Archives of Academic Emergency Medicine. 2024; 12(1): e43

REVIEW ARTICLE

Immature Platelet Fraction and Acute Coronary Syndrome; a Systematic Review and Meta-Analysis

Elmira Jafari Afshar[1], Vahid Shahnavaz[1**], Hamed Talakoob[1], Parnaz Kafialqora[1], Aryan Madady[1], Shamimeh Pourbahrighesmat[1], Amirhossein Tayebi[1], Mohammadhossein MozafaryBazargany[2], Niloofar Gholami[1], Aryan Ayati[3], Parham Samimisedeh[1*], Hadith Rastad[1†], Hossein Karim[1]**

1. Cardiovascular Research Center, Alborz University of Medical Sciences, Alborz, Iran.
2. Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.
3. Tehran Heart Center, Cardiovascular Diseases Research Institute, Tehran University of Medical Sciences, Tehran, Iran.

Received: February 2024; Accepted: March 2024; Published online: 23 April 2024

Abstract: **Introduction:** Immature Platelet Fraction (IPF) is a measure of the proportion of reticulated platelets (RPs) to all platelets in circulation. IPF may have both prognostic and diagnostic values in patients with Acute Coronary Syndrome (ACS). This study aims to comprehensively summarize the diagnostic utility of IPF levels in patients with ACS, specifically focusing on its ability to differentiate between different subtypes of ACS. **Methods:** We conducted a systematic search in online databases including MEDLINE, Scopus, and Google Scholar up to March 4th 2024, to identify relevant studies. The random-effect model, employing inverse variance for mean differences (MD) and Mantel-Haenszel methods for odds ratios (OR) were utilized to combine the data. Joanna Briggs Institute (JBI) appraisal tool was employed to assess the quality of included studies. **Results:** Our systematic review contains 15 articles with a total sample size of 2,030 ACS patients. Pooled analysis revealed significant differences in IPF levels of ACS patients compared to healthy controls (MD (95%CI): 2.85 (0.86, 4.85), P-value = 0.004) and stable angina patients (MD (95%CI): 0.58 (0.23, 0.92), P-value < 0.001). Subgroup comparisons within ACS patients demonstrated higher IPF levels in myocardial infarction (MI) vs. unstable angina (UA) (MD (95%CI): 1.81 (0.41, 3.22), P-value = 0.01), ST elevation MI (STEMI) vs. non-ST elevation (NSTEMI) ACS (MD (95%CI): 0.74 (0.31, 1.17), P-value < 0.001), and NSTEMI vs. UA (MD (95% CI): 1.07 (0.24, 1.90), P-value = 0.01). **Conclusion:** IPF levels could increase in patients with ACS, particularly during the acute phase of STEMI. This suggests that IPF may be a useful biomarker for early diagnosis of ACS. Additionally, IPF levels may help differentiate between ACS subtypes.

Keywords: Acute coronary syndrome; Mean platelet volume; Myocardial infarction; Immature platelet fraction; Reticulated platelet

Cite this article as: Jafari Afshar E, Shahnavaz V, Talakoob H, et al. Immature Platelet Fraction and Acute Coronary Syndrome; a Systematic Review and Meta-Analysis. Arch Acad Emerg Med. 2024; 12(1): e43. https://doi.org/10.22037/aaem.v12i1.2292.

1. Introduction

Reticulated Platelets (RP) are immature large platelets that are newly released into circulation. They are more metabolically active than mature platelets, as they have more megakaryocyte-driven RNA content, which can lead them to synthesize proteins [1]. Immature Platelets Fraction (IPF) is a proportion of RPs to all platelets in the circulation, and it can reflect the platelets turnover and thrombopoiesis [2]. IPF can be utilized to differentiate between bone marrow failure and peripheral destruction in patients with thrombocytopenia[3]. It can also evaluate treatment response and bone marrow recovery in conditions causing bone marrow suppression [1, 4, 5].

Interestingly, several studies have explored the association between IPF levels and cardiometabolic diseases. In this regard, studies reported increased IPF levels in patients with cardioembolic stroke, diabetes mellitus, Coronary Artery Disease (CAD), and Acute Coronary Syndrome (ACS)[6-8].

Previous investigations have explored the role of IPF as both prognostic and diagnostic markers in patients with ACS[9-12]. Regarding prognosis, evidence suggested that elevated IPF levels may worsen outcomes in ACS patients[12, 13]. Additionally, some studies suggested that elevated IPF levels could reduce the effect of anti-platelet drugs including P2Y12 inhibitors and aspirin, and may increase the risk of platelet aggregation and thrombosis[14-16].

Furthermore, IPF may have an additive value in early diag-

*Corresponding Author: Parham Samimisedeh; Cardiovascular Research Center, Alborz University of Medical Sciences, Alborz, Iran. Email: parham-samimimd@gmail.com, TEL: 09122716812, 0000-0002-3667-8142.

†Corresponding Author: Hadith Rastad; Cardiovascular Research Center, Alborz University of Medical Sciences, Alborz, Iran. Email: h.rastad91@gmail.com, TEL: 09101748813. ORCID: 0000-0001-9037-0782.

** Elmira Jafari Afshar & Vahid Shahnavaz equally contributed to this work and shared the first author.

nosis of ACS, as its levels rise in the acute phase of the disease[11, 17-19]; however, studies reported controversial results in this regard[20-22] [23]. Moreover, its possible role in distinguishing different subtypes of ACS remains unclear [24, 25]. Therefore, the objective of this study is to provide a comprehensive summary of the existing evidence regarding the diagnostic utility of IPF levels in ACS, specifically emphasizing its ability to differentiate between various types of ACS.

2. Methods

2.1. Study design and setting

This systematic review was conducted following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines[26]. We included all original studies assessing the role of IPF levels in ACS in terms of prediction, diagnosis, subtype differentiation, and clinical outcomes.

Institutional committee ethical approval was not required as this study is a systematic review of previously published articles.

2.2. Search strategy

A systematic search was conducted on online databases, including MEDLINE and Scopus, up to March 4^{th} 2024, using a combination of the related keywords in two domains:

1) Immature Platelet Fraction (IPF)

2) Acute Coronary Syndrome (ACS)

We used the Boolean operator "OR" to connect key terms within each domain and the "AND" operator for connecting domains.

In addition, we manually screened the first 100 pages of Google Scholar and the reference list of the relevant articles for any possible additional citations. The detailed search strategy of each database is presented in Supplementary table 1.

All citations from retrieved documents were imported into EndNote software (version X9.3.2, Captivate Analytics, California USA), and then, duplicate articles were removed.

2.3. Study selection

Two researchers (E. JA. & V. SH.) independently screened the titles, and abstracts, followed by the full texts of the imported articles to find eligible studies; any disagreement was resolved via discussion.

To be included in our review study, studies had to report IPF levels in ACS patients and meet the following criteria:

1. Being written in English

2. Having an observational study design, including cohort, cross-sectional, or case-control studies

3. Assessing the association of IPF levels with at least one of the following endpoints in ACS: the risk, diagnosis, subtypes, and clinical prognosis

4. Quantitative synthesis-oriented studies must measure IPF levels before ACS medical or device-based treatment at the acute phase and/or assess the prognostic values of IPF at follow-up

2.4. Exclusion criteria

1. Animal studies, in-vitro studies, and review articles

2.5. Data extraction

We extracted the following data from the full text of included articles into "Data extraction form" in Microsoft Excel (Version 2016, Microsoft Corp., Redmond, WA, USA): First author's name, publication year, country, study design, sample size, age, gender, types of instrument used to measure IPF, serum levels of IPF, mean platelet volume (MPV),Immature Platelet Counts (IPC), and clinical outcomes of ACS.

2.6. Quality assessment

We used the Joanna Briggs Institute (JBI) appraisal tool checklists adapted for cross-sectional studies to assess the quality of included studies[27].

2.7. Statistical analysis

Our primary goal for meta-analysis was to compare the IPF levels between ACS patients and controls (either healthy subjects or stable angina), or between different ACS subgroups.

A random-effect or fixed-effect model was used to pool the effect sizes based on the heterogeneity size of the Mean Differences (MD) across studies; the random-effect model was used when I2 > 75% or the P-value < 0.01 (from Q-test). We used the inverse variance method to pool the MDs of IPF levels between the ACS and control groups and also between different ACS subgroups.

We also used the Mantel-Haenszel method to pool reported odds ratios and corresponding standard errors. Egger's linear regression test of funnel plot asymmetry was used to check the publication bias between studies. We performed our meta-analysis using the Meta package in R-studio software (version 4.3.1).

3. Results

Our comprehensive systematic search of databases identified 603 publications; after removing duplicates (n = 80), studies were screened based on their title/abstracts (n = 523), followed by the full texts (n = 42). Finally, we included 15 articles that met our eligibility criteria [10-13, 15, 18-25, 28-30] in our review of which, 11 were included in our quantitative synthesis. The detailed study selection process is presented in Supplementary figure 1.

3.1. Characteristics of included studies

Of the 15 studies included in our review, 7 studies involved a control group (healthy subjects (n=3)[18, 19, 23], stable angina (n=3)[11, 22, 24], or both (n=1)[28]), and the remaining studies (n=8) solely evaluated IPF levels in ACS patients. Seven studies performed a subgroup comparison between different subtypes of ACS. Furthermore, MPV values were re-

ported in 12 included studies alongside IPF levels, of which four studies employed a control group (either stable angina or healthy subjects)(Table 1).

The included articles were published between 2008 and 2021, and all studies adopted a cross-sectional (n = 9) or prospective cohort (n = 6) design; however, for our meta-analysis, we chose to treat all studies as cross-sectional, focusing exclusively on baseline IPF measurements that captured a cross-sectional snapshot of IPF levels at the beginning of each study. Studies were mainly conducted in Italy (n = 3), Denmark (n = 2), Indonesia (n = 2), and Israel (n = 2), followed by the USA, Taiwan, Pakistan, Egypt, Germany, and Spain (n = 1 each).

The total sample size of ACS patients was 2,030, ranging from 44 to 372 patients across studies. The mean age (standard deviation; SD) of the ACS groups varied between 57 (11.6) and 76 (9.8) years in the included studies. Notably, the male gender consistently predominated in all studies within the ACS groups (Table 1).

Except for one study[19], which used the conventional flow cytometry technique, all studies utilized an automated hematological analyzer (Sysmex Corporation, Kobe, Japan) to measure IPF levels (Table 1).

3.2. Risk of bias assessment

The risk of bias assessment of the included studies using the JBI appraisal tool revealed that all studies scored between six to eight points, indicating the high quality of the studies. Supplementary table 2 provides further details on the risk of bias assessment results.

3.3. Qualitative synthesis

Comparison of platelet turnover indicators between ACS and stable angina groups

Four studies compared the indicators of platelet production and turnover including IPF levels or IPF& MPV between the ACS and stable angina patients [11, 20, 22, 28]. All studies aimed to evaluate the diagnostic value of these platelet indicators in distinguishing ACS from stable angina in patients with typical chest pain and/or positive stress tests. Three out of these four studies reported significantly higher IPF values in the ACS group compared to the stable angina patients; while, in one study there were no significant differences in IPF levels between the two groups[20]. Of the three studies that also compared MPV values between the ACS and stable angina patients, only one study reported significantly higher MPV values in the ACS patients[11], while the other two studies found no significant differences between the two groups.

Moreover, in the aforementioned study which reported higher values of MPV in the ACS group, a binary logistic regression revealed that MPV can be utilized as an independent predictor in patients with ACS (OR: 5.08, 95% CI: (1.9, 13.5), P-value < 0.001). Alongside the comparison of IPF and MPV levels between ACS and stable angina patients, Grove et al. [28] also compared these platelet parameters and platelet counts in three ACS subgroups including ST-Elevation Myocardial Infarction (STEMI), Non-ST-Elevation Myocardial Infarction (NSTEMI), and Unstable Angina (UA), and also with healthy subjects as a control group. Their results suggest that the highest value of IPF was seen in the STEMI patients and IPF levels followed a decreasing trend from STEMI to NSTEMI, unstable angina, and stable angina patients, respectivel Interestingly there were no significant differences between stable angina patients and healthy individuals regarding IPF levels. Furthermore, their results revealed no significant differences in MPV levels and platelet counts between the three ACS subgroups (STEMI, NSTEMI, and UA) or between the ACS and stable angina patients or healthy subjects. Overall, ACS subgroup analysis in terms of IPF levels was conducted in seven studies out of all included studies. In five studies there was a significant decreasing trend for IPF levels from STEMI to NSTEMI and UA patients, respectively[18, 19, 25, 28, 29]; however, no significant differences were found in the other two studies[21, 24].

Comparison of platelet turnover indicators between ACS and healthy subjects

All three studies with healthy subjects as a comparison group reported significantly higher IPF levels in the ACS group[18, 19, 23]. Additionally, Khalifa et al. [19] performed a binary logistic regression analysis and concluded that IPF is an independent risk factor for acute coronary syndrome disease (OR: 1.09, 95% CI: (1.01, 19.8), P-value = 0.04). Gonzales et al. [18] also compared MPVs and platelet counts between the ACS and healthy controls and found that although MPVs were significantly higher in the ACS groups, platelet counts were greater in healthy controls.

Platelet turnover indicators in ACS subjects without a control group

Among the included studies, eight of them exclusively reported on IPF levels in ACS patients. Out of these, four studies specifically compared IPF results between different ACS subgroups, while the remaining three studies provided IPF results for the ACS group as a whole without subgroup analysis. In studies that compared the IPF levels in ACS subgroups, two studies reported significantly higher IPF levels in STEMI compared to NSTEMI and unstable angina patients[25, 29]; however, in the other two studies which compared IPF values between STEMI and NSTEMI patients, no significant differences were found[21, 24].

MPV comparisons among different ACS subgroups were examined in three of the mentioned studies. Among them, one study reported the highest MPV value in patients with STEMI, while the lowest MPV value was observed in patients with unstable angina[29]. However, the other two studies did not find any significant differences in MPV between the ACS subgroups[21, 25].

Studies exclusively included in the qualitative synthesis

Four studies did not meet the inclusion criteria for quantitative synthesis in our study. Among these, three studies reported IPF levels and MPV in ACS patients without a con-

trol group or subgroup analysis. Funk-Jensen et al. [30] measured platelet turnover indices including IPF, Immature Platelet Count (IPC), and MPV at various time points following STEMI. The study reported a significant decrease in IPF, IPC, and MPV from before Primary Percutaneous Intervention (PPCI) to twelve hours after the procedure. However, no significant differences were observed in these indices between the 12-hour and 3-month post-PPCI periods. Their results suggested increased IPF levels at the acute phase of STEMI.

Another time-course study conducted by Fabbri et al.[15], investigated IPF and High fluorescent IPF (H-IPF) levels in patients with acute coronary syndrome (ACS), specifically focusing on those with late High Platelet Reactivity (HPR) at 6 and 12 months after ACS. The study found significantly higher IPF and H-IPF levels in patients with late HPR (HPR at 6 or 12 months) compared to those without HPR. Cesari et al.[10], reported a direct correlation between IPF levels and the rate of cardiovascular death among patients with ACS at 12-month follow-up based on their receiver operating characteristic (ROC) curve analysis; the IPF cut-off of 3.3% could be utilized to predict 12-month mortality in patients with ACS.

In another study conducted by Cesari et al.[12], they compared IPF and high-fluorescent IPF levels between patients with acute coronary syndrome (ACS) and healthy subjects. However, due to the blood samples being obtained after a percutaneous coronary intervention (PCI) procedure, we were unable to include this study in our meta-analysis. The PCI procedure has the potential to introduce confounding factors that could interfere with the results of other studies included in the analysis. Nevertheless, the Cesari et al. [12] study revealed significantly higher levels of IPF and high-fluorescent IPF in patients with ACS compared to the reference group. No significant differences were found regarding MPV in this study.

Quantitative synthesis

Given the high heterogeneity observed between studies, we utilized the random-effect model to pool the mean differences in IPF levels between comparison groups. Significantly higher levels of IPF were observed in ACS groups compared to both healthy controls (mean differences (MD) (95% CI): 2.85 (0.86, 4.85), P-value = 0.004) and stable angina patients (MD (95% CI): 0.58 (0.23, 0.92), P-value < 0.001) (Figure 1). Furthermore, we compared mean IPF levels between patients with MI and UA, and between those with STEMI and non-ST elevation ACS (NSTEACS). Our analysis revealed significantly higher levels of IPF in the MI group compared to the unstable angina group (MD (95% CI): 1.81 (0.41, 3.22), P-value = 0.01) (Figure 2), and in the STEMI group compared to NSTEACS (MD (95% CI): 0.74 (0.31, 1.17), P-value < 0.001) (Figure 2).

We also conducted pairwise analysis between three ACS subgroups regarding the levels of IPFs; the results revealed STEMI patients had higher mean IPF levels compared to NSTEMI (MD (95% CI): 0.49 (0.17, 0.81), P-value = 0.002) and UA patients (MD (95% CI): 2.57 (0.33, 4.81), P-value = 0.02). Moreover, higher levels of IPF were detected in NSTEMI patients than in UA patients (MD (95% CI): 1.07 (0.24, 1.90), P-value = 0.01).

The comparison of MPV between patients with ACS and healthy subjects revealed no significant differences (MD (95% CI): 0.19 (- 0.15, 0.54), P-value = 0.27). Similarly, no significant differences in MPV were observed when comparing ACS patients to those with stable angina (MD (95% CI): 0.26 (- 0.19, 0.72), P-value = 0.21). Furthermore, the comparison of MPV between patients with MI and UA yielded non-significant results (MD (95% CI): 0.25 (- 0.25, 0.75), P-value = 0.34), as did the comparison between STEMI and NSTEACS patients (MD (95% CI): 0.09 (- 0.22, 0.39), P-value = 0.18) (Figure 3).

Egger's test for publication bias showed no significant publication bias in any of our analyses.

4. Discussion

Our meta-analysis revealed a significant increase in levels of IPF in patients with ACS compared to both stable angina patients and healthy subjects. However, no significant differences were observed in MPV values among these groups. Furthermore, when we compared IPF levels between different subgroups of ACS, we found that STEMI patients exhibited significantly higher IPF levels compared to other ACS subtypes upon admission. Notably, there was a decreasing trend in IPF levels observed from STEMI to NSTEMI and then UA.

ACS is a critical condition that demands immediate diagnosis and treatment. While biomarkers like troponin, CK-MB, myoglobin, and BNP are commonly used for diagnosing ACS, their sensitivity and specificity are limited in early detection[6].To enhance accuracy and efficiency in early ACS diagnoses, researchers have investigated other biomarkers such as IPF and MPV[8, 29]. However, the association between IPF and ACS diagnosis is still being investigated. Immature platelets have been found to be larger, more metabolically active, and enzymatically active compared to smaller mature platelets. They also possess a higher thrombotic potential. In-vitro studies have shown that immature platelets have a stronger and faster adhesion response when exposed to collagen, adenosine diphosphate, and thrombin[17]. These findings indicate that immature platelets have the potential to significantly impact ischemic cardiovascular events and could serve as a valuable diagnostic tool for ACS[28]. Additionally, larger platelets are known to generate higher levels of thromboxane A2 and serotonin, which activate other platelets.

They also contain elevated levels of -granules, releasing various proteins like platelet-derived growth factors, transforming growth factor , platelet factor 4, and P-selectin. Immature platelets are considered markers of increased platelet turnover, which can impact platelet aggregation during

platelet inhibitor treatment [31]. Moreover, Elevated levels of IPF have been linked to increased inflammation and oxidative stress, which can result in heightened reactivity and a greater tendency to form blood clots compared to mature platelets. In this regard , higher IPF levels may be associated with elevated levels of inflammatory markers like C-reactive protein (CRP) and interleukin-6 (IL-6). During the inflammatory process triggered by ACS, the increased platelet turnover and activation may release immature platelets into circulation, leading to higher IPF levels[32]. IPF is a potential biomarker for early diagnosis of ACS. Elevated IPF levels indicate an ongoing inflammatory process, which could be indicative of ACS. Measuring IPF levels may help identify patients at high risk of developing ACS, even if they initially test negative for troponin I (TnI). Additionally, IPF levels are positively correlated with TnI levels, suggesting that IPF may be a useful complement to TnI testing[11].. The difference in IPF levels we found between STEMI and NSTEMI/unstable angina may be due to differences in the degree of inflammation, the extent of tissue damage, time course, platelet activation, and endothelial dysfunction[18, 28].

Multiple studies have demonstrated that IPF can serve as a valuable biomarker for predicting the risk of major adverse cardiovascular events (MACE) in patients with ACS[11, 14, 32]. Elevated IPF levels could suggest an insufficient response to treatment or potential platelet resistance. Monitoring IPF can assist in making clinical decisions regarding adjustments to the antiplatelet regimen or exploring alternative treatments. However, it is important to consider IPF alongside other parameters, and further studies are needed to fully understand its clinical utility[33]. MPV is another potential biomarker that shows promise in diagnosing and predicting ACS. Studies have indicated that elevated MPV levels are linked to increased platelet activity and have a significant impact on hemostasis. Furthermore, higher MPV levels have been associated with adverse clinical outcomes and impaired angiographic reperfusion in patients with myocardial infarction[8, 17].

4.1. Limitations and strengths

The current research investigating the utility of IPF and MPV as potential biomarkers for diagnosing and predicting ACS encounters several limitations. Firstly, there exists a dearth of established cut-off values for IPF and MPV levels across various subtypes of ACS. This lack of standardized thresholds hampers their clinical applicability as diagnostic markers. Secondly, considerable heterogeneity persists among studies, notably in terms of the devices utilized and the timing between symptom onset/diagnosis of ACS and the measurement of IPF and MPV. This heterogeneity may obscure findings and complicate data interpretation. Hence, there is a pressing need for original studies conducted under uniform conditions, which would afford greater clarity regarding the diagnostic utility of IPF and establish definitive cut-off values.

On a positive note, it is noteworthy that all studies included in our analysis exhibited high quality, bolstering the reliability and robustness of our findings.

5. Conclusions

Evidence suggests that there may be a correlation between increased levels of IPF and ACS, particularly in those with STEMI, indicating that IPF could potentially be useful in the early detection of ACS patients.

6. Declarations

6.1. Acknowledgments

The researchers would like to express their appreciation for the support and assistance provided by the Clinical Research and Development Units at Kamali and Rajaee Hospitals affiliated with Alborz University of Medical Sciences.

6.2. Conflict of interest

The authors declare that there are no conflicts of interest.

6.3. Funding

This research did not receive any funding.

6.4. Authors' contribution

Elmira Jafari Afshar, Parham Samimisedeh, and Hadith Rastad contributed equally to the conceptualization and design of the study. Parnaz Kafialqora, Vahid Shahnavaz, Aryan Madady, and Shamimeh Pourbahrighesmat performed data collection and analysis. Hamed Talakoob, Amirhossein Tayebi, Mohammadhossein MozafaryBazargany, and Niloofar Gholami contributed to the interpretation of results. Aryan Ayati, Parham Samimisedeh, Hadith Rastad, and Hossein Karim provided critical revisions and intellectual input. All authors reviewed and approved the final version of the manuscript.

6.5. Data Availability

The data supporting the findings of this study are available upon request.

6.6. Using artificial intelligence chatbots

There was no utilization of artificial intelligence chatbots in this study.

References

1. Gurbel, P.A., et al., Platelet-mediated thrombosis: from bench to bedside. Circulation research, 2016. 118(9): p. 1380-1391.
2. Shaikh, M.S. and S.A. Ali, Clinical utility of immature platelet fraction-an advanced parameter in laboratory hematology. Journal of the College of Physicians and Surgeons, 2016. 26(9), 798-799.

3. Briggs, C., et al., Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. British journal of haematology, 2004. 126(1): p. 93-99.
4. Barsam, S.J., et al., Platelet production and platelet destruction: assessing mechanisms of treatment effect in immune thrombocytopenia. Blood, The Journal of the American Society of Hematology, 2011. 117(21): p. 5723-5732.
5. Ali, I., C. Graham, and N.C. Dempsey-Hibbert, Immature platelet fraction as a useful marker in the etiological determination of thrombocytopenia. Experimental Hematology, 2019. 78: p. 56-61.
6. Chacko, S., et al., The role of biomarkers in the diagnosis and risk stratification of acute coronary syndrome. Future science OA, 2017. 4(1): p. FSO251.
7. Nardin, M., et al., Impact of active smoking on the immature platelet fraction and its relationship with the extent of coronary artery disease. European journal of clinical investigation, 2020. 50(2): p. e13181.
8. Aryanto, D., et al. Diagnostic value of mean platelet volume (MPV) to troponin T inpatients with acute coronary syndrome. in IOP Conference Series: Earth and Environmental Science. 2018. IOP Publishing.
9. Welder, D., et al., Immature platelets as a biomarker for disease severity and mortality in COVID-19 patients. British Journal of Haematology, 2021. 194(3): p. 530-536.
10. Cesari, F., et al., Reticulated platelets predict cardiovascular death in acute coronary syndrome patients. Thrombosis and haemostasis, 2013. 109(05): p. 846-853.
11. Huang, H.-L., et al., Clinical utility of mean platelet volume and immature platelet fraction in acute coronary syndrome. biomedical journal, 2019. 42(2): p. 107-115.
12. Cesari, F., et al., Relationship between high platelet turnover and platelet function in high-risk patients with coronary artery disease on dual antiplatelet therapy. Thrombosis and haemostasis, 2008. 99(11): p. 930-935.
13. Freynhofer, M.K., et al., Platelet turnover predicts outcome after coronary intervention. Thrombosis and Haemostasis, 2017. 117(05): p. 923-933.
14. Ibrahim, H., et al., Immature platelet fraction (IPF) determined with an automated method predicts clopidogrel hyporesponsiveness. Journal of thrombosis and thrombolysis, 2012. 33: p. 137-142.
15. Fabbri, A., et al., A time course study of high on treatment platelet reactivity in acute coronary syndrome male patients on dual antiplatelet therapy. Thrombosis Research, 2015. 136(3): p. 613-619.
16. Guthikonda, S., et al., Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. Journal of Thrombosis and Haemostasis, 2007. 5(3): p. 490-496.
17. Faber, J., et al., Immature platelets and risk of cardiovascular events among patients with ischemic heart disease: a systematic review. Thrombosis and Haemostasis, 2021. 121(05): p. 659-675.
18. Gonzalez-Porras, J.R., et al., The role of immature platelet fraction in acute coronary syndrome. Thrombosis and haemostasis, 2010. 103(01): p. 247-249.
19. Khalifa, K.A., M.A. Helwa, and A.M.M. Mousa, Reticulated platelets in acute coronary syndrome patients. Menoufia Medical Journal, 2017. 30(3): p. 880.
20. Berny-Lang, M.A., et al., Do immature platelet levels in chest pain patients presenting to the emergency department aid in the diagnosis of acute coronary syndrome? International journal of laboratory hematology, 2015. 37(1): p. 112-119.
21. Yahud, E., et al., Immature platelet fraction over time and clinical outcomes in patients with acute myocardial infarction. International Journal of Laboratory Hematology, 2021. 43(5): p. 966-972.
22. Cohen, A., et al., Immature platelets in patients hospitalized with Covid-19. Journal of Thrombosis and Thrombolysis, 2021. 51(3): p. 608-616.
23. Ijaz, S., et al., Levels of Immature Platelet Fraction in Patients of Acute Coronary Syndrome. Journal of Sharif Medical and Dental College Lahore, Pakistan 2021; Vol 7(01):37-42.
24. Bernlochner, I., et al., Impact of immature platelets on platelet response to ticagrelor and prasugrel in patients with acute coronary syndrome. European heart journal, 2015. 36(45): p. 3202-3210.
25. Paramita, P., A. Nurulita, and R. Pakasi, Analysis of immature platelet fraction and mean platelet volume in acute coronary syndrome patient. Young, 2019. 1: p. 2.
26. Page, M.J., et al., The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Bmj, 2021. 372.
27. Institute, J.B., The Joanna Briggs Institute Critical Appraisal Tools for Use in JBI Systematic Reviews Checklist for Analytical Cross Sectional Studies. North Adelaide, Australia The Joanna Briggs Institute, 2017.
28. Grove, E.L., A.-M. Hvas, and S.D. Kristensen, Immature platelets in patients with acute coronary syndromes. Thrombosis and haemostasis, 2009. 101(01): p. 151-153.
29. Indriastuti, E., Y. Hernaningsih, and Y.N. Indrasari, Immature Platelet Fraction as A Potential Marker To Differentiate Types of Acute Coronary Syndrome. Indonesian Journal of Clinical Pathology and Medical Laboratory, 2020. 27(1): p. 16-21.
30. Funck-Jensen, K.L., et al., Increased platelet aggregation and turnover in the acute phase of ST-elevation myocardial infarction. Platelets, 2013. 24(7): p. 528-537.
31. Handtke, S. and T. Thiele, Large and small platelets—(When) do they differ? Journal of Thrombosis and Haemostasis, 2020. 18(6): p. 1256-1267.
32. Bongiovanni, D., et al., Immature platelet fraction predicts adverse events in patients with acute coronary syndrome: the ISAR-REACT 5 reticulated platelet substudy. Arteriosclerosis, Thrombosis, and Vascular Biology, 2023. 43(2): p. e83-e93.

33. Gumiężna, K., et al., Immature platelet fraction in cardiovascular diagnostics and antiplatelet therapy monitoring. Cardiology Journal, 2023; 30(5):817-824.

Table 1: Patient characteristics and details of platelet turnover parameters in included studies

	Article	Study type	Groups	N	Male % (N)	Age (year)	Instrument	IPF Mean (SD)	MPV Mean (SD)	Main findings†	More findings
Articles with a control group (stable CAD)	Cohen et al. 2020 Israel	Prospective cohort	ACS	100	82 (82)	59.35 (12.8)	Sysmex XN--3000	4.8 (2.8)	NR	↑IPF in AMI > Control	IPF: AMI COVID-19 group > Stable angina ↓ IPC: AMI COVID-19 group > Stable angina ↓
			Control	64	64.1 (41)	66.23 (11.4)		3.77 (1.8)	NR		
	Huang et al. 2019 Taiwan	Cross-sectional	AMI	53	NR	NR	Sysmex 5000 XE	NR	10.7 (0.79)	↑IPF in ACS > Control ↑MPV in ACS > Control MPV between AMI and UA	MPV had an independent predictive value for ACS OR (95% CI): 5.08 (1.0, 13.5), P-value < 0.001 ROC curve: the cutoff value of MPV to diagnose ACS: 10.55 fL AUC: 0.73 (0.63, 0.83) Sensitivity: 54.2% Specificity: 82.8 Mortality rate with higher IPF levels in both ACS and control groups was higher than in patients with lower IPF levels
			UA	10	NR	NR		NR	11.1 (0.75)		
			ACS	63	NR	NR		3.7 (2.64)	10.7 (0.8)		
			Control	41	NR	NR		3.1 (2.7)	10.0 (0.64)		
	Berny-Lang et al. 2014 USA	Cross-Sectional	ACS	44	77 (34)	62 (16)	Sysmex 2100 XE	5.0 (2.8)	11.5 (0.7)	IPF between ACS and Control MPV between ACS and Control	IPC: ACS Control
			Control	236	64 (151)	59 (14)		4.6 (2.7)	11.3 (1.0)		
	Lerkevang Grove et al. 2008 Denmark	Cross-sectional	STEMI	177	73 (129)	65 (13)	Sysmex 2100 XE	3.71 (1.77)	10.6 (1.0)	↑IPF in STEMI > NSTEMI/UA > Control (stable angina and healthy subjects) IPF between healthy subjects and stable angina patients MPV between STEMI, NSTEMI, UA, Control Plt between STEMI, NSTEMI, UA, Control	↑IPF in active smokers > non-smokers Linear regression between IPF and MPV: R = 0.81, P-value – 0.0001) Multivariate linear regression analysis demonstrated that IPF significantly differed between groups and the difference was independent of other variables
			NSTEMI	69	73 (50)	67 (15)		3.16 (1.64)	10.5 (1.0)		
			UA	113	45 (59)	63 (14.3)		2.79 (1.33)	10.5 (1.0)		
			ACS	359	66.2 (238)	65 (14.3)		3.31 (1.66)	10.5 (1.0)		
			Control (Stable angina)	39	79 (30)	65 (7)		2.87 (1.44)	10.6 (0.7)		
			Control (Healthy subject)	22	68 (15)	35 (11)		2.51 (1.27)	10.5 (0.6)		

Table 1: Patient characteristics and details of platelet turnover parameters in included studies (continue)

	Article	Study type	Groups	N	Male % (N)	Age (year)	Instrument	IPF Mean (SD)	MPV Mean (SD)	Main findings†	More findings
Articles with a control group (Healthy population)	Ijaz et al. 2021 Pakistan	Cross-Sectional	ACS	85	52.9 (45)	57 (11.6)	Sysmex XN 1000	8.71 (6.2)	NR	↑IPF in ACS > control	-
			Control	85	55.2 (47)	54.1 (9.8)		3.83 (1.6)	NR		
	Gonzalez-zPorras et al. 2010 Spain	Case-Control study	STEMI	129	72.1 (93)	66.7 (12.4)	Sysmex 2100 XE	5.84 (3.3)	11.1 (0.9)	↑IPF in ACS > Control ↑IPF in STEMI > NSTEACS > Control ↑MPV in ACS > Control ↑Plt count in Control > ACS	Elevated IPF levels were directly correlated with elevated MPV levels Increased platelet counts were associated with decreased hemoglobin levels
			NSTEMI and UA	73	79.5 (58)	68.28 (12.6)		4.73 (2.14)	11 (0.8)		
			ACS	202	74.75 (151)	67.27 (12.46)		5.42 (2.9)	11.06 (0.86)		
			Control	202	74.8 (151)	67.5 (11.3)		3.9 (4.7)	10.7 (0.8)		
	Khalifa et al. 2016 Egypt	Cross-sectional study	AMI	33	78.8 (26)	62 (7.2)	Flow cytometry	6.29 (5.73)	NR	↑IPF in ACS > Control ↑IPF in AMI > Unstable angina ↑IPF STEMI > NSTEMI > UA > Control	↑IPC ACS > Control ↑IPC AMI > Unstable angina ↑IPC STEMI > NSTEMI > UA > Control Elevation in IPF and IPC levels were correlated with smoking, hypertension, DM, and previous CV intervention Increases in MPV levels were directly correlated with increases in IPF and IPC levels. Binary logistic regression analysis for independent risk: IPF is an independent risk factor for acute cardiovascular disease (OR: 1.9 (95% CI: 1.01, 19.8) (P-value = 0.04)
			STEMI	19	NR	NR		8.63 (6.23)	NR		
			NSTEMI	14	NR	NR		4.12 (2.1)	NR		
			UA	17	58.8 (10)	60 (7.2)		1.9 (0.8)	NR		
			ACS	50	72 (36)	61.3 (7.2)		5.2 (0.5)	NR		
			Healthy control	15	80 (12)	57.4 (7.9)		0.8 (1.1)	NR		
Articles without the control group but comparing platelet parameters between ACS subgroups	Yahud et al. 2020 Israel	Prospective cohort	STEMI	54	90.7(49)	57.6 (12.1)	Sysmex XN-3000	4.7 (2.66)	11.1 (1.7)	IPF between NSTEMI and STEMI MPV between NSTEMI and STEMI	↓ IPF after PCI Elevated IPF levels were associated with increased risk of MACE The binary logistic regression model showed a significant predictive value of IPF on the third day after PCI: HR: 1.6 (95% CI: 1.02, 2.59) P-value = 0.04
			NSTEMI	46	71.7(33)	61.6 (10.1)		5.12 (2.44)	10.9 (1.1)		

Table 1: Patient characteristics and details of platelet turnover parameters in included studies (continue)

	Article	Study type	Groups	N	Male % (N)	Age (year)	Instrument	IPF Mean (SD)	MPV Mean (SD)	Main findings†	More findings
			ACS	100	82	59.5 (11.3)		4.89 (2.55)	11.0 (1.4)		
	Indriastuti et al. 2010 Indonesia	Cross-Sectional	STEMI	30	70 (21)	60.8 (11.6)	Sysmex XN-1000	4.52 (2.47)	10.6 (1.07)	↑IPF in STEMI > NSTEMI > UA ↑MPV in STEMI > NSTEMI > UA Plt between STEMI, NSTEMI, UA	↑ PDW in STEMI > UA ↑ PDW in NSTEMI > UA PDW between STEMI and NSTEMI
			NSTEMI	25	56 (14)	57.9 (8.6)		3.47 (2.47)	10.24 (0.94)		
			UA	24	58.3 (14)	54.5 (14.1)		2.02 (0.82)	9.76 (1.03)		
			ACS	79	62 (49)	57.9 (11.8)		3.43 (2.32)	10.26 (1.07)		
	Bernlochner et al. 2015 Germany	Prospective Cohort	STEMI	53	NR	NR	Sysmex 5000 XE	4.1 (1.98)	NR	IPF between STEMI and NSTEMI	IPF between Prasugrel and Ticagrelor group MPV between Prasugrel and Ticagrelor group
			NSTEMI	71	NR	NR		3.78 (1.81)	NR		
			ACS	124	81.4 (101)	64.9 (11.8)		3.97 (2.08)	11.47 (1.05)		
	Paramita et al. 2019 Indonesia	Cross-sectional	STEMI	30	NR	NR	Sysmex XN-1000	3.1 (1.98)	9.9 (0.74)	↑IPF in STEMI > NSTEMI > UA MPV between STEMI, NSTEMI, UA	IPF levels were directly correlated with MPV levels
			NSTEMI	30	NR	NR		2.41 (1.17)	10.59 (0.81)		
			UA	7	NR	NR		1.8 (1.46)	9.43 (0.76)		
			ACS	67	73.1 (49)	NR		2.65 (1.64)	10.16 (0.86)		
Studies without a control group	Cesari et al. 2013 Italy	Prospective cohort	ACS	229	67.24 (154)	76 (9.8)	Sysmex 2100 XE	2.97 (1.64)	11.2 (1.0)	↑IPF levels were associated with ↑Cardiovascular death	There was no correlation between MPV level and Cardiovascular death ↑IPF and H-IPF were correlated with ↑cardiovascular death ↑MPV was correlated with ↑IPF and ↑H-IPF ↑Platelet counts were correlated with ↓IPF, ↓H-IPF, and ↓MPV ROC analysis: IPF cut-off for predicting 12-month mortality: 3.3%, specificity: 61.8%, sensitivity: 63.6%, P-value = 0.02
	Cesari et al. 2008 Italy	Cross-sectional	ACS	372	75 (279)	69.6 (10.2)	Sysmex 2100 XE	4.12 (2.6)	11.02 (0.92)	↑IPF in ACS > healthy subjects ↑H-IPF in ACS > healthy subjects	↑MPV was correlated with ↑IPF and ↑H-IPF ↑IPF and H-IPF were correlated ↓Hemoglobin
	Fabbri et al. 2015 Italy	Prospective Cohort	ACS	101		66.26 (12.03)	Sysmex 2100 XE	2.5 (1.98)	11.54 (3.1)	↑IPF (follow-up) in late HPR > no HPR	↑IPF, ↑ H-IPF, and ↑MPV at 6 and 12 months were present in patients with late HPR.
	Funk-Jensen et al. 2012 Denmark	Prospective Cohort	STEMI	55	75 (41)	60 (12)	Sysmex 2100 XE	4.22 (1.8)	11.3 (1.14)	↑IPF in the acute phase of STEMI	↓IPF and ↓MPV in follow-up (3 months) compared to baseline

Table 1: Patient characteristics and details of platelet turnover parameters in included studies (continue)

† The arrow direction shows significant differences in findings between groups of each study, with upward arrows indicating increase in values and downward arrows indicating decrease in values, and indicating no significant differences between study groups
ACS: Acute coronary syndrome; IPF: Immature platelet fraction; AMI: Acute myocardial infarction; AUC: Area under curve; CI: Confidence interval; SD: standard deviation; fL: femtoliter: H-IPF: High fluorescent IPF; HPR: High on-treatment platelet reactivity; HR: Hazard ratio; IPC: Immature platelet count; MPV: Mean platelet volume; MACE: Major adverse cardiovascular events; NSTEMI: Non-ST segment elevation myocardial infarction; OR: Odds ratio; PCI: Percutaneous coronary intervention; Plt: Platelet; ROC curve: Receiver operating characteristic curve; STEMI: ST-segment elevation myocardial infarction; UA - Unstable angina; NR: not reported; DM: diabetes mellitus; PDW: Platelet distribution width.

Supplementary table 1: Detailed systematic search strategy

Question	Can IPF levels be utilized to distinguish ACS patients with stable angina/healthy subjects? Is there any difference between ACS subtypes, regarding IPF levels?
Title	Immature platelet fraction and acute coronary syndrome; a systematic review and meta-analysis
PICOT	P: Patients with ACS I: Immature platelet fraction levels C: Patients with stable coronary artery disease and/or healthy subjects O: Differences in IPF levels between different group T: Observational studies
Keywords	immature platelet fraction, IPF, reticulated platelet, reticulated platelet fraction, Acute Coronary Syndrome, ACS, Myocardial Infarction, MI, STEMI, NSTEMI, unstable angina
Search strategy (Searched up to 4^{th} March 2024)	Scopus: (TITLE-ABS-KEY ("Immature platelet fraction") OR TITLE-ABS-KEY ("IPF")) AND (TITLE-ABS-KEY ("Acute coronary syndrome") OR TITLE-ABS-KEY ("ACS") OR TITLE-ABS-KEY ("Myocardial infarction") OR TITLE-ABS-KEY ("Unstable angina") OR TITLE-ABS-KEY ("Non-ST elevation ACS") OR TITLE-ABS-KEY ("STEMI") OR TITLE-ABS-KEY ("NSTEMI") OR TITLE-ABS-KEY ("MI"))
	PubMed: (((((immature platelet fraction) OR (IPF)) OR (immature platelet)) OR (reticulated platelet)) OR (reticulated platelet fraction OR (young platelet))) AND ((((((("Acute Coronary Syndrome"[Mesh]) OR ("Myocardial Infarction"[Mesh])) OR ("Angina, Unstable"[Mesh])) OR (STEMI)) OR (NSTEMI)) OR (ACS))

IPF: Immature Platelet Fraction; ACS: Acute Coronary Syndrome; MI: myocardial infarction; STEMI: ST elevation MI; NSTEMI: non-ST elevation MI.

Supplementary table 2: Quality and risk of bias assessment of included studies according to JBI critical appraisal tool

Included Studies	JBI quality assessment criteria								Total Score (%)
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	
Indriastuti et al., 2010	Y	Y	Y	Y	Y	Y	Y	Y	8/8 (100)
Berny-lang et al., 2014	Y	Y	Y	Y	Y	Y	Y	Y	8/8 (100)
Gonzalez-Porras et al., 2010	U	Y	Y	Y	Y	Y	Y	Y	7/8 (87)
Lerkevang Grove et al., 2008	Y	Y	Y	Y	Y	Y	Y	Y	8/8 (100)
Ijaz et al., 2021	Y	Y	Y	Y	Y	Y	Y	Y	8/8 (100)
Cesari et AL., 2008	Y	Y	Y	Y	Y	Y	Y	Y	8/8 (100)
Paramita et al., 2019	Y	Y	Y	Y	N	NA	Y	Y	6/8 (87)
Khalifa et al., 2017	Y	Y	N	Y	Y	NA	Y	Y	6/8 (75)
Yahoud et al, 2020	Y	Y	Y	Y	Y	N	Y	Y	7/8 (87)
Cesari et al., 2013	Y	Y	Y	Y	Y	Y	Y	Y	8/8 (87)
Cohen et al., 2020	Y	Y	N	Y	Y	N	Y	Y	6/8 (87)
Bernlochner et al., 2015	Y	Y	Y	Y	Y	N	Y	Y	7/8 (87)
Fabbri et al., 2015	Y	Y	Y	Y	Y	Y	Y	Y	8/8 (100)
Funk-Jensen et al., 2012	Y	Y	N	N	Y	Y	Y	Y	6/8 (75)
Haung et al.,2019	Y	Y	Y	Y	Y	N	Y	Y	7/8 (87)

Note: Y - Yes, N - No, U – Unclear, NA-Not applicable
Q1= Were the criteria for inclusion in the sample clearly defined?
Q2= Were the study subjects and the setting described in detail?
Q3= Was the exposure measured in a valid and reliable way?
Q4= Were objective, standard criteria used for measurement of the condition?
Q5= Were confounding factors identified?
Q6= Were strategies to deal with confounding factors stated?
Q7= Were the outcomes measured in a valid and reliable way?
Q8= Was appropriate statistical analysis used?

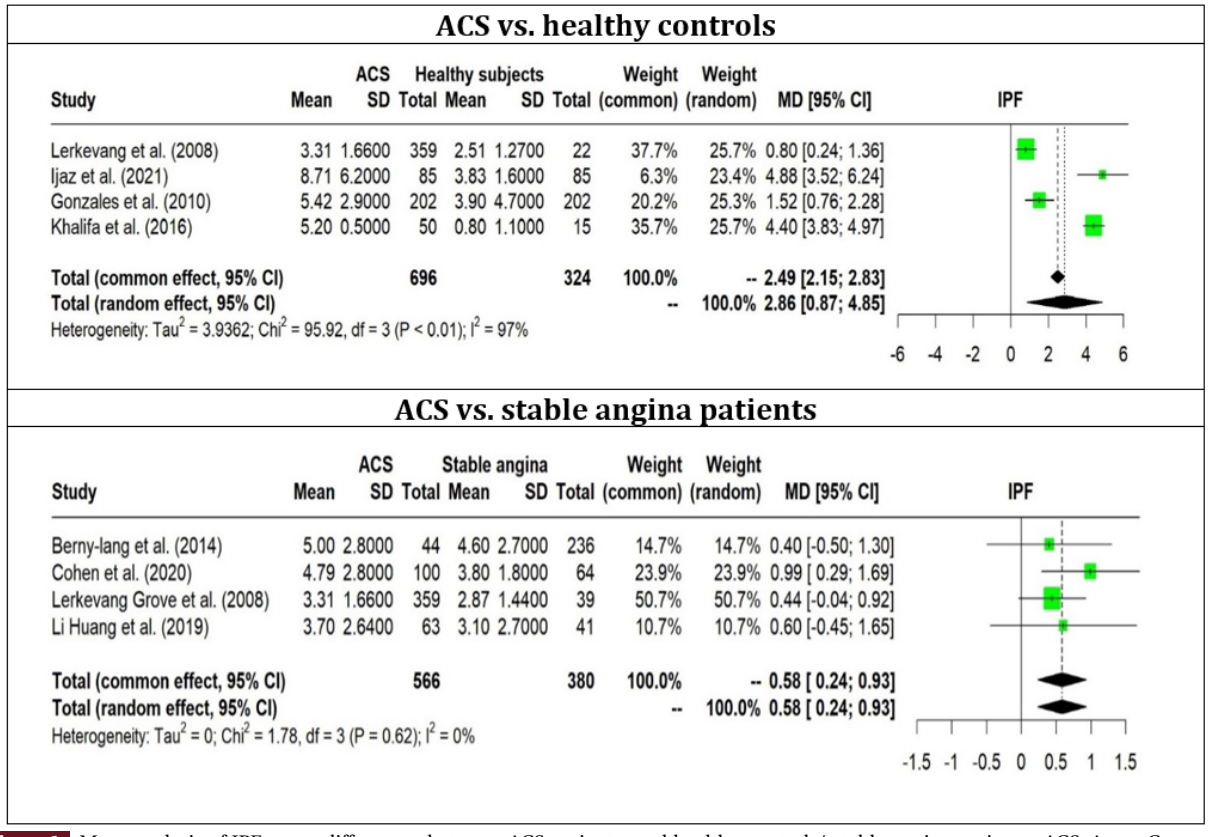


Figure 1: Meta-analysis of IPF mean differences between ACS patients, and healthy controls/ stable angina patients. ACS: Acute Coronary Syndrome; CI: Confidence Interval; IPF: Immature Platelets Fraction; MD: Mean Differences; SD: Standard Deviation.

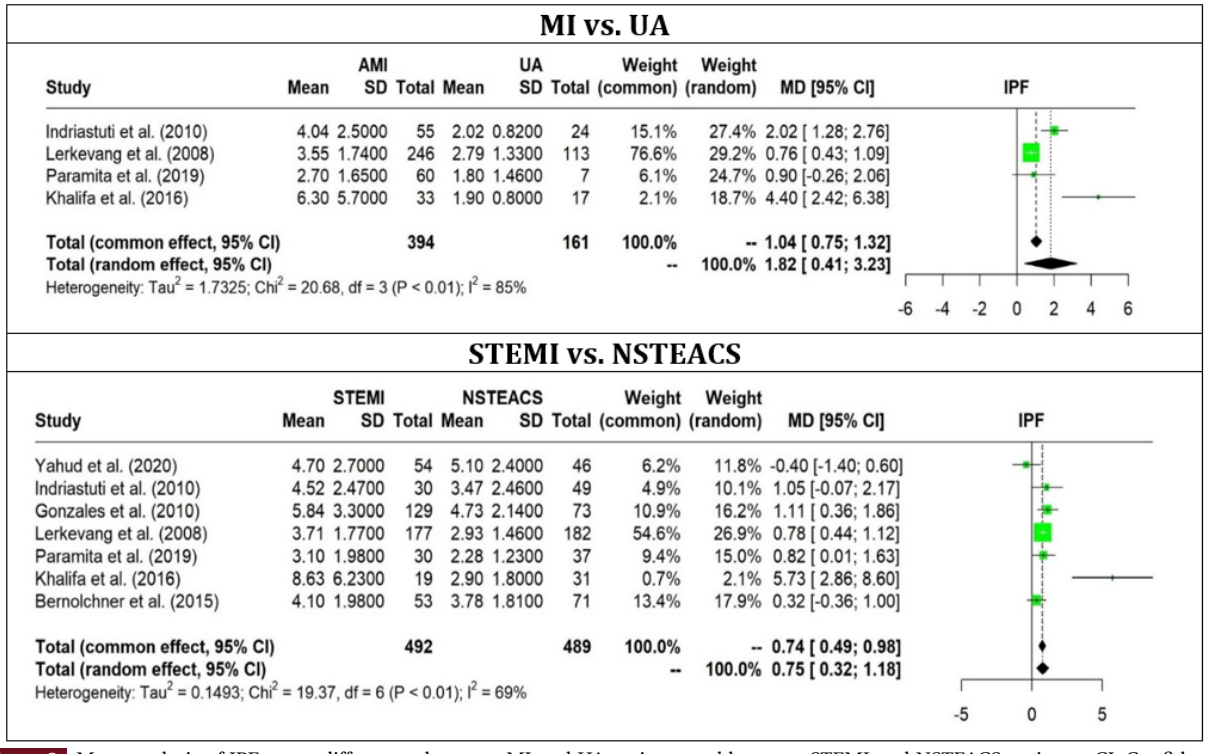


Figure 2: Meta-analysis of IPF mean differences between MI and UA patients and between STEMI and NSTEACS patients. CI: Confidence Interval; MD: Mean Differences; MI: Myocardial Infarction; NSTEACS: Non-ST Elevation Myocardial Infarction (MI); SD: Standard Deviation; STEMI: ST-Elevation Myocardial Infarction; UA: Unstable Angina; IPF: Immature Platelets Fraction; AMI: Acute Myocardial Infarction.

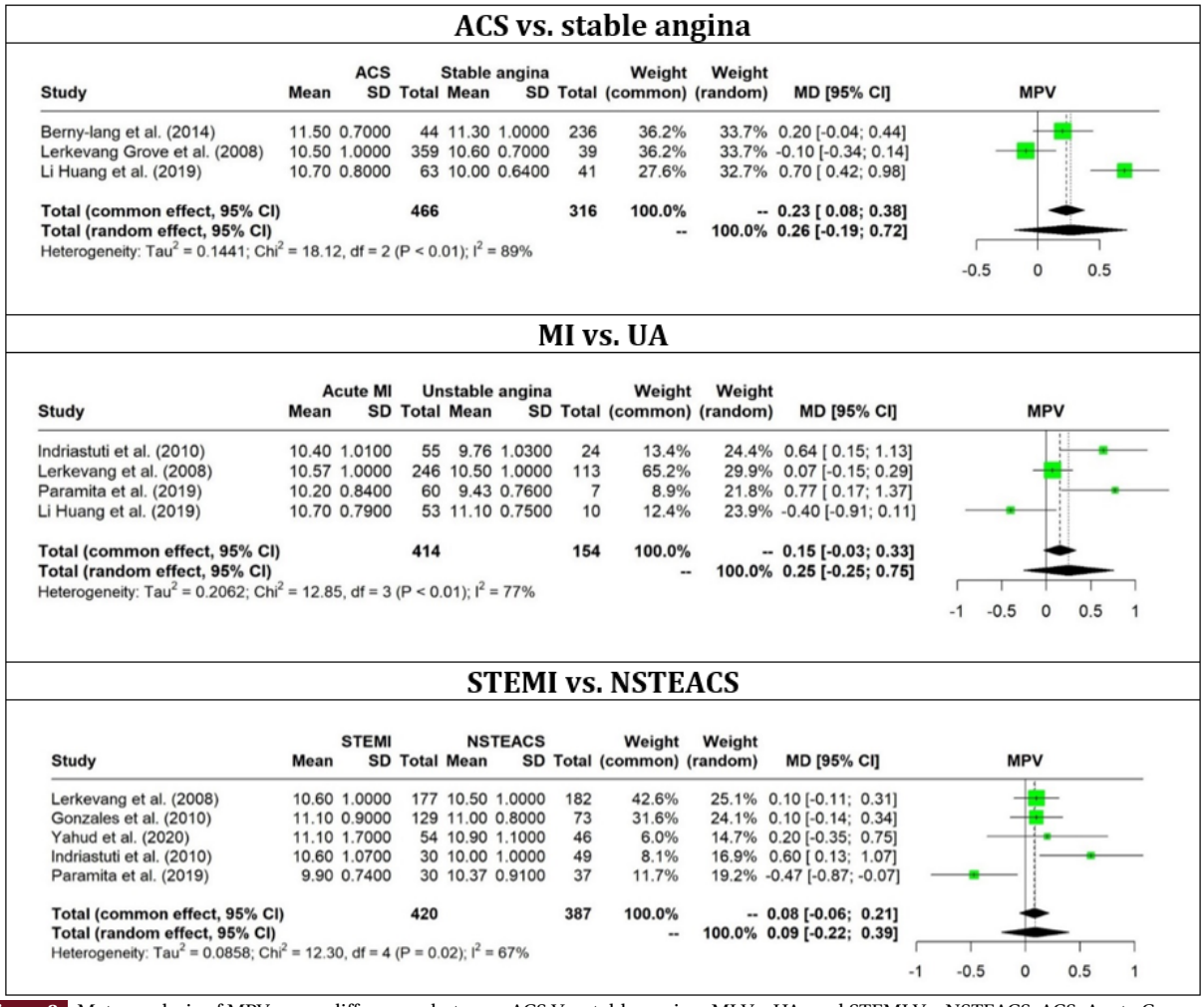
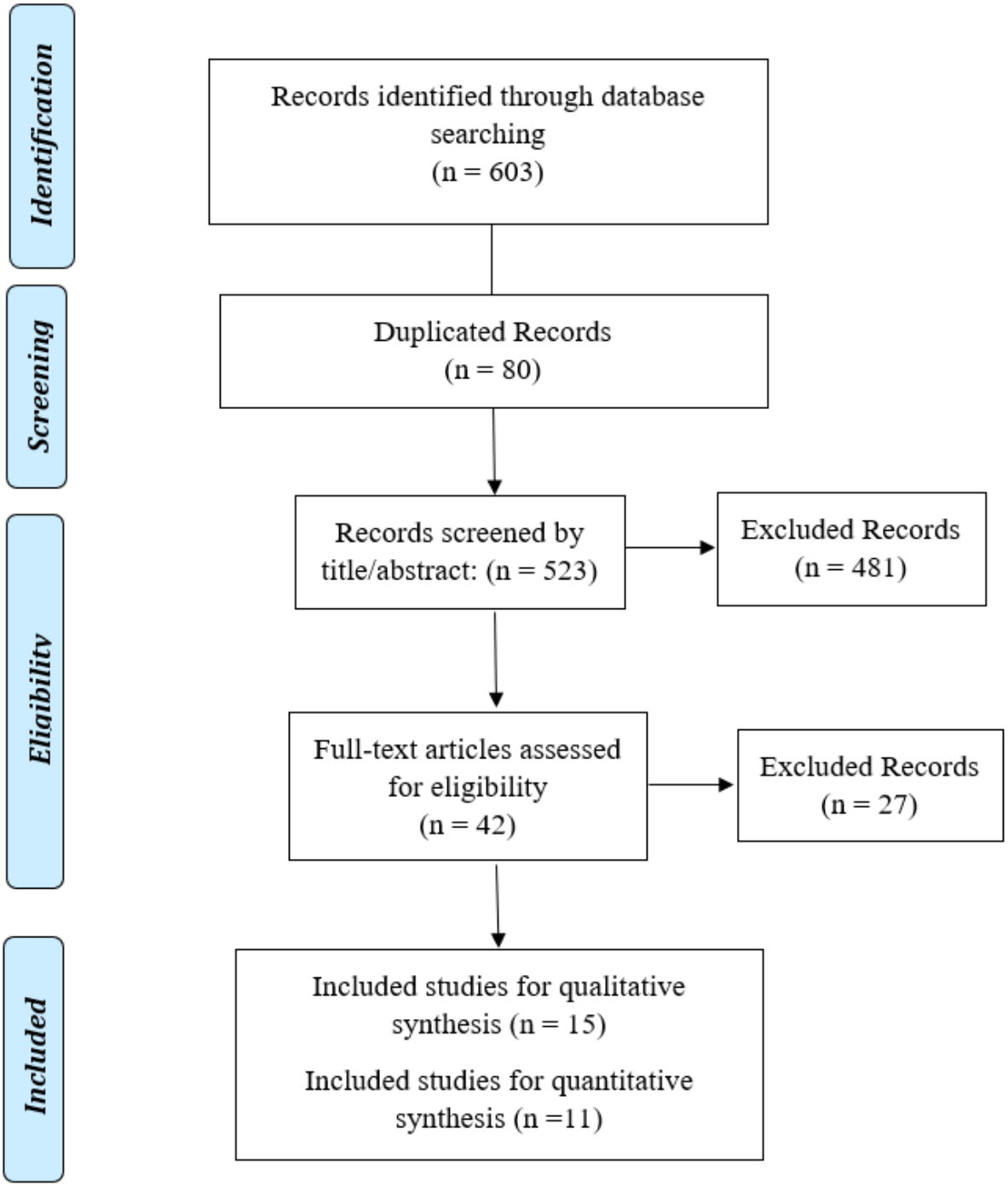


Figure 3: Meta-analysis of MPV mean differences between ACS Vs. stable angina, MI Vs. UA, and STEMI Vs. NSTEACS. ACS: Acute Coronary Syndrome; CI: Confidence Interval; MD: Mean Differences; MI: Myocardial Infarction; MPV: Mean Platelet Volume; NSTEACS: Non-ST Elevation Acute Coronary Syndrome; SD: Standard Deviation; STEMI: ST-Elevation Myocardial Infarction; UA: Unstable Angina.



Supplementary Figure 1: Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flowchart of literature search and selection process