with PV. These mutations are located in region between the SH2 and JH2 and result in the gain-of function and activation of JAK2 similar to the V617F mutation. The pleoitropic natures of JAK2 exon 12 mutations include missense, deletion, and insertion mutations in codons 538 – 543 of JAK2. To date exon 12 mutations have only been identified in patients with JAK2V617F- negative PV and not in ET or PMF. In addition, most patients with exon 12 mutations present with isolated erythrocytosis without thrombosis or leukocytosis *(*Scott, *et al*., 2007*)*.

More recently (December 2013), mutations in the *CALR* gene have been reported in a significant proportion of MPN patients (~70% of *JAK2*-nonmutated ET and ~85% of JAK2-nonmutated PMF) *(*Klampfl, *et al*., 2013; Nangalia, *et al*., 2013; Ha and Kim, 2015*)*. Calreticulin is a protein with multiple functions including roles in cell proliferation and apoptosis. Mutations occurring in exon 9 are most commonly small deletions or insertions with or without substitutions. The mutations result in a frameshift to the alternative reading frame, and the resultant mutant proteins have a novel amino acid sequence at the C-terminus. Pathologically, *CALR* mutations are confined to ET, PMF, and refractory anemia with ringed sideroblasts associated with marked thrombocytosis (not seen in PV). Clinically, patients with *CALR* mutations were reported to have higher platelet counts, lower leukocyte and hemoglobin levels, lower risk of thrombosis, and a more indolent clinical course compared to those with *JAK2* mutations *(*Rumi, *et al*., 2014; Choi, *et al*., 2015*)*. The significance of the *CALR* mutant allele burden is not known. Therefore the JAK-STAT activation might be the central theme in MPN pathogenesis; driver mutations in MPN are often mutually exclusive and include *JAK2*, *MPL* and *CALR*. *JAK2* and *MPL* mutations are believed to directly activate JAK-STAT whereas *CALR* mutations might do the same indirectly, although mouse models suggest a primary effect on platelet production. Mutations or DNA sequence variants other than *JAK2*, *MPL* or *CALR* occur in a substantial number of patients with all three MPN. The pathogenetic role of these other mutations is incompletely understood and might include cooperation with the driver mutations in facilitating disease progression *(*Tefferi, 2015*)*.

The genetics of MPN suggest that there are additional disease alleles in MPN patients in addition to those within the JAK-STAT pathway. For example, studies have shown

in MPN patients with a clonogenic cytogenetic abnormalities have cytogenetically abnormal clones with and without the JAK2V617F allele. In addition, approximately one half of JAK2- mutant MPN patients that undergo leukemic transformation paradoxically transform to JAK2 wild-type AML *(*Edward, *et al*., 2014*)*. TET2 (ten eleven translocation), a putative tumor suppressor gene located on 4q24, can be affected by an array of frameshift, nonsense and missense mutations *(*Francesco, *et al*., 2011*)*. TET2 mutations, first described in 2008 *(*Tefferi, 2010*)*, and in a large cohort of MPN patients, TET2 mutations were detected in 16% of PV, 5% of ET, 17% of PMF, 14% of post-PV MF, 14% of post-ET MF and 17% of blast phase MPN; but TET2 mutations are also described in other myeloid malignancies such as myelodisplastic syndromes (MDS) *(*Francesco, *et al*., 2011*)*.

Shortly after the discovery of TET2 mutations in the myeloid cancers, somatic, loss-of –function mutations were identified in the ASXL gene in the patients with myeloid malignancies with and without TET2 mutations *(*Edward, *et al*., 2014*)*. ASXL1 (Additional Sex Combs-like 1) is located on 20q11.1 and belongs to a family of three identified members that encode poorly characterized proteins regulating chromatin remodeling, enhancing transcription of certain genes while repressing the transcription of others. Mutations, mainly frameshift and nonsense, occur within exon 12. Both TET2 and ASXL1 alterations lead to an increase in the program of self-renewal in MPN progenitors through modifications of DNA and histone regulation. Mutations within ASXL1 are found in 8% of MPN, 11% of MDS, 43% of chronic myelomonocytic leukemia, 7% of de novo AML, and 47% of secondary AML *(*Francesco, *et al*., 2011*)*.

Most recently, a series of studies have identified recurrent somatic mutations in MPN, MDS, and AML patients, including IDH1/2 mutations, EZH2 mutations and DNMT3A mutations. In each case these mutations are not specific to MPN, as they are observed often more commonly in patients with MDS and AML *(*Edward, *et al*., 2014*)*.

Although less common (<10% of MPN cases) additional mutations in MPN have also been described in the intracellular signaling gene (eg; Casitas B.cell lymphoma) [CBL]. CBL is an adapter protein that has also been shown to possess E3 ubiquintin ligase activity and it serve to negatively regulate JAK2 signaling through

ubiquintation and subsequently internalization of growth factor receptors. In MPN cells, mutations that inactivate or cause truncation of CBL result in enhanced downstream signaling through JAK/STAT and modulation of cell cycle, proliferation and apoptosis-related proteins *(*Ross, 2012*)*. Mutations in this gene are more frequent in juvenile myelomonocytic leukemia (17%) than in MPN (6% in PMF) *(*Francesco, *et al*., 2011*)*.

LNK mutations have been detected in 6% to 13% of chronic phase and blast phase of MPL and occur within a hot spot in the plsckstrin homology domain of exon2 *(*Gery, *et al*., 2009*)*. LNK, located on 12q24.12, encodes for LNK, a plasma membrane-adaptor protein whose functions include inhibition of wild type and mutant JAK2 signaling. In fact, LNK mutations in JAK2V617F-negative MPN are associated with myeloid progenitor expansion and cytokine responsive pSTAT5 and pSTAT3 expression, which phenocopies activating mutations in JAK2 *(*Tong, *et al*., 2005*)*. It's intriguing that LNK-deficient mice exhibit increased number of megakaryocytes and erythrocyte progenitors, as well as an expanded hematopoietic stem cell pool with enhanced self renewal. Loss of function mutations of LNK situated within exon 2 has been described at low frequency in ET and PMF, and in erythrocytosis with low erythropoietin *(*Pardanani, *et al*., 2010*)*. suppressor of cytokine signaling (SOCS) proteins are also negative regulators of JAK signaling, the expression of which is inactivated by mutation or through CpG island hypermethylation of SOCS1 or SOCS3 promoter in MPN cells, leading to excessive cytokine signaling. Although mutations in the SOCS genes have been described in MPN, their occurrence is rare, and their role in pathogenesis of MPN is controversial *(*Ross, 2012*)*.

### 1.2.7 Classical BCR/ABL-negative chronic myeloproliferative neoplasms (MPNs)

The term classical BCR/ABL-negative chronic myeloproliferative neoplasms (MPNs) includes three clonal disease arising in a pluripotent hemopoietic stem cell that share clinical, hematological, and biological features: polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) *(*Srdan, *et al*., 2011*)*. These related entities characterized by proliferaion of one or more of the myeloid lineages with the variably common features of erythrocytosis, granulocytosis, and/or thrombocytosis in peripheral blood (BP) and/or variable bone marrow (BM) fibrosis. Generally are diseases of older individual; however, ET and PMF have been reported in children. This category also possesses tendency toward organomegaly (i.e., hepatosplenomegaly), thrombosis, and bleeding *(*Cherie and Philip, 2010*)*. There are considerable overlaps in the morphologic features between the various entities in this category of MPN, and proper diagnostic classifications depend on clinical and molecular characterization *(*Stefan and Kagop, 2011*)*. The BM usually hypercellular with blast count of<10%. Although these diseases are heterogeneous, they are characterized by increased blood cell production related to cytokine hypersensitivity, virtually normal cell maturation, and progressive evolution to BM failure with an end point of fibrosis, or leukemia *(*Cherie and Philip, 2010*)*. In contrast to CML, where the bcr-abl fusion protein leads to constitutive tyrosine kinase activity that plays a key role in pathogenesis, the molecular pathogenesis of the BCR/ABL-negative chronic myeloproliferative neoplasms(MPNs) has been poorly understood until the recent discovery of the somatic Janus kinase 2(JAK2) mutations in 2005 *(*Greil, *et al*., 2010*)*. More than 95% of patients with PV and approximately 60% of patients with ET and MF were found to have a G to T substitution in exon 14 of JAK2, leading to a valine to a phenylalanine change at position 617 (JAK2-V617F) *(*Harper, 2012*)*, therefore The 2008 World Health Organization (WHO) classification incorporated JAK2 V617F mutation status into the diagnosis of BCR-ABL negative MPN *(*Huijsmans, *et al*., 2011*)*. The V617F point mutation within the JH2 domain disrupts its autoinhibitory function *(*Ross, 2012*)*, leading to constitutive activation of the JAK2 tyrosine kinase activity and a consequent loss of control in cell proliferation and growth *(*Cherie and Philip, 2010*)*.

### 1.2.7.1 Polycythemia Vera (PV)

Polycythemia Vera is primary bone marrow disorder where excessive number of red cells produced *(*Tiziano and Ayalew, 2012*)*. The first description of PV was by Vaquez in 1892. Osler, in 1903, published the first series of patients, identifying prominent clinical features setting PV away from other erythrocytoses. Considerable information has been gathered about PV since the work of these pioneers, much of it due to the work of the Polycythemia Vera Study Group (PVSG), which was set in 1967 with the aim of optimizing diagnosis and management of PV. The recent discovery of the V617F mutation in the pseudokinase domain of the tyrosine kinase JAK2, in nearly all cases of PV, represents a major advance in our understanding of this disorder *(*Hoffbrand, *et al*., 2011*)*. The annual incidence of PV is 1-3 per 100,000 population, men are more likely to be affected than women with ratio 1.2:1 *(*Srdan, *et al*., 2011*)*. The average age at diagnosis is approximately 60 years, and patients younger than 20 years are rarely encountered *(*Giuseppe, *et al*., 2009*)*.

#### 1.2.7.1.1 Pathophysiology

The central pathological feature of PV is an expansion in the total red cell mass, although elevations in the platelet and/or neutrophil counts are relatively common. Erythropoiesis in PV is autonomous and does not rely on erythropoietin (Epo). Plasma levels of this hormone are reduced in PV patients, and PV progenitor cells, unlike normal ones, can survive in vitro and give rise to erythroid colonies (BFU-E) in the absence of added Epo (endogenous erythroid colonies). PV erythroid progenitors show increased sensitivity to Epo *(*Hoffbrand, *et al*., 2011*)*. Moreover, subsequent studies revealed that marrow and blood cells from patients with PV were hypersensitive not only to Epo but also to several other hematopoietic growth factors, including interleukin 3 (IL-3), stem cell factor (SCF), thrombopoietien, granulocyte-macrophage colony-stimulating factor (GM-CSF) and insulin-like growth factor-1 (IGF-1). These findings suggest that events downstream from receptor engagement might be responsible for endogenous erythroid colony formation in PV *(*Hui, 2007*)*. A previous study has demonstrated that inhibitors of JAK2 can repress the Epo-independent differentiation of erythroid progenitors in PV, and constitutive activation of STAT3 has also been reported in patients with PV. JAK2 therefore represented a logical target for identifying the molecular abnormalities of PV *(*Hui, 2007*)*. In 2005,

a unique acquired mutation V617F in JAK2 has been identified in vast majority patients with PV. The mutation affects the noncatalytic 'pseudo-kinase' domain (JH2) of JAK2, and disrupts its autoinhibitory function, resulting in constitutive activation of JAK2 mediated signaling pathways leading to uncontrollable proliferation *(*Donal, *et al*., 2006; Ross, 2010*)*. The JAK2-V617F have been detected in around 95% in PV patients, and most of the remainder has activating mutations of the JAK2 in the region of exon 12 *(*Srdan, *et al*., 2011*)*. The exon 12 mutations have only been identified in patients with JAK2V617F- negative PV and not in ET or PMF. In addition, most patients with exon 12 mutations present with isolated erythrocytosis without thrombosis or leukocytosis *(*Ross, 2010*)*.

**Erythropoietin Independent Erythroid Colonies (EEC):** When cultured in serum, but in the absence of EPO the hematopoietic progenitors form burst forming units of erythroid (BFU-E) – a phenomenon referred to as (EEC's). This phenomenon can be observed in virtually all patients with PV and a large proportion of patients with ET and PMF. Progenitors from patients with reactive and familial erythrocytosis do not form EEC's in the absence of EPO. The capability of EEC formation was accepted as a minor criterion in the WHO diagnostic criteria. However, the EEC assay, although consistent and concise has not been widely introduced in the diagnostic setup outside large laboratory units *(*Thomas, 2007*)*.

**Erythropoietin (EPO):** The plasma concentration of EPO is subnormal in patients with PV at diagnosis as compared to normal controls and cases of secondary erythrocytosis (SE) and relative polycythemia. This subnormal level of EPO seems to persist after phlebotomy treatment, although a slight increase is observed in some patients. A subgroup of ET patients also displays subnormal EPO levels. Measurements of plasma EPO concentrations can be used in the diagnostic work-up in patients suspected of a CMPD, and a subnormal plasma EPO concentration is a minor criterion in the WHO classification *(*Thomas, 2007*)*.

#### 1.2.7.1.2 Clinical features

Thrombosis is the most common serious complication of PV. Untreated PV patients run a greatly increased risk of thrombosis, which can be arterial, venous or microvascular *(*Hoffbrand, *et al*., 2011*)*. Whole blood viscosity increases dramatically when the hematocrit (Hct) exceeds 45 %. The elevated Hct has been shown to be a

significant risk factor associated with an increased risk of arterial and venous thrombosis, which is the dominant morbidity in patients with PV. Lowering the Hct by phlebotomy markedly reduces the thromboembolic risk. In patients with PV the prevalence rates of major thrombosis at time of diagnosis is 34-39%, whereas the corresponding figures at follow-up is 8-19 % *(*Thomas, 2007*)*. After approximately 10 years disease the clinical phenotype changes in about 10-15 % of PV patients, being featured by the appearance or enhancement of constitutional symptoms, a decline in phlebotomy requirements in concert with rising leukocyte counts and an increasing spleen size due to myeloid metaplasia *(*Ralph and Edward, 2011*)*. The total red cell mass is often still massively increased in spite of a falsely normal or low Hb *(*Judith and Stefan, 2012*)*. This phenomenon is partly caused by pooling of red blood cells in the spleen and hemodilution consequent to an increased plasma volume *(*Srdan, et al., 2011*)*. The phenotype has traditionally been referred to as the "spent phase" of PV *(*Levine, et al., 2005*)*. A disease state with pancytosis, splenomegaly and bone marrow fibrosis can remain unaltered for many years, and is known as a 'transitional myeloproliferative disorder' *(*Hoffbrand, et al., 2011*)*. Patients diagnosed in this transitional disease state may be misclassified as PMF when the presenting features are slight anemia, huge splenomegaly together with a fibrotic bone marrow. In these patients the total red cell mass is nevertheless increased and the venous Hb concentration and Hct misleadingly low or sub-normal because of hemodilution *(*Thomas, 2007*)*. Furthermore clinical features like facial or conjunctival a plethora, hypertension, hepatomegaly, cutaneous ulcers and gouty features are also presenting in PV patients. Disease progression leads to massive hepatosplenomegaly, marrow fibrosis in the majority and transformation into acute leukemia in 20% to 25% cases *(*Munjal, et al., 2012*)*.

Management include: Phlebotomies for all to maintain a haematocrit levels at less than 45% in men and more than 42 in women, use of low dose aspirin 100 mg daily in all reduces the incidence of thrombosis and risk of cardiovascular death, and cytotoxic drugs like hydroxyurea are usually reserved for high-risk patients with risk of vascular events, older than 60 years of age and lowering platelet count and reducing the spleen size *(*Munjal, et al., 2012*)*.

#### 1.2.7.1.3 Diagnosis

PV is characterized by excessive number of red cells produced *(*Tiziano and Ayalew, 2012*)* erythrocytosis, reflected by elevated red cell mass, an increased Hb concentration as well as an elevated (Hct) *(*Faramarz, 2008*)*. Together with the increased red cell mass some degree of thrombocytosis and leukocytosis are often apparent *(*Thomas, 2007*)*.The world health organization (WHO) has now defined diagnosis criteria for PV *(*Tiziano and Ayalew, 2012*)* (Table 1.6). Diagnosis requires the presence of both major criteria and one of minor criterion or the presence of the first major criterion and two of the minor criteria *(*Tiziano and Ayalew, 2012*)*.

**Table 1.6** WHO Diagnostic Criteria for Polcythemia vera (PV)

| |
|---|
| Major Criteria |
| 1. Hemoglobin >18.5 g/dl in men, 16.5 g/dl in women or Hb/Hct>99$^{th}$ percentile of reference range for age, sex, or altitude of residence or Hb >17g/dl (men), >15g/dl (women) if associated with a sustained increase of ≥2g/dl from baseline that cannot be attributed to correction of iron deficiency or elevated red cell mass more than 25% above mean normal predicted values. |
| 2. Presence of JAK2V617F or other functionally similar mutation such as JAK2 exon 12. |
| Minor Criteria |
| 1. Bone marrow trilineage myeloproliferative. |
| 2. Subnormal serum erythropoietin level. |
| 3. Endogenous erythroid colony (EEC) formation in vitro. |
| Diagnosis requires the presence of both major criteria and one of minor criterion or the presence of the first major criterion and tow of the minor criteria. |

### 1.2.7.2 Essential thrombocythemia (ET)

Essential thrombocythemia is an MPN that involves primarily the megakaryocytes linage, it characterized by sustained thrombocytosis (>450×$10^9$L) in the peripheral blood and increase number of large megakaryocytes with staghorn-like nuclei in a normocellular bone marrow *(*Baxter, *et al*., 2005*)*. It is a rare disorder with an estimated incidence of about 0.6 -2.5 persons per 100,000 *(*Christoph, *et al*., 2008*)*. The disease can occur at any age including in children; most cases occur in the sixth decade of life, although a second peak particularly in women occurs at approximately 30 years of age *(*Srdan, *et al*., 2011*)*.

#### 1.2.7.2.1 Pathophysiology

Although the JAK2-V617F gain-of-function mutation has a pivotal role in the diagnosis of Philadelphia chromosome-negative myeloproliferative neoplasms ($Ph^-$ MPN), it only occurs in 50%-60% in patients with ET *(*Kim, *et al*., 2015*)*. Another clonal mutation such as in the gene encoding the thrombopoietin receptor (*MPL*) has been detected in approximately 5% of patients with ET. *MPL* encodes the receptor for thrombopoietin, the major regulator of platelet production. Somatic MPL W515L/K/A mutations are gain-offunction mutations inducing constitutive signaling of the downstream JAK/STAT pathway, lead to megakaryocytes proliferation and platelet production *(*Stefan and Kagop, 2011; Cabagnols, *et al*., 2015*)*. Recently, somatic mutations in exon 9 of calreticulin (*CALR*), were identified as addition to our knowledge of pathogenesis of MPNs. *CALR* mutations present in about 20% of patients with ET and PMF but very rarely in PV, and in ET it is (with very few exceptions) not present in *JAK2*- or *MPL*-positive patients *(*Gunner, 2015*)*. *In vitro* studies on the molecular pathogenesis of *CALR* mutations in MPN have shown controversial results in regard to the involvement and/or activation of the JAK/STAT signaling pathway *(*Klampfl, *et al*., 2013; Nangalia, *et al*., 2013*)* and the exact pathogenesis of *CALR* mutations are not yet completely understood at the present time *(*Shivarov, *et al*., 2014*)*. Therefore, the frequencies of *JAK2*V617F, *CALR*, and *MPL* mutations are approximately 60%, 22%, and 3%, respectively. This leaves about 15% of patients with ET that are wild-type for all 3 mutations (ie, are triple-negative). It should be noted that other nonspecific mutations might coexist with the

aforementioned MPN-specific mutations, and their incidence and relevance are currently being studied *(*Ayalew and Tiziano, 2015*)*.

#### 1.2.7.2.2 Clinical features

Although many patients are asymptomatic at the time of diagnosis, symptoms and complications of ET include splenomegaly, bleeding and thrombosis especially with platelet count above $1000\times10^9$/L, neurologic symptoms such as headache, paresthesias, and transient ischemic attacks, erythromelalgia or digital ischemia *(*Jones, *et al*., 2005*)*. Thrombosis can be life-threatening, so it is important to identify patients at risk for thrombotic complications. Thrombosis risk assessment is still a matter of debate as different research groups use different risk criteria. To date, the most widely used are the conventional evidence-based criteria. Based on them, high-risk factors for thrombosis are age > 60 years and a history of previous thrombosis *(*Ruta, *et al*., 2015*)*. The risk of leukemia transformation is 1.4% and myelofibrosis occurs in 3.8% of patients in first decade of the disease duration. The survival of patients with ET appears comparable to the general population in the first 10 years of diagnosis, but decreases with longer duration of disease as the risk of leukemia transformation and development of myelofibrosis increases *(*Alvin and Hillard, 2012*)*. Current drug therapy for ET is not curative, and it has not prolonged survival or prevented disease transformation to AML or post-ET MF. In other words, drug therapy has limited value in modifying the natural history of the disease. The goal of the therapy is to control platelet count to decrease the risk of thrombosis and bleeding *(*McClure, *et al*., 2006*)*. Low-dose aspirin is recommended for young asymptomatic (low-risk) patients except for patients with platelet counts above $1500\times10^9$/L (due to risk of bleeding from qualitative platelet defect) or with acquired von Willebrand syndrome. Hydroxyurea is usually started in asymptomatic patients with platelet count above $1500\times10^9$/L, but there are no clear guidelines on management of extreme thrombosis in asymptomatic low-risk patients *(*Alvin and Hillard, 2012*)*.

#### 1.2.7.2.3 Diagnosis

ET usually involves primarily the megakaryocytic lineage. Characteristic features are sustained thrombocytosis ($> 450 \times 10^9$ /L), megakaryocytic proliferation, and JAK2 mutation. Platelets in ET have a quantitative defect and show abnormalities in platelet

aggregation studies and acquired von Willebrand's syndrome can occur with extremely high platelets counts *(*Alvin and Hillard, 2012*)*. Bone marrow reveals megakaryocyte proliferation with large and mature megakaryocytes in the bone marrow *(*Thiele and Kvasnicka, 2009*)*. When evaluating thrombocytosis, the detection of *JAK2*V617F, *CALR*, or *MPL* mutations confirms the presence of an underlying MPN but their absence does not rule out the possibility since up to 20% of patients with ET might be triple-negative (i.e. negative for all three mutations) *(*Ayalew and Tiziano, 2015*)*. It is also important to note that other *JAK2*/*CALR*/*MPL* mutated MPN (or myelodysplastic syndromes [MDS]/MPN) can mimic ET in their presentation; these include prefibrotic PMF *(*Tefferi, *et al*., 2014*)* and refractory anemia with ring sideroblasts with marked thrombocytosis (RARS-T) *(*Schmitt, *et al*., 2008*)*. Therefore, bone marrow examination is often necessary to make an accurate morphologic diagnosis of ET and distinguish it from other myeloid neoplasms, especially from prefibrotic PMF; megakaryocytes in ET are large and mature-appearing whereas those in prefibrotic PMF display abnormal maturation with hyperchromatic and irregularly folded nuclei *(*Kvasnicka and Thiele, 2010*)*. A recent large international study confirmed the prognostic relevance of distinguishing ET from pre-fibrotic PMF *(*Tefferi, 2008*)*. The 2008 WHO classification, limited the following criteria (Table 1.7) to diagnosis of ET patients:(i) sustained Platelet count $\geq 450\times10^9$/L;(ii) bone marrow biopsy showing increased proliferation of megakaryocytes, with increased number of enlarged mature megakaryocytes; (iii) clinical presentation not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm; (iv) presence of JAK2V617F (or other clonal marker) or no evidence of reactive thrombosis in the absence of AK2V617F *(*Munjal, *et al*., 2012*)*.

**Table 1.7** WHO Diagnostic Criteria for Essential Thrombocythaemia (ET) *(*WHO, 2008)

| Diagnosis requires meeting all four criteria |
|---|
| 1) Sustained Platelet count $\geq 450\times10^9/L^*$ |
| 2) Bone marrow biopsy specimen showing proliferation mainly of the Megakaryocytic lineage with increased numbers of enlarged mature megakaryocytes. No or little granulocyte or erythroid proliferation. |
| 3) Not meeting WHO criteria for PV, † PMF, ‡ CML, § MDS‖ or other myeloid neoplasm |
| 4) Demonstration of JAK2V617F or other clonal marker. Or in absence of JAK2V617F, no evidence of reactive thrombosis. ¶ |

* Sustained during the work-up process.

† Requires the failure of iron replacement therapy to increase hemoglobin level to the polycythemia vera range in the presence of decreased serum ferritin. Exclusion of polycythemia vera is based on hemoglobin and hematocrit levels, and red cell mass measurement is not required.

‡ Requires the absence of relevant reticulin fibrosis, collagen fibrosis, peripheral blood leukoerythroblastosis, or markedly hypercellular marrow accompanied by megakaryocyte morphology that is typical for primary myelofibrosis—small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering.

§ Requires the absence of BCR-ABL1

‖ Requires the absence of dyserythropoiesis and dysgranulopoiesis

¶ Causes of reactive thrombocytosis include iron deficiency, splenectomy, surgery, infection, inflammation, connective tissue disease, metastatic cancer, and lymphoproliferative disorders.However, the presence of a condition associated with reactive thrombocytosis does not exclude the possibility of ET if other criteria are met.

### 1.2.7.3 Primary Myelofibrosis (PMF)

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by stem cell-derived clonal myeloproliferation, abnormal cytokine expression, bone marrow fibrosis, anemia, splenomegaly, extramedullary hematopoiesis (EMH), constitutional symptoms, cachexia, leukemic progression, and shortened survival *(*Nangalia, *et al*., 2013*)*. Extramedullary hematopoiesis is a well recognized phenomenon of this disease process *(*Ghulam and Abdulraheem, 2016*)*. PMF occurs at a frequency of 0.5 to 1.5 per 100,000. It occurs at equal frequency in males and female and the age of onset is usually after the 6$^{th}$decade *(*Tefferi, 2000*)*. Median reported survival in various studies range between 3.5 to 5 years *(*Mohamad, *et al*., 2013*)*. Post MF is found to develop in 25-50% of PV and 2-3% of ET after approximately 15-20 years of follow up *(*Nisha, 2015*)*. PMF has been reported rarely in infants and in younger patients with or without a family history of PMF and these cases appear to have a better outcome than adults *(*Tefferi, 2000*)*.

#### 1.2.7.3.1 Pathophysiology

PMF pathogenesis involves aberrant activation of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, which may result from somatic mutations directly affecting JAK activity, excessive cytokine stimulation, and/or epigenetic modifications of chromatin structure interfering with normal regulation of gene expression *(*Savona, 2014*)*. The JAK2-V617F gain-of-function mutation was identified in about half of PMF patients. JAK2V617F homozygosity via 9pLOH has been associated with more severe phenotype. Interestingly, 9pLOH is more common in PMF and PV, However, the contribution of 9pLOH in the pathogenesis of MPNs remains unclear *(*Akada, *et al*., 2014*)*. The *CALR* mutations frequencies are estimated at 20–25% in PMF patients *(*Tefferi, *et al*., 2014*)*. Somatic MPL W515L/K/A mutations have been described in 7% of patients with PMF *(*Chul, *et al*., 2015*)*. Recent evidence suggests that mutations outside of the JAK-STAT pathway such as *TET2* and *ASXL1* also have important roles in pathogenesis *(*Cross, 2011; Mascarenhas, *et al*., 2011*)*. The pathophysiology of extramedullary hematopoiesis is thought to be associated with the constitutive mobilization of CD34+ cells into the peripheral blood. This dysregulation of hematopoietic stem cell (HSC) trafficking

likely ultimately leads to the seeding of extramedullary sites *(*Leibundgut, *et al*., 2006*)*.

**1.2.7.3.2 Clinical features**

Up to 25% of patients may be asymptomatic at the time of diagnosis when their enlarged spleen is noted on exam. Most patients with PMF present with enlarged spleen, fatigue and constitutional symptoms such as fever, sweats weight loss, and progressive abdominal symptoms, as well as other general chronic debilitating complaints *(*Alvin and Hillard, 2012*)*. Anemia develops with disease progression *(*Munjal, *et al*., 2012*)*. Patient with PMF have problem due to cytopenias and extramedullary hematopoiesis. Thrombotic and bleeding complications (from thrombocytopenia, dysfunctional platelets or DIC) can also occur. Extramedulary hematopoiesis can cause organ damage including CNS sites and organ failure *(*Jones, *et al*., 2005*)*. The PMF-associated consequences and medical complications often result in premature death from infection, thrombohemorrhagic events, cardiac or pulmonary failure, and leukemic transformation *(*Mohamad S, *et al*., 2013*)*.

For management, Interferon-alpha may ameliorate some features for a certain period. Blood transfusions become the most important supportive care once significant anemia sets in *(*Munjal, *et al*., 2012*)*. The only curative treatment for PMF is allogeneic stem cell transplantation, supportive treatment alleviating symptoms, but has little impact on the relatively poor survival of PMF patients. It is for this reason that the future development of therapeutic JAK2 inhibitors is most eagerly awaited for these patients, rather than for those with PV or ET who have much better prognosis *(*Hoffbrand, *et al*., 2011*)*.

**1.2.7.3.3 Diagnosis**

Peripheral blood analysis of patients with PMF can show tear drop cells, nucleated red blood cells, and immature myeloid cells, megathrombocytes and fragments of megakaryocytes *(*Alvin and Hillard, 2012*)*. Other laboratory findings include leucocytosis or leukopenia, thrombocytosis or thrombocytopenia and circulating myeloblasts, increased serum lactate dehydrogenase and low cholesterol are also observed. Abnormalities on blood film examination at diagnosis may include a leukoerythroblastic blood film, left shifted granulocytes and red cell anisopoikilocytosis *(*Nisha, 2015*)*. Bone marrow aspirate is usually difficult to obtain due to marrow fibrosis. The bone marrow biopsy specimen usually shows

hypercellular bone marrow, increased number of megakaryocytes, varying number of reticulin, and increased vascularity *(*Alvin and Hillard, 2012*)*. Acoording to 2008 WHO classification, the diagnosis of PMF is made in patients fitting three major criteria (Table 1.8).

**Table 1.8** WHO Diagnostic Criteria for primary myelofibrosis (PMF)

| **Diagnosis requires meeting all 3 major criteria and 2 minor criteria** |
|---|
| Major Criteria |
| 1. Megakaryocyte proliferation and atpyia,* accompanied by either reticulin and/or collagen fibrosis, or in its absence increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis |
| 2. Not meeting WHO criteria for PV, † BCR-ABL-positive CML, ‡ MDS, § or other myeloid neoplasm. |
| 3. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive marrow fibrosis. ǁ |
| Minor Criteria |
| 1. Leucoerythroblastosis. ¶ |
| 2. Increased serum LDH. ¶ |
| 3. Anemia. ¶ |
| 4. Palpable splenomegaly. ¶ |

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

† Requires the failure of iron replacement therapy to increase hemoglobin level to the polycythemia vera range in the presence of decreased serum ferritin. Exclusion of polycythemia vera is based on hemoglobin and hematocrit levels. Red cell mass measurement is not required

‡ Requires the absence of BCR-ABL1

§ Requires the absence of dyserythropoiesis and dysgranulopoiesis

ǁ It should be noted that patients with conditions associated with reactive myelofibrosis are not immune to primary myelofibrosis, and the diagnosis should be considered in such cases if other criteria are met

¶ Degree of abnormality could be borderline or marked

### 1.2.7.4 Others non classical BCR-ABL -negative MPNs

The BCR-ABL -negative MPNs include not only PV, ET, and PMF, but also, non classical MPNs *(*Srdan, *et al*., 2011*)*, such as chronic neutrophilic leukemia, chronic eosinophilic leukemia-not otherwise specified, mast cell disease, and unclassified MPNs *(*Tefferi, 2010*)*. A large proportion of these cases show tyrosine kinase activation as a consequence of gene fusions mediated by chromosome translocation or deletions *(*Munjal, *et al*., 2012*)*. The remaining cases are either reactive proliferation or are still awaiting molecular characterization.

#### 1.2.7.4.1 Chronic Neutrophilic Leukemia (CNL)

CNL is a rare MPN characterized by peripheral leukocytosis (>25×$10^9$L), with a predominance of mature neutrophils (greater than 80% mature granulocytes), but without increase in circulating myeloblast, eosinophils, or basophils and without dysplastic features in any linage *(*Stefan and Kagop, 2011*)*. Bone marrow biopsy reveals hyperplasia of granulocytic lineage without involvement of other series, and there is an absence of fibrosis or myelodysplastic features *(*Alessandro, *et al*., 2009*)*, an increased Myeloid: Erythroid (M:E) ratio, blast are not increased. The vast majority of cases of CNL are karyocytically normal lacking BCR-ABL1 rearrangement *(*Rosane, *et al*., 2012*)*. Given the potential for evolution to acute leukemia or progressive refractory leukocytosis, allogeneic stem cell transplantation may be appropriate for younger patients (Alessandro, *et al*., 2009*)*.

#### 1.2.7.4.2 Mast cell disease

Mastocytosis is due to clonal proliferation of mast cells that accumulate in different organs such as the bone marrow, gastrointestinal tract, liver, spleen and lymph nodes. The disease is heterogeneous, varying from itchy skin lesions that can regress spontaneously to widespread and aggressive infiltration with general symptoms. It can occur at any age. It is classified according to the distribution into cutaneous form, indolent systemic disease associated with clonal hematologic damage, and systemic aggressive mast cell leukemia, mast cell and mast cell extracutaneous sarcoma *(*Rosane, *et al*., 2012*)*. Life expectancy is nearly normal in indolent forms of SM but is significantly shortened in aggressive SM. The bone marrow is almost universally involved in SM and is characterized by dense, multifocal aggregates of

morphologically and immunophenotypically abnormal mast cells, preferentially in a perivascular location, and is often accompanied by increased eosinophils. Serum levels of tryptase are typically high and represent a clinically useful disease- related marker *(*Alessandro, *et al*., 2009*)*. The *kit* D816V mutation is detected in virtually all patients with SM. Therapeutic options are represented by interferon-alpha or cytotoxic drugs, such as cladribine, but clinical responses are limited *(*Alessandro, *et al*., 2009*)*.

### 1.2.8 Chronic myelogenous leukemia (CML)

CML was first described as early as 1845. Relatively soon after Dameshek's description of myeloproliferative neoplasm in 1950, a series of rapid advances in cytogenetics techniques resulted in numerous discoveries related to human chromosomes *(*Greil, *et al*., 2010*)*, one of these landmark discoveries was the Philadelphia chromosome in CML, the first described structural chromosome abnormalities, by Nowell and Hungerford in 1960 *(*Domnita, 2010*)*.

Chronic myelogenous leukemia account for 7 – 20% of leukemias has an estimated incidence of one to two per 100.000 worldwide *(*Francesca, *et al*., 2014*)*. The median age at diagnosis is in the fifth and sixth decades *(*Tefferi, *et al*., 2011*)*.

#### 1.2.8.1 The BCR-ABL1 fusion and pathogenesis of CML

The Ph chromosome is a pathognomonic marker of CML, that results from a reciprocal chromosomal translocation between the ABL gene on chromosome 9 with BCR gene on chromosome 22 t (9;22), resulting in The chimeric oncogene BCR-ABL *(*Najia, et al., 2014*)*. The presence of the BCR-ABL1 fusion transcript in CML cells was demonstrated in 1985 by Shtivelman et al. The BCR-ABL1 fusion, gives rise to a constitutively activated and unregulated cytoplasmic tyrosine kinase that causes uncontrolled proliferation and differentiation of hematopoietic cells *(*Francesca, *et al*., 2014*)*. The resultant bcr-abl fusion protein plays a key role in pathogenesis of CML, by constitutive tyrosine kinase (TYK) activity *(*Cherie and Philip, 2010*)*. Their cellular effect is exerted through activation of multiple signal transduction pathways that transduce oncogenic signals *(*Najia, *et al*., 2014*)*.

#### 1.2.8.2 Clinical and laboratory features

At diagnosis patients most often complain of fatigue, bleeding tendencies, weight loss, sweets, and symptoms related to splenomegaly such as left upper quadrant pain,

sensation of mass, abdominal fullness, and/or swelling *(*Srdan, *et al*., 2011*)*. Bone pain and infection are less common *(*Stefan and Kagop, 2011*)*. The most common laboratory findings at presentation are granulocytic leukocytosis, and anemia, with or without thrombocytosis. Average values range 174-225×$10^9$L for white blood cell count, 9.7-10.3g/dl for hemoglobin, and 430-485 ×$10^9$L for platelets. There is generally a loose inverse relation between the leukocyte count and severity of anemia. The granulocytes are left-shifted in the peripheral blood, with full maturation to neutrophils, a prominence of myelocytes, relatively few blasts compared acute leukemias. Absolute basophilia is virtually invariant feature, and absolute eosinophilia and monocytosis are present in the majority of cases *(*Harper, *et al*., 2012*)*. Although leukocytosis is the rule, exceptional cases may have normal cell count and present instead with markedly elevated platelet count, myelofibrosis, or anemia. The bone marrow is markedly hypercellular with marked predominance of granulocytes and thick paratrabecular cuffs of immature granulocytic precursors. Blast count for less than 10% of cellularity in chronic phase *(*Huijsmans C, et al., 2011*)*.

#### 1.2.8.3 Diagnosis

Diagnosis easily established by the presence of leukocytosis, basophilia, eosinophilia, and the Philadelphia chromosome. However, in approximately 10% of CML patients, the Philadelphia chromosome cannot be detected by standard cytogenetic studies. In these cases, molecular analysis by Southern blots analysis, reverse transcriptase/polymerase chain reaction (RT-PCR), or fluorescent in situ hybridization (FISH) is needed for demonstration of fused BCR-ABL *(*Maher and Emil, 2000*)*. Treatment of CML has changed dramatically over the last decades.

#### Treatment of CML

Therapy with busulphan was initiated in the 1950s but was then replaced by hydroxyurea, followed by introduction of IFN-a in the early 1980s, the first drug to induce a marked cytogenetic response in CML patients. Treatment with curative intention was subsequently realized through allogeneic stem cell transplantation (SCT) in the 1980s, and this became the treatment of choice for younger patients with HLA-matched donors. The therapy has since been revolutionized by arrival of imatinib that was first used in clinical trials starting in 1998 *(*Srdan, *et al*., 2011*)*. Imatinib has now emerged as a front-line therapy in the treatment of CML patients

with increased survival advantages compared to previously available treatment regimens *(*Sverre and Mitelman, 2009*)*. The imatinib compound bind to the ATP-binding pocket of ABL1 domain, this blocks the tyrosine kinase activity and inhibits both BCR/ABL1-mediated autophosphorylation and substrate phosphorylation, resulting in abrogated downstream cell signaling and reduced proliferation of the BCR/ABL1-positive cell *(*Maher and Emil, 2000*)*.

## 1.3 Rationale

The discovery of an acquired somatic point mutation (JAK2-V617F) in classic BCR/ABL-negative MPNs, namely, PV, ET and PMF have lead to new insights into the molecular pathogenesis of these disorders. Many crucial changes in myeloproliferative neoplasms field have been occurred in regards to achieving a correct diagnosis, improving classification and predicting prognosis based on the JAK2V617F mutation. Few data regarding MPNs mutations are available in Sudan. The results of this study could be a new important data on the prevalence, diagnosis, and affect of mutational load on clinical phenotypes of Sudanese MPNs patients.

## 1.4 Objectives of the study

### 1.4.1 General Objectives

To detect JAK2-V617F mutation in Sudanese patients with myeloproliferative neoplasms

### 1.4.2 Specific Objectives

The specific objectives of current study were

- To determine the hematological parameters (Hb, Hct, RBCs, WBCs, and Plt) in MPNs patients.
- To detect JAK2-V617F mutation among MPNs patients.
- To investigate the differences of hematological parameters between JAK2-V617F positive patients and JAK2 wild type MPNs patients.
- To estimate the percentage of mutant allele in JAK2V617F-positive MPNs patients.
- To detect the frequency of homozygous mutant allele patients for PV, ET and PMF.
- To examine the association of quantitative JAK2-V617F allele burden with hematologic characteristics.

# CHAPTER TWO

## Materials and Methods

### 2.1 Study design

A cross sectional study

### 2.2 Study Area

Radioisotopes Centre Khartoum (RICK) in Khartoum State-Sudan

### 2.3 Study population

A total 259 patients with MPNs (PV, ET and PMF) were involved. In addition11 healthy adult individuals were included.

**Inclusion criteria:** Patients with PV, ET or PMF diagnosed according to WHO criteria were included.

**Exclusion criteria:** Patients who not meet WHO diagnostic criteria for PV, ET and PMF were excluded.

### 2.4 Patient Samples

Peripheral blood samples from each subject were obtained. Blood collected in vacationer tubes containing EDTA anticoagulant under aseptic procedure. Blood samples were refrigerated before nucleic acid extraction was preformed.

### 2.5 Procedures

DNA was isolated from peripheral blood Leukocytes by innuPREP kit. Allele specific PCR was used to screen the JAK2-V617F, and The quantitative real-time polymerase chain reaction (qRT-PCR) technology(Ipsogen JAK2 MutaQuant Kit, Germany) was used to determinate the percentage of mutated alleles in genomic DNA among JAK2-V617F positive MPNs.

#### 2.5.1 Complete Blood Count

Full automated blood analyzer (Sysmex KX-21N) was used to obtain hematologic paraters (Hb, Hct, RBCs, WBCs, and Plt) for each samples.

### 2.5.2 DNA Extraction

Genomic DNA extraction from peripheral blood leukocytes was carried out following the protocol of innuPREP kit (Analytik Jena/Germany). The innuPREP Blood DNA Mini Kit is a highly efficient tool for directly isolating DNA from whole blood samples of up to 400 µl. The innuPREP Blood DNA Mini Kit is fast and easy to use. Isolation of genomic DNA free of inhibitors or impurities can be completed in just 24 minutes. Briefly, the basic principle is the use Spin Filter. The steps of procedure were homogenization and lysis of whole blood, binding of Nucleic Acid to the Spin Filter, wash of bound Nucleic Acid, finally elution of Nucleic Acid.

**Protocol: DNA isolated from blood sample**

1- Into a 1.5 ml receiver tube, we added 250µl the blood sample, 400µl Lysis solutions (BLS) and 25µl Proteinase K. Tube content was mixed vigorously by pulsed vortex for 5 second and incubated at 70 C for 10 minutes. Alternatively mixing by vortex 3-4 times was done during lysis. To remove RNA from samples, 4µl of RNase solution (100 mg/ml) was added after lysis time, and then mixed shortly and incubated 5 minutes at room temperature.
2- 200 µl of binding solution TBS was added to lysed sample, mixed by vortex.
3- We applied the sample into the Spin Filter located in a 2.0 ml receiver tube. Closed the cap and centrifuged at 10.000x g (12.000 rpm) for 2 minutes. Then we discard the receiver tube with the filtrate. The Spin filter was placed into a new 2.0 ml receiver tube.
4- 500µl of washing solution HS was added into the Spin Filter, centrifuged at 10.000x g (12.000 rpm) for 1 minute. Then we discarded the receiver tube with the filtrate, and placed the spin filter into a new 2.0 ml receiver tube.
5- We opened the Spin Filter and add ed750µl of washing solution MS, closed the cap and centrifuge at 10.000x g (12.000 rpm) for 1 minute. Then discard the receiver tube with the filtrate and placed the spin filter into a new 2.0 ml receiver tube. Repeat the washing step with washing solution MS once again.
6- We discarded the receiver tube with the filtrate after the third washing step and placed the Spin Filter into a new 2.0 ml receiver tube.
7- The tube was centrifuge at Max. Speed for 2 minutes to remove all traces ethanol. Then we discarded the 2.0 ml receiver tube.

8- The Spin Filter was placed into 1.5ml Elution tube. Carfully the cap of the Spin Filter was opened and 200 µl of Elution buffer was added, Incubated at room temperature for 1 minute. Centrifuged at 6.000x g (8.000rpm) for 1 minute. The extracted DNA stored at -20C before used for polymerase chain reaction. DNA quantity was determined by measuring the optical density (OD) at 260 nm spectrophotometry.

### 2.5.3 The JAK2-V617F mutation detection by AS-PCR

Mutation analysis of *JAK2* V617F was performed using allele-specific PCR, based on amplification refractory mutation system (ARMS) technique. Allele-specific polymerase chain reaction (AS-PCR) is a conceptually simple SNP genotyping strategy. The rationale of AS-PCR is that a single nucleotide mismatch at the 3′ extremity of the annealed forward primer renders Taq polymerase unable to extend the primer. So, the absence of the specific PCR product reveals a deviation from the wild-type DNA sequence. The sensitivity of ARMS technique is 1% to 2%. It uses two primer pairs to specifically amplify the normal and mutant sequences in a single reaction and followed just by gel electrophoresis.

***PCR mix***

Amplifications were done in a total volume of 25 µL PCR mix containing 12.5 µL of TaqMan Universal Master Mix, 5 µL of nuclease-free PCR grade water, 2.5 µL of primers and probes mix and 50 ng/µL of DNA template.

***AS-PCR primers***

PCR primers were forward wild-type 5'-TCC TCA GAA CGT TGA TGG CAG-3' and reverse wild-type 5'-ATT GCT TTC CTT TTT CAC AAG AT-3', forward mutant 5'-GCA TTT GGT TTT AAA TTA TGG AGT ATA TG-3' and a reverse mutant 5'-GTT TTA CTT ACT CTC GTC TCC ACA AAA-3'.

***PCR conditions***

The PCR conditions used were denaturation at 95℃ for 1 min, annealing at 58℃ for 40 s, and extension at 72℃ for 45 s.

***Agarose gel electrophoreses***

Products were electrophoresed on agarose gels and visualized using ethidium bromide staining.

***Result interpretation***

Products were 453bp (for both *JAK2* V617F-positive and *JAK2* V617F-negative), 279bp (for *JAK2* V617F-positive) and 229bp (*JAK2* V617F-negative)

### 2.5.4 Quantification of the allele burden of the *JAK2* V617F mutation

For quantitative analysis of JAK2 V617F mutation, we used RQ-PCR using JAK2 MutaQuant™ (Ipsogen Inc., New Haven, CT). The use of qPCR permits the accurate quantification of PCR products during the exponential phase of the PCR amplification process. Quantitative PCR data can be rapidly obtained, without post-PCR processing, by real-time detection of fluorescent signals during and/or subsequent to PCR cycling, thereby drastically reducing the risk of PCR product contamination.

This assay exploits the qPCR double-dye oligonucleotide hydrolysis principle. During PCR, forward and reverse primers hybridize to a specific sequence. A double-dye oligonucleotide is contained in the same mix. This probe, which consists of an oligonucleotide labeled with a 5' reporter dye and a downstream, 3' quencher dye, hybridizes to a target sequence within the PCR product. qPCR analysis with hydrolysis probes exploits the 5'→3' exonuclease activity of the *Thermus aquaticus* (*Taq*) DNA polymerase. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer.

During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'→3' exonuclease activity of the DNA polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR (Figure 2.1). This process occurs in every cycle and does not interfere with the exponential accumulation of product. The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and hence amplified during PCR. Because of these requirements, nonspecific amplification is not detected. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.

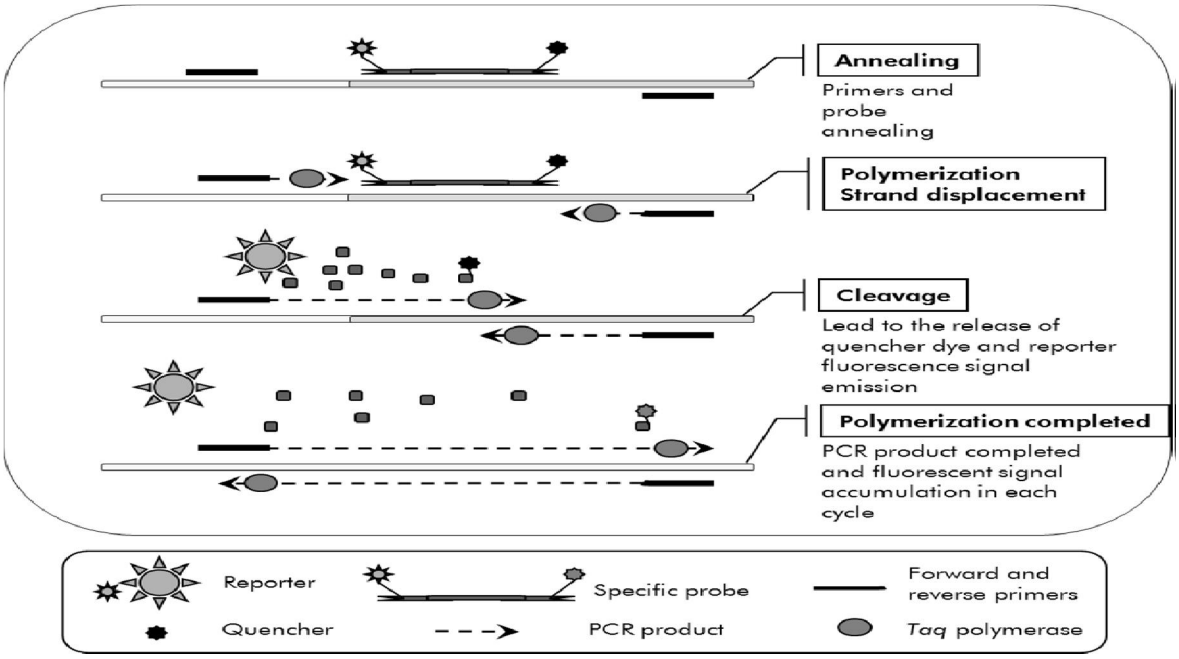



**Figure 2.1 Reaction principles.**

The quantitative allele specific PCR technology used in this assay kit allows a sensitive and accurate detection of SNPs. This technique is based on the use of specific forward primers, for the wild-type and the V617F allele. Only perfect match between primer and target DNA allows extension and amplification in the PCR (see Figure 2.2).

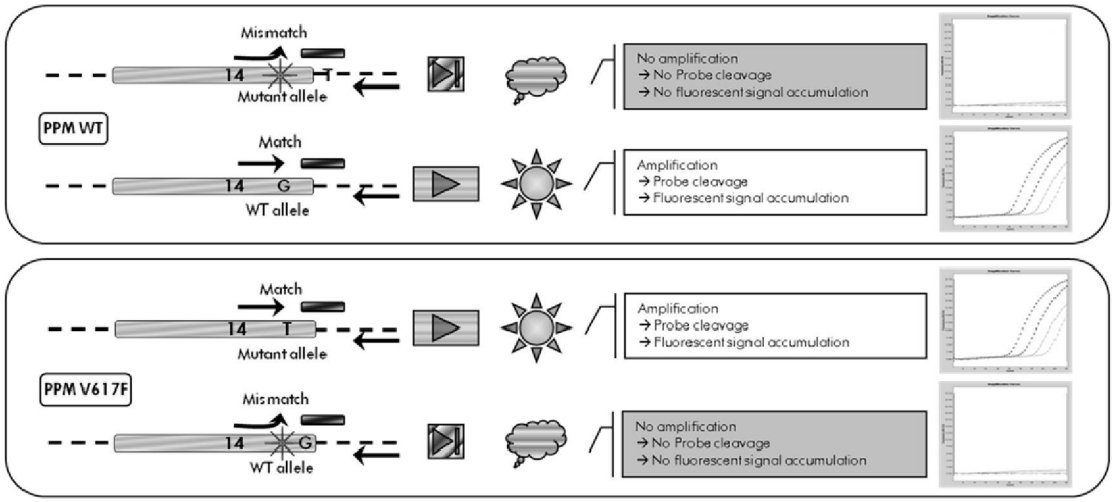



**Figure 2.2 Allele specific qPCR**.

Use of wild-type or the V617F primers and probe mix allows the specific detection of the wild-type or mutated allele in two separate reactions conducted using the same sample. Results can be expressed as percentage of VF copies among total JAK2 copies.

| ***ipsogen* JAK2 Muta*Quant* Kit (Kit contents)** |
|---|
| V617F positive control (100% V617F allele) |
| V617F negative control (100% wild-type allele) |
| M1-VF Standard Dilution, 50 copies (5 x 101 V617F copies/5 µl) |
| M2-VF Standard Dilution, 500 copies (5 x 102 V617F copies/5 µl) |
| M3-VF Standard Dilution, 5000 copies (5 x 103 V617F copies/5 µl) |
| M4-VF Standard Dilution, 50,000 copies (5 x 104 V617F copies/5 µl) |
| WT-1 Standard Dilution, 50 copies (5 x 101 wild-type copies/5 µl) |
| WT-2 Standard Dilution, 500 copies (5 x 102 wild-type copies/5 µl) |
| WT-3 Standard Dilution, 5000 copies (5 x 103 wild-type copies/5 µl) |
| WT-4 Standard Dilution, 50,000 copies (5 x 104 wild-type copies/5 µl) |
| Primers and Probe Mix JAK2 WT |
| Primers and Probe Mix JAK2 V617F |
| TaqMan Universal PCR Master Mix |
| Nuclease-free PCR grade water |

**Protocol: qPCR on Rotor-Gene Q Real time insturoment**

1. All kit components were thawed and placed on ice.
2. The qPCR mix Prepare according to the number of samples being processed as described in table 2.1

**Table 2**.1 Tables describe the pipetting scheme for the preparation of one reagent qPCR mix

| Component | V617F pre-mix 1 reaction (µl) | WT pre-mix 1 reaction(µl) |
|---|---|---|
| TaqMan Universal PCR Master Mix,2x | 12.5 | 12.5 |
| Primers and probemix, PPM-VF25x | 1.0 | - |
| Primers and probe mix, PPM-WT25x | - | 1.0 |
| Nuclease-free PCR grade water | 6.5 | 6.5 |
| Sample (to be added at step 4) | 5.0 | 5.0 |
| Total volume | 25 | 25 |

qPCR reactions were performed in a final volume of 25 µl mix which containing 12.5 µL of TaqMan Universal PCR master mix, 1 µL of IPSOGEN PPM- VF or wild type primers and probe mix, and 6.5 µL of nuclease-free water, and 5 µL genomic DNA was used per well.

3. We dispense 20 µl of the qPCR pre-mix (VF or WT) per tube.
4. 5 µl of the material to be quantified (25 ng sample genomic DNA or control) was added into corresponding tube (total volume 25 µl).
5. Contents mixed gently, by pipetting up and down.
6. The tubes were placed in the thermal cycler according to the manufacturer recommendations.
7. Rotor-Gene Q instrument with the thermal cycling programed as indicated in Table 2.2.

Table 2.2 Temperature profile

| Mode of analysis | Quantitation |
|---|---|
| Hold | Temperature: 50 deg<br>Time: 2 mins |
| Hold 2 | Temperature: 95°C<br>Time: 10 mins |
| Cycling | 50 times<br>95°C for 15 secs<br>62°C for 1 min with acquisition of FAM fluorescence in channel Green: Single |

8. The thermal cycling program was started, as indicated in Table 2.
9. Then selected "Slope Correct" in Rotor-Gene instrument for the analysis.

**Results calculation**

Data for the threshold cycle ($C_T$) and crossing point ($C_P$) values can be exported from the qPCR instrument and pasted into an Excel® file for analysis. These values can then be used to calculate the mean value for $C_P$ and $C_T$ and the standard mean $C_T$ values can be plotted to obtain a standard curve for both the wild-type and V617F standards using the following equation and table 2.3.

y = Mean $C_P$; x = $\log_{10} C_N$ where $C_N$= gene copy number in the 5 µl sample

**Table 2.3** Quantitative data for the wild-type and V617F standards

| Standard | Copy number (CN) | log10 CN |
|---|---|---|
| M1-VF | $5 \times 10^1$ VF | 1.7 |
| M2-VF | $5 \times 10^2$ VF | 2.7 |
| M3-VF | $5 \times 10^3$ VF | 3.7 |
| M4-VF | $5 \times 10^4$ VF | 4.7 |
| WT-1 | $5 \times 10^1$ WT | 1.7 |
| WT-2 | $5 \times 10^2$ WT | 2.7 |
| WT-3 | $5 \times 10^3$ WT | 3.7 |
| WT-4 | $5 \times 10^4$ WT | 4.7 |

The standard curve equations can then be used to calculate V617F and WT $\log_{10}$ copy numbers in the unknown samples. The V617F standard curve equation should be used to transform raw $C_P$/ $C_T$ value means (obtained with PPM-VF) for the unknown and control samples, into JAK2 V617F copy numbers ($CN_{V617F}$).

$$\log_{10} CN_{V617F} = \frac{(\text{Mean } C_{pV617F} - \text{Standard curve intercept}_{V617F})}{\text{Standard curve slope}_{V617F}}$$

The wild-type standard curve equation should be used to transform raw mean $C_P/C_T$ value (obtained with PPM-WT) for the unknown and control samples, into JAK2 wild-type copy numbers ($CN_{WT}$).

$$\log_{10} CN_{WT} = \frac{(\text{Mean } C_{pWT} - \text{Standard curve intercept}_{WT})}{\text{Standard curve slope}_{WT}}$$

**Expression of the results**

Results expressed as the percentage of JAK2 V617F as follows.

$$\text{JAK2 V617F \%} = \frac{CN_{V617F}}{(CN_{V617F} + CN_{WT})} \times 100$$

**Positive and negative controls**

The positive control or PC-VF should give a JAK2 V617F percentage that is higher than 99.9%. The negative control or NC-VF should give a JAK2 V617F percentage that is lower than 0.1%.

### 2.6 Data collection

Data were collected using following tools:

1- Laboratory investigations.
2- Patient records.
3- A structured questioner.

### 2.7 Data Analysis

Statistical analyses were performed using the SPSS version 11.2.

P.value significant at $< 0.05$

# CHAPTER THREE

## 3. Results

The current study included 259 (161 male, and 98 female) patients with Ph-negative MPNs; 159 with PV, 55 with ET and 45 with PMF. At diagnosis the mean age was 49.9±15.10, 49.3±14.46, and 57±16.64 years for PV, ET and PMF respectivly. All patients were screened for JAK2-V617F mutation. The JAK2-V617F mutation was detected in 71% of all patients with MPNs. The prevalence of the mutation in ET and PMF were 56.4% and 51.1% respectively. 81.7% of patients with PV were harbored the JAK2-V617F mutation (Figuer 3.1). JAK2-V617F was not detected in 11 healthy adult people. At diagnosis, the mean leukocytes count for MPNs was 11.07±8.87; ET had highest mean WBCs 15.58±12.30. PV had highest mean RBCs, Hb, and Hct (7.03±4.04, 17.16±1.93, and 50.78±6.77), while ET have highest mean platelets count 1121.98. Demographic and hematologic features were summarized in (Table 3.1) and (Table 3.2).

**Table 3.1** Age and gender according to myeloproliferative neoplasm (MPN) subtypes

| | No of patients | Female | Male | Female/Male | Age at diagnosis (yr,mean±SD) |
|---|---|---|---|---|---|
| **PV** | 159 | 51 | 108 | 1:2.1 | 49.91±15.10 |
| **ET** | 55 | 29 | 26 | 1:0.89 | 49.30±14.46 |
| **PMF** | 45 | 18 | 27 | 1:1.5 | 57.00±16.64 |

PV, Polycythemia Vera; ET, Essential thrombocythemia; PMF, Primary Myelofibrosis

**Table 3.2** Hematologic parameters according to myeloproliferative neoplasm (MPN) subtypes

| Characteristics | PV | ET | PMF | Total |
|---|---|---|---|---|
| **WBC ($\times 10^9$/L, mean±SD)** | 9.23±5.79 | 15.58±12.30 | 12.05±10.78 | 11.07±8.87 |
| **RBC ($\times 10^9$/L, mean±SD)** | 7.03±4.04 | 4.68±1.61 | 4.01±1.41 | 6.01±3.56 |
| **Hb (g/dL, mean±SD)** | 17.16±1.93 | 11.18±2.56 | 9.89±2.59 | 14.63±3.90 |
| **Hct (%, mean±SD)** | 50.78±6.77 | 33.48±7.70 | 29.28±7.59 | 43.38±11.82 |
| **Platelet ($\times 10^9$/L, mean±SD)** | 421.91±270.52 | 1121.98±359.86 | 242.20±206.27 | 539.35±418.86 |
| **NO of jak2v617f positive (%)** | 130(81.7%) | 31(56.4%) | 23(51.1%) | 184(71%) |

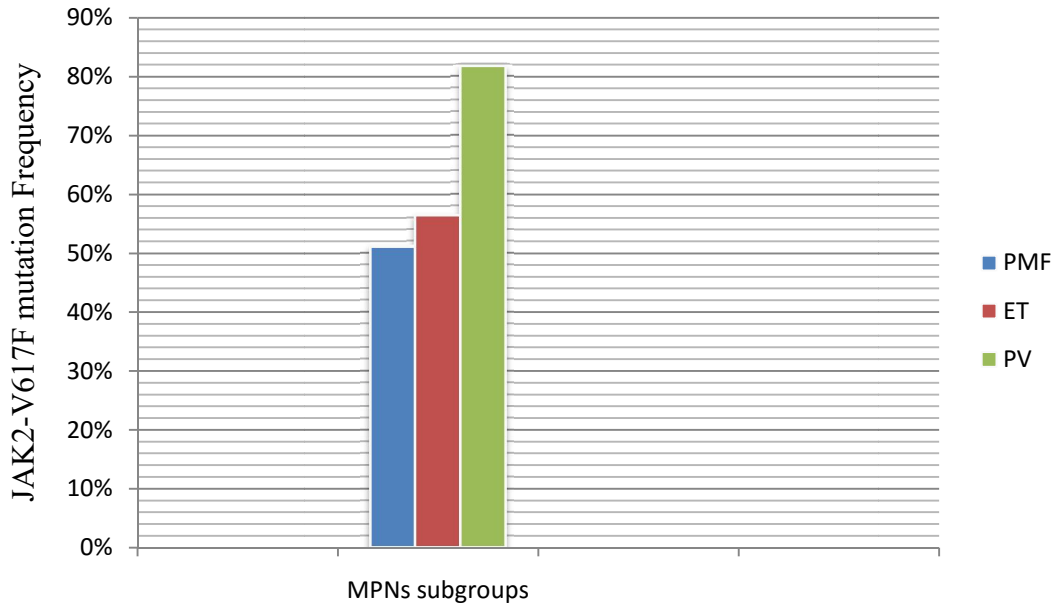



**Figure 3.1** Distribution of JAK2-V617F mutations among MPNs subgroups

At diagnosis, the presence of JAK2-V617F mutation was found to be significantly associated with high Hb (*P*=.039) and Hct (*P*=.04) in PMF patients. The JAK2-V617F positive ET patients were older than ET patients without mutation (*P*=.033). There was association between JAK2-V617F mutation and erythrocytosis in PV patients (*P*= .040). No significant associations were detected between JAK2-V617F mutation and WBCs, Hb, Hct, or platelet count in PV, whoever JAK2V617F-positive PV patients had high WBCs, Hb, Hct and low platelets count. No significant differences in WBCs, RBCs, Hb, Hct, or platelet count were observed between JAK2-V617F positive and JAK2-V617F negative ET patient, although ET patients who harbor the mutation have highest WBCs, RBCs, Hb, Hct, and lowest platelet count. Although JAK2V617F-positive PV and PMF patients had high mean of age, but the difference was not significant statistically. These results were summarized in (Table 3.3).

**Table 3.3** Hematologic parameters associated with the JAK2 V617F mutation status

| Characteristics | PV | | | ET | | | PMF | | |
|---|---|---|---|---|---|---|---|---|---|
| | JAK2 WT | JAK2-V617F | P.value | JAK2 WT | JAK2-V617F | P.value | JAK2 WT | JAK2-V617F | P.value |
| **Age at diagnosis** | 45.40± | 50.83± | .089 | 44.62± | 52.93± | .033* | 54.31± | 59.56± | .296 |
| **(yr, mean±SD)** | 14.20 | 15.16 | | 14.31 | 13.72 | | 16.55 | 16.68 | |
| **WBC** | 9.01± | 9.27± | .830 | 13.24± | 17.39± | .218 | 11.20± | 12.95± | .592 |
| **(×10$^9$/L, mean±SD)** | 5.27 | 5.91 | | 12.05 | 12.38 | | 10.30 | 11.43 | |
| **RBC** | 6.73± | 8.49± | .040* | 4.77± | 4.616± | .719 | 3.64± | 4.40± | .075 |
| **(×10$^9$/L, mean±SD)** | 1.23 | 9.45 | | 1.44 | 1.75 | | 1.19 | 1.55 | |
| **Hb** | 17.46± | 17.10± | .383 | 11.14± | 11.24± | .891 | 9.11± | 10.70± | .039* |
| **(g/dL, mean±SD)** | 1.70 | 1.97 | | 2.82 | 2.24 | | 2.02 | 2.90 | |
| **Hct** | 50.51± | 52.09± | .272 | 33.43± | 33.55± | .957 | 27.02± | 31.65± | .040* |
| **(%, mean±SD)** | 7.10 | 4.77 | | 8.48 | 6.74 | | 5.88 | 8.55 | |
| **Platelet** | 432.13± | 371.92± | .293 | 1161.62± | 1091.29± | .477 | 272.04± | 213.65± | .348 |
| **(×10$^9$/L, mean±SD)** | 285.74 | 173.82 | | 394.67 | 333.84 | | 222.64 | 189.83 | |

P.value significant at <0.05

A JAK2 V617F allele burden was measured for 184 of the 259 patients; 130 PV, 31 ET and 23 IMF. Patient with PMF showed highest burden, then PV patients and ET patient had the lowest burden. The mean allele burden of JAK2 V617F was (69.3% ± 32.8) in PMF, (60.2% ± 33.4) in PV and (31.5% ± 31.1) in ET. These differences between the groups studied were statistically significant ($P$=0.00). Homozygous JAK2 V617F mutation was defined as JAK2 mutant allele exceeded 50%. The majority of JAK2V617F-positive ET patients were heterozygous, while of JAK2V617F- homozygous allele was frequently detected in PV patients. The prevalence of JAK2V617F mutation with high allele burden (i.e. allele burden exceeded 50%) was 85 (53.4.0 %) of 159 PV patients, 7 (12.7%) of 55 ET patients and 14 (31.1%) of 45 PMF patients. differences between the groups studied were statistically significant (P=0.00). These results were summarized in (Table 3.4**)**

**Table 3.4** JAK2 V617F Mutation status among myeloproliferative neoplasm (MPN) subtype

| Disease subtypes | Total No. of subjects | JAK2 wild type N(%) | JAK2V617F heterozygous N(%) | JAK2V617F homozygous N(%) (P=.000) | Mean of JAK2 V617F Allele burden (P=.000) |
|---|---|---|---|---|---|
| **PV** | 159 | 29(18.24%) | 45(28.3%) | 85(53.46%) | 60.26±33.46 |
| **ET** | 55 | 24(43.64%) | 24(43.64%) | 7(12.72%) | 31.58±31.13 |
| **PMF** | 45 | 22(48.89%) | 9(20%) | 14(31.11%) | 69.35±32.83 |

P.value significant at <0.05

The JAK2V617F-positive PV patients with high allele burden showed significantly highest leukocytes count (*P*=.006), and lowest platelets count (*P*=.000) if compared with PV patients with low allele burden. No significant differences in age, Hb, RBCs or Hct between PV patients homozygous for JAK2V617F mutation, JAK2V617F heterozygous PV patients.(Table 3.5).

**Table 3.5** Associations of hematologic parameters with the JAK2 V617F allele burden in PV patients.

| **PV** | JAK2V617F heterozygous | JAK2V617F homozygous | P.value |
|---|---|---|---|
| No of patients | 45 | 85 | |
| Age (years, mean±SD) | 52.48±14.04 | 49.97±15.72 | .369 |
| WBC ($\times 10^9$/L, mean±SD) | 8.27±4.74 | 11.22±7.36 | .006 |
| RBC ($\times 10^9$/L, mean±SD) | 6.88±1.16 | 6.65±1.26 | .313 |
| Hb (g/dL, mean±SD) | 17.05±1.59 | 17.13±2.15 | .824 |
| Hct (%, mean±SD) | 50.42±4.81 | 50.56±8.06 | .931 |
| Platelet ($\times 10^9$/L, mean±SD) | 627.15±337.05 | 331.26±189.88 | .000 |

P.value significant at <0.05

Although JAK2V617F-positive ET patients with high allele burden have highest erythrocytes count when compared with low allele burden ET patients, but the difference statistically was not significant. No associations between JAK2-V617F allele burden and WBCs, Hb, Hct or platelets count in ET patients (Table 3.6).

**Table 3.6** Associations of hematologic parameters with the JAK2 V617F allele burden in ET patients

| **ET** | **JAK2V617F heterozygous** | **JAK2V617F homozygous** | **P.value** |
|---|---|---|---|
| No of patients | 24 | 7 | |
| Age at diagnosis (yr, mean±SD) | 54.16±12.97 | 48.71±16.42 | .364 |
| WBC ($\times 10^9$/L, mean±SD) | 17.07±12.00 | 18.48±14.56 | .769 |
| RBC ($\times 10^9$/L, mean±SD) | 3.48±1.22 | 4.94±1.76 | .051 |
| Hb (g/dL, mean±SD) | 10.10±2.36 | 11.45±2.91 | .272 |
| Hct (%, mean±SD) | 30.15±7.02 | 34.39±8.76 | .252 |
| Platelet ($\times 10^9$/L, mean±SD) | 1091.29±333.84 | 1110.70±221.88 | .865 |

P.value significant at <0.05

Significant difference in platelets counts between JAK2V617F-positive PMF patients with high allele burden and PMF patients with low mutational load was observed, higher allele burden PMF patients had lowest platelets count. No significant differences were observed between JAK2-V617F allele burden and WBCs, RBCs, Hb, or Hct in PMF patients. These results summarized in (Table 3.7)

**Table 3.7** Associations of hematologic parameters with the JAK2 V617F allele burden in PMF patients

| PMF | JAK2V617F heterozygous | JAK2V617F homozygous | P.value |
|---|---|---|---|
| No of patients | 9 | 14 | |
| Age at diagnosis (yr, mean±SD) | 58.44±14.49 | 60.28±18.44 | .803 |
| WBC ($\times 10^9$/L, mean±SD) | 9.87±9.91 | 13.25±11.16 | .456 |
| RBC ($\times 10^9$/L, mean±SD) | 3.30±0.84 | 4.19±1.50 | .082 |
| Hb (g/dL, mean±SD) | 8.75±2.24 | 9.68±1.56 | .288 |
| Hct (%, mean±SD) | 26.06±6.64 | 28.52±4.40 | .340 |
| Platelet ($\times 10^9$/L, mean±SD) | 329.55±184.36 | 139.14±157.44 | .015 |

P.value significant at <0.05

# CHAPTER FOUR

# 4. Discussion, Conclusion and Recommendations

## 4.1 Discussion

The discovery of an acquired somatic point mutation (JAK2-V617F) in classic BCR/ABL-negative MPNs, namely, PV, ET and PMF has opened new opportunities with regards to achieving a correct diagnosis, improving classification and perhaps predicting prognosis. An important consequence of the identification of this mutation was the modification of the World Health Organization classification (2008) and diagnostic algorithms for classical Philadelphia-negative MPNs.

The JAK2-V617F mutation is the most frequent mutation associated with Philadelphia-negative MPNs; it was detected in >70% of MPNs *(*Baxter, *et al*., 2005; Zhu *et al*., 2011; Duletic, *et al*., 2012; Ha *et al*., 2012*)*. The frequency of the *JAK2*-V617F mutation varied between the MPNs subtypes, it can be found in approximately 95% cases of PV and in approximately 50-60% cases of ET or PMF *(*Baxter, *et al*., 2005; Shirane, *et al*., 2015*)*. In numerous published studies, the *JAK2*-V617F mutation was detected in 80% - 97.0% in PV *(*Zhu, *et al*., 2011; Jones A, 2005; Ebid, *et al*., 2015; Ayad and Nafea, 2011; Speletas, *et al*., 2007*)*.

In agreement with these reports, in this study the JAK2-V617F mutation have been detected in 71% of patients with MPNs. The prevalence of the mutation was 81.7% in PV, 56.4% in ET, and 51.1% in PMF. Also the findings of this study were similar to the results obtained by Baxter et al. *(*Baxter, *et al*., 2005*)*; they were detected the JAK2 mutation in 29 (57%) of 51 with ET, eight (50%) of 16 with idiopathic myelofibrosis, and 71 (97%) of 73 patients with PV. Similarly, Ayad et al. *(*Ayad and Nafea, 2011*)* detected the JAK2-V617F mutation in 81.4% of PV, 50% of ET, 46.1% of PMF. The study by Karkucak et al. *(*Karkucak, *et al*., 2012*)* was in line with this study. They found that 80% of the PV group and 42% of the ET group were positive for the JAK2-V617F mutation. Also this study was consistent with the study by Zhu et al, who detected the mutation in 83.9%, 60% in PV and ET, respectively *(*Zhu, *et al*., 2011*)*.

In summary, in this study the prevalence of JAK2-V617F mutation in Sudanese MPNs patients is consistent with previous studies of prevalences of JAK2-V617F mutation in MPN patients worldwise.

The finding of the JAK2-V6217F mutation in several related disorders led to the hypothesis that its presence or absence or its quantity could modify the disease phenotype. In clinical studies, correlations of JAK2-V617F mutation with parameters such as age, white blood cell (WBC) count, Hb concentration, Hct, platelet count, spleen size, disease duration, pruritus, and fibrosis in subjects with MPN have been evaluated frequently *(*Campbell, *et al*., 2005*)*. In numerous studies, the JAK2-V617F mutation has been variably associated with higher Hb, WBC, older age and unchanged or decreased platelet count *(*Speletas, et al., 2006; Kittur, *et al*., 2007; Benmoussa, *et al*., 2009; Yonal, *et al*., 2015*)*. Another study by Campbell *et al* showed that patients with the mutation present with higher hemoglobin levels, higher white cell counts in PV *(*Campbell, *et al*., 2005*)*. In the study of Singh *et al*, it has been showed that JAK2V617F negative patients with PMF were significantly associated with more severe anemia, younger age and thrombocytopenia *(*Singh, *et al*., 2015*)*. Several studies *(*Rudzki, *et al*., 2007; Yonal, *et al*., 2015*)* revealed positive correlation with the JAK2-V617F mutation and higher Hb, WBC, older age and lowest platelet count in patients with ET. In contrast other studies *(*Ha, *et al*., 2012; Sultan and Irfan, 2015*)* showed no significant correlations between V617F mutation and patient age, white blood cell count, Hb, Hct, or the platelet count for ET or PMF patients.

In this study, significant association between older age and JAK2-V617F positive ET patients was observed. The JAK2V617F-positive PMF patients had high Hb level and high Hct in comparison with JAK2V617F-negative ET patients, these differences were statistically significant (*P*=.039),(*P*=.04). Also significant association was observed between JAK2V617F-positive PV patients and erythrocyte count (*P*= .040). In contrast, No significant differences in WBCs, RBCs, Hb, Hct, or platelet count were observed between JAK2-V617F positive and JAK2-V617F negative ET patient, even though ET patients who harbor the mutation have highest WBCs, RBCs, Hb, Hct, and lowest platelet count. No significant differences were detected between JAK2-V617F mutation and WBCs, Hb, Hct, or platelet count in PV, whoever JAK2V617F-positive PV patients had slightly high WBCs, Hb, Hct and low platelets

count. Although JAK2V617F-positive PV and PMF patients had high mean of age, but the difference was not significant statistically. The findings in current study were consistent with all above previous published studies of MPN patients in Europe, the USA and Asia.

Many questions regarding the mechanism underlying the ability of the identical somatic mutation in JAK2 to contribute to 3 related, but clinically distinct, disorders remains unknown [Ross L, 2008], but clinical, biological and pathological data have led to three potential hypotheses, One of them called the gene-dosage hypothesis, postulates a correlation between disease phenotype and the proportion of JAK2 (V617F) mutant alleles introducing the concept of allele burden, that is the ratio between mutant and wild type JAK2 in hematopoietic cells *(*Francesco and Elisa, 2008*)*.

Several experimental and clinical observations supported the possibility that the burden of JAK2 mutation has an influence on disease phenotype through the level of activation of downstream JAK/STAT signaling pathways. First, there are substantial differences in the median burden of V617F allele in peripheral blood granulocytes among individual MPNs; the highest levels are found in PV and the lowest in ET patients with most PMF patients displaying intermediate to high levels *(*Passamonti, *et al*., 2006; Vannucchi, *et al*., 2008a*)*. Furthermore, homozygosity for the JAK2 V617F mutation, which follows mitotic recombination of the short arm of chromosome 9, is displayed by approximately 30% of PV or PMF patients as opposite to 2-4% of ET. Variable proportion of wild-type, heterozygous and homozygous progenitors are present in most patients with PV, while homozygous progenitors are reported as being rare in ET *(*Campbell, *et al*., 2006a*)*. Erythroid progenitors harbouring the V617F mutation are more sensitive to erythropoietin than normal erythroid progenitors, and most erythropoietin-independent erythroid colonies are made up of homozygous progenitors *(*Delhommeau, *et al*., 2007*)*. Conceivably, duplication of the mutant allele is expected to result in a level of JAK2/STAT activation higher than in cells harbouring one mutant and one wild-type allele and in a greater activation of downstream genes *(*Rakesh and Agrawal, 2005; Kralovics, *et al*., 2005a*)*. This could be due to the loss of competition between normal and mutated allele *(*Hookham, *et al*., 2007; Alessandro, *et al*., 2011*)*. Potential influence of the JAK2-V617F allele burden on disease phenotype in patients with a myeloproliferative neoplasm has been the

target of intensive research in recent years. The published studies regarding the impact of the mutational load on phenotypic properties in PV, ET and PMF have reported variable results. Several studies *(*Larsen, *et al*., 2007; Alshemmari, *et al*., 2014*)* have shown that the mean of JAK2 V617F allele burden was significantly different among MPNs subgroup, highest in PMF, and lowest in ET, the PV had intermediate V617F burden. In another study *(*Sang, *et al*., 2013*)* the mean allele burden of JAK2 V617F for ET (21.7%) was significantly lower than that for PV (56.88%) and PMF (56.16%). Also in study by Antonioli E et al, the median V617F allele burden in 165 patients with ET was 24% *(*Antonioli, *et al*., 2008*)*.

In agreement of all above studies, in this study the mean allele burden of JAK2 V617F was (69.3% ± 32.8) in MF, (60.2% ± 33.4) in PV and (31.5% ± 31.1) in ET. These differences between the groups studied were statistically significant ($P$=0.00).

In several studies *(*Levine, *et al*., 2005; Kralovics R, *et al*., 2005; Rajnai, *et al*., 2006; Yonal, *et al*., 2015*)*, homozygous genotype was found rarely in ET, and most frequent in PV and PMF. The prevalence of homozygous reported in PMF patients in several studies was (22% - 31%)*(*Kralovics, *et al*., 2005, Yonal, *et al*., 2015*)*. In PV patients homozygous genotype reported frequencies were in range (27% - 49%) *(*Kralovics, *et al*., 2005; Vannucchi , *et al*., 2007; Hamidah, *et al*., 2012; Payzin, *et al*., 2014*)*. A lowest (2.2% - 6.0%) frequency of homozygous genotype has been reported in ET *(*Kralovics, *et al*., 2005; Rajnai, et al., 2006; Antonioli, *et al*., 2008; Yonal, *et al*., 2015*)*. In summary all above studies has been identified that the prevalence of JAK2V617F-positive patients with high allele burden was greater in PMF or PV compared to ET.

In this study, among 55 patients with ET 7(12.7%) were homozygous, in 45 MF patients 14(31.1%) were homozygous, and 85(53.4%) were homozygous among 159 patients with PV. The findigs of this study was in line with all above previous studies, whoever our prevalence regarding ET and PV were slightly higher than in above studies, but coordinated with the hypothesis that frequency of homozygous genotype different significantly among the three subgroups. And also in comparison, there were studies detected the prevalence of ET homozygous higher than in our studies, in study of Chao et al; 18 were homozygous of 98 patients with ET, study by Hamidah et al; they detected the mutation in 52.9% cases of ET, of which 36.4% were homozygous

for the mutant allele *(*Chao, *et al*., 2009; Hamidah, *et al*., 2012*)*. The variations between theses result most probably due to differences in the nature of study sample and technique used to quantify mutation.

In numerous studies, the JAK2V617F allele has been variably associated with higher Hb, WBC, older age and unchanged or decreased platelet count *(*Kittur, *et al*., 2007; Vannucchi, *et al*., 2007; Benmoussa, *et al*., 2009; Yonal, *et al*., 2015*)*. Another study by Larsen, *et al* showed significantly higher levels of white blood cell counts (WBC), as well as lower platelet counts in homozygous PV patients compared to their heterozygous counterparts *(*Larsen, *et al*., 2007*)*. In the study of Singh *et al*, it has been that Patients who were homozygous for JAK2V617F mutation were associated with thrombocytosis and had higher mean total leukocyte count *(*Singh, *et al*., 2015*)*. In contrast other study *(*Ha, et al., 2012*)* showed no significant correlations between V617F allele burden and patient age, white blood cell count, Hb, Hct, or the platelet count for PV, ET, or PMF patients. Regard to PV, statistical comparison between JAK2 (V617F) heterozygotes and homozygotes did not reveal any significant associations in age, gender, leukocyte or platelet count. But homozygote patients displayed a significantly higher hemoglobin level at the time of diagnosis in study by Tefferi, *et al (*Tefferi, et al., 2006*)*.

In this study the JAK2V617F-positive PV patients with high allele burden showed significantly highest leukocytes count ($P$=.006), and lowest platelets count ($P$=.000) if compared with PV patients with low allele burden. No significant differences in age, Hb, RBCs or Hct between PV patients homozygous for JAK2V617F mutation, JAK2V617F heterozygous PV patients. No associations were observed between JAK2-V617F allele burden and WBCs, Hb, Hct or platelets count in ET patients. Although JAK2V617F-positive ET patients with high allele burden have highest erythrocytes count when compared with low allele burden ET patients, but the difference statistically not significant. Significant difference in platelets counts between JAK2V617F-positive PMF patients with high allele burden and PMF patients with low mutational load was observed, JAK2V617F-positive PMF patients had lowest platelets count. No correlations were observed between JAK2-V617F allele burden and WBCs, RBCs, Hb, or Hct in PMF patients. In summary the findings in current study were revealed that the JAK2 V617F allele burden positively affect

erythrocytosis and leucocytosis in JAK2V617F- positive MPN patients, unchanged or decreased platelet count. Overall these observations have been detected by the vast majority of previous published studies.

## 4.2 Conclusion

The current study demonstrated the JAK2V617F mutational status and allele burden, and find out the correlation of mutation allele with clinicohematological characteristics in Sudanese MPNs patients. It concluded that

- The *JAK2*-V617F mutation could be frequently detected in the Sudanese patients with MPNs, vast majority of polycythemia patients and around half of essential thrombocythemia and primary myelofibrosis have the mutation.
- The presence of JAK2-V617F mutation was found to be significantly associated with high Hemoglobin and Hematocrit in PMF patients, and associated with older age, erythrocytosis in ET and PV patients respectively.
- The majority of PV patients have homozygous JAK2-V617F allele, and higher JAK2V617F mutational burden is related with higher leukocyte count and lower platelets count. Higher allele burden in PMF correlates with low platelets count. The JAK2V617F allele burden different significantly among MPNs subgroups, patient with PMF showed highest burden, while ET patient had the lowest burden.

### 4.3 Recommendations

- Further studies on larger population of Sudanese MPN patient might improve the analysis of significance of *JAK2* V617F alleles with clinical or phenotypic subgroups of MPN patients.
- Cohort studies on large population with MPN are required to explain the effect of allele burden on survival, treatment and other hematologic complications (e.g thrombosis)
- Future genetic studies may lead to better understand the molecular pathogenesis of these MPNs, to create more accurate genetic models of MPNs and to develop molecularly targeted therapies for patients with MPNs.

## References


Adamson JW, Fialkow PJ, Murphy S, Prchal JF, and Steinmann L.(1976). Polycythemia vera: stem-cell and probable clonal origin of the disease. N Engl J Med; 295:913-916

Aggarwal R, Lu J, Pompili VJ, and Das H. (2012) Hematopoietic Stem Cells: Transcriptional Regulation, Ex Vivo Expansion and Clinical Application. Curr Mol Med 12(1):34-49

Akada H, Akada S, Hutchison E and Mohi G. (2014). Loss of wild-type Jak2 allele enhances myeloid cell expansion and accelerates myelofibrosis in Jak2V617F knock-in mice. Leukemia; 28(8):1627-35 10

Alessandro M, Lisa P, and Paola G. (2011). JAK2 allele burden in the myeloproliferative neoplasms: effects on phenotype, prognosis and change with treatment. Ther Adv Hematol 2(1) 21-32

Alessandro M. Vannucchi P, and Ayalew T. (2009). Advances in Understanding and Management of Myeloproliferative Neoplasms, CA CANCER J CLIN; 59:171-191

Alexandra W, Rene E, Karoline G, Catherine R, Serge H, Iris B, Bernd D, claude H, and Fred S. (2013) JAK2-V617F-induced MAPK activity is regulated by PI3K and acts synergistically with PI3K on the proliferation of JAK2-V617F-positive cells. 2:3, e24574; Landes Bioscience

Alister C.(2002) The Jak-Stat Pathway in Hematopoiesis and disease. Molecular Biology Intelligence Unit 20. Lands bioscience. P 10

Alshemmari SH, Rajaan R, Ameen R, Al-Drees MA, and Almosailleakh MR. (2014) JAK2V617F allele burden in patients with myeloproliferative neoplasms. Ann Hematol. 93(5):791-6

Alvin H and Hillard M. (2012). Concise Guide to Hematology, first edition, chapter 1. Blackwell publishing Ltd. P9

Amittha W and Barbara K. (2009) Molecular Basis of Hematopoiesis. Chapter 1, Springer P2

Amy J and Nicholas C. (2013). Inherited predisposition to myeloproliferative neoplasms; Ther Adv Hematol 4(4) 237–253

Amy J. (2010).The molecular pathogenesis of myeloproliferative neoplasms. University of Southampton Research Repository. P15-20

Ana P, Nair A, and Michael J. (2011). Dysregulation of janus kinases and signal transducers and activators of transcription in cancer. Am J Cancer Res;1(6):806-816

Angela T, David W, and Alexander D. (2007) A Simple, Rapid, and Sensitive Method for the Detection of the JAK2 V617F Mutation. Am J Clin Pathol; 127:977-981

Angus T and Michael L. (2003). The Cytokine Handbook, Fourth edition. Elsevier Science Ltd, P86

Antonioli E, Guglielmelli P, Poli G, Bogani C, Pancrazzi A, Longo G, Ponziani V. et al. (2008) Influence of JAK2V617F allele burden on phenotype in essential thrombocythemia. Haematologica. 93(1):41–48.

Ayad M and Nafea D.(2011) Acquired mutation of the tyrosine kinase JAK2V617F in Egyptian patients with myeloid disorders. Genet Test Mol Biomarkers; 15(2):17-21

Ayalew T and Tiziano B. (2015) Essential Thrombocythemia and Polycythemia Vera: Focus on Clinical Practice. Mayo Clin Proc; 90(9):1283-1293

Ayalew T and Tiziano B. (2015) Polycythemia vera and essential thrombocythemia: 2015 update on diagnosis, risk-stratification and management. American Journal of Hematology**,** Volume 90, Issue 2, pages 162–173

Ayalew T, Juergen T and James V. (2009). The 2008 World Health Organization Classification System for Myeloproliferative Neoplasms Cancer; 115:3842–7. VC American Cancer Society

Bang S, Jeong Y, Jiyoon P, Soo J Park Se, Eun M, Pil Park, Yiel S, Eun Cho, Dong Shin and Jae Lee. (2006) Diagnostic Usefulness of the Janus Kinase 2 Mutation in non BCR/ABL Myeloproliferative Disorders. Korean Journal of Internal Medicine: 21:219-224

Barbara D, Hakim H, Friedel N, Bruno V, and Philippe J. (2010) A Real-Time Polymerase Chain Reaction Assay for Rapid, Sensitive, and Specific Quantification of the JAK2V617F Mutation Using a Locked Nucleic Acid-Modified Oligonucleotide. Journal of Molecular Diagnostics, 12(4)

Basel. (2008) Studies of JAK2 Mutations in Myeloproliferative Disorders; Chapter 1 P 15-18.

Baxter J, Scott M, Campbell J, East C, Fourouclas N, Swanton S, Vassiliou G, Bench A, Boyd E, Curtin N,Scott M, Erber W, and Green A. (2005). Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 365: 1054–1061

Benmoussa A, Dehbi H, Fehri S, Quessar A, and Nadifi S.(2009) JAK2-V617F mutation in Moroccan patients with myeloproliferative disorders: contribution, diagnosis and therapeutic prospects. PatholBiol (Paris); 59(4):e89-92.

Bociek R and Armitage J.(1996). Hematopoietic growth factors, Cancer journal for clinicians 46: 165-184

Bogani C, Bartalucci N, Martinelli S, Tozzi L, Guglielmelli P, and Bosi A. (2013) mTOR inhibitors alone and in combination with JAK2 inhibitors effectively inhibit cells of myeloproliferative neoplasms. PLoSOne; 8(1):e54826

Cabagnols X, Fabrizia F, Florence P, Kahia M, Philippe J, and Christophe J. (2015) Presence of atypical thrombopoietin receptor (MPL) mutations in triple negative essential thrombocythemia patients. Blood: 127( 3): 333 - 342

Campbell J, Scott L, Buck G, Wheatley K, East L, Marsden, Duffy A, Boyd E, Bench A, Scott M, Vassiliou, Milligan D, Smith S, Erber W, Bareford D, Wilkins B, Reilly J, Harrison C, and Green.(2005) Definition of subtypes of

essential thrombocythaemia and relation to polycythaemiavera based on JAK2 V617F mutation status: a prospective study. Lancet; 366: 1945–1953

Campbell P, Baxter E, Beer P, Scott L, Bench A, Huntly B. (2006a) Mutation of JAK2 in the myeloproliferative disorders: Timing, clonality studies, cytogenetic associations, and role in leukemic transformation. Blood 108: 3548–3555 [PubMed]

Chao HY, Shen YM, Zhang R, Feng YF, Cen JN, and Yao L.(2009) Quantitative analysis for JAK2 mutation in 98 patients with essential thrombocythemia and its clinical significance. Journal of experimental hematology/ Chinese accusations of pathphysiology 17(3):665-9.

Cherie H and Philip T. (2010) Molecular Pathology of Hematolymphoid Diseases. Springer Science, P395-403

Choi CW, Bang SM, Jang S, Jung CW, Kim HJ, Kim HY, Kim S, Kim Y, Park J, and Won J. (2015) Guidelines for the management of myeloproliferative neoplasms. Korean J Intern Med; 30(6):771-788

Christoph W, Nicholas C, Richard A, and Andreas R. (2008). Comparison of mutated JAK2 and ABL1 as oncogenes and drug targets in myeloproliferative disorders; Leukemia; 22(7): 1320–1334

Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, Kutok J, Clark J, Galinsky I, Griffin JD, Cross NC,Tefferi A, Malone J, Alam R, Schrier SL, Schmid J, Rose M, Vandenberghe P, Verhoef G, Boogaerts M, Wlodarska I,Kantarjian H, Marynen P, Coutre SE, Stone R, and Gilliland D.(2003). A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med; 348:1201–14[PubMed]

Coussens L, Beveren C, Smith D, Chen E, Mitchell R, Isacke C, Verma I and Ullrich A. (1986). Structural alteration of viral homologue of receptor protooncogene fms at carboxyl terminus; Nature 320, 277-280

Cross N. (2011) Genetic and epigenetic complexity in myeloproliferative neoplasms. Hematol Am Soc Hematol Educ Prog; 2011: 208–214

Cumano A and Godin I. (2007) Ontogeny of the hematopoietic system. Annu. Rev. Immunol. 25, 745-785 [PubMed]

Dameshek W. (1951) Some speculations on the myeloproliferative syndromes. Blood; 6:372-5.

Delhommeau F, Pisani DF, James C, Casadevall N, Constantinescu S, and Vainchenker W.(2006) Oncogenic mechanisms in myeloproliferative disorders. Cell Mol Life Sci; 63(24):2939-2953.

Delhommeau F, Dupont S, Tonetti C, Masse A, Godin I, and Le Couedic J. (2007). Evidence that the JAK2 G1849T (V617F) mutation occurs in a lymphomyeloid progenitor in polycythemia vera and idiopathic myelofibrosis. Blood 109: 71–77 [PubMed]

Delhommeau F, Jeziorowska D, Marzac C, and Casadevall N.(2010) Molecular aspects of myeloproliferative neoplasms. Int J Hematol; 91:165–173.

Dino V, Enrico C, Giovanna M, Massimo F, Elisabetta B, Marco B, Solero GP, Ambrosetti A, Quaresmini G,Pizzolo G. (2009). Comparison of JAK2V617F mutation assessment employing different molecular diagnostic techniques; Blood Transfus; **7**: 204-9

Domnita C. (2010) Hematopayhology, Genomic Mechanisms of Neoplastic Disaese. 1st ed. Springer Science

Donal M, Melanie P, Mary F. (2006). JAK2 V617F: A Single Mutation in the Myeloproliferative Group of Disorders. Ulster Med J; 75 (2) 112-119

Drew P and John G. (2010). Molecular Hematology, third edition, chapter 9; Myeloproliferative disorders. Black Well publishing

Duletic AN, Dekanic A, Hadzisejdic I, Kusen I, Matusanllijas K, Grohovac D, Grahovac B and Jonjic N. (2012). JAK2-v617F mutation is associated with clinical and laboratory features of myeloproliferative neoplasms. CollAntropol ; 36(3):859-65

Ebid G, Ghareeb M, Salaheldin O, Kamel M.(2015). Prevalence of the frequency of JAK2-V617F mutation in different myeloproliferative disorders in Egyptian patients International Journal of Clinical and Experimental Pathology; 8(9):11555-11559

Edward P, Charles L, Frank J. (2014). Molecular Oncology; JAK2 and myeloproliferative neoplasms, Cambridge University Press. chapter 76, p 818.

El Kassar N, Hetet G, Li Y, Briere J, and Grandchamp B.( 1995). Clonal analysis of haemopoietic cells in essential thrombocythaemia. Br J Haematol; 90:131-137

Fabio S and Srdan. (2010). JAK2 Inhibitors: What's the true therapeutic potential? Blood Rev 25(2):53-63

Faramarz N, Nageh P, Sophie S, and Wayne G. (2013) Atlas of hematophathology; morphology, immunophenotype, cytogenetics, and molecular approaches, First edition. Elsevier Ltd

Faramarz N, Nagesh P, Wayne G. (2008). Hematopathology; chronic myeloproliferative diseases, 1st ed, chapter 9. Elsevier Ltd, P155

Fialkow P, Gartler S, and Yoshida A. (1967). Clonal origin of chronic myelocytic leukemia in man. Proc.Natl. Acad. Sci. U.S.A. 58, 1468–1471

Francesca F, Emma C, Natalia C, Gloria P, Sergio M, Angela R, Ettore C, Anna B, Francesco E,Francesco Lo, Clara N, and Giuseppe C. (2014). A highly specific q-RT-PCR assay to address the relevance of the JAK2WT and JAK2V617F expression levels and control genes in Ph-negative myeloproliferative neoplasms Ann Hematol 93:609–616

Francesco P and Elisa R. (2009) Clinical relevance of JAK2 (V617F) mutant allele burden. Haematologica 94(1): 7–10

Francesco P, Margherita M, Domenica C, Mario C. (2011) Myeloproliferative neoplasms: From JAK2 mutations discovery to JAK2 inhibitor therapies. Oncotarget; 2: 485 – 490

Furitsu T, Tsujimura T, Tono T, Ikeda H, Kitayama H, Koshimizu U, Sugahara H, Butterfield H, Ashman K and Kanayama Y. (1993). Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. J Clin Invest; 92:1736–44

Galloway J and Zon L. (2003). Ontogeny of hematopoiesis: examining the emergence of hematopoietic cells in the vertebrate embryo. Curr. Top. Dev. Biol. 53, 139-158 [PubMed]

Gary H and David C.(2011) Hematopoietic growth factors in Oncology.1st edition. Springer Science. P 3-10

Gery S, Cao Q, Gueller S, Xing H, Tefferi A, and Koeffler HP. (2009) Lnk inhibits myeloproliferative disorder-associated JAK2 mutant, JAK2V617F. J Leukoc Biol, 85(6):957-965.

Ghoreschi K, Laurence A, and John J. (2009). Janus kinases in immune cell signaling. Immunol Rev; 228(1): 273–287

Ghulam R and Abdulraheem Y. (2016). Primary Myelofibrosis Presenting as Extramedullary Hematopoiesis in a Transplanted Liver Graft: Case Report and Review of the Literature. Case Rep Hematol: 9515404.

Giuseppe T, Rosanna Di, Roberto S, Daniela Z, Donata B, Paolo R, Renzo R, Giovanni R, Roberta S, Cristina T and Nunzio T. (2009) The JAK2V617F tyrosine kinase mutation in blood donors with upper-limit haematocrit levels Blood Transfus; 7: 111-116

Godfrey AL, Chen E, Pagano F, Ortmann CA, Silber Y, Bellosillo B, Renzo R, Giovanni R, Roberta S, Cristina T, and Nunzio T. (2012) JAK2V617F-homozygosity arises commonly and recurrently in PV and ET, but PV is characterized by expansion of a dominant homozygous subclone. Blood 120(13):2704–2707.

Golub R, Barker F, Lovett M, and Gilliland G.(1994) Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell 77, 307-316

Greil R, Pleyer L, Neuriter D, Faber V. (2010). Chronic Myeloid Neoplasias and Clonal overlap Syndromes. Spinger Wien NewYork, P1-2

Groner B. (2007). Targeted interference with Signal Transduction Events-Recent results in cancer research vol,172. Spriner Berlin New York

Gunner B. (2015) Advances and challenges in the management of essential thrombocythemia. TherAdvHematol. 6(3): 142–156

Ha J S, Kim Y K, Jung S I, Jung H R, and Chung I S. (2012) Correlations between Janus Kinase 2 V617F Allele Burdens and Clinicohematologic Parameters in Myeloproliferative Neoplasms. Annals of Laboratory Medicine; 32(6):385-391

Ha JS and Kim YK. (2015) Calreticulin exon 9 mutations in myeloproliferative neoplasms. Ann Lab Med; 35:22–27. [PubMed]

Hamidah NH, Farisah NR, Azlinda AB, Wong FL, Das S, Fadillah SA.(2012) A study of JAK2 (V617F) gene mutation in patients with chronic myeloproliferative disorders Clin Ter; 163(2):109-13

Harper G, Dale M, Elizabeth O. (2012). Myelofibrosis: it's complicated. Adv Hematol 3(3) 131–146

Hoffbrand A, Daniel C, Edward G, and Anthony R.(2011) Postgraduate Hematology, Sixth edition Published by Wiley Blackwell, p686

Hoffbrand A, Moss P, Pettit J.(2006) Essential Haematology, Fifth edition Wiley Blackwell

Hookham M, Elliott J, Suessmuth Y, Staerk J, Ward A, Vainchenker W. (2007). The myeloproliferative disorder-associated JAK2 V617F mutant escapes negative regulation by suppressor of cytokine signaling 3. Blood 109: 4924–4929 [PubMed]

Hui-Chi Hsu. (2007). Pathogenetic Role of JAK2 V617F Mutation in Chronic Myeloproliferative Disorders; J Chin Med Assoc 70 (3)

Huijsmans C, Poodt J, Savelkoul P, and Hermans M. (2011). A real-time polymerase chain reaction assay for rapid, sensitive, and specific quantification of the JAK2V617F mutation using a locked nucleic acid-modified oligonucleotide; J Mol Diagn; 13(5):558-64.

IpekYonal H, AynurDaglar A, BasakAkadam T, Ceylan Y, Meliha N, AkifSelim Y. (2015). The Burden of JAK2V617F Mutated Allele in Turkish Patients with Myeloproliferative Neoplasms. JCl in Med Res; 7(3):161–170.

James V, Thiele J, Daniel A, Richard B, Michael B, Anna P.(2009). The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. BLOOD; 114(5)

James C, Delhommeau F, Marzac C, Teyssandie I, Le Couedic J-P, Giraudier S , Roy L , Saulnier P , Lacroix L , Maury S , Tulliez M , Vainchenker W , Ugo V and Casadeval N. (2006) Detection of JAK2 V617F as a first intention diagnostic test for erythrocytosis. Leukemia 20:350–353.

Janice P, Peter H, John M, Robert A.(2003) Hematopoietic growth factors in the supportive care and treatment of patients with hematologic malignancies, Neoplastic diseases of blood, 4th edition P1123. Cambridge university press

Jason S, Kristin M, Douglas A.(2004) The JAK/STAT signaling pathway Journal of Cell Science 117: 1281-1283

Jones A, Kreil S, Zoi K, Curtis C, Zhang L, Score J. (2005). Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders; Blood, 106:2162-2168.

Jones D. (2010). Neoplastic Hematopathology: Contemporary Hematology. Humana Press, Chapter 9 P 177

Judith S and Stefan N. (2012). The JAK-STAT pathway and hematopoietic stem cells from the JAK2 V617F perspective; Landes Bioscience 1(3)

Karkucak M, Yakut T, Ozkocaman V, Ozkalemkas F, Ali R, Bayram M, Gorukmez O, Ocakoglu G.(2012) Evaluation of the JAK2-V617F gene

mutation in Turkish patients with essential thrombocythemia and polycythemia vera. MolBiol Rep; 39(9):8663-7

Kile B, Alexander W, Nicola N, Martin H, and Hilton D.(2002) The SOCS box: a tale of destruction and degradation. Trends. Biochem. Sci. **27**, 235–241

Kim B, Young Cho, Mi-Hyun, Seongsoo Jang, Eul-Ju Seo, Hyun-Sook, Kim D, Lee J, Je-Hwan, Lee K, Park Y, Jong L, and Park C. (2015) JAK2 V617F, MPL, and CALR Mutations in Korean Patients with Essential Thrombocythemia and Primary Myelofibrosis. J Korean Med Sci. 30(7): 882–888

Kim H, Choi H, Kim Y, Kim J, Shin J, Suh S . (2013) Allelic Expression Imbalance of JAK2 V617F Mutation in BCR-ABL Negative Myeloproliferative Neoplasms. PLoS ONE 8(1)

Kittur J, Knudson RA, Lasho TL, Finke CM, Gangat N, Wolanskyj AP. (2007) Clinical correlates of JAK2V617F allele burden in essential thrombocythemia. Cancer; 109(11):2279-84

Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al.(2013) Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med; 369:2379–2390. [PubMed]

Kralovics R, Passamonti F, Buser A, Teo S, Tiedt R, Passweg J, Tichelli A, Cazzola M and Skoda R. (2005a) A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 352: 1779–1790 [PubMed]

Kvasnicka H and Thiele J. (2010). Myeloproliferative neoplasms: The 2008 WHO classification. Am J Hematol; 85(1):62–69.

Lacout C, Pisani DF, Tulliez M, Gachelin FM, Vainchenker W, Villeval JL. (2006) JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. Blood 108(5):1652–1660.

Larsen TS, Pallisgaard N, Moller MB, Hasselbalch HC.(2007) The JAK2 V617F allele burden in essential thrombocythemia, polycythemia vera and

primary myelofibrosis--impact on disease phenotype. Eur J Haematol. 79(6):508-15.

Leibundgut E, Horn M, Brunold C, Pfanner-Meyer B, Marti D, Hirsiger H. (2006) Hematopoietic and endothelial progenitor cell trafficking in patients with myeloproliferative diseases. Haematologica; 91(11):1465–1472. [PubMed].

Levine R, Belisle C, Wadleigh M, Zahrieh D, Lee S, Chagnon P. (2006). X-inactivation-based clonality analysis and quantitative JAK2V617F assessment reveal a strong association between clonality and JAK2V617F in PV but not ET/MMM, and identifies a subset of JAK2V617F-negative ET and MMM patients with clonal hematopoiesis. Blood, 107:4139-4141.

Levine R, Wadleigh M, Cools J, Ebert B, Wernig G, Huntly B. (2005) Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 7: 387–397

Lippert E, Boissinot M, Kralovics R, Girodon F, Dobo I, Praloran V. (2006). The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. Blood 01-013540

Maher A and Emil J.(2000) Molecular Defects in Chronic Myeloproliferative Disorders .Molecular Medicine 6(7): 555–567, 2000

Mamatha R, Anagha D, and Martin S. (2012) Targeting JAK2 in the therapy of myeloproliferative neoplasms. Expert Opin Ther Targets 16(3): 313-324

Manula T, Katia B,Lidia S, Judith V, Bruno P. (2010) Embryonic origin of human hematopoiesis. Int. J. Dev. Biol. 54: 1061-1065

Martha W and Ayalew T. (2010) Classification and diagnosis of myeloproliferative neoplasms according to the 2008 World Health Organization criteria. Int J Hematol 91:174-179

Marty C, Lacout C, Martin A, Hasan S, Jacquot S, Birling M And Villeval J. (2010) Myeloproliferative neoplasm induced by constitutive expression of JAK2V617F in knock-in mice. Blood 116(5):783–787.

Mascarenhas J, Mughal T, and Verstovsek S.(2012) Biology and Clinical Management of Myeloproliferative Neoplasms and Development of the JAK Inhibitor Ruxolitinib. Current Medicinal Chemistry19, 4399-4413

Mascarenhas J, Roper N, Chaurasia P, and Hoffman R. (2011) Epigenetic abnormalities in myeloproliferative neoplasms: a target for novel therapeutic strategies. Clin Epigenet ; 2: 197–212

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

McGrath K, Frame J, Fromm G, Koniski A, Kingsley P, Little J. (2011) A transient definitive erythroid lineage with unique regulation of the β-globin locus in the mammalian embryo. Blood 117, 4600-4608 [PubMed]

Mehta J, Wang H, Iqbal SU, Mesa R. (2014) Epidemiology of myeloproliferative neoplasms in the United States. Leuk Lymphoma; 55(3):595-600

Mohamad S, Belal F, Ahmad Z, Mohammad M, Vishal R, Ramon V. (2013). Comprehensive review of JAK inhibitors in myeloproliferative neoplasms .Ther Adv Hematol 4(1) 15–35

Moliterno AR, Williams DM, Rogers O, and Spivak JL.(2006) Molecular mimicary in chronic myeloproliferative disorders. Reciprocity between quantitative JAK2V617F and Mpl expression. Blood 108: 3913-3915

Mughal T, Kris V, Nicholas J, and Verstovsek S. (2014). Myelofibrosis-associated complications: pathogenesis, clinical manifestations, and effects on outcomes. International Journal of General Medicine: 7 89–101

Munjal P, Agarwal K, Gupta P, Kamath A , Nadkar M. (2012 ). Text Book of medicine; Association of physician of India

Najia T, Mohammed S, Rubina G, and Moinuddin J. (2014) Frequency of JAK2 V617F mutation in patients with Philadelphia positive Chronic Myeloid Leukemia in Pakistan. Med Sci. 30(1)

Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC. (2013) Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med; 369:2391–2405. [PubMed]

Neha B, Priya K, Matthew K, Sachie M, Kaitlyn S, Raajit R. (2014) Improved targeting of JAK2 leads to increased therapeutic efficacy in myeloproliferative neoplasms. Blood; 123(13): 2075–2083

Nisha R. (2015) Genomic diversity in myeloproliferative neoplasms; focus on myelofibrosis. Transl Pediatr. 4(2):107–115

Oka T, Ouchida M, Koyama M, Ogama Y, Takada S, Nakatani Y.(2002). Gene silencing of the tyrosine phosphatase SHP1 gene by aberrant methylation in leukemias/lymphomas Cancer Res; 62:6390-6394

Orkin S and Zon L. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. Cell 132, 631-644 [PubMed]

Paik E and Zon L. (2010) Hematopoietic development in the zebrafish. Int. J. Dev. Biol. 54, 1127-1137 [PubMed]

Palis J and Yoder M. (2001). Yolk-sac hematopoiesis: the first blood cells of mouse and man. Exp. Hematol. 29, 927-936 [PubMed]

Pardanani A, Lasho T, Finke C, Oh ST, Gotlib J, and Tefferi A. (2010) LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. Leukemia 24(10):1713-8

Passamonti F, Rumi E, Pietra D, Della P, Boveri E, Pascutto C. (2006) Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. Blood 107: 3676–3682 [PubMed]

Payzin KB, Savasoglu K, Alacacioglu I, Ozdemirkiran F, Mutlu BB, Bener S. (2014). JAK2 V617F mutation status of 232 patients diagnosed with chronic myeloproliferative neoplasms. Clin Lymphoma Myeloma Leuk; 14(6):525-33

Pecquet C, Diaconu C, Staerk J. (2012) Thrombopoietin receptor down-modulation by JAK2 V617F: restoration of receptor levels by inhibitors of pathologic JAK2 signaling and of proteasomes. Blood 119(20):4625–4635.

Peter H, John M, Janice P, Robert A. (2013). Neoplastic Diseases of Blood, Fifth edition. Springer Science

Pikman Y, Lee BH, Mercher T, McDowell E, Ebert B, Gozo M. (2006) MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med; 3:e270.

Qiaofang C, Pin Lu, Amy V, Nicholas C, Richard T, and Lynn Y.(2007) Amplification Refractory Mutation System, a Highly Sensitive and Simple Polymerase Chain Reaction Assay, for the Detection of JAK2 V617F Mutation in Chronic Myeloproliferative Disorders. J Mol Diagn; 9(2): 272–276

Rajnai H, Bodor C, Reiniger L, Timár B, Csernus B, Szepesi A.(2006) Novel method in diagnosis of chronic myeloproliferative disorders; detection of JAK2 mutation. Orv Hetil. 147(45):2175-9

Rakesh K and Agrawal D. (2005) Controlling cytokine signaling by constitutive inhibitors. Biochem Pharmacol; 70:649-657

Rakesh K. (2008). Novel role of the JAK-STAT pathway in mediating the effects of atypical antipsychotics on 5-$HT_{2A}$ receptor signaling. University of Kansas. ProQuest Information and Learning Company

Ralph A and Edward A. (2011). Functining of Transmembrane Receptors in Cell Signaling, First edition. Elsevier Inc

Reilly J T. (2008) Pathogenetic insight and prognostic information from standard and molecular cytogenetic studies in the BCR-ABL-negative myeloproliferative neoplasms (MPNs). Leukemia 22, 1818–1827

Rosane B, Vassallo J, Maria de, Sandra X, Katia P, Ana C. (2012) Philadelphia-negative chronic myeloproliferative neoplasms. Rev Bras Hematol Hemoter; 34(2):140-9

Ross L. (2008). JAK2V617F: you can't have too much. Blood; 111(8): 3913.

Ross L. (2012). Non CML Diseases, Hematology/Oncology clinics of north amwerica, Volume 26, Number 5

Rudzki Z, Sacha T, Stoj A, Czekalska S, Wójcik M, Skotnicki AB. (2007). The gain-of-function JAK2 V617F mutation shifts the phenotype of essential thrombocythemia and chronic idiopathic myelofibrosis to more "erythremic" and less "thrombocythemic": a molecular, histologic, and clinical study. Int J Hematol; 86:130–136. [PubMed]

Rumi E, Pietra D, Ferretti V. (2014) JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood; 123:1544–1551.[PubMed]

Ruta D, Rolandas G, Elona J, and Rima G. (2015) Thrombotic risk assessment in 185 WHO-defined essential thrombocythemia patients: single center experience**.** Contemp Oncol (Pozn); 19(5): 396–399

Sang H, Hyun Chi, Young Cho, Seongsoo J, and Chan P. (2013). The allele burden of JAK2 V617F can aid in differential diagnosis of Philadelphia Chromosome-Negative Myeloproliferative Neoplasm. Blood Res; 48:128-32.

Savona MR. (2014) Are we altering the natural history of primary myelofibrosis? Leuk Res; 38(9):1004-12

Schmidt M, and Dikic I. (2005) The Cbl interactome and its functions. Nat. Rev. Mol. Cell Biol. 6, 907–918

Schmitt H, Teo S, Olschewski M. (2008). JAK2V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica 93(1):34–40.

Scott L, Tong W, Levine R, Scott M, Beer P, Stratton M, Futreal A, Erber W, McMullin M, Harrison C, Warren A, Gilliland D, Lodish H, and Green A.

(2007). JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med, 356:459–468.

Shirane S, Araki M, Morishita S, Edahiro Y, Takei H, Yoo Y, Choi M, Suami Y, Hironaka Y. Noguchi M, Koike M, Noda N, Ohsaka A and Komatsu N.(2015) JAK2, CALR, and MPL mutation spectrum in Japanese patients with myeloproliferative neoplasms. Haematologica; 100(2):e46-8. [PubMed]

Shivarov V, Ivanova M, and Tiu RV.(2014) Mutated calreticulin retains structurally disordered C terminus that cannot bind Ca(2+): some mechanistic and therapeutic implications. Blood Cancer J; 4:e185. [PubMed]

Shuai K and Bin L. (2003). Regulation OF JAK–STAT Signaling in the Immune System; Nature; 3(11)

Shuai K. (200) Modulation of STAT signaling by STAT-interacting proteins. Oncogene 19, 2638–2644

Singh N, Sazawal S, Upadhyay A, Chhikara S, Mahapatra M, and Saxena R. (2015) Correlation of JAK2V617F mutational status in primary myelofibrosis with clinico-hematologic characteristics and international prognostic scoring system scoring: a single center experience. Indian J PatholMicrobiol; 58(2):187-91

Smith BR. (1990) Regulation of hematopoiesis. Yale J Biol Med; 63(5): 371–380.

Speletas M, Katodritou E, Daiou C, Mandala E, Papadakis E, Kioumi A. (2007) Correlations of JAK2-V617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leuk Res; 31(8):1053-62.

Srdan V, Judith E, and Ayalew T. (2011) Myeloproliferative Neoplasms, Biology and therapy. Springer Science

Stefan F and Kagop K. (2011) Leukemias Principles and practice of therapy. Wiley Blackwell

Stefan O and Walter R. (2008) Hematopoietic growth factors, Encyclopedia of molecular pharmacology, Volume one, 2nd edition. Springer-verlag Berlin Heidelberg New York p579

Sultan S and Irfan SM. (2015) Acquired JAK-2 V617F Mutational Analysis in Pakistani Patients with Essential Thrombocythemia. Asian Pac J Cancer Prev; 16(16):7327-30.

Sverre H and Mitelman F. (2009). Cancer Cytogenetics; Chromosomal and molecular Genetic aberrations of tumor cells. 3rd edition. Wiley Blackwell

Tariq M, Kris V, Nicholas J, and Srdan V. (2014) Myelofibrosis-associated complications: pathogenesis, clinical manifestations, and effects on outcomes**.** Int J Gen Med.; 7: 89–101.

Tefferi A and Gilliland D. (2007) Oncogenes in Myeloproliferative Disorders. Cell Cycle; 6:550-566.

Tefferi A, Lasho TL, Schwager SM.(2006) The clinical phenotype of wild-type, heterozygous, and homozygous JAK2(V617F) in polycythemia vera. Cancer; 106:631-635

Tefferi A, Noel P, Curtis A. (2011). Uses and Abuses of JAK2 and MPL Mutation Tests in Myeloproliferative Neoplasms Journal of Molecular Diagnostics, 13 (5)

Tefferi A, Skoda R, Vardiman J. (2009). Myeloproliferative neoplasms: contemporary diagnosis using histology and genetics. Nature Reviews Clinical Oncology 6, 627-637

Tefferi A, Thiele J, Orazi A. (2007). Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. Blood; 110:1092-1097.

Tefferi A, Thiele J, Vardiman J, Vannucchi AM, Barbui T. (2014) An overview on CALR and CSF3R mutations and a proposal for revision of

WHO diagnostic criteria for myeloproliferative neoplasms. Leukemia; 28:1407–1413. [PubMed]

Tefferi A. (2000) Myelofibrosis with myeloid metaplasia. N Engl J Med; 342:1255-65. [PubMed]

Tefferi A. (2006). Classification, Diagnosis and Management of Myeloproliferative Disorders in the JAK2 V617F Era; American Society of Hematology. P 240-245

Tefferi A. (2008) Mutant molecules of interest in myeloproliferative neoplasms: introduction. Acta Haematol; 119(4):192–193

Tefferi A. (2010). Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. Leukemia 24, 1128–1138

Tefferi A. (2015) Myeloproliferative neoplasms: A decade of discoveries and treatment advances. American Journal of Hematology; 91(1)

Thiele J and Kvasnicka H. (2009). The 2008 WHO diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Curr Hematol Malig Rep; 4:33–40. [PubMed]

Thomas D and Mathias M.(2012). Jak-Stat signaling: From Basics to Disease. Springer-Verlag wien. P 239-258

Thomas L. (2007).The JAK2 V617F mutation in Philadelphia chromosome negative chronic myeloproliferative disorders -Impact on clinical phenotype. University of Southern Denmark. P 10-22

Tiedt R, Hao-Shen H, and Sobas M. (2008) Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. Blood 111:3931–3940.

Titmarsh GJ, Duncombe AS, McMullin MF, O'Rorke M, Mesa R, De Vocht F, et al.(2014) How common are myeloproliferative neoplasms? A systematic review and meta-analysis. Am J Hematol; 89(6):581-7.

Tiziano B and Ayalew T. (2012) Myeloproliferative Neoplasms, Critical concepts and management. Springer Science. P 37-45

Tong W, Zhang J, Lodish HF. (2005) Lnk inhibits erythropoiesis and Epo-dependent JAK2 activation and downstream signaling pathways. Blood, 105(12):4604-4612.

Tsukamoto N, Morita K, Maehara T, Okamoto K, Sakai H, Karasawa M.(1994). Clonality in chronic myeloproliferative disorders defined by X-chromosome linked probes: demonstration of heterogeneity in lineage involvement. Br J Haematol; 86:253-258

Vannucchi A, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, Barosi G, Ruggeri M, Specchia G, Lo-Coco, Delaini F, Villani F, Finotto S, Ammatuna E, Alterini R, Carrai V, Capaccioli G, Lollo S, Liso V, Rambaldi A, Bosi1 A, and Barbui T. (2008a) Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. Blood 112: 844–847 [PubMed]

Vannucchi A, Guglielmelli P, Antonioli E, Mappa S, Pancrazzi A, Bogani C. (2006). Inconsistencies in the association between the JAK2(V617F) mutation and PRV-1 over-expression among the chronic myeloproliferative diseases. Br J Haematol 132: 652–654 [PubMed]

Vannucchi AM, Antonioli E, Guglielmelli P, Longo G, Pancrazzi A, Ponziani V. (2007) Prospective identification of high-risk polycythemia vera patients based on JAK2 (V617F) allele burden. Leukemia; 21:1952–1959.

Vannucchi AM, Antonioli E, Guglielmelli P, Rambaldi A, Barosi G, Marchioli R.(2007) Clinical profile of homozygous JAK2V617F mutation in patients with polycythemia vera or essential thrombocythemia; 110: 840–846.

Verstovsek S and Tefferi A. (2011). Myeloproliferative Neoplasms, Biology and therapy. Springer Science. P 1-40

Wernig G, Mercher T, Okabe R, Levine R, Lee B, and Gilliland D.(2006) expression of JAK2V617F causes a polycythemia vera like disease associated

with myelofibrosis in a murine bone marrow transplant model. Blood 107: 4274-4281

William E. (2013). Fundamental Immunology, Seventh edition. Wolter Kluwer

William V, Delhommeau F, Stefan N, Olivier A. (2011) New mutations and pathogenesis of myeloproliferative neoplasms. Blood, 118(7)

Yonal I, Daglar A, AkadamTeker B, Ceylan Y, Nalcacı M, Yavuz AS, Sargin F.(2015) Impact of JAK2V617F Mutational Status on Phenotypic Features in Essential Thrombocythemia and Primary Myelofibrosis. Turk J Haematol-33(2); 94-101

Yoshimura A, Ohkubo T, Kiguchi T, Jenkins NA, Gilbert DJ, Copeland NG, Hara T, and Miyajima A. (1995) A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. EMBO J. 14**,** 2816–2826

Zhe Li, Shu Xing, Shaofeng W, Wanting T, Zhizhuang Joe. (2007) Characterization of a Highly Effective Protein Substrate for Analysis of JAK2V617F Activity, Exp Hematol; 35(11): 1624–1632

Zhu JF, Liu Y, Liu P, Jia MF, Cheng J, Zhao L. (2011) JAK2V617F mutation in the patients with myeloproliferative disorder and its relation with clinical characteristics. Journal of experimental hematology/ Chinese association of pathophysiology; 19(4):916-20

## APPENDICS

**Appendix I**

**Questionnaire**

Sudan University of Science and Technology

College of Graduate studies

Department of Hematology and immunohematology

**Questionnaire**

**Date …….....**

**Serial number** ☐

**Name ……**

**Age …….**

**Sex** **Male** ☐ **Female** ☐

**Residence…….**

Tribe…….

Phone No ☐

**Present Complaint**

-……

-……

-……

**Date of first complaining……..**

**Diagnosis**

**PV** ☐ **ET** ☐ **PMF** ☐

**Date of diagnosis……...**

**Clinical finding**

**Laboratory findings at diagnosis**

| | |
|---|---|
| **Hemoglobin** | |
| **RBC** | |
| **WBC** | |
| **PCV** | |
| **Platelets count** | |

*Thank you very much!*

## Appendix II

### Consent form

| |
|---|
| The isotope centre hospital director asked for permission to collect sample for research and he agreed, then all patients were informed and their agreements were obtained. |

**Appendix III**

**Blood DNA extraction kit**

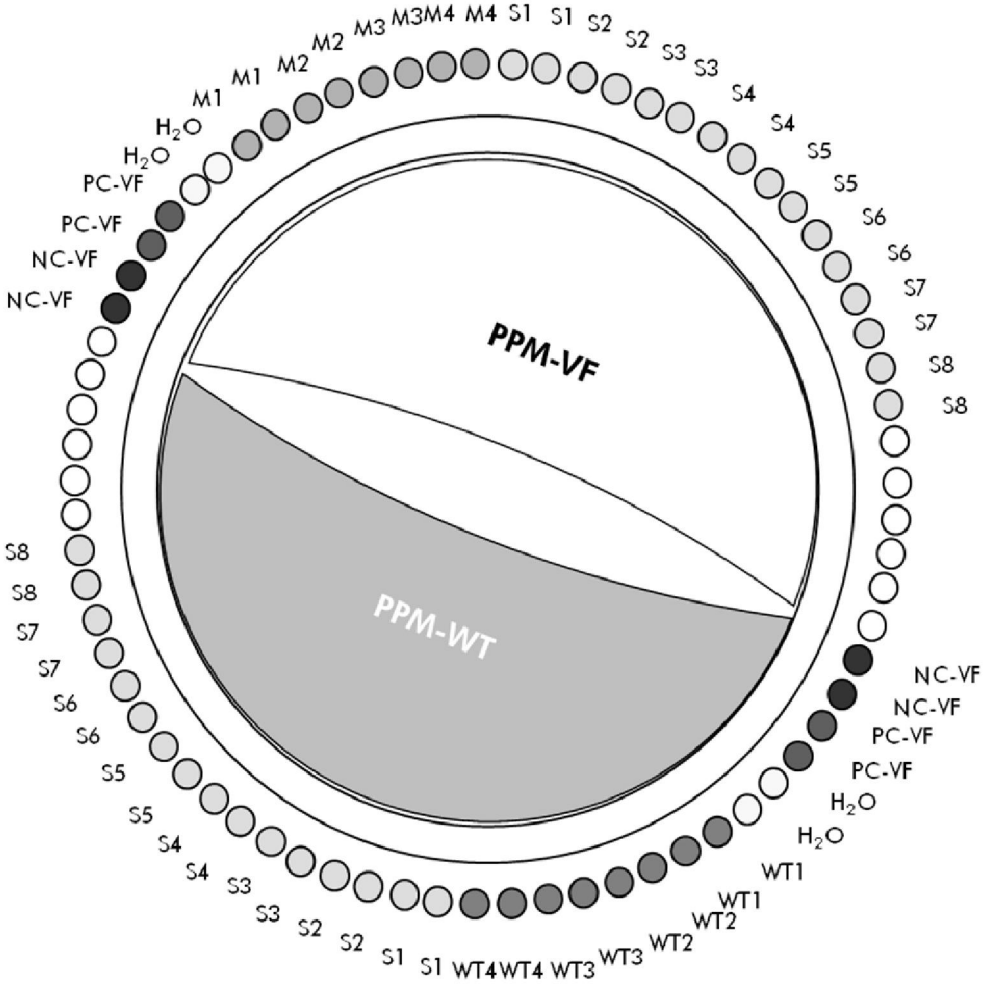
innuPREP Blood DNA Mini Kit


Mini Kit is a highly efficient tool for directly isolating DNA from whole blood samples of up to 400 µl. The innuPREP Blood DNA Mini Kit is fast and easy to use. Isolation of genomic DNA free of inhibitors or impurities can be completed in just 24 minutes. Briefly, the basic principle is the use Spin Filter.The innuPREP Blood DNA Mini Kit should be stored dry at room temperature (14-25) and is stable for at least 12 months under these conditions

**Kit components**

- Lysis solutions (BLS)
- Binding solutions (TBS)
- Proteinase K
- Washing solution HS
- Washing solution MS
- Elution Buffer
- Spin filter
- Receiver tubes 2.0 ml & Elution tubes 1.5 ml
- 96-99.8 ethanol
- ddH2O
- RNase A (100 mg/ml)

**Appendix IV**




- qPCR on Rotor-Gene Q instruments with 72-tube rotor

**Appendix V**

**Master mix Preparations**

**Preparation of Washing solution HS**

Add 70 ml of 96-99.8% ethanol to bottle washing solution HS (70 ml) to reach total volume 140 ml. mix thoroughly and keep the bottle always firmly closed

**Preparation of Washing solution MS**

Add 140 ml of 96-99.8% ethanol to bottle washing solution MS (60 ml) to reach total volume 200 ml. mix thoroughly and keep the bottle always firmly closed

**Preparation of Proteinase K**

Dissolve 1.5ml of Proteinase K by adding 1.5 ml of ddH2O mix thoroughly and store at – 20 C

**AS- PCR mix**

Amplifications were done in a total volume of 25 µL PCR mix containing 12.5 µL of TaqMan Universal Master Mix, 5 µL of nuclease-free PCR grade water, 2.5 µL of primers and probes mix and 50 ng/µL of DNA template.

**Preparation of qPCR mix**

Table describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A premix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPM-VF or PPM-WT).

| Component | V617F pre-mix 1 reaction (µl) | WT pre-mix 1 reaction(µl) |
|---|---|---|
| TaqMan Universal PCR Master Mix,2x | 12.5 | 12.5 |
| Primers and probemix, PPM-VF25x | 1.0 | - |
| Primers and probe mix, PPM-WT25x | - | 1.0 |
| Nuclease-free PCR grade water | 6.5 | 6.5 |
| Sample (to be added at step 4) | 5.0 | 5.0 |
| Total volume | 25 | 25 |

**Appendix VI**

**Primers sequences**

JAK2-V617F AS-PCR primers

| | |
|---|---|
| Forward wild-type | 5'-TCC TCA GAA CGT TGA TGG CAG-3' |
| Reverse wild-type | 5'-ATT GCT TTC CTT TTT CAC AAG AT-3' |
| Forward mutant | 5'-GCA TTT GGT TTT AAA TTA TGG AGT ATA TG-3' |
| Reverse mutant | 5'-GTT TTA CTT ACT CTC GTC TCC ACA AAA-3' |

## Appedix VII

## Publications

## Paper I

The Prevalence of JAK2-V617F Mutation in Sudanese Patients with Chronic Myeloproliferative Neoplasms

Abkar et al.; BJMMR, 14(5): 1-7, 2016

Article no.BJMMR.24741

## Paper II

Correlations of JAK2V617F Mutational status with Clinicohematologic Characteristics in Sudanese Patients with Primary Myelofibrosis

[Abkar HM, Hassan FM, Mohamed BA. Correlations of JAK2V617F Mutational status with Clinicohematologic Characteristics in Sudanese Patients with Primary Myelofibrosis. N Y Sci J 2016;9(3):7982].

(online). http://www.sciencepub.net/newyork. 14.

doi:10.7537/marsnys09031614.

*British Journal of Medicine & Medical Research*
*14(5): 1-7, 2016, Article no.BJMMR.24741*
*ISSN: 2231-0614, NLM ID: 101570965*

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# The Prevalence of *JAK2*-V617F Mutation in Sudanese Patients with Chronic Myeloproliferative Neoplasms

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author HMA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author BAM managed the literature searches, analyses of the study performed the spectroscopy analysis and author FMH managed the experimental process, identified the species of plant. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BJMMR/2016/24741
Editor(s):
(1) Paulo Ricardo Gazzola Zen, Departament of Clinical Medicine, Federal University of Health Sciences of Porto Alegre (UFCSPA), Brazil.
Reviewers:
(1) Sylvie Hermouet, Institut de Recherche en Sante de l'Universite de Nantes, Nantes, France.
(2) Burak Uz, Gazi University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/13690

***Original Research Article***

***Received 31st January 2016***
***Accepted 2nd March 2016***
***Published 14th March 2016***

## ABSTRACT

Myeloproliferative neoplasms (MPNs) are clonal malignant diseases that represent a group of conditions including polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF). The *JAK2*-V617F mutation has been described as a frequent genetic event in majority of patients with MPNs. The aim of this study was to determine the frequency of *JAK2*-V617Fmutation in Sudanese patients with myeloproliferative disorders referred to Isotope Center, and to investigate the differences of laboratory parameters between patients with *JAK2*-V617F positive myeloproliferative neoplasms (MPNs) and *JAK2* wild type MPNs. A total of 259 patients with MPNs; 159 with polycythemia vera (PV), 55 with essential thrombocytosis (ET) and 45 with primary myelofibrosis (PMF), and11 healthy adult individuals were enrolled in this study from March 2013 to November 2015. DNA was isolated from peripheral blood leukocytes by innuPREP kit, and *JAK2*-V617F mutation gene detected by allele specific PCR. The *JAK2*-V617F mutation was

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detected in 71% (184/259) patients with MPNs. The prevalence of the mutation was 81.7% in PV, 56.4% inET, and 51.1% in PMF. Mutation was not detected in 11 healthy adult people. The presence of *JAK2*-V617F was not associated with Hb level, Hct, or the platelet count for PV and ET, whoever the mutation positively correlated with high Hb (*P*=.039), Hct (*P*=.04) in PMF patients, and with high erythrocytes count in PV. The *JAK2*-V617F mutation can be frequently detected in the Sudanese patients with MPNs.

*Keywords: JAK2-V617F mutation; Sudanese patients; chronic myeloproliferative neoplasm; polycythemia vera; essential thrombocythemia.*

*Key points: 1. JAK2-V617F was most frequent mutation detected in the classic BCR-ABL1- negative Chronic Myeloproliferative Neoplasms.*
*2. The JAK2-V617F was not detected in healthy people.*

## 1. INTRODUCTION

Myeloproliferative neoplasms (MPNs) are clonal disorders of the myeloid linage that clinically present as an excess of cells from one or more terminally differentiated myeloid compartments [1]. According to 2008 World Health Organization (WHO) classification of hematological neoplasms [2,3], MPNs are subclassified into eight separate entities; chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia, chronic eosinophilic leukemia-not otherwise specified, mastocytosis and MPN unclassifiable. Chronic myeloid leukemia (CML), PV, ET, and PMF are considered 'classic' MPNs [4]. As CML is invariably and specifically associated with *BCR-ABL1*, the other three (PV, ET and PMF) are operationally dubbed as '*BCR-ABL1*-negative MPN' [5]. The most important discovery associated with classical *BCR-ABL1*-negative MPNs was the identification of *JAK2*-V617F mutation in 2005 [6,7], the mutation results from a somatic G to T mutation involving JAK2 exon 14, which leads to nucleotide change at position 1849 and the substitution of valine to phenylalanine at codon 617. The JAK2 is a cytoplasmic, non receptor, tyrosine kinase that via its association with cytokine receptors serves as a signaling mediator for hematopoietic cytokines such as erythropoietin (Epo), and thrombopoietin (Tpo) to regulate cell proliferation and growth [8]. V617F mutation affects the noncatalytic 'pseudo-kinase' domain (JH2) of *JAK2* and disrupts its autoinhibitory function, resulting in constitutive activation of JAK2-mediated signaling pathways, leading to growth factor independent autonomous proliferation of hematopoietic precursors [8,9]. *JAK2*-V617F was found in the majority of patients with PV and approximately half of the patients with ET andPMF [6,7,10]. Several studies revealed association of the *JAK2*-V617F mutation with clinical and hematological characteristics in MPNs. The aim of our study was to determine the frequency *JAK2*-V617F mutation, and to analyze the association of mutated *JAK2* gene with laboratory characteristics among Sudanese MPNs patient. We used allele specific polymerase chain reaction (PCR) for the detection of *JAK2*-V617F mutation.

## 2. MATERIALS AND METHODS

### 2.1 Study Population (Patients)

Over a period of about 2 years (2013-2015), a total of 259 patients with chronic MPNs; 159 with PV, 55 with ET and 45 with PMF, and 11 healthy adult individuals were randomly enrolled. Hematologic data obtained from patient records and hematological diagnoses and subtyping results were reconfirmed according to the 2008 WHO criteria.

### 2.2 Blood Sampling and DNA Extraction

5 ml of peripheral blood from each subject was collected in EDTA K3 containing vacutainer tube. Genomic DNA extraction from peripheral blood leukocytes was carried out following the protocol of innuPREP kit (Analytik Jena/Germany), then it was stored at –20℃ until the PCR was performed.

### 2.3 Analysis of the *JAK2*-V617F Mutation

Mutation analysis of *JAK2*-V617F was performed using allele-specific PCR. Amplifications were done in a total volume of 25 µL PCR mix containing 12.5 µL of TaqMan Universal Master Mix, 5 µL of nuclease-free PCR grade water, 2.5 µL of primers and probes mix and 50 ng/µL of DNA template. PCR primers were; forward wild-type 5'-TCC TCA GAA CGT TGA TGG CAG-3' and reverse wild-type 5'-ATT GCT TTC CTT TTT CAC AAG AT-3', forward mutant 5'-GCA TTT GGT TTT AAA TTA TGG AGT ATA TG-3' and a reverse mutant 5'-GTT TTA CTT ACT CTC GTC

TCC ACA AAA-3'. The PCR conditions used were denaturation at 95℃ for 1 min, annealing at 58℃ for 40 s, and extension at 72℃ for 45 s. Products were electrophoresed on agarose gels and visualized using ethidium bromide staining.

### 2.4 Statistical Analysis

Statistical analyses were performed using the SPSS version 11.2.

## 3. RESULTS

The *JAK2*-V617F mutation was detected in 71% (184/259) patients with MPNs. The prevalence of the mutation in subtypes were 81.7% in PV, 56.4% inET, and 51.1% in PMF. *JAK2*-V617F was not detected in 11 healthy adult people. At diagnosis, the presence of *JAK2*-V617F was found to be significantly associated with high Hb (*P*=.039) and Hct (*P*=.04) in PMF patients. *JAK2*-V617F positive ET patients were older than ET patients without the mutation (*P*=.033). But no significant correlations were detected between *JAK2*-V617F mutation and white blood cell count among the three groups. No significant differences in Hb, Hct, or platelet count were observed between *JAK2*-V617F positive and *JAK2*-V617F negative PV and ET patient. These results are summarized in (Tables 1 and 2).

## 4. DISCUSSION

A somatic single point mutation (V617F) in the *JAK2*gene is the most commonly described mutation associated with Philadelphia-negative MPNs; it was detected in >70% of MPNs [6,11,12,13]. The frequency of the *JAK2*-V617F mutation varied between the MPNs subtypes, it can be found in approximately 95% cases of PV and in approximately 50–60% cases of ET or PMF [6,10]. In numerous published studies, the *JAK2*-V617F mutation was detected in 80% - 97.0% in PV [13,14,15,16,17]. In agreement with these reports, we have detected the *JAK2*-V617F mutation in 71% (184/259) of patients with MPNs. The prevalence of the mutation was 81.7% in PV, 56.4% in ET, and 51.1% in PMF. Our results were similar to the results obtained by Baxter et al. [6]; they were detected the *JAK2* mutation in 29 (57%) of 51 with ET, eight (50%) of 16 with idiopathic myelofibrosis, and 71 (97%) of 73 patients with PV. Similarly, Ayad et al. [16] detected the *JAK2*-V617F mutation in 81.4% of PV, 50% of ET, 46.1% of PMF. The study by Karkucak et al. [18], was in line with our study. They found that 80% of the PV group and 42% of the ET group were positive for the *JAK2*-V617F mutation. Also our study was consistent with the study by Zhu et al. [13]; who detected the mutation in 83.9%, 60% in PV and ET, respectively. In numerous studies, the *JAK2*-V617F mutation has been variably associated with higher Hb, WBC, older age and unchanged or decreased platelet count [19,20,21,22]. Another study by Campbell et al. [23] showed that patients with the mutation present with higher hemoglobin levels, higher white cell counts in PV. In the study of Singh et al. [24], it has been showed that *JAK2*-V617F negative patients with PMF were significantly associated with more severe anemia, younger age and thrombocytopenia. Several studies [25,26] revealed positive correlation with the *JAK2*-V617Fmutation and higher Hb, WBC, older age and lowest platelet countin patients with ET. In contrast other studies [27,28] showed no significant correlations between V617F mutation and patient age, white blood cell count, Hb, Hct, or the platelet count for ET or PMF patients. In our study, we have observed significant association between older patients with *JAK2*-V617F positive ET, with high Hb (*P*=.039) and Hct (*P*=.04) in PMF patients, and high erythrocyte count in PV patients. In contrast, no significant differences in Hb, Hct, or the platelet count between V617F positive and V617F negative patients for PV and ET were detected. Overall our study of the *JAK2*-V617F status of Sudanese MPN patients is consistent with the above studies of MPN patients in Europe, the USA and Asia.

**Table 1. Hematologic parameters according to myeloproliferative neoplasm (MPN) subtypes**

| Characteristics | PV | ET | PMF | Total |
|---|---|---|---|---|
| No of patients | 159 | 55 | 45 | 259 |
| Age at diagnosis (yr,mean±SD) | 49.91±15.10 | 49.30±14. 46 | 57.00±16.64 | 52.07±15.40 |
| WBC (×$10^9$/L, mean±SD) | 9.23±5.79 | 15.58±12.30 | 12.05±10.78 | 11.0 7±8.87 |
| RBC (×$10^9$/L, mean±SD) | 7.03±4.04 | 4.68±1.61 | 4.01±1.41 | 6.01±3.5 6 |
| Hb (g/dL, mean±SD) | 17.16±1.93 | 11.18±2.56 | 9.89±2.59 | 14.63±3.90 |
| Hct (%, mean±SD) | 50.78±6.77 | 33.48±7.70 | 29.28±7.59 | 4 3.38±11.82 |
| Platelet (×$10^9$/L, mean±SD) | 421.91±270.52 | 1121.98±359.86 | 242.20±20 6.27 | 539.35±418.86 |
| NO of *JAK2*-V617F positive (%) | 130(81.7%) | 31(56.4%) | 23(51.1%) | 184(71%) |

*Abbreviation: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit*

**Table 2. Hematologic parameters associated with the JAK2 V617F mutation status**

| Characteristics | PV | | | ET | | | PMF | | |
|---|---|---|---|---|---|---|---|---|---|
| | JAK2 WT | JAK2 V617F | P | JAK2 WT | JAK2 V617F | P | JAK2 WT | JAK2 V617F | P |
| No of patients | 29 | 130 | | 24 | 31 | | 22 | 23 | |
| Age at diagnosis (yr, mean±SD) | 45.40±14.20 | 50.83±15.16 | .089 | 44.62±14.31 | 52.93±13.72 | .033* | 54.31±16.55 | 59.56±16.68 | .296 |
| WBC ($\times10^9$/L, mean±SD) | 9.01±5.27 | 9.27±5.91 | .830 | 13.24±12.05 | 17.39±12.38 | .218 | 11.20±10.30 | 12.95±11.43 | .592 |
| RBC ($\times10^9$/L, mean±SD) | 6.73±1.23 | 8.49±9.45 | .040* | 4.77±1.44 | 4.616±1.75 | .719 | 3.64±1.19 | 4.40±1.55 | .075 |
| Hb (g/dL, mean±SD) | 17.46±1.70 | 17.10±1.97 | .383 | 11.14±2.82 | 11.24±2.24 | .891 | 9.11±2.02 | 10.70±2.90 | .039* |
| Hct (%, mean±SD) | 50.51±7.10 | 52.09±4.77 | .272 | 33.43±8.48 | 33.55±6.74 | .957 | 27.02±5.88 | 31.65±8.55 | .040* |
| Platelet ($\times10^9$/L, mean±SD) | 432.13±285.74 | 371.92±173.82 | .293 | 1161.62±394.67 | 1091.29±333.84 | .477 | 272.04±222.64 | 213.65±189.83 | .348 |

*Abbreviation: WT, wild type; P*, significant*

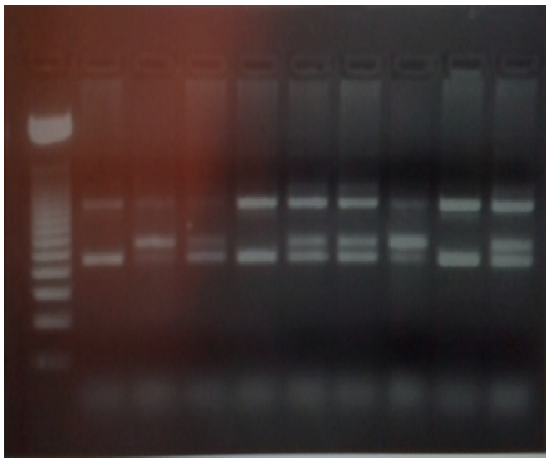

**Fig. 1. Detection of *JAK2*-V617F by allele specific PCR. Products were electrophoresed on agarose gels and visualized using ethidium bromide staining. Expected products are 453bp (for both *JAK2*-V617F-positive and *JAK2*-V617F-negative), 279bp (for *JAK2*-V617F-positive) and 229bp (*JAK2*-V617F-negative)**

## 5. CONCLUSION

The *JAK2*-V617F mutation can be frequently detected in the Sudanese patients with MPNs, and our study is consistent with previous published studies of MPN patients in Europe, the USA and Asia.

## CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this paper.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Edward P, Charles L, Frank J. Molecular Oncology: Causes of cancer and targets for treatment. Cambridge University Press; 2014. P818.ISBN978-0-521-87662-9.
2. Amy V, Nicholas C. Inherited predisposition to myeloproliferative neoplasms. Ther Adv Hematol. 2013;4(4):237-253. DOI: 10.1177/2040620713489144 PMCID: PMC3734902.
3. Dan J, Cameron Y. Neoplastic hematopathology: Experimental and Clinical Approches. Humana press; 2010. ISBN978-1-60761-383-1.e ISBN978-1-60761-384-8.

   DOI: 10.1007/978-160761-384-8
4. Dameshek W. Some speculations on the myeloproliferative syndromes. Blood. 1951;6:372-5.
5. Tefferi A, Vardiman J. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia. 2008;22(1):14-22. DOI: 10.1038/sj.leu.2404955
6. Baxter E, Scott L, Campbell P, East C, Fourouclas N, Swanton S. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365:1054-1061.
7. Kralovics R, Passamonti F, Buser A, Teo S, Tiedt R, Passweg J. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779-1790.
8. Ross L. Non CML myeloproliferative neoplasms. Hematology/Oncology Clinics of North America. 2012;26(5).
9. James C, Ugo V, Le Couedic J, Staerk J, Delhommeau F, Lacout C. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemiavera. Nature. 2005;434.
10. Shirane S, Araki M, Morishita S, Edahiro Y, Takei H, Yoo Y et. JAK2, CALR, and MPL mutation spectrum in Japanese patients with myeloproliferative neoplasms. Haematologica. 2015;100(2):e46-8.

    [PubMed] [Ref list].
11. Duletic N, Dekanic A, Hadzisejdic I, Kusen I, Matusanllijas K, Grohovac D, et al. JAK2-v617F mutation is associated with clinical and laboratory features of myeloproliferative neoplasms. Coll Antropol. 2012;36(3):859-65.

    PMID: 23213945 [PubMed - indexed for MEDLINE].

12. Ha S, Kim K, Jung I, Jung R, Chung S. Correlations between Janus Kinase 2 V617F allele burdens and clinicohematologic parameters in myeloproliferative neoplasms. Annals of Laboratory Medicine. 2012;32(6):385-391. DOI: 10.3343/alm.2012.32.6.385.

13. Zhu JF, Liu Y, Liu P, Jia MF, Cheng J, Zhao L, et al. JAK2V617F mutation in the patients with myeloproliferative disorder and its relation with clinical characteristics. 2011;19(4):916-20.

14. Jones A. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005;106:2162–2168. [Pubmed].

15. Ebid GT, Ghareeb M, Salaheldin O, Kamel MM. Prevalence of the frequency of JAK2 (V617F) mutation in different myeloproliferative disorders in Egyptian patients International Journal of Clinical and Experimental Pathology. 2015;8(9): 11555-11559.

16. Ayad MW, Nafea D. Acquired mutation of the tyrosine kinase JAK2V617F in Egyptian patients with myeloid disorders. Genet Test Mol Biomarkers. 2011;15(1-2):17-21.

DOI: 10.1089/gtmb.2010.0093. Epub 2010 Oct 29.

17. Speletas M, Katodritou E, Daiou C, Mandala E, Papadakis E, Kioumi A, et al. Correlations of JAK2-V617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leuk Res. 2007;31(8):1053-62. Epub 2006 Oct 12.

18. Karkucak M, Yakut T, Ozkocaman V, Ozkalemkas F, Ali R, Bayram M, et al. Evaluation of the JAK2-V617F gene mutation in Turkish patients with essential thrombocythemia and polycythemia vera. MolBiol Rep. 2012;39(9):8663-7.

DOI: 10.1007/s11033-012-1721-x.

Epub 2012 Jun 22.

19. Yonal I, Daglar A, Akadam B, Ceylan Y, Nalcacı M, Yavuz AS, et al. Impact of JAK2V617F mutational status on phenotypic features in essential thrombocythemia and primary myelofibrosis. Turk J Haematol. 2015;27. Epub 2015 Apr 27.

20. Kittur J, Knudson RA, Lasho TL, Finke CM, Gangat N, Wolanskyj AP, et al. Clinical correlates of JAK2V617F allele burden in essential thrombocythemia. Cancer. 2007;1,109(11):2279-84.

21. Benmoussa A, Dehbi H, Fehri S, Quessar A, Nadifi S. JAK2-V617F mutation in Moroccan patients with myeloproliferative disorders: Contribution, diagnosis and therapeutic prospects. Pathol Biol (Paris). 2011;59(4):e89-92.

DOI: 10.1016/j.patbio.2009.06.005. Epub 2009 Nov 24.

22. Speletas M, Katodritou E, Daiou C, Mandala E, Papadakis E, Kioumi A, et al. Correlations of JAK2-V617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leuk Res. 2007;31(8):1053-62. Epub 2006 Oct 12.

23. Campbell J, Scott M, Buck G, Wheatley K, East L, Marsden T, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemiavera based on JAK2 V617F mutation status: A prospective study. Lancet. 2005;366:1945-1953. PMID:16325696 [PubMed - indexed for MEDLINE].

24. Singh N, Sazawal S, Upadhyay A, Chhikara S, Mahapatra M, Saxena R. Correlation of JAK2V617F mutational status in primary myelofibrosis with clinico-hematologic characteristics and international prognostic scoring system scoring: A single center experience. Indian J Pathol Microbiol. 2015;58(2):187-91.

DOI: 10.4103/0377-4929.155311

25. Ipek H, Aynur A, Basak T, Ceylan Y, Meliha N, Akif Y, et al. The burden of JAK2V617F mutated allele in Turkish patients with Myeloproliferative Neoplasms. J Clin Med Res. 2015;7(3):161-170.
DOI: 10.14740/jocmr2047w.

26. Rudzki Z, Sacha T, Stój A, Czekalska S, Wójcik M, Skotnicki AB, et al. The gain-of-function JAK2 V617F mutation shifts the phenotype of essential thrombocythemia and chronic idiopathic myelofibrosis to more "erythremic" and less "thrombocythemic": A molecular, histologic, and clinical study. Int J Hematol. 2007;86(2):130-6.

27. Ha JS, Kim YK, Jung SI, Jung HR, Chung IS. Correlations between Janus kinase 2 V617F allele burdens and

clinicohematologic parameters in myeloproliferative neoplasms. Ann Lab Med. 2012;32(6):385-91.
DOI: 10.3343/alm.2012.32.6.385.
Epub 2012 Oct 17.

28. Sultan S, Irfan SM. Acquired JAK-2 V617F mutational analysis in Pakistani patients with essential thrombocythemia. Asian Pac J Cancer Prev. 2015;16(16):7327-30.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://sciencedomain.org/review-history/13690*

# Correlations of JAK2V617F Mutational status with Clinicohematologic Characteristics in Sudanese Patients with Primary Myelofibrosis

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**Abstract:** Primary myelofibrosis (PMF) is a *BCR*/*ABL*-negative myeloproliferative neoplasm (MPNs) characterized by dysregulated kinase signaling and release of abnormal cytokines. About 50-60% of PMF patients have been reported to have acquired somatic mutation in Janus kinase 2 gene (*JAK*-V617F mutation). The aim of this study was to fine out correlation between the *JAK2*-V617F mutational status and the clinicohematologic characteristics in Sudanese patients with PMF. 45 patients with PMF were involved in this study. Allele specific PCR was used to detected the *JAK2*-V617F, and quantitative real-time polymerase chain reaction (qRT-PCR) was used to determinate the percentage of mutated alleles in genomic DNA among *JAK2*-V617F positive MPNs. The *JAK2*-V617F mutation was detected in 51.1%. The mean allele burden of *JAK2*-V617F for positive patients was 69.3%. The prevalence of patients with high allele burden (i.e. *JAK2*-V617F allele burden exceeded 50%) was 31.1%. *JAK2*-V617F mutation associated with high Hb (P=.039), Hct (P=.04) in PMF patients. *JAK2* V617F allele burden was correlate with lower platelet count in (*P=.015)*.

[Abkar HM, Hassan FM, Mohamed BA. **Correlations of JAK2V617F Mutational status with Clinicohematologic Characteristics in Sudanese Patients with Primary Myelofibrosis.** *N Y Sci J* 2016;9(3):79-82]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 14. doi:10.7537/marsnys09031614.

**Keywords:** *JAK2*-V617F mutation; Primary myelofibrosis; Myeloproliferative neoplasms; JAK2V617F allele burden

## 1. Introduction

Primary myelofibrosis (PMF) is a clonal stem cell disorder characterized by chronic myeloproliferation, atypical megakaryocytic hyperplasia, and bone marrow fibrosis. The disorder manifests clinically as anemia, splenomegaly due to extramedullary hematopoiesis (EMH), leukoerythroblastosis, and constitutional symptoms (Tefferi, 2014). Along with polycythemia vera (PV) and essential thrombocythemia (ET), PMF considered as classic Philadelphia (Ph) negative MPNs (Amy and Nicholas, 2013) (Dan and Cameron, 2010). approximately half of the patients with PMF have been reported to have an acquired somatic mutation, results from G to T mutation involving JAK2 exon 14, which leads to nucleotide change at position 1849 and the substitution of valine to phenylalanine at codon 617 (Baxter et al., 2005) (Kralovics et al., 2005) (Shirane et al., 2015).

The JAK2 is a cytoplasmic, non receptor, tyrosine kinase that via its association with cytokine receptors serves as a signaling mediator for hematopoietic cytokines such as erythropoietin (Epo), and thrombopoietin (Tpo) to regulate cell proliferation and growth (Ross, 2012). *JAK2*-V617F mutation affects the noncatalytic 'pseudo-kinase' domain (JH2) of JAK2 and disrupts its autoinhibitory function, resulting in constitutive activation of JAK2- mediated signaling pathways, leading to growth factor independent autonomous proliferation of hematopoietic precursors (Ross, 2012) (James et al 2005).

Correlation of *JAK2*-V617F mutation with clinical and hematological characteristic in MPNs haves demonstrated in several studies and reported variable results. In this current study we aimed to determine the relationship between JAK2 V617F mutational statuses, JAK2 V617F allele burden with clinicohematologic characteristics in Sudanese patients with PMF.

## 2. Material and Methods

**Study population (patients):** This cross sectional study, extended from 2013 to 2015. Forty-five patients diagnosed to have PMF according to the World Health Organization (WHO) criteria (James et al, 2009) were enrolled. Hematologic data obtained from patient's records.

**Blood sampling and DNA extraction:** 5 ml of peripheral blood from each subject was collected in EDTA K3 tube. Genomic DNA extraction from peripheral blood leukocytes was carried out following the protocol of innuPREP kit (Analytik Jena/Germany).

**Analysis of the *JAK2* V617F mutation**: The presence of JAK2-V617F mutation was assessed in 45 patients with PMF by Allele specific PCR. Amplifications were done in a total volume of 25 µL PCR mix containing 12.5 µL of TaqMan Universal Master Mix, 5 µL of nuclease-free PCR grade water, 2.5 µL of primers and probes mix and 50 ng/µL of DNA template. PCR conditions used were denaturation at 95℃ for 1 min, annealing at 58℃ for 40 s, and extension at 72℃ for 45 s. Products were electrophoresed on agarose gels and visualized using ethidium bromide staining. 23 PMF patients positive for *JAK2*-V617F mutation were analyzed for *JAK2*-V617F mutational load by using *JAK2* Muta*Quant*™ (Ipsogen Inc., New Haven, CT). qPCR reactions were performed in a final volume of 25 µl mix which containing 12.5 µL of TaqMan Universal PCR master mix, 1 µL of IPSOGEN PPM- VF or wild type primers and probe mix, and 6.5 µL of nuclease-free water, and 5 µL genomic DNA was used per well. The RQ-PCR conditions used were as follows: 50℃ for 2 min, heating at 95℃ for 10 min, and 50 cycles of 95℃ for 15 s and 63℃ for 90 s. The RQ-PCR was performed using a Rotor-Gene Q 5plex HRM instruments. Standard curves for both V617F and wild type were constructed using either a V617F or a wild-type plasmid of known value, provided by the manufacturer. The equation was calculated for each curve, and these equations were used to calculate the copy number of V617F and wild-type alleles in unknown samples. The allele burden of *JAK2* V617F is expressed as the percentage of V617F copies compared with the sum of V617F and wild-type copies.

**Statistical analysis**: Statistical analyses were performed using the SPSS version 11.2

## 3. Results

Forty-five PMF patients were enrolled in this study. At diagnosis the mean age was 57.00 years, mean hemoglobin 9.89 g/dL, RBC $4.01\times10^9$/L, WBC$12.05\times10^9$/L and mean platelets count was $242.20\times10^9$/L.

Out of all patients, 23(51.1%) patient were JAK2V617F-positive, while 22(48.9%) were wild-type. The median allele burden of *JAK2*-V617F for positive patients was 69.3%. Patients divided into homozygous (had allele burden exceeded 50%) and heterozygous in which mutational load equal or less than 50%. Of 23 patients who were positive for *JAK2*-V617F mutation, 14 patients were homozygous 60.9% correspond 31.1% of whole patients.

The presence of *JAK2*-V617F mutation significantly associated with high Hb (P=.039) and Hct (P=.04). There were no significant differences in median age at presentation, total leukocyte count or erythrocyte count between JAK2V617F-positive and JAK2V617F-negative PMF patients (Table 1).

Homozygous patients had lowest platelets count compare with heterozygous *PMF patients* (*P=.015)*. There were no significant differences in mean age, WBC or Hb between Homozygous and heterozygous *PMF patients (Table 2*).

Table 1. Hematologic characteristics associated with the JAK2 V617F mutation status in PMF patients. P*, significant

| Characteristics | Total | JAK2 Wild-type | JAK2- V617F | P |
|---|---|---|---|---|
| No of patients | 45 | 22(48.9%) | 23(51.1%) | |
| Age at diagnosis (yr, mean±SD) | 57.00±16.64 | 54.31±16.55 | 59.56±16.68 | .296 |
| WBC ($\times10^9$/L, mean±SD) | 12.05±10.78 | 11.20±10.30 | 12.95±11.43 | .592 |
| RBC ($\times10^9$/L, mean±SD) | 4.01±1.41 | 3.64±1.19 | 4.40±1.55 | .075 |
| Hb (g/dL, mean±SD) | 9.89±2.59 | 9.11±2.02 | 10.70±2.90 | .039* |
| Hct (%, mean±SD) | 29.28±7.59 | 27.02±5.88 | 31.65±8.55 | .040* |
| Platelet ($\times10^9$/L, mean±SD) | 242.20±206.27 | 272.04±222.64 | 213.65±189.83 | .348 |

Table 2. Associations of hematologic parameters with the JAK2 V617F allele burden in PMF patients

| Characteristics | JAK2V617F- heterozygous | JAK2V617F- homozygous | P |
|---|---|---|---|
| No of patients | 9(39.1%) | 14(60.9%) | |
| Age at diagnosis(yr,mean±SD) | 58.44±14.49 | 60.28±18.44 | .803 |
| WBC ($\times10^9$/L, mean±SD) | 9.87±9.91 | 13.25±11.16 | .456 |
| RBC ($\times10^9$/L, mean±SD) | 3.30±0.84 | 4.19±1.50 | .082 |
| Hb (g/dL, mean±SD) | 8.75±2.24 | 9.68±1.56 | .288 |
| Hct (%, mean±SD) | 26.06±6.64 | 28.52±4.40 | .340 |
| Platelet ($\times10^9$/L, mean±SD) | 329.55±184.36 | 139.14±157.44 | .015* |

## 4. Discussions

The *JAK2* V617F acquired somatic mutation is the most commonly described mutation associated with MPN. In PMF patients it have been detected in range (50-60%) (Levine et al, 2205) (Sultan and Irfan, 2015)]. Prevalence of JAK2V617F mutation in PMF patients in our study was 51.1%, similar to the reported data worldwide. The median allele burden of *JAK2*-V617F for positive patients was 69.3%, similar to findings by *Larsen TS* et al (Larsen et al., 2007) and Sang P et al (Sang et al., 2013). In our study, the homozygosity among JAK2V617F positive PMF patients was 60.9% correspond 31.1% of whole patients. Similarly these result were reported in numerous published studies (Singh et al., 2015) (Ipek et al., 2015) (Kralovics et al., 2005) (Yonal et al., 2015).

In numerous studies, the JAK2V617F mutation has been variably associated with higher indices of erythropoiesis, unchanged or decreased platelet count, increased bone marrow fibrosis, older age, and longer duration of disease (Kralovics et al.,2005) (Rudzki et al., 2007) (Speletas et al.,2007). The studies by Yonal I et al (Yonal et al., 2015), Benmoussa A et al (Benmoussa et al., 2011) and Ayad W et al (Ayad and Nafea, 2010) showed association between JAK2-V617F mutation and higher levels of haemoglobin and hematocrit in PMF patients. Similarly we have observed significant differences in Hb and Hct between JAK2V617F-positive and JAK2V617F-negative PMF patients. Also we observed no significant differences in mean age, WBCs, RBCs or platelets between mutated and un mutated patients, these results were consistent with the observations reported by Ha S et al (Ha et al., 2012), Sultan S et al (Sultan and Irfan, 2015) and Campbell J et al (Campbell et al., 2005).

Correlation analysis in our study showed association between JAK2V617F homozygous genotype and low platelets count (*P=.015)*, this observation was comparable with results in several studies [Yonal et al., 2015) (Rudzki et al., 2007). In contrast several studies [(Sultan and Irfan, 2015) (Ha et al., 2012), showed no correlations between JAK2V617F allele burden and mean age, WBCs, Hb, or Hct in PMF mutated patients. Similarly we observed on significant differences in Hb, WBCs, or mean age between homozygous genotype and heterozygous genotype groups. Over all the findings in our study were comparable with to the reported data worldwide.

**Acknowledgements:**

Authors are grateful to the Department of Hematology al isotope centre hospital-Sudan (Dr. ihsan, Dr. Dalal G sid) for helping us in the data collection.

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## References

1. Tefferi A. Primary myelofibrosis: 2014 update on diagnosis, risk-stratification, and management. Am J Hematol 2014 Sep; 89(9):915-25. doi: 10.1002/ajh.23703. PMID:25124313.
2. Amy V, Nicholas C. Inherited predisposition to myeloproliferative neoplasms. Ther Adv Hematol, 2013, 4(4) 237–253. doi:10.1177/2040620713489144 PMCID: PMC3734902.
3. Dan J, Cameron Y. Neoplastic hematopathology: Experimental and Clinical Approches. Humana press 2010, ISBN978-1-60761-383-1.e ISBN978-1-60761-384-8. doi:10.1007/978-160761-384-8.
4. Baxter E, Scott L, Campbell P, East C, Fourouclas N, Swanton S. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet, 2005, 365: 1054–1061.
5. Kralovics R, Passamonti F, Buser A, Teo S, Tiedt R, Passweg J. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med, 2005, 352: 1779–1790.
6. Shirane S, Araki M, Morishita S, Edahiro Y, Takei H, Yoo Y et. JAK2, CALR, and MPL mutation spectrum in Japanese patients with myeloproliferative neoplasms. Haematologica. 2015 Feb; 100(2):e46-8.[PubMed] [Ref list].
7. Ross L. Non CML myeloproliferative neoplasms. Hematology/oncology clinics of North America. October 2012. Volume 26. Number 5.
8. James C, Ugo V, Le Couedic J, Staerk J, Delhommeau F, Lacout C. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemiavera. Nature, 2005, 434.
9. James V, Thiele J, Daniel A, Richard D, Michael J, Anna P et al. The 2008 revision of theWorld Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. BLOOD, 30 JULY 2009 _ VOLUME 114, NUMBER 5

10. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7:387–397
11. Sultan S, Irfan SM. JAK-2 V617F Mutational Analysis in Primary Idiopathic Myelofibrosis: Experience from Southern Pakistan. Asian Pac J Cancer Prev. 2015;16(17):7889-92.
12. Larsen TS, Pallisgaard N, Møller MB, Hasselbalch HC. The JAK2 V617F allele burden in essential thrombocythemia, polycythemia vera and primary myelofibrosis--impact on disease phenotype. Eur J Haematol. 2007 Dec; 79(6):508-15. Epub 2007 Oct 23.
13. Sang H, Hyun Chi, Chan P. The allele burden of JAK2 V617F can aid in differential diagnosis of Philadelphia Chromosome-Negative Myeloproliferative Neoplasm. Blood Res. 2013 Jun; 48(2): 128–132.
14. Singh N, Sazawal S, Upadhyay A, Chhikara S, Mahapatra M, Saxena R. Correlation of JAK2V617F mutational status in primary myelofibrosis with clinico-hematologic characteristics and international prognostic scoring system scoring: a single center experience. Indian J Pathol Microbiol. 2015 Apr-Jun;58(2):187-91. doi:10.4103/0377-4929.155311.
15. Ipek Y, Aynur D, Basak A, Ceylan Y, Meliha N, Akif S et al. The Burden of JAK2V617F Mutated Allele in Turkish Patients with Myeloproliferative Neoplasms. Med Res. 2015 March; 7(3).
16. Yonal I, Daglar A, Akadam B, Ceylan Y, Nalcacı M, Yavuz AS et al. Impact of JAK2V617F Mutational Status on Phenotypic Features in Essential Thrombocythemia and Primary Myelofibrosis. Turk J Haematol. 2015 Apr 27; Epub 2015 Apr 27.
17. Rudzki Z, Sacha T, Stój A, Czekalska S, Wójcik M, Skotnicki AB, et al. The gain-of-function JAK2 V617F mutation shifts the phenotype of essential thrombocythemia and chronic idiopathic myelofibrosis to more "erythremic" and less "thrombocythemic": A molecular, histologic, and clinical study. Int J Hematol 2007;86:130-6.
18. Speletas M, Katodritou E, Daiou C, Mandala E, Papadakis E, Kioumi A et al. Correlations of JAK2-V617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leuk Res. 2007 Aug; 31(8):1053-62. Epub 2006 Oct 12.
19. Benmoussa A, Dehbi H, Fehri S, Quessar A, Nadifi S. JAK2-V617F mutation in Moroccan patients with myeloproliferative disorders: contribution, diagnosis and therapeutic prospects. Pathol Biol 2011 Aug;59(4):e89-92. doi:10.1016/j.patbio.2009.06.005. Epub 2009 Nov 24.
20. Ayad MW, Nafea D. Acquired mutation of the tyrosine kinase JAK2V617F in Egyptian patients with myeloid disorders. Genet Test Mol Biomarkers. 2011 Jan-Feb;15(1-2):17-21. doi: 10.1089/gtmb.2010.0093. Epub 2010 Oct 29.
21. Ha JS, Kim YK, Jung SI, Jung HR, Chung IS. Correlations between Janus kinase 2 V617F allele burdens and clinicohematologic parameters in myeloproliferative neoplasms. Ann Lab Med. 2012 Nov;32(6):385-91. doi:10.3343/alm.2012.32.6.385. Epub 2012 Oct 17.
22. Campbell PJ, Griesshammer M, Döhner K, Döhner H, Kusec R et al. V617F mutation in JAK2 is associated with poorer survival in idiopathic myelofibrosis. Blood. 2006 Mar 1;107(5):2098-100. Epub 2005 Nov 17.

3/13/2016