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Underestimation of unfractionated heparin therapy assessment due to platelet activation when using partial-draw (pediatric) citrate collection tubes

Céline Desconclois [a], Valérie Eschwège [b], Valérie Proulle [a], Tewfik Boutekedjiret [a], Marie Dreyfus [a], Pierre Toulon [c,*]

[a] Service d'Hématologie Biologique, CHU Bicêtre, AP-HP, Université Paris-Sud, Le Kremlin-Bicêtre, France
[b] Service d'Hématologie Biologique, CHU Saint-Antoine, AP-HP, Paris, France
[c] Université Nice Sofia-Antipolis, CHU Saint Roch, Service d'Hématologie Biologique, Nice, France

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ABSTRACT

The "so-called" pediatric tubes are often used when collecting smaller blood volume is necessary, particularly in pediatric patients or in case of difficult/recurrent sampling. The aim of this multicenter study was to compare coagulation test results evaluated in evacuated polymer tubes containing 0.109 M citrate (1 vol./9 vol.) specifically designed to allow either a partial (2.0 mL,"pediatric") or a total (3.5 mL) filling. No significantly relevant discrepancy was found between routine coagulation test results in both tubes collected from untreated patients and from patients on vitamin K antagonist or low molecular weight heparin. In contrast, aPTT was significantly shorter and anti-FXa activity was significantly lower in partial-draw than in full-draw tubes collected from 46 patients receiving unfractionated heparin (UFH). This discrepancy was likely related to increased platelet activation in partial-draw tubes, as suggested by higher platelet factor 4 plasma concentrations and platelet P-Selectin expression in partial-draw than in full-draw citrate tubes. To confirm this hypothesis, we then evaluated partial-draw tubes containing CTAD, a mixture of anticoagulant and antiplatelet agents. In 25 patients on UFH, aPTT and anti-FXa activity were not significantly different in partial-draw CTAD tubes and in full-draw citrate tubes. In conclusion, despite increased platelet activation, samples collected into partial-draw citrate tubes allow accurate routine coagulation testing in all patients but those requiring UFH assessment, in which their use could lead to significant underestimation of anticoagulation. In such cases, partial-draw tubes containing CTAD could be validly used to monitor heparin therapy as well as to perform routine coagulation testing.

Introduction

Sampling small volumes of blood may be necessary, particularly in pediatric patients, or in case of difficult or recurrent venipunctures. To achieve that goal, different options are available from the collection tube manufacturers. Beside tubes with a smaller than usual external volume and tubes with a reduced internal volume [1], most manufacturers developed tubes with the same external and internal dimensions (diameter and length) than the usual ones but with a reduced vacuum allowing collection of smaller blood volumes. In such partial-draw tubes, the volume of citrate anticoagulant solution was adjusted to ensure the proper 9 to 1 blood-to-anticoagulation solution volumes ratio. If the impact of underfilling full-draw citrate collection tubes on coagulation test results has been extensively investigated [2–7], very few publications evaluated the potential impact of collecting blood into specifically designed partial-draw collection tubes [8–11]. Actually, two publications dealing with blood collection for coagulation testing into partial-draw tubes dated back to the nineties [8,10]. Both focused on the potential impact of their use in patients treated with unfractionated heparin (UFH) and concluded that unfilled tubes were unsuitable for monitoring treatments with UFH [8,10]. One was published by an Australian group which evaluated unusual 6.7 mL collection tubes filled with 4.5 or 1.8 mL blood (0.11 M citrate anticoagulation solution volume was 0.5 and 0.2 mL respectively) [8]. The other was about standard 5 mL evacuated tubes of unknown origin and material containing either 0.3 mL or 0.5 mL 3.8% citrate anticoagulation solution volume and a vacuum adapted to allow collection of either 2.7 or 4.5 mL blood respectively [10]. Recently, a significant underestimation of heparin

Abbreviations: Anti-FXa, anti-activated factor X (activity); aPTT, activated partial thromboplastin time; CTAD, citrate, theophylline, adenosine, dipyridamole; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; FV, (coagulation) factor V; INR, international normalized ratio; IU, international unit; LMWH, low molecular weight heparin (derivative); MFI, mean fluorescence intensity; PF4, platelet factor 4; PT, prothombin time; SD, standard deviation; UFH, unfractionated heparin; VKA, vitamin K antagonist.

* Corresponding author at: CHU de Nice, Hôpital Pasteur, Service d'Hématologie Biologique, 30, avenue de la Voie Romaine, CS 51069, F-06001 Nice Cedex 1, France. Tel.: +33 4 92 03 87 09; fax: +33 4 92 03 85 95.
E-mail address: toulon.p@chu-nice.fr (P. Toulon).

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anticoagulation assessment was reported using commercially available evacuated partial-draw collection tubes [11].

Aim of the study

The aim of this multicenter study involving three hematology laboratories was to verify if a currently commercially available partial-draw citrate tube could be validly used, instead of a full-draw tube, to evaluate coagulation in patients with special emphasize on those on traditional anticoagulant therapy. As little was known about such modern tubes, we decided to evaluate not only global assays, but also specific clotting factor assays such as fibrinogen and more importantly factor V which is known to be sensitive to preanalytical conditions [12], and the chromogenic substrate-based anti-FXa activity assay which was found to be altered in previous publication [8–11]. For that purpose, we compared coagulation test results in plasma obtained from blood collected into evacuated polymer collection tubes containing 0.109 M citrate (1 vol./9 vol.) with the same external and internal dimensions that were designed to allow either a partial (2.0 mL, partial-draw tubes) or a full (3.5 mL, full-draw tubes) filling, and to further investigate if any difference was demonstrated.

Patients, materials and methods

Description of the tubes

The studied vacuum polymer sandwich tubes were specifically designed by the manufacturer (Greiner Bio-One, Kremsmünster, Austria) to have a nominal volume of 2.0 mL (partial-draw tubes) or 3.5 mL, (full-draw tubes) as defined according to the ISO 6710 norm [13]. Both had the same external and internal dimensions, were made of the same material (polyethylene terephtalate as the outer part and polypropylene as the inner part) and were closed with identical rubber stopper and safety cap. The ratio of blood to anticoagulant solution was 9 to 1 in both tubes. The Vacuette® Ref. 454321 contained 0.20 mL of a 0.109 M (3.2%) sodium citrate solution [14] and the vacuum was adapted to allow 1.8 mL blood to be collected (partial-draw citrate tubes). The Vacuette® Ref. 454327 contained 0.35 mL of a 0.109 M (3.2%) sodium citrate solution and the vacuum was defined to allow the collection of 3.15 mL blood (full-draw citrate tubes). In the second part of the study, we evaluated another evacuated partial-draw polymer tube (Vacuette® Ref. SA1022) containing 0.20 mL of a mixture of anticoagulant and antiplatelet agents i.e. 75.4 mmol/L tri-Na citrate, 34.8 mmol/L citric acid, 15 mmol/L theophylline, 3.74 mmol/L adenosine, and 0.20 mmol/L dipyridamole (CTAD), and the vacuum was adapted to allow 1.8 mL blood to be collected (partial-draw CTAD tubes). It was identical to the previously described tubes regarding material, rubber stopper, and safety cap, except color. Single lots of partial-draw citrate tubes (lot #A110812), full-draw citrate tubes (lot #A070804), and partial-draw CTAD tubes (lot #A020801) were used in all centers throughout the study.

Evaluated samples

In the first part of the study, one one partial-draw and one full-draw citrate citrate tubes were simultaneously collected by direct venipuncture from a total of 287 adult patients who were prescribed coagulation tests in the three participating centers (A, B, and C). Patients aged below 18 years old, known anemic patients (hemoglobin below 8.0 g/dL) and pregnant women were excluded. The study was performed in accordance of the Declaration of Helsinki, after being approved by the local Ethics Committee. Ninety patients were treated with vitamin K-antagonists (VKA), 46 patients were treated with UFH and 43 patients with subcutaneous injections of low molecular weight heparin derivatives (LMWH) for prophylaxis purpose or with full dose regimen. All of the patients on LMWH were treated with enoxaparin but two who received full dose regimen of tinzaparin (175 IU/kg SC once a day) for the treatment of pulmonary embolism. All patients on UFH or LMWH had detectable anti-activated factor X (anti-FXa) activity i.e. $\geq$0.05 IU/mL. Samples from 108 patients without any anticoagulant treatment were also studied, as well as samples from 9 hemophiliac A patients with factor VIII levels ranging from <1% to 14%, and from 8 patients with liver failure. In addition, healthy volunteers were sampled for investigating potential platelet activation.

In the second part of the study, blood samples were collected into one partial-draw CTAD tubes in addition to the full-draw and partial-draw citrate tubes from a total of 76 adult patients in two centers (A and C). Twenty five patients were treated with UFH and had anti-FXa activity $\geq$0.05 IU/mL, whereas 51 patients were without any anticoagulant treatment.

Tested tubes were collected from the patients in a random order and sent simultaneously to the local hemostasis laboratory at each participating center and centrifuged at 3,000 x g at 18 °C for 15 min, according to the current recommendations [7,15,16]. Plasma was analyzed within one hour. Incomplete doublets/triplets of tubes (n = 1), incompletely filled tubes i.e. less than 90% of their nominal volume (n = 2), hemolyzed or coagulated samples (n = 2) were excluded from the study.

Methods

All these routine coagulation tests were locally performed at each participating centers (A, B, and C) using their own routine procedures i.e. reagents and coagulometer (Table 1). Prothrombin time (PT) was expressed in seconds and as the patient-to-control ratio) or as the international normalized ratio (INR) in patients on VKA, and activated partial thromboplastin time (aPTT) was expressed in seconds and as the patient-to-control ratio. Fibrinogen (in g/L) was measured according to Clauss [17]. Coagulation factor V (FV, expressed in international unit per mL, IU/mL) was measured using one-stage clotting assays. Anti-FXa activity (in IU/mL) when applicable, was measured using chromogenic substrate-based assays. Platelet factor 4 (PF4) was evaluated using a commercially available ELISA kit (Asserachrom PF4, Stago, Asnières, France) [18], according to the recommendations of the manufacturer, on the PR 3100 microplate reader (Bio-Rad Life Science, Marnes-La-Coquette, France). Test results were expressed in IU/mL. Platelet P-Selectin expression, expressed in mean fluorescence intensity (MFI), was evaluated by flow cytometry (FACSCAN, Becton-Dickinson, Meylan, France) [19,20].

Table 1
Reagents and instruments used in the three different centers (A, B, and C) for the measurement of prothrombin time (PT)/international normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen (Fg) evaluated according to Clauss, clotting factor V (FV) evaluated using one-stage clotting assays, and Anti-FXa activity evaluated using chromogenic substrate-based assays.

Center	Test	Reagents	Analyzer
A	PT/INR	Innovin (Siemens)	STA-R (Stago)
	aPTT	Automated APTT (TCoag)	
	Fg	Fibrinogen (Siemens)	
	FV	STA Deficient V (Stago) + Innovin (Siemens)	
	Anti-FXa	Biophen Heparin 6 (Hyphen Biomed)	
B	PT/INR	Thromborel S (Siemens)	ACL TOP (IL)
	aPTT	Automated APTT (TCoag)	
	Fg	FibriQuick (TCoag)	
	FV	STA-Deficient V (Stago) + Thromborel S (Siemens)	
	Anti-FXa	Biophen Heparin 6 (Hyphen Biomed)	
C	PT/INR	HemosIL RecombiPlasTin 2G (IL)	ACL TOP (IL)
	aPTT	Platelin LS (TCoag)	
	Fg	HemosIL Fibrinogen-C (IL)	
	FV	Coagulation FV Deficient (Siemens) + HemosIL RecombiPlasTin 2G (IL)	
	Anti-FXa	Biophen Heparin 6 (Hyphen Biomed)	