Reducing the determination errors of the international normalized ratio by using plasma cogulation calibrators

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Abstract
The objective was to use of a minimum number of ISI (International Sensitivity Index) to determine the International Normalized Ratio (INR). This specific calibration methods allowed to calculate ISI for all other thromboplastines comparative with the reference thromboplastine. Bigger values than 1 show a decrease sensitivity, as compared with the reference thromboplastine. The Prothrombin Time (PT) values are not the same in all laboratory because of differences in sensitivity of thromboplastins used. To standardize the same results in any laboratory, an INR (International Normalised Ratio) constant has been introduced. Estimation of thromboplastine sensitivity is useful in any laboratory involved in the medical control of therapy with AVK, being also helpull for the clinicians to find out a suitable curative dealing.
Keywords: *reference thromboplastin, ISI, INR, plasma calibrants, oral anticoagulants.*

Introduction

In 1977, (World Health Organization) WHO introduced the IRP 67/40 (International Reference Preparation) preparation as a reference for all marketed thromboplastin preparations. In 1985, the International Committee for Standardization in Hematology (ICSH) and the International Committee on Thrombosis and Hemostasis (ICTH) recommended the use of INR for the prothrombin time of patients treated with vitamin K antagonists.

The standard ISI calculation procedure adopted by BCR (Community Bureau of Reference) consists in determining prothrombin time for 60 samples from patients treated with vitamin K antagonists and 20 samples from patients considered healthy using two thromboplastins - one to be researched and the other to (IRP 67/40) reference (1,2)

The first reference thromboplastin recommended by the WHO (IRP 67/40) received an ISI of 1.0 and is a benchmark for all the other used thromboplastins (and subsequent reference preparations).

To facilitate clotting tests in the medical laboratory, since 1991 it has been recommended by the to practice a simplified version of the calibration procedure: Thrombosis Reference Centre System ISI Calibrant plasma kit. By this method, a set of 18 plasma which covers the therapeutic range corresponding to oral anticoagulants is prepared from normal plasma by adsorption on barium sulfate of coagulation factors synthesized in the presence of vitamin K(3)

The Prothrombin Time (PT**)** in an individual with one or more deficiencies of a clotting factor will vary with the type of thromboplastin (e.g. rabbit, human, bovine etc) used in the assay. This difference in sensitivities is known as the sensitivity index. Individual thromboplastins can be calibrated against an international WHO reference thromboplastin (International Reference Preparation or IRP) to assign them an International sensitivity index or ISI.

Material and method

Reagents and instruments

Reagents: HemosIL PT Fibrinogen Recombinat (Instrumentation Laboratory), Siron LS (Technoclone).

Plasma calibrants: RecombiPlasTin (Instrumentation Laboratory), Technoplastin His (Technoclone) – ISI 1.1-1.2.

Coagulation analyzer: ACL 100 (Instrumentation Laboratory), Trombotimer (Behnk Elektronic)

Methods

Statistical analysis of lyophilized plasma levels of coagulation factor was performed by Labquality and Hematrom laboratories.

Results and discussions

The reference thromboplastins used in the calibration of the coagulometers cause errors if it does not correspond to the recommended reference thromboplastin quality (IRP 67/40).

Errors in coagulation tests are due to coagulometer or plasma calibrats and the reagents used.

The results of clotting tests allow us to identify errors due to coagulometer, plasma calibrats or the reagents used.

For this purpose, we analyzed the coagulation tests performed within the external quality control.

Coagulation tests performed on the ACL 100 coagulometer (Plasma calibrants: RecombiPlasTin – Instrumentation Laboratory, ISI 1.2. Reagents: HemosIL PT Fibrinogen Recombinat – Instrumentation Laboratory) were performed. The performance of reagents and instruments used was evaluation by number of resultats, value of mean, standard deviation and coefficent of variation.

Coagulation tests performed on the Trombotimer – Behnk Elektronic (Plasma calibrants: Technoplastin His – Technoclone ISI 1.1. Reagents: Siron LS – Technoclone) were performed. The performance of reagents and instruments used was evaluation by number of resultats, value of mean, standard deviation, coefficent of variation and Z score.

Some coagulometers induce reporting errors (INR). For this reason, it is necessary to use the thromboplastins used in the calibration of the coagulometers, the equivalent quality of the international reference thromboplastin. This error may be controlled by assigning a System ISI- that is, a corrected ISI specific for the combination or System of coagulometer and thromboplastin reagent in local use (4). One approach to the calculation of the System ISI is to determine the prothrombin time, with the local coagulometer/thromboplastin combination, of a set of calibrant plasmas and to relate these values to those obtained manually with reference thromboplastins, by orthogonal regression (5).

Different types of calibrators have different calibrating systems or calculating interfaces and may not be valid for some reagent-instrument combinations because of inconsistency in the reporting of INR (6,7,8).

Figure 1 shows the INR of the plasmas obtained with reference thromboplastins. Panel A reveals the considerable variation in INR of the adsorbed plasmas. BCT 441 gave the tightest range and lowest values (2.05-3.95) whereas RBT 90 gave the widest range and highest values (2.2-9.1).

Panel B, in contrast, demonstrates the relatively good agreement in INR of the frozen coumarin plasmas shown by the thromboplastins used.[9]

The adsorbed plasmas have a calibrated value assigned following prothrombin time testing by the manual method, using reference thromboplastins, including WHO International Reference Preparations (IRP) and BCR Certified Reference.

Materials using IRP BCT 441 (human brain, plain) and RBT 90 (rabbit brain, plain). Coumarin plasmas were obtained from patients stabilised on oral anticoagulant therapy. RBT 1303, which had been calibrated previously against BCT 441 and RBT 90, was also used (9).

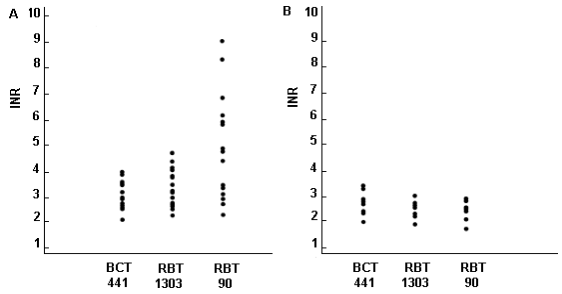

Figure 1: INR of the plasmas obtained with reference thromboplastins

However, the study results[10] of Hwa Jeen Lee suggest that the commercial local calibrators could provide more standardized INR results than the uncorrected INR after investigated the variability of INR values obtained through four INR determination methods in terms of six commercial thromboplastins in 30 validation plasmas from patients treated with warfarin anticoagulation therapy (Figure 2).

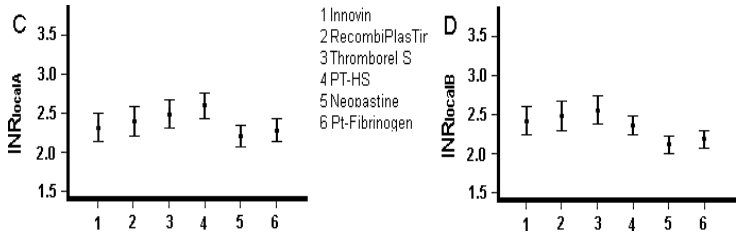

Figure 2: INR of the plasmas with warfarin anticoagulation therapy

Dispersion of the international normalized ratio (INR) in calibration plasma from patients receiving warfarin treatment (n = 30) among six different thromboplastins**. A**, INRWHO was obtained by ISIWHO calibrated by the World Health Organization (WHO) method**. B**, INR uncorrected was obtained by the manufacturer-informed international sensitivity index (ISI). **C**, INR localA was obtained by the ISI determined by the HemosIL ISI Calibrate (Instrumentation Laboratory, Lexington, MA). **D**, INR local B was obtained by the ISI determined by the PT-Multi Calibrator (Siemens Healthcare Diagnostics, Marburg, Germany). The value of the coefficient of variation (CV) represents total percent CV across six thromboplastins: A, 3.96%; B, 10.17%; C, 7.09%; and D, 7.80 (10).

Conclusions

1. The variation in INR adequately inflates the clotting tests was performed.
3. The international normalized ratio (INR)/international sensitivity index (ISI) system was introduced to standardize clotting tests variation with the use of different thromboplastin reagents.
4. Prothrombin time (PT), activated partial thromboplastin time (APTT) is widely used in monitoring of oral anticoagulant therapy, liver diseases, anesthesia, surgery.

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