Platelet function monitoring tests in the evaluation of platelet antagonists efficacy

Teste de monitorizare a funcției plachetare în evaluarea eficacității antagoniştilor plachetari

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Abstract

The development of pharmacologic agents that inhibit platelet function leads to the similar development of laboratory methods for evaluation of therapeutic efficacy, low response or drug resistance and monitoring of complications represented by minor or major bleeding. Specific and non-specific platelet antagonists act by inhibiting one or several platelet functions. Platelet antagonists are in relation with function inhibition agents that inhibit platelet adhesion, activation, aggregation and secretion.

Platelet functions monitoring has many applications during surgery, in haematology, in cardiovascular disease and research applications. High spontaneous aggregability, low response or resistance to anti-platelet drugs are risk factors for thromboembolic events. In these conditions many methods were developed for evaluation of platelet and platelet function starting with simple tests like blood smear, platelet count, mean platelet volume, in vivo bleeding time, in vitro bleeding time to more specific and standardised methods such as: Rapid Platelet Function Assay, determination of eicosanoids specific platelets metabolites, flow-cytometric determination of vasodilator-stimulated phosphoprotein phosphorylation, light transmission platelet aggregometry, impedance aggregometry - multiple electrode platelet aggregometry. The last method - impedance aggregometry seems to be the best suited for the clinical monitoring of anti-platelet therapy because it analyzes the platelet function in whole blood, similarly to the in vivo conditions, it is sensitive for all the three classes of commonly used platelet function inhibitors, provides a wide range of aggregation tests, it is standardised and cost effective in comparison with previously used methods.

Key words: *platelet function monitoring, platelet antagonists, drug resistance*

Rezumat

Descoperirea unor noi agenți terapeutici eficienți în inhibarea funcțiilor plachetare a determinat dezvoltarea unor metode de laborator performante care să evalueze eficiența terapeutică, rezistența la tratament şi să urmărească evoluția complicațiilor reprezentate de sângerări majore sau minore. Antagoniştii plachetari spe-

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cifici sau nespecifici acționează prin inhibarea uneia sau mai multor funcții plachetare fiind clasificați în agenți inhibitori ai aderării, activării, agregării şi secreției plachetare.

Monitorizarea funcțiilor plachetare are aplicații multiple în timpul intervențiilor chirurgicale, în hematologie, în cardiologie şi în cercetare. Hiperagregabilitatea plachetară spontană şi rezistența la tratamentul antiplachetar reprezintă factori de risc pentru evenimente tromboembolice. În aceste condiții s-au dezvoltat numeroase metode de evaluare a trombocitelor şi functiilor trombocitare incluzând teste simple ca examinarea frotiului sangvin, numărătoarea trombocitelor, volumul plachetar mediu, timpul de sângerare in vivo, timpul de sângerare in vitro şi metode complexe reprezentate de metoda determinării rapide a funcțiilor plachetare, determinarea metaboliților eicosanoidelor specifice plachetare, determinarea prin citometrie de flux a fosforilării fosfoproteinei stimulate prin vasodilatatie, agregometrie turbidimetrică, agregometria prin impedanță a sângelui integral. Ultima metoda- agregometria prin impedanță a sângelui integral este cea mai completă în monitorizarea clinică a terapiei antiplachetare deoarece evaluează funcțiile plachetare în sângele integral, mediul real al trombocitelor in vivo, este o metoda sensibilă pentru cele trei clase de antiagregante plachetare cel mai frecvent administrate, are cele mai multe teste disponibile, este o metodă standardizată şi cost eficientă în comparație cu alte metode de evaluare a funcțiilor plachetare.

Cuvinte cheie: monitorizarea funcțiilor plachetare, antagonişti plachetari, rezistența la tratament

The development of pharmacologic agents that inhibit platelet function led to the similar development of laboratory methods for evaluation of therapeutic efficacy, low response or drug resistance and monitoring of complications represented by minor or major bleeding. Specific and non-specific platelet antagonists inhibit one or several platelet functions. Platelet antagonists are classified in relation to the function they inhibit in:

- **Agents that inhibit platelet adhesion:** anti von Willebrand factor (vWF) monoclonal antibodies, aurintricarboxylic acid, glycoprotein IIb/IIIa (GP IIb IIIa) receptor antagonists.
- **Agents that inhibit platelet activation:** prostacyclin, prostaglandin E_1, prostanoid analogs (Iloprost, Beraprost, Cicaprost, Ciprostene), thromboxane/endoperoxide receptor antagonists, platelet-activating factor (PAF) antagonists.
- **Agents that inhibit platelet aggregation:** nitric oxide/nitric oxide donors, apyrase, GPIIb/IIIa receptor antagonists, aspirin and other nonststeroidal anti-inflammatory drugs (NSAID), dipyridamole, ticlopidine, clopidogrel, prasugrel, ridogrel, omega - 3 fatty acids, cilostazol, ketanserin, and in evaluation new adenosine diphosphat (ADP) $P2Y_{12}$ receptor inhibitors - Cangrelor, AZD6140 and protease activated receptor (PAR-1) antagonists – E5555, SCH-530348. Protease activated receptor (PAR-1) is a thrombin receptor activating peptides (TRAP) on platelets.
- **Agents that inhibit platelet secretion:** calcium channel antagonists, protease activated receptor (PAR-1), antagonists – E5555, SCH-530348.

Platelet functions monitoring has many applications: during surgery - control of platelet function, detection of aspirin and clopidogrel previous treatment by platelet aggregation measurement, monitoring of transfusion regimen; in haematology- assessment of inherited and acquired platelet disorders; research applications- anti-platelet drugs, effect of medication on platelet function, experimental models and animal models; in cardiovascular disease: control of compliance, control of the individual response to antiplatelet treatment and assessement of drug resistance.[1-3,5]

High spontaneous aggregability, low response or resistance to anti-platelet drugs are risk factors for thromboembolic events. Patients with high spontaneous aggregability have high risk for major acute cardiac events after myocardial infarction. Aspirin resistance is associated with a greater than threefold increase in the risk of major acute cardiovascular events in comparison with normal aspirin response patients. Low response to aspirin is associated

with an increased incidence of myocardial infarction related with percutaneous coronary intervention (PCI). Combined low response to aspirin and clopidogrel after PCI increases the risk for early (0-30 days after stent implantation) stent thrombosis, PCI-related myocardial infarction and composite major acute cardiovascular events at 6 months and during long term follow up. Low response to clopidogrel is associated with early stent thrombosis acute myocardial infarction and cardiovascular death after coronary stent implantation. Lack of responsiveness to clopidogrel is a strong independent predictor of stent thrombosis in patients receiving sirolimus- or paclitaxel- eluting stents. [7,9,12,15,17,21]

In these conditions many methods were developed for evaluation of platelet and platelet function starting with simple tests like blood smear, platelet count, mean platelet volume, in vivo bleeding time (Ivy and Mielke method), in vitro bleeding time (Kratzer and Born method) to more specific and standardised methods: Rapid Platelet Function Assay (RPFA), determination of eicosanoids specific platelet metabolites; flow-cytometric determination of vasodilator-stimulated phosphoprotein (VASP) phosphorylation; light transmission platelet aggregometry (Born aggregometry); impedance aggregometry[2].

Platelet count and mean platelet volume distribution profile could be determinate by modern haematology analyzers. Normal distribution of platelet volume is between 1.7 and 25.5 μm^3 with a mean value of 6 μm^3. An increased platelet volume is a prognostic factor for major acute cardiac events after acute myocardial infarction [12].

***In vivo* bleeding time** (Ivy and Mielke method) represents the time between the moment of a skin incision and the stop of bleeding. This test globally evaluates platetelet function, platelet-vascular wall interaction and vascular integrity and it is prolonged in case of dysfunction or damage of these components. Bleeding time prolongation appears when platelet count is < 100000/mm^3 or when platelet functions are inhibited (anti-platelet drugs, uraemia, liver dysfunction). A normal bleeding time for the Ivy method is less than five minutes from the time of the stab until all bleeding from the wound stops. Some texts extend the normal range to eight minutes. Normal values for the template method range up to eight minutes, while for the modified template methods, up to 10 minutes are considered normal. Normal for the Duke method is three minutes. Although bleeding time evaluates primary haemostasis, fibrinogen and plasma coagulation factors (V, VII, VIII, and IX) deficiency can affect the results. Bleeding time is useful to evaluate the efficacy of anti-platelet drugs: aspirin, clopidogrel and other thienopiridines, GPIIb/IIIa receptor antagonists and for the diagnosis of congenital or acquired platelet dysfunction: Glanzmann-Naegeli thrombastenia, Bernard-Soulier syndrome, von Willebrand-Jürgens disease, storage and release defects, cyclooxigenase deficiency [17].

***In vitro* bleeding time** (Kratzer and Born method) can be determinated with Platelet Function Assay -100 (PFA- 100), a global test for platelet function and vWF testing. Normal value of this test is 1-2 minutes and can be modiffied by GPIb receptor antagonists, GPIIb/IIIa receptor antagonists, von Willebrand factor (vWF) antagonists, aspirin, clopidogrel. In vitro bleeding time has a higher sensitivity when compared to in vivo bleeding time. When testing aspirin resistance and clopidogrel induced platelet inhibition, PFA-100 is less specific than aggregation based methods[3, 7].

Rapid Platelet Function Assay – RPFA- (Accumetrics® Verify now®) is a cartridge based, fully automated platelet function assay, with cartridges for thrombin receptor activated peptide (TRAP), aspirin and $P2Y_{12}$ - the most important adenosine diphosphate (ADP) receptor on platelet surface inhibited by clopidogrel. RPFA is a turbidimetric-based optical

detection system, measures changes in light transmission automatically and thus platelet-induced aggregation. The method is useful for quick detection of congenital or acquired platelet dysfunction, and for the evaluation of anti-platelet drugs efficacy: aspirin, clopidogrel and other thienopiridines, GPIIb/IIIa receptor antagonists in patients with acute coronary syndrome treated with platelet antagonists[3, 9].

Eicosanoids specific platelets metabolites determination - Platelets are activated by arachidonic acid, which is converted by the platelet cyclooxygenase (COX) to the potent platelet agonist thromboxane A_2 (TxA_2) which is unstable and is transformed in the more stable thromboxane B_2 (TxB_2). TxB_2 can be measured in plasma to evaluate cyclooxygenase platelet activation, while its metabolites can be measured in urine. This in an indirect, not cost effective, not standardised method and can't be used for monitoring aspirin and other nonsteroidal anti-inflamatory drugs treatment [17].

Flow-cytometric determination of vasodilator-stimulated phosphoprotein (VASP) phosphorylation is dependent on the activation level of the platelet $P2Y_{12}$ receptor, which is targeted by clopidogrel. Vasodilator-stimulated phosphoprotein (VASP) is an intraplatelet actin regulatory protein. When compared to other methods, flow-cytometry analyses specific changes in size, granularity and surface antigen expression on individual platelets. It can evaluate platelet receptors status using monoclonal antibody against GPIIb/IIIa, CD 41 (integrin alpha 2b), PAC-1 (procaspase-activating compound 1) LIBS (ligand-induced binding sites) fibrinogen-RIBS (receptor-induced binding sites) [3,4]. Some limitations of the flow-cytometry assay for platelet function were described: activation of $P2Y_{12}$ by ADP initiates other signaling pathways involving molecules such as phosphoinositide-3-kinase and Rap1b (low molecular weight guanosine triphosphatase known to promote integrin-dependent adhesion) that activate and stabilise GPIIb/IIIa independent of VASP phosphorylation; 16C2 antibody for VASP phosphorylate a serine residue that is also phosphorylated by cyclic guanosine monophosphate (cGMP)-dependent kinases that are not regulated by $P2Y_{12}$. The 16C2 antibody is a specific antibody for VASP, an established substrate of both cyclic adenosine monophosphate (cAMP) and cGMP-dependent protein kinase only when serine 239 is phosphorylated. [11, 14].

Light transmission aggregometry -LTA- (Born Aggregometry) is a method based on the variation of the optical density of a particle suspension depending on the particle number and size. Light transmission aggregometry after ADP addition to the platelet rich plasma (PRP) was considered as the current gold standard to asses platelet function in response to clopidogrel. In Born aggregometry and single platelet counting methods, platelets aggregate in the liquid phase. This presumably happens only in severely ill patients (e.g. during heparin induced trombocytopenia type II and disseminate intravascular coagulation), as coagulation and platelet aggregation in-vivo occur on surfaces (vascular injuries / inflammated vessels /atheromatous plaques). The technique is labor-intensive and restricted to specialised laboratories and has several disadvantages including sample preparation. The assay is time consuming, requires training to a high level of technichal proficiency; the methods for aggregation assay vary among laboratories, depending on the selection of agonists, agonist concentration, platelet concentration, time between sampling and analysis, centrifugation speed in preparing PRP with threefold difference in centrifugation speed (1000 rpm, 10 minutes when Chronolog Analyser is used, 720 g, 2 min for Bio/Data PAP4 Analyser and 200 g, 10 min for APACT Analyser). The assay can't be performed at the bedside and the blood samples must be quickly sent to an onsite laboratory. Other disadvantage is the fact that platelet functions are not similar in vitro and in vivo. All this data demonstrate that light transmission aggregometry is a

method with low standardisation posibilities.[6,7] .

Impedance aggregometry by Cardinal and Flower is used for the assessment of platelets functions in whole blood. It is based on the principle that blood platelets are non-thrombogenic in their resting state and expose receptors on their surface when they are activated, which allow them to attach on vascular injuries sites and artificial surfaces. The fact that aggregation in impedance aggregometry by Multiplate platelet function analysis occurs on surfaces is a major difference when compared to methods such as Born aggregometry and single platelet counting. The Multiplate Analyser is a multiple platelet function analyser and the method is usually named multiple electrode platelet aggregometry (MEA). This method functions with 5 independent channels, integrated computer, Windows XP based software, automatic analysis and documentation, electronic pipetting available and can detect the effects of aspirin, clopidogrel, GP IIb/IIIa antagonists and sensitivity for direct ADP receptor antagonists.

The principle of Multiplate analysis is based on the fact that platelets get sticky upon activation and therefore have a tendency to adhere and aggregate on metal sensor wires in the Multiplate test cell. When activated platelets adhere onto the sensor wires, the electrical resistance between the wires rises and is continuously recorded. Every Multiplate test cell has two independent sensor units, each consisting of 2 silver coated highly conductive copper wires and transformed to arbitrary aggregation units (AU) that are plotted against time. The instrument detects the impedance change of each sensor separately. The areas under the aggregation curve detected by each channel are compared, and if the difference is higher than 20% (vs. the mean curve) the measurement has to be repeated; the duplicate sensor serves as an internal control. [4,10]

The main application of the Multiplate system using the different test procedures is the monitoring of platelet function inhibitors. Several tests with different sensitivity towards the various anti-platelet agents are available. All Multiplate tests are affected by a blockade of the GPIIb/IIIa receptor. This shows that the binding of platelets to the metal sensors is dependent on the GPIIb/IIIa receptor[13-15] (*Table 1*).

Multiplate continuously records platelet aggregation. The increase of impedance by the attachment of platelets onto the Multiplate sensors is transformed into arbitrary aggregation units (AU) and plotted against time. Three parameters are calculated: the most important parameter is the area under the aggregation curve (AUC). It is affected by the total height of the aggregation curve as well as by its slope and is best suited to express the overall platelet activity. Two more parameters are calculated for research use: the aggregation (the height of the curve) and the velocity (the maximum slope of the curve). Two curves are assessed using the two independent sensors in the test cell. The parameters calculated by the software are the mean values of the parameters determined with each curve (*Figure 1*).

The unit of the AUC is AU * min (as the y-axis is the aggregation, expressed in Aggregation units (AU) and the x-axis is time, expressed in minutes). The AUC can be expressed in U (1 U corresponds to 10 AU*min). The correlation coefficient (cc) between the values of the 2 individual curves is determined. The analysis is accepted when the cc is at least 0.98. The difference from the mean curve (DIF) is calculated based on the AUC values of the 2 individual measured curves. The analysis is accepted when the difference is lower than 20% (vs. the mean value of the 2 curves).

ASPItest – Aspirin test - platelets are activated by arachidonic acid, which is converted by the platelet cyclooxygenase (COX) to the potent platelet agonist thromboxane A2. Arachidonic acid alone is not a platelet agonist. Therefore the platelet activation in ASPItest allows a very sensitive and specific detection of aspirin action. Arachidonic acid is the physio-

Table 1. Multiple electrode platelet aggregometry sensitivity to anti-platelet agents

Test	Activation by	Sensitivity	Not sensitive for	Reference range
ASPItest	Arachidonic acid: is converted to TxA_2 by platelet own cyclooxygenase	Aspirin ++ GpIIb/IIIa antagonists+	Clopidogrel, vWF	74-136 U
ADPtest	ADP: binds onto platelet ADP receptors	Clopidogrel+ GpIIb/IIIa antagonists+	Aspirin, vWF	53-122 U
ADPtest HS	ADP + prostaglandin E_1 – enhances the sensitivity of the assay for clopidogrel	Clopidogrel++	Aspirin, vWF	31-107 U
TRAPtest	TRAP-6 (thrombin receptor activating peptide) is a potent agonist which mimics the platelet activated action of thrombin	GpIIb/IIIa antagonists+	vWF, Aspirin, Clopidogrel	94-156 U
COLtest	Collagen activates platelet and triggers a release of arachidonic acid from the platelet membrane which is converted to TxA_2 by platelet own cyclooxygenase	Aspirin ++ GpIIb/IIIa antagonists+	Clopidogrel, vWF	46-116 U
RISTOtest	Ristocetin – vWF dependent platelet activation via GpIb receptor RISTOhigh: 0.77 mg/ml final concentration RISTOlow: 0.2 mg /ml final concentration	Aspirin Bernard-Soulier syndrome Severe vW disease	Mild vW disease	RISTOhigh: 90-201 U RISTOlow: 1-34 U

ADP - adenosine diphosphate, vWF - von Willebrand factor, TxA_2 - thromboxane A_2, GPIIbIIIa - Glycoprotein IIbIIIa

logical substrate of the platelet COX. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) can block the platelet COX, resulting in a reduced aggregation in ASPItest. However, the potency of Aspirin is higher compared to NSAID. ASPItest can also be reduced in case of administration of other antiplatelets antagonists, platelet disorders, e.g. in hematological disorders, thrombocytopenia,. Higher ASPItest values in patients treated with aspirin demonstrate aspirin resistance, a normal response to aspirin therapy is with aggregation values below the cut- off (30 U in patients treated with aspirin). By definition, aspirin therapy should block arachidonic acid induced aggregation. Potential strategies to treat an aspirin lack of response would be either an increase of the aspirin dose (most likely treating aspirin non-response because of reduced aspirin absorption), an increase in frequency of aspirin administration (in case of suspected elevated turn-over of platelets in the body), or switching the medication to a different drug (e.g. clopidogrel).

ADPtest - Adenosine diphosphate test - platelets are activated by ADP, which triggers several receptors on the platelet surface. Clopidogrel and related drugs block the $P2Y_{12}$ ADP receptor. ADPtest can also be reduced when other drugs interfering with platelet aggregation are taken, or in case of thrombocy-

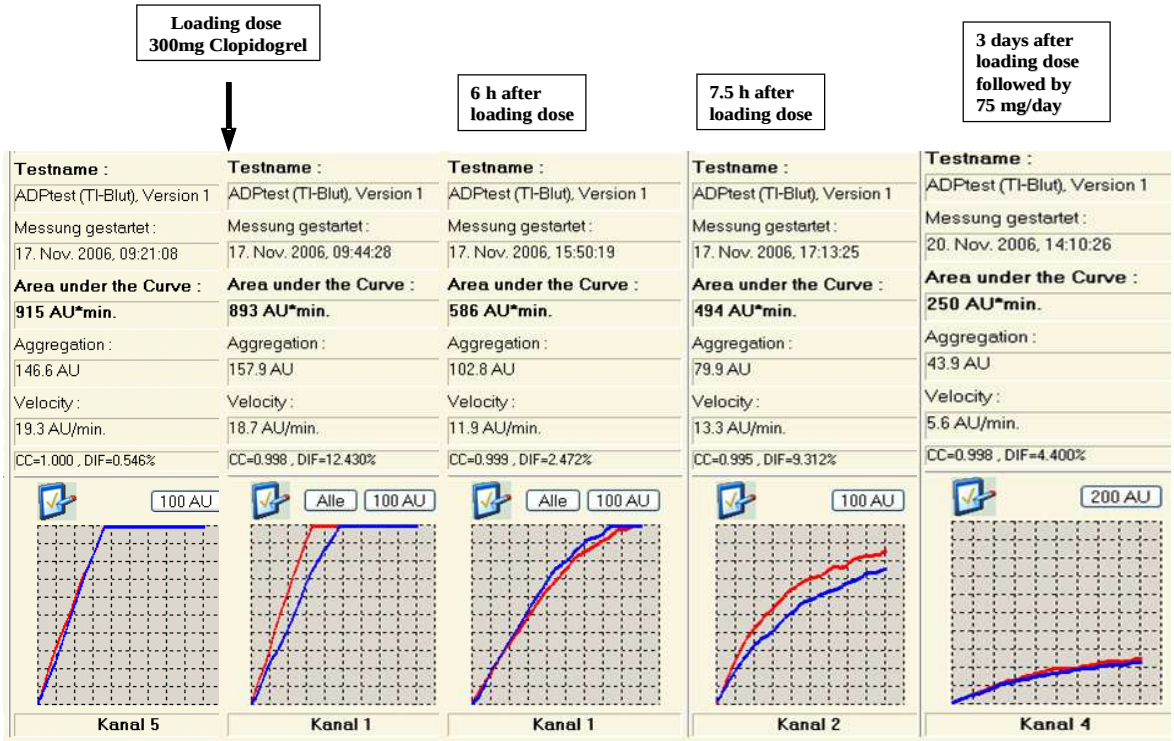

Figure 1. Examples of ADPtest results in patient treated with clopidogrel.

topenia, platelet or other hematological disorders. An ingestion of aspirin leads typically to no or only a minor inhibition of aggregation in ADPtest. However some patients may also have a reduced platelet aggregation, due to other drugs interfering with platelet activity or due to comorbidities. Higher ADPtest values in patients treated with clopidogrel show an incomplete or no platelet inhibition and a normal response determine aggregation values below the cut-off (50 U in patients treated with clopidogrel). Patients non-responsiveness to clopidogrel could be treat with an increase of the clopidogrel dose, an increase in frequency of clopidogrel administration or switching the medication to aspirin or Aggrastat for short-term use.[15]

ADPtest HS - adenosine diphosphate test high sensitivity - In ADPtest under clopidogrel therapy an incomplete blockade of aggregation is often seen. This may be due to the fact that ADP not only triggers the $P2Y_{12}$ ADP receptor on the platelet surface (i.e. the receptor that is blocked by clopidogrel), but also other ADP receptors (which are not affected by clopidogrel). A combination of ADP and a physiological platelet inhibitor (prostaglandin E_1 = PGE_1) can be more sensitive for the detection of the action of clopidogrel than the use of ADP alone. The binding of ADP to the $P2Y_{12}$ receptor reduces the level of cAMP in the platelet which enhances the release of calcium from endogenous sources. The increase of intracellular calcium then leads to the activation and aggregation of the platelet. PGE_1 reduces the mobilisation of calcium and thus inhibits platelet aggregation. As clopidogrel also reduces the platelet activation by ADP, clopidogrel and PGE_1 act synergistically. Agregation values in patients with normal response to clopidogrel are below the cut-off (25 U in patients treated with clopidogrel)[8].

COLtest - Collagen test - leads to platelet activation via the platelet collagen receptor. For suitable platelet activation endoge-

nous arachidonic acid is released from the platelet phospholipids and is transformed to the platelet agonist thromboxane A_2 by cyclooxygenase. Cyclooxygenase is blocked by aspirin, and therefore COLtest is sensitive to aspirin action. However this mechanism also explains why COLtest is less specific towards the action of aspirin compared to ASPItest. In ASPItest a defined amount of arachidonic acid is used as the activator, while in COLtest platelet are activated through endogenous TXA_2 and other TXA_2 independent mechanisms.

RISTOtest - Ristocetin test- Ristocetin is an anti-microbial substance which foms complexes with von Willebrand factor (vWF). In this complex vWF changes its conformation in a way that allows it to bind to platelets. Ristocetin is used on Multiplate in 2 concentrations: RISTOhigh: 0.77 mg/ml final concentration RISTOlow: 0.2 mg /ml final concentration. This allows the detection of samples with enhanced tendency for Ristocetin induced aggregation, especially von Willebrand disease (vWD) type IIB and samples with an absent or severely reduced response to Ristocetin (Bernard Soulier-Syndrome, vWD Type III, severe vWD Type I or II).

Conclusions

Platelet aggregation inhibition by clopidogrel may be suboptimal in 4-30% of patients. Optical aggregometry is used to assess clopidogrel's anti-platelet effects by inhibition of ADP-induced aggregation in platelet rich plasma. Red blood cells are an important source of ADP and, thus, are known to modulate platelet function. The whole blood aggregation by impedance method assesses platelet function in a physiological condition. The comparison of clopidogrel response by impedance aggregometry, by optical method in platelet rich plasma (PRP) and by Platelet Function Analyzer (PFA-100™) demonstrated that whole blood aggregation is more sensitive in detecting clopidogrel effect compared with the platelet rich plasma method and the PFA-100™ was unable to detect clopidogrel effect in the majority of the subjects[7].

Tight relationships between aggregation and cytometric quantification of platelet markers in whole blood, allow to predict aggregation response to ADP from flow data in patients treated with aspirin alone or with aspirin plus clopidogrel. Some limitations of the flow-cytometry assay for platelet function were described: activation of $P2Y_{12}$ by ADP initiates other signaling pathways and 16C2 antibody is specific antibody for VASP - an established substrate of both cAMP- and cGMP-dependent protein kinase (that are not regulated by $P2Y_{12}$) when serine 239 is phosphorylated.

Other disadvantages are represented by need of high sample stability, a long time for results availability, high costs of flow cytometer, request of experienced personel.[11, 14].

Impedance aggregometry - multiple electrode platelet aggregometry seems to be the best suited for the clinical monitoring of anti-platelet therapy because it analyzes platelet function in whole blood, similarly to in vivo conditions, it is sensitive for all three classes of commonly used platelet antagonists: COX inhibitors (aspirin, NSAID), ADP receptor antagonists (Clopidogrel, Prasugrel, Cangrelor) and GP IIb/IIIa antagonists (Abciximab, Tirofiban, Integrilin), provides a wide range of aggregation tests (ASPItest, ADPtest, ADPtest HS, TRAPtest, COLtest, RISTOtest), it is standardised and cost effective in comparisson with previously used methods[18-20].

Abbreviations

16C2 antibody = specific antibody for vasodilator-stimulated phosphoprotein (VASP)- an established substrate of both cAMP- and cGMP-dependent protein kinase only when serine 239 is phosphorylated.

ADPtest HS = adenosine diphosphate test high sensitivity

ADPtest = adenosine diphosphate test
ASPItest = Aspirin test
AU = aggregation units
AUC = area under the aggregation curve
AZD6140, E5555, SCH-530348 – platelets antagonists in clinical evaluation (phase II and III clinical trials)
CD41 = integrin alpha IIb
COLtest = colagen test
COX = cyclooxygenase
GP IIb/IIIa = Glycoprotein IIb/IIIa
LIBS = ligand-induced binding sites
LTA = Light transmission aggregometry
MEA = multiple electrode platelet aggregometry
$P2Y_{12}$ = the most important adenosine diphosphate receptor on platelet surface
PAC-1 = Procaspase-activating Compound 1
PAF = Platelet-activating factor
PAR-1 = protease activated receptor
PCI = percutaneus coronary intervention
PFA = Platelet Function Assay
PG E_1 = prostaglandin E_1
PRP = platelet rich plasma
Rap1b = low molecular weight guanosine triphosphatase known to promote integrin-dependent adhesion
RIBS = receptor-induced binding sites
RISTOtest = Ristocetin test
RPFA = Rapid Platelet Function Assay
TRAP = thrombin receptor activated peptide
TRAPtest = thrombin receptor activated peptide test
TxA_2, TxB_2= thromboxane A_2, B_2
VASP = vasodilator-stimulated phosphoprotein
vWD = von Willebrand disease
vWF = von Willebrand factor

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