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Review

Clinical monitoring of the antithrombotic treatment

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ABSTRACT

Consequences of thrombosis, whether in the arterial or in the venous system, or in the left atrium in patients with atrial fibrillation, are the most common causes of death. Therefore, great attention is given to the prevention and treatment of thrombosis in the present. The aim of this review article is to summarize the current knowledge on the laboratory and clinical monitoring of the antithrombotic treatment.

Contents

1. Introduction

Consequences of thrombosis, whether in the arterial or in the venous system, or in the left atrium in patients with atrial fibrillation, are the most common causes of death. Therefore, great attention is given to the prevention and treatment of thrombosis in the present.

The aim of this review article is to summarize the current knowledge on the laboratory and clinical monitoring of antithrombotic (i.e. anticoagulant, antiplatelet and fibrinolytic) treatment. For practical reasons, attention is limited to problems of anticoagulant and antiplatelet treatment monitoring.

2. Monitoring of anticoagulant treatment

The use of traditional anticoagulants is linked to strict laboratory control of their efficiency in terms of both insufficient and excessive dosage. The oldest drugs used for anticoagulation – warfarin and heparin – have a very narrow therapeutic window, and therefore their correct dosing is closely linked with the need for accurate laboratory control. In a case of inadequate dosage, the patient is in the risk of thromboembolic complications, on the contrary, in a case of higher levels may occur bleeding complications (Fig. 1).

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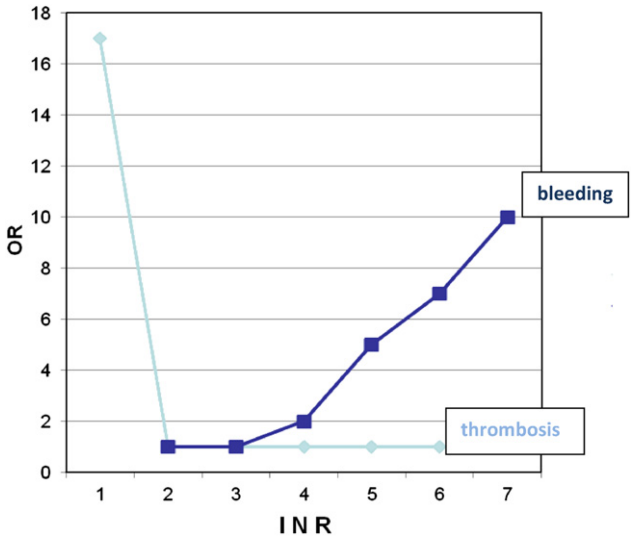

Fig. 1 – Therapeutic range of warfarin. OR = odds ratio, INR = international normalized ratio.

Warfarin remained for many decades the only oral anticoagulant. A number of studies showed a significant effect of warfarin versus placebo in patients with atrial fibrillation, and warfarin is effective in thromboembolic prevention also in patients after valve replacement. Warfarin full dose was significantly more effective in patients with atrial fibrillation as compared to low-dose warfarin, aspirin and a combination of aspirin with clopidogrel. Acetylsalicylic acid alone is not effective either in preventing systemic embolism in patients with atrial fibrillation or in preventing pulmonary embolism. A combination of acetylsalicylic acid with clopidogrel is more effective in preventing systemic embolism in atrial fibrillation than acetylsalicylic acid alone, but significantly less effective than warfarin [1]. The dosing of warfarin is very difficult and is associated with the need for strict laboratory control by the prothrombin test (PT), better expressed as International Normalized Ratio (INR). Only two-thirds of the patients (and in higher age groups, even less) are actually treated by warfarin, the percentage of patients in a therapeutic range of anticoagulation in the studies ACTIVE and SPORTIF was only between 64% and 68%, and in the RE-LY study between 41% and 77%. The problems of warfarin dosage are caused by the presence of genetic polymorphisms, drug interactions or by the frequent changes in the absorption due to food interactions. From the above-mentioned reasons only half of the eligible patients (and in higher age categories even less) actually receive the drug [1]. The optimum range with maximum antithrombotic effect and minimum bleeding complications is INR value between 2.0 and 3.0 (Fig. 1). In the RE-LY study in warfarin open branch was shown, that if the patients were divided into quartiles according to the percentage results in the effective range of anticoagulation, the mortality rate was directly related to the degree of success of anticoagulant treatment.

From indirect inhibitors of thrombin (i.e. requiring the presence of antithrombin) is still clinically used unfractionated heparin (UFH). Monitoring of its effectiveness is carried out using activated partial thromboplastin test (APTT) (partial because of the absence of tissue factor in activation mixture).

Table 1 – Target values for the evaluation of the effectiveness of heparin.

aPTT 0.9–1.1	Reference value
aPTT 2–2.5 × reference value	Adequate treatment by unfractionated heparin

Table 2 – The target values of the ACT for assessing the effectiveness of heparin.

ACT 120–180s	Normal values
ACT 200–350s	Therapeutic values

The time to blood coagulation is measured in oxalate or citrate sample after adding phospholipid, an activator and calcium (normally 25–39 s), effectivity of treatment is also expressed as the ratio of the measured to control sample (Table 1).

In the GUSTO study with 29,656 patients, Granger et al. [2] have shown that the values of APTT between 50 and 70 s are associated with the lowest incidence of both ischemic and bleeding complications.

Activated Clotting Time or Activated Coagulation Time (ACT) is used for anticoagulation monitoring in the procedures requiring administration of heparin, like cardiovascular surgery [3,4], hemodialysis or coronary interventions [5] (Table 2). The test is carried out directly at the operating theater or in the catheterization laboratory and the results are available immediately.

The ACT is a quantitative test of coagulation based on the same principle as APTT, the clotting is, however, initiated in the whole blood using a contact activator without the addition of phospholipids and calcium. It is used to monitor the anticoagulant effect of high doses of heparin. Normal values of the ACT are 80–140 s. The therapeutic range varies according to the reason for heparin administration and is 200–350 s for coronary interventions and 400–500 s for cardiosurgical procedures. The ACT does not correlate with other tests, but shows rather the degree of the prevention of thrombus creation. The results are very sensitive to the correct methodology, to the number and function of the platelets, the degree of hypothermia, hemodilution and may be also affected by some drugs, such as aprotinin. Thorough compliance with the manufacturer's instructions and the proper methodology is important, the blood collection must not be made from the entry sites, which have been contaminated by heparin administration and the obtained blood sample must be processed immediately – injected into the test tube, the activator added and the timer activated.

Ferguson and the co-authors have demonstrated in the early 1990s in the group of 1469 patients with PCI after standard, weight-adjusted heparin dose that the complications were more common in patients with ACT < 250 s than in those with the ACT > 300 [6,7]. ACT is also the appropriate test to evaluate the anticoagulant activity of low molecular weight heparin dalteparine after intravenous administration [8].

The EASY study evaluated the fate of the patients after PCI by radial approach treated by combination of abciximab, clopidogrel and aspirin. The final ACT of the 330 s was connected with the smallest peri-PCI damage and with the best prognosis during

Table 3 – The target values of the anti-Xa for assessing the effectiveness of LMWH.

Anti-Xa	
0.5–1.2 IU/ml	Therapeutic value for b.i.d. administration
>1.6 IU/ml	Increased risk of bleeding

the next 3 years, and without an increased risk of bleeding [9]. The use of combination of antithrombotic treatment with the active dose of newer antiplatelet drugs shows that for the implementation of coronary interventions with stenting it is safe to administer even lower doses of heparin with target ACT 200 s, or even just below this level [10]. This may not apply for STEMI or PCI without stenting.

In another study evaluating major bleeding complications after primary PCI for STEMI, a higher ACT was associated with an increased occurrence of bleeding (284±63 vs. 248±57 s, $P = 0.007$), multi-factorial analysis showed the value of the ACT > 250 s as being associated with significantly higher incidence of bleeding and increased 6-month mortality [11].

Treatment by the low molecular weight heparins – LMWH (enoxaparine, nadroparin, dalteparin, etc.) can be monitored using anti-Xa activity (blood sample 3–4 h after administration of heparin) (Table 3).

The results of OASIS-5 study showed that the treatment by enoxaparine can be associated with increased risk of bleeding, especially for older patients and those with reduced glomerular filtration rate. For enoxaparine administration it is therefore appropriate to determine or calculate the glomerular filtration rate (the calculation is available on www.nephron.com) with the following enoxaparine dose adjustment:

- GF > 50 ml/min – dose adjusted according to the weight;
- GF 30–50 ml/min – reduced dose (appropriate anti-Xa checks);
- GF < 30 ml/min – adjust dose according to the anti-Xa (in some countries LMWH contraindicated).

Therapeutic range of anti-Xa is between 0.5 and 1.2 (values > 1.6 means an increased risk of bleeding).

From the newer anticoagulants – indirect factor Xa inhibitor, fondaparinux (a synthetic pentasaccharide) has a more favourable ratio of efficiency and bleeding risk than low molecular weight heparins and does not need routine monitoring, albeit it is possible to measure the level of anti-Xa.

Direct intravenous thrombin inhibitor bivaluridin has the same efficiency and fewer bleeding complications in comparison to the combination of heparin and abciximab (REPLACE-2 study with 6010 patients with elective PCI and ACUITY study with 13,819 patients with medium and high risk acute coronary syndrome). Its effectiveness can be monitored using both ACT and APTT measurements.

One of the newer antithrombotic drug is dabigatran etexilate, an oral, direct thrombin inhibitor, which is effective after the conversion to its active form by serum esterase (independent of cytochrome P-450). Dabigatran inhibits both free thrombin and thrombin bound to fibrin and thrombin induced platelet aggregation. Dabigatran etexilate – after oral administration – is quickly absorbed and metabolized to dabigatran in liver and plasma. Maximum plasma concentration is achieved in 1–2 h, with half-life 14–17 h, 80% is eliminated by the kidneys. Dabigatran etexilate does not have specific antidote, its half-life is short, however, and its effects can be non-specifically reversed by higher doses of PROTHROMPLEX TOTAL EA (includes the factor II, factor VII, factor IX and factor X).

Dabigatran efficacy is influenced neither by drugs nor dietary interactions nor by genetic polymorphisms. In the prevention of thromboembolism in patients with atrial fibrillation, the standard dose is twice daily 150 or 110 mg and it is not necessary to monitor routinely the efficiency. Dabigatran prolongs APTT (APTT is appropriate for the correction of the supranormal values), APTT > 80 s at the trough (immediately before the next dose) increases the risk of bleeding). APTT is capable of only semiquantitative assessment of the anticoagulant activity of direct thrombin inhibitors (including dabigatran) and is not, however, suitable for to its determination in high concentrations. The high concentrations of direct thrombin inhibitors should be assessed by determining the ECT or dilute TT (Hemoclot®). APTT rises linearly with plasmatic concentrations of dabigatran with plateau at concentrations of ≥200 ng/ml. After supratherapeutic dose of dabigatran (400 mg t.i.d.), APTT was 2–3fold both at the lowest and at the highest concentrations of dabigatran (>400–500 ng/ml). During the chronic treatment with dabigatran 150 mg b.i.d., the ratio is around 2. Twelve hours after the last dose of dabigatran at the trough APTT is around 1.5. APTT poorly reflects the range of concentrations, which can occur in patients [12].

The effectiveness of direct thrombin inhibitors, including dabigatran, can be determined by using the relatively sensitive indicators like Ecarin Clotting Time (ECT) and Thrombin Time (TT). More recently, HEMOCLOT test (CE mark) was introduced for the evaluation of direct thrombin inhibitors. The test is currently calibrated and approved for hirudin, dabigatran and argatroban. The tests are based on the chronometric method: provide the clotting time and after appropriate calibration also plasmatic dabigatran concentration can be determined. Dabigatran does not affect the prothrombin time (PT) or INR in clinically used doses.

Determination of INR in the case of warfarin is not dependent on the time of blood sampling due to the prolonged biological half-life (half-life of S-warfarin is 18–35 h and R-warfarin 20–70 h). On the other hand, the tests to determine the anticoagulant activity of dabigatran should be carried out at trough just before the intake of the following dose due to its short biological half-life.

Thrombin time directly reflects the activity of thrombin generation in plasma and is linearly proportional to plasma concentrations of direct thrombin inhibitors. Its problem is too high sensitivity, so due to the lack of standardization of reagents in different laboratories, values in higher plasma concentrations frequently exceed the maximum calibration of the coagulometers.

Diluted thrombin time (Hemoclot®)-Hemoclot® Thrombin Inhibitor assays (Hyphen BioMed, Neuville-sur-Oise, France) allows quantitative determination of direct thrombin activity in plasma. The test is based on the inhibition of constant and

defined concentration of thrombin. Coagulation in the test plasma is initiated by adding highly purified α-thrombin. The test can be used for any direct thrombin inhibitor. The relationship between the time of coagulation and the concentration of dabigatran is linear. The most accurate is a direct calibration by lyophilized dabigatran standard, the calibration by hirudin is less accurate [12].

Ecarin Clotting Time (ECT) is specific to the measurement of thrombin generation. The trigger is snake venom Ecarin activation of prothrombin and generation of meizothrombin, an unstable thrombin precursor. As the direct thrombin inhibitors are able to inhibit meizothrombin activity similar to that of thrombin, ECT allows direct measurement of their efficacy. A linear relationship between ECT and plasmatic concentrations of direct of thrombin inhibitors was documented both in healthy volunteers and patients. ECT ratio of 2–4 was found after administration of 150 mg dabigatran b.i.d. Although clinical experience favours ECT before APTT for the evaluation of the effect of direct thrombin inhibitors, its use is rather limited to research purposes due to the lack of wide availability of the standardized and easy-to-use commercial kits [12].

Prothrombinase-induced clotting test (PiCT) [13,14] is used for monitoring the efficacy of fondaparinux, dabigatran and rivaroxaban, this test was also used for the monitoring of activity of heparin, low molecular heparins or hirudin for many years.

PiCT (Pentapharm, Basel, Switzerland) is an assay sensitive to the inhibitors of factor Xa and factor IIa. The test is based on the addition of factor Xa and the snake venom RVV (Russell Viper Venom factor V activator) with a specific activation of factor V and phospholipids in the platelet-poor plasma. The mixture is recalcified after incubation and time to coagulation is measured. It is highly sensitive and linearly proportional for unfractionated heparin, low molecular weight heparins, hirudin and argatroban. Fondaparinux has non-linear response.

Parenteral direct thrombin inhibitors hirudin, lepirudin, bivalirudin may be monitored using APTT, ECT or diluted thrombin time (Hemoclot®) (sampling is required 4 h after administration). Another option is to perform PiCT.

3. Monitoring of the antiplatelet treatment

Antiplatelet treatment is recently undergoing very rapid development, and today two new drugs are registered and clinically available – in addition to the classic ones. This could allow greater individualization of the antiplatelet treatment.

Indications and dosage of the antiplatelet treatment so far is based rather on the standard dose according to the clinical diagnosis than on controlled strategy determined in accordance with individually measured efficiency. Treatment with most commonly used antiplatelet drugs – acetylsalicylic acid and clopidogrel – may, however, be from different reasons ineffective in considerable proportion of the patients.

High residual on-treatment platelet reactivity is associated with a higher incidence of thrombotic complications [15–17] and on the contrary, there is also a laboratory documented boundary of low aggregability with increased incidence of bleeding complications [17].

Nevertheless, the evidence that the assessment of the laboratory efficiency and consequent amendment of antiplatelet treatment would improve clinical outcome, is still scarce: the study of Bonella and coworkers suggests that using the VASP it was possible to document that 25% of the patients had even after treatment with prasugrel persistent high platelet reactivity associated with the deterioration of prognosis [18]. The introduction of new and more effective antiplatelet drugs – prasugrel and ticagrelor – to the clinical testing and practice, however, led to the initiation of new multicentral randomized studies, which compare the routine administration of newer more effective drugs with a strategy of therapy escalation only in patients with persistent high residual platelet reactivity. Such studies are ongoing, like the ARCTIC study, in which patients are randomized either to routine prasugrel or to the determination of the residual platelet reactivity and the change of clopidogrel for prasugrel only in patients with high residual platelet reactivity.

4. Evaluation of platelet aggregability

Light transmission aggregometry (LTA), VerifyNow (turbidimetric optical detection system) and MULTIPLATE (multiple electrode impedance aggregometry) are currently considered to be the only reproducible, reliable, and prognostically relevant methods for the evaluation of platelet aggregability [16].

LTA assesses the light transmittance during aggregation in platelet poor plasma (PPP) and platelet rich plasma (PRP) after stimulation by an activator (arachidonic acid, collagen, adenosindiphosphate, ristocetin, epinephrine, cationic propylgalat, etc.) (Fig. 2). The results are expressed as the maximum aggregation (AU), the rate of the aggregation (AU/min or %/min) or as the area under the curve (AU*min). For patients on antiplatelet treatment the result is expressed as a percentage of platelet aggregability from the values without treatment [19]:

(1−residual aggregation/baseline aggregation) × 100

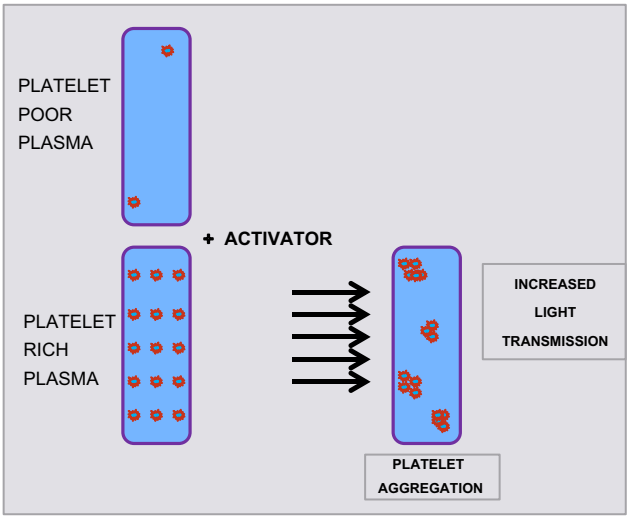

Fig. 2 – LTA measures the penetration of light during the platelet aggregation in plasma. The platelet poor plasma (PPP) and platelet rich plasma (PRP) after stimulation by activator (arachidonic acid, collagen, ADP, ristocetin, epinephrine, cationic propylgalate, etc. are evaluated).

Specific tests with the activation using the arachidonic acid (high concentration up to 1 μM), depend on the formation of endogenous thromboxane and assess the ability of ASA to block the COX-1 pathway with subsequent inhibition of thromboxane A_2 (TXA_2) formation. Because even complete inhibition of COX-1 activity may be bypassed via few other platelets activating pathways, less specific functional tests can detect high platelet reactivity even when COX-1 is completely blocked [20,21]. Less specific activators, such as collagen, ristocetin, epinephrine or cationic propylgalat assess therefore also the pathway independent of COX-1 effects of ASA. An increased turnover of platelets, transitional expression of COX-2 in the newly formed platelets or extra-platelet TXA2 resources may result in an increased platelet reactivity during the ASA treatment [22].

The lack of suppression of platelet function during treatment is associated with a variety of causes, including non-compliance, insufficient dosage, poor absorption from the gastrointestinal tract, interactions with other drugs or some predefined genetic polymorphisms.

Increased platelet reactivity is more common in patients with acute coronary syndrome [23] and is related to an adverse outcome [15]. It may be related to the elevated markers of inflammation [15,24,25], or to the increased activity of the tissue factor [26].

VerifyNow is used to evaluate the effect of antiplatelet therapy by ASA, thienopyridines (clopidogrel, prasugrel, ticlopidin, etc.), and GP IIb/IIIa inhibitors (abciximab, eptifibatide) (Fig. 3). VerifyNow Aspirin Test – the activator is arachidonic acid, result is expressed in Aspirin Reaction Units (ARU), the cut off value is usually 550 ARU, when values ≥550 ARU mean normal aggregability, while values <550 mean adequate platelets inhibition by ASA. This test cannot be performed in patients treated with other antiplatelet drugs such as clopidogrel, prasugrel or inhibitors of GP IIb/IIIa receptor.

VerifyNow P_2Y_{12} test – ADP is used as an activator, the test is focused on the evaluation of platelet aggregability in patients already treated with clopidogrel (TEST). Therefore aggregation after modified activator of thrombin receptor – iso-TRAP (Thrombin Receptor Activating Peptide) – is used as a control value in the reference channel (BASE). The test result is given as the absolute P_2Y_{12}-Reaction-Units (PRU) and as percentage inhibition (% inhibition), calculated as follows:

$$100-(\text{TEST}/\text{BASE}\times 100)$$

or

$$[(\text{BASE}-\text{TEST})/\text{BASE}]\times 100$$

VerifyNow GP IIb/IIIa test – uses iso-TRAP (Thrombin Receptor Activating Peptide) as an activator, the values are given in Platelet Aggregation Units (PAU).

MULTIPLATE evaluates the platelets function in whole blood. Platelets aggregate on metal sensors and increase electrical resistance measured between the pair of sensors (Fig. 4).

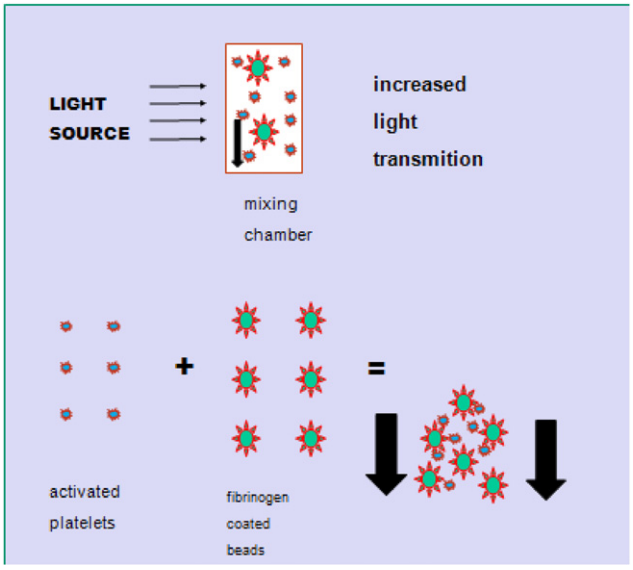

Fig. 3 – VerifyNow assesses the changes in light penetration after aggregation of activated platelets to the particles (beads) covered with fibrinogen.

Table 4 – Cut-off values for MULTIPLATE (AUC = area under the curve, U = unit, HS = high sensitivity – sensitized by prostaglandin E1).

Activator	AUC (U)	Cut-off (U)
ASPItest (arachidonic acid, 15 mM)	74–136	30
ADPtest (adenosidiphosphat 0.2 mM)	53–122	50
ADPtest HS (ADP 0.2 mM sensitized by prostaglandin E1)	31–107	25
TRAPtest (thrombin receptor activating peptide, 1 mM)	94–156	30

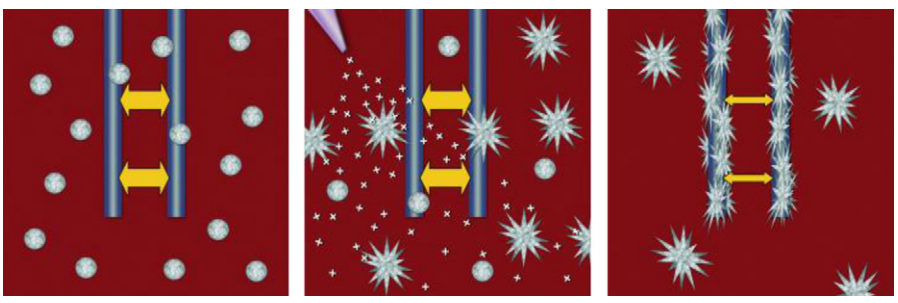

Fig. 4 – The MULTIPLATE system is based on the principle that the platelets aggregate at the surface of the metallic sensors and subsequently increase the electrical resistance measured between two of these sensors.

ASPItest, ADPtest and TRAPtest are used to evaluate the effects of ASA, ADP inhibitors and GP IIb/IIIa inhibitors, respectively.

For ASPItest – activator is arachidonic acid, cut-off values for sufficient blockade of platelet by ASA is $\leq$50 U, values above 50 U mean ineffective treatment by ASA (Table 4).

ADPtest is suitable for the assessment of treatment by clopidogrel, ticagrelor, prasugrel or ticlopidin, cut-off value for sufficient blockade is $\leq$45 U, values above 45 U mean ineffective treatment. The initial experience suggests that the evaluation of the effect of the new ADP inhibitor ticagrelor may need a different cut-off values and a higher dose of ADP – i.e. 0.6 μmol/l.

TRAPtest evaluates the effect of treatment by GP IIb/IIIa inhibitors, ASA and inhibitors of ADP, the values $\leq$60 U indicate sufficient blockade, values above 60 U mean ineffective treatment.

Determination of residual platelet reactivity during antiplatelet treatment cannot be regarded as a test for the occurrence of thrombosis in the arterial system (such as troponin is the marker of myocardial necrosis), but it is a test showing an increased risk of thrombotic complications (such as, for example, hyperlipoproteinemia is a risk factor for the atherosclerosis development and progression). The prerequisites to arterial thrombosis are usually both an increased residual platelet reactivity and a substrate like non-endothelised stent struts, an atherosclerotic plaque rupture, etc. Available data indicate that sufficient suppression of platelet reactivity reduces to a minimum the risk of arterial thrombotic complications.

We documented good short-term reproducibility of the platelet function tests, in the medium and long term horizons, however, the results may be influenced by a whole series of other variables, it is therefore warranted to repeat the test in the course of the time [27].

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