CLINICAL CHARACTERISTICS AND ITS ASSOCIATION WITH DRIVER GENE MUTATIONS (*JAK2* V617F, *CALR*, AND *MPL*) IN MYELOPROLIFERATIVE NEOPLASMS

DR RAZAN HAYATI ZULKEFLEE

DISSERTATION SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PATHOLOGY (HAEMATOLOGY)

UNIVERSITI SAINS MALAYSIA

2022

CLINICAL CHARACTERISTICS AND ITS ASSOCIATION WITH DRIVER GENE MUTATIONS (*JAK2* V617F, *CALR*, AND *MPL*) IN MYELOPROLIFERATIVE NEOPLASMS

DR RAZAN HAYATI ZULKEFLEE

DISSERTATION SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PATHOLOGY (HAEMATOLOGY)

UNIVERSITI SAINS MALAYSIA

2022

ACKNOWLEDGEMENTS

In the name of ALLAH, the Most Gracious, the Most Merciful,

First and foremost, Alhamdulillah, all praise and prayers to Allah S.W.T, gave me blessing, strength, health, and patience in completing this journey. It has been an intense experience for me, and it would not be possible without the support and guidance from many special people.

Foremost, I would like to express my utmost sincere gratitude to my supervisor, Dr. Zefarina Zulkafli. I was fortunate to have a mentor who cared so much about the progress of my study. Similarly, I would like to extend a profound thank you to my co-supervisors, Associate Prof Dr Muhammad Farid Johan, Associate Prof Dr Azlan Husin, Dr Md Asiful Islam and special thanks to Dr Anis Kausar Ghazali from Biostatistics and Research Methodology for the guidance and direction given throughout the period making this study a reality.

I am utterly grateful especially to my amazing parents Dr Zulkeflee Ismail and Dr Rosline Hassan. They have raised me and lending their hands in whatever way they could during this demanding period. To my husband Dr Muhammad Amiro Rasheeq Mohd Radzi, who has been by my side, believing me and let me fly my wings, thank you so much. To my baby girl, Maryam, and more to come, you are the reasons behind my every dream. With boundless love and gratitude, thank you for being so understanding and wonderful.

I am thankful to my faithful and hardworking colleagues who have supported me throughout this journey. We've gone through good times and hard times together. I would like to also convey my appreciation to these following peoples, for their contribution: 1) All the lecturers in the Hematology Department of Hospital USM 2) All staff in the hematology lab of Hospital USM, especially to Puan Nurul Ameera Samat

I only ask that ALLAH bless all of you with His incredible goodness in ways that only He can.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION AND SYMBOLS

AKT	Protein kinase B
BCR-ABL1	Ca2+ binding sites and devoid of a KDEL retention
BM	Bone Marrow
Ca+	Calcium
CALR	Calreticulin
CSF	Colony stimulating factor
DNA	Deoxyribonucleic acid
ERK	Extracellular signal regulated kinase
ET	Essential thrombocythemia
Hb	Hemoglobin
HCT	Hematocrit
HUSM	Hospital Universiti Sains Malaysia
IL-3	Interleukin 3
JAK2	Janus Kinase 2
JAK2 V617F	Janus Kinase 2 V617F
KDEL	Amino acid sequence
KDEL ER	Amino acid sequence receptor
LDH	Lactate dehydrogenase
MPL	Myeloproliferative Leukemia gene
MPNs	Myeloproliferative neoplasms

NOS	Not otherwise specified
PCR	Polymerase chain reaction
Plt	Platelet
PMF	transcription (STAT) protein
PV	Polycythemia vera
qPCR	Quantitative polymerase chain reaction
SCF	Stem cell factor
STAT	Signal transducer and activator of transcription
STAT3	Signal transducer and activator of transcription 3
STAT5	Signal transducer and activator of transcription 5
TN MPNS	Triple negative myeloproliferative neoplasm
TPO	Thrombopoietin
TPO R	Thrombopoietin receptor
WBC	White blood cell

ABSTRAK

Pengenalan: Mutasi *JAK2* V617F, *CALR* dan *MPL* mengesahkan diagnosis kanser neoplasma mieloproliferatif (MPNS). Kajian ini bertujuan mengetahui profil mutasi genetik *JAK2* V617F, *CALR* ekson 9 Jenis 1 (52 bp *deletion*) dan Jenis 2 (5 bp *insertion*), dan *MPL* W515 L / K dalam kalangan pesakit MPN di Malaysia dan menghubungkaitkan mutasi ini dengan parameter klinikal dan hematologi.

Kaedah: Mutasi *JAK2* V617F, *CALR*, dan *MPL* dianalisis kepada 159 orang pesakit MPNS di Malaysia dengan menggunakan reaksi rantai polimerase spesifik alel, termasuk 76 polisetemia vera (PV), 41 trombositemia esensial (ET), 42 mutasi mielofibrosis primer (PMF), dan demografi serta parameter klinikal dan heamtologi di dapatkan.

Keputusan: Hasil kajian menunjukkan daripada jumplah keseluruhan kes MPN 73.6% mempunyai *JAK2* V617F, 5.66% mempunyai *CALR*, dan 27.7% tidak mengandungi sebarang mutasi. Mutasi *MPL* W515L / K tidak dapat dikesan dalam kalangan pesakit MPN. Dalam ET dan PMF, jenis kepradominanan ialah mutasi *CALR* Jenis 1. Serum LDH mempunyai tahap yang lebih tinggi kepada PMF berbanding dengan PV dan ET. PV mempunyai risiko yang lebih tinggi untuk berkembang menjadi pasca-mielofibrosis PV berbanding dengan ET. Pembentukan trombotik pada awal diagnosis 40.9% adalah tinggi berbanding dengan peratusan global. Hanya seorang pesakit PMF mengalami mutasi *CALR* yang bertukar menjadi leukemia mieloid akut.

Kesimpulan: Mutasi *JAK2* V617F dan *CALR* memainkan peranan yang penting dalam ujian diagnostik. Oleh itu, setiap pesakit yang disyaki mempunyai penyakit MPN harus diperiksa untuk mengenal pasti mutasi ini.

ABSTRACT

Background: Mutations of *JAK2*V617F, *CALR*, and *MPL* genes confirm the diagnosis of myeloproliferative neoplasms (MPNs). This study aims to determine the genetic profile of *JAK2*V617F, *CALR* exon 9 Type 1 (52 bp deletion) and Type 2 (5 bp insertion), and *MPL* W515 L/K genes among Malaysian patients and correlate these mutations with clinical and hematologic parameters in MPNs.

Methods: Mutations of *JAK2*V617F, *CALR*, and *MPL* were analyzed in 159 Malaysian MPNs patients using allele-specific polymerase chain reaction, including 76 polycythemia vera (PV), 41 essential thrombocythemia (ET), and 42 primary myelofibrosis (PMF), and the demographics including clinical and laboratory data of the patients were retrieved.

Results: The result showed that 73.6% *JAK2*V617F, 5.66% *CALR*, and 27.7% were triple-negative mutations. No *MPL* W515L/K mutation was detected. In ET and PMF, the predominance type was the *CALR* Type 1 mutation. Serum LDH showed higher in mutated *CALR* PMF compared to PV and ET. PV has a higher risk of evolving to post PV myelofibrosis compared to ET. A thrombotic event at initial diagnosis of 40.9% was high compared to global incidence. Only one PMF patient had a *CALR* mutation that transformed to acute myeloid leukemia.

Conclusion: *JAK2*V617F and *CALR* mutations play an important role in diagnostics. Hence, every patient suspected of having a myeloproliferative neoplasm should be screened for these mutations.

CHAPTER 1

INTRODUCTION

1.1. Definition of Myeloproliferative Neoplasms

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders that harbor somatic mutations characterized by the excessive production of one or more terminally differentiated myeloid lineages. MPNs are classified into (i) chronic myeloid leukemia (BCRABL1-positive), (ii) polycythemia vera (PV), (iii) essential thrombocythemia (ET), (iv) primary myelofibrosis (PMF), (v) chronic neutrophilic leukemia, (vi) chronic eosinophilic leukemia - not otherwise specified (NOS), and (vii) MPN - unclassifiable. Although most of the MPNs are indolent, MPNs have the potential to become thromboembolic and/or bleeding events and can transform into acute leukemia or myelofibrosis [1]–[3].

1.2. Clinical manifestation and diagnosis

Clinical manifestations of MPNs result from clonal proliferation (overproduction) of myeloid cells leading to an increase in the production of abnormal blood cells, depending on the subtypes. PV is clinically described by panmyelosis characterized by erythrocytosis, leukocytosis, and thrombocytosis, frequently with splenomegaly and seldom with myelofibrosis [4]. The hallmark of driver gene mutation in PV is *JAK2* V617F mutation while the minority (3-5%) is *JAK2* Exon 12 mutation [5].

ET is manifested by sustained thrombocytosis and megakaryocytic hyperplasia in the bone marrow leading to thrombosis and hemorrhage [6]. The most common driver gene mutations identified are *JAK2* V617F in 50–60% of cases, *Calr*eticulin (*CALR*) mutations in 30%, and *MPL* (Myeloproliferative Leukemia) mutations in less than 5% of cases [5].

The classical presentation of PMF is the continuous proliferation of mainly granulocytic and megakaryocytic cells in the bone marrow, thus leading to bone marrow fibrosis, splenomegaly and extramedullary hematopoiesis [7]. The most common driver gene mutations involved are *JAK2* V617F in 50–60%, with *CALR* in 30% and *MPL* in 8% of cases [5].

The outcome and progression of MPNs differ according to subtypes; PV and ET are more indolent than PMF. Over 20 years, the incidence of myelofibrosis transformation occurs 26% and 19.9% in PV and ET, respectively. Whereas the leukemic transformation of 20 years is 7.9–17% for PV and 8.1% for ET, accordingly [5].

Diagnosis of MPNs is made based on the WHO criteria illustrated in table 1 below [8].

Table 1: WHO Updated 2016 Criteria for Polycythemia Vera, Primary myelofibrosis and Essential thrombocythemia

	Polycythemia Vera	**Primary myelofibrosis**	**Essential thrombocythemia**
	The diagnosis requires either 3 major criteria or 2 major criteria with minor criterion	The diagnosis of prefibrotic/early primary myelofibrosis requires all 3 major criteria and at least 1 minor criterion.	The diagnosis requires either all the major criteria or the first 3 major criteria with 1 minor criterion
Major criteria	1. Hb level >16.5 g/dL in men or > 16.0 g/dL in women, or haematochrit > 49% in men; >48% in women or red cell mass >25% from predicted value 2. BM biopsy shows hypercellularity for age with trilineage growth, panmyelosis including prominent erythroid, granulocytic and megakaryocytic proliferation with pleomorphic, mature megakaryocytes 3. Presence of *JAK2* V617F or *JAK2* exon 12 mutation	1. Presence of megakaryocytic proliferation and atypia (accompanied by either reticulin or collagen fibrosis grades 2 or 3) 2. WHO criteria for ET, PV, BCR-ABL1 + CML, MDS or other myeloid neoplasms is not met 3. Presence of *JAK2* V617F, *CALR* or *MPL* mutation	1. PLT count $\geq 450 \times 109/L$ 2. BM biopsy shows proliferation, mainly of the megakaryocyte lineage, mature megakaryocytes with hyperlobulated nuclei (no significant increase or left shift in neutrophils granulopoiesis or erythropoiesis and increase in reticulin fibers) 3. Not meeting WHO criteria for BCR-ABL1 + CML, PV, PMF, myelodysplastic syndromes (MDS) or other myeloid neoplasms 4. Presence of *JAK2* V617F, *CALR* or *MPL* mutation
Minor criteria	Subnormal serum erythropoietin level	1. Anemia not due to comorbid condition 2. Leukocytosis $\geq 11 \times 109/L$ 3. Palpable splenomegaly 4. Increased lactate dehydrogenase (LDH) 5. Leucoerythroblastosis	1. Presence of a clonal marker 2. Absence of evidence of reactive thrombocytosis

1.3. Epidemiology of MPNs

The incidence of all MPNs amongst Asian populations, estimated at 12-15 per 100 000, is higher than that of the white population (2.7 per 100 000) [9][10]. For the subtypes of MPNs, ET was the commonest (1.03 per 100 00), followed by PV (0.84 per 100 000), and PMF was 0.47 per 100 000. In Malaysia, the incidence increases with age, with a mean age at diagnosis of 54.2 years predominantly male and Chinese ethnic [11].

Mutations of *JAK2*V617F, *CALR*, and *MPL* account for over 90% of MPNs cases and are usually mutually exclusive to the diagnosis. The hallmark diagnostic driver gene mutation in MPNs is *JAK2*V617F. While the second most common mutation, mainly in ET and PMF, is *CALR* mutation which has been detected in 25% of MPNs patients. *MPL* mutation was reported in 4-6% of ET and PMF patients [12].

However, in approximately 15% of ET and less than 10% of PMF, driver mutations are still unknown and these patients are thus defined as triple-negative MPNs (TN-MPNs) [13].

1.4. Genetic pathogenesis and target genes in myeloproliferative neoplasm

Recently, genetic mutations are increasingly being recognized as an essential diagnostic marker in MPNs. Acquired mutations in genes encoding for tyrosine kinase *JAK2*, the thrombopoietin (TPO) receptor *MPL* and calreticulin (*CALR*) leading to hyperactivation of the Janus kinase2 (*JAK2*)/signal transducer and activation of transcription (STAT) signaling pathway has been identified as an important impact on the pathogenesis of MPNs [14]. There are three target

genes mutations involved in the pathogenesis of MPNs. They are *JAK2*, *CALR* and *MPL* genes mutation.

a) *JAK2* gene

The *JAK2* gene is mapped to chromosome 9p24.1 in humans [15]. In normal hematopoietic cells, *JAK2* is characterized by the presence of two homologous kinases: one is an enzymatically active kinase domain (JAK homology 1; JH1) and the other comprise of a catalytically inactive pseudokinase domain (JH2) that inhibits the kinase activity of *JAK2* following ligand binding [16].

The most common mutation involves the substitution of valine with phenylalanine at position 617 in the JH2 domain (*JAK2*-V617F) located in exon 14, resulting in disruption of normal inhibitory function towards the JH1 kinase domain. This mutation also induces excessive myeloid progenitor cell proliferation and activity by directly activating the JH1 domain through the SH2-JH2 link leading to downstream activation of intracellular signaling via the activation of transcription (STAT) protein signaling and mitogen-activated protein kinase and phosphoinositide 3-kinase signaling pathways [17].

For the *JAK2* V617F mutation to affect hematopoietic progenitor cells, the presence of receptors for erythropoietin, thrombopoietin or granulocyte-colony stimulating factor (CSF) is essential and may aggravate the functional activity and enhanced sensitivity to cytokines and hematopoietic growth factors, such as interleukin 3 (IL-3), stem cell factor (SCF), granulocyte-macrophage CSF and insulin-like growth factor-1 [16].

There is an established link between *JAK2*V617F mutation and classic *BCR-ABL* negative myeloproliferative neoplasms, however, the association has neither been invariable nor specific. Mutations in the *JAK2* gene accounts for 96% in PV, 55% in ET, and 65% in PMF, respectively [18]. However, *JAK2*V617F mutations are also infrequently associated in other types of MPNs, MDS/MPN, and even in BCR-ABL positive MPN, chronic myeloid Leukemia [19]. No *JAK2*V617F mutations were identified among healthy population [16].

Clinical implications of *JAK2*V617F mutation involves the facilitation of diagnosis, available targeted therapy and prognostic biomarkers. It acts as valuable diagnostic marker to distinguish clonal from reactive causes. However, even if the criteria concerning the driver mutations is not met, the diagnosis still can be made based on clinical, pathologic and morphologic/histologic features. [8]

*JAK2*V617F mutation can be detected via qualitative and quantitative methods. Qualitative assay that is commonly used involves allele specific oligo nucleotide polymerase chain reaction. While, the widely available assays for quantitative method is via quantitative polymerase chain reaction (qPCR) which has been shown to have superior sensitivity compared with the latter. For the quantitative assays, the cut-off mutant allele burden is an important parameter to prevent false positive, especially if it is too low, given the case that *JAK2*V617F can be found in 1% of normal individuals [20]. Thus, the threshold of 1-3% is considered sufficient to identify a mutant *JAK2*V617F allele.

The main target of drug developments regarding MPNs is the inhibition of Janus kinase enzymes. The first JAK targeted therapy was the initial use of Ruxolitinib to treat myelofibrosis

but has since been expanded to PV and ET. Ruxolitinib blocks the JAK1 and subsequently inhibits the STAT phosphorylation pathway [5]. This treatment is specific against the JAK and it appears as a symptoms reduction. Several new targeted molecular therapies specifically against *JAK2* are still under study and clinical trials.

b) CALR

The calreticulin (*CALR*) chaperone protein is mapped on chromosome 19p13 in humans and contains 9 exons. The *CALR* is a multifunctional protein that resides mainly in the endoplasmic reticulum and cytoplasm. Structurally, it has three domains: an amino domain (N-terminal ER) which act as chaperone function through lectin-(glycan) binding domain and for signal sequence, a P domain with high-affinity Ca2+ binding sites, and a carboxyl domain (negatively charged C-terminal tail) with low-affinity Ca2+ binding sites and devoid of a KDEL retention motif [21]. It has several functions, including calcium homeostasis, a chaperone in interacting with *MPL* and *JAK2* /STAT signaling, controlling cell proliferation and survival and acting as a perturbation for STAT3, STAT5, AKT, MAPK and PTK2 (FAK) cell signalling [22] .

Mutations in the *CALR* gene are documented in 28 % of MPNs cases, specifically in ET and MF [23]. It is the second most common mutation after *JAK2*V617F and provides a new molecular diagnostic marker in ET and MF. The most common mutations involved either at 52–base pair deletions(type 1) or 5–base pair insertions(referred to as type 2) are restricted to exon 9 which generated a +1 base-pair frameshift, eliminating most of C-terminal domain and disrupted Ca++ binding, and lack of the KDEL ER retention motif [24]. The *CALR* mutants exert function by augmenting TPO-R initiation and concurrently bind to the extracellular N-glycosylation residues to activate the downstream pathway. The signal transducer and activator

of transcription will activate type 1 mutations and subsequently cause in vitro cytokine independent growth. On the other hand, type 2 mutations was found to increase platelet count and is postulated to harbor a role in megakaryocytes maturation [25].

CALR mutants have a role in diagnostic importance and prognostic information about thrombotic potential and leukemic transformation. Molecular analysis of this driver mutation is part of the major criterion in the newest revision of the World Health Organization classification of hematopoietic tumors [8].

It also has distinct clinical characteristics and outcomes compared to *JAK2* V617F and *MPL* mutant patients. Clinically, *CALR* in ET is a favorable mutation associated with the younger age group and higher in males and is associated with a lower incidence of thrombotic events when compared to *JAK2* V617F and *MPL* mutations [24]. However, it is related with higher platelet counts ($P<0.001$) and lower hemoglobin levels with higher incidence of transformation to myelofibrosis than other mutations ($P = 0.03$) [26]. Similarly, *CALR* in MF are associated with younger age group and is related with anemia, leukocytosis and higher platelet counts.

There are also significant clinical implications between type 1 and type 2 mutations. Type 1 mutations are PMF preponderance while type 2 mutations are frequently found in ET. Type 2 in ET displays higher platelet count whereas type 1 in ET tends to MF progression [27].

c) MPL

MPL (Myeloproliferative leukaemia) gene is mapped to chromosome 1 p34 and has 12 exons that resides at the juxtamembrane region of the thrombopoietin receptor protein (TPOR). It consists of 2 types of cytokine receptors domain: a transmembrane domain and a cytoplasmic domain. *MPL* acts as regulators for thrombopoietin (TPO) levels, giving negative feedback mechanism by eliminating TPO bound to the *MPL* receptor. *MPL* and TPO are 2 vital proteins for hematopoietic stem cell self-renewal and DNA repair [5]. The binding of TPO to *MPL* receptor will initiate signaling through *JAK2*.

Acquired mutations in *MPL* lead to cytokine-independent growth and thrombopoietin hypersensitivity, leading to phosphorylation of *JAK2*, STAT3, STAT5, AKT, and ERK [28].

Several mutations of *MPL* were already documented including W515L (tryptophan-to-leucine substitution) and W515K (tryptophan-to-lysine substitution) are the most commonly involved mutations that occur within exon 10, leading to the loss of an auto-inhibition of the thrombopoietin receptor resulting in continuous activation. It was initially discovered in PMF negative BCR-ABL patients in 2006. This mutation was reported in 4-6% of ET and PMF individuals.

1.5 Triple negative MPNs

Triple negative is defined as negative for mutations in all three genes which are found in 2% in PV and 10-15% of cases in ET and PMF. It has been found to be related with poor prognosis especially in PMF [29].

1.6 Rationale of Study

To date, there is limited data exist on the prevalence of driver mutation genes (*JAK2* V617F, *CALR* and *MPL*) in the association between clinical and laboratory profiles MPNs in Malaysia. Additionally, by knowing the driver genes involved, patients may benefit from targeted gene therapy. The targeted gene therapy may improve the clinical states and increase the survival outcome for the patients. It is helpful to recognize which genetic mutation is involved in patients, whether *JAK2* V617F, *CALR* and *MPL* because they determine the clinical features, complications, and survival of MPNs. *JAK2* V617F-mutant ET has a high risk of thrombosis. By contrast, *CALR*-mutant ET has a lower risk of thrombosis but a relatively high risk of myelofibrosis transformation (especially the condition associated with type 1 mutation) [30]. Research shows that genetic testing will help to determine the phenotype and prognosis of MPNs. For instance, a patient diagnosed as essential thrombocytosis with *CALR* mutation has a better prognosis. Furthermore, these mutations also can act as additional biomarkers to differentiate between reactive causes and clonal disorder.

This study aims to determine the genetic profile of *JAK2* V617F, *CALR* exon 9 Type 1 (52 bp deletion) and Type 2 (5 bp insertion), and *MPL* W515 L/K genes among Malaysian patients using allele-specific polymerase chain reaction and to correlate these mutations with clinical and laboratory parameters.

This was a cross-sectional study over five years. This study was approved by the Universiti Sains Malaysia Research Ethics Committee (USM/JEPeM 19100646) and carried out in accordance with the Declaration of Helsinki. In this study, 159 MPNs clinical and laboratory data were obtained from the Unit record and Hematology Laboratory of Hospital Universiti Sains Malaysia (Hospital USM) from 2016 until 2020. Mutational analyses (*CALR* and *MPL*)

were performed. The results were analyzed by Statistical Package for the Social Sciences (SPSS) Statistics for Windows (Version 25.0; Armonk, NY, IBM Corp.).

The dissertation was arranged according to Format B (manuscript ready format) according to guideline by Postgraduate Office, School of Medical Sciences (2016). The following chapter contains the objectives of this study. Chapter 3 is the manuscript entitled "Clinical and Laboratory Features of *JAK2* V617F, *CALR*, and *MPL* Mutations in Malaysian Patients with Classical Myeloproliferative Neoplasm (MPNs)" that has already published under International Journal Environmental Research and Public Health. 2021. Chapter 4 contains the study protocol that had been submitted and obtained ethical approval from Jawatankuasa Etika Penyelidikan Manusia (JEPeM), Universiti Sains Malaysia. The appendices contain elaboration of methodology and additional references. The raw data is included in the attached CD.

CHAPTER 2

OBJECTIVE

2.1 General Objective

To study the driver gene mutations (*JAK2* V617F, *CALR* and *MPL*) involvement in myeloproliferative neoplasms and it's association with the clinical and laboratory features.

2.2 Specific Objectives

1. To determine the prevalence of driver gene mutations *JAK2*V617F, *CALR* and *MPL* among myeloproliferative neoplasms
2. To determine the association between *JAK2*V617F and *CALR* mutational status with clinical and hematology laboratory parameters in Myeloproliferative neoplasm
    a. Splenomegaly
    b. Thrombosis
    c. Mean Hemoglobin level
    d. Mean total white cell count
    e. Mean platelet count

**Both objectives have been covered in the manuscript; (Chapter 3)

CHAPTER 3

MANUSCRIPT

Published : Zulkeflee RH, Zulkafli Z, Johan MF, Husin A, Islam MA, Hassan R. Clinical and Laboratory Features of *JAK2* V617F, *CALR*, and *MPL* Mutations in Malaysian Patients with Classical Myeloproliferative Neoplasm (MPNS). Int J Environ Res Public Health. 2021 Jul 16;18(14):7582. doi: 10.3390/ijerph18147582. PMID: 34300032; PMCID: PMC8307561., (JCR Q1/ (ISI-indexed, IMPACT FACTOR 3.39)

(See 5.3: Evidence of submission/ accepted for publication; Page 116)

3.1 Title Page

Clinical and Laboratory Features of *JAK2* V617F, *CALR*, and *MPL* Mutations in Malaysian Patients with Classical Myeloproliferative Neoplasm (MPNs)

Razan Hayati Zulkeflee[1,2], Zefarina Zulkafli[1,2,*], Muhammad Farid Johan[1], Azlan Husin[2,3], Md Asiful Islam[1], Rosline Hassan[1,2,*]

[1] Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Malaysia; rhayatiz@usm.my (R.H.Z.); faridjohan@usm.my (M.F.J.); asiful@usm.my (M.A.I.)

[2] Hospital Universiti Sains Malaysia, Kubang Kerian 16150, Malaysia; azlanh@usm.my

[3] Department of Internal Medicine, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Malaysia

** Correspondence: zefarinazulkafli@gmail.com (Z.Z.); roslin@usm.my (R.H.)*