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REGULAR ARTICLE

Changes in molecular markers of hemostatic and fibrinolytic activation under various sampling conditions using vacuum tube samples from healthy volunteers ☆

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Sampling condition

Abstract

Molecular makers such as thrombin-antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2), soluble fibrin (SF), and D-dimer, are useful markers in the diagnosis and assessment of various thrombotic conditions. These markers are measured in plasma after blood sampling. Difficult blood sampling is known to falsely elevate plasma TAT levels. However, it is not known exactly why this occurs. In the present study, we examined how levels of molecular markers of haemostatic and fibrinolytic activation change under various sampling conditions using vacuum tube samples from healthy volunteers.
When blood was sampled continuously by taking 10 consecutive vacuum tube samples following application of a tourniquet, blood sampling resulted in an accurate assessment of these molecular makers.
When blood was sampled continuously by taking vacuum tube samples every one minute over a total of 9 minutes to investigate possible changes in the levels of the molecular markers over time, plasma levels of TAT, SF, and F1+2 gradually increased with time. Plasma levels of TAT, F1+2, and SF increased beyond the normal range over

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the course of nine minutes. When blood was sampled using three alternative methods, which varied in terms of the duration of needle puncture (sampling B), duration of tourniquet use (sampling C), or both (sampling A), plasma TAT and SF levels were significantly increased with all three methods, compared to control samples. Plasma F1+2 levels were significantly increased with sampling methods A and B, compared to control samples, but not with sampling method C. On the other hand, plasma D-dimer levels were not significantly altered by any of the sampling methods.
In conclusion, the results suggest that molecular markers of haemostatic and fibrinolytic activation, except for D-dimer, may be affected by sampling method, particularly the duration of needle puncturing. Therefore, care needs to be taken when using TAT, F1+2, and SF levels to diagnose and estimate activation of the coagulation system.
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Introduction

Thrombotic diseases are often induced by activation of blood coagulation and sustained thrombin generation in vivo under pathologic conditions. Therefore, molecular markers that estimate thrombin generation are useful in the diagnosis of thrombotic disease. Molecular markers such as thrombin-antithrombin complex (TAT) and prothrombin fragment 1+2 (F1+2) are known to indicate thrombin production in vivo [1-3]. Soluble fibrin (SF) exists as soluble protein containing a fibrin monomer known to associate with fibrinogen, thus indicating fibrin monomer generation [4]. D-dimer is a marker of reactive fibrinolysis of cross-linked fibrin [5,6]. When these molecular markers are elevated in plasma, blood coagulation is thought to be activated in vivo [7-9]. Thus, these molecular markers are essential in estimating the severity of various thrombotic conditions.

These markers are measured using plasma samples. During blood sampling, the duration of sampling is sometimes increased when patients have difficult access or when unskilled operators sample blood from patients. When blood sampling is difficult, false elevations of plasma TAT levels are known to occur. However, the specific factors contributing to this artifact remain unclear. In particular, the duration of tourniquet use and duration of needle puncture warrant further investigation as possible causes.

In this study, we examined changes in the levels of various molecular markers of hemostatic and fibrinolytic activation under various sampling conditions using vacuum tube samples from healthy volunteers.

Methods

Healthy Subjects

Samples of blood were collected from five healthy volunteers (two men and three women, aged 24-32 y.o.). Informed consent was obtained from all participants.

Blood samples

Samples of blood (5 mL each) were collected from an antecubital vein using vacuum tubes containing 3.8% trisodium citrate (one volume of citrate to nine volumes of blood) following application of a tourniquet. A 21G sampling needle was used. Various sampling conditions were investigated as described below.

Sampling method I (Fig. 1-I): Blood was sampled continuously from an antecubital vein using vacuum tubes following immediate application of a tourniquet. Ten samples were taken within three minutes.

Sampling method II (Fig. 1-II): Blood was sampled continuously at one minute intervals (from zero to nine minutes) from an antecubital vein using vacuum tubes following application of a tourniquet.

Sampling method III included three variations, classified as subtypes A, B and C (Fig. 1-III).

III - A: Blood was sampled nine minutes after puncture of an antecubital vein following application of a tourniquet for 9 minutes.

III - B: An antecubital vein was punctured following application of a tourniquet, after which the tourniquet was removed for 9 minutes. After 9 minutes, the tourniquet was re-applied and the blood collected into vacuum tubes.

III - C: An antecubital vein was punctured by a blood sampling needle 9 minutes after initial application of a tourniquet.

In short, sampling method A involved a prolonged duration of needle puncture and tourniquet use. Sampling method B involved a prolonged duration of needle puncture, while sampling method C involved prolonged tourniquet use only.

Since we did want to reveal the extent of sampling method available to measure coagulation makers, we determined the interval of 9 minutes.

After sampling, the tubes were centrifuged at 2,000 ×*g* for 15 min. Plasma was separated, and stored at −80 °C until analyzed.

Laboratory methods

Plasma TAT levels were determined by time resolution fluorescence immunoassay (TR-FIA) using LPIA-A700 (Mitsubishi Chemical Medience, Japan). Plasma SF levels were determined by latex immunoassay using latex reagent containing IF-43 antibody (Mitsubishi Chemical Medience, Japan).

Plasma F1+2 levels were determined by enzyme-linked immunosorbent assay using BEP-III produced from Dade Behring,

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