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Review

Platelet response to aspirin: Leading the way towards individualized therapy

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ABSTRACT

Aspirin remains the most widely used drug in patients with cardiovascular disease to prevent acute ischemic events. Its daily administration reduces the risk of stroke, myocardial infarction and death by approximately 25%. However, inhibition of platelet aggregation by aspirin is variable, the cause of this phenomenon being most likely multifactorial. Several intrinsic mechanisms may contribute to modulating platelet response to aspirin, in addition to various extrinsic factors not related to platelet activation pathways. Moreover, the currently available platelet function assays target different pathways of platelet activation, and may also contribute to the variability of the measured platelet response to aspirin. Therefore, judicious use of these assays, through better understanding of their value and limitations in assessing platelet function, as well as a better understanding of the various factors influencing platelet aggregation, should lead the way towards better individualized antiplatelet therapy, offering both adequate inhibition of platelet aggregation and reduced risk of bleeding.

KEYWORDS: aspirin, antiplatelet therapy, pharmacology, platelet function testing, response variability

1. INTRODUCTION

Cardiovascular diseases are the leading cause of death in the industrialized world, with coronary artery disease (CAD) and stroke accounting for over 70% of cardiovascular mortality [1]. Antiplatelet therapy represents the cornerstone of preventive cardiovascular medicine by inhibiting platelet aggregation, an important step in the pathogenesis of atherothrombosis [2].

Platelet function is modulated by numerous factors, which directly or indirectly influence the ability of aspirin to inhibit platelet aggregation. Moreover, the assays currently available to assess platelet aggregation are not all equivalent in their ability to measure the effect of aspirin and correlate poorly amongst themselves [3-5]. In light of this, individualization of aspirin therapy based on specific patient characteristics and platelet mechanisms known to modulate platelet function is an attractive option for patients to receive the best preventive therapy against

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cardiovascular ischemic events. Additionally, treatment effect should be assessed with validated, sensitive, and specific assays. In this article, we sought to 1) evaluate the current tools used for the assessment of aspirin's response; 2) discuss the mechanisms by which platelet response may be modulated; and 3) review the characteristics that may put patients at greater risk of reduced aspirin efficacy.

2. Clinical efficacy of aspirin

Although aspirin has been on the market for over a century, it remains the most widely used antiplatelet agent, mainly due to its low cost, favourable adverse effect profile, and effectiveness [2]. Through irreversible acetylation of the platelet cyclooxygenase (COX)-1 enzyme (Figure 1), aspirin inhibits thromboxane (Tx) A_2-dependent platelet aggregation [6, 7], and reduces the risk of stroke, myocardial infarction and death by approximately 25% [2]. This means that the remaining 75% of recurrent events are not prevented by daily aspirin therapy. Platelet response to aspirin has been repeatedly shown to be variable [8], although estimates about the prevalence of aspirin resistance are still unreliable and highly dependent on the platelet function assay used and the population studied [3, 9-12]. Several studies have shown that patients biochemically identified as aspirin resistant using *in vitro* platelet function assays exhibit significantly higher risks of recurrent cardiovascular events compared with patients who are identified as aspirin sensitive [13-17].

3. Measuring aspirin's efficacy

Because of the mechanism of action of aspirin, measurement of platelet aggregation, which may occur in response to several agonists, has become a valuable tool to evaluate the efficacy of the drug [18]. Considerable cross-talk occurs between the various pathways leading to platelet aggregation (Figure 1); the relative efficacy of aspirin to inhibit platelet aggregation depends on the platelet agonists involved [e.g. arachidonic acid (AA), adenosine diphosphate (ADP), epinephrine, collagen, etc.] [19]. To assess the aspirin-sensitive COX pathway-dependent platelet aggregation, AA, the precursor of TxA_2, is the preferred agonist [19]. Alternatively, when other agonists are used, platelet aggregation may be induced despite effective COX inhibition by aspirin [3]. Whereas most agonists in low concentrations (i.e. ADP 1-3 µM, epinephrine 5 µM, or collagen 1 µg/mL) require synergistic activity of the COX pathway to induce measurable irreversible platelet aggregation, higher concentrations of these agonists induce platelet activation directly through their respective platelet receptor pathway, thus bypassing platelet inhibition by aspirin (Figure 1) [19]. Accordingly, their use in high concentrations to assess platelet response to aspirin is usually inappropriate.

A number of platelet function assays have been developed over the years to quantify platelet aggregation, either directly through measurement of TxA_2 metabolite concentrations, or indirectly through changes in platelet surface in response to activation (Table 1). These include point-of-care devices that are attractive in terms of labour and ease of use, but limited to pre-selected agonists. Although their popularity grew to evaluate aspirin resistance in clinical trials [20, 21], most available assays were not designed or validated specifically for this indication. Notwithstanding, some platelet function assays, TxA_2-specific or not, can identify sub-groups of patients at increased risk of adverse cardiovascular events despite aspirin administration [17, 22-28]. The principal advantages and limitations inherent to the most commonly used assays are discussed below.

3.1. Quantification of platelet aggregation

3.1.1. Optical aggregometry

By far the most widely used platelet function assay, optical or light transmission aggregometry (LTA) is the established gold standard for assessment of platelet aggregation [20, 21]. After addition of a platelet agonist to platelet-rich plasma, luminosity is measured as aggregation occurs [18, 21]. Although LTA may be considered among the most platelet-specific of all available techniques [29], it is time consuming, requires technical expertise and displays poor inter-laboratory standardization and reproducibility, which limits its usefulness as a clinical decision-making assay [3].

3.1.2. Whole blood aggregometry (Chrono-Log WBA® and Multiplate®)

Alternatively, aggregation may be measured in whole blood by quantifying the electrical

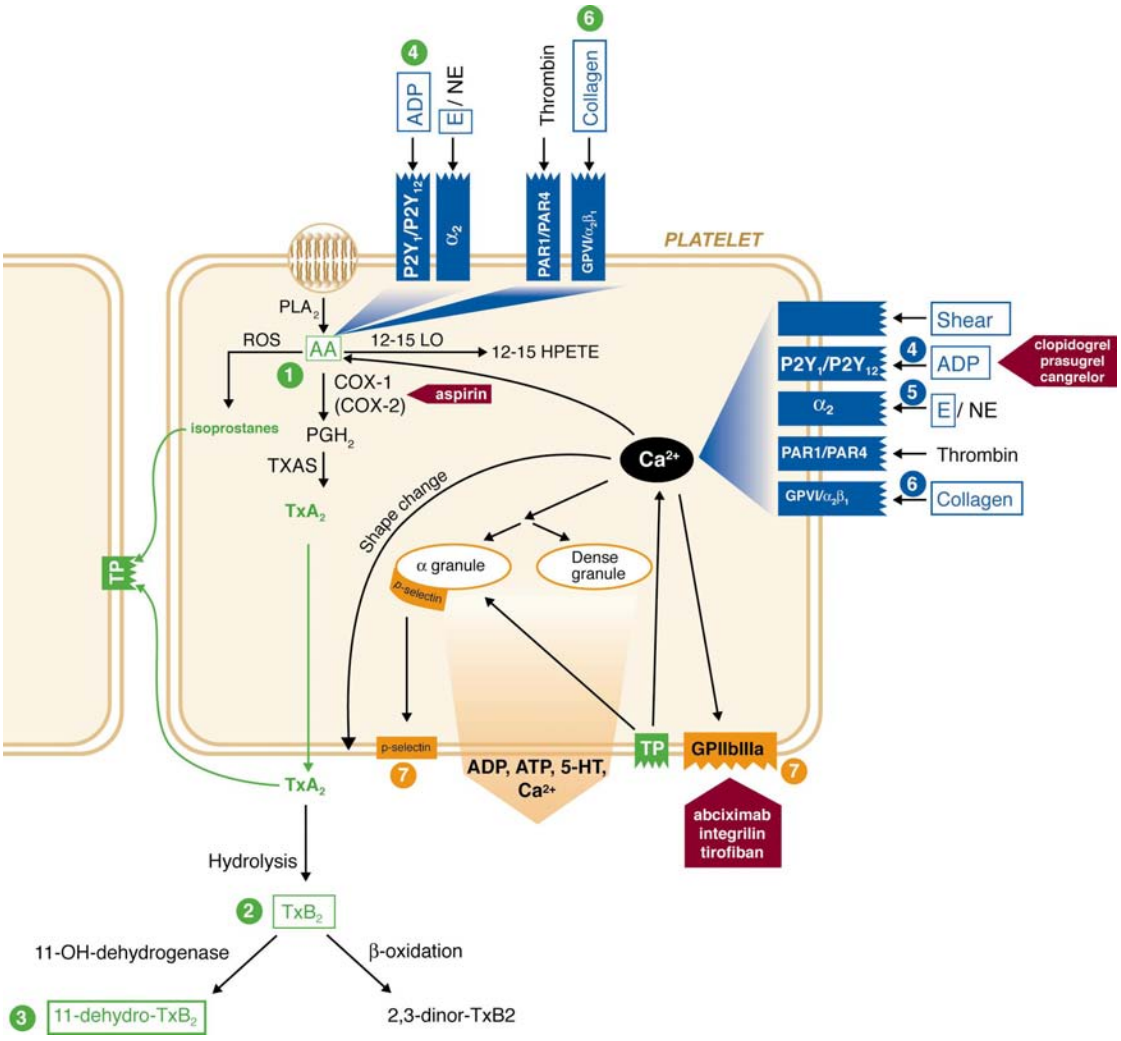

Figure 1. Pathways leading to platelet activation and targeted platelet function assays. Considerable cross-talk takes place between different pathways of platelet activation, resulting in a synergistic effect of platelet agonists. Platelet activation increases intracellular calcium concentration through stimulation of several receptors (including $P2Y_1/P2Y_{12}$, α_2, PAR1/PAR4, GPVI/$\alpha_2\beta_1$, TP), leading to platelet shape change and degranulation. Additionally, it activates PLA_2, thus promoting TxA_2 generation from AA. Alternatively, AA can undergo conversion by the lipooxygenase pathway or through nonenzymatic oxidative modifications, and generate platelet activating molecules (e.g. lipid hydroperoxides and isoprostanes). Antiplatelet agents act on distinct pathways of platelet aggregation, including the COX pathway, the ADP-sensitive pathway and the GP IIb/IIIa receptor. As aspirin inhibits platelet COX-1, it abolishes TxA_2-dependent platelet aggregation, as well as produces a relative inhibition of aggregation induced by other agonists through abolishment of a synergistic loop. Because other pathways of platelet activation remain active, aggregation in response to other agonists in high concentrations may occur. The different numbers refer to various platelet function assays that can be used to evaluate platelet function, the agonist involved or the measured element, as presented in Table 1. The green numbers indicate COX-specific assays; the blue ones refer to alternative platelet activation pathways; and the yellow ones point to platelet elements that can be measured to evaluate the activation state of platelets. Platelet function tests targeted at the COX pathway are most appropriate in measuring aspirin's antiplatelet efficacy. Direct measurement of serum TxB_2 may be best suited for quantification of platelet response to the pharmacological effect of aspirin. AA: arachidonic acid; ADP: adenosine diphosphate; ATP: adenosine triphosphate; Ca^{2+}: calcium; COX: cyclooxygenase; E: epinephrine; HPETE: hydroperoxyeicosatetraenoic acid; LO: lipooxygenase; NE: norepinephrine; PGH_2: prostaglandin H_2; PLA_2: phospholipase A_2; ROS: reactive oxygen species; TP: thromboxane receptor; Tx: thromboxane; TXAS: thromboxane synthase; 5-HT: serotonin.

Table 1. Platelet function assays frequently used to assess response to aspirin.

	Laboratory testing (agonist)	**Point-of-care assays (agonist)**
Specific COX-pathway assays	❶❹❻ LTA (AA, low-dose ADP or collagen) ❶ WBA (AA) ❷ Induced serum TxB_2 concentrations ❸ Urinary $dTxB_2$ concentrations	❶ VerifyNow Aspirin® (AA) ❶ Chrono-Log WBA® (AA) ❶ TEG® (AA) ❶ IMPACT® (AA) ❸ AspirinWorks™
Unspecific assays	❹❻ LTA (high-dose ADP; high-dose collagen) ❹ WBA (high-dose ADP) ❼ Flow cytometry - p-selectin, activated GP IIb/IIIa receptors	❹❻ PlateletWorks® (collagen or ADP) ❺/❻ PFA-100® (collagen/epinephrine)

AA: arachidonic acid; ADP: adenosine diphosphate; $dTxB_2$: 11-dehydro-thromboxane B_2; LTA: light transmission aggregometry; TEG: thromboelastography; TxB_2: thromboxane B_2; WBA: whole blood aggregometry. Low-dose ADP refers to ADP 1-3 µM; low-dose collagen to 1-3 µg/mL; high-dose ADP to 5-20 µM; high-dose collagen to 5-10 µg/mL. The numbers refer to Figure 1.

impedance between two electrodes immersed in blood in the presence of a platelet agonist [19, 21]. The main advantage of this technique is that measurements are undertaken in a physiological milieu and that it is also available as a point-of-care assay. However, the mechanisms leading to increased platelet aggregation in such a milieu may bypass inhibition by aspirin due to transcellular prostanoid formation by monocytes or through direct stimulation of degranulation by erythrocytes [30-32]. Hence, although highly clinically relevant, whole blood aggregometry may not be as sensitive to aspirin inhibition as aggregometry in platelet-rich plasma [33].

3.1.3. VerifyNow Aspirin®

The VerifyNow Aspirin® is a whole blood point-of-care assay based on turbidimetric detection, in which light transmittance is captured as platelets aggregate on beads coated with human fibrinogen following exposure to AA as platelet agonist [34]. Unlike most other assays, it was specifically designed and US Food and Drug Administration (FDA)-approved to detect platelet inhibition by aspirin [18]. VerifyNow Aspirin® was found to correlate well with non-COX dependent platelet function tests using various agonists, suggesting that it may identify a generalized high platelet reactivity phenotype [35]. Nevertheless, the use of this test comes with several limitations. This assay is performed in whole blood, which, as discussed previously, may be clinically relevant but less sensitive to detect aspirin's response. Further studies are needed to determine its ability to predict the risk of bleeding and to justify the current cut-off level for aspirin resistance set at 550 Aspirin Reaction Units [36, 37].

3.1.4. Platelet function analyzer (PFA-100®)

The PFA-100® device, FDA-approved to detect platelet dysfunction, assesses platelet aggregation under high shear, mimicking platelet-rich thrombus formation after injury to a small vessel wall under flow conditions [18, 21]. Although it has been one of the most widely used point-of-care assays to evaluate platelet response to aspirin [38], its epinephrine and collagen-based methodology renders this test unspecific to the aspirin-sensitive COX pathway, thus less suitable for quantification of aspirin's response.

3.1.5. PlateletWorks® (Platelet count drop or platelet ratio)

The point-of-care PlateletWorks® assay is FDA-approved to measure platelet aggregation during cardiac interventional procedures. This assay measures the reduction in the number of free platelets in whole blood following aggregation in response to either ADP or collagen [21]. The test has seldom been used for quantification of aspirin's effect and appears to be less sensitive and less able to detect aspirin's effect and to predict clinical outcome than LTA [5, 35].

3.1.6. Thromboelastography (TEG®)

The Thromboelastograph Platelet-Mapping System® is FDA-approved for assessment of platelet function in patients who have received platelet inhibiting drugs, such as aspirin. The assay determines the strength of a clot formed in whole blood following addition of AA, between a rotating cup and a suspended pin [18]. Although its use remains limited, it has been shown to be of low sensitivity to platelet inhibition by aspirin [39].

3.1.7. Cone and plate(let) analyzer (IMPACT®)

This assay does not measure platelet aggregation but rather shear-induced platelet adhesion, produced by the rotation of a cone in a standardized polystyrene-coated cup [18, 21]. Although the availability of AA as the agonist enables monitoring of aspirin therapy, its use in assessing platelet response to aspirin has rarely been reported in the literature [40].

3.2. Measurement of TxA_2 metabolites

3.2.1. *In vitro* induction of TxB_2 production

TxA_2 synthesis can be determined by measuring the concentration of its stable metabolites, such as TxB_2, in serum or plasma following platelet aggregation induced by letting whole blood clot or by adding a platelet agonist to platelet-rich plasma sample (AA, collagen, etc.) [41]. This technique measures directly the ability of aspirin to interact with its pharmacological target, and is best suited to quantify platelet response to aspirin [42]. However, normal ranges of response are not known with precision, which makes this technique difficult to interpret.

3.2.2. Urinary concentration of 11-dehydro-TxB_2 (AspirinWorks™)

In recent years, measurement of TxA_2 metabolites in urine has gained popularity following publication of a report associating increased risk of cardiovascular events in patients with high levels of urinary 11-dehydro-TxB_2 [23]. Although it theoretically assesses the degree of inhibition of platelet COX-1 by aspirin, urinary concentrations of TxA_2 metabolites are a global index of TxA_2 synthesis, which may originate from other blood elements (e.g. erythrocytes and monocytes) and renal biosynthesis [9]. Accordingly, because high levels of 11-dehydro-TxB_2 in urine despite daily aspirin therapy may be a reflection of a larger non-platelet production as opposed to increased platelet activity, this technique might not be optimal for measuring the antiplatelet effect of aspirin.

3.3. Activation-dependent changes in platelet surface

Flow cytometric evaluation of platelet characteristics after labelling activation markers with monoclonal antibodies can be used to assess aspirin's effect. The most commonly used markers of platelet activation include epitopes of P-selectin (expressed on the platelet surface after granule secretion) and of the activated GP IIb/IIIa receptor [19, 21]. Although this technique is powerful in assessing platelet function, its interpretation remains subjective and normal ranges of response have not been defined, rendering the technique exploratory and, for the most part, qualitative.

In summary, depending on the methodology used, different results of platelet response to aspirin may be obtained, which may not accurately reflect the true efficacy of aspirin but rather a state of activation that bypasses the aspirin-specific COX-1 pathway. Therefore, proper understanding of the mechanisms and sensitivities of the various methodologies is necessary before clinical extrapolation is possible.

4. Factors modulating platelet response

As discussed previously, difficulties in accurately evaluating platelet response to aspirin are inherent to the methodologies used, but are also a reflection of the multiple activation pathways that modulate platelet response (Figure 1). The following paragraphs discuss several factors that may influence platelet function (Table 2).

4.1. Intrinsic factors modulating platelet response to aspirin

4.1.1. Platelet COX-1 modification

Platelet response to aspirin may be altered by several mechanisms. The inability of aspirin to interact with its pharmacological target on platelet COX-1 due to structural changes to the target protein, constitutes one such mechanism [43].

Table 2. Factors modulating platelet response.

Intrinsic factors modulating platelet response to aspirin
Platelet COX-1 modification
Platelet COX-1 regeneration
Platelet COX-2 expression
Increased platelet turnover
Factors modulating platelet function independent of aspirin therapy
TxA2-independent platelet activation
Platelet hypersensitivity
Endothelial dysfunction and oxidative stress
Extrinsic factors leading to apparent low aspirin response
Non-adherence to therapy
Reduced bioavailability
Drug interactions

From an environmental perspective, both glucose and aspirin compete for platelet COX-1, but the latter cannot inhibit COX-1 after it has been glycosylated [43]. Hence, increased COX-1 glycosylation in poorly controlled diabetic patients can contribute to impaired platelet response to regular doses of aspirin [43-45].

The structure or activity of COX-1 could also be altered through genetic variation of the COX-1 coding gene. Indeed, a common haplotype affecting the promoter region of the gene (-842A/G) and exon 2 (50C/T) was found to modulate platelet response to aspirin, with carriers of the -842G - 50T alleles presenting higher platelet aggregation and TxA_2 levels despite aspirin administration [46, 47]. However, other studies have reported discrepant results where this haplotype was not associated with reduced platelet inhibition by aspirin [48-50]. Thus, this association remains unclear.

In effect, platelet response to aspirin could be subject to platelet environmental or genetic alteration of the COX enzyme, however none of these mechanisms account fully for the variability in platelet responsiveness to aspirin.

4.1.2. Platelet COX-1 regeneration

Being anuclear, platelets have long been considered incapable of regenerating the COX-1 enzyme following acetylation by aspirin [6]. This dogma has been challenged by recent reports of the presence of mRNA in platelets coding for a multitude of proteins and of *de novo* protein synthesis in stimulated platelets [51-53]. Evangelista *et al.* demonstrated *ex vivo* that platelets treated with aspirin retained the capacity to synthesize sufficient TxA_2 to induce platelet aggregation within 24 hours of aspirin administration [54]. This occurred through generation of new and active COX-1. If COX-1 regeneration were found *in vivo*, it could explain the variation in platelet response to aspirin observed in certain individuals.

4.1.3. Platelet COX-2 expression

COX is present in 2 distinct isoforms in humans. While COX-1 is constitutively expressed and takes part in many house-keeping functions, COX-2, mostly absent at rest, is inducible through cytokine signalling, and often accompanies inflammatory states [55]. While megakaryocytes are capable of COX-1 and COX-2 expression, mature platelets usually express COX-1 exclusively. However, in certain clinical conditions associated with high platelet regeneration, a significant portion of newly generated platelets may co-express COX-2 [55, 56]. Like COX-1, COX-2 can transform AA into TxA_2 [55]. Because aspirin is 170-fold more potent in inhibiting COX-1 than COX-2, administration of cardioprotective doses

of aspirin does not allow for effective COX-2 inhibition [6]. Consequently, TxA_2 formation may not be fully inhibited by daily low-dose aspirin administration and may lead to platelet aggregation despite aspirin therapy in patients displaying platelet COX-2 activity [57].

4.1.4. Increased platelet turnover

In addition to platelet COX-1 regeneration and COX-2 expression, increased platelet turnover results in the introduction into the bloodstream of a large number of newly formed aspirin-naïve platelets which may further contribute to increased TxA_2-induced platelet aggregation. Because irreversible COX-1 acetylation by aspirin is classically known to inhibit TxA_2-induced platelet aggregation for the platelet lifespan (7 to 10 days), recovery of platelet function following aspirin administration is highly dependent upon generation of new platelets from megakaryocytic cells. As thrombopoiesis can increase more than 10-fold under conditions of increased demand (such as inflammation or platelet consumption in thrombosis) [58], it can rise above the usual daily platelet turnover rate of approximately 10-15% providing enough young platelets to overcome aspirin-induced platelet inhibition during the 24-hour dosing interval [6]. This has been observed in patients with essential thrombocythemia [59, 60], in diabetic patients [61], in healthy volunteers [33], as well as in CAD patients [62-65], suggesting that the once-daily aspirin dose may no longer be the optimal standard dosing for all patients [66]. The same phenomenon has been observed in CAD patients on aspirin and clopidogrel, suggesting that increased platelet turnover may explain the suboptimal platelet inhibition despite dual antiplatelet therapy [67].

4.2. Factors modulating platelet function independent of aspirin therapy

4.2.1. TxA_2-independent platelet activation

In order for platelet aggregation to occur, several platelet agonists need to act synergistically with TxA_2 to induce sustained platelet aggregation [68]. Although almost complete suppression of TxA_2 formation through the AA pathway occurs with aspirin, other platelet agonists may allow platelet aggregation to occur through other pathways [41]. For example, stress increases circulating levels of catecholamines, which activate platelets through stimulation of α_2-adrenoreceptors [69]. Acting through an alternate pathway, epinephrine can bypass TxA_2-induced platelet aggregation and modulate platelet function despite aspirin administration in patients such as those undergoing mental, physical, or cigarette smoke-induced stress [70-72].

4.2.2. Platelet hypersensitivity

Alternatively, platelets may become hypersensitive to some agonists, such as ADP or collagen, which can result in platelet aggregation despite aspirin administration [73, 74]. Although mechanisms responsible for platelet hypersensitivity remain mostly unknown, genetic variations in platelet receptors or enzymes regulating platelet function may be operative.

A specific polymorphism of the platelet $P2Y_1$ ADP receptor (1622A/G) has been shown to increase platelet reactivity to ADP in a study on healthy volunteers [75], but this finding could not be replicated in a study on stable aspirin-treated CAD patients [76]. Notwithstanding, this same polymorphism has been shown to be associated with insufficient inhibition of AA-induced platelet aggregation in stable CAD patients on daily aspirin therapy. The mechanism underlying this phenomenon remains unknown [76].

The collagen receptors GPIa/IIa and GPVI have also been studied. The C807T polymorphism of the GP Ia subunit has been the most thoroughly investigated, although the published results have been conflicting. In a Chinese population of 200 patients suffering from atherosclerotic disease, the 807T allele was found to confer a 3.8 times increased risk of insufficient platelet inhibition by aspirin [77]. This association could not however be replicated in most other investigations [47, 78, 79]. The T13254C polymorphism of the collagen GPVI receptor has also been examined in this context. The T13254C genotype affected the aspirin response evaluated by PFA-100® in one study [49], but not in a second report [80]. Also, no association was found when using LTA as the platelet function assay. Thus, mutations in the collagen receptors are unlikely to contribute significantly to variability in platelet responsiveness to aspirin.

Finally, a dimorphism of the β_3 allele of the $\alpha_{2b}\beta_3$ integrin, the final step in platelet aggregation irrespective of the pathway activated, is also suspected of modulating platelet response to aspirin [79, 81]. However, the number of conflicting reports on the association between polymorphisms in the GPIIb/IIIa receptor and platelet responsiveness to aspirin makes this association speculative [82]. Further and longer-term studies are needed to clarify this issue.

In summary, although modifications in genes encoding for platelet receptors and enzymes may alter platelet response to aspirin even though they do not affect platelet COX-1 directly, their individual contributions are likely to be minor. Nevertheless, cumulative contributions cannot be eliminated.

4.2.3. Patient conditions associated with increased platelet aggregation

In addition to known factors related to platelet generation or function, a number of patient conditions have been associated with increased platelet aggregation through mechanisms that sometimes remain hypothetical and probably unrelated to aspirin's efficacy to inhibit COX-1.

One such condition is diabetes. Patients suffering from diabetes often display abnormal response to aspirin [44]. Mechanisms that may explain this lack of susceptibility to the action of aspirin's effect include competitive platelet COX-1 glycosylation, COX-2 over-expression induced by a pro-inflammatory state, increased platelet turnover, endothelial dysfunction, and oxidative stress [43, 44, 83, 84].

Similarly, platelet aggregation is positively correlated with increasing body mass index resulting in inadequate platelet inhibition in obese patients despite aspirin therapy [85, 86]. Possible mechanisms underlying this higher platelet reactivity in obese patients may include depletion of cAMP and cGMP, two cyclic nucleotides involved in the intrinsic inhibition of platelet aggregation. Platelet depletion of cAMP and cGMP has been observed in obese patients, and impaired synthesis of these inhibitory molecules due to reduced activation of their specific kinases may lead to generation of platelets with a lower threshold for platelet activation [85, 86]. As a result, platelets are more easily stimulated by platelet agonists, which may explain the relative inefficacy of aspirin to thoroughly inhibit platelet function in this population.

Recently, aspirin resistance was found to be markedly more frequent among patients with nephrotic syndrome, and tended to correlate with the biochemical and clinical parameters of the disease [87]; however the exact causative mechanism remains unknown.

4.3. Extrinsic factors leading to apparent low aspirin response

Aspirin's apparent inefficacy to inhibit platelet aggregation may be caused by issues not related to platelet pathophysiology, such as reduced aspirin bioavailability or drug interactions. The use of enteric-coated aspirin preparations results in significantly lower bioavailability of the drug, which may lead to reduced antiplatelet efficacy [88]. In addition to formulation issues, compliance is of crucial importance when assessing platelet response to aspirin, as it has been shown that the majority of patients considered aspirin low responders were in fact non-compliant to the prescribed therapy [89].

Drug interactions can further impair the efficacy of aspirin. For instance, competition between aspirin and certain non-steroidal anti-inflammatory drugs (NSAID), such as ibuprofen or naproxen, for the binding of COX-1 results in reduced antiplatelet activity [90, 91]. By reversibly binding platelet COX-1, these agents limit access of aspirin to its binding site. Because their binding capacity outlasts aspirin's activity in the blood-stream, COX-1 is restored to its active state when the reversible liaison of NSAID to COX-1 is terminated, enabling normal TxA_2 production and platelet aggregation [90].

5. Individualization of antiplatelet therapy

Recent advances in pharmacogenetics, and the abundance of data about the variability of platelet response to antiplatelet therapy make treatment individualization according to specific patient characteristics an appealing strategy. Such patient characteristics may include genetic make-up and individual risk of bleeding or thrombosis. Current research suggests that such features may be useful for the establishment of clinical risk scores to help in the process of risk stratification and

identification of patients who may benefit from tailored antiplatelet therapy [92]. Individualization of antiplatelet therapy implies the proper selection of the antiplatelet drug(s) regimen (including the selection of the doses, the dosing intervals, and the duration of treatment), the evaluation of the need for a combination therapy, and the necessity of special follow-up measures according to specific patient characteristics. For example, recent studies have raised doubts about the ability of the standard once-daily aspirin dosing to provide sustainable optimal platelet inhibition in patients having higher than normal platelet turnover, calling for appropriate investigation of the clinical utility of a twice daily regimen in this population [33, 35, 59, 61, 63, 65]. Recovery of COX-dependent platelet aggregation within the 24-hour dosing interval was observed in healthy subjects receiving once-daily aspirin doses and may be attributed to higher platelet turnover [33]. Accelerated TXA_2 biosynthesis, through enhanced COX-2 activity and faster regeneration of unacetylated COX-1, was reported in most patients with essential thrombocythemia despite receiving aspirin once daily [59]. In a recent study by Spectre *et al.*, platelet inhibition was significantly higher in patients with type-2 diabetes following administration of a twice-daily 75-mg aspirin dose than once-daily 75-mg or 320-mg doses, as measured by LTA, WBA and IMPACT® [93]. Rocca *et al* also reported that, in patients with type-2 diabetes having a high baseline on-aspirin COX-1 recovery rate, a twice-daily aspirin regimen, but not a doubled once-daily dose, could overcome the abnormal COX-1 recovery rate observed with once-daily aspirin doses in this population [61]. Similarly, higher on-aspirin platelet reactivity was detected in CAD patients with an increased platelet turnover, indicating that once-daily dosing of aspirin might not adequately inhibit platelet aggregation in CAD patients with an increased platelet turnover, notably diabetics [65].

Before individualization of antiplatelet therapy becomes integrated into routine clinical practice, our ability to accurately predict the risk of both thrombosis and bleeding must be refined. Debate remains about the clinical value and the variability of platelet function tests, but recent studies have consistently reported a considerable association between platelet reactivity as measured by some of these tests and the incidence of major adverse cardiovascular events [13, 17]. These findings support the potential of platelet function testing to become a reliable tool for the identification of patients with a particularly high risk of thrombosis. Prediction of the risk of bleeding, however, remains a challenge. Currently, aside from some suggestion in the literature that certain platelet function tests might be useful [13, 94], there is a lack of simple, precise and accessible platelet function tests that allow a clinical estimation of the risk of bleeding. Therefore, the validation of the safety of a personalized antiplatelet regimen may remain questionable until appropriate tools become available.

CONCLUSION

Given the benefit provided by aspirin administration in secondary prevention of acute ischemic events, ensuring its efficacy is of paramount importance. Monitoring the efficacy of aspirin by platelet function testing may provide the means for personalized antiplatelet therapy. However, different platelet function assays exploit distinct pathways of platelet response that are not all sensitive to inhibition by aspirin. Platelet function may be modulated by various factors that may or may not be influenced by the presence of aspirin and some platelet function assays may identify sub-groups of patients with an increased risk of major adverse cardiovascular events due to sustained platelet aggregation despite aspirin use. However, the current literature does not allow implementation of specific guidelines or recommendations with regard to the selection of a platelet function assay of choice in specific clinical settings. Thus, platelet function testing remains a research tool, and until large clinical trials on adjusting aspirin treatment according to platelet function assays are conducted, clinical judgement should direct practice in instances where insufficient platelet inhibition by aspirin is suspected.

PRACTICAL APPROACH

In the absence of systematic screening guidelines, clinical expertise should guide the decision of whether to test platelet response and the choice of the most informative test. The following situations

may warrant platelet function testing: 1) High-risk acute coronary syndrome patients undergoing percutaneous coronary intervention (PCI), notably in the presence of stent thrombosis and in potentially critical situations (e.g. left main stenting, last patent vessel, multiple stents deployed, etc.); 2) Patients presenting sub-optimal results following PCI (suboptimal stent deployment, small vessels, bifurcation, floating struts, etc.).

In addition to the contribution of genetic polymorphisms to a state of platelet hyperactivity, other non-genetic factors may also indicate the necessity of platelet function testing even in the absence of the aforementioned clinical risk factors for stent thrombosis. Geisler *et al.* developed the PREDICT risk score, an objective tool for the identification of patients at higher risk of thrombo-ischemic events post-PCI based on non-genetic factors such as age, current acute coronary syndrome, reduced left ventricular function and comorbidities (diabetes and chronic renal failure) [95].

The choice of the platelet function test to be employed remains a question of clinical expertise as well. To date, LTA_{AA} seems to provide a clinically pertinent measure of platelet response to aspirin, allowing a good discrimination between responders and non-responders, with little overlap around the cut-off value compared to other tests [3].

In light of the current literature, research should aim at finding ways to better individualize therapy to ensure that patients are treated with agents that best tackle the specific mechanisms by which their platelet function is affected. Let us remember, one size does not always fit all.

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REFERENCES

1. Pamukcu, B. 2007, J. Thromb. Thrombolysis, 23(3), 213.
2. Antithrombotic Trialists' Collaboration, 2002, BMJ, 324(7329), 71.
3. Lordkipanidzé, M., Pharand, C., Schampaert, E., Turgeon, J., Palisaitis, D. A. and Diodati, J. G. 2007, Eur. Heart J., 28, 1702.
4. Harrison, P., Segal, H., Silver, L., Syed, A., Cuthbertson, F. C. and Rothwell, P. M. 2008, Platelets, 19(2), 119.
5. Lennon, M. J., Gibbs, N. M., Weightman, W. M., McGuire, D. and Michalopoulos, N. 2004, J. Cardiothorac. Vasc. Anesth., 18(2), 136.
6. Awtry, E. H. and Loscalzo, J. 2000, Circulation, 101(10), 1206.
7. Patrono, C., Coller, B., FitzGerald, G. A., Hirsh, J. and Roth, G. 2004, Chest, 126(3 Suppl.), 234S.
8. Patrono, C., Garcia Rodriguez, L. A., Landolfi, R. and Baigent, C. 2005, N. Engl. J. Med., 353(22), 2373.
9. Hankey, G. J. and Eikelboom, J. W. 2006, Lancet., 367(9510), 606.
10. Grove, E. L., Hvas, A. M., Johnsen, H. L., Hedegaard, S. S., Pedersen, S. B., Mortensen, J. and Kristensen, S. D. 2010, Thromb. Haemost., 103(6), 1245.
11. Gurbel, P. A., Bliden, K. P., DiChiara, J., Newcomer, J., Weng, W., Neerchal, N. K., Gesheff, T., Chaganti, S. K., Etherington, A. and Tantry, U. S. 2007, Circulation, 115(25), 3156.
12. Harrison, P., Segal, H., Blasbery, K., Furtado, C., Silver, L. and Rothwell, P. M. 2005, Stroke, 36(5), 1001.
13. Breet, N. J., van Werkum, J. W., Bouman, H. J., Kelder, J. C., Ten Berg, J. M. and Hackeng, C. M. 2010, J. Thromb. Haemost., 8, 2140.
14. Snoep, J. D., Hovens, M. M., Eikenboom, J. C., van der Bom, J. G. and Huisman, M. V. 2007, Arch. Intern. Med., 167(15), 1593.
15. Chen, W. H., Cheng, X., Lee, P. Y., Ng, W., Kwok, J. Y., Tse, H. F. and Lau, C. P. 2007, Am. J. Med., 120(7), 631.
16. Krasopoulos, G., Brister, S. J., Beattie, W. S. and Buchanan, M. R. 2008, BMJ, 336(7637), 195.
17. Frelinger, A. L. 3rd, Li, Y., Linden, M. D., Barnard, M. R., Fox, M. L., Christie, D. J., Furman, M. I. and Michelson, A. D. 2009, Circulation, 120, 2586.
18. Michelson, A. D., Frelinger, A. L. 3rd and Furman, M. I. 2006, Am. J. Cardiol., 98(10 Suppl. 1), S4.
19. Rand, M. L., Leung, R. and Packham, M. A. 2003, Transfus Apher. Sci., 28(3), 307.

20. Michelson, A. D. 2004, Circulation, 110(19), e489.
21. Harrison, P. 2005, Blood Rev., 19(2), 111.
22. Chen, W. H., Lee, P. Y., Ng, W., Tse, H. F. and Lau, C. P. 2004, J. Am. Coll. Cardiol., 43(6), 1122.
23. Eikelboom, J. W., Hirsh, J., Weitz, J. I., Johnston, M., Yi, Q. and Yusuf, S. 2002, Circulation, 105(14), 1650.
24. Gum, P. A., Kottke-Marchant, K., Welsh, P. A., White, J. and Topol, E. J. 2003, J. Am. Coll. Cardiol., 41(6), 961.
25. Yilmaz, M. B., Balbay, Y., Caldir, V., Ayaz, S., Guray, Y., Guray, U. and Korkmaz, S. 2005, Thromb. Res., 115(1-2), 25.
26. Cuisset, T., Frere, C., Quilici, J., Barbou, F., Morange, P. E., Hovasse, T., Bonnet, J. L. and Alessi, M. C. 2006, J. Thromb. Haemost., 4(3), 542.
27. Marcucci, R., Paniccia, R., Antonucci, E., Gori, A. M., Fedi, S., Giglioli, C., Valente, S., Prisco, D., Abbate, R. and Gensini, G. F. 2006, Am. J. Cardiol., 98(9), 1156.
28. Pamukcu, B., Oflaz, H., Oncul, A., Umman, B., Mercanoglu, F., Ozcan, M., Meric, M. and Nisanci, Y. 2006, J. Thromb. Thrombolysis, 22(2), 103.
29. Gurbel, P. A., Becker, R. C., Mann, K. G., Steinhubl, S. R. and Michelson, A. D. 2007, J. Am. Coll. Cardiol., 50, 1822.
30. Santos, M. T., Valles, J., Marcus, A. J., Safier, L. B., Broekman, M. J., Islam, N., Ullman, H. L., Eiroa, A. M. and Aznar, J. 1991, J. Clin. Invest., 87(2), 571.
31. Valles, J., Santos, M. T., Aznar, J., Osa, A., Lago, A., Cosin, J., Sanchez, E., Broekman, M. J. and Marcus, A. J. 1998, Circulation, 97(4), 350.
32. Patrignani, P. 2003, Thromb. Res., 110(5-6), 281.
33. Perneby, C., Wallen, N. H., Rooney, C., Fitzgerald, D. and Hjemdahl, P. 2006, Thromb. Haemost., 95(4), 652.
34. Hillman, R. S. 2006, J. Am. Coll. Cardiol., 47(12), 2565.
35. Dichiara, J., Bliden, K. P., Tantry, U. S., Chaganti, S. K., Kreutz, R. P., Gesheff, T. B., Kreutz, Y. and Gurbel, P. A. 2007, Platelets, 18(6), 414.
36. Nielsen, H. L., Kristensen, S. D., Thygesen, S. S., Mortensen, J., Pedersen, S. B., Grove, E. L. and Hvas, A. M. 2008, Thromb. Res., 123(2), 267.
37. Coleman, J. L. and Alberts, M. J. 2006, Am. J. Cardiol., 98(6), 838.
38. Feuring, M., Schultz, A., Losel, R. and Wehling, M. 2005, Semin. Thromb. Hemost., 31(4), 411.
39. Blais, N., Pharand, C., Lordkipanidze, M., Sia, Y. K., Merhi, Y. and Diodati, J. G. 2009, Thromb. Haemost., 102(2), 404.
40. Spectre, G., Brill, A., Gural, A., Shenkman, B., Touretsky, N., Mosseri, E., Savion, N. and Varon, D. 2005, Platelets, 16(5), 293.
41. Maree, A. O., Curtin, R. J., Dooley, M., Conroy, R. M., Crean, P., Cox, D. and Fitzgerald, D. J. 2005, J. Am. Coll. Cardiol., 46(7), 1258.
42. Born, G. and Patrono, C. 2006, Br. J. Pharmacol., 147(Suppl. 1), S241.
43. Watala, C., Pluta, J., Golanski, J., Rozalski, M., Czyz, M., Trojanowski, Z. and Drzewoski, J. 2005, J. Mol. Med., 83(2), 148.
44. Watala, C., Golanski, J., Pluta, J., Boncler, M., Rozalski, M., Luzak, B., Kropiwnicka, A. and Drzewoski, J. 2004, Thromb. Res., 113(2), 101.
45. Kobzar, G., Mardla, V. and Samel, N. 2011, Platelets, 22(5), 338.
46. Maree, A. O., Curtin, R. J., Chubb, A., Dolan, C., Cox, D., O'Brien, J., Crean, P., Shields, D. C. and Fitzgerald, D. J. 2005, J. Thromb. Haemost., 3(10), 2340.
47. Gonzalez-Conejero, R., Rivera, J., Corral, J., Acuna, C., Guerrero, J. A., and Vicente, V. 2005, Stroke, 36(2), 276.
48. Hillarp, A., Palmqvist, B., Lethagen, S., Villoutreix, B. O. and Mattiasson, I. 2003, Thromb. Res., 112(5-6), 275.
49. Lepantalo, A., Mikkelsson, J., Resendiz, J. C., Viiri, L., Backman, J. T., Kankuri, E., Karhunen, P. J. and Lassila, R. 2006, Thromb. Haemost., 95(2), 253.
50. Voora, D., Horton, J., Shah, S. H., Shaw, L. K. and Newby, L. K. 2011, Am. Heart J., 162(1), 166.
51. McRedmond, J. P., Park, S. D., Reilly, D. F., Coppinger, J. A., Maguire, P. B., Shields, D. C. and Fitzgerald, D. J. 2004, Mol. Cell Proteomics, 3(2), 133.

52. Denis, M. M., Tolley, N. D., Bunting, M., Schwertz, H., Jiang, H., Lindemann, S., Yost, C. C., Rubner, F. J., Albertine, K. H., Swoboda, K. J., Fratto, C. M., Tolley, E., Kraiss, L. W., McIntyre, T. M., Zimmerman, G. A. and Weyrich, A. S. 2005, Cell, 122(3), 379.
53. Zimmerman, G. A. and Weyrich, A. S. 2008, Arterioscler Thromb. Vasc. Biol., 28(3), s17.
54. Evangelista, V., Manarini, S., Di Santo, A., Capone, M. L., Ricciotti, E., Di Francesco, L., Tacconelli, S., Sacchetti, A., D'Angelo, S., Scilimati, A., Sciulli, M. G. and Patrignani, P. 2006, Circ. Res., 98(5), 593.
55. Rocca, B., Secchiero, P., Ciabattoni, G., Ranelletti, F. O., Catani, L., Guidotti, L., Melloni, E., Maggiano, N., Zauli, G. and Patrono, C. 2002, PNAS, 99(11), 7634.
56. Cipollone, F., Rocca, B. and Patrono, C. 2004, Arterioscler Thromb. Vasc. Biol., 24(2), 246.
57. Weber, A. A., Zimmermann, K. C., Meyer-Kirchrath, J. and Schror, K. 1999, Lancet., 353(9156), 900.
58. Kaushansky, K. 2005, J. Clin. Invest., 115(12), 3339.
59. Dragani, A., Pascale, S., Recchiuti, A., Mattoscio, D., Lattanzio, S., Petrucci, G., Mucci, L., Ferrante, E., Habib, A., Ranelletti, F. O., Ciabattoni, G., Davi, G., Patrono, C. and Rocca, B. 2010, Blood, 115, 1054.
60. Lloyd-Jones, D., Adams, R., Carnethon, M., De Simone, G., Ferguson, T. B., Flegal, K., Ford, E., Furie, K., Go, A., Greenlund, K., Haase, N., Hailpern, S., Ho, M., Howard, V., Kissela, B., Kittner, S., Lackland, D., Lisabeth, L., Marelli, A., McDermott, M., Meigs, J., Mozaffarian, D., Nichol, G., O'Donnell, C., Roger, V., Rosamond, W., Sacco, R., Sorlie, P., Stafford, R., Steinberger, J., Thom, T., Wasserthiel-Smoller, S., Wong, N., Wylie-Rosett, J. and Hong, Y. 2009, Circulation, 119(3), e21.
61. Rocca, B., Santilli, F., Pitocco, D., Mucci, L., Petrucci, G., Vitacolonna, E., Lattanzio, S., Mattoscio, D., Zaccardi, F., Liani, R., Del Ponte, A., Ferrante, E., Martini, F., Davì, G. and Patrono, C. 2010, Circulation, 122, A12233 (abstract).
62. Guthikonda, S., Lev, E. I., Patel, R., Delao, T., Bergeron, A. L., Dong, J. F. and Kleiman, N. S. 2007, J. Thromb. Haemost., 5(3), 490.
63. Lordkipanidze, M., Pharand, C., Schampaert, E., Palisaitis, D. A. and Diodati, J. G. 2011, Int. J. Cardiol., 150(1), 39.
64. Henry, P., Vermillet, A., Boval, B., Guyetand, C., Petroni, T., Dillinger, J. G., Sideris, G., Bal Dit Sollier, C. and Drouet, L. 2010, Thromb. Haemost., 105(2), 336.
65. Grove, E. L., Hvas, A. M., Mortensen, S. B., Larsen, S. B. and Kristensen, S. D. 2011, J. Thromb. Haemost., 9(1), 185.
66. Lordkipanidzé, M. 2011, Thromb. Haemost., 105(2), 209.
67. Cesari, F., Marcucci, R., Caporale, R., Paniccia, R., Romano, E., Gensini, G. F., Abbate, R. and Gori, A. M. 2008, Thromb. Haemost., 99(5), 930.
68. Freedman, J. E. 2005, Circulation, 112(17), 2725.
69. Anfossi, G. and Trovati, M. 1996, Eur. J. Clin. Invest., 26(5), 353.
70. Hung, J., Lam, J. Y. T., Lacoste, L. and Letchacovski, G. 1995, Circulation, 92(9), 2432.
71. Christiaens, L., Macchi, L., Herpin, D., Coisne, D., Duplantier, C., Allal, J., Mauco, G. and Brizard, A. 2003, Thromb. Res., 108(2-3), 115.
72. Sestito, A., Maccallini, A., Sgueglia, G. A., Infusino, F., Larosa, C., Aurigemma, C., Crea, F. and Lanza, G. A. 2005, Thromb. Res., 116(1), 25.
73. Kawasaki, T., Ozeki, Y., Igawa, T. and Kambayashi, J.-I. 2000, Stroke, 31(3), 591.
74. Macchi, L., Christiaens, L., Brabant, S., Sorel, N., Allal, J., Mauco, G. and Brizard, A. 2002, Thromb. Res., 107(1-2), 45.
75. Hetherington, S. L., Singh, R. K., Lodwick, D., Thompson, J. R., Goodall, A. H. and Samani, N. J. 2005, Arterioscler Thromb. Vasc. Biol., 25(1), 252.
76. Lordkipanidze, M., Diodati, J. G., Palisaitis, D. A., Schampaert, E., Turgeon, J. and Pharand, C. 2011, Thromb. Res., 128(1), 47.
77. Su, G., Wang, Z. and Ding, Y. 2007, J Huazhong Univ. Sci. Technolog. Med. Sci., 27(6), 664.

78. Fontana, P., Nolli, S., Reber, G. and de Moerloose, P. 2006, J. Thromb. Haemost., 4(4), 813.
79. Macchi, L., Christiaens, L., Brabant, S., Sorel, N., Ragot, S., Allal, J., Mauco, G. and Brizard, A. 2003, J. Am. Coll. Cardiol., 42(6), 1115.
80. Kunicki, T. J., Williams, S. A., Nugent, D. J., Harrison, P., Segal, H. C., Syed, A. and Rothwell, P. M. 2009, Thromb. Haemost., 101(1), 123.
81. Papp, E., Havasi, V., Bene, J., Komlosi, K., Czopf, L., Magyar, E., Feher, C., Feher, G., Horvath, B., Marton, Z., Alexy, T., Habon, T., Szabo, L., Toth, K. and Melegh, B. 2005, Ann. Pharmacother, 39(6), 1013.
82. Goodman, T., Ferro, A. and Sharma, P. 2008, Br. J. Clin. Pharmacol., 66(2), 222.
83. Fateh-Moghadam, S., Plockinger, U., Cabeza, N., Htun, P., Reuter, T., Ersel, S., Gawaz, M., Dietz, R. and Bocksch, W. 2005, Acta Diabetol., 42(2), 99.
84. Davi, G., Ciabattoni, G., Consoli, A., Mezzetti, A., Falco, A., Santarone, S., Pennese, E., Vitacolonna, E., Bucciarelli, T., Costantini, F., Capani, F. and Patrono, C. 1999, Circulation, 99(2), 224.
85. Tamminen, M., Lassila, R., Westerbacka, J., Vehkavaara, S. and Yki-Jarvinen, H. 2003, Int. J. Obes. Relat. Metab. Disord., 27, 907.
86. Russo, I., Del Mese, P., Doronzo, G., De Salve, A., Secchi, M., Trovati, M. and Anfossi, G. 2007, Clin. Chem., 53(6), 1053.
87. Akoglu, H., Agbaht, K., Piskinpasa, S., Falay, M. Y., Dede, F., Ozet, G. and Odabas, A. R. 2012, Nephrol. Dial. Transplant., 27(4), 1460.
88. Cox, D., Maree, A. O., Dooley, M., Conroy, R., Byrne, M. F. and Fitzgerald, D. J. 2006, Stroke, 37(8), 2153.
89. Schwartz, K. A., Schwartz, D. E., Ghosheh, K., Reeves, M. J., Barber, K. and DeFranco, A. 2005, Am. J. Cardiol., 95(8), 973.
90. Catella-Lawson, F., Reilly, M. P., Kapoor, S. C., Cucchiara, A. J., DeMarco, S., Tournier, B., Vyas, S. N. and FitzGerald, G. A. 2001, N. Engl. J. Med., 345(25), 1809.
91. Capone, M.L., Sciulli, M. G., Tacconelli, S., Grana, M., Ricciotti, E., Renda, G., Di Gregorio, P., Merciaro, G. and Patrignani, P. 2005, J. Am. Coll. Cardiol., 45(8), 1295.
92. Geisler, T. and Gawaz, M. 2009, Hamostaseologie, 29(4), 360.
93. Spectre, G., Arnetz, L., Ostenson, C. G., Brismar, K., Li, N. and Hjemdahl, P. 2011, Thromb. Haemost., 106(3), 491.
94. Mortensen, J., Poulsen, T. S., Grove, E. L., Refsgaard, J., Nielsen, H. L., Pedersen, S. B., Thygesen, S. S., Hvas, A. M. and Kristensen, S. D. 2008, Scand. J. Clin. Lab. Invest., 68(8), 786.
95. Geisler, T., Grass, D., Bigalke, B., Stellos, K., Drosch, T., Dietz, K., Herdeg, C. and Gawaz, M. 2008, J. Thromb. Haemost., 6(1), 54.