Identifying Poor Antiplatelet Drug Response and its Risks Early on

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Platelets are important cells for repairing endothelial lesions, initiating thrombus formation after vascular damage and modulating wound healing. They are also important in the formation of blood clots and so help to reduce the flow of blood through the circulatory system. Early analysis of *in vivo* platelet reactivity is important for detecting developing thrombotic events. For most of these patients, these analyses also serve as a forecast of future complications and help to evaluate the efficacy of antiplatelet medication.

Patients with acute coronary syndromes often have high immature platelet counts [1, 2] that the body produces to compensate for platelet loss caused by platelet aggregation due to atherosclerosis. Immature platelets have been found to play an important role in risk assessment and therapy monitoring of coronary artery diseases. The immature platelet count (IPF#) is a new diagnostic parameter and the IPF# value specifically reflects the absolute number of newly produced platelets in peripheral blood.

IMMATURE PLATELETS ARE MORE REACTIVE THAN MATURE ONES AND HAVE INCREASED PROTHROMBOTIC POTENTIAL

Younger, immature platelets with a greater density and residual amount of RNA (historically called 'reticulated' platelets) are more reactive since they can produce and release more thrombogenic substances (e. g. thromboxane TX) and can express more specific surface receptors (e. g. glycoproteins GPIIb/IIIa, P-selectin (CD62P)), which are important platelet activation markers. A higher prothrombotic potential of immature platelets when compared with mature ones has been documented in several publications [3-6].

A study by Stratz *et al.* (2016) showed that patients with higher IPF# values had higher platelet reactivity. A significant correlation was observed between immature platelet count and adenosine diphosphate-induced platelet reactivity (***Fig. 1***) [5].

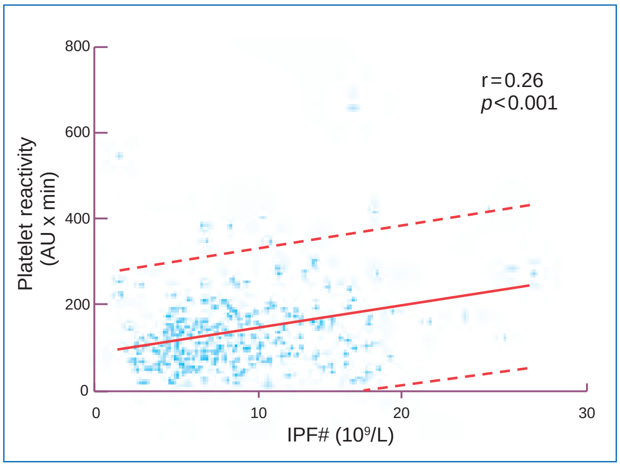

Fig. 1 *Correlation of the immature platelet count (IPF#) with platelet reactivity. Adapted from Stratz et al.* [5].

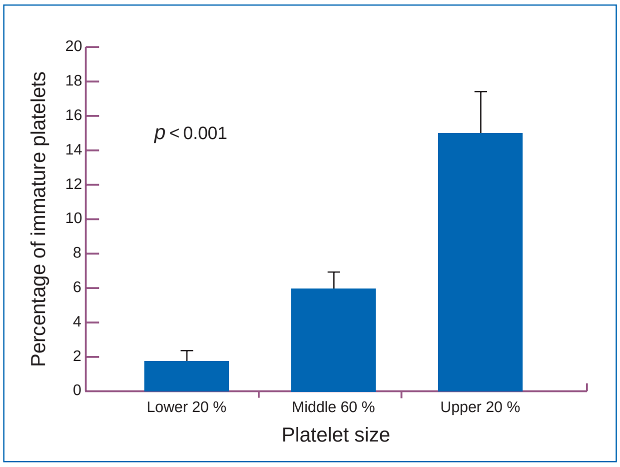

Fig. 2 *Percentage of immature platelets in the lower 20%, middle 60% and upper 20% pool size. Adapted from Guthikonda et al.* [6].

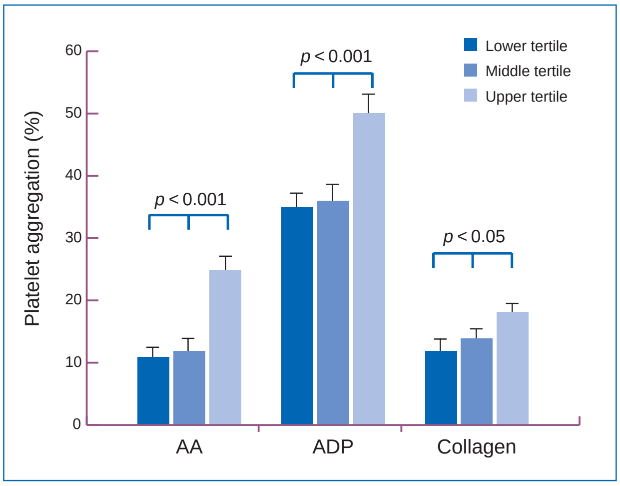

Fig. 3 *Platelet aggregation in response to arachidonic acid (AA), adenosine diphosphate (ADP) and collagen. Tertiles according to platelet size. As described above, the upper tertile reflects a high concentration of immature platelets. Adapted from Guthikonda et al.* [6].

The study from Guthikonda *et al.* (2008) found that the proportion of circulating immature platelets correlates strongly with platelet activation and aggregation. Ninety patients were stratified into tertiles according to platelet size and the proportion of immature platelets determined by immune flow cytometry. A greater percentage of immature platelets were in the pool of large platelets (upper 20%) compared with the pool of small platelets (lower 20%; 15.4% and 1.7%, respectively, ***Fig. 2***). Greater expression of both GPIIb/IIIa (5.7 versus 2.1) and P-selectin (7.8 versus 4.6) were found in the 'large' pool compared with the 'small' platelet pool. Platelet aggregation - determined by light transmission aggregometry (LTA) - in response to 5-µmol/L adenosine diphosphate (ADP), 1.5-mmol/L arachidonic acid (AA), or 1-µg/mL collagen was significantly higher in the upper tertile of platelets compared with both the middle and lower tertiles (***Fig. 3***) [6].

THE IMMATURE PLATELET COUNT IS A PREDICTOR OF ANTIPLATELET THERAPY SUCCESS

Coronary artery disease and acute coronary syndromes (ACS) are the most common cause of death in the Western world. ACS are caused by the formation of a thrombus in the location of an atherosclerotic plaque in the coronary arteries, which occludes coronary circulation. Although novel therapies for atherosclerosis are under investigation, platelet inhibition remains the cornerstone of medical therapy for ACS because there is broad clinical evidence that antiplatelet drugs reduce cardiovascular risk. Aspirin alone or in combination with $P2Y_{12}$ inhibitors (dual antiplatelet therapy) constitute the cornerstone in treatment and secondary prevention of ACS. Antiplatelet drugs are an essential preventive tool in patients with coronary artery disease. Numerous studies have shown inter-individual variability in response to aspirin therapy. It seems that once-daily dosing of aspirin is inadequate for some patients [7], since platelet function and synthesis of platelet thromboxane A2 recovers during a 24-hours dosing interval [7-9]. High-risk patients (e.g. diabetics), patients with severe atherosclerosis or with increased platelet turnover might benefit from different antiplatelet regimens [8,10-12]. An increased immature platelet count was identified as a key factor associated with insufficient platelet inhibition in response to aspirin, clopidogrel and prasugrel treatment [7, 13-16].

Immature platelets are more resistant to functional inhibition by aspirin and $P2Y_{12}$ receptor antagonists.

Aspirin is 170-fold more potent in inhibiting COX-1 than COX-2. It has been proposed that one possible explanation for aspirin resistance is residual thromboxane generation via platelet COX-2. Newly formed platelets produce COX-2 [18], which means that in conditions associated with increased platelet turnover, the large population of immature platelets generates elevated COX-2 concentrations that may be sufficient to produce detectable concentrations of thromboxane despite aspirin therapy. Several studies have also revealed that patients with an increased immature platelet count showed higher residual platelet reactivity compared to patients with physiological immature platelet counts [6,7,10,13].

The study of Guthikonda *et al.* (2007) evaluated the role of immature platelets in the antiplatelet effects of aspirin. Sixty healthy volunteers had platelet studies performed before and 24 hours after the administration of a single 325 mg dose of aspirin. Subjects were divided into tertiles based on the percentage of immature platelets determined in whole blood using immune flow cytometry. Immature platelets were found to be associated with diminished antiplatelet effects of aspirin and increased aspirin resistance, due to increased reactivity and uninhibited COX-1 and COX-2 activity. The incidence of aspirin resistance was significantly higher in the uppermost tertile (45 %) than in the lowest tertile (5 %) [13].

A potential and interesting consequence of the reduced effectiveness of aspirin in patients with high platelet turnover was that shorter aspirin dosing intervals could be beneficial for such patients, since the reactivity of immature platelets would be expected to be countered by the availability of aspirin (aspirin has a short half-life). Indeed, Pascale *et al.* (2012) concluded that the increased megakaryopoiesis accounts for a shorter-lasting antiplatelet effect of low-dose aspirin through faster renewal of platelet COX-1 and -2, and impaired platelet inhibition can be resolved by modulating the aspirin dosing interval rather than the dose [14]. The authors found that a twice-daily aspirin dosing interval reduced aspirin resistance compared to once-daily dosing.

The immature platelet count (IPF#) is a biomarker of residual platelet reactivity and predictor of the efficacy of antiplatelet therapy.

Table 1 *The antiplatelet effect of aspirin was reduced in coronary artery disease patients with increased IPF#. AA = arachidonic acid; ADP = adenosine diphosphate; RPR = residual platelet reactivity. Adapted from Grove et al.* [10].

AA 1.0 mM	**No RPR (n = 58)**	**+ RPR (n = 58)**	***p*-value**
Platelet count (10^9/L)	205 (186 – 234)	254 (237 – 305)	< 0.0001
MPV (fL)	10.8 ± 0.9	11.0 ± 0.8	0.038
IPF (%)	3.0 (2.0 – 4.2)	3.4 (2.3 – 4.9)	0.256
IPF# (10^9/L)	6.0 (4.5 – 9.1)	8.4 (6.4 – 12.4)	< 0.001
Collagen 1.0 µg/mL	**No RPR (n = 58)**	**+ RPR (n = 61)**	
Platelet count (10^9/L)	194 (178 – 234)	250 (219 – 309)	< 0.0001
MPV (fL)	10.8 ± 0.8	11.1 ± 0.9	0.055
IPF (%)	3.1 (2.1 – 4.3)	3.4 (2.5 – 4.8)	0.207
IPF# (10^9/L)	6.1 (4.6 – 8.1)	8.4 (6.1 – 13.5)	< 0.0001
ADP 10 µM	**No RPR (n = 58)**	**+ RPR (n = 61)**	
Platelet count (10^9/L)	194 (176 – 215)	262 (234 – 320)	< 0.0001
MPV (fL)	10.9 ± 0.8	11.0 ± 1.0	0.746
IPF (%)	2.8 (2.2 – 4.2)	3.4 (2.0 – 4.8)	0.579
IPF# (10^9/L)	5.6 (4.5 – 7.0)	8.4 (5.8 – 13.6)	< 0.0001
VerifyNow® Aspirin	**No RPR (n = 59)**	**+ RPR (n = 58)**	
Platelet count (10^9/L)	214 (192 – 267)	238 (208 – 283)	0.055
MPV (fL)	11.0 ± 0.9	11.0 ± 0.9	0.860
IPF (%)	3.0 (2.1 – 4.3)	3.4 (2.5 – 4.7)	0.245
IPF# (10^9/L)	6.6 (5.0 – 8.9)	8.2 (5.4 – 11.7)	0.060

Grove *et al.* (2011) investigated the impact of platelet turnover on the antiplatelet effect of aspirin in patients with stable coronary artery disease (CAD). Platelet turnover was evaluated by measuring immature platelets in 177 stable CAD patients on aspirin monotherapy. As shown in ***Table 1***, the antiplatelet effect of aspirin was reduced in CAD patients with increased IPF# [10].

Stent thrombosis is a dangerous complication of coronary stenting. The study of Wurtz *et al.* (2010) including 117 patients previously undergoing percutaneous coronary intervention found that patients with previous stent thrombosis had a reduced antiplatelet effect of aspirin due to a higher residual platelet aggregation [7].

A study from Guthikonda *et al.* (2008) found that the proportion of circulating immature platelets correlates strongly with a response to antiplatelet therapy in patients with stable CAD. Ninety patients were stratified into tertiles according to their values for immature platelets (%) determined by immune flow cytometry. As represented in ***Fig. 4***, the frequency of low response to aspirin was significantly higher in the upper tertile (53 %) compared with the middle (10 %) and lower (17 %) tertiles. The frequency of low response to clopidogrel was also higher in the upper tertile (50 %) compared with the other two tertiles (20 % and 13 % in the middle and lower tertiles, respectively) (***Fig. 4***) [6].

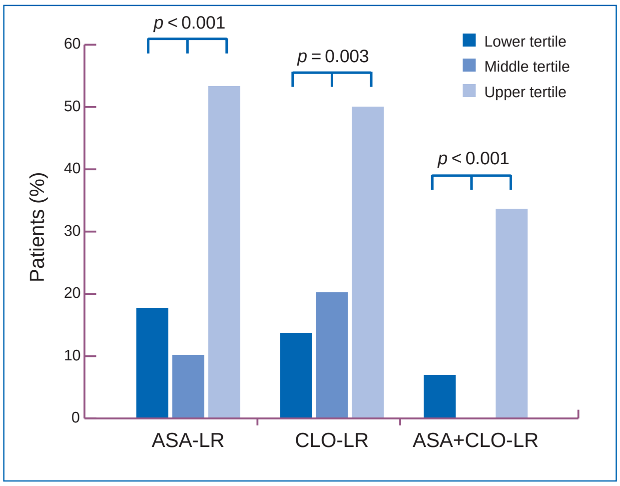

Fig. 4 *Percentage of patients with low responses to aspirin (ASA-LR), clopidogrel (CLO-LR) and both aspirin and clopidogrel (ASA+CLO-LR) in tertiles of immature platelets. Adapted from Guthikonda et al.* [6].

To determine whether immature platelets modulate the antiplatelet effects of clopidogrel, Ibrahim *et al.* (2012) evaluated 29 healthy volunteers before and one week after a 75 mg daily dosing of clopidogrel, and the volunteers were stratified into tertiles based on their immature platelet counts determined by immune flow cytometry. A higher percentage of patients with a low response to 5 μM ADP after clopidogrel was found in the uppermost tertile than in the lowest tertile of immature platelets (54% versus 23%, respectively) [15].

The aim of a study by Perl *et al.* (2014) was to determine whether response to prasugrel is associated with the proportion of circulating immature platelets in patients with ST-segment elevation myocardial infarction (STEMI). Sixty-two patients were included in the study. At the early point in time, levels of immature platelets obtained by immune flow cytometry were strongly correlated with platelet reactivity when evaluated by the $P2Y_{12}$ assay and multiple electrode aggregometry. The upper tertile of immature platelets displayed higher platelet reactivity compared with the middle and lower tertiles. Similar results with strong correlations between immature platelets and platelet reactivity were noted at 30 days post primary percutaneous intervention (***Fig. 5***) [16].

Several other studies similarly showing a high correlation of platelet aggregation with increased IPF or IPF# values in patients with coronary artery disease treated with ticagrelor, prasugrel or dual antiplatelet therapy were published recently [4,17,18].

AN INCREASED IMMATURE PLATELET COUNT IS ASSOCIATED WITH THE RISK OF THE OCCURRENCE OF ADVERSE CARDIOVASCULAR EVENTS

The main causes of atherothrombosis include disturbed blood flow, endothelial cell injury and hypercoagulability. These factors may also contribute to the transition from stable CAD into ACS. The important underlying mechanism of these diseases involves atherosclerosis. Platelets are key elements in ACS as they are the main component of thrombi in patients with ACS. As the platelets are consumed in thrombus formation the platelet count is compensated by an increased production of platelets and so increased immature platelet counts are seen in peripheral blood. Several authors reported an association between increased immature platelet fraction or count values and an increased risk of serious cardiovascular events [1,2,19-21].

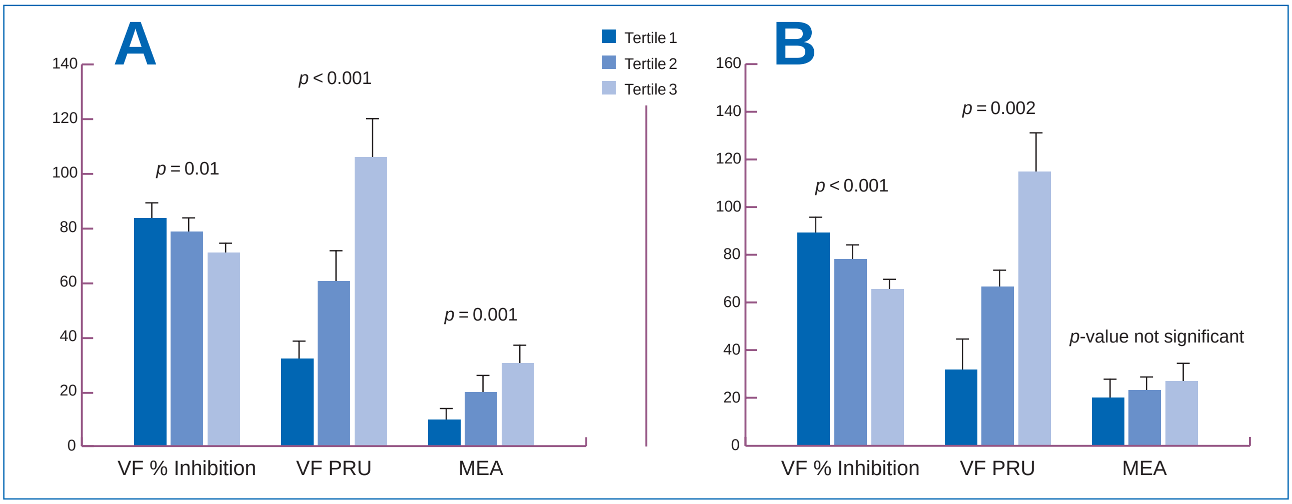

Fig. 5 *Assessment of platelet reactivity by immature platelet tertile was done by comparisons across the three tertiles after percutaneous intervention at days 2–4 (A) and day 30 (B). VF: VerifyNow $P2Y_{12}$ platelet function assay; PRU: $P2Y_{12}$ reaction units; MEA: multiple electrode aggregometry. Adapted from Perl et al.* [16].

Table 2 *A high IPF# tertile was associated with higher rates of major adverse cardiovascular events compared with the intermediate and low tertiles. Adapted from Ibrahim et al.* [22].

		IPF# tertile (10^9/L)			
		Lowest	Middle	Highest	
	Total (n = 89)	(1.364 – 5.836) (n = 30)	(5.836 – 9.272) (n = 29)	(9.272 – 27.520) (n=30)	*p*-value
Death	10 (11.2)	1 (3.3)	3 (10.3)	6 (20)	0.047
NSTEMI	11 (12.4)	1 (3.3)	3 (10.3)	7 (23.3)	0.023
Hospitalization for angina	7 (7.9)	2 (6.7)	1 (3.4)	4 (13.3)	0.175
Revascularization	6 (6.7)	1 (3.3)	1 (3.4)	4 (13.3)	0.116
MACE (composite)	30 (33.7)	5 (16.7)	7 (24.1)	18 (60)	< 0.001

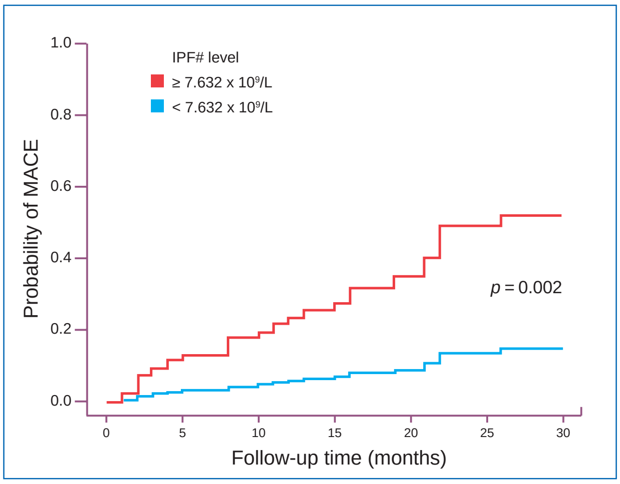

Fig. 6 *The level of IPF# ≥ 7.632 x 10^9/L was associated with an increased risk of major adverse cardiovascular events. Adapted from Ibrahim et al.* [22].

A publication from Ibrahim *et al.* (2014) found a strong association of IPF# with major adverse cardiovascular events (MACE) [22]. In the prospective cohort study in patients with CAD, patients were followed up for the composite endpoint of MACE, defined as a composite of all-cause mortality, myocardial infarction, unplanned revascularisation or hospitalisation for angina. Eighty-nine patients were followed up for a median of 31 months. Stratification into the high IPF# tertile was associated with higher rates of MACE compared with the intermediate and low tertiles (60 % versus 24 % versus 17 %, respectively) (***Table 2***). Time-dependent receiver operating characteristic analysis revealed that an IPF# level ≥ 7.632 x 10^9/L was 70.7 % sensitive and 82.1 % specific for MACE. The patients with an IPF# level ≥ 7.632 x 10^9/L were more likely to experience a MACE (odds ratio: 4.65) (***Fig. 6***).

CONCLUSION AND CLINICAL INTERPRETATION

The evaluation of the effectiveness of antiplatelet medication and forecast of future cardiovascular complications are very important for many patients. The immature platelets are more reactive compared to mature platelets and have a higher prothrombotic potential. Consumption of platelets in thrombus formation is compensated by releasing immature platelets with a higher aggregation potential. The increased immature platelet reactivity reduces the potency of several antiplatelet drugs to inhibit their aggregation potential.

The increased immature platelet count (IPF#) is a new haematological diagnostic parameter available from a routine blood laboratory test, which can be performed together with the complete blood count. IPF# is a biomarker of poor antiplatelet drug response due to residual platelet reactivity and has a better predictive value compared to traditional platelet function tests [23]. The immature platelet count can be used for determining the risk of adverse cardiovascular events.

Reference

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