

Variability of coagulation testing between two separate laboratories: implications for diagnosis and therapeutic monitoring

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Abstract

Despite introduction of some measures to achieve a more effective standardization in routine coagulation testing, there are evidences that harmonization among different clinical laboratories has not been fully achieved as yet. To evaluate whether differences in instruments and reagents between two separate laboratories might influence the clinical management of patients with hemostatic disturbances, we analyzed prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and d-dimer in 84 consecutive outpatients on oral or heparin anticoagulant therapy. Despite statistically significant differences could be observed for all the parameters tested, Passing & Bablok analysis yielded acceptable results. The extent of measurement deviation always exceeded the current analytical quality specifications for desirable bias, when available. The percentage of patients in the therapeutic range or outside the respective reference range was significantly different between laboratories for APTT and fibrinogen, but not for PT and d-dimer. In conclusion, results of our investigation testify that some further efforts are needed to achieve harmonization of routine coagulation testing among laboratories.

Key Words: Coagulation testing, Assay Methodology, Prothrombin time, D-dimer, Fibrinogen, Activated Partial Thromboplastin Time.

Introduction

Coagulation testing is essential to ensure accurate diagnosis and appropriate treatment of patients with hemostasis disturbances. Although an efficient standardization of the procedures employed for specimen collection, handling and storage is pivotal to achieve accuracy and precision of results, the lack of satisfactory harmonization in routine coagulation testing among different laboratories is an additional matter of concern, as it might exert a considerable influence on the clinical management of patients. Despite several attempts were made to overcome most preanalytical and analytical problems, some factors, including the off-

site blood collection, still affect the reliability of results. In particular, there is strong evidence that the overall bias for prothrombin time (PT), activated thromboplastin time (APTT), fibrinogen and d-dimer measurements might largely depend upon individual reagents and analytical techniques¹⁻⁵.

Materials and Methods

To evaluate whether different instruments and reagents might modify the clinical management, we analyzed PT, APTT, fibrinogen and d-dimer in 84 consecutive outpatients on oral or heparin anticoagulant therapy. Venipunctures were performed in the morning of the same day on fastened patients, by a single practiced phlebotomist. All phases of the sample collection were accurately standardized, including an identical time of tourniquet placement, the use of needles of the same dimension (20G) and vacuum tubes of the same lot (Becton-Dickinson, Oxford, UK). After collection into siliconized vacuum tubes containing 0.109 mol/L buffered sodium citrate, samples were gently mixed and centrifuged at 3000 \times g for 10 min at 10 °C; plasma was separated and stored in aliquots at -70 °C until measurement. In case of unsatisfactory attempts, results were excluded from the statistical evaluation. According to these rules, data of three patients originally enrolled were excluded and the final study population consisted on 81 subjects (46 women, 35 men; mean age: 51 years), selected to cover all the clinically significant ranges of coagulation values. Coagulation measurements were simultaneously performed on aliquots of identical specimens by two separate laboratories, employing different instruments and reagents. The first laboratory, designed as Center 1 (Vicenza), used a STA-R® Hemostasis System (Diagnostica Stago, Parsippany, NJ), employing Sta® Neoplastin Plus (Suspension of Thromboplastin, rabbit brain, Diagnostica Stago) for PT, Sta® APTT (lyophilized cephalin prepared from rabbit cerebral tissues and silica, Diagnostica Stago) for APTT and STA Fibrinogen (Diagnostica Stago) for fibrinogen measurement. D-dimer levels were measured immunoturbidimetrically on STA-R®

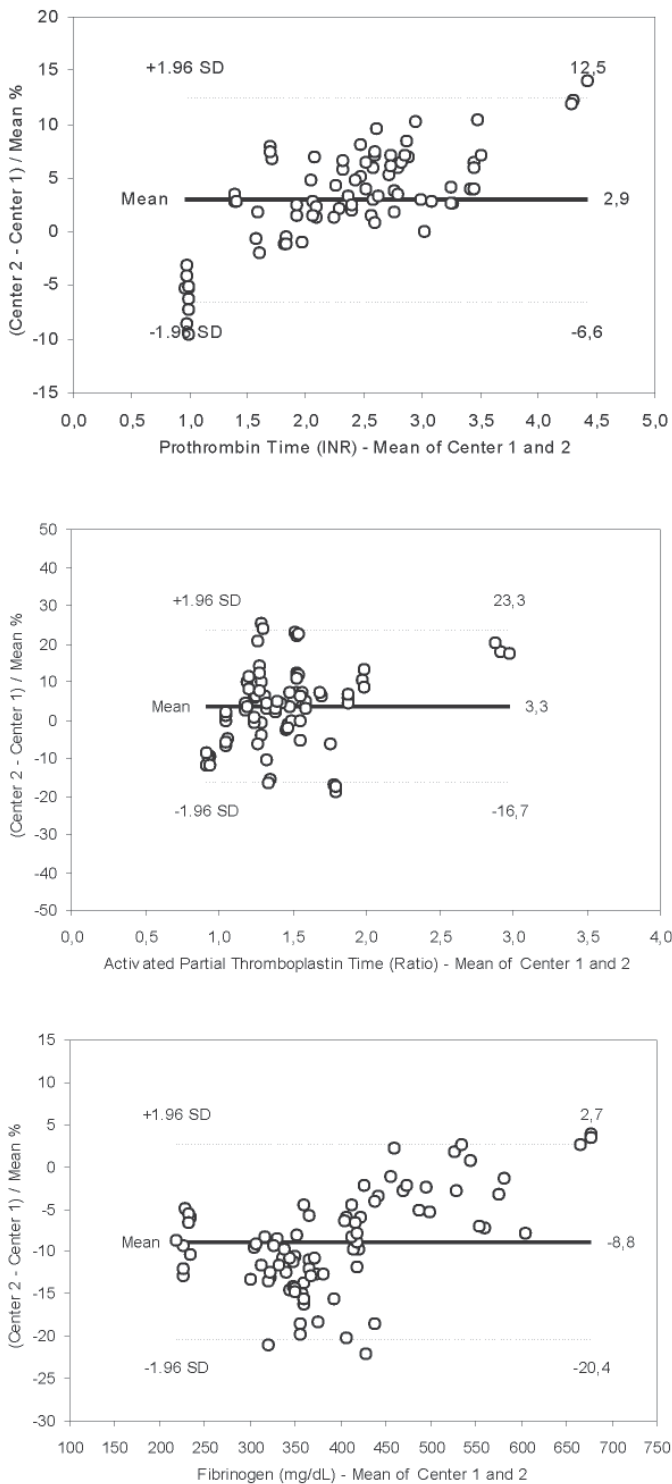


Figure 1. Bland & Altman plots for the independent measurements of prothrombin time, activated partial thromboplastin time and fibrinogen on identical samples measured in two separate laboratories by different instruments and reagents. Plot differences are expressed as percentage of averages.

with the STA Liatest D-DI immunoassay (Diagnostica Stago). The second laboratory (Center 2, Verona), performed the measurements on a Behring Coagulation System (BCS, Dade-Behring, Deerfield, IL), employing proprietary reagents: Thromborel S (lyophilized human placental thromboplastin), Pathromptin SL (Vegetable phospholipid with micronized silica) and Multifibrin U, for PT, APTT

and fibrinogen, respectively. Plasma d-dimer was measured with the Vidas DD, a rapid, quantitative automated ELISA with fluorescent detection, on the Mini Vidas Immunoanalyzer (bioMerieux, Marcy l'Etoile, Francia). Both laboratories are accredited to the Italian Federation of Centers for the Anticoagulation Surveillance (FCSA) and both PT reagents are calibrated against the international reference thromboplastin preparation. All calibrations were performed according to the instructions provided by the manufacturers. Analytical imprecision, expressed in terms of mean inter-assay coefficient of variation (CV), was quoted by all manufacturers as being comprised between 2 and 5%. Statistical calculations included the mean and standard deviation (SD) of paired differences with results reported as mean \pm SD. Paired Student's t test was used to determine statistically significant differences between measurements. Results were further compared by Passing-Bablok regression analysis and Bland-Altman plots. The Bland & Altman plot was used to compare the results of the independent measurements on samples and plot differences were finally reported as percentage of averages. We selected this graphical option, as it appears more suited to verify the potential increase in variability of the differences as the magnitude of the measurement increases⁶. As d-dimer products comprise a broad range of structural motifs, raw numerical values obtained with different immunoassays are barely comparable. Such variations arise from the heterogeneous reactivity of different monoclonal antibodies for d-dimer containing degradation products⁷. For this reason, the statistical comparison of data between the two assays was not feasible.

Results

Results of the evaluation are synthesized in table 1. Despite statistically significant differences could be observed for all the parameters tested, Passing & Bablok analysis yielded acceptable results, displaying slope values of 0.89, 0.815, 1.06 and correlation coefficients of 0.995 and 0.933 and 0.980 for PT, APTT and fibrinogen measurements, respectively. The extent of measurement deviation is represented in Bland & Altman plots and the 95% agreement interval in the set of differences between samples (Figure 1). The mean proportional biases and relative 95% intervals of confidence (C.I.) were +2.9% (-6.6% to +12.5%), +3.3% (-16.7% to +23.3%) and -8.8% (-20.4% to +2.7%) for PT, APTT and fibrinogen, respectively, thus always exceeding the current analytical quality specifications for desirable bias (2.0% for PT, 2.3% for APTT and 4.8% for fibrinogen)⁸. Although the bias observed for APTT measurements was homogeneously distributed over the whole clinical relevant measuring range, PT and fibrinogen measurements displayed a consistent trend toward underestimation of lower values and overestimation of higher values in Center 2. After stratifying results according to the ideal respective therapeutic indications (from 2.0 to 3.0 for PT and from 1.5 to 2.5 for APTT), the percentage of patients in the therapeutic range were significantly different by chi square analysis for APTT (23.5% versus 35.8%, $P=0.03$), but not for PT (48.1% versus 46.9%, $P=0.82$). Accordingly, the percentage of patients outside the reference range (150-400 mg/dL for fibrinogen) or excee-

Table 1.

Statistical analysis of coagulation testing on aliquots of identical samples tested simultaneously in two separate laboratories, employing different instruments and reagents. Results are compared by paired Student's t-test and Passing & Bablock regression analysis. Values are compared to the current analytical quality specifications for desirable bias derived from biologic variation (8).

	<i>Patients (n)</i>	<i>Center 1</i>	<i>Center 2</i>	<i>Desirable bias</i>	<i>CV</i>	<i>t-test</i>	<i>Regression analysis</i>	
PT (INR)	81	2.26±0.78	2.37±0.89	±2.0	3.5%	<0.001	y = 1.12x - 0.17	r = 0.995
APTT (Ratio)	81	1.42±0.35	1.48±0.44	±2.3	6.0%	0.002	y = 1.23 - 0.26	r = 0.933
Fibrinogen (mg/dL)	81	409±100	377±108	±4.8	6.6%	<0.001	y = 1.06x - 0.56	r = 0.980

ding the relative diagnostic thresholds (<500 µg/L for Vidas d-dimer and <400 mg/L for Liatest D-DI) was significantly different for fibrinogen (46.4% versus 32.1%, $P<0.001$), but not for d-dimer (15.5% versus 20.2%, $P=0.24$).

Discussion

Although several strategies were attempted over the past decades to standardize and harmonize routine coagulation measurements, including the adoption of thromboplastin calibration against the primary WHO reference preparation for the INR assay and the preparation of an international reference material for d-dimer testing^{7,9}, the agreement among commercial assays is as yet almost unsatisfactory²⁻⁵. There is consolidated evidence that both the preanalytical and analytical phases might be crucial in the clinical management of patients with coagulation disorders. In the present investigation, we further demonstrate that routine coagulation testing in separate laboratories, by different instruments and reagents, might occasionally generate misleading clinical information. Such an evidence highlights a concerning ineffectiveness of current standardization strategies in coagulation testing, especially for APTT and fibrinogen measurements, which may ultimately compromise both the diagnostic and therapeutic approaches, narrowing the appropriate application of anticoagulation guidelines. Although the comparison of most parameters between two different laboratories did not fulfilled the major analytical criteria in terms of analytical quality specifications for desirable bias, major discordance was observed from data of APTT and fibrinogen testing. Surprisingly, a satisfactory clinical agreement was achieved in d-dimer measurement. As different d-dimer immunoassays recognize heterogeneous components of cross-linked fibrin degradation products, a certain amount of disomogeneity of results is somehow expectable. To overcome this problem, investigators recently focused on identifying specific diagnostic thresholds for each commercial immunoassay, based on test efficiencies, rather than attempts at standardization. Taken together, results of our investigation testify the effectiveness of these efforts. In fact, despite the poor comparability of numeric values between the two Centers, due to the different analytical approach, we confirm that when a reliable, assay-specific diagnostic threshold is established, the clinical management of patients is not likely to be substantially influenced¹⁰.

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