The Value of Local ISI Calibration in Correcting the Variability in INR Determination

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Abstract : We have compared the International Normalized Ratio (INR) determination using the manufacturers' stated International Sensitivity Index (ISI) with an alternative method using local calibration of ISI with the calibrator plasma. It was found that the variability of the INR was less when the results were expressed by INR using local calibration of ISI. The results indicated that the local ISI calibration might reduce the variability in the INR determination.

เรื่องย่อ : ประโยชน์ของการหาค่า ISI ในห้องปฏิบัติการเพื่อแก้ไขความคลาดเคลื่อนในการรายงานค่า INR

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คณะผู้รายงานได้ทำการศึกษาเปรียบเทียบการรายงานผล INR โดยการคำนวณจากค่า ISI ที่ได้จากการกำหนดโดยบริษัทที่จำหน่ายน้ำยากับ INR ที่คำนวณจากการค่า ISI ที่หาในห้องปฏิบัติการ ผลการศึกษาพบว่าการรายงานผล INR โดยใช้ค่า ISI ที่ได้จากการหาในห้องปฏิบัติการมีความแตกต่างกันระหว่างกลุ่มที่ทำการศึกษาน้อยกว่า ดังนั้นการหาค่า ISI ในห้องปฏิบัติการอาจจะมีประโยชน์ในการรายงานผล INR

INTRODUCTION

The International Normalized Ratio or INR system was introduced to eliminate inter-laboratory differences in test results caused by the use of thromboplastins with different sensitivities.[1,2] This system defines the International Sensitivity Index (ISI) of a reagent in relation to a reference material. The INR is then calculated by raising the prothrombin time ratio to the power of an ISI. The ISI system was originally designed for manual tests and, in some cases, the ISI is influenced by the use of coagulometers.[3-8] One approach to correcting for the instrument effects on ISI is to perform a local calibration of the reagent/instrument combination.[9,10] Conventional WHO ISI calibration is based on 60 abnormal samples combined with 20 normal samples. This is clearly not a practical method for most laboratories because of the workload and the availability of the samples. Many

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laboratories are unable to determine the local ISI and tend to use the ISI issued by the manufacturers of the thromboplastin reagents with the consequent risk of inter-laboratory variability. The alternative use of lyophilized calibration plasmas for the local ISI calibration has been suggested in a few studies.[11-13]

In the present study, we study the variability in INR measured by two different thromboplastins and three different coagulometers and assess the usefulness of lyophilized calibration plasmas for local ISI calibration to reduce the variability.

MATERIALS AND METHODS

Plasma samples

Plasma samples from 20 healthy subjects and 5 patients treated with warfarin were used in the study. Patients were selected to span an INR interval of 1-4 based on the determination carried out with the thromboplastin routinely used in our laboratory (Thromborel S). The blood was collected in 3.2% sodium citrate. After centrifugation of the blood, the plasma was transferred to plastic tubes and stored at -70° C until use.

Design of the study

Three instruments and two thromboplastin reagents were used in the study. The two thromboplastin reagents were from the brain of a rabbit (Neoplastin C, Stago and Thromborel S, Dade Behring). The study comprised six groups of instrument-reagent combinations. Each group was asked to determine the prothrombin time in duplicate of warfarin plasma. In addition, each group provided a mean normal prothrombin time (MNPT) based on calculation of the geometric mean of 20 normal plasmas.

Each group participating in the study performed ISI calibration for each instrument-reagent specific using the INR calibrator plasma. Lyophilized INR calibrator plasmas from normal subjects and from patients on oral anticoagulant treatment were obtained from Dade Behring (PT calibration plasma kit). The INR values for PT calibration plasma determined by the Austrian external quality control program were 0.99, 1.99, 2.96 and 3.67. Plasmas were reconstituted and the calibration was performed according to the manufacturer's instructions.

Prothrombin time results were expressed as (a) INR using the instrument-specific ISI values supplied by the manufacturers (M-INR) and (b) locally calibrated INR with the use of calibrator plasma (C-INR).

RESULTS

1. Instrument-reagent combination

Six instrument-reagent combinations were included in the study, as shown in Table 1.

2. Local ISI calibration with calibrator plasma

The results of local ISI calibration in all groups except group 1 were different from the manufacturers' stated ISI. Table 2 shows the comparison of the manufacturers' stated ISI with the ISI values obtained from the local calibration with calibrator plasma.

3. Variability in M-INR and C-INR

The mean, standard deviation and coefficient of variation of M-INR and C-INR are shown in Table 3. The coefficient of variation for M-INR and C-INR in each plasma ranged from 1.12 % to 3.03 % and 1.1 % to 2.8 %, respectively. The coefficient of variation for C-INR is lower than that of M-INR in all plasma.

DISCUSSION

It has been emphasized that, after 30 years of attempts at standardization of the prothrombin time, there is still inter-laboratory variation in INR determination. Undoubtedly, one component of the variation is the presence of coagulometers, which can influence the thromboplastin reagent ISI in an unpredictable fashion.

This study demonstrated that the coagulometers have an effect on ISI of thromboplastins. In the six thromboplastin-instrument combinations that were studied, the ISI of the thromboplastin provided by the manufacturer was different from the values obtained by the calibrator plasma. Not only did they have different effects on the stated ISI of the thromboplastins; the coagulometer effects varied considerably with different instruments of the same make.

Table 1. Reagents and instruments used by the study group.

Group	Thromboplastin	Instrument
1	Thromborel S	Sysmex, CA 500
2	Thromborel S	Sysmex, CA 500
3	Neoplastin C	Sysmex, CA 500
4	Thromborel S	Sysmex, CA 50
5	Neoplastin C	Sysmex, CA 50
6	Neoplastin C	Stago

Table 2. Comparison of local calibration ISI values with manufacturers'stated ISI.

Group	Thromboplastin	Manufacturers' stated ISI	Local calibration ISI
1	Thromborel S	1.07	1.07
2	Thromborel S	1.07	1.03
3	Neoplastin C	1.26	1.0
4	Thromborel S	1.0	1.06
5	Neoplastin C	1.0	1.08
6	Neoplastin C	1.26	1.2

Table 3. Comparison of INR using the instrument-specific ISI values supplied by the manufacturers (M-INR) and locally calibrated INR with the use of calibrator plasma (C-INR) in 5 plasma determined by 6 study group.

	M-INR			C-INR		
	Mean	SD	CV(%)	Mean	SD	CV(%)
Plasma 1	1.12	0.08	1.12	1.1	0.09	1.1
Plasma 2	2.4	0.21	2.4	2.32	0.12	2.32
Plasma 3	2.65	0.24	2.65	2.47	0.15	2.47
Plasma 4	3.03	0.48	3.03	2.8	0.15	2.8
Plasma 5	2.33	0.19	2.33	2.27	0.12	2.27

The variable effect on ISI of instruments reduces the reliability of INR reported from different laboratories. It is suggested that local calibration of ISI will solve these problems. In this study, we compared the conventional INR estimation with the INR using local ISI calibration. It was found that the INR estimates obtained by local calibration of ISI agree closely with each other in all study groups as demonstrated by less CV in all plasma. These findings indicate that the effects of coagulometers on the ISI of thromboplastins may be corrected by local ISI calibration using calibrator plasma. The main advantage of local system ISI determination with the plasma calibrator over conventional thromboplastin

calibration is that it eliminates the need for parallel performance of the prothrombin time with a manual technique. In addition, an international reference preparation (IRP) for thromboplastin is not required. Finally, the plasma samples from a minimum of 60 long-term patients stabilized on warfarin within the therapeutic range required for conventional calibration are not needed.

However, we found that the difference between conventional INR estimation and INR using local ISI calibration is not significant. This may be due to a few varieties of plasma used in the study. Moreover, the thromboplastin that we used in the study is high-sensitivity reagent and the plasma was in the low INR range. It has been suggested that the coumarin plasma with the high INR demonstrated the relationship between high ISI and imprecision in prothrombin time testing.[14,15]

In conclusion, we found variability in INR determination from different study group and determined that local calibration of ISI with calibrator plasma may reduce this variability.

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