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Immature Platelets in Hematologic Malignancies: A Meta-Analysis

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Abstract. Thrombocytopenia is a common hematological complication in patients with malignant hematological diseases, particularly after chemotherapy or hematopoietic stem cell transplantation (HSCT). This condition increases the risk of bleeding and necessitates strict hematological monitoring and platelet transfusions. Immature Platelet Fraction (IPF), a parameter reflecting thrombopoiesis, has emerged as a potential biomarker for predicting platelet recovery. This meta-analysis evaluates the role of IPF and IPF% in predicting platelet recovery in patients with hematological malignancies post-chemotherapy and HSCT. A systematic literature search was conducted across multiple databases, including PubMed, EMBASE, and Cochrane Library, from 2014 to 2024. Studies were selected based on predefined criteria, including patient population, intervention, and outcomes related to platelet recovery. The analysis revealed that IPF% increases earlier than other hematological parameters, with a peak occurring 1–11 days before platelet recovery. However, significant heterogeneity ($I^2 = 99\%$) was observed among studies, indicating variability in methodologies and patient populations. Despite promising results, the clinical application of IPF% requires further validation to establish optimal cutoff values and standardize measurement techniques. This meta-analysis highlights the potential of IPF% as a predictive biomarker for platelet recovery, while emphasizing the need for more robust studies to enhance its reliability in clinical practice.

Keywords: *Immature Platelet Fraction (IPF), Thrombocytopenia, Platelet Recovery, Hematological Malignancies, Chemotherapy, Hematopoietic Stem Cell Transplantation (HSCT).*

A. INTRODUCTION

Thrombocytopenia is a common hematologic complication in patients with hematologic malignancies, particularly following chemotherapy or hematopoietic stem cell transplantation (HSCT). This condition increases the risk of bleeding and requires close hematologic monitoring and interventions such as platelet transfusions. Clinical guidelines generally recommend platelet transfusions when the count drops to 5–20 × 10^9/L, and at higher levels for patients undergoing invasive procedures. Therefore, predicting the timing of platelet recovery is a crucial aspect in determining optimal transfusion strategies and preventing complications from prolonged thrombocytopenia (Young, 2018).

One parameter that can be used to predict platelet recovery is the immature platelet count, also known as reticulated platelets. These platelets are newly released from the bone marrow, are larger in size, and contain more RNA than mature platelets. The number of immature platelets reflects thrombopoietic activity and thus can serve as an indicator of platelet regeneration after chemotherapy and HSCT. Immature platelet measurement was initially performed using flow cytometry with fluorescent dyes such as thiazole orange, which binds to RNA. Several studies have shown that immature platelet counts measured with this method correlate with the level of thrombopoiesis. Therefore, this parameter holds potential for use in the differential diagnosis of platelet production disorders and as a predictor of platelet recovery following chemotherapy and HSCT (Yang, 2024).

However, the clinical application of immature platelet measurement faces significant challenges. Flow cytometry techniques lack a universal standard, resulting in significant variability in reference ranges across different methods and laboratories. This limits the use of immature platelets as a routine parameter in managing patients with thrombocytopenia (Jeon, 2020).

With advances in technology, automated detection methods for immature platelets are now available through fluorescence-based hematology analysis systems, such as the reticulocyte detection (RET) channel in the XE-2100 hematology analyzer (Sysmex, Japan). This method uses a polymethine RNA dye to detect immature platelets and expresses the results as the Immature Platelet Fraction (IPF), reported both as an absolute value and as a percentage of total platelets (IPF%). The advantages of this method include its ability to provide real-time measurement results as part of routine complete blood count analysis, with better reproducibility and stability compared to conventional flow cytometry (Benlachgar, 2020).

Several independent studies have shown that IPF or IPF% measurement can be used for the differential diagnosis of thrombocytopenia and the evaluation of thrombopoiesis. Furthermore, elevated IPF% levels have been found to correlate with platelet recovery following chemotherapy and HSCT. Therefore, IPF has potential as a more practical biomarker in predicting platelet recovery in patients with hematologic malignancies.

Although various studies have evaluated the utility of IPF in this context, discrepancies remain in the findings reported, particularly regarding the relationship between IPF% levels and the timing of platelet recovery. Hence, a more comprehensive analysis is required to synthesize existing findings and evaluate the reliability of IPF as an indicator of platelet recovery.

This meta-analysis aims to evaluate the role of the Immature Platelet Fraction (IPF) and IPF% as biomarkers in predicting platelet recovery in patients with hematologic malignancies following chemotherapy and hematopoietic stem cell transplantation (HSCT). In addition, this study analyzes the relationship between IPF% levels and platelet recovery time to determine a clinically applicable cutoff value. By identifying an optimal cutoff, IPF% is expected to serve as a more reliable indicator for estimating the timing of platelet recovery and assisting in decision-making regarding platelet transfusion. Moreover, this meta-analysis compares the accuracy of various immature platelet detection methods, including flow cytometry and the automated RET channel system, in assessing platelet regeneration. By comparing the advantages and limitations of each method, this study aims to provide insight into the most efficient and accurate techniques for clinical application.

B. METHODS

1. Data Sources and Search Strategy

This meta-analysis was conducted in accordance with the Cochrane and PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A systematic literature search was performed in PubMed, EMBASE, ScienceDirect, Google Scholar, and the Cochrane Library to identify relevant studies published between 2014 and 2024. The keywords used included the following combinations:

"Immature platelet fraction" OR "reticulated platelets" OR "IPF" AND "hematologic malignancies" OR "chemotherapy-induced thrombocytopenia" OR "stem cell transplantation" AND "platelet recovery" OR "thrombopoiesis" OR "flow cytometry" OR "RET channel method".

The complete search strategy is presented in Figure 1. In addition, manual screening of the reference lists of selected studies was conducted to ensure that no relevant articles were missed.

Table 1. Database Search Strategy

Database	Search Strategy	Number of Results (Hits)
PubMed	("immature platelet fraction" OR "reticulated platelets" OR "IPF") AND ("hematologic malignancies" OR "chemotherapy-induced thrombocytopenia" OR "stem cell transplantation") AND ("platelet recovery" OR "thrombopoiesis" OR "flow cytometry" OR "RET channel method")	25
EMBASE	('immature platelet fraction'/exp OR 'reticulated platelets'/exp OR 'IPF'/exp) AND ('hematologic malignancies'/exp OR 'chemotherapy-induced thrombocytopenia'/exp OR 'stem cell transplantation'/exp) AND ('platelet recovery'/exp OR 'thrombopoiesis'/exp OR 'flow cytometry'/exp OR 'RET channel method'/exp)	30
Cochrane Library	("immature platelet fraction" OR "reticulated platelets" OR "IPF") AND ("hematologic malignancies" OR "chemotherapy-induced thrombocytopenia" OR "stem cell transplantation") AND ("platelet recovery" OR "thrombopoiesis" OR "flow cytometry" OR "RET channel method")	5
Web of Science	TS= ("immature platelet fraction" OR "reticulated platelets" OR "IPF") AND TS= ("hematologic malignancies" OR "chemotherapy-induced thrombocytopenia" OR "stem cell transplantation") AND TS= ("platelet recovery" OR "thrombopoiesis" OR "flow cytometry" OR "RET channel method")	20
Scopus	TITLE-ABS-KEY ("immature platelet fraction" OR "reticulated platelets" OR "IPF") AND TITLE-ABS-KEY ("hematologic malignancies" OR "chemotherapy-induced thrombocytopenia" OR "stem cell transplantation") AND TITLE-ABS-KEY ("platelet recovery" OR "thrombopoiesis" OR "flow cytometry" OR "RET channel method")	22

2. Study Selection and Eligibility Criteria

Studies were included based on the following criteria:

a. Study population: Patients with hematologic malignancies experiencing thrombocytopenia due to chemotherapy or hematopoietic stem cell transplantation (HSCT).
b. Intervention: Measurement of IPF and IPF% using either flow cytometry or an automated RET channel-based method.
c. Study design: Randomized controlled trials (RCTs), cohort studies, or case-control studies.
d. Reported outcomes: At least one of the primary parameters, such as time to platelet recovery, correlation between IPF% levels and platelet recovery, or comparison of the accuracy of immature platelet detection methods.

Studies were excluded if:

a. They were non-clinical studies (e.g., in vitro studies or animal research).
b. They involved fewer than 50 participants.

c. They did not use a consistent standard method for measuring IPF/IPF%.
d. The full text was unavailable or outcome data were insufficient.

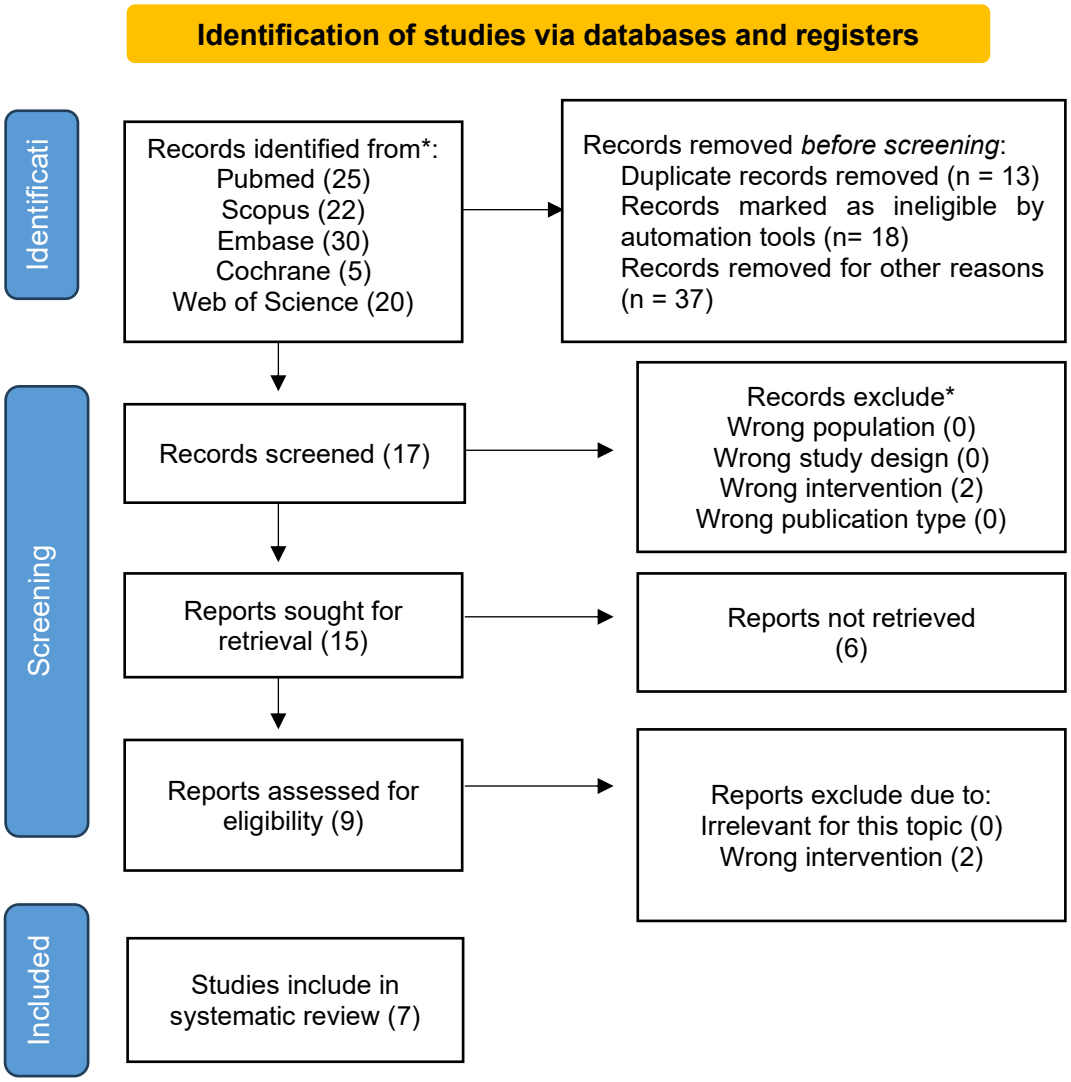

Figure 1. Study Selection Flow Diagram

3. Data Extraction and Risk of Bias Assessment

Two independent reviewers extracted the data using a standardized form. Collected variables included patient characteristics, type of hematologic malignancy, chemotherapy or HSCT regimen, IPF/IPF% detection methods, and the relationship between IPF% and platelet recovery.

Disagreements in data extraction were resolved through discussion with a third reviewer. Risk of bias was assessed using the Cochrane Risk of Bias Tool for randomized controlled trials (RCTs) and the JBI Critical Appraisal Tool for observational studies.

4. Primary Parameters and Outcomes

The primary outcomes analyzed in this meta-analysis included:

a. Time to platelet recovery following chemotherapy or HSCT.
b. The relationship between IPF% levels and platelet recovery time to determine the optimal cutoff value.

c. Accuracy of immature platelet detection methods, including flow cytometry and the automated RET channel method.

5. Statistical Analysis

All statistical analyses were performed using Review Manager (RevMan) version 5.4. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for categorical variables. Heterogeneity was assessed using the I^2 statistic, with $I^2 > 50\%$ indicating substantial heterogeneity. A fixed-effect model was used for low heterogeneity ($I^2 < 50\%$), while a random-effects model was applied otherwise. Sensitivity analysis was conducted to assess the robustness of the results, and potential publication bias was also evaluated. Statistical significance was defined as $p < 0.05$.

C. RESULTS AND DISCUSSION

1. IPF and How the Fraction Is Determined by Analyzers

The Immature Platelet Fraction (IPF) is the percentage of immature platelets relative to the total platelet count. Immaturity is determined by two main characteristics: large platelet size and high fluorescence intensity. First-generation automated analyzers used a reticulocyte/platelet channel that measured the immature platelet fraction relative to the total platelet count using flow cytometry. Second-generation analyzers employ a dedicated platelet channel (PLT-F), which provides more sensitive and specific results. Various fluorochromes have been tested, but the latest instruments use Oxazine dye. High fluorescence intensity in immature platelets occurs due to the interaction between platelet RNA and the dye. This interaction requires perforation of the platelet membrane by a reagent, allowing staining with Oxazine. Data analysis is performed using specialized algorithm-based software (IPF software) (Benlachgar,2014).

2. IPF Reference Values

Several studies have proposed reference ranges for physiologic immature platelet percentages. These reference intervals are important parameters that vary across studies. Table 1 summarizes 33 studies that evaluated IPF using Sysmex XE/XN instruments in healthy adult populations. The suggested ranges span from 0.3% to 17.8%. The large variation across studies raises questions about the reliability of these intervals. One of the most notable studies, conducted according to CLSI guidelines and involving a large population, was by Jung H et al., who reported IPF values of 0.5–3.2% for men and 0.4–3.0% for women in a study of 2,039 healthy adults in Korea. KO YJ et al. reported an average IPF of 1.6% (range 0.3–7.4) in 2,104 healthy adults in Korea. Joergensen MK et al. reported reference IPF values of 1.3–9.0% in 1,674 healthy adults in Denmark (Benlachgar,2014).

Following the introduction of newer-generation Sysmex devices with a dedicated platelet channel, Young Jin Ko et al. compared the XE and XN analyzer generations. They concluded that the XN series had higher and broader IPF reference intervals. The results and characteristics of four studies on IPF using the XN series in healthy adults are summarized in Table 1, with IPF ranges between 0.7–10.1%. Some studies also reported sex-specific reference intervals (Benlachgar,2014).

Reference values for IPF differ in pediatric populations; however, studies in this group are limited due to the challenges of selecting healthy subjects. As illustrated in **Table 2**, for the neonatal age group, the reference range for IPF is 1.5–5.9%. The largest study was conducted retrospectively, involving 8,967 neonates with normal platelet counts. Results were presented as curves from birth, based on gestational age, and over the first 90 days of life, using the 5th to 95th percentile reference intervals. The use of age-specific intervals appears to be more appropriate for pediatric patients with thrombocytopenia. Studies on both adult and pediatric

populations have several limitations, particularly in the methodological differences used to determine reference intervals (Benlachgar,2014).

3. Stability of IPF Determination

Many factors affect the stability of IPF results, including the condition/disease, type of anticoagulant, storage temperature, and duration of storage. The majority of studies prefer blood samples collected in K2-EDTA. In healthy individuals, IPF values remain stable at room temperature for up to four days when stored in K2-EDTA, acid-citrate-dextrose (ACD), and citrate-theophylline-adenosine-dipyridamole (CTAD). At 4°C, IPF values progressively increase in various anticoagulants, including K2-EDTA, ACD, CTAD, and sodium fluoride. This increase can be predicted and corrected using a simple algorithm. Nishiyama et al. also reported high stability with the Sysmex XE-5000 using the CTAD anticoagulant in patients with chronic immune thrombocytopenia. In several other studies, IPF measurements remained stable at room temperature for 24–48 hours after blood collection.

4. Relationship Between Immature Platelet Parameters (Reticulocytes) and MPV

Mean platelet volume (MPV) is a parameter that reflects the average size of platelets. Thrombocytopenia with high MPV has been reported in diseases with increased destruction or consumption of platelets. In contrast, low MPV associated with thrombocytopenia is found in diseases with platelet production disorders. However, MPV variation should be interpreted cautiously as most data come from retrospective studies with small populations, without prospective validation of MPV cut-off standards. IPF, which reflects large platelets with high fluorescence intensity, has been found in many studies to correlate positively with MPV and immature platelets. However, Meintker et al. found a weak correlation between the two, while Richards EM et al. did not report any correlation. Meintker et al. concluded that MPV cannot replace IPF. Similar conclusions have been reported in children for differentiating between immune thrombocytopenia and acute lymphoblastic leukemia. Several factors influence this correlation, such as the type of instrument used (Sysmex or CD Sapphire), the mechanism of thrombocytopenia, total platelet count, and sample storage conditions.

5. Contribution of IPF in Clinical Practice for Hematologic and Non-Hematologic Disorders

a. IPF and Immune Thrombocytopenia (ITP)

Patients with ITP have an increased number of large platelets and high MPV values. This increase may lead to falsely elevated IPF values. Some studies have shown increased IPF in adult patients with ITP, with no difference between pediatric and adult populations or between genders. In ITP patients, there is a strong correlation between platelet count and IPF, with IPF returning to normal values during remission.

b. IPF in Thrombotic Thrombocytopenic Purpura (TTP)

Thrombocytopenia in microangiopathy syndromes occurs due to increased platelet destruction and consumption in the peripheral circulation. High IPF values have been reported in TTP patients. Briggs et al. reported high IPF in all 11 TTP patients, with an average value of 17.2% (range 11.2–30.9%). TTP remission was associated with the normalization of IPF values. Hong H. reported that increased IPF and decreased absolute immature platelet count (A-IPC) were significant in 18 idiopathic TTP patients.

c. IPF in Disseminated Intravascular Coagulation (DIC)

In DIC, the percentage of immature platelets (IPF-%) is estimated to increase. A study by Hong KH et al. showed a correlation between IPF and DIC scores, and that IPF can predict mortality in this condition. However, another study by Cannavo I. et al. found increased IPF in

only 7 of 18 DIC patients, with the increase correlating with the severity of DIC and thrombocytopenia.

d. IPF in Aplastic Anemia

In aplastic anemia (AA) with thrombocytopenia as the main manifestation, differential diagnosis from other causes is difficult without invasive procedures. Studies show that IPF-% increases in AA, but not as high as in immune thrombocytopenia (ITP). Several studies have established threshold values to differentiate the primary mechanisms of thrombocytopenia: peripheral destruction vs bone marrow hypoproduction. Jung H. et al. established an IPF threshold value of 7.3% to differentiate ITP from AA, with a sensitivity of 54% and specificity of 92.2%.

e. IPF in Chemotherapy-Induced Thrombocytopenia and Stem Cell Transplantation

Standard chemotherapy causes thrombocytopenia due to impaired central production. IPF increases 2–3 days before platelet recovery, both after chemotherapy and stem cell transplantation. This can prevent unnecessary platelet transfusions and serve as an indicator of successful engraftment following both autologous and allogeneic stem cell transplantation. In patients undergoing bone marrow transplantation with a non-myeloablative regimen, platelet recovery occurs more quickly with an IPF threshold of 10%. However, a study by Meintker L. et al. found no predictive value of IPF for platelet recovery after intensive chemotherapy, possibly due to dilution effects from platelet transfusions.

f. IPF in Myelodysplastic Syndromes (MDS)

Sugimori N. et al. reported increased IPF-% in 51 MDS patients, with no correlation to platelet count. Some patients with normal platelet count still exhibited high IPF, which was associated with chromosomal abnormalities (monosomy 7). Therefore, the IPF parameter should be interpreted alongside other parameters from hematology analysis devices, such as white blood cell precursors and white blood cell differentiation.

g. IPF in Cardiovascular Diseases

Several studies have reported increased IPF in patients with coronary artery disease, especially during the acute phase of acute coronary syndrome (ACS), with higher increases in smokers and diabetic patients. Lopez-Jimenez RA et al. found that ACS patients with IPF >6.2% had a higher probability of mortality. However, some studies did not find a relationship between IPF and clinical outcomes in patients undergoing coronary angiography. Additionally, IPF has been studied as an indicator of response to antiplatelet therapy, but results vary depending on the measurement method and time of assessment. In cyanotic congenital heart disease (CCHD), patients with thrombocytopenia have a median IPF of 16.3%. The mechanism of thrombocytopenia in CCHD is not fully understood, but the increase in IPF suggests a peripheral destruction mechanism.

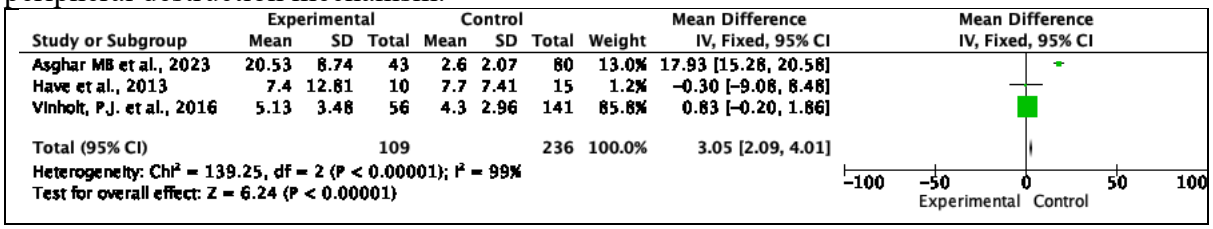

Figure 2. Forest Plot of Immature Platelet Fraction in Hematologic Malignancies

This forest plot presents the results of a meta-analysis from three studies comparing the Immature Platelet Fraction (IPF) between the experimental and control groups. The studies included are Asghar MB et al. (2023), Have et al. (2013), and Vinholt PJ et al. (2016). The results show that the experimental group had a higher IPF compared to the control group, with a combined mean difference of 3.05 (95% CI: 2.09–4.01), which is statistically significant ($Z = 6.24$, $P < 0.00001$). The study by Asghar MB et al. (2023) contributed the most to the overall

effect with a striking difference (20.53 ± 8.74 vs. 2.6 ± 2.07). Meanwhile, Have et al. (2013) did not show a significant difference (7.4 ± 12.81 vs. 7.7 ± 7.41), and Vinholt PJ et al. (2016) reported a small difference (5.13 ± 3.48 vs. 4.3 ± 2.96). However, the very high heterogeneity (I^2 = 99%) indicates substantial variation between the studies, which may be attributed to differences in methodology, patient characteristics, or other factors. Therefore, although the results suggest that IPF is higher in the experimental group, further interpretation is needed to understand the factors contributing to the high variation among the studies.

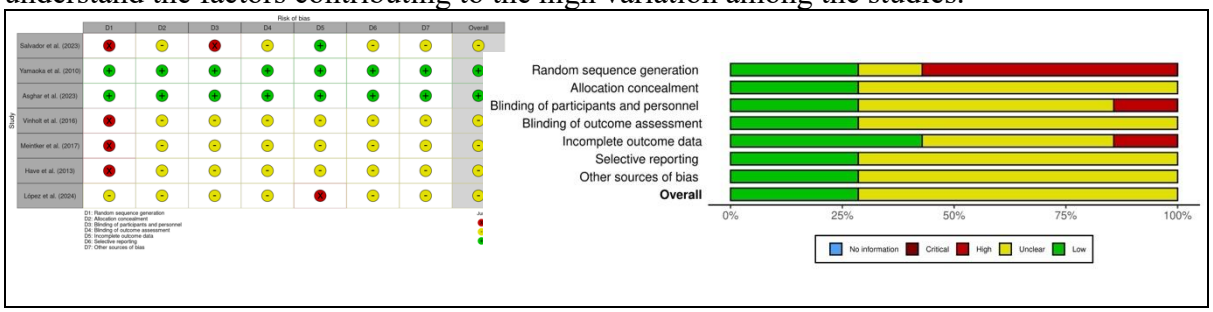

Figure 3. ***Risk of Bias***

h. Risk of Bias Analysis and Conclusion

Based on the risk of bias analysis, there is variability in the level of bias across the studies analyzed. The studies by Salvador et al. (2023), Vinholt et al. (2016), Meintker et al. (2017), and Have et al. (2013) been found to have a high risk of bias in random sequence generation (D1), indicating that their randomization methods may have been inadequate or not clearly described. In contrast, the studies by Yamaoka et al. (2010) and Asghar et al. (2023) demonstrated a low risk of bias in this domain.

Regarding allocation concealment (D2), most studies showed an unclear risk, which could potentially affect the validity of participant allocation. For blinding of participants and personnel (D3), a high risk of bias was noted in Salvador et al. (2023), suggesting that participants or healthcare providers may have been aware of the treatment being administered. Other studies tended to show an unclear level of bias in this domain.

In terms of blinding of outcome assessment (D4), most studies also had an unclear risk of bias due to insufficient information on the blinding procedures used in outcome evaluation. All studies had a low risk of bias for incomplete outcome data (D5), except López et al. (2024), which showed a high risk, likely due to missing or inadequately reported data.

For selective reporting (D6) and other sources of bias (D7), most studies demonstrated an unclear risk, indicating possible issues in data reporting and other potential biases. Overall, the studies with the lowest risk of bias were Yamaoka et al. (2010) and Asghar et al. (2023), whereas Salvador et al. (2023), Vinholt et al. (2016), and Meintker et al. (2017) had a higher risk of bias, particularly in terms of randomization and blinding.

To improve the quality of future research, more detailed descriptions of randomization methods, allocation concealment, and blinding procedures for participants and healthcare personnel are necessary.

D. CONCLUSION

This meta-analysis evaluated the role of *Immature Platelet Fraction* (IPF) and IPF% as biomarkers for predicting platelet recovery in patients with hematologic malignancies, particularly following chemotherapy or hematopoietic stem cell transplantation (HSCT). Overall, while IPF% shows promise as a predictive tool for platelet recovery and assessment of thrombopoiesis, its application in clinical practice still faces challenges, including variability in reference ranges, measurement methods, and potential confounding factors. Therefore, further studies are needed to establish optimal cutoff values and to assess the reliability of IPF%

in various clinical contexts. With such advancements, IPF% could become a more reliable tool in managing patients with thrombocytopenia, aiding in decisions regarding platelet transfusion, and reducing the risk of complications associated with prolonged thrombocytopenia.

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