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Mitigation of Device-Associated Thrombosis and Thromboembolism Using Combinations of Heparin and Tirofiban

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Abstract: Combined anti-platelet–anticoagulant therapy is increasingly being used to reduce the risk of device-induced thrombosis and thromboembolism. However, direct quantitative confirmation of the effectiveness of this combination approach is lacking. This study was undertaken to quantify the effects of various combinations of heparin (anticoagulant) and tirofiban (antiplatelet agent) on device-induced thrombosis and thromboembolism using a coronary stent as a prototype device. Adult sheep were implanted with ex vivo carotid–carotid shunts containing replaceable tubing segments in which nitinol stents were deployed. Nine combinations of heparin (average activated clot time = 129, 199, and 355 seconds) and tirofiban (0%, 50%, and 100% platelet inhibition) were tested at random with three replicates per animal. Thrombus weight on the stent at the end of each experiment (1 hour) was measured, and emboli released from the stent were continuously monitored during the experiment using a light scattering microemboli detector. With no tirofiban, increasing the heparin concentration was associated with a decreased endpoint thrombus weight ($p < .05$) but with a slight (non-significant) increase in the number of downstream thromboemboli. However, the presence of tirofiban decreased both thrombus weight and thromboemboli numbers ($p < .05$), regardless of the heparin concentration. In the presence of medium or high tirofiban, an increase of heparin from low to medium levels also decreased both thrombus weight and thromboemboli numbers ($p < .05$). Heparin alone does not provide adequate protection against thromboembolism (and may actually increase it by reducing thrombus cohesive strength). However, the combination of heparin and tirofiban is effective in reducing both thrombus and thromboemboli, and an optimal combination may exist. **Keywords:** thromboembolism, platelets, anticoagulant, anti-platelet, stent. *JECT. 2006;38:230–234*

Platelet adhesion when consolidated with a dense fibrin network leads to an organized thrombus on blood contacting devices. This can cause device dysfunction and/or occlusion of the blood vessel in which the device is deployed, or thrombi may dislodge and emboli traveling downstream may occlude distal vessels leading to ischemia.

Anticoagulants such as heparin are routinely used to mitigate the risk of thrombosis (1). These agents typically inhibit key reactions in the clotting cascade and suppress fibrin formation (2). However, anticoagulants only inhibit the clotting pathway and generally do not have an inhibitory effect on platelets, which may be equally important in initiating and sustaining device thrombosis (3). Moreover, several reports have suggested that heparin, the most widely used anticoagulant, activates platelets (4), which may exacerbate device-associated thrombosis and thromboembolism (TTE).

Therefore, to reduce the risk of thrombosis, antiplatelet agents are often used in conjunction with anticoagulants. This strategy has proven to be effective in interventional procedures such as stenting (5,6), where the development of thrombi on the device can be fatal. Furthermore, platelet inhibitors such as glycoprotein IIb/IIIa antagonists have been used in conjunction with heparin during cardiopulmonary bypass (7), and attempts have been made to combine an oral adenosine diphosphate (ADP) receptor antagonist (clopidogrel) and heparin during hemodialysis to reduce the risk of thrombosis and TTE (8). Although the combination of antiplatelet agents and anticoagulants is being increasingly adopted to reduce the risk of thrombosis, no specific attempts have been made to directly examine and quantify the effect of this strategy on device-associated thrombosis and TTE.

This study was designed to study the effects of various combinations of heparin (anticoagulant) and tirofiban

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(antiplatelet agent) on thrombosis and TTE, using a coronary stent as a prototype device in ovine ex-vivo carotid arterial shunts (9). A light scattering microemboli detector (LSMD) developed by the investigators was used to continuously monitor thromboemboli released from the device in real time (10).

MATERIALS AND METHODS

Shunt Components

To establish an ex vivo shunt, one end of a polyurethane tubing segment (6.4 mm ID; Cole-Palmer, Vernon Hills, IL) was attached to a 2.5-cm-long, 6-mm-ID Dacron graft (Gore, Flagstaff, AZ) using a polyurethane adhesive (pellethane in dimethyl acetamide 1%–10% weight/volume). This graft-tubing combination was implanted (described below) and represented the permanent portion of the shunt. Two days after the implant, the experiments with the stents and the pharmacological agents were started. During the experiments, segments of 3.2-mm-ID Tygon tubing (Cole-Palmer) containing a 3.5-mm coronary stent (deployed at 6 ATM) were connected to the implanted shunt using tapered 6.4- to 3.2-mm heparin-coated connectors (9) (heparin benzalkonium chloride coating solution; Celsus Laboratories, Cincinnati, OH; connectors; Baxter Healthcare, Bentley Division, Irvine, CA). An ultrasonic flow probe (Model 109; Transonic Systems, Ithaca, NY) was clamped to monitor the flow, and the light-scattering microemboli detector was placed distal to the stent to monitor emboli (Figure 1). The separation of the stent-tubing segments from the permanent portion of the shunt allowed convenient multiple exchanges of the test segment after the conclusion of each experiment.

Animal Preparation and Surgical Procedure

The ex vivo studies were conducted in four adult male sheep weighing ~70–100 kg. Food and water were withheld for 12 and 6 hours before surgery, respectively. DuraPen penicillin (20,000 U/kg SQ, Agrilabs, St. Joseph, MO) and tetracycline (2000 mg orally, Ivax Pharmaceuticals, Inc., Miami, FL) were administered before anesthesia. Atropine (2.2 mg/kg IV, Neogen Corporation, Lexington, KY) and Pentothal (15 mg/kg IV, Abbot Laboratories, North Chicago, IL) were used for induction of anesthesia, and Halothane (Halocarbon, River Edge, NJ) was used to maintain anesthesia. After anesthesia, the left or right carotid artery was exposed, clamps were placed on the exposed carotid artery ~2 in apart, and the artery was cut between the clamps. To each cut end of the artery, a 6.0-mm-ID Dacron graft (with associated polyurethane tubing mentioned above) was sutured end-to-end with 6-0 Prolene (Ethicon, Somerville, NJ). The animal was anticoagulated with a single bolus of 5000 units of heparin to prevent acute clotting of the graft and other components. The clamps were slowly removed, and the anastomoses were checked for leaks. The shunts were tunneled under the skin and exteriorized (Figure 1). The two open ends of the tubing were primed with saline and connected with a 6.4-mm heparin-coated coupler, allowing the blood to flow through the shunt. After waiting for 5–10 minutes to assure that hemostasis was achieved and that there was no graft or suture line bleeding, the incision was closed, allowing ~8 in of the shunt tubing to be accessible from outside. Heparin infusion (bag concentration, 20,000 U/L; drip rate, 10–100 mL/h) was adjusted to maintain the activated clotting time (ACT) above 200 seconds postoperatively. Anesthesia was withdrawn, and the animals were allowed to recover for 48 hours. During the 2-day post-operative period torbugesic (0.1 mg/kg IV every 4 hours), penicillin (20,000 U/kg SQ every 48 hours) and heparin (dosage as required to achieve target clotting times of 1.5 times the baseline) were administered. All animals used in this study were treated in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington, DC, 1996).

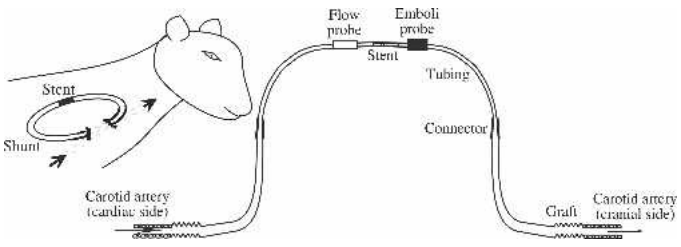

Figure 1. Schematic of the ovine shunt model.

Thrombosis and Thromboembolism Experiments With Heparin and Tirofiban

Target anticoagulation levels as indicated in Table 1 were achieved by varying heparin dosages (continuous intravenous drip) and characterized based on ACT. Low levels of the anticoagulant (Table 1) were targeted to be as close to the baseline as possible without risking widespread shunt occlusion, the medium levels were 1.5–2 times the baseline, and the high level was targeted to be 3–3.5 times the baseline. Similarly, target platelet inhibition levels were achieved by adjusting tirofiban to achieve 0%, 50%, and 100% inhibition of platelet aggregation (tirofiban loading dose = 0.2 mg/kg in 60 mL 0.9% sodium chloride administered over 10 minutes, followed by a continuous intravenous drip rate adjusted to achieve desired inhibition of platelet aggregation). The platelet aggregation was evaluated by the whole blood aggregometer (WBA; Model 591–592 Chronolog, Havertown, PA) using ADP (Sigma-Aldrich, St. Louis, MO) at 10 μmol/L final concentration. A new stent was used for each heparin–tirofiban combination. Table 1 summarizes the various drug combinations tested.

The order of the nine combinations in Table 1 was ran-

Table 1. Anticoagulant and antiplatelet combinations.

Low Heparin	Medium Heparin	High heparin
No platelet inhibition		
Low heparin, no tirofiban	Med. heparin, no tirofiban	High heparin, no tirofiban
ACT range = 119–156 seconds	ACT range = 148–239 seconds	ACT range = 212–444
Avg. ACT = 128 seconds	Avg. ACT = 187 seconds	Avg. ACT = 313 seconds
Avg. platelet inhibition = 0%	Avg. platelet inhibition = 0%	Avg. platelet inhibition = 0%
'Control'		
Medium platelet inhibition		
Low heparin, med. tirofiban	Med. heparin, med. tirofiban	High heparin, med. tirofiban
ACT range = 95–177 seconds	ACT range = 149–223 seconds	ACT range = 226–398 seconds
Avg. ACT = 127 seconds	Avg. ACT = 191 seconds	Avg. ACT = 316 seconds
Avg. measured platelet inhibition = 56% (range = 41–76%)	Avg. measured platelet inhibition = 38% (range = 29–44%)	Avg. measured platelet inhibition = 44% (range = 15–85%)
High platelet inhibition		
Low heparin, high tirofiban	Med. heparin, high tirofiban	High heparin, high tirofiban
ACT range = 96–177 seconds	ACT range = 201–258 seconds	ACT range = 267–635 seconds
Avg. ACT = 131 seconds	Avg. ACT = 225 seconds	Avg. ACT = 452 seconds
Avg. measured platelet inhibition = 90% (range = 82–100%)	Avg. measured platelet inhibition = 89% (range = 76–100%)	Avg. measured platelet inhibition = 89% (range = 81–100%)

ACT, activated clotting time; Avg, average.

domized between animals, and stent thrombosis and thromboembolism were tested three times at each drug combination in each animal. Although past studies have shown that the stent is the dominant source of thromboemboli in this model (9), a segment of tubing (without a stent) was tested at each drug combination to establish the baseline thromboemboli from the shunt (suture line, Dacron graft, and connectors). This "background" thromboemboli number was later subtracted from the number of thromboemboli detected by the LSMD during the experiments with the stents.

Each experiment typically lasted 60 minutes and represented the average amount of time required to reduce the flow through the stented conduit from the starting flow of 75–125 mL/min to <20 mL/min (caused by thrombotic occlusion) in the "low-heparin + no tirofiban" control. The amount of endpoint thrombus on the stents was quantified gravimetrically on retrieval as described previously (11).

Statistical Analysis

The data were analyzed using the analysis of variance (ANOVA) function in Microsoft Excel. The level of significance (α) was 0.05, and this was considered to be a randomized complete block design study. A complete set of conditions with three replications each was tested in each of the four animals (blocks) used in the study.

RESULTS

There was no significant reduction in the number of thromboemboli with increasing heparin concentration in the absence of tirofiban, even though there was a significant decrease in thrombus weight (Figure 2A and B).

In the experiments with medium dose of tirofiban, increased heparin concentration resulted in decreased thrombus weight and thromboemboli number (Figure 3A and B).

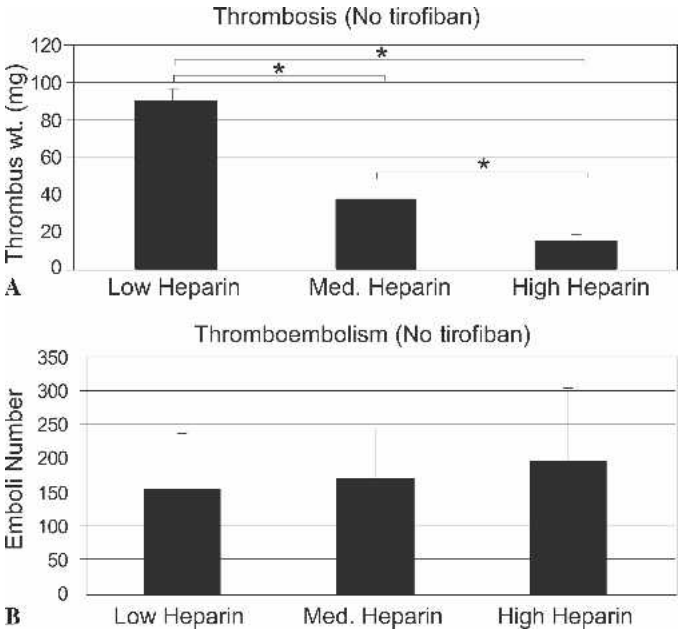

Figure 2. (A) Thrombus weight and (B) thromboemboli detected with increasing heparin doses (without tirofiban; $n = 12$; 4 animals × 3 replicates per animal). Note that there was a significant decrease in thrombus weight ($p < .05$, ANOVA for all pairwise differences), but no concurrent decrease in the number of thromboemboli with increasing heparin dose alone.

At each heparin concentration, increasing the concentration of tirofiban resulted in a decrease in thrombus weight and in thromboemboli numbers (Figure 4A and B).

The combination of medium heparin and medium tirofiban doses resulted in substantial inhibition of thrombosis and thromboembolism (compared with low heparin and no tirofiban). Only marginal additional inhibition was

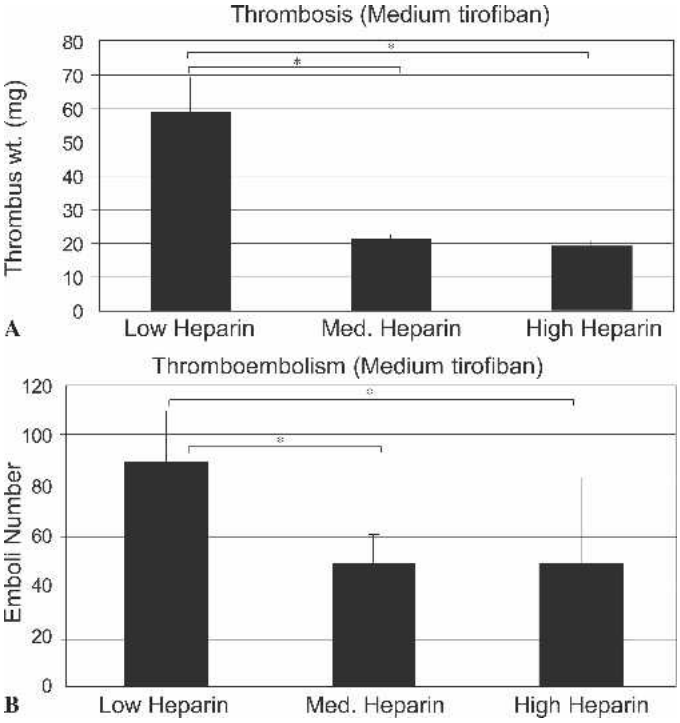

Figure 3. (A) Thrombus weight and (B) number of thromboemboli with increasing heparin doses (with medium tirofiban; $n = 9$; 3 animals × 3 replicates per animal; $p < .05$ for low vs. medium and low vs. high heparin; no significant difference between medium and high heparin). There was a significant decrease in thrombus weight, with a concurrent decrease in the thromboemboli number with increasing heparin dose from low to medium heparin. The high heparin (with medium tirofiban) did not result in any further decrease in thrombosis or thromboembolism.

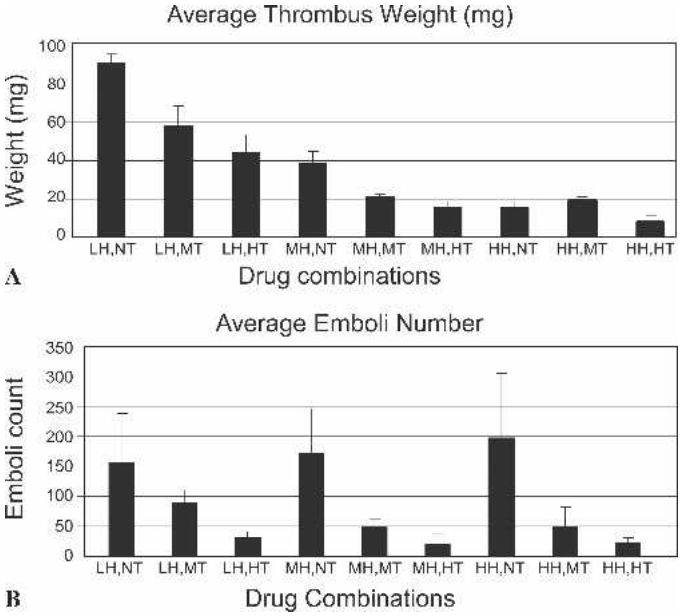

Figure 4. (A) Thrombus weight and (B) thromboemboli numbers for all combinations of heparin and tirofiban ($n = 9$–12). At each heparin concentration, increasing the concentration of tirofiban resulted in a decrease in thrombus weight and the number of thromboemboli. HH, high heparin; HT, high tirofiban; LH, low heparin; MH, medium heparin; MT, medium tirofiban; NT, no tirofiban.

apparent with further increases in the doses of the drugs (e.g., high heparin and high tirofiban; Figures 3 and 4). No significant signs of bleeding were observed at any level of drug or drug combinations.

DISCUSSION

This study revealed an important, but hitherto unexamined, aspect of device thromboembolism. The observation that increasing the concentration of heparin resulted only in the decrease of device thrombosis without an accompanying decrease in thromboemboli is significant. One possible explanation for this observation is that the systemic heparin presumably mitigates the consolidation of the thrombus by fibrin and does not have any direct inhibitory effect on platelets. Thus, it is possible that heparin merely serves to weaken thrombi growing on a device and may not represent an effective or comprehensive antithrombotic strategy for blood contacting devices. This notion is supported by the fact that the number of thromboemboli did not decrease, but showed an increasing trend (although statistically insignificant) with increasing heparin concentrations. This apparent increase in the number of thromboemboli may have been caused by the development of less organized thrombi in the presence of higher concentrations of heparin, making them more susceptible to embolization.

Such an observation has important ramifications in extracorporeal circulation (e.g., cardiopulmonary bypass [CPB] and hemodialysis), where the release of thromboemboli from the surface of devices and conduits may be as detrimental as formation of a thrombus on the device. The neurological deficits experienced by a sizable fraction of patients undergoing CPB (12) may be explained, in part, by such thromboemboli entering the neurovasculature (13). Based on the results of this study, it may be postulated that the incidence of cerebrovascular thromboemboli may be exacerbated by the high concentrations of heparin used in CPB. Some investigators have recently attempted to use anti-platelet agents in conjunction with anticoagulants during CPB and have reported improved clinical outcomes (7). These reports, in conjunction with the results of this study, support the strategy of combining anticoagulants and anti-platelet agents during extracorporeal circulation of blood.

Such a paradigm has not been adequately explored for patients undergoing hemodialysis, where they are routinely exposed to heparin for 3–4 hours per dialysis session, with multiple sessions per week. Some of these patients are known to have hemostatic deficiencies. These patients may benefit from a more balanced approach that uses a combination of heparin and a short-acting antiplate-

let agent. Although there is no direct evidence linking such systemic hemostatic deficiencies to incomplete protection against device thrombosis, it may be postulated that a more comprehensive protection against device thrombosis may reduce circulating levels of activated or degraded hemostatic components resulting from thrombi (e.g., fibrinogen fragments and fibrin degradation products that have been implicated in hemostatic deficiencies in uremic patients) (14).

Although a combination of an anti-platelet agent and an anticoagulant may be beneficial, it should be noted that there seems to be an optimal combination of the drugs that results in the effective inhibition of the thrombotic/thromboembolic process. In this study, it is noteworthy that only minor additional benefit was observed when tirofiban dose was increased beyond that which resulted in 50% inhibition of platelet function and increasing the heparin dose beyond that which resulted in 1.5–2 times the baseline ACT. Because recent studies have shown significant individual variations in the sensitivity to certain antiplatelet agents (15), it would be prudent to monitor the platelet and clotting functions and adjust the doses in each patient to ensure that the inhibitions are in the optimal range to avoid overdosing (which may lead to bleeding) or inadequate dosing (which may fail to prevent thrombosis and thromboembolism).

The above discussion not withstanding, it could be argued that free stream thromboemboli that result from thrombi that have been weakened by heparin may not represent a significant clinical risk. It is possible that the supposedly weaker thromboemboli may be more easily disintegrated by the lytic system and that the pathological consequences of such entities may be transient, if any. However, it is reasonable to presume that eliminating thromboemboli altogether may be more desirable than accepting weakened thromboemboli, especially in patients with an impaired lytic system.

A limitation of this study is that it was conducted with sheep, which may not represent the risk of thromboemboli in humans. Also, a metallic stent was used as a prototype device and the focal point of thromboembolic events. It is possible that the polymeric components used in CPB and hemodialysis circuits are more hemocompatible and may not be associated with sufficient thromboembolic events to cause concern. These limitations represent the justification to corroborate the results from this study in other animal experiments using typical ECC components followed by clinical confirmation.

In summary, increasing the concentration of heparin alone resulted in a decrease in endpoint device thrombosis but not in a decrease in thromboembolism. Only a combination of tirofiban (an anti-platelet agent) and heparin (the anticoagulant) resulted in concurrent decrease in endpoint thrombosis and thromboembolism.

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REFERENCES

1. Hilt T, Bayat M. Drugs used to limit blood surface interactions. Crit Care Nurs Clin North Am. 2002;14:7–16.
2. Bick RL, Frenkel EP, Walenga J, Fareed J, Hoppensteadt DA. Unfractionated heparin, low molecular weight heparins, and pentasaccharide: Basic mechanism of actions, pharmacology, and clinical use. Hematol Oncol Clin North Am. 2005;19:1–51.
3. Gorbet MB, Sefton MV. Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. Biomaterials. 2004;25:5681–703.
4. Poncz M. Mechanistic basis of heparin-induced thrombocytopenia. Semin Thorac Cardiovasc Surg. 2005;17:73–9.
5. Tendera M, Wojakowski W. Role of antiplatelet drugs in the prevention of cardiovascular events. Thromb Res. 2003;15:355–9.
6. Tamberella MR, Furman MI. The role of platelet inhibition in the drug-eluting stent era. Coron Artery Dis. 2004;15:327–9.
7. Koster A, Fischer T, Gruendel M, et al. Management of heparin resistance during cardiopulmonary bypass: The effect of five different anticoagulation strategies on hemostatic activation. J Cardiothorac Vasc Anesth. 2003;17:171–5.
8. Kaufman JS, O'Connor TZ, Zhang JH, et al. Randomized controlled trial of clopidogrel plus aspirin to prevent hemodialysis access graft thrombosis. J Am Soc Nephrol. 2003;14:2313–21.
9. Sukavaneshvar S, Zheng Y, Rosa GM, Mohammad SF, Solen KA. Thromboembolization associated with sudden increases in flow in a coronary stent ex vivo shunt model. ASAIO J. 2000;46:301–4.
10. Solen K, Sukavaneshvar S, Zheng Y, et al. Light-scattering instrument to detect thromboemboli in blood. J Biomed Opt. 2003;8:70–9.
11. Sukavaneshvar S, Solen KA, Mohammad SF. An in-vitro model to study device-induced thrombosis and embolism: evaluation of the efficacy of Tirofiban, aspirin, and dipyridamole. Thromb Haemost. 2000;83:322–6.
12. Nollert G, Reichart B. Cardiopulmonary bypass and cerebral injury in adults. Shock. 2001;16(Suppl 1):16–9.
13. Raymond PD, Marsh NA. Alterations to haemostasis following cardiopulmonary bypass and the relationship of these changes to neurocognitive morbidity. Blood Coagul Fibrinolysis. 2001;12:601–18.
14. Mezzano D, Tagle R, Panes O, et al. Hemostatic disorder of uremia: the platelet defect, main determinant of the prolonged bleeding time, is correlated with indices of activation of coagulation and fibrinolysis. Thromb Haemost. 1996;76:312–21.
15. Cattaneo M. Aspirin and clopidogrel: efficacy, safety, and the issue of drug resistance. Arterioscler Thromb Vasc Biol. 2004;24:1980–7.