ESJ Natural/Life/Medical Sciences

Platelets and Inflammation: Relations between Platelet Counts and Markers of Inflammation

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Doi:10.19044/esj.2021.v17n37p108

Submitted: 27 August 2021
Accepted: 02 October 2021
Published: 31 October 2021

Cite As:
Sonar N., Bafna V., Singh P., Khadas I. & Chavan M. (2021). *Platelets and Inflammation : Relations between Platelet Counts and Markers of Inflammation.* European Scientific Journal, ESJ, 17(37), 108.
https://doi.org/10.19044/esj.2021.v17n37p108

Abstract

Platelets, or thrombocytes are small non-nucleated cells that are primarily responsible for the formation of the platelet plug, and consequently in primary hemostasis. However, their role is not limited only to a response to cell injury, but they also respond to systemic inflammation. Due to factors such as a shared lineage with other cells of the blood, recent studies showing the molecular expression of immune modulators on platelets, and the release of inflammatory molecules leading to chemoattraction by responder molecules. These implications may also go deeper into the pathogenesis of cancers in the human body, and quantification of that relation, platelets with inflammation, is the aim of the study.

Keywords: Platelets, Inflammation, Hematology, Oncology, Signaling

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

Aim

To evaluate the changes in platelet counts with respect to inflammation in systemic inflammatory states, and mount a correlational analysis showing quantitative estimation of changes in platelet values. In this study, based on the inflammatory markers recorded, a scatter plot will be charted, with linear trendlines, and correlational analysis showing results of white blood cell (WBC) counts with platelets, as well as Vitals such as Temperature (*suggestive of fever or hypothermia*), Pulse (*suggestive of tachycardia, or bradycardia*), and Respiratory rate with platelets. Additionally, this article will also be reporting the prevalence of Abnormal platelet counts in the presence of inflammation.

Materials and Methods

Patients coming to the outpatient department (the office), as well as those admitted in the hospital were selected. A total of 377 patients were selected to be the sample size. The following details were collected from the patients. Patient information was limited to initials, vitals such as temperature, pulse, respiratory rate, white cell count, and platelet count. Inflammation presence was based on the presence of the Systemic Inflammatory Response Syndrome (SIRS) Criteria. This was chosen to be the primary inclusion criteria.

Results

A total of 413 patient forms were obtained, however, some patients would not fulfill the SIRS criteria, and thus, 377 patients' data was finalized for further analysis.

Title	Data	Comments
Total number of patients	413	
Actual sample size	377	
% of patients fulfilling SIRS criteria	91.28%	These are out of the 413 that were selected.
Correlation of white blood cells with Platelets		White blood cells (x) with Platelets (y)
R	0.42	at p value <0.001
R^2	0.17	
Correlation of Temperature with Platelets		
R	-0.08	at p value 0.16 (not significant)
R^2	0.004	
Correlation of Pulse with Platelets		Pulse rate (x) with Platelets (y)
R	-0.06	
R^2	0.003	at p value 0.25 (not significant)
Correlation of Respiratory Rate(RR) with Platelets		RR (x) and Platelets (y)

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

R	0.06	at p value 0.24 (not significant)
R^2	0.001	
Total number of patients who had high platelet counts	75	
Prevalence of High Platelet Count in inflammation	19.89%	Inflammation refers to SIRS positive patients

In the background of inflammation, a weak positive correlation between the platelet counts and white blood cells exists (r=0.42), which was statistically significant. The prevalence of elevated platelet counts in inflammation (SIRS criteria positive patients) was 19.89%. The relationship of Temperature, Pulse and Respiratory Rate was not found to be statistically significant.

Introduction

Inflammation can be looked at as an active state of the body. It is a state where the body actively enhances expression of proteins, and certain molecules which are the pro-inflammatory markers, to modulate and counter an foreign substance. Robbins and Cotran's pathology defines inflammation as a response of vascularized tissue to infection and damaged tissue (Ferrero-Miliani, L. et al., 2006). So essentially, we have a state on exposure to these molecules, pathogen or damage associated (in some literature, they are termed as PAMP and DAMP), which our body responds to by releasing certain markers (predominantly the tumor necrosis factor-alpha (TNF-alpha)), the march of cells such as white blood cells and changes in clinical parameters such as temperature, pulse and respiration (Levy, M. et al., 2003) Platelets, or thrombocytes are non-nucleated cells that are historically, and primarily known for hemostasis, and thrombosis. They number around 150,000 to 350,000 per microliter of blood. Platelets contain various granules, and arrays for synthesizing inflammatory molecules, which are the primary reason for their activity (Sonmez, O., & Sonmez, M., 2017). Now the question arises, is how do platelets act in inflammation?

There are multiple theories acting on this, such as the cell lines of inflammatory cells, such as white blood cells and platelets are of a common origin (McDonald, T. P., & Sullivan, P. S. ,1993). So when inflammatory processes demand the body to increase the production of white cells, it can be expected that platelets may also rise. In fact, in animals such as fishes and birds, platelets directly play a central role in inflammation (Levin, J., no date). Furthermore, platelets express on their surface Toll-like-Receptor (TLR) molecules, which are directly involved in the detection of an antigen (Clemetson, K.J., 2009). This action means platelets can behave like sentinels, or first responders to the presence of an inflammatory agent.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

Platelet granules contain and release chemoattractants, platelets also express adhesion molecules such as selectins, and CD-40 receptors (Henn, V. et al.,1998), which cause interactions of platelets with the inflammatory cells, thus mounting an inflammatory response. Activated vascular endothelium, in response to TNF-alpha, releases Nitric Oxide (NO), which stimulates the generation, and activation of platelets (Nathan, C., 2002)(Yamamoto, K, et al., 1999). And finally, when activated by Interleukins like IL-1, IL-1B, and IL-6, gene expression through NF-KB, leading to transcription of inflammatory genes, and translation of eicosanoids like prostaglandins and leukotrienes (Gawaz, M., 2005)

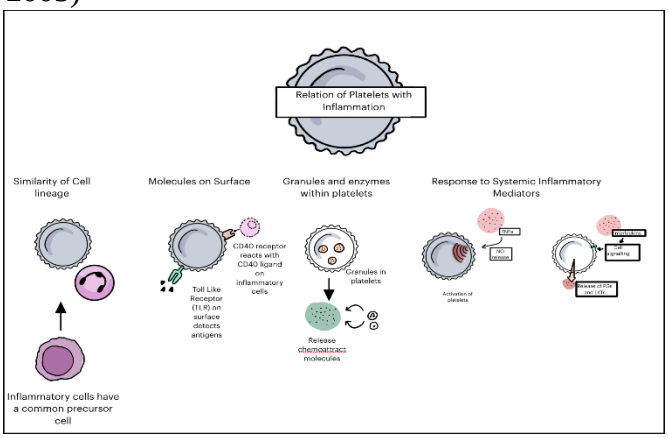

Fig 1. *Relation of Platelets with Inflammation*

The final question arises of how to link the two entities (platelets, and inflammation) together. The major issue here is, how can it be said that an inflammatory process is there? Going back to the definition, it is extremely non-specific. The concept of inflammation is a generalized, non-specific response to an invading molecular pattern, in order to mount a versatile defense action. So a very nonspecific, but highly sensitive criteria (Vincent, L. et al., 2013), which was the Systemic Inflammatory Response Syndrome (SIRS) Criteria was used. SIRS criteria was historically used for sepsis, but it's use in sepsis has faded (Boka, K., 2021), but its fundamental value in identifying an inflammatory state remains the same. This includes the recording of vitals, such as temperature, pulse and respiratory rate, with lab findings of white cell counts. Due to patients being readily given analgesics and antipyretics, fever alone cannot be seen as a sign of inflammation. Therefore, this criteria can be used, with its sensitivity being a sentinel to rule out the absence of inflammation.

The multi-faceted ways by which platelets are involved in inflammation, leads to believe there must be some correlation clinically of these theories (Chakraborty, R.K. et al., 2021). So what is looked for is a relative rise or fall, a change in the value of platelets based on the presence of

inflammation. The logical sequence should be that when there is an excess of said inflammatory products and cells, the platelet counts should be changing, whether they cross the threshold or not. The various amounts of targets that are provided here, from gene expression, to surface molecules, have immense potential for development of biologicals to target therapy against a variety of states, such as infections, chronic inflammatory states, autoimmune conditions, and neoplastic states (Franco, A., et. al, 2015).

Aim and Objectives

Aim

To find and quantify the changes in platelet count with respect to inflammatory markers.

Objectives

Correlation of values of Temperature, Pulse, Respiratory Rate, and white cell count with Platelet counts. A secondary objective of prevalence of raised platelets in SIRS positive state (state of inflammation) is added.

Methodology

Permission and clearance from the ethical committee was obtained prior to the conduct of this study.

Type of study: Question-form based, observational study.

Site of study: Office (outpatient department), Wards (inpatient department) and Intensive Care Units (ICUs).

Study Participants: Patients in Hospital (Hospital name is not disclosed for privacy reasons)

Study duration: 6 months

Sample size: 377 (minimum) [Based upon prevalence of 33% - 50%, 95% confidence interval with 5% margin of error].

Inclusion Criteria

Males and Females above the age of 18 who were willing to give consent were chosen.

Exclusion criteria

Patients not willing to participate or give informed consent/assent. Patients having diseases that falsely elevate platelets, such as Mixed

Cryoglobulinemia, Acute Leukemias (such as APL), or any myeloproliferative disorders. Patients with Thalassemias, and Hemolytic anemias are excluded from analysis (Bleeker, S., & Hogan, J., 2011). Pediatric age groups below 18 years of age were excluded, as children tend to have a higher heart rate and respiratory rate, therefore, falsely qualifying positive for the SIRS criteria, even when they may be completely normal (Fleming, S. et al., 2011). Also, pregnant females, and those who are postpartum up to 6 weeks are excluded, due to volume expansion, and physiological changes driving changes in cell counts (Shehata, N. et al, 1999).

Materials Used

Google Form: A Google Form recording the following data was created, and extrapolated into a spreadsheet. Patient details were limited only to the name in initial format, as a measure to protect the patient's privacy.

The data recorded were Temperature of the Patient in degrees Fahrenheit, Pulse of Patient in Beats per minute, Respiratory Rate of Patient in respirations per minute, white blood cell Count (WBC) of the patient sent as per criteria in thousands per mL (the criteria refers to a standard aseptic precaution based procedure, with sample collection in a EDTA tube (for all CBC measurements)) and Platelet Count of the patient sent as per criteria. After this, a question for the Experimenting Physician : *Are two or more out of four criteria deranged? (Is SIRS criteria met?)*. Finally, a question for the Patient : *I can confirm that I have been explained the purpose, the information collected for this experiment. I hereby authorize these individuals to collect, and analyze my data for educational purposes.*

Procedure

Permission from the Institutional Ethics Committee was obtained. The procedures followed were in accordance with the ethical standards of the committee on human experimentation and with the Declaration of Helsinki, adopted by the 18th World Medical Assembly, revised in October 2008. After obtaining ethical committee and authority's permission, patients were approached during their visit in the office, or after rounds in the hospital wards and the intensive care unit (ICU). The purpose of study and instructions for filling the form will be explained and informed consent was obtained. Eligible patients were asked the respective questions, and file record or the EMR was used to obtain results after permission from the patient. Based upon the responses from the form, further evaluation was carried out to find whether they met the SIRS criteria.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

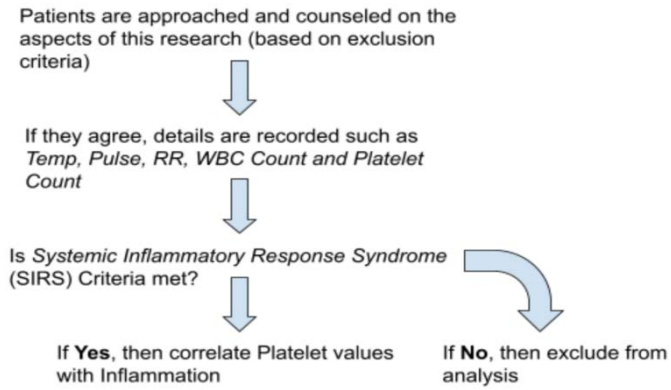

Fig. 2. Simplified Diagram on our Approach Protocol

The Systemic Inflammatory Response Syndrome (SIRS) diagnostic criteria

This table compares the diagnostic criterion with the questions used in the forms.

Temperature >100.4 deg Fahrenheit (>38 deg Celsius) or <96.8 deg Fahrenheit (<36 deg Celsius), **Heart Rate or Pulse** >90 beats per minute, **Respiratory Rate** >22 respirations per minute or arterial pressure of carbon dioxide less than 32 mm of Hg, and **White blood cell Count (WBC)** > 12,000 per cubic milliliter, <4,000 per cubic milliliter or > 10% bands (Bone, C. et al., 1992).

Patients who fulfilled **two or more** of the SIRS diagnostic criteria for sepsis or severe sepsis will be rated as having significant inflammation, and chosen for further analysis (Levy, M. et al., 2003).

Statistical Analysis

Data was entered in Microsoft Excel and will be statistically analyzed using PSPP. Linear regression with trendlines are set for each of the 4 criteria as they are compared with the Platelet values.

Resources Attached

For the congruence of our research, and to maintain transparency, the documents, such as the Google Form and Raw data are available.

Implications of the Study

To put forth and understand the concepts of platelets as inflammatory molecules, and to shine a light on the non-thrombotic roles of platelets. Real world applications, such as potential targets for inflammatory states or neoplastic states could be identified, even though hypothetical. Analysis of values of routinely used parameters, such as white cell counts, temperature and more, in relation to platelets. And finally, Quantification of the

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

aforementioned analysis allows a deeper understanding of the platelet count trends with inflammation.

Discussion

The first part of the discussion involves how patients were analyzed. Patients were basically screened by the diagnosis made, which was available to the physicians during pre-rounds time. Due to the overwhelming presence of the COVID-19 outbreak during this time, it was relatively easier to find patients with inflammation, here screened by the SIRS criteria in the wards and the ICUs. To some degree, approaching patients with '-itis' in their diagnosis, the researchers could narrow down the patients that we needed to approach. This does not always translate to inflammation, but was a guide to selecting patients.

The criteria's extremely non-specific nature was appreciated when upon analysis, it was noted that clinical parameters such as temp, pulse and resp rate do not synchronously rise with each other or with the WBC counts. Some patients were clinically not performing well, but had normal WBC counts. This multi-faceted approach eliminated the bias which a single measurement could create. Now, there is a rule called the Leibermeister's rule, which states that temperature, pulse and respiration all follow increments together. However, since multiple sources vary with the ratios of the three, and clinically, there are too many variations for this to happen. For example, patients may be anxious to see the doctors, which may raise their pulse rate. Alternatively, a patient may be on round-the-clock antipyretics, such as acetaminophen (paracetamol), which continually provides an afebrile state or low fever state. To minimize these effects, or to standardize the results, a decision was made to take all vitals during pre-round times, when blood samples were sent.

The next part of the discussion was the validity and quality control of the measurements taken. For these a 3-tier approach was taken. The first tier were the most standardized results, which would be the platelet count ad white cell count. These would be least subject to bias and clinical or subjective errors. The next tier includes pulse and temperature. These are relatively objective, with pulse can be taken by a pulse oximetry or manually, and temperature readings by various thermometers. The multiple ways, however, mean that they cannot be standardized really, as physicians will have different preferences. The last tier includes respiratory rate, which is the most subjective, and most prone to error. In fact, due to the subjective nature, we decided to mainly focus on white cell count vs platelet count. Now remember, this white cell count is in a background of inflammation only, as we have screened through the SIRS criteria for all said values.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

The figures here show the significant result of white blood cells with platelet values, and a linear trend is observed. The other comparisons, being statistically insignificant, are grayed out.

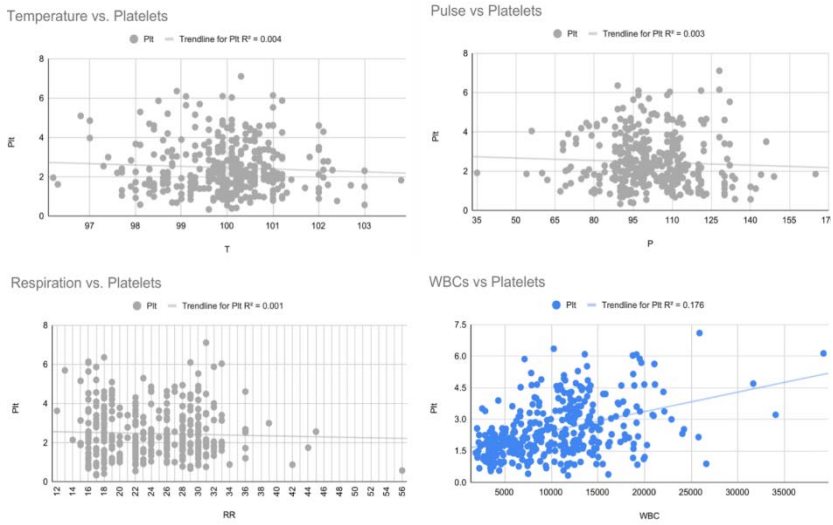

Figure 3. The Relationship of SIRS Markers with Platelet Counts

Compared to the theoretical part, which should have led to all parameters rising, the experiment's results yield a different result. Due to this, the researchers realized that some questions remained unanswered. The theory that inflammatory cells share the same lineage, but why do they not follow a perfect correlation? Why are there variations in the amount of platelet release? Another question came up was, how differently do platelets behave in acute and chronic inflammation? Is there a time gap, or a specific period after which the genesis of platelets begins? The team also notes that the clinical criteria, such as temperature, pulse, and respiration yielded non-significant results. It seems that either the clinical recording may be flawed, for example the calculation of respiratory rate may have subjective inter-observer variations or external factors play a big role in changing their values.

These questions are only the seeds to further research venues, as our team uncovers more facts and data about the relation of platelets in inflammation.

Conclusion

The data here showed only one objective parameter (white blood cells) rising with platelet counts, with a weak positive correlation, in the background of inflammation. With supporting theories from recent years, that platelets are involved in some way, in the process of inflammation. The various pathways of thrombocytes in inflammation were followed. A generalized topic such as inflammation, which is a part of all medical disciplines, is quantified here,

with respect to the changes in platelet values. By comparing the values of the clinically relevant, and routinely used criteria, the strength of these parameters makes our understanding deeper into the molecular pathogenesis of inflammation. This has clinical applications varying from potential prevention of chronic diseases such as atherosclerosis, to identifying novel treatments to delay the progression of cancers.

Acknowledgements

Our sincere gratitude extends to all the patients , who have participated in this study. We wish them luck for their future endeavors. We also thank our hospital, and the reader for giving their time to our study.

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