The clinical utility of IPF in thrombocytopenia
Platelets

• Platelets are the smallest blood cells which are released by cytoplasmic blebbing of megakaryocytes with an average size of 2um.
• Normal values: 150-400 x10^9/L
• A megakaryocyte can form thousands of platelets.
• The freshly released platelets contain RNA, which most likely is not produced by the platelets themselves but originates from the megakaryocyte.
• These young platelets have been termed “immature platelets” or, synonymously, “reticulated platelets” or “high fluorescent platelets”.
• After 1-2 days immature platelets lose most of their RNA while developing into “mature” platelets, which have a life-span in the circulation of approximately one week.
Production of platelets
What is thrombocytopenia?

It is a reduction in platelets count below normal values.

It can be broadly categorized into 2 causes:

• Increased peripheral destruction/consumption of platelets

• Impaired Bone Marrow Production
Causes of thrombocytopenia

1. Increased Peripheral Destruction/consumption
   • Immune mechanism such as AITP, etc.
   • Increased Consumption such as DIC, TTP etc.
Causes of thrombocytopenia

2. Decreased BM Production
   • BM failure (i.e. aplastic anemia, MDS)
   • BM infiltration (i.e. tumor, fibrosis, leukaemia)
   • BM toxins (i.e. chemotherapy, infections as HIV, CMV)
How to evaluate thrombocytopenia?

• FBP including the counts and the blood smear.
• Bone marrow aspirate and trephine biopsy for cases where the cause of thrombocytopenia is suspected to be marrow related.
IMMATURE PLATELET FRACTION (IPF)
IPF- reticulated platelets

- First described by Ingram & Coopersmith (1969) in peripheral blood of dogs following acute blood loss, and they referred them to platelets with increased RNA content
- Newly released platelets which contains RNA that can be stained supravitally or with fluorescent dyes
- Larger and more reactive than mature platelets
- They are analogous to reticulocyte which is the red blood cell precursor
- **They reflect the rate of thrombopoiesis**
- Can be quantitated by microscopy and flow cytometry
How the idea started?

- The RNA inside the reticulated platelets can be measured using flow cytometry and a fluorescent dye binding to RNA, like thiazole orange.
- In the early 90’s it was demonstrated by Kienast and Schmitz that in the clinical condition of thrombocytopenia due to increased consumption or destruction of platelets (with normal or elevated numbers of megakaryocytes in the bone marrow) the percentage of platelets stained with thiazole orange was significantly higher than in cases of thrombocytopenia due to impaired platelet production (with reduced bone marrow megakaryocytes).
- This observation suggested that the measurement of immature platelets could be very helpful to facilitate the differential diagnosis of thrombocytopenia and to monitor thrombocytopenic conditions, e.g. after cytotoxic chemotherapy.
On the Sysmex XE-2100 haematology analyser platelets can be measured in the optical (fluorescence) reticulocyte/platelet channel.

Platelets and immature platelet fraction (IPF) are counted in the same channel.

The RNA in the cells is stained using a proprietary fluorescent dye containing polymethine and oxazine.

The cells pass through a laser beam and the forward scattered light and fluorescence of the cells are measured.

The reference range is approximately 1 to 5% of the total platelet count.
The clinical utility of IPF

It can help to distinguish between causes of thrombocytopenia due

• Increased consumption/destruction - **High IPF**
• Decreased production (marrow failure) – **Low IPF**
• It predicts the timing of platelet recovery (after transplantation or chemotherapy)
• It can save platelet transfusions
  – help to save a precious resource
  – help to avoid potential infection risk
IPF in AITP

• In patients with autoimmune thrombocytopenic purpura (AITP) platelet count is usually reduced due to peripheral destruction of the platelets by autoantibodies.

• Thus, thrombopoiesis is increased as a compensatory reaction to the peripheral thrombocytopenia.

• Very high values for IPF%
  – As long as PLT are consumed massively, the IPF% remains high and there will be no recovery of the platelet count
  – Patients in remission generally have near normal IPF% values
Despite different national guidelines, the investigation of the bone marrow to diagnose ITP remains controversial. Although it is not generally required by the guidelines, some authors perform bone marrow examination also for certain patients under 60 year of age.

In these cases the measurement of IPF% could demonstrate a proper thrombopoietic function of the bone marrow and avoid some of these invasive procedures.

On the contrary, it has been suggested that all patients with suspected ITP without significantly elevated IPF% should undergo bone marrow examination.
IPF in DIC

- Studies showed that IPF% was normal or elevated in DIC patients.
- Hence, IPF% might not be sensitive enough to detect DIC in all cases, however, high values of IPF% within the respective clinical setting will support or suggest the diagnosis of DIC.
Cytotoxic chemotherapy is myelosuppressive causing suppression to all marrow precursor cells including the megakaryoblasts and the megakaryocytes leading to thrombocytopenia.

The platelet count falls here due to a marrow problem. **IPF% will be on the low side.** However, as soon as the bone marrow recovers, **IPF% will rise.**

Thus, IPF can help to assess marrow recovery in these situations.
High cost, limited availability of donors, potential formation of anti-platelet antibodies and a small risk of infection are the main issues associated with platelet transfusions.

In addition, platelet transfusion temporarily suppresses thrombopoietin production and, thereby, thrombopoiesis.

Although in many countries guidelines suggest platelet transfusions when the platelet concentration falls below 20,000/ul or 10,000/ul, the necessity of this automatism has been challenged and it has been argued that many prophylactic platelet transfusions are clinically unnecessary.

A rise of IPF% proves that the bone marrow has recovered again and that a rise in platelets is expected within a short time. Therefore, it is suggested that in patients after cytotoxic chemotherapy/peripheral blood stem cell transplantation when IPF is already elevated and they are neither septic nor bleeding, platelet transfusions should be deferred even at platelet concentrations below 10,000/uL.

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IPF & Dengue fever

- IPF% can be used to evaluate the recovery of platelets in patients with dengue.
- It holds a great promise of becoming a reliable future guide for decisions concerning platelet transfusions.
Thrombocytopenia in dengue fever

- Thrombocytopenia in patients with dengue may cause a steep fall in platelet count, warranting platelet transfusion.
- However, unnecessary transfusions are best avoided due to heightened risk from alloimmunization, immunosuppression, transmission of infectious diseases, and graft-vs.-host disease.
- Clearly, a transfusion may be avoided when the platelet count is set to rise.
- This brings us to the issue of how we can reliably predict the rise in the platelet count.
- IPF% holds great promise of being this predictor.
Thrombocytopenia of moderate degree is a usual finding associated with dengue, the reasons for which are multifactorial, which include

• early transient marrow suppression with damage to megakaryocytes,
• platelet aggregation to endothelial cells targeted by dengue fever viruses,
• hemophagocytosis,
• and finally, immune destruction of platelets, with dengue antibody complexes being found on their membrane.
Prophylactic platelet transfusion may be given at level of <20 x 10^9/L in the absence of bleeding manifestations.

However, according to WHO, it should be noted that prophylactic platelet transfusions for severe thrombocytopenia in otherwise hemodynamically stable patients have not been shown to be effective and are not necessary.

Because no clear criteria exist for management of thrombocytopenia in patients with dengue, IPF% can be used by the treating physician to predict the recovery of platelets in patients with dengue, so as to avoid unnecessary blood transfusion.
• Usually in patients with dengue, at a certain point, the IPF% starts rising even though the platelets might be falling. This is due to a combination of pathogenetic mechanisms; the peripheral destruction, which lowers the platelet count, stimulates the marrow to produce more platelets which causes an increase in the IPF.

• It was found that when the IPF starts going up (rising trend), 93.75% of the patients showed recovery within 24–48 h of the rise. After a certain point when the IPF has peaked, then the platelets start coming up and IPF starts falling.

• This fall in the IPF is a strong predictor of an impending rise in platelet count.

• It was observed that 100% patients show a recovery within 24 h of the fall.
Assuming that we may not have follow-up IPF data for all patients in a clinical scenario, it was shown in one study that it is possible to evaluate the platelet recovery time based on a single time point IPF cut-off value of 10%.

This study found that 93.75% of the patients show platelet recovery within 24–48 h if the IPF is more than 10%.

Therefore, measurement of IPF% should be considered as a routine practice to evaluate and monitor thrombocytopenia in patients with dengue.
A fully automated rapid IPF counting using the sysmex technology provides clinically useful data on thrombopoietic activity.

Results are available at the same time as the results of the FBC using an EDTA-anticoagulated sample.
Take home messages

• The simplicity of the measurement along with its availability in an automated mode on a cell counter, makes the measurement of IPF% potentially useful for detecting evidence of increased platelet production and helpful for the initial evaluation of patients with thrombocytopenia.
Take home messages

- IPF% can potentially be useful as a predictor of platelet recovery in some bone marrow failure syndromes.
- The IPF% provides a valuable diagnostic method and should become a standard routine parameter in the diagnosis and monitoring of thrombocytopenic patients.
RESEARCH IN USM
A total of (51) Healthy blood donors were recruited for this study. FBC was done for each volunteer including the IPF. All samples were analyzed within 2-4 hours after collection. The results of the study showed that the mean IPF value was 3.4%. The IPF% ranged from 1.6-6.7%. Our results were similar to previous studies done worldwide.
More research

- The clinical utility of IPF in dengue fever
- The clinical utility of IPF in thrombocytopenic patients.
References


• Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia, Carol Briggs, Stefan Kunka, Dan Hart, Shinichiro Oguni and Samuel J. Machin, British Journal of Haematology, 126, 93–99
Thank you… Terima Kasih…. شكرا جزيلا