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Platelet-mapping assay for monitoring antiplatelet therapy during mechanical circulatory support in children: A retrospective observational study

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Abstract

Introduction: The complex hemostatic changes associated with Berlin Heart (BH) implantation in children require a challenging antithrombotic treatment. The aim of this retrospective analysis was to evaluate the thromboelastography (TEG)-platelet mapping (PM) assay to monitor antiplatelet therapy in children implanted with a BH.

Methods: TEG-PM was performed in 4 BH-implanted patients receiving dipyridamole and aspirin, and 9 healthy volunteers. Patients' antiplatelet therapy was adjusted to TEG-PM results. Light transmission aggregometry (LTA) was also available for 2 of these patients.

Results: Between 2009 and 2014, 4 BH-implanted patients received a dual antiplatelet therapy monitored by TEG-PM. In 2 patients, 18 of 34 tracings were atypical, because the maximum amplitude due to fibrin never stabilized, which made difficult antiplatelet therapy adjustment as recommended by BH's guidelines. To overcome this difficulty, TEG-PM and LTA were next performed in parallel. However, both methods led to different decisions to adjust antiplatelet therapy in 57% of the cases. In order to better understand this atypical tracing, TEG-PM was also performed in 9 volunteers and surprisingly 3 of them had the same atypical tracing. This atypical tracing was corrected by adding apyrase, suggesting that adenosine diphosphate (ADP) participates to spontaneous platelet activation in heparinized samples. In addition, we evidenced a high variability in the responses of TEG-PM with ADP in volunteers.

Conclusions: Antiplatelet therapy monitoring in BH-implanted children remains challenging, as TEG-PM is sensitive to several preanalytical and analytical conditions.

KEYWORDS

pediatrics, platelet aggregation inhibitors, platelet function tests, platelets, thromboelastography, ventricular-assist device

Essentials

- Thromboelastography (TEG)-platelet mapping (PM) assay is used for antiplatelet-drug monitoring.
- The TEG-PM was evaluated for dypiridamole and aspirin monitoring in 4 BH-implanted children.
- Some TEG-PM tracings were atypical leading to wrong results.
- The TEG-PM has to be improved for antiplatelet-drug monitoring during ventricular assistance.

Ventricular assist devices (VADs) are essential for the management of patients with terminal heart failure or cardiomyopathy, awaiting heart transplantation or myocardial recovery.[1–4] In 1992, with the introduction of miniaturized pumps for clinical use, the Berlin Heart (BH) device became the first pulsatile VAD available for small children.[5,6] Mechanical assistance in pediatrics has considerably evolved over a decade, providing excellent results, even for small children.[7] However, device and/or systemic thrombosis and hemorrhagic events remain the major causes of mortality and morbidity with complication rates (mainly neurologic) ranging from 20% to 60%.[2,7–11]

The hemostatic changes associated with BH implantation are complex and require a challenging antithrombotic treatment.[11] Thus, an antithrombotic protocol combining anticoagulant and antiplatelet molecules is recommended.[5] Unfractionated heparin (UFH) is started soon after VAD implantation and is relayed by vitamin K antagonists or low-molecular-weight heparin once the patient's condition is fully stabilized. Within 2 to 10 days after VAD implantation, platelet inhibitors (dipyridamole or clopidogrel and aspirin) are introduced. The bitherapy is continued until the VAD is removed.

Determining hemostatic function in these patients is essential, but standard coagulation tests performed in plasma are not designed to evaluate global hemostatic function. Thus, BH's guidelines recommend using whole blood thromboelastography (TEG) and (TEG)-platelet mapping-(PM) to monitor anticoagulation and platelet inhibition in children with a BH.[5,12,13] TEG-PM is designed to specifically evaluate platelet inhibition secondary to antiplatelet therapy. Although this assay has been shown to correlate with light transmission aggregometry (LTA) and to have a predictive value of bleeding risks in patients undergoing percutaneous coronary intervention[14,15], some concerns with the design of TEG-PM assay have been recently raised.[16,17]

The aim of this retrospective analysis was to evaluate the TEG-PM assay to monitor antiplatelet therapy in children implanted with a BH.

PATIENTS AND METHODS

Patients and healthy volunteers

This retrospective study was approved by the ethical committee (N° 2016-05-03) CPP Ile de France II (Chairperson Prof Marie-France Mamzer-Bruneel).

Seven patients were implanted with a BH (Mediport Kardiotechnik, Berlin, Germany) in our institution between 2009 and 2014. After BH implantation[18], antithrombotic treatment was monitored according to the BH reference center's recommendations, partly described by Karimova et al.[7] Inlet and outlet cannulae and artificial devices were monitored daily for micro thrombi which could potentially migrate to the brain.

Blood samples were obtained from the French Blood Bank Institute (Etablissement Français du Sang, Paris, France N°13/CABANEL/008) from nine healthy volunteers after they had given their written informed consent and after it had been verified that they had no increased bleeding tendency and had not taken medication that could decrease platelet function.

Hemostasis monitoring

Blood from patients and healthy volunteers was collected in Vacutainer tubes (BD Vacutainer System, Paymouth, UK) containing 0.109 mol L^{-1} sodium citrate or 15 U mL^{-1} heparin. The blood was drawn from the patients' arterial line following the recommendations of avoiding heparin contamination and sample dilution[19] or from the antecubital vein of healthy volunteers. To avoid platelet activation, blood specimens were transported to the laboratory by hand delivery (the pneumatic tube system was never used).[20,21] Blood was analysed within 30 minutes after sampling.

Conventional tests

Coagulation tests (fibrinogen, anti-Xa activity, international normalized ratio (INR), antithrombin) were run according to standardized procedures on ACLTop (Werfen, Bedford, MA, USA).

TEG-PM

This technique was performed according to the manufacturer's instructions using the platelet-mapping kit (Haemonetics, Braintree, MA, USA).[22] For the standard TEG reaction, kaolin-treated citrated blood (360 µL) was transferred into a prewarmed cup containing heparinase and calcium chloride (20 µL) to measure thrombin-induced platelet-fibrin clot-strength. Under these conditions, maximum amplitude (MA) is dependent on fibrin and platelet contributions ($MA_{thrombin}$).

To eliminate the thrombin effect on platelet activation, heparinized blood (340 µL) is used and activated by reptilase in the presence of factor XIIIa (activator F) (10 µL), enabling the strength of the fibrin clot (MA_{fibrin}) to be specifically measured. The addition of platelet agonists (10 µL), either 1 mmol L^{-1} AA or 2 µmol L^{-1} ADP to activator F ensures determination of the strength of the platelet-fibrin clot (MA_{ADP} or MA_{AA}). The difference between $MA_{ADP\ or\ AA}$ and $MA_{thrombin}$ is considered as platelet inhibition (%) and the results are expressed as the percentage of

TABLE 1 Antiplatelet therapy-monitoring with TEG-PM according to the Berlin Heart manufacturer's platelet-inhibition protocol

Platelet inhibition with:	Dose change	Repeat test
dipyridamole (ADP)		
G_{ADP} 30-20	Increase 100%	Next day
G_{ADP} 20-15	Increase 50%	Next day
G_{ADP} 15-10	Increase 25%	48 hours
G_{ADP} 10-6	None	D1-D7: every other day D8-D14: twice a week After D15: once a week
G_{ADP} 6-3	Decrease 10%	48 hours
G_{ADP} 3-0	Hold next dose; after bleeding stops, decrease 50%	Next day
aspirin (AA)		
0-30%	Increase 100%	Next day
31-50%	Increase 50%	Next day
50-69%	Increase 25%	48 hours
70-95%	None	D1-D7: every other day D8-D14: twice a week After D15: once a week
96-100%	Decrease 10%	48 hours
100% if patient is bleeding	Hold next dose. After bleeding stops, decrease 50%	Next day

Dx, x days postimplantation; AA, arachidonic acid; ADP, adenosine disphosphate.

platelet inhibition, calculated as followed: $100-[(MA_{ADP\ or\ AA}-MA_{fibrin})/(MA_{thrombin}-MA_{fibrin})\times100]$. The strength of the platelet-fibrin clot can also be expressed as G ($G_{ADP\ or\ AA}$), calculated from MA according to the formula $G=(5000\times MA_{ADP\ or\ AA})/(100-MA_{ADP\ or\ AA})$ expressed in dynes cm^{-2}.

The BH's guidelines recommend starting platelet inhibitors according to some of these parameters, namely when $MA_{thrombin}$ is >56 mm, G_{ADP} >6 dynes cm^{-2} and AA platelet inhibition <70%. The dipyridamole dose should be adjusted to the G_{ADP} value: for G_{ADP} 6 to 10 dynes cm^{-2}, no dose change is required; otherwise the dose must be adjusted as described in Table 1. The aspirin dose must be adjusted to the percentage of platelet inhibition in the presence of AA: when inhibition is 70–95%, no dose change is required; otherwise the dose must be adjusted (Table 1).

Light transmission aggregometry (LTA)

Platelet-rich plasma (PRP) and autologous platelet-poor plasma (PPP) were obtained by centrifugations of citrated blood as recommended.[23] A photometric method on an 8-channel aggregometer (PAP-8E, Bio/Data Corporation, Horsham, PA, USA) was used.

Platelet-rich plasma was incubated 2 minutes at 37°C and was then stirred at 122 *g* for 2 minutes before adding saline, ADP or AA (5 µmol L^{-1} and 1 mmol L^{-1}, respectively). The platelet response was recorded during 5 minutes. Platelet aggregation was quantified as the percentage of maximal optical change.

In the last version of the BH's guidelines, when ADP-induced platelet aggregation is <50%, no dose change of dipyridamole is required; when AA-induced platelet aggregation is <30%, no dose change of aspirin is required.

TEG-PM in healthy volunteers

TEG-PM assay was also performed in healthy volunteers using activator F with standard ADP and AA concentrations (ie, 2 µmol L^{-1} and 1 mmol L^{-1}, respectively) or 10 µmol L^{-1} ADP.

In addition, control tests were run with activator F alone (without any platelet activators) after incubating blood with 5 U mL^{-1} of apyrase (an ectoenzyme with ADPase and ATPase activities) (Sigma Aldrich, St Louis, MO, USA) for 15 minutes, with 10 µg mL^{-1} of eptifibatide (glycoprotein (GP)-IIb/IIIa inhibitor) for 2 minutes or with saline.

Statistical analyses

Data are presented as medians [range]. A linear-regression analysis was computed with Statview 4.0 software (SAS institute, Cary, NC, USA).

RESULTS

Characteristics of patients

Demographic and clinical patients' characteristics are presented in Table 2. Six patients were affected by dilated cardiomyopathy with end-stage heart failure and were assisted with a bi-VAD; one patient had myocarditis-induced dilated cardiomyopathy and was supported by a left VAD. The median duration of the support was 54[6-107] days. Among patients with bi-VAD, 4 were successfully transplanted and 2 died. The patient assisted by a left BH was successfully explanted.

TABLE 2 Characteristics of patients

Patients	Age (years)	Etiology of heart failure	Antiplatelet therapy	Duration of support (Days)	Complications/outcome
1	3	Dilated cardiomyopathy with end-stage heart failure	N	10	Heart transplantation
2	12	Dilated cardiomyopathy with end-stage heart failure	N	18	Multiorgan failure. Death
3	10	Hereditary heart failure with dilated cardiomyopathy and end-stage heart failure	Y	47	1 thrombus (D2) and 1 microthrombus (D8). One ventricle changed twice. Heart transplantation
4	9	Dilated cardiomyopathy with end-stage heart failure	N	6	Pulmonary embolism. Death
5	5	Myocarditis-induced new onset dilated cardiomyopathy	Y	66	BH explantation. Myocardial recovery
6	10	Dilated cardiomyopathy with end-stage heart failure	Y	71	Heart transplantation
7	3	Dilated cardiomyopathy with end-stage heart failure	Y	107	Right Ventricle changed (thrombus) once. v-v ECMO. Heart transplantation

ECMO, extracorporeal membrane oxygenation; v-v, venous-venous; yrs, years; BH, Berlin Heart; Dx, x days postimplantation; Y, yes; N, no.

Anticoagulation and platelet inhibition after BH implantation

Conventional tests were monitored at least daily (not shown). UFH was started 24–48 hours after BH implantation. The heparin dose was adjusted to achieve a target anti-Xa range of 0.35–0.5 IU mL^{-1}. For 6 patients, heparin was maintained during the whole assistance period. Antithrombin was monitored daily and human antithrombin (Aclotine) administration was adjusted to achieve a target value above 70%. The combined administration of heparin and antithrombin resulted in consistent therapeutic anticoagulation for all patients. No significant bleeding event was observed. Vitamin K antagonist (warfarin) was used in only one patient (patient 3) with an initial dose of 0.2 mg kg^{-1} d^{-1} after 40 days of assistance; the dose was adjusted to maintain the INR between 2.7 and 3.5. Patients who were without bleeding and hemodynamically stable (patients 3, 5, 6, and 7) received dual antiplatelet therapy (Table 3). Aspirin was given between 6 and 9 days postimplantation when TEG-PM platelet inhibition with AA decreased below 70% according to BH's guidelines. On the contrary, dipyridamole was started between 5 and 47 days postimplantation to limit the risk of thrombosis, although the 6 dynes cm^{-2} threshold for G_{ADP} indicated in BH's guidelines was reached for none of these patients.

Platelet inhibition monitoring

In the 4 patients receiving antiplatelet therapy (Table 3), TEG-PM was repeated every 2 days during the first week and then twice weekly. The number of TEG-PM determinations was 15^{7-20} per patient.

Surprisingly, the correlation between MA_{fibrin} and fibrinogen level was weak (r=.17; non significant, n=47). Moreover, in 2 patients (patients 3 and 7), PM tracings were atypical. Indeed, MA_{fibrin} (red tracing, Figure 1) never stabilized, generating an atypical tracing (Figure 1A) compared to control (Figure 1B). This anomaly was also observed for MA_{ADP}.

TABLE 3 Platelet inhibition monitoring

			TEG-PM				LTA				
								Aggregation (%)			
Patients	Dipyridamole (Delay)	Aspirin (Delay)	n	G_{ADP} (dynes/cm^2)	AA Aggregation inhibition (%)	Atypical pattern	n	ADP	AA	Agreement between TEG-PM and LTA for ADP	Agreement between TEG-PM and LTA for AA
3	D47	D9	20	2.1 [0.2-5.1]	51 [16-100]	Yes	0	N/A	N/A	N/A	N/A
5	D5	D7	7	1.9 [1.6-5.2]	70 [0-79]	No	0	N/A	N/A	N/A	N/A
6	D7	D8	11	2.3 [1.8-4.7]	42 [3.4-79]	No	6	49 [28-77]	68 [0-79]	3/6	4/6
7	D6	D6	14	5.4 [2-9.4]	75 [0-89]	Yes[a]	9	91 [50-100]	8 [0-89]	1/9	5/9

Dx, x days postimplantation. N/A, none applicable; LTA, light; TEG-PM, Thromboelastography-Platelet-Mapping; n, number.
[a]After the first observation, MA_{fibrin} (maximum amplitude) was obtained with eptifibatide.

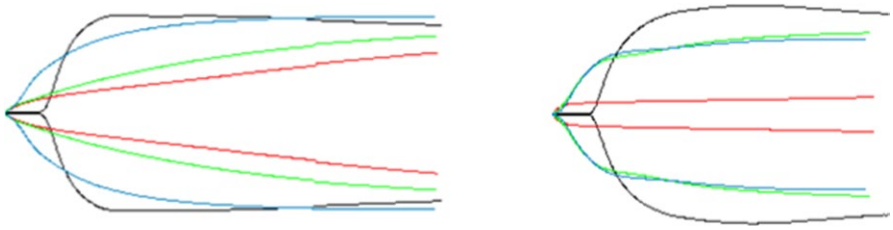

FIGURE 1 TEG-PM tracings. Left panel presents one example of atypical tracings, obtained for patient 3 on day 2 postimplantation, compared to typical tracing obtained with a control sample (right). Black tracing with kaolin-activated blood with heparinase cup ($MA_{thrombin}$); red tracing with activator F (MA_{fibrin}); green tracing with activator F + ADP (MA_{ADP}); blue tracing with activator F + arachidonic acid (MA_{AA}). MA, maximum amplitude

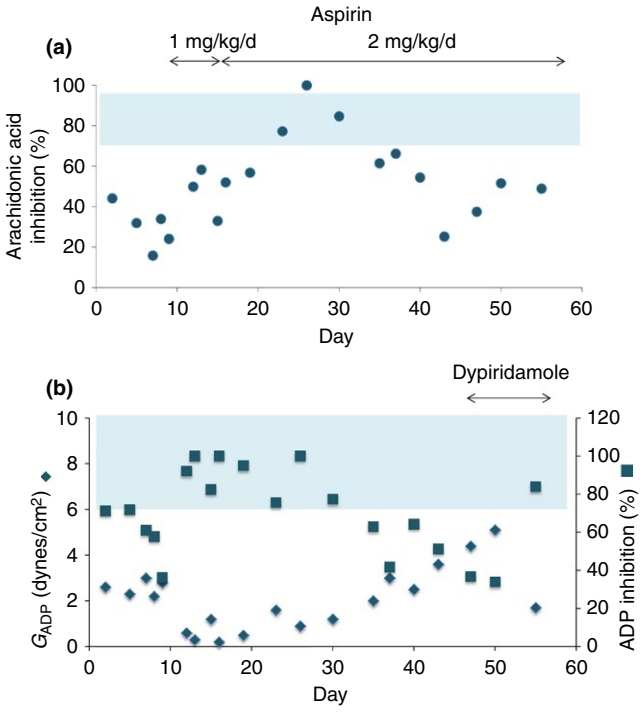

FIGURE 2 TEG-PM obtained for patient 3 in the presence of arachidonic acid (AA) (A) or ADP (B). (A) Percentage of platelet inhibition in response to AA according to the day after Berlin Heart implantation is used to monitor aspirin treatment (circles). (B) The clot strength G in response to ADP is used to monitor dipyridamole treatment (diamonds). Percentage of platelet inhibition in the presence of ADP is also represented (square, right scale). The blue rectangular zone delimits the therapeutic interval for percentage of platelet inhibition with AA (A) and G_{ADP} (B)

Patient 3 was the first BH-implanted patient receiving antiplatelet therapy managed with TEG-PM in our institution. The results of the TEG-PM in the presence of AA and ADP according to the treatment with aspirin and dipyridamole for this patient are shown in Figure 2. Although G_{ADP} is required in the BH's guidelines for the adjustment of dipyridamole, for a better understanding, we also calculated the percentage of ADP platelet inhibition (Figure 2). During the first 9 days, despite the fact that AA platelet inhibition didn't reach 70% no antiplatelet therapy was given, because the targets given for $MA_{thrombin}$ and G_{ADP} were not reached [BH's guidelines]) (Figure 2B). On day 9, in the absence of any bleeding, 1 mg kg^{-1} d^{-1} aspirin was started, because $MA_{thrombin}$ increased and AA platelet inhibition remained under 70% (Figure 2A). Moreover, a left ventricular thrombus appeared in the right ventricle on day 8, although anti-Xa and antithrombin activities were 0.49 UI mL^{-1} and 91%, respectively. On day 15, higher aspirin dose (2 mg kg^{-1} d^{-1}) was needed to achieve the desired 70% inhibition. However, AA platelet inhibition was very unstable during the following days (day 15 to day 55). Dipyridamole was introduced later than aspirin because, until day 9 postimplantation, the G_{ADP} value remained stable, fluctuating between 2.2 and 3 dynes cm^{-2} (Figure 2B), indicating spontaneous low platelet sensitivity to ADP. During follow-up, G_{ADP} ranged from 0.2 to 5.1 dynes cm^{-2}, (corresponding to 34% to 100% ADP platelet inhibition). Although the G_{ADP} threshold value (6 dynes cm^{-2}) recommended for dipyridamole initiation was never reached, to limit the risk of thrombosis, dipyridamole was started 47 days postoperatively at the initial dose of 1 mg kg^{-1} every 6 hours, and maintained until transplantation.

For patient 7, presenting the same atypical pattern, MA_{fibrin} was repeated in the presence of eptifibatide. Indeed, since 2012, BH's guidelines recommends to perform MA_{fibrin} in the presence of eptifibatide when an atypical tracing is generated in the standard conditions. This resulted in an improvement of the correlation between fibrinogen level and MA_{fibrin} (r=.28 vs .54 in standard conditions or in the presence of eptifibatide, respectively).

The monitoring of children receiving dual antiplatelet therapy was also done with LTA for the last 2 patients (6 and 7, Table 2). For patient 7, because of abnormal tracing in the classic conditions, MA_{fibrin} with eptifibatide was taken into account for the comparison with LTA. Overall, 15 (6 for patient 6 plus 9 for patient 7) LTA and TEG-PM were performed in parallel. LTA and TEG-PM inhibition results are shown in Table 3. The concordance between both methods was 60% for AA (9/15) but only 27% for ADP (4/15). In all cases of discordant results for ADP, TEG-PM showed platelet inhibition (G_{ADP} 3.8 [1.8-9.4] dynes cm^{-2}) while LTA showed high platelet reactivity (platelet aggregation: 81.5 [28-100]%).

TEG-PM in healthy volunteers

To improve our understanding of the TEG-PM results, in particular the atypical tracings and the high discordances between TEG-PM and LTA with ADP, PM was also done for healthy volunteers in different conditions. Under standard conditions, median [range] of MA values

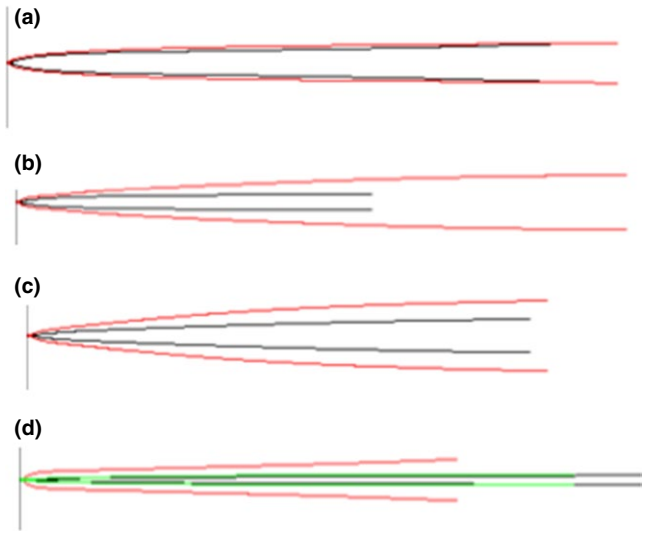

FIGURE 3 Effect of apyrase and eptifibatide on maximum amplitude of control samples due to fibrin formation. For each control, TEG with activator F was done with vehicle (red), apyrase (black) (control A, B, C, D), and eptifibatide (green) (control D). A is representative of 5 healthy volunteers

were as follow: $MA_{thrombin}$ 64 [58-71] mm, (n=9); MA_{ADP} 43 [21-70.8] mm and MA_{AA} 57.1 [54.4-74] mm (n=6). While AA platelet inhibition was low (median: 7.1%) and homogeneous [0–15.6%], ADP platelet inhibition was high (median: 37%) and showed high variability [1.9–68%]. We wondered if the high variability of ADP platelet inhibition in healthy subjects free of any antiplatelet treatment could be explained by the analytical conditions, in particular by the low concentration of ADP used in the test (2 µmol L^{-1}). Indeed, an interindividual variability in ADP-induced platelet aggregation was previously reported, suggesting that 2 µmol L^{-1} ADP could be insufficient to reach full platelet activation in some subjects.[24] We thus compared the effect of ADP 10 µmol L^{-1} and 2 µmol L^{-1} in TEG-PM for 4 healthy volunteers and observed a decrease in ADP platelet-inhibition (37% [25-49%] vs 52% [37-68%] with 10 and 2 µmol L^{-1}, respectively), even though the inhibition remains high.

Surprisingly, an atypical tracing was also observed in 3 healthy volunteers (Figure 3B,C,D). Again, tracings normalized when TEG-PM was performed in the presence of apyrase, suggesting that, even in healthy volunteers, ADP-induced platelet activation interferes on MA_{fibrin}. Likewise, platelet inhibition after addition of eptifibatide (Figure 3D), in one volunteer, also decreases and stabilizes MA_{fibrin} demonstrating a role of platelet aggregation in this unusual pattern.

DISCUSSION

This retrospective analysis underlines the difficulties of monitoring antiplatelet therapy with TEG-PM in children implanted with pediatric BH.

An antithrombotic protocol including platelet inhibitors is essential during mechanical circulatory support to prevent thromboembolic complications.[5,25] Anticoagulant monitoring is relatively well-standardized, albeit sometimes more difficult in children than in adults.[26] In contrast, antiplatelet-drug monitoring remains an issue.

In general, bridged patients have suppressed platelet and coagulation functions during the acute phase after device implantation, with more frequent bleeding. This first phase is usually followed by a hypercoagulable phase during which anticoagulation and platelet inhibitors are required. These complex and clinically unpredictable hemostatic changes explain why BH recommends using the TEG-PM assay, which is presented as a convenient tool to provide an analogous profile of platelet function over time that can accurately monitor platelet antagonists in whole blood.[14,27,28] BH provides algorithms specifying the dipyridamole and aspirin dose changes according to G_{ADP} and the percentage of platelet inhibition in the presence of AA, respectively. However, no correlative studies on TEG-PM results and patient outcomes have been reported. Moreover, some publications highlighted the weaknesses of TEG-PM.[16,22,29,30]

Four out of the 7 patients implanted with BH in our series were treated by antiplatelet therapy after BH implantation. The first BH-implanted patient with antiplatelet therapy monitored by the TEG-PM in our institution showed hypocoagulable status at least until 9 days postimplantation, with low $MA_{thrombin}$, low G_{ADP} and high platelet inhibition with AA TEG-PM, despite no antiplatelet therapy. However, 1 thrombus developed during that period. From the beginning of TEG-PM monitoring, our ability to make therapeutic decisions was compromised by the unusual tracings obtained with activator F. We wondered how MA_{fibrin} was determined, mostly because MA_{fibrin} never stabilized with questionable numerical information provided by the TEG software (MA_{fibrin} and calculated percentage of platelet inhibition). Nelles and Chandler described this pattern demonstrating that platelet activation occurred in 28% of heparinized blood samples, generating a rising unstable MA_{fibrin} curve and, consequently, an overestimation of the percentages of ADP and AA inhibition.[16] In that study, patients were critically ill (79% had left VAD). Eptifibatide use corrected the tracing and decreased the percentage of platelet inhibition. Our study shows that atypical pattern also occurred in critically ill children. Spontaneous platelet activation due to the patient's extreme hypercoagulability and/or conditions of blood-sampling (through central arterial lines) might explain this tracing. Likewise, severe qualitative platelet dysfunction was reported with ADP TEG-PM in children supported with ECMO[31], but in that study no information was given on MA_{fibrin} tracings.

Herein, we showed that platelet activation also occurred in samples from healthy volunteers since adding apyrase or eptifibatide (a GPIIb/IIIa platelet inhibitor) to activator F, reduced the MA_{fibrin}. Nelles et al. have clearly shown that heparin used to prevent thrombin generation at baseline (MA_{fibrin}) induces platelet activation[16] and our results with apyrase suggest that ADP, an important physiological agonist of platelets, participates in the spontaneous platelet activation in heparinized samples. This unstable MA_{fibrin} rise probably also explains the weak correlation between MA_{fibrin} and fibrinogen level. When the MA_{fibrin} curve rises, the manufacturer now recommends adding

GPIIb/IIIa platelet inhibitor to activator F to block platelet aggregation, thereby preventing activated platelet participation in the activator-induced clot. However, spontaneous activated platelets remain present during TEG-PM in presence of ADP or AA leading to inappropriate MA_{ADP} and inappropriate G_{ADP}. The ability of TEG-PM to specifically and accurately reflect platelet sensitivity to ADP or AA becomes questionable after addition of a GPIIbIIIa inhibitor to the activator F. Patients' increasing MA_{fibrin} probably overestimated the percentage of platelet inhibition in the presence of ADP, which is very high in healthy subjects free of any antiplatelet treatment.

In the last version of the BH's guidelines, the target of platelet inhibition for LTA is given in addition to the target of TEG-PM, probably because of the complexity of interpreting TEG-PM data. Although LTA is time consuming, operator-dependent, and is not recommended by the International Society on Thrombosis and Haemostasis to monitor antiplatelet therapy[23], LTA is often used as a reference method in studies evaluating platelet function tests in patients on antiplatelet therapy. TEG-PM has been proven to correlate with LTA.[14] However, when TEG-PM and LTA were conducted simultaneously (patients 6 and 7), the results often led to different decisions for adjusting the doses of dipyridamole and to a lower extent of aspirin. Whether LTA is more appropriate than TEG-PM for monitoring patients with BH on antiplatelet therapy remains to be demonstrated.

Another concern is the reference values of TEG-PM for subjects without platelet inhibitor. According to TEG-PM results in healthy volunteers, a platelet inhibition ADP-dependent was evidenced while they do not receive any antiplatelet therapy. Indeed, ADP platelet inhibition exceeded 40% for 3 of 6 healthy volunteers. In the first publications on TEG-PM, the percentage of ADP platelet inhibition for patients without antiplatelet therapy was high.[32] More recently, Gosselin et al. reported a high percentage of platelet inhibition in normal, drug-naïve donors, especially with ADP TEG-PM, but also to a lesser extent with AA TEG-PM.[17] In our study, using a higher ADP concentration, MA_{ADP} increased for 3 out of 4 healthy volunteers. These results are explained by the interindividual variability in ADP-induced platelet aggregation, as previously shown in a study on 98 healthy volunteers, among whom 2 phenotypic groups of subjects with high or low responsiveness to 2 μmol L^{-1} ADP could be identified.[24] However, the percentage of ADP platelet inhibition, also decreasing, remains high in healthy subjects free of antiplatelet therapy when 10 μmol L^{-1} ADP was used.

Our study has several limitations. The implantation of BH in children, although steadily increasing, is still rare and our study is limited by its small sample size and heterogeneity of patients. Moreover, healthy volunteers were not run in parallel of patients, and blood collection was not standardized between patients and healthy volunteers. Thus, no comparison is allowed between patients and volunteers, but this was not the aim of this study.

Results of TEG-PM in healthy volunteers improved our understanding of patients' results by showing that spontaneous platelet activation involving ADP occurs in heparinized blood samples. This compromises TEG-PM's ability to specifically reflect platelet inhibition secondary to antiplatelet therapy with ADP inhibitors. Our conclusion is consistent with a study on 40 healthy volunteers and volunteers taking daily antiplatelet therapy that compared 5 platelet-function tests, and concluded that TEG-PM is the least suited to monitor the effects of antiplatelet agents.[30] Unfortunately, due to the necessary blood volume, such a study comparing different platelet-function test is not possible in pediatrics.

CONCLUSION

Antiplatelet drug management by monitoring TEG parameters is difficult in VAD-bearing children and requires individual decision-making according to each patient's clinical status. Given the broad variability of TEG-PM results, its sensitivity to several preanalytical and analytical conditions, and the lack of validation of its use in clinical settings, we believe that TEG-PM has to be improved for antiplatelet-drug monitoring during ventricular assistance.

AUTHOR CONTRIBUTIONS

Planned and designed the study: DL, CBL. Conducted the experiments: MC, CG, TBR, TP. Acquisition of data: MC, CG, TBR, PP, OR, TP. Analyzed and interpreted data: MC, CG, TBR, DL, DB, CBL. Statistical analysis: MC, TBR, DL. Wrote the manuscript: CG, DL, CBL. Edited the manuscript: MC, DB, TBR, TP, PP, OR.

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RELATIONSHIP DISCLOSURES

None of the authors have any disclosures relevant to this paper.

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