Differences in Activated Partial Thromboplastin Time (APTT) measures depending on the device used in blood samples drawn (direct Vacutainer® versus Butterfly needle): A Randomized Comparative Study

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ABSTRACT

Background: Most laboratory errors occur during the pre-analytical phase and are often related to the blood drawn equipment. No studies have evaluated yet the influence of the most common vein puncture devices on activated partial thromboplastin time (APTT). We aimed to compare APTT when venous blood samples are drawn with two different needle devices (direct Vacutainer® versus butterfly needle).

Methods: Consecutive adult healthy volunteers were prospectively enrolled and assigned to receive two antecubital peripheral blood punctures from both arms, using the two devices. The blood drawn sequence (i.e. right or left arm first) and the first device to be used (i.e. direct vacuntainer® or butterfly needle first) were randomly established.

Results: Forty healthy volunteers (mean age 42.9 years; SD 7.5; range 27-58), comprising 25 (62.5%) women, were recruited. APTT obtained with the two blood drawn devices significantly and positive correlated (Spearman rho=0.943; p<0.001). However, mean APTT was significantly more prolonged in blood samples drawn with butterfly needles than in those obtained with direct vacutainer® (34.01 ± 4.4 vs. 33.63 ± 3.8 seconds, respectively; p=0.013). No differences in APTT were found when samples obtained from right and left arms were compared.

Conclusion: The type of blood drawn equipment significantly influences APTT, which is prolonged when a butterfly needle is used. An increased contact time between blood and butterfly tube plastic material would favor a premature consumption of plasma coagulation factors before mixing with anticoagulants in the collection tube. The appropriately chosen blood drawn device is thus essential to avoid pre-analytical errors.

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Background:

Activated partial thromboplastin time (APTT) is a common hematologic test, routinely used to study the intrinsic coagulation pathway factors. It also allows for the studying of the common pathway for monitoring treatment with unfractionated heparin and assessing the presence of lupus anticoagulant or other inhibitors (1). Because of that, its use is widely spread in clinical practice at several settings.

Several causes are recognized to prolong the APTT, including a deficiency of function or an inhibition of any of the coagulation factors (except for factor VII), von Willebrand disease (VWD), factor VIII deficiency, and serum antibodies against plasmatic proteins bound to anionic phospholipids constituting lupic anticoagulant (2,3). Drugs prolonging APTT include heparin, direct thrombin inhibitors, and direct factor Xa inhibitors. Warfarin has a weak effect on most of APTT reagents, but will increase the sensitivity of APTT to the heparin effect (4). An APTT over 25 to 35 seconds to form blood clots is required performing to address the presence of coagulopathies. These must involve assessing the activity of coagulation factors VIII, IX, XI and XII, presence of lupus anticoagulant, anticardiolipin anticoagulant, Immunoglobulin (Ig) G, IgM and a test of mixtures (by mixing normal plasma with the problem plasma) (5,6).

Irregular results in coagulation tests are frequent in clinical practice: a systematic review revealed that 4% to 15% of patients with no risk factors for bleeding presented altered results in preoperative analyses (7). Despite the low predictive value for perioperative bleeding that isolated analytical APTT or prothrombin time enlargement have in patients with no bleeding history, these alterations may constitute an unwarranted cause of delayed surgery and transfusion of fresh frozen plasma (7). Although these results might not imply changes in the clinical management of patients, they warrant repeat coagulation tests (6,8).

Alterations in coagulation results may originate at any phase of the laboratory process: Possibilities of error have been documented in the pre-analytical, the analytical and even the post-analytical phases. Among the pre-analytical errors able to alter blood test results, the technique of blood sample collection has been limitedly assessed in literature. Specifically, the election of the adequate blood drawn collection device might impact on the accuracy of laboratory test results, and several studies have demonstrated that hematological and clinical chemistry parameters greatly depend on the puncture device used, including vacutainer® versus syringe, and also butterfly needle, as well the size and type of needles (10,11,12). In hospital environments, direct vacutainer®-based devices have been imposed as the reference method for venous blood drawn (14,15), whereas the butterfly needle (generally with the smallest needle caliber) is reserved for very small caliber veins and to diminish pain during blood extraction in children (15). The selection of the final device depends on individual conditions and is usually decided under different nurse criteria, not always based on the best evidence practice demonstrated (16).

Because no study to date has compared direct vacutainer® with butterfly needles in terms of its potential in inducing alterations in blood coagulation tests related with venous puncture technique, this research aims to analyze whether APTT results depend on the selection of blood drawn device to provide further evidence in improving clinical practice

Methods

Study design and setting

A prospective randomized comparative study was developed in a consecutive series of healthy adults to assess the concordance in APTT results in peripheral venous blood samples drawn with direct vacutainer® and butterfly needle. Participants were healthy adult volunteers consecutively recruited in February 2016 in the hematology outpatient clinic at the Hospital Virgen de Altagracia, located in Castilla-La Mancha region, at central Spain. Participants were recruited from patients' companions and healthcare professionals who agreed to participate and provided informed consent. Clinical evaluation and review of previous analytical results in clinical records in the same hospital were performed before inclusion.

Exclusion criteria consisted in having a medical background of congenital protein C or S deficiencies, disseminated intravascular coagulation, deficiency in clotting factors II, V, VII, X or XII, Glanzmann's disease, Hemophilia A, Hemophilia B, Hemophilia C or factor XI deficiency, idiopathic thrombocytopenic purpura, VWD, as

well as age under 18 or denial of consent to participate. In addition, to be included, all participants were required to have normal hematologic and coagulation test results, documented in at least two different determinations within the previous 5 year period.

The study protocol supporting this research was approved by the institutional review board at Hospital General La Mancha Centro.

Study performance and random sequence generation

After signing the informed consent form, participants who fulfilled inclusion criteria underwent two consecutive blood samples drawn by peripheral vein puncture, which was done according to the local laboratory protocol to ensure correct extraction and processing of samples. Participants were assigned to be punctured in an antecubital vein at both arm flexures and with one or the other of the devices randomly, by the same registered nurse. Vacutainer® Elipse™ were compared to BD safety lok™ butterfly needles, both inserted in a plastic transparent holder. A 20-gauge (0.8 mm) needle caliber was used throughout the study in every device. Light blue cap, 3.2% sodium citrate, 2.7 mL collection tubes were used throughout. All needles and tubes were manufactured by Becton Dickinson and company, which had no role in the present research.

In order to prevent selection bias, a random list was obtained with the EPIDAT v3.2 software, available for free on the Internet, to determine the sequence of puncture (right or left arm first) as well as the puncture device to be used first. The allocation sequence was concealed from the nurse responsible for puncturing participants until they provided consent. Both the registered nurse performing the puncture and the participant were un-blinded to the interventions and the sequence of procedures.

After sample drawn, collection tubes were labeled according to the puncture arm and the type of puncture device used, and sent to the hospital laboratory for immediate simultaneous processing for analysis, according to standard clinical methods: Briefly, after centrifugation for 5 minutes at 3000 rpm, each pair of samples were analyzed in an ACL TOP 500 CTS analyzer (Werfen). A scheme for the research procedure is shown in Figure 1.

Figure 1.Flow chart.
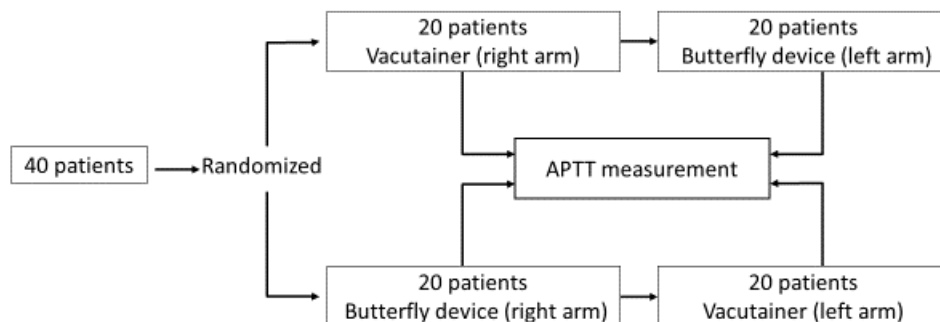
Because of the lack of previous studies, a potential difference in APTT values obtained with each blood drawn device was not available in literature. Therefore the study was performed according to a two-stage sequential test design, with the possible enlarging of the sample size according to the results of and intermediate analysis scheduled by the

time 30 patients were enrolled. After the intermediate analysis being available, final sample size was determined by the probabilistic sampling method for paired samples, assuming a statistical power of 90% and a 95% confidence level.

Data Analysis

Management of the data was performed anonymously, using codes assigned to each of the subjects participating in this study. The identification of each patient was only available for the researchers responsible for the study exclusively for the purposes of the present investigation. An excel-based registry was designed and completed with demographic, protocol details and analytical results from all patients, including identification number, age and gender, arm punctured with each device, APTT results in the samples obtained with direct vacutainer® and with butterfly needle, respectively.

Quantitative variables were summarized with statistics of central tendency and dispersion; qualitative variables were expressed as proportions. APTT values obtained from each extraction device were compared with Ji^2 tests, and their correlations by means of Cohen's Spearman Rho and Student-t test. All analyses were performed with the SPSS statistic package v18.0 (SPSS Inc. Chicago, Ill); a p value less than 0.05 was considered as significant.

Results

A total of 40 healthy volunteers with a mean age of 42.9 years (SD 7.5; range 27- 58) were recruited, 25 of whom (62.5%) were women. Half of participants (20) were randomized to be punctured firstly in the right arm; vacutainer™ was randomly used for the first blood drawn in twenty subjects. The main characteristics of the participants included in the study are summarized in Table 1.

Characteristics	N (%)
Mean Age (years), (SD; range) (years)	42.95 (7.5; 27 – 58)
Gender (number of female / male) (%)	25 (62.5%) / 15 (37.5%)
ATTP Vacutainer®, mean (SD) (seconds)	33.63 (3.8)
ATTP Butterfly, mean (SD) (seconds)	34.01 (4.4)

APTT was more prolonged in blood samples obtained with butterfly needles in 28 participants (70%), whereas the opposite happened in the 12 (30%) remaining. A statistically significant and positive correlation between APTT results obtained by direct Vacutainer® Elipse™ and BD safety lok™ butterfly needles punctures was also found (Spearman rho= 0.943; p <0.001).   Figure 2.
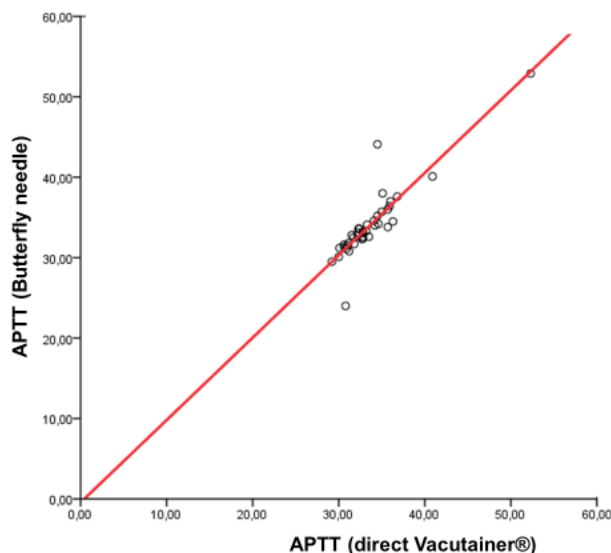
Mean APTT results in blood samples obtained with the aid of butterfly needles were slightly but significantly higher than those obtained with direct vacutainer® system (33.63 ±3.8 *vs*. 34.01 ± 4.4 seconds, respectively; p=0.013), with APTT in the samples obtained with butterfly needles overall exceeding 0.39 seconds over the direct vacutainer®. These differences remained significant in the subgroup of women (33.8 ± 4.4 *vs*. 34.1 ± 4.4; p=0.027) but not in males (33.3 ± 2.8 *vs*. 33.9 ± 4.6; p=0.279).

Finally, no differences in APTT results were found when samples obtained from right and left arms were compared (p = 0.372).

Discussion:

This randomized comparative study demonstrated a significantly more prolonged APTT when blood samples are obtained with a butterfly-type needed device as compared with the direct vacutainer® system. This research therefore, highlights the importance of choosing the proper blood drawn device in optimizing the analytical processing and accuracy of results.

Laboratory processing consists of sequences of consecutively performed procedures divided into three phases - pre-analytical, analytical and post-analytical-, all of them subjected to several possibilities of error. Previous review papers have revealed that between 40 and 70% of all errors in the determination of laboratory results are produced during the pre-analytical phase (17,18,19,20,21), warranting the greatest attention regarding the variations in the preparation and collection of laboratory samples to reduce its impact on the final analytical result, as well as on the diagnosis, treatment and therapeutic decisions made on patients. A recent review on the main pre-analytical quality errors occurred during blood drawn summarizes them into clotted samples, insufficient blood quantity, incorrect sample (coagulation profile or incomplete blood vial count), lacking a label and incorrect labeling as the most common ones (22). Each of these errors can be avoided by a proper communication, training of and cooperation among the staff, and computerization of the laboratories, including simplification of specimen routing and tracking (23-25). Since an incorrect collection of blood samples may cause abnormal results in analyses, basic standards have been developed and are provided by hematology laboratories and hemotherapy services (26-28).

Despite causes of an alteration in the APTT being well known (29,30), the potential influence of the technique and device used for blood drawn had not been analyzed before. To our knowledge, the current is the first study that determines how coagulation tests, specifically APTT, are affected by blood drawn devices. It has been documented that vascular access devices, such as catheters and needles, exert shear forces on blood flow and create a predisposition to cell lysis, which alters the plasma composition and the function of its components. Interactions between blood specimens and collection tubes (which may contain additives, stoppers, lubricants, surfactants, and separator gels) are able to alter analyte stability, constituting a potential source of errors during the pre-analytical phase, which decrease laboratory efficiency with detrimental effects on patient care (31,32). We hypothesize that butterfly needles significantly increase APTT by altering blood stability when interacting with device components. The design of butterfly needles include a metal needle connected to a long, small caliber, 19-cm length tube made of polypropylene polymers, which the blood has to go through before being collected in the tube and mixed with preservative anticoagulant substances (usually sodium citrate). A small needle caliber further favors an increased contact time between the blood and the plastic materials of the tube, thus activating coagulation cascades and prematurely consuming part of the plasma coagulation factors before this process could be interrupted by the sodium citrate contained in the collection tube. In direct vacutainer® system, blood is drawn directly from the vein into the collection tube through the needle, thus avoiding premature consumption of plasma coagulation factors, and likely providing a more accurate measure of the APTT.

According to our results, no differences for each needle device were found in APTT when blood drawn from both arms were compared, thus reinforcing the idea of the indifference from where the blood samples are obtained. However, we found that the significantly longer APTT in blood samples drawn with butterfly needle compared to direct vacutainer® were also reproduced in the subgroup of female volunteers participating in this study, but not among male subjects. The higher number of women included in our study sample is a plausible explanation for the statistical differences.

The main strength of our research derives from the consecutive recruitment of healthy participants and the random allocation of participants to be punctured with each of the devices in both arm antecubital flexures with the same needle-size. Full adherence to local laboratory protocols and the fact that one single registered nurse carried out

all the venous punctures to reduce operator-dependent variability, together with the simultaneous processing and analysis of each pair of blood samples, to ensure analytical homogeneity, constitutes additional methodological strengths. However, some limitations of this study should be also acknowledged, including the single-centre inclusion of participants and the limited sample size considered. In addition, no additional analytic value to APTT was determined, therefore impacting on the wide generalization of our results.

To conclude, we show with this randomized comparative study that the type of blood drawn device significantly influences APTT, which is prolonged in the case of using butterfly needle compared to direct vacutainer®. An appropriate choose of the vein puncture device for blood analysis by the responsible nurse is thus essential to avoid pre-analytical errors, specifically in the case of coagulation tests, therefore reducing the repetition of analysis, avoiding diagnostic and therapeutic delay and reducing health-care related costs..

Acknowledgements

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