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Polymorphonuclear-Mononuclear Ratio in Peripheral Blood as Hematologic Malignancy Predictor in Pancytopenia

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ABSTRACT

Pancytopenia is a laboratory finding of decreased hematological cells characterized by hemoglobin of <13.5 g/dL for males or <11.5 g/dL for females, leukocytes of <4x109/L and platelets of <150x109/L. The data from Dr. Moewardi Hospital reported 56 cases of pancytopenia in 2020. Follow-up tests such as reticulocyte test, Bone Marrow Puncture (BMP), or bone marrow biopsy are needed to determine the cause of pancytopenia. This study aimed to assess the performance of the Pm/M ratio of peripheral blood as a screening instrument to predict the cause of pancytopenia. A cross-sectional study was carried out on pancytopenia patients undergoing laboratory tests at the Clinical Pathology Laboratories of Dr. Moewardi Hospital from January 2020 to June 2021. The cut-off point of the Pm/M ratio was determined by ROC and AUC curves. The results were presented in a 2x2 table. The Pm/M ratio <0.91 as a predictor of hematological malignancy had a sensitivity of 82.9%, specificity of 82.9%, PPV 82.9, NPV 82.9%, LR positive 4.833 and LR negative 0.207. The Pm/M ratio can be used as a screening biomarker to predict the cause of pancytopenia before performing BMP and to distinguish between hematological malignancy and non-hematological malignancy.

Keywords: Pm/M ratio, pancytopenia, BMP

INTRODUCTION

Pancytopenia is a laboratory finding of anemia, leukopenia, and thrombocytopenia caused by various causes and various disease processes, especially those involving bone marrow.[1-3] The data issued by Dr. Moewardi Hospital in 2020 reported that there were 56 cases of pancytopenia. Laboratory has a pivotal role in assisting clinicians to make a diagnosis and provide appropriate therapy. Follow-up tests such as reticulocyte count, Bone Marrow Puncture (BMP), or bone marrow biopsy are needed to identify the cause of pancytopenia. A study in India reported that most pancytopenia was caused by hypersplenic conditions (33.17%) and aplastic anemia (13.9%). In addition, infection (10.8%), megaloblastic anemia (9%), leukemia (9%), non-Hodgkin's lymphoma (2.4%), splenomegaly with malaria parasite infestation (2.4%), Multiple Myeloma (MM) (2.4%), Myelodysplastic Syndrome (MDS) (2.4%) and Systemic Lupus Erythematosus (SLE) (0.6%) may also contribute to the occurrence of pancytopenia.[1,2]

Laboratory tests and physical examinations have a pivotal role in assisting clinicians to make a diagnosis and prescribe appropriate therapy. Reticulocyte count, BMP, and bone marrow biopsy are required to investigate the cause of pancytopenia. A previous study reported that bone marrow biopsy and immunophenotyping had a diagnostic value of 83.4% in pancytopenia cases.[4]

A complete blood count is an affordable, simple, and widely used test to diagnose various disorders as well as monitor therapy responses. Polymorphonuclear-mononuclear ratio (Pm/M) has never been applied to predict the cause of pancytopenia either in hematological or non-hematological malignancies. Therefore, this study analyzed the performance of the Pm/M ratio of peripheral blood in predicting the etiology of pancytopenia.

METHODS

A cross-sectional study in patients with pancytopenia who underwent initial BMP at the Clinical Pathology Laboratories of Dr. Moewardi Hospital between January 2020 and July 2021. Patients who agreed to participate in this study signed the informed consent. Patients whose contradicting BMP results between two observers were excluded. The characteristics of research subjects were taken from physical examination and medical records.

Pancytopenia in this study was determined based on a complete blood count for hemoglobin, leukocytes, and absolute platelet count using an automated hematology analyzer. A peripheral blood smear was used to determine leukocyte differential counting to the buffy coat sample of the research subjects who needed peripheral blood smear examination. Pm/M ratio was calculated based on the ratio of the percentages of mature polymorphonuclear cells to mononuclear cells (lymphocyte, monocyte, and blast cells) in peripheral blood smear. Following peripheral blood smear examination, BMP was carried out to detect hematologic malignancy or non-malignancy. Bone Marrow Puncture (BMP) preparations were interpreted by staff and senior staff of clinical pathology laboratories of Dr. Moewardi Hospital with expertise in hematology. The Kappa test was used to analyze the agreement between both observers; a cut-off from Kappa coefficient ≥ 0.6 was reported as a good value. The Pm/M ratio was then calculated. Hematologic malignancies determined in this study were myelodysplastic syndrome (MDS), Multiple Myeloma (MM), Monoclonal Gammopathy of Undetermined Significance (MGUS), and leukemia. In addition, non-hematologic malignancies determined in this study were aplastic anemia, peripheral response, and hyperactive marrow.

The numerical data were presented in mean±standard deviation and analyzed using an independent T-test, whereas categorical data were analyzed using a Chi-Square test. The statistical results with a p-value of <0.05 were reported as significant. The cut-off value of the Pm/M ratio was determined with Receiver Operating Characteristic (ROC) and Area Under Curve (AUC). A diagnostic test of a 2×2 table was used to assess sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), accuracy, and precision.

RESULTS AND DISCUSSIONS

There were 70 pancytopenia patients who met inclusion criteria consisting of 35 patients with hematological malignancy and 35 patients without

Table 1. Characteristics of research subjects based on clinical findings

Parameter	Hematological Malignancy (n=35)	Non-Hematological Malignancy (n=35)	p-value
Age[1] (year)	46.00 (3.00-73.00)	55.00 (4.00-76.00)	0.066
Gender[2]			0.811
Female	18 (51.4%)	17 (48.6%)	
Male	17 (48.6%)	18 (51.4%)	
Clinical manifestation[3]			
Weakness	21 (60%)	30 (85.7%)	0.016*
Fever	6 (17.1%)	2 (5.7%)	0.259
Bleeding	5 (14.3%)	4 (11.4%)	1.000
Pale	3 (8.6%)	2 (5.7%)	1.000
Bone pain	3 (8.6%)	0	0.239
Organomegaly[3]			
Hepatomegaly	6 (17.1%)	1 (2.9%)	0.106
Splenomegaly	6 (17.1%)	4 (11.4%)	0.495
Lymphadenopathy	1 (2.9%)	1 (2.9%)	1.000
No organomegaly	26 (74.3%)	30 (85.7%)	0.232
Comorbid[3]			
Diabetes mellitus	2 (5.7%)	1 (2.9%)	1.000
Heart failure	2 (5.7%)	0	0.493
Liver failure	2 (5.7%)	1 (2.9%)	1.000
Skin infection	0	2 (5.7%)	0.493
Fracture	0	1 (2.9%)	1.000
Gastritis	0	2 (5.7%)	0.493
No comorbid	29 (82.9%)	28 (80%)	0.601

Note: [1] Data were shown in median (min-max) using Mann-Whitney test; [2] Data were shown in mean+SD, independent T-test,
[3] Data presented in frequency distribution (%), Chi-Square test, *significance α=5%

hematological malignancy. Characteristics of research subjects in both groups were comparable, except for the clinical manifestation of weakness. More patients in non-hematological malignancy group experienced weakness than those in the malignancy group (p-0.016) (Table 1).

Hematological tests performed in all patients revealed that the platelet count of patients with hematological malignancy was significantly higher than that of the non-hematological malignancy group (p-0.005). Patients with hematological malignancy had a lower percentage of polymorphonuclear cells ranging from 2% to 72% than those without hematological malignancy, which ranged between 5% and 83% (p=< 0.001). In addition, the mean value of mononuclear cell percentage was significantly higher (65%) in the hematological malignancy group than that of the non-hematological group (42%) (p=<0.001).

Hypocellularity was more common in the non-hematological malignancy group (n=31) than in the hematological malignancy group (n=9) (p=<0.001), whereas normocellularity (n=13) and hypercellularity (n=13) were more frequent in a hematological group than non-hematological malignancy group (n=4 and 0, respectively) (p=0.012 and <0.001, respectively) (Table 2).

The ROC curve result of the Pm/M ratio analysis showed an AUC of 0.821 with a 95% Confidence Interval (CI) = 71.8%-92.4% and a p-value of <0.001. The cut-off value of the Pm/M ratio of 0.91 had a sensitivity of 82.9% (95% CI=74.0%-91.7%) and specificity of 82.9% (95% CI=74.0%-91.7%). Based on the cut-off of the ROC curve, the Pm/M ratio of < 0.91 indicated hematological malignancy (Figure 1).

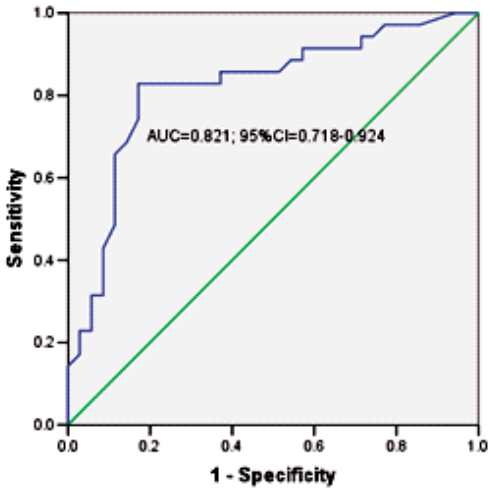

Figure 1. Receiver operating characteristic curve of Pm/M ratio as a predictor of hematological malignancy

Table 2. The characteristics of research subjects based on laboratory and BMP results

Parameter	Hematological Malignancy (n=35)	Non-Hematological Malignancy (n=35)	p-value
Hematology			
Hemoglobin[1] (g/dL)	8.20 (2.00-10.40)	7.60 (3.30- 10.90)	0.565
White blood cell[2] (/uL)	2487.43 ±1109.67	2271.12 ±1127.20	0.421
Platelet[1] (/uL)	36,000 (3,000-131,000)	13,000 (1,000- 95,000)	0.005*
Polymorphonuclear[1](%)	35.00 (2.00-72.00)	58.00 (5.00-83.00)	<0.001*
Mononuclear[1] (%)	65.00 (28.00-98.00)	42.00 (17.00-95.00)	<0.001*
Cellularity			
Hypocellular	9 (25.7%)	31 (88.6%)	<0.001*
Normocellular	13 (37.1%)	4 (11.4%)	0.012*
Hypercellular	12 (34.3%)	0	<0.001*
Diagnosis			
MDS	9 (25%)		
AML	8 (23%)		
ALL	8 (23%)		
MPAL	6 (17%)		
MM	2 (6%)		
MGUS	2 (6%)		
Hypoplasia		31 (88%)	
Hyperactive marrow		4 (12%)	

Note: MDS: myelodysplastic syndrome, AML: Acute Myeloid Leukemia, ALL: Acute Lymphoblastic Leukemia, MPAL: Mixed Phenotype Acute Leukemia, MM: Multiple Myeloma, MGUS: Monoclonal Gammopathy of Undetermined Significance, g: gram, dL: deciliter, uL: microliter, BMP: Bone Marrow Puncture

Pancytopenia is not a primary disease; however, its laboratory findings as a consequence of a disease process may affect the bone marrow. It can result from impaired bone marrow production, peripheral destruction, or a combination of both. There were 35 patients with hematological malignancy among 70 patients diagnosed with pancytopenia in this study. In addition, a total of 62.9% and 25% of patients suffered from leukemia and MDS, respectively. These MDS patients were dominated by those aged > 60 years old (55.6%). This finding was supported by a study of Sallman *et al*., which reported that MDS commonly occurs at the median age of 71 years old, and its incidence increases after the age of 60 years and it rarely occurs at a young age.[5] The occurrence of MDS is associated with the gene mutation process. The difference in the frequency of gene mutations between elderly and young people indicates that older people have a higher incidence of gene mutations. Mutations of tet methylcytosine dioxygenase 2 (TET2) and serine/arginine-rich splicing factor 2 (SRSF2) genes were significantly more common in people at age > 70 years.[6] The number of mutations in MDS increases linearly with age and patients at age > 50 years have more mutations in TET2, SRSF2, and deoxyribonucleic acid (DNA) methyltransferase 3a (DNMT3A) genes.

Microscopic examination of bone marrow detected plasma cells in the bone marrow of four patients (12%) in our study. There were 2 monoclonal gammopathy of undetermined significance (MGUS) patients with plasma cell counts of 0.4% and 4% and two MM patients with plasma cell counts of 18.4% and 45.2%, respectively. Plasma cell disorders are progressive disorders. Plasma cells migrate to the bone marrow via chemokine (C-X-C motif) ligand (CXCL12) and Stromal Derived Factor-1 (SDF-1) binding. Bone marrow expressing SDF-1 together with IL-7 has the potential to develop B-cells to become naive B-cells and immature B-cells. The progression of plasma cell clones explains the occurrence of cytopenia.[7] Pancytopenia in MM and MGUS is associated with plasma cell proliferation, fas-ligand-mediated apoptosis or cytokine-mediated bone marrow failure, or erythropoietin deficiency due to renal failure.[8]

A total of 31 of 35 patients with non-hematological malignancies in this study were predominantly hypoplasia, whereas the other 4 patients were categorized as hyperactive marrow. Most of these hypoplastic patients were between 18 and 76 years old (n=29). The incidence of aplastic anemia mostly occurs at the age of > 60 years old. Therefore, this age group was also reported as an independent risk factor of aplastic anemia. The cause of aplastic anemia in old age is idiopathic; however, according to several biological aspects indicate that mutations occur in old age and cytogenetic abnormalities are found in younger patients.[9,10]

Some drugs such as non-steroid anti-inflammation drugs (NSAIDs), chemotherapy drugs, anti-epileptic drugs, steroids, and chloramphenicol can cause aplastic anemia. The aplastic mechanism may occur through decreased P-gp activity in aplastic anemia. Hematopoietic cells express P-gp, which can protect cells from toxic compounds. Decreased P-gp function results in cell damage due to toxic effects and drug accumulation in the cytoplasm.[11,12]

Decreased hemoglobin levels in hematological malignancies and non-hematological malignancies result from an inflammatory process or chronic disease correlated to iron deficiency. Iron homeostasis is regulated by hepcidin, a hormone, which can bind to ferroportin. Chronic inflammatory conditions will increase hepcidin and lead to degradation of ferroportin resulting in iron deficiency and anemia.[13]

The most common type of bone marrow cellularity in cases of hematological malignancies and non-hematological malignancies was hypocellular with percentages of 25.7% and 88.6%, respectively. Hypocellularity in the group of non-hematological malignancies might be due to aplasia, drugs, radiation, and infection.[14] Age influences reducing the percentage of cellularity; therefore, it must still be considered in establishing the diagnosis.[15] Gene mutations are associated with hypocellularity in leukemia. From a molecular perspective, mutations in the Renin-Angiotensin System (RAS) and fms like tyrosine kinase 3 (FLT3) (2% and 4%, respectively) are associated with hypocellular leukemia.[16]

There were 6 cases of bone marrow hypoplasia, which had a Pm/M ratio of < 0.9. False positives in non-hematological malignancies are affected by the administration of macrolide antibiotics such as Azithromycin and Erythromycin resulting in neutrophil apoptosis.[17] Other classes of antibiotics, which have been shown to cause neutropenia are beta-lactam antibiotics such as Penicillins, Cephalosporins, and Carbapenems. This is because of declined granulopoiesis and induced antibody formation (haptens) towards neutrophils. Hapten molecule emergence can occur within hours to days after drug administration and neutropenia is reversible.[18,19]

There were 6 cases of hematological malignancy with a Pm/M ratio of > 0.9, namely three MDS cases,

one Acute Myeloid Leukemia (AML) case, one Mixed Phenotype Acute Leukemia (MPAL) case, and one MM case. False negative in hematological malignancy cases is influenced by several comorbidities like heart disease and diabetes mellitus. Cardiovascular disease interferes with several mechanisms, which regulate neutrophil production, thereby increasing the expressions of IL-3, IL-5, and the Granulocyte Colony Stimulating Factor (G-CSF) receptor that stimulates cells to proliferate. Neutrophils in bone marrow express a lot of chemokine C-X-C motif receptor 4 (CXCR4) on the cell surface and interact with SDF-1.[20] The interaction of CXCR4-SDF1 results in an increase in neutrophils affecting the formation of atherosclerotic plaque.[21,22] Diabetes mellitus can affect neutrophils in response to chemokines and neutrophil migration to endothelial cells. Neutrophil migration in endothelial cells from the vessel wall can lead to tissue damage and an inflammatory process. Expression of CXCR1 and CXCR2 on the surface of neutrophil cells then induces macrophages to produce CXCL2 in diabetes mellitus patients.[23]

Two patients with non-hematological malignancy transformed into hematological malignancy within 2-4 months from hypoplasia to acute lymphoblastic leukemia (ALL) and MPAL. Both patients were at the age of <14 years old with a Pm/M ratio of 0.2% and 0.9%, respectively. The most common causes of the transformation of aplastic anemia into leukemia are mutations in the phosphatidylinositol glycan anchor and loss of human leukocyte antigen class 1 alleles which are pathognomonic of bone marrow failure. Another parameter that can be observed in this transformation process is blast cells.[24]

Acute leukemia is characterized by chromosomal damage and gene mutations, which have a significant role in the pathogenesis of the disease. Epigenetic modifications including DNA methylation and histone modifications contribute to the leukemogenic phenotype as well as the presence of micro ribonucleic acid (miRNA) in leukemia. A chromosome 8:21 translocation is common in AML, whereas a chromosome 11:23 translocation is common in ALL.[25]

When hematological malignancy is suspected, the physician usually suggests a bone marrow assessment. This morphologic assessment is recommended by European Leukemia Net (ELN), the National Comprehensive Cancer Network (NCCN), and World Health Organization (WHO) 2016 guidelines. Bone marrow puncture findings are used for establishing the diagnosis as well as prognosis. Another evaluation is immunophenotyping. The immunophenotyping by the flow cytometry has been found to be similar in peripheral blood and bone marrow blasts.[26]

This study analyzed the potential of the Pm/M ratio from peripheral blood to predict hematological malignancy and the cause of pancytopenia. This is a non-invasive procedure, which may help physicians to screen pancytopenic patients. However, further study is required as this was a single-center study, and its findings might not be applied to other populations. No exclusion of comorbid diseases such as diabetes mellitus, cardiac, liver, and renal disease, which might influence the Pm/M ratio, and no analysis of treatment history were limitations in this study.

CONCLUSION AND SUGGESTIONS

It was concluded that a Pm/ M ratio of < 0.91 can predict hematological malignancy as it had good sensitivity, specificity, PPV, NPV, PLR, and NLR of 82.9%, 82.9%, 82.9%, 82.9%, 4.833, and 0.207, respectively. Therefore, Pm/M ratio can be applied as a screening biomarker to predict the etiology of pancytopenia as well as to distinguish between hematological malignancy and non-hematological malignancy. Further multi-center study with more detailed analysis is required.

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