Journal Of Cardiovascular Disease Research
ISSN: 0975-3583,0976-2833 VOL15, ISSUE6, 2024

Original Research

Role of immature Platelet fraction as an early indicator of sepsis in intensive care unit: A Prospective Study

[1]Dr. Anshul Jain, [2]Dr. Vijay Kumar

[1]DNB General Medicine (PD Hinduja National Hospital and Medical Research Centre, Mumbai), Senior Medical Officer, Gandhi Medical College, Bhopal
[2]Assistant Professor, Department of Tuberculosis and Respiratory Diseases, RKDF Medical College and Research Centre, Bhopal

Corresponding Author
Dr. Vijay Kumar
Assistant Professor, Department of Tuberculosis and Respiratory Diseases, RKDF Medical College and Research Centre, Bhopal

Received: 21May 2024 Accepted:10June 2024

Abstract:
Aim: To assess the hypothesized that platelet activation markers triggered by common infections may help predicting occurrence of sepsis in specific ICU patient populations.
Material and Methods: The present prospective study was conducted in patients >18 years of age, admitted at P.D. Hinduja Hospital and Medical Research Center in the Intensive care unit (ICU) with acute febrile illness. Any patient fulfilling the inclusion criteria, admitted as an in-patient to this hospital during a period of 12 consecutive months was included in the study. **Group I** Patients Admitted in ICU with fever and proven to have sepsis (confirmed by positive blood culture) **Group II** Patients admitted in ICU with fever and having no proven sepsis (blood culture is negative), The q-SOFA score was recorded with IPF was compared with PCT and blood culture. q-SOFA score was compared in both the groups.
Results:q - SOFA score comparison- group I ,**q - SOFA** = 2 or >2(83.60 ./.) as compared to group II q - sofa =1 (91.9./.) with statistically significant. P value = 0.001 All the parameters of SIRS criteria viz. temperature (^oC), HR (beats/min), breath rate (/min), WBC ($x10^3$/ul) and immature forms (%) were comparable among the study groups as p>0.05. Sensitivity, specificity, negative predictive value and positive predictive value in diagnosis of sepsis (considering culture as gold standard) was 77 %,76%,75%,70.5%respectively.
Conclusion: Using IPF initially to recognize patients of sepsis, can lead to reduction in morbidity and mortality of these patients. Measurement of the IPF is simple, and cost-effective. It can be done as part of a CBC on most automated hematology analyzers.
Keywords: Immature Platelet Fraction**,** Procalcitonin,Sepsis, SequentialOrganFailureAssessment

INTRODUCTION:
Sepsis is a clinical syndrome defined as life threatening organ dysfunction caused by a dysregulated host response to infection, Sepsis and the inflammatory response that ensues can lead to multiple organ dysfunction syndrome and death.
Septic shock: define as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone, Patient requires vasopressors to maintain a Mean Arterial Pressure (MAP) of 65 mm Hg or greater and a lactate level greater than 2 mmol/L after adequate volume resuscitation**.**
Systemic Inflammatory Response Syndrome (SIRS):is an exaggerated defense response of the body to a noxious stressor (infection, trauma, surgery, acute inflammation, ischemia or reperfusion, or malignancy) to localize and then eliminate the endogenous or exogenous source of the insult. These were also removed from sepsis definition in recognition of its lack of specificity. While some patients with infections may fit the SIRS criteria, SIRS may also occur in multiple noninfectious disease states such as trauma, burns, surgery or pancreatitis. The sepsis syndrome triad includes infection, the patient's individual response to that infection, and the resulting organ dysfunction. Objectively, SIRS is defined by the satisfaction of any two of the criteria below:
1. Body temperature higher than 38 degrees Celsius (100.4-degreeFahrenheit) or lower than 36 degrees Celsius (96.8-degreeFahrenheit)
2. Heart rate greater than 90 beats per minute
3. Respiratory rate greater than 20 breaths per minute, or partial pressure of CO2 (paCO2) less than 32 mm Hg
4. White blood cell (WBC) count greater than 12000 or less than 4000/micro liters or over 10% immature forms and bands[1-3]Sepsis is the main cause of mortality in Intensive Care Unit (ICU) setting despite improvements in

treatment and care of critically ill patients. Delay in the recognition and diagnosis of sepsis contributes to the high mortality in an ICU setting, reported from 27 to 54%.[4]There are multiple novel markers for early detection of sepsis in admitted patients. Some of these markers are commonly employed in the detection of sepsis like C-Reactive protein (CRP), serum Procalcitonin (PCT) and Blood Cultures. However, blood cultures may not always pick up the organism in an overtly septic patient owing to many reasons such as low sample volume, low levels of bacteremia and temporal variations in bacteremia.[5]

Sepsis syndrome is associated with microcirculatory changes which eventually lead to multi organ dysfunction. Diffuse inflammation associated with sepsis leads to activation of coagulation factors and platelets. lnflammation and coagulation are thus interrelated, hence the inclusion of coagulation markers in early diagnosis of sepsis[5]. Immature Platelet fraction measures recently produced megakaryocytes from the bone marrow that are released in to circulation. It has been shown that 20-30% of patients admitted to ICUs have low platelet counts.[6]IPF percentage can differentiate between thrombocytopenia due to bone marrow failure or due to increased peripheral platelet destruction. IPF % increases when platelet production rises and declines when production falls.[7]Increased platelet activity can be diagnosed by a raised platelet volume, which, in turn, suggests an increased prothrombotic state associated with adverse outcomes in ICU patients. Factors such as thromboxane A2 have prothrombotic properties and are produced during hemostasis by activated platelets. It increases platelet aggregation as well as stimulates activation of new platelets. Large platelets have increased platelet activity as they produce large amounts of thromboxane A2[9].

Increased IPF% has also been related to thrombotic events including disseminated intravascular coagulation, thus becoming a prognostic marker of this condition[10] and is commonly used in the neonatal ICU to investigate the cause of thrombocytopenia in the 1st days of life[11].

One recent study showed that Immature Platelet Fractions (IPF) could predict sepsis occurrence in critically ill subjects[12]. Further, in severe trauma, platelet activation and leukocyte-platelet aggregate formation have been incriminated in the pathogenesis of tissue lesions leading to organ failure[13]. Hence the present prospective observational study hypothesized that platelet activation markers triggered by common infections may help predicting occurrence of sepsis in specific ICU patient populations.

MATERIAL AND METHODS

The present prospective study was conducted in patients >18 years of age, admitted at P.D. Hinduja Hospital and Medical Research Center in the Intensive care unit (ICU) with acute febrile illness.

Any patient fulfilling the inclusion criteria, admitted as an in-patient to this hospital during a period of 12 consecutive months was included in the study.

Patients were divided into two groups:--

Group I

Patients Admitted in ICU with fever and proven to have sepsis (confirmed by positive blood culture)

Group II

Patients admitted in ICU with fever and having no proven sepsis (blood culture is negative)

Inclusion criteria:-

1. Core temperature >38 degree Celsius (^{0}C) or <36 (^{0}C)
2. Heart Rate > 90 beats per minute
3. Respiratory Rate > 24 per minute
4. WBC Count >/= 12000 per cu mm blood or </=4000 per cu mm blood or >/= 10% bands

Exclusion criteria

1. Patients not willing for the study
2. Patients with Chronic Kidney Disease, hematological and solid organ malignancies, pancreatitis, trauma and burn victims
3. Patients on growth factors
4. Patients of acute myocardial Infarction
5. Patients with autoimmune Disorders

q-SOFA – (quick Sequential Organ Failure Assessment) SCORE:-

The qSOFA score (also known as quick SOFA) is a bedside prompt that may identify patients with suspected infection who are at greater risk for a poor outcome outside the (ICU), if score is ≥ 2. It uses three criteria, assigning one point for low blood pressure (SBP≤100 mmHg), high respiratory rate (≥22 breaths per min), or altered mentation (Glasgow coma scale <15). qSOFA score was done in all patients.

Data Collection:

- All descriptive data, consisting of demographics, diagnosis, clinical and laboratory data, and sepsis severity scores were recorded i.e. gender, age, type of admission (surgical or medical), history of diabetes and cardiovascular disease, previous treatment by vasopressor, prophylactic antibiotics, aspirin, and anticoagulants.
- The q-SOFA score was computed in all patients. The following laboratory parameters were also assayed: platelets, Immature Platelet Fraction (IPF), procalcitonin (PCT), blood culture, bilirubin, d-dimer, lactate, creatinine.
- IPF was compared with PCT and blood culture. q-SOFA score was compared in both the groups.

Results:

Males were comparatively more than females in both the groups studied with statistically insignificant difference. Mean age in group I and II was 58.4±7.27 and60.09±8.12 years respectively.Age and gender distribution was comparable among both group I and group II (table 2). The control group was age and gender matched with the study group.

Table 3 shows Diabetes and cardiovascular disease was seen more in group I as compared to group II, though no significant difference was found. Prophylactic antibiotics were used by 85.25% in group II while the same was observed in 60.66% of the subjects in group I. A significant difference was observed between the 2 groups $p<0.05$)

83.60 % of the cases with fever with proven sepsis had q-sofa =2 or >2 while 91.9 % of the cases with fever and no sepsis had q-sofa = 1. The difference was highly statically significant on applying chi square test . P value was less than 0.001. As the q- sofa score is non continuous data the chi square test is applicable in this case. Table 4

Table 5 All the parameters of SIRS criteria viz. temperature (oC), HR (beats/min), breath rate (/min), WBC (x103/ul) and immature forms (%) were comparable among the study groups as $p>0.05$in all. (table 8). Hence they were not able to differentiate between the 2 groups.

Mean immature platelet fraction (%) was found to be higher in group I (6.1±2.79) as compared to group II (3.37±2.30) with statistically significant difference when compared using t test. Similarly, immature reticulocyte fraction (%) was found to be more in group I (19.87±13.33) as compared to group II (13.05±7.14), though no significant difference was noted (table 6).

Table 7 shows and figure 1 shows the Sensitivity, specificity, negative predictive value and positive predictive value in diagnosis of sepsis (considering culture as gold standard) was 80.5%, 74.6%, 96.2% and 48.10% respectively. The cut-offs values were derived from the ROC analysis

Table 1: Gender and age distribution among the study groups

Variables	Group I (Fever+Sepsis)		Group II (Fever+ No Sepsis)		p value
	N=61	%	N=61	%	
Gender					
Male	47	77.05	49	80.33	0.83
Female	14	22.95	12	19.67	
Age, Mean±SD	58.4±7.27		60.09±8.12		0.36

Table 2: Co-morbidities and clinical data among the study groups

Variables	Group I (Fever+Sepsis)		Group II (Fever+ No Sepsis)		p value
	N=61	%	N=61	%	
Diabetes	12	19.67	7	11.48	0.20
Cardiovascular Disease	43	70.49	32	52.46	0.12
Vasopressor before the admission	6	9.84	4	6.56	0.58
Prophylactic antibiotics	37	60.66	52	85.25	0.026
Aspirin	36	59.02	29	47.54	0.29

Table:3- q-sofa score among the study and control groups at admission

Group	q – SOFA		p value
	q- SOFA >2 or =2	q - SOFA=1	
Group I (Fever+Sepsis)	51(83.6)	10(16.4)	0.007*
Group II (Fever+ No Sepsis)	5(8.1)	56 (91.9)	

*: statistically significant

Table 4: Comparison of SIRS criteria among the study groups

SIRS Criteria	Group I (Fever+Sepsis)		Group II (Fever+ No Sepsis)		p value
	Mean	SD	Mean	SD	
Temperature (°c)	38.6	0.4	37.3	1.6	0.08
HR (beats/min)	112.8	10.2	109.9	8.53	0.17
Breath Rate (/min)	24.88	11.32	27.14	12.7	0.59
WBC ($x10^3$/ul)	15.67	7.7	16.39	9.1	0.42
Immature forms (%)	5.81	7.6	10.29	8.15	0.13

Table 5: Comparison of advanced hematological parameters among the study groups

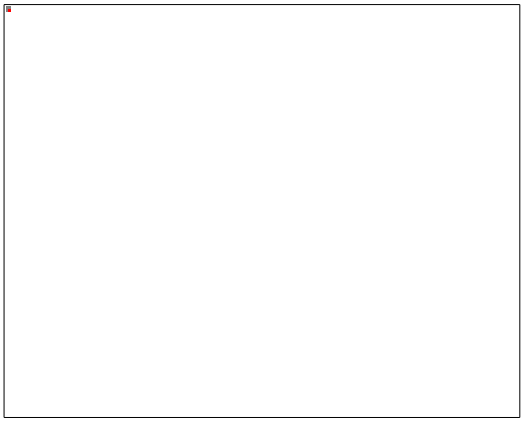
Profile	Group I (Fever+Sepsis)		Group II (Fever+ No Sepsis)		p value
	Mean	SD	Mean	SD	
Immature platelet fraction (%)	6.1	2.79	3.37	2.30	0.005*
Immature reticulocyte fraction (%)	19.87	13.33	13.05	7.14	0.10

* statistically significant

Table 6: Diagnostic efficacy of immature platelet fraction (%) in diagnosis of sepsis

Variables	Value (%)	95% CI
Sensitivity	77./.	58.20-86.35
Specificity	76./.	53.4-81.8
Negative Predictive Value	75./.	88.7-98.8
Positive Predictive Value	70.5./.	25.5 to 62.7

Figure 1: ROC curve w.r.t. Ipf (Immature Platelet Fraction) in diagnosing sepsis



Discussion:

This study was conducted to determine whether IPF can be used as an early indicator of sepsis. Our patients were randomly distributed into two groups i.e. Group I (patients admitted in ICU with fever and proven to have sepsis) and Group II (patients admitted in ICU with fever and having no proven sepsis).

Males were comparatively more as compared to females in both the study groups with statistically insignificant difference. Mean age in group I and II was 58.4±7.27 and 60.09±8.12 years respectively. Age and gender distribution was comparable among both the study groups in this study. Similar age and gender distribution was reported by Hubert RME et al[15] in their study.In this study, admission from medical wards was found to be more in group II while admission from surgical wards was more in group I. Reason for admission viz. cardiac surgery, acute brain injury, trauma and ventilation was reported among 50.82%, 29.51%, 11.48%, 8.20% in Group I and 80.33%, 3.28%, 13.11%, 3.28% in group II respectively. Similar category and reason for admission among the study subjects was mentioned by Hubert RME et al[15] in their study.Mean±SD of the q-SOFA score was comparatively much higher in group I (2.03±0.42) as compared to group II (1.00±0.28). When q-sofa score was compared statistically among group I and II using chi square test, statistically significant difference was noticed as p value was <0.05 in this study. Hubert RME et al[15] in their study too showed that q-sofa score was comparatively much higher in fever with sepsis subjects as compared to subjects who had fever without sepsis.

Procalcitonin (PCT) is a biomarker which is released in response to bacterial infections and can be used to differentiate the etiology of infectious processes. It can be employed as a tool to guide appropriate antibiotic therapy and thus has a role in antibiotic stewardship. Procalcitonin (PCT) is a protein that consists of 116 amino acids. It is the peptide precursor of calcitonin, a hormone that is synthesized by the parafollicular C Cells of the thyroid and involved in calcium homeostasis. Procalcitonin arises from endopeptidase-cleaved Preprocalcitonin. The reference value for procalcitonin in adult is less han 0.1 ng/mL. Levels greater than 0.25 ng/mL can indicate the presence of an infection[16].PCT is <0.1 in normal individuals. When PCT was compared between the two groups, it was found to be higher in group I (Mean = 5.71) than group II (Mean = 1.24). This difference is statistically significant.PCT can be used to as a decision parameter for antibiotic dosage. If PCT is 0.1 to 0.25, it indicates that bacterial infection is present but use of antibiotics is discouraged.When the PCT is in the range of 0.2 to 0.5, bacterial infection is present and antibiotic is recommended.If the PCT is more than 0.5, then bacterial infection is present with an increased chance of occurrence of sepsis and antibiotic is strongly recommended.In our study, lactate (p>0.05) was comparable among both the study groups. Mean lactate (mmol/l) was found to be more in group I (3.93±2.01) as compared to group II (1.18±0.51) with statistically significant difference when compared using t test. According to Hubert RME et al[15], when patients with non-complicated sepsis were compared with patients with severe sepsis/septic shock, IPF and lactate were the only significantly different clinical and laboratory parameters. Neither platelet counts, C-reactive protein, nor any other individual laboratory or clinical parameters, including IRF, were found to be insignificantiy different between these two populations at the time of ICU admission.The mechanism behind the association of IPF with sepsis and sepsis severity remains to be determined. We believe that higher IPF could be part of the ongoing inflammatory response to sepsis, in which platelets have been shown to play an important role51. Accordingly, it has been demonstrated that platelets express many types of TLR receptors52, which are key regulators of the innate immune response to pathogens. Activation of these receptors leads to the release of IL-1b and formation of neutrophil extracellular traps (NETs) 53-54 which trap invading pathogens in infection sites. Therefore, higher IPF values in patients with sepsis compared to healthy individuals, as well as its association with sepsis severity might reflect the formation and recruitment of newly formed platelets, as part of a TLR-mediated mechanism triggered by infections. Alternatively, higher IPF levels in sepsis, and especially in patients with severe sepsis, might be a reflection of ongoing disseminated intravascular coagulation (DIC), which results in compensatory platelet production. In fact, IPF has been previously correlated with DIC scores in a cohort of critically-ill patients.Mean IPF (%) was found to be higher in group I (6.1±2.79) as compared to group II (3.37±2.30) with statistically significant difference when compared using t test. Similarly, immature reticulocyte fraction (%) was found to be more in group I (19.87±13.33) as compared to group II (13.05±7.14), though no significant difference was found in the present study. However, Hubert RME et al[15] in their study showed that IPF and IRF could not discriminate SIRS from sepsis. Roberto Alberto De Blasi et al[12] in their study mentioned that new finding in their observational prospective study conducted over 7 days in critically ill adult patients admitted to a general ICU with no sepsis criteria is that IPF% can identify those patients in whom sepsis will develop over the ensuing 6 days. This easily measured cellular variable reflecting the thrombopoietic rate, which is rarely used in general ICUs, has relatively low diagnostic sensitivity (56.2 %) but high specificity (90.0 %) as an early marker predicting the onset of sepsis. Another innovative finding in their study was that unlike the commonly used markers related to infection or inflammation (PCT, CRP), IPF% is unrelated to cause of infection or clinical cause of sepsis criteria but is significantly related to the development of sepsis.

In a recent study, De Blasi et al[12] showed that the IPF was capable of predicting the development of sepsis up to 3 days before sepsis become clinically manifest, with IPF values above 4.7% presenting a specificity of 90.0% and a sensitivity of 56.2% for sepsis development. In this study, IPF also showed to be the only variable correlated with the development of sepsis. In another study that evaluated the association between high IPF values and the presence of bloodstream infections, Di Mario et al[8] showed that the samples with positive blood cultures had significantly higher mean IPF values (4.86%) than samples with negative blood cultures (1.79%).

IPF and IRF are laboratory parameters derived from the CBC in automated hematological analyzers available in most healthcare services8. As such, they could represent readily available and low-cost sepsis biomarkers, accessible to several healthcare units.

Limitations and Strength of our study

The relatively small number of patients may have limited the statistical power of the study

Though, D-dimer levels were available for a subgroup of patients, but a formal evaluation of the association of IPF with DIC was not possible in our study. On the other hand, the unbiased patient selection from two independent ICUs (medical , surgical) and the adequate characterization of sepsis diagnosis and severity scores, and the inclusion of major surgery patients more closely resembling a real-life scenario in which a sepsis biomarker would be used are important strengths of our study.Although additional studies are necessary to more precisely understand the biological rationale behind the association of IPF and sepsis, IPF should be considered a type 0 biomarker, according to a previously published classification[16-17]. Type 0 biomarkers include markers

of the natural history of a disease and correlate longitudinally with known clinical indices such as symptoms over the full range of disease states.

Conclusion:

IPF values obtained within 24 hours from ICU admission are higher in patients with sepsis compared to individuals without sepsis and correlate well with the culture report. Since IPF values are higher in febrile patients with proven sepsis than in non-sepsis patients, one can well differentiate these 2 groups. Thus, it can be used as an early marker of sepsis in the ICU setting. These patients can benefit from prompt antibiotic therapy which would have been delayed as blood culture reports take at least 24 hours to be available for action by the clinician. Therefore, using IPF initially to recognize patients of sepsis, can lead to reduction in morbidity and mortality of these patients.

Measurement of the IPF is simple, and cost-effective. It can be done as part of a CBC on most automated hematology analyzers.

Reference:

1.EPISEPSIS Study Group. EPISEPSIS: a reappraisal of the epidemiology and outcome of severe sepsis in Frenh intensive care units. Intensive Care Med. 2004;30:580-8.

2.Levy MM, Dellinger RP, Townsend SR, Linde-Zwirble WT, Marshall JC, Bion J, Schorr C, et al. The Surviving Sepsis Campaign: results of an international guideline-based performance improvement program targeting severe sepsis. Intensive Care Med. 2010;36(2):222-31.

3.Muller B, Becker KL, Schachinger H, Rickenbacher PR, Huber PR, Zimmerli W, et al. Calcitonin precursors are reliable markers of sepsis in a medical intensive care unit. Crit Care Med 2000;28:977–983.

4.Ayres SM. SCCM'S new horizons conference on sepsis and septic shock. Crit Care Med 1985;13:864–866.

5.Zimmerman GA, McIntyre TM, Prescott SM, Stafforini DM. The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. Crit Care Med. 2002; 30(5):S294–S301.

6.Greinacher A, Selleng K. Thrombocytopenia in the intensive care unit patient. Hematology 2010, AM SocHematolEduc Program 2010;2010(1):135-43.

7.Briggs C, Kunkal S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF %) in peripheral thrombocytopenia. Br J Haematol 2004;126: 93-99

8.Di Mario A, Garzia M, Leone F, Arcangeli A, Pagano L, Zini G. Immature platelet fraction (IPF) in Hospitalized patients with neutrophlilia and suspected bacterial infection. J Infect 2009;59: 201-206.

9.Yoo H, Ku SK, Kim SW, Bae JS. Early diagnosis of sepsisusing serum hemoglobin subunit Beta. Inflammation2015;38:394-9.

10.Hong KH, Kim HK, Kim JE, Jung JS, Han KS, Cho HI. Prognosticvalue of immature platelet fraction andplasmathrombopoietin in disseminatedintravascular coagulation. BloodCoagul Fibrinolysis 2009; 20:409–414.

11.Brown RE, Rimsza LM, PastosK,Young L, Saxonhouse MA, Bailey M,Lawrence RM, Sola-Visner MC. Effects of sepsis on neonatalthrombopoiesis. Pediatr Res 2008; 64:399–404.

12.De Blasi RA, Cardelli P, Costante A, SandriM, Mercieri M, Arcioni R. Immature platelet fraction in predictingsepsis in critically ill patients. Intensive Care Med 2013; 39:636–643.

13.Ogura H, Kawasaki T, Tanaka H, Koh T, Tanaka R, Ozeki Y et al (2001) Activated platelets enhance microparticleformation and platelet-leukocyte interaction in severe trauma and sepsis. J Trauma 2001; 50:801–809.

14.Hubert RM, Rodrigues MV, Andreguetto BD, Santos TM, de Fátima Pereira Gilberti M, de Castro V, Annichino-Bizzacchi JM, Dragosavac D, Carvalho-Filho MA, De Paula EV. Association of the immature platelet fraction with sepsis diagnosis and severity. Scientific reports. 2015;5(1):1-6.

15.Assinger A, Schrottmaier WC, Salzmann M, Rayes J. Platelets in sepsis: an update on experimental models and clinical data. Frontiers in immunology. 2019;1 0:1687.

16.Lesko LJ, Atkinson Jr AJ. Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. Annual review of pharmacology and toxicology. 2001; 41:347.

17.Frank R, Hargreaves R. Clinical biomarkers in drug discovery and development. Nature reviews Drug discovery. 2003;2(7):566-80.