Significance of Local International Sensitivity Index Systems for Monitoring Warfarin and Liver Function

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Key Words: International normalized ratio; International sensitivity index; Local calibration; Commercial local calibrator; Liver disease

ABSTRACT

Objectives: Use of a local calibrator has been recommended for standardization of the international normalized ratio (INR) and international sensitivity index (ISI). We investigated the performance of two commercial local calibrators for warfarin monitoring and determined the significance of liver-specific INR.

Methods: ISI values were determined using the World Health Organization (WHO) method and two commercial local calibrators. Liver-specific ISI was determined using plasma samples from patients with liver cirrhosis and normal controls.

Results: In warfarin monitoring, the two local ISIs determined by the two local calibrators showed better consistency than uncorrected ISI, although they were inferior to the ISIs calibrated using the WHO method. Alternative calibration using calibration plasma from patients with liver cirrhosis instead of warfarinized plasma reduced the INR variability.

Conclusions: Local ISI determined by a commercial local calibrator improved INR standardization among thromboplastins. The alternative ISI calibration using liver-specific calibration plasma is expected to reduce INR variability for the evaluation of liver function.

Prothrombin time (PT) is widely used in monitoring warfarin, disseminated intravascular coagulation, and liver diseases.1,2 The international normalized ratio (INR)/international sensitivity index (ISI) system was introduced to standardize PT variation with the use of different thromboplastin reagents.3,4 The ISI of each thromboplastin represents a responsiveness to the reduction of coagulation factors.5 The ISI varies according to the type of coagulometer and thromboplastins used. Recently, some manufacturers have provided “instrument-specific” and “thromboplastin-specific” ISIs, which have been shown to provide insufficiently standardized INR values.5 Thus, determination of a local ISI for each combination of coagulometer and thromboplastin has been recommended for clinical laboratory practice.7

Several methods of ISI calibration have been developed.5 The World Health Organization (WHO) method for ISI calibration has not been feasible in most clinical laboratories.
because plasma samples from 60 anticoagulated patients and
20 healthy controls are required. The European Concerted
Action on Anticoagulation (ECAA) has recommended a sim-
plified WHO calibration method using 20 artificially depleted
lyophilized plasma samples and seven normal lyophilized
plasma samples with certified PT values. Direct INR was
also proposed, which requires more than three pooled plasma
samples and a normal pooled plasma sample, with certified
INR values. The establishment of a PT/INR line would
allow for easy determination of a patient’s INR values from
his or her measured PT value without the need to measure
ISI and mean normal prothrombin time (MNPT). Although
direct INR calibration methods may improve interlaboratory
variability, the implementation of direct INR in individual
laboratories has been restricted due to a lack of certified
plasma samples. Recently, commercially certified plasmas
for the determination of local ISI have been introduced, with
the advantages of simplifying procedures and improving INR
accuracy. The Clinical and Laboratory Standard Institute
(CLSI) has recommended verification or assignment of a local
ISI value to each combined thromboplastin/coagulometer sys-
tem using certified plasmas in clinical laboratories. However,
different commercial certified plasmas may induce different
ISI estimates, which may cause INR variation.

In addition to oral anticoagulant monitoring, INR has
been used to assess disease severity in other coagulopathies,
including liver disease. While PT prolongation in patients
treated with warfarin is a result of decreased factor II, VII,
IX, and X levels, PT prolongation in patients with liver
disease may be caused by deficiencies in all coagulation fac-
tors. Since the patterns of coagulation factor deficiency differ
according to the underlying disease, conventional ISI cal-
ibration using plasma from warfarin-treated patients may not
yield consistent INR values among various thromboplastins
as demonstrated previously. Since the INR of patients with
liver disease has been adopted in the model for the end-stage
liver disease (MELD) score, which is generally used to priori-
tize patients for liver transplantation, it is important to note
that inconsistency in INR determination could lead to serious
erroneous decisions, especially in recipient prioritization. Use
of calibration plasma samples from patients with liver disease
has been investigated as a method of reducing INR variation
according to different thromboplastins.

Although several commercial local calibrators have been
made available to clinical laboratories, there is limited infor-
mation regarding the consistency of local ISIs determined
using different commercial calibrators compared with the
WHO method. Therefore, to investigate INR variation for
warfarin monitoring, we determined local ISI values (ISIlocalA
and ISIlocalB) of six commonly used commercial thrombopa-
stins by using two local calibrators and compared them with
the reference ISI calculated by WHO calibration (ISIWHO) and
manufacturer-informed ISI (ISIuncorrected). In addition, we
determined an alternative ISI specific for liver disease (ISIliver)
and evaluated the applicability of liver-specific INR (INRliver)
to the MELD score in liver disease.

Materials and Methods

Study Population and Blood Samples

Blood was collected from 90 individuals on a stable war-
farin dosage (41 men and 49 women; mean age, 65 years; age
range, 32-90 years), 76 patients with liver cirrhosis (42 men and
34 women; mean age, 52 years; age range, 33-88 years), and
20 normal healthy controls (10 men and 10 women; mean age, 55
years; age range, 37-69 years). The patients on warfarin treat-
ment showed a stable INR range (1.5-4.5), and their clinical
conditions included prosthetic heart valves (n = 42), atrial fibril-
lation (n = 20), prosthetic heart valves with atrial fibrillation (n
= 15), and thromboembolism (n = 13). Liver cirrhosis was diag-
nosed on the basis of clinical, laboratory, or radiologic findings.
Exclusion criteria for cirrhotic patients were inherited bleeding
or thrombotic disorders, warfarin or heparin use within 7 days
of blood collection, or body weight less than 30 kg. The MELD
score, used for the evaluation of cirrhosis severity, was calcu-
lated as follows: MELD score = 10[0.957 ln(creatinine, mg/dL)
+ 0.378 ln(bilirubin, mg/dL) + 1.12 ln(INR) + 0.643]. Clinical
and demographic data were collected from medical records.
This study was approved by the Institutional Review Board of
Seoul National University Hospital.

Peripheral blood samples were collected in 3.2% sodium
citrate. Following centrifugation (1550g for 15 minutes) at
room temperature, plasma was separated into aliquots and
stored at –70°C until testing.

Reagents and Instruments

We used six commercial PT reagents Table I, including
Innovin (lot number 539189; Siemens Healthcare Diagnos-
tics, Marburg, Germany), RecombiPlasTin 2G (N0720740;
Instrumentation Laboratory, Lexington, MA), Thromborel S
(545480; Siemens Healthcare Diagnostics), PT-Fibrinogen HS
Plus (N1017745; Beckman Coulter, Miami, FL), Neoplastine
Cl Plus (107920; Diagnostica Stago, Asnieres, France), and
PT-Fibrinogen (N0429644; Beckman Coulter). We arbitrarily
used one lot (N0817013, ISI 1.03) of RecombiPlasTin 2G
(Instrumentation Laboratory) as a reference thromboplastin
to assign ISI values calibrated using the WHO method. Plasma
levels of coagulation factors II, V, VII, and X were determined
using a one-stage clotting assay (HemosIL; Instrumentation
Laboratory). All tests were performed on the ACL 3000 coag-
ulation analyzer (Beckman Coulter, Fullerton, CA). Bilirubin
and creatinine values were measured using either the Hitachi
7600 analyzer (Hitachi High-Technologies, Tokyo, Japan) or
Toshiba 200FR (Toshiba Medical Systems, Tokyo, Japan).

Table I
The ISI WHO values of six thromboplastins were determined using the WHO reference method.5 PT testing for each thromboplastin was performed using plasma samples from 60 warfarin-treated patients (24 men and 36 women; mean age, 65 years; age range, 32-86 years) and 20 healthy controls. A natural logarithm scaled plot compared the PT of reference thromboplastins on the vertical axis with the PT of the working thromboplastins on the horizontal axis. The ISI values of the working thromboplastins were defined as the slope of the orthogonal regression lines multiplied by the ISI of the reference thromboplastin. An acceptable coefficient of variation (CV) for the slope of calibration line was considered 3% or less.

Two local ISIs (ISIlocalA, ISIlocalB) were defined using two commercial calibration kits, including local calibrator A (lot number N0302097, HemosIL ISI Calibrate; Instrumentation Laboratory) and local calibrator B (lot number 41662, PT-Multi Calibrator; Siemens Healthcare Diagnostics). We arbitrarily defined a local ISI determined by local calibrator A as “ISIlocalA.” Local calibrator A comprised four INR-certified lyophilized plasmas. The PTs of calibrant plasmas were tested in duplicate over 3 days using the ACL 3000 according to the CLSI guideline.7 A log-scaled orthogonal regression line was generated by plotting certified INRs on the vertical axis, and the PTs were measured on the horizontal axis.5 From the slope and y-intercept of the calibration line, the local ISI and MNPT were derived as follows: ISI = 1/slope, and MNPT = 10y-intercept.21 We also determined “ISIlocalB” by using local calibrator B, which has a set of six pooled lyophilized plasmas with certified INR values according to the method above.5,7,21

### Table 1

<table>
<thead>
<tr>
<th>Thromboplastin</th>
<th>ISIWHO</th>
<th>ISIuncorrected</th>
<th>ISIlocalA</th>
<th>ISIlocalB</th>
<th>ISIliver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human recombinant</td>
<td>0.85</td>
<td>0.92</td>
<td>1.02</td>
<td>–16.67</td>
<td>0.91</td>
</tr>
<tr>
<td>Innovin</td>
<td>0.97</td>
<td>0.66</td>
<td>1.02</td>
<td>–4.90</td>
<td>0.99</td>
</tr>
<tr>
<td>RecombiPlasTin 2G</td>
<td>1.05</td>
<td>1.23</td>
<td>0.96</td>
<td>9.38</td>
<td>1.16</td>
</tr>
<tr>
<td>Human placenta</td>
<td>1.21</td>
<td>1.35</td>
<td>1.16</td>
<td>–12.93</td>
<td>1.10</td>
</tr>
<tr>
<td>Neoplastine Cl Plus</td>
<td>1.80</td>
<td>1.70</td>
<td>1.79</td>
<td>0.56</td>
<td>1.59</td>
</tr>
<tr>
<td>Rabbit brain</td>
<td>1.01</td>
<td>1.35</td>
<td>1.16</td>
<td>–12.93</td>
<td>1.10</td>
</tr>
<tr>
<td>PT-Fibrinogen HS Plus</td>
<td>1.21</td>
<td>1.40</td>
<td>1.29</td>
<td>–6.20</td>
<td>1.15</td>
</tr>
<tr>
<td>PT-Fibrinogen</td>
<td>1.80</td>
<td>1.70</td>
<td>1.79</td>
<td>0.56</td>
<td>1.59</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; ISI, international sensitivity index; PT, prothrombin time.

The ISI WHO values of six thromboplastins were determined using the WHO reference method.5 PT testing for each thromboplastin was performed using plasma samples from 60 warfarin-treated patients (24 men and 36 women; mean age, 65 years; age range, 32-86 years) and 20 healthy controls. A natural logarithm scaled plot compared the PT of reference thromboplastins on the vertical axis with the PT of the working thromboplastins on the horizontal axis. The ISI values of the working thromboplastins were defined as the slope of the orthogonal regression lines multiplied by the ISI of the reference thromboplastin. An acceptable coefficient of variation (CV) for the slope of calibration line was considered 3% or less.

### INR Performance for Warfarin Monitoring

#### Determination of ISIWHO

The ISIWHO values of six thromboplastins were determined using the WHO reference method.5 PT testing for each thromboplastin was performed using plasma samples from 60 warfarin-treated patients (24 men and 36 women; mean age, 65 years; age range, 32-86 years) and 20 healthy controls. A natural logarithm scaled plot compared the PT of reference thromboplastins on the vertical axis with the PT of the working thromboplastins on the horizontal axis. The ISI values of the working thromboplastins were defined as the slope of the orthogonal regression lines multiplied by the ISI of the reference thromboplastin. An acceptable coefficient of variation (CV) for the slope of calibration line was considered 3% or less.

#### Determination of Two Local ISIs (ISIlocalA, ISIlocalB)

Two local ISIs were defined using two commercial calibration kits, including local calibrator A (lot number N0302097, HemosIL ISI Calibrate; Instrumentation Laboratory) and local calibrator B (lot number 41662, PT-Multi Calibrator; Siemens Healthcare Diagnostics). We arbitrarily defined a local ISI determined by local calibrator A as “ISIlocalA.” Local calibrator A comprised four INR-certified lyophilized plasmas. The PTs of calibrant plasmas were tested in duplicate over 3 days using the ACL 3000 according to the CLSI guideline.7 A log-scaled orthogonal regression line was generated by plotting certified INRs on the vertical axis, and the PTs were measured on the horizontal axis.5 From the slope and y-intercept of the calibration line, the local ISI and MNPT were derived as follows: ISI = 1/slope, and MNPT = 10y-intercept.21 We also determined “ISIlocalB” by using local calibrator B, which has a set of six pooled lyophilized plasmas with certified INR values according to the method above.5,7,21

### Validation of INR Consistency for Warfarin Monitoring

To evaluate the INR consistency among the six thromboplastins, we used plasma from 30 warfarinized samples (17 men and 13 women; mean age, 64 years; age range, 39-90 years), which were different from plasma samples used for the ISI calibration. MNPT for each thromboplastin was determined by calculating the geometric mean of the PT from 20 normal healthy controls.14 Each INR was calculated from the assigned ISI and MNPT using the following equation: INR = (patient PT/MNPT)ISI.

### INR Performance for Evaluation of Liver Disease

#### Measurement of Coagulation Factor Levels

Plasma levels of coagulation factors II, V, VII, and X, as well as fibrinogen, were measured in 45 patients treated with oral anticoagulation therapy and 45 patients with liver cirrhosis.

#### Determination of Liver-Specific ISI (ISIliver)

ISIliver values of the six thromboplastins were determined using 60 samples from patients with liver disease (35 men and 25 women; mean age, 52 years; age range, 33-88 years) and 20 healthy controls according to the WHO reference method, with some modification.5 ISIliver of each thromboplastin was defined as the slope of the orthogonal regression lines multiplied by the ISI of the reference thromboplastin.
Validation of INR and MELD Consistency

Sixteen samples from patients with liver cirrhosis (seven men and nine women; mean age, 51 years; age range, 33-66 years), which were different from those used for ISI liver determination, were selected. The INR values (INR\textsubscript{uncorrected}, INR\textsubscript{WHO}, and INR\textsubscript{liver}) of the 16 patients were calculated from the assigned ISI values (ISI\textsubscript{uncorrected}, ISI\textsubscript{WHO}, and ISI\textsubscript{liver}) and MNPT for each thromboplastin. MELD scores were also calculated for the 16 patients.

Statistical Analysis

Statistical analysis was performed using MedCalc Statistical Software, version 12 (MedCalc Software, Mariakerke, Belgium) and SPSS, version 19 (SPSS, Chicago, IL). The variation in both ISI and INR values across six thromboplastins was estimated by calculating the percentage CV: \[\text{standard deviation/mean} \times 100\]. The differences in the INR or MELD score between calibration methods were investigated using an independent \(t\) test. The difference in coagulation factor levels between clinical conditions was determined using an independent \(t\) test. \(P\) values less than .05 were considered statistically significant.

Results

INR Performance for Warfarin Monitoring

Differences in ISI Values

ISI values were calculated using four different methods and six commercial thromboplastins (Table 1). The CV of the slope, representing the precision of calibration, was acceptable (<3%) in all cases. The ISI\textsubscript{uncorrected} values of two human recombinant thromboplastins—Innovin and RecombiPlasTin 2G—were higher than their corresponding ISI\textsubscript{WHO}. The ISI\textsubscript{uncorrected} values of rabbit brain–origin thromboplastins also tended to be higher than their corresponding ISI\textsubscript{WHO} and ISI\textsubscript{local} of Innovin and Thromborel were slightly higher than their corresponding ISI\textsubscript{WHO} while the ISI\textsubscript{local} of PT-Fibrinogen tended to be much lower than their corresponding ISI\textsubscript{WHO}. The differences in ISI\textsubscript{WHO} and ISI\textsubscript{local} of Innovin and Thromborel were significantly lower than their corresponding ISI\textsubscript{WHO}.

Differences in INR Values Across Six Thromboplastins

We 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. Table 2. The between-thromboplastin total CV of the INR was significantly higher in INR\textsubscript{uncorrected} (CV, 10.17) than in INR\textsubscript{WHO} (3.96), INR\textsubscript{localA} (7.09), or INR\textsubscript{localB} (7.80). Compared with INR\textsubscript{uncorrected}, the between-thromboplastin total CVs of commercial calibration kits (INR\textsubscript{localA} and INR\textsubscript{localB}) were significantly decreased. In human-type thromboplastins, the CVs of INR\textsubscript{localA} (4.38) and INR\textsubscript{localB} (3.87) were significantly decreased compared with those of INR\textsubscript{uncorrected} (13.07). However, in rabbit thromboplastins, only the CV of INR\textsubscript{localB} (5.93) was lower than that of INR\textsubscript{uncorrected} (6.93). Compared with INR\textsubscript{WHO}, the INR\textsubscript{uncorrected} showed considerably more dispersion of INR values across the six thromboplastins. The dispersion patterns tended to be reduced in INR\textsubscript{localA} and INR\textsubscript{localB} compared with INR\textsubscript{uncorrected}.

INR Performance for Evaluation of Liver Function

Differences in Coagulation Factor Levels in Terms of Clinical Conditions

Plasma levels of coagulation factors were investigated with regard to clinical conditions. Table 3. Fibrinogen level was lower in patients with liver disease than in those...
undergoing warfarin anticoagulation therapies. The levels of vitamin K–dependent coagulation factors (II, VII, and X) were markedly decreased in patients treated with warfarin anticoagulation therapy compared with patients with liver cirrhosis. However, the level of vitamin K–independent coagulation factor V was higher in patients undergoing warfarin anticoagulation therapy than in patients with liver cirrhosis.

**Determination of ISIliver**

When ISIliver values were determined, the CVs of the slope were acceptable (<3%) in the ISIliver of all six thromboplastins (Table 1). For Innovin, the ISIliver (0.99) was higher than the ISIWHO (0.85) but lower than the ISIuncorrected (1.02).

In the case of RecombiPlasTin 2G, there was no significant difference between the ISIliver and ISIuncorrected. In three rabbit brain thromboplastins, the ISIliver tended to be lower than the ISIuncorrected.

**Differences in INRs for Patients With Liver Cirrhosis**

The variability in INR values (INRuncorrected, INRWHO, and INRlocal) was investigated in 16 validation plasma samples from patients with liver cirrhosis (Table 4). The between-thromboplastin total CV of INRlocal (7.93) was lower than that of INRWHO (9.31) and INRuncorrected (11.51). When evaluating the INR variability according to the origin of the thromboplastin used, the between-thromboplastin

![Figure 1](image-url) 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, INRuncorrected was obtained by the manufacturer-informed international sensitivity index (ISI). C, INRlocalA was obtained by the ISI determined by the HemosIL ISI Calibrate (Instrumentation Laboratory, Lexington, MA). D, INRlocalB 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%. Open circles refer to mean values, and the bar limits represent the 95% confidence interval.
human CV of INR\textsubscript{liver} (5.61) was significantly lower than that of INR\textsubscript{uncorrected} (11.77), although the CV of INR\textsubscript{WHO} (5.46) was also low. The between-thromboplastin rabbit CV of INR\textsubscript{liver} (6.52) was shown to better standardize than that of INR\textsubscript{WHO} (9.26) and INR\textsubscript{uncorrected} (7.84). Figure 2 shows the dispersion of INR variation when INRs were applied to calibration plasma from patients with liver cirrhosis. Compared with the dispersion of INR\textsubscript{WHO} (Fig. 2A), INR\textsubscript{uncorrected} showed more dispersion of INR values across six thromboplastins (Figure 2B). However, INR\textsubscript{liver} showed greater standardization than both INR\textsubscript{WHO} and INR\textsubscript{uncorrected} (Figure 2C).

**Differences in MELD Scores in Patients With Liver Cirrhosis**

The MELD scores were also calculated from the INRs of six thromboplastins in 16 calibration plasma samples from patients with liver cirrhosis Table 5. The between-thromboplastin total CV of MELD\textsubscript{liver} (7.02) was significantly lower than that of MELD\textsubscript{uncorrected} (8.88) but similar to that of MELD\textsubscript{WHO} (7.01). The between-thromboplastin human CV of MELD\textsubscript{liver} (4.25) was markedly lower than that of MELD\textsubscript{uncorrected} (10.27). In addition, the between-thromboplastin rabbit CV of MELD\textsubscript{liver} (5.79) showed a similar quality of standardization as MELD\textsubscript{uncorrected} (5.68), although it was superior to that of MELD\textsubscript{WHO} (7.20).

### Table 3

**Plasma Levels of Coagulation Factors According to Clinical Condition**

<table>
<thead>
<tr>
<th>Coagulation Factor</th>
<th>Normal Controls (n = 20)</th>
<th>Warfarin Anticoagulation (n = 45)</th>
<th>Liver Disease (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>333.1 (40.4)</td>
<td>333.0 (81.6)</td>
<td>195.0 (76.1)(a)</td>
</tr>
<tr>
<td>Factor II, IU/dL</td>
<td>114.8 (7.7)(a)</td>
<td>23.1 (10.0)</td>
<td>34.2 (14.2)(a)</td>
</tr>
<tr>
<td>Factor V, IU/dL</td>
<td>123.4 (14.7)(a)</td>
<td>86.6 (21.1)</td>
<td>34.4 (20.4)(a)</td>
</tr>
<tr>
<td>Factor VII, IU/dL</td>
<td>99.6 (25.4)(a)</td>
<td>20.8 (11.1)</td>
<td>28.9 (19.7)(a)</td>
</tr>
<tr>
<td>Factor X, IU/dL</td>
<td>116.2 (20.5)(a)</td>
<td>15.5 (9.0)</td>
<td>41.1 (17.8)(a)</td>
</tr>
</tbody>
</table>

\(a\) P < .05 vs warfarin anticoagulation.

### Table 4

**Variability of INR Values Determined Using Three Calibration Methods and Six Commercial Thromboplastins in Patients With Liver Cirrhosis**

<table>
<thead>
<tr>
<th>Thromboplastin</th>
<th>INR\textsubscript{WHO}(b)</th>
<th>INR\textsubscript{uncorrected}(c)</th>
<th>INR\textsubscript{liver}(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Human CV(e)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innovin</td>
<td>2.08 (1.68-2.60)</td>
<td>2.42 (1.86-3.16)</td>
<td>2.36 (1.83-3.05)</td>
</tr>
<tr>
<td>RecombiPlasTin 2G</td>
<td>2.25 (1.83-2.77)</td>
<td>2.36 (1.90-2.93)</td>
<td>2.28 (1.85-2.81)</td>
</tr>
<tr>
<td>Thromborel S</td>
<td>2.09 (1.67-2.61)</td>
<td>1.96 (1.60-2.40)</td>
<td>2.25 (1.73-2.77)</td>
</tr>
<tr>
<td>% Rabbit CV</td>
<td>9.26</td>
<td>7.84</td>
<td>6.52</td>
</tr>
<tr>
<td>PT-Fibrinogen HS Plus</td>
<td>2.17 (1.81-2.53)</td>
<td>2.43 (1.98-2.89)</td>
<td>2.14 (1.79-2.48)</td>
</tr>
<tr>
<td>Neoplastine Cl Plus</td>
<td>2.48 (2.08-3.02)</td>
<td>2.64 (1.93-3.27)</td>
<td>2.22 (1.91-2.65)</td>
</tr>
<tr>
<td>PT-Fibrinogen</td>
<td>2.39 (1.81-3.20)</td>
<td>2.38 (1.81-3.18)</td>
<td>1.99 (1.61-2.52)</td>
</tr>
<tr>
<td>% Total CV(f)</td>
<td>9.31</td>
<td>11.51</td>
<td>7.93</td>
</tr>
</tbody>
</table>

\(e\) CV, coefficient of variation; INR, international normalized ratio; ISI, international sensitivity index.

\(\ast\) Values are presented as mean INR (range).

\(\ast\) INR value calculated by ISI\textsubscript{WHO}.

\(\ast\) INR value calculated by ISI\textsubscript{uncorrected}.

\(\ast\) INR value calculated by ISI\textsubscript{liver}.

\(\ast\) Coefficient of variation of INR values across three human thromboplastins.

\(\ast\) Coefficient of variation of INR values across all six thromboplastins.

### Discussion

Since INR has been designed to standardize PT values to monitor therapeutic responses to warfarin therapy, it is important to yield a consistent INR result worldwide.\(\ast\) However, it has been shown that a major inconsistency in INR results from local variations in ISI values determined using different thromboplastins and coagulometers. Even the same thromboplastin and coagulometer combination may show some difference in ISI value.\(\ast\) Hence, to improve INR consistency, local ISI calibration has been recommended by using a local calibrator set with certified PT/INR values that are traceable to an international standard.\(\ast\)

Since some commercial local calibrators have recently become available in clinical fields, there was a need to evaluate the performance of these commercial local calibrators. Our study demonstrated that local ISI values, calibrated by two commercial calibrators, improved INR standardization across six different thromboplastins compared with the uncorrected ISI provided by the manufacturer, although the local ISIs did not show better standardization than the ISI calculated by the WHO method. A previous study showed that local ISI calibration improved INR consistency.\(\ast\) However, the authors of that study used an ECAA calibrator, which is a local calibrator;
therefore, the performance of commercial local calibrators could not be estimated. Another recent study reported that use of commercial calibrator plasma may not be valid for some reagent-instrument combinations because of inconsistency with the uncorrected INR. However, our results suggest that the commercial local calibrators could provide more standardized INR results than the uncorrected INR.

In our study, local calibrator B generally showed better-harmonized INR values than local calibrator A in human thromboplastins, although both local calibrators were able

<table>
<thead>
<tr>
<th>Thromboplastin</th>
<th>MELD&lt;sub&gt;WHO&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>MELD&lt;sub&gt;uncorrected&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>MELD&lt;sub&gt;liver&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Human CV&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.48</td>
<td>10.27</td>
<td>4.25</td>
</tr>
<tr>
<td>Innovin</td>
<td>18.65 (3.53-31.41)</td>
<td>20.25 (5.40-33.11)</td>
<td>19.98 (5.07-32.84)</td>
</tr>
<tr>
<td>RecombiPlasTin 2G</td>
<td>19.54 (4.46-31.41)</td>
<td>20.03 (5.03-31.87)</td>
<td>19.66 (4.59-31.52)</td>
</tr>
<tr>
<td>Thromborel S</td>
<td>18.65 (3.67-30.48)</td>
<td>17.97 (2.87-29.83)</td>
<td>19.47 (4.77-32.75)</td>
</tr>
<tr>
<td>% Rabbit CV&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.20</td>
<td>5.68</td>
<td>5.79</td>
</tr>
<tr>
<td>PT-Fibrinogen HS Plus</td>
<td>19.19 (3.91-33.20)</td>
<td>20.41 (5.28-34.67)</td>
<td>19.02 (3.72-32.98)</td>
</tr>
<tr>
<td>Neoplastine Cl Plus</td>
<td>20.61 (5.24-34.94)</td>
<td>21.30 (6.01-35.79)</td>
<td>18.44 (3.91-33.51)</td>
</tr>
<tr>
<td>PT-Fibrinogen</td>
<td>20.05 (5.73-35.97)</td>
<td>19.98 (5.65-35.90)</td>
<td>18.14 (3.38-33.29)</td>
</tr>
<tr>
<td>% Total CV&lt;sup&gt;g&lt;/sup&gt;</td>
<td>7.01</td>
<td>8.88</td>
<td>7.02</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; MELD, model for end-stage liver disease; ISI, international sensitivity index.

<sup>a</sup> Values are presented as mean MELD score (range).

<sup>b</sup> MELD score calculated by ISI<sub>WHO</sub>.

<sup>c</sup> MELD score calculated by ISI<sub>uncorrected</sub>.

<sup>d</sup> MELD score calculated by ISI<sub>liver</sub>.

<sup>e</sup> Coefficient of variation of MELD scores across three human thromboplastins.

<sup>f</sup> Coefficient of variation of MELD scores across three rabbit thromboplastins.

<sup>g</sup> Coefficient of variation of MELD scores across all six thromboplastins.
to reduce the INR variability more than the uncorrected INR. As demonstrated in previous studies, different types of calibrators have different calibrating systems or calculating interfaces. Local calibrator A used four certified plasmas from the plasma of healthy donors or patients on stable warfarin therapy. The assigned INR value was in the range of 0.9 to 5.0. It was designed for HemosIL PT reagents (RecombiPlasTin 2G, PT-Fibrinogen HS Plus, and PT-Fibrinogen) on coagulometers from the Instrumentation Laboratory in conjunction with the ISI web software. Local calibrator B used six certified plasmas, which were derived from pooled plasma of healthy human donors and depleted pooled human plasma. The INR value, which was dependent on the PT reagent, was in the range of approximately 0.83 to 6.0. It was designed for Siemens reagents (Dade Innovin and Siemens Thromborel S) on coagulometers from Siemens. Therefore, further study is required to investigate the performance of commercial local calibrators with respect to their affiliated thromboplastins and coagulometers.

Although the INR is generally used to monitor warfarin therapy, it has also been applied to the MELD score to assist in the prioritization of patients for liver transplantation. INR showed a high imprecision and inaccuracy among the MELD components. As mentioned in previous studies, warfarin INR may be unsuitable for measuring coagulation status in patients with liver disease. For INR standardization in the evaluation of liver cirrhosis, it is important to understand the difference in coagulation factor levels in each clinical condition. Our study demonstrated that there was a large difference in coagulation factors between warfarin anticoagulation and liver cirrhosis (Table 3). As expected, vitamin K–dependent coagulation factors (II, VII, and X) were markedly decreased in the warfarin anticoagulation group, while a greater decrease in vitamin K–independent coagulation factors (fibrinogen and factor V) was observed in the liver cirrhosis group.

Since the thromboplastin reagents have different responsiveness to different levels of coagulation factors, we attempted to investigate an alternative calibration that was specific for liver cirrhosis. We found that INR$_{\text{liver}}$ was more consistent than INR$_{\text{uncorrected}}$, especially in human thromboplastin. However, in rabbit brain–derived thromboplastins, INR$_{\text{liver}}$ exhibited little improved consistency compared with INR$_{\text{uncorrected}}$. INR standardization could be improved using an alternative ISI specific for liver cirrhosis by using calibration plasmas from patients with liver cirrhosis. Consistent with our result, previous studies have reported that alternative calibration using plasma samples from patients with liver cirrhosis was able to resolve INR variability between thromboplastins.

This study has some limitations. First, we arbitrarily defined a commercial human recombinant thromboplastin (RecombiPlasTin 2G, lot number N0817013) instead of a WHO reference thromboplastin as a reference thromboplastin in the WHO calibration method. Since this study focused on the variability of INR results among six different thromboplastins, the absolute ISI value calibrated with the WHO calibration method was not important to evaluate consistency in our results. Nonetheless, use of the real WHO reference thromboplastin may be considered for future study. Second, “like-to-like” calibration should be performed. In other words, human-based thromboplastins should be locally calibrated using certified plasmas with PT/INR values that have been assigned with a human international reference preparation because species-specific artifacts may cause interference in the calibration procedure. However, since there was no available commercial local calibrator that included a rabbit-origin reference thromboplastin, we inevitably applied the local calibration of rabbit thromboplastins to two commercial local calibrators that had been assigned with a human reference thromboplastin. Therefore, our results tended to show relatively poor standardization of INR results for rabbit thromboplastins. In the future, it is expected that a commercial local calibrator assigned a rabbit-origin reference thromboplastin will be available. Third, this study focused only on INR consistency according to the thromboplastins, not the coagulometer, because one coagulometer was used. Future studies need to evaluate the effect of coagulometers on ISI calibration.

In summary, the local ISI values (ISI$_{\text{localA}}$ and ISI$_{\text{localB}}$) calculated using two local calibrators showed better consistency among six thromboplastins than the manufacturer-informed ISIs (ISI$_{\text{uncorrected}}$). However, the local ISI values did not show better consistency than the ISI calculated by the WHO method (ISI$_{\text{WHO}}$) for warfarin monitoring. The plasma coagulation factor levels in the liver cirrhosis group were significantly different from those in the warfarin anticoagulation group. The alternative calibration, using calibration plasma samples from patients with liver cirrhosis instead of calibration plasma samples from patients on warfarin anticoagulation therapy, could reduce the variability in INR and MELD scores in patients with liver cirrhosis.

In conclusion, INR is better standardized using the WHO calibration method and commercial local calibrators compared with the uncorrected manufacturer-informed INR. Considering that the WHO calibration method is impractical in terms of availability despite its excellent performance, clinical laboratories can obtain relatively reliable INR values by adapting the commercial calibrator, which can be simply applied with lower cost and labor. In addition, the alternative calibration using liver-specific calibration plasma samples may reduce INR variability in patients with liver disease. However, the alternative calibration method requires liver-specific calibration plasma samples and significant labor in a clinical laboratory. It is expected that commercial calibrators for alternative liver-specific calibration will be developed in the future.
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