CHAPTER 1

INTRODUCTION

1.1. Background

Myeloid malignancies are stem cell-derived and clonal disorders and consist of three wide-ranging clinicopathologic categories: acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPNs). MPNs were first acknowledged by William Damashek in 1951. The classic MPNs were polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF) and chronic myelogenous leukemia (CML). They were initially grouped together based on their common phenotype of proliferation. Because of their similarities in increasing mature peripheral blood cells and overlapping phenotype, diagnosis has been difficult to be established in the past. It is believed that classic MPNs came from similar unknown mechanism.[1]

The important issues in the course of MPNs are thrombosis, hemorrhage, evolution to post-polycythemic or post-thrombocythemic myelofibrosis and AML transformation.[2] Thrombosis and bleeding are the leading causes of morbidity in MPNs.[3] In one-third of MPNs patients, early vascular events constitutes first disease manifestation.[4] Even though thrombosis is the most frequent complication in MPNs, but bleeding is more observed in ET.[5]

As is the nature of the proliferating marrow, chromosomal aberrations were largely found in hematologic malignancies, and the findings were pathognomonic. Starting from this point, cytogenetic has become a valuable tool in the assessment of cancer - in diagnosis, therapeutic guidance, and as a prognostic marker.[6,7] The understanding of the molecular pathogenesis of myeloid malignancies, has fundamentally derived from the identification and characterization of recurrent chromosomal translocations, defined as Philadelphia chromosome, which results from a reciprocal translocation between the long arms of the chromosomes 9 and 22 t(9;22)(q34;q11). However, in many patients with myeloproliferative neoplasms (MPNs), Philadelphia chromosomes were not found. Conversely, no specific abnormality has been identified to date. The frequency of cytogenetic abnormalities in the Philadelphia-negative MPNs varies from approximately 40% in PMF to 3% in ET.[8] The spectrum of aberrations is heterogeneous, ranging from gains and losses of genetic material to structural changes including unbalanced translocations.[9] The role of cytogenetic abnormalities as a prognostic marker in PMF has been suggested, both at the time of diagnosis and later during disease course.[10]

In molecular level, Philadelphia chromosome is derived from two new genes fusion, BCR-ABL on the 22q- and the reciprocal ABL-BCR on 9q-. The identification of the BCR-ABL gene and consequent protein led to the production of small-molecule drugs, proposed to hinder BCR-ABL tyrosine kinase activation by competitive binding at the adenosine triposhpate (ATP)-binding site. The first tyrosine kinase inhibitor (TKI) was imatinib mesylate (IM), and launched into

clinical practice in 1998.[11] IM blocks the ATP binding pocket on the BCR-ABL tyrosine kinase thus inhibits activation of the enzyme which involved in the pathogenesis of CML. It has been reported that it induces up to 60% major cytogenetic remission and 95% complete hematologic responses, and also prevents 89% patients' progression to blast crisis.[12] Imatinib turn out to be the first choice drug in chronic phase CML, as a result of its high efficacy, low toxicity and capacity to preserve strong hematological and cytogenetic responses.[11]

Important recent discoveries have identified a central role of protein tyrosine kinase (PTK) in the pathogenesis of MPNs. Several groups reported the discovery of JAK2 V617F mutation in early 2005.[13-15] Baxter et al found a single base substitution, guanine to thymine change at 1849, which resulted in the change of valine to phenylalanine in exon 14 of the pseudokinase domain of tyrosine kinase JAK2 (Janus Kinase 2) gene in 97% PV, 57% ET and 50% PMF.[13] This mutation results in a gain of function due to the constitutive activation of tyrosine kinase-dependent cellular signaling pathways, particularly of the JAK-STAT (Signal Transducers and Activators of Transcription) pathway. The pathway is principal in regulation of cell proliferation, differentiation and apoptosis in hematopoiesis.[15]

In MPNs patients with confirmed JAK2 V617F mutation, it has been associated with older age at diagnosis (ET and PMF),[18] higher hemoglobin level (ET and PMF),[19] leukocytosis (ET and PMF),[18] lower platelet count (ET), larger spleen size (PV, ET and PMF),[20] the need for spelenectomy,[20] and leukemic

transformation[20]. Patients with mutation have been associated with shorter survival in PMF, but less likely to require blood transfusion.[21]

JAK2 V617F mutation as a common genetic aberration in PV, ET, and PMF had pointed the possibility of using tyrosine kinase as a valid therapy target, which could follow the efficacy of IM and other tyrosine kinase inhibitors in CML.[2,5] Novel JAK2 inhibitors are under development.[16] Class I inhibitors were intentionally developed as JAK2 inhibitors, and Class II inhibitors were initially developed as other target kinases inhibitors but later found to obtain JAK2 inhibitory effect.[17]

This study described the prevalence of JAK2 V617F mutation and cytogenetic abnormality in Semarang MPNs patients.

1.2. Research Question

What is the the prevalence of JAK2 V617F mutation and cytogenetic abnormality in Semarang MPNs patients?

1.3. Objectives

1. 3. 1. General Objective

To know the prevalence of JAK2 V617F mutation and cytogenetic abnormality in Semarang MPNs patients.

1. 3. 2. Specific Objective

1.3.2.1. To know the prevalence of JAK2 V617F mutation in Semarang MPNs patients.

1.3.2.2. To describe chromosomal abnormality in Semarang MPNs patients using conventional cytogenetic analysis.

1.4. Benefits

1.4.1. Provide supporting data about molecular and cytogenetic markers for MPNs which improve the precision of MPNs diagnosis.

1.4.2. Provide supporting data to enrich the understanding of the pathogenesis of MPNs.

1.4.3. Provide supporting data about the phenotype of MPNs patients.

1.4.4. Identification of JAK2 V617F will be beneficent as a therapy target of JAK2 inhibitors and/or tyrosine kinase inhibitors.

1.5. Originality

This study is the first to analyze the JAK2 V617F mutation and cytogenetic profile in MPNs patients in Indonesia.

Table 1. List of previous associated studies

No.	Author, Title of Publications	Method	Result
1.	Bacher U, Schnittger S, Kern W, et al. Distribution of cytogenetic abnormalities in myelodysplastic syndromes, Philadelphia negative myeloproliferative neoplasms, and the overlap MDS/MPNs category. [8] Ann Hematol 2009;88(12):1207–13	Chromosome banding & molecular analysis of MPNs, MDS, and MPNs/MDS.	+9 and gain of 9p were more frequent in MPNs. Isolated del(5q) were more in MDS. Trisomy 8, 21 and del(20q) were comparably frequent. JAK2 V617F & NRAS mutation showed overlap in varying proportions.
2.	Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005 Mar 19-25;365(9464):1054-61.	Bidirectional sequencing of JAK2 exons from peripheral-blood granulocytes, T cells, or both. Allele-specific PCR, molecular cytogenetic studies, microsatellite PCR, Affymetrix SNPs array analyses, and colony assays.	First identification of JAK2 V617F mutation in 97% PV, 57% ET, 50% and PMF in Caucasian (UK).

3.	Sazawal S, Bajaj J, Chikkara S, et al Prevalence of JAK2 V617F mutation in Indian patients with chronic myeloproliferative disorder.[18] Indian J Med Res 2010 Oct;132:423-7.	Mutation screening in patients with PV, ET, PMF using PCR and restriction enzyme based assay.	Mutation was found in 68% CMPD, 82% PV, 70% ET, and 52% PMF in Indian population.