INTRODUCTION

Thrombocytopenia is a disease that can cause life-threatening bleeding.\(^1\) The causes of thrombocytopenia can be divided into four groups: increased destruction of platelets in the circulation, impaired platelet distribution, pseud thrombocytopenia, and decreased production ability of the bone marrow (production disorders).\(^2\) A complete blood count shows only the total platelet count, but unable to provide information about the cause of thrombocytopenia.\(^3\)

Bone marrow aspiration (BMA) is frequently used to investigate the cause of thrombocytopenia. Examination of a bone marrow smear can provide a reliable diagnosis. However, this BMA examination is invasive and painfully.\(^4\) Young platelets released by the bone marrow are larger and more reactive than mature platelets. It contains more RNA. Due to their similarity to reticulocytes (young erythrocytes), these young platelets are often called reticulated (RP) or immature platelets (IP).\(^5\) The number of RP correlates with the process of thrombopoiesis, which the number of RP will increase with the increasing of megakaryocyte production and decrease with the decreasing of megakaryocyte production. The RP act as a real-time marker of megakaryocytes.\(^6\)

After the Sysmex tool (Kobe, Japan) introduced immature platelet fraction (IPF) which assess the amount of IP using a hematology analyzer based on RNA staining as an automatic platelet counter.\(^7\) \(^8\) Research regarding the ability of IPF% as a diagnostic test tool to detect production disorders in thrombocytopenia is still very limited. Researchers are interested in conducting diagnostic tests on the ability of IPF% as a diagnostic test tool to detect production disorders in thrombocytopenia.

METHODS

This research is a diagnostic test. This research was conducted at Analytical Laboratory of Prof. Dr. I GNG Ngeorah Hospital, Denpasar, Udayana University. Thrombocytopenia patients aged at least 18 years with thrombocytopenia (platelet <10\(^9\)/L) and willing to participate by signing an informed consent were included in this study. The sample is divided into two groups: production disorders and non-production disorders. The patients who were taking antiplatelets,
received platelet concentrate transfusions 48 hours before blood samples were taken, cirrhosis of the liver, SLE who received immunosuppressants, dengue infection with an onset of fever less than 6 days, and sepsis with worsening clinical status were excluded.

The research material for determining IPF% levels was blood samples taken from the patient's cubital vein. Complete blood count and IPF% were performed using an Automated Hematology Analyzer Sysmex-XN 3000. The estimated sample size for hypothesis testing in this study was calculated using the sample size formula for diagnostic test research (Dahlan, 2010). Research precision was set at 8.5%. The sensitivity of the desired diagnostic test marker was determined to be 87%. Based on sample calculations, the number of samples in this study was 81 people.

Variable Immature platelet fraction is the proportion of young platelets contained in the circulation. Variable thrombocytopenia due to suspected production disorders is disease groups consisting of diagnoses (ALL, AML, CML, MDS AA, Post-chemotherapy Cancer). Variable thrombocytopenia due to non-production disorders is a production disorder, a group of diseases that are not included in the group of production disorders.

Data analysis in this study consisted of descriptive statistical analysis, receiver operating curve (ROC) analysis, and diagnostic tests. Descriptive statistical analysis aims to describe the characteristics of the subjects and research variables as a whole. ROC analysis aims to assess the ability of IPF% as variables used to detect production disruptions in thrombocytopenia and assess the best cut point of IPF% to detect production disorders in thrombocytopenia. Ability to detect etiology is assessed based on the area under the curve (AUC) and is said to have good ability if AUC ≥0.7. Diagnostic tests are carried out by making a 2x2 cross tabulation between IPF% that has been categorized as ≥cut-off and <cut-off according to cause of thrombocytopenia between production disorders and non-production disorders.

RESULT

In this study, the number of subjects in this research consisted of 81 samples. The subjects consisted of 48 (59.3%) men and 33 (40.7%) women. Median value of sample age is 43 (18-80) years. The proportion of thrombocytopenic subjects divided into the production disorder group was 49 (60.5%) and the non-production disorder group was 32 (39.5%). The diagnosis of thrombocytopenia due to production disorders uses the BMA gold standard. The proportion of causes of thrombocytopenia in the production disorders group is: AML 21 (25.9%), ALL 3 (3.7%), CML 1 (1.2%), post-chemotherapy cancer 4 (4.9%), aplastic anemia 10 (12.3%), and MDS 9 (11.1%) subjects.

The median platelet value in all study samples was 19.0 x10^3/µL (1-92), while the median IPF% value for the entire research sample is 9.4% (0.3-49.2). Platelet values in the production disorder group were not significantly different from those in the non-production disorder group (15.5 x10^3 /µL (1-85) vs 24 x10^3/µL (1-92), p <0.148). We get different results if we compare the IPF% value in the production disruption group, which is significantly lower than the non-production disruption (3.1% (0.3-21.4) vs 13% (2.0-49.2), p<0.001).

The highest median IPF% value in the production disorder group was at AML 4.2% (0.9-21). Meanwhile, the lowest median IPF% value was found in the post-chemotherapy 2.0% (1.2-3.8). In the non-production disorder group, the highest median IPF% value was found in ITP group 22% (6.2-49.2). Meanwhile, the lowest median IPF% value was found in the sepsis group 10.2% (2-21). The results of calculations using ROC analysis in this study obtained an AUC of 0.917 (95% confidence interval (CI): 0.853-0.981, p<0.001).

In this study, the best threshold value for IPF% was found to be 7.5%. The IPF% 7.5 value was converted into a dichotomous scale, namely; IPF% value ≥7.5% is non-production disorder group and <7.5% production disorder group. The results of the analysis set a cut-off value of 7.5% at sensitivity 83.7%, specificity 84.4%, PPV 89.1%, NPV 77.1%, LR+ 5.53, LR- 0.19, prevalence 60.4%, and 83.9% accuracy.

DISCUSSION

ROC curve analysis in this study obtained an AUC value of 0.917 (95% (CI): 0.853-0.981). The AUC results of this study were between 0.90<AUC<1.00, it was classified as very good for differentiating between the two groups studied. It means IPF% can be used to differentiate between production disruption groups and non-production disruption groups. This can also be interpreted that IPF% can detect production disorders in thrombocytopenia. The AUC results which show very good values are equivalent to other studies; Abe (AUC 0.921 (95% (CI): 0.849-0.994)), Zoe V (AUC 0.910), and Kibum (AUC 0.931).

The optimal IPF% cut-off value of 7.5% was determined based on considerations to obtain a sensitivity value of 83.7% and a specificity of 84.4%. The IPF% sensitivity 83.7% shows the ability of IPF% to detect which individuals suffer from production disorders from the entire population who actually experience production disorders of 83.7%. The IPF% specificity 84.4% shows the ability of IPF% to detect which individuals do not suffer from production disorders from those who really do not suffer from production disorders is 84.4%. This research also determined the PPV IPF% value is 89.1%. This value shows that if a person's IPF% result is <7.5, the probability of that individual actually suffering from production disorders is 89.1%. The IPF% NPV 77.1% indicates that if the IPF% result is ≥7.5, the probability that the individual really does not suffer from production disruption is 77.1%.

The cut-off results obtained in this study are very different from two other recent studies; 1) Zoe V (2019), IPF% cut-off value of 13% has specificity and PPV of 100%, with sensitivity of 64% and NPV of 58%.2) Kibum (2020), with an IPF% cut-off value of 2.3% has a sensitivity of 95.5%, specificity 73.5%, PPV 78.9%, and NPV 96.3%. Zoe uses a high IPF% cut-off value of up to ≥13 to predict peripheral thrombocytopenia, because researchers are trying to get specificity and NPV of 100%. However, this reduces the IPF% sensitivity to only 64%. This is different from Kibum which uses an IPF% cut-off value of <2.3 to obtain a
high sensitivity of 95.5% in differentiating thrombocytopenia due to hyperproduction. However, Kibum’s research only determined a low specificity value, namely 73.5%.

The cut-off value of this study resembles Abe’s research in 2006 which used an IPF% cut-off value of >7.7% to obtain sensitivity 87%, specificity 93%, PPV 77.6%, and NPV 95.9%, in determining thrombopoietic activity of thrombocytopenic patients. This research has a higher PPV value than Abe’s research 89.1%. The PPV IPF% value of 89.1% shows that thrombocytopenia patients with IPF% <7.5% actually suffer from production disorders of 89.1%.9,12

This research obtained an LR+ IPF% value of 5.53. The positive likelihood ratio shows the ratio between the probability of a positive test in individuals who suffer from production disorders and the probability of a positive test in individuals who do not suffer from production disorders. This result is a good result because getting a value >1 indicates a positive test result in the population group with greater production disruption than in the group without production disruption. This research obtained an LR- IPF% value of 0.19. The negative likelihood ratio shows the ratio between the probability of a negative test result in individuals suffering from production disorders and the probability of negative test results in individuals who do not suffer from production disorders. The LR- IPF% result of 0.19 in this study is good because the smaller the LR- value, the better. The IPF% accuracy value in this study was 83.9%, which is very good. The accuracy value shows the IPF%’s ability to correctly detect all subjects tested.

The results of this study show the reliability of IPF% in detecting production disorders in thrombocytopenia patients at health centers that have the Sysmex automatic tool for assessing IPF%.

The limitation of this research is the difficulty of finding samples in the non-production disruption group. Not all patients undergo BMA because management is in accordance with clinical practice guidelines for each existing diagnosis. The diagnosis of samples from the non-production disorder group did not use the gold standard for each diagnosis, but only used working bias. Validation cohort for Further research needs to be carried out to confirm the ability of IPF% in detecting production disruptions in thrombocytopenia

CONCLUSION
From this study, it can be concluded that the IPF% cut-off value set in this study is <7.5% to detect production disorders in patients with thrombocytopenia. This IPF% cut-off value was determined to obtain sensitivity of 83.7%, specificity of 84.4%, PPV of 89.1%, NPV of 77.1%, LR+ 5.53, LR- 0.19, and accuracy of 83.

CONFLICT OF INTEREST
The authors declare that no competing financial, professional, or personal interests might have affected the performance or presentation of the work described in this manuscript.

ETHICAL STATEMENT
The study was conducted following the Declaration of Helsinki and approved by The Research Ethics Committee of the Faculty of Medicine, Universitas Udayana, with number 1014/UN14.2.2.VII.14/ LT/2022.

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AUTHOR CONTRIBUTION
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REFERENCES