technical note
Haematologica 1997; 82:630-633

*Advances in Basic, Laboratory and Clinical Aspects of Thromboembolic Diseases**

USE OF PLASMAS FROM DONORS UNDER ORAL ANTICOAGULANT TREATMENT FOR THE EXPRESSION OF INR VALUES

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Prothrombin time (PT) is used worldwide for monitoring oral anticoagulant treatment. In 1983, the INR (*International Normalized Ratio*) was introduced by the WHO[1] as a means of estimatating the PT in a reagent-free manner.

The use of the INR has clear benefits. It constitutes a significant advance towards standardization of PT, it allows for comparison of international clinical studies and therefore establishes international indications for therapeutical ranges and improves comparability from one laboratory to another.

Nevertheless, several problems are associated with INR. The *International Sensitivity Index* (ISI) has been shown to depend on the coagulometers used,[2,3] and the INR is influenced by the value taken for PT normal plasma, which can either be the PT in seconds of normal pooled plasma (NPP) or, in accordance with the recommendations set forth in 1994 by the ICTH, the *Mean Normal Prothrombin Time* (MNPT). The use of calibrated plasmas[4] can limit these drawbacks and allow for new INR calculation models.

Lyophilized plasmas are used as precision and accuracy controls.[5-7] Lyophilized plasmas with artificially reduced prothrombin complex factors can be used as precision control (*system control*) but show higher variation in INR values obtained with different thromboplastins (see ref. #8,9). Plasmas from donors under oral anticoagulant treatment (AK plasmas), however, can be used as precision and accuracy control in PT determinations. The establishment of a set of calibrated AK plasmas offers a new tool for the standardization of the INR and allows for better interlaboratory comparability.

Lyophilized AK plasmas

AK plasmas have low values of prothrombin complex factors and vitamin K-dependent inhibitors and also contain PIVKA proteins. Therefore, they tie in perfectly with the principle of equivalence (*like vs. like*).[10] AK plasmas are produced on a small scale by *RELAC* (The Netherlands) and the *UK Reference Laboratory for Anticoagulant Reagents and Control*. Commercially, they are available from *Immuno AG* (Austria). The production and quality requirements for lyophilized control plasmas in the normal range (see ref. #11-13) also apply to AK plasmas.

In the AK plasmas produced by Immuno, a large number of single plasma donations are pooled to obtain a distribution of the coagulation factors reflecting the patient's overall situation as closely as possible. The plasmas are obtained from donors under stable oral anticoagulation with an INR between 1.8 and 4.5.

AK plasmas are used in proficiency tests, which are part of the national external quality assessment programs (EQAS) in different countries. In Austria INR proficiency tests have been performed since 1988 by Öquasta, the *Austrian Society of Quality Assurance and Standardization of Diagnostic and Medical Investigations*. In the PT proficiency tests in Austria, we were able to prove that it is possible to assess participants by using different thromboplastins and instruments, and it is possible to determine target values from the proficiency test results by taking the mean value of valid data (after outlier elimination) from more than 200 laboratories.[14] The cooperation between EQAS institutions, manufacturers and laboratories is of mutual benefit (Figure 1).

Use of calibrated plasmas

Prothrombin time as the oldest coagulation test dates back to 1935 when it was introduced by R. Quick. Since the 1960's, it has been used for the control of oral anticoagulant treatment. The results are given differently, either as PT in seconds or as a ratio (patient plasma/normal plasma), or in percent of normal plasma. They are also influenced by the kind and quality of the thromboplastin used.

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**This paper was presented at the Second International Winter Meeting on Basic, Laboratory and Clinical Aspects of Thromboembolic Diseases, held in La Thuile, Italy, on March 17-23, 1996. The Meeting organizers (A. D'Angelo, A. Girolami & F. Piovella) have acted as Guest Editors and assumed the responsibility for peer review of this manuscript.*
Received July 22, 1997; accepted September 5, 1997.

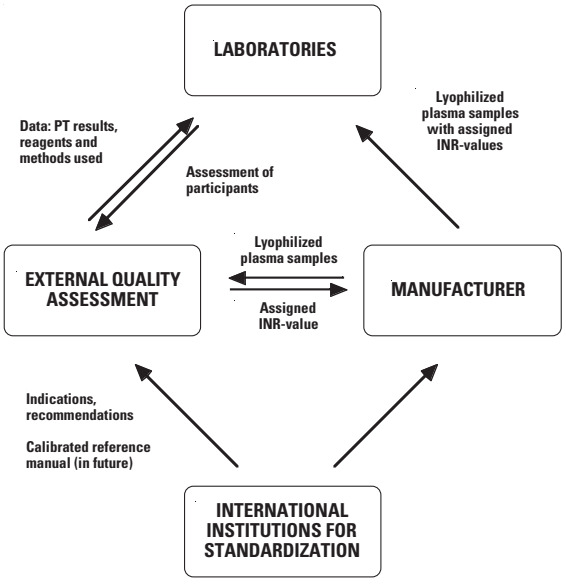

Figure 1. Cooperation between International Insitutions for Standardization, EQAS, laboratories and manufacturers.

Therefore, Quick himself wrote in 1957 that "*many of the adverse criticisms of the method can be traced to the use of insatisfactory thromboplastin reagents*".[15] Consequently, the introduction of the INR/ISI scheme by the WHO in 1983 meant a big step forward. Ten years later, in 1993, Houbouyan *et al*. proposed the use of calibrated plasmas,[3] and introduced this procedure in a French proficiency test together with a 2-point calibration curve.

Susequently, we proposed a set of four calibrated plasmas, one lyophilized NPP and three AK plasmas with INR target values that covered the whole therapeutical range.[16] The calibrator set can be used to draw an INR curve. In a log-log system a linear relationship exists between the INR and the PT value in seconds. The normal plasma value or the 100% value is given by the MNPT through a calibration procedure. The slope of the linear relationship corresponds to 1/ISI (Figure 2). According to this definition:

$$INR = \left\{\frac{PT_{pp}}{PT_{np}}\right\}^{ISI} \qquad \textit{(formula 1)}$$

(INR: International Normalized Ratio; ISI: International Sensitivity Index; PT: prothrombin time (in seconds); PP: patient plasma; NP: normal plasma)

the following mathematical transformation can be done:

$$\log(INR) = \log(PT_{pp}) \cdot ISI - \log(PT_{np}) \cdot ISI \qquad \textit{(formula 2)}$$

$$\log(PT_{pp}) = (1/ISI) \cdot \log(INR) + \log(PT_{np}) \qquad \textit{(formula 3)}$$

The INR reference curve can be drawn by plotting the mean values of the PT on double log paper. The ratio to MNPT or the INR is given on the X-axis; on the coordinate the coagulation time in seconds is given. The INRs of the patient plasmas can then be read off directly or calculated from the linear relationship according to *formula 3*.

This set of calibrated plasmas can also be used to determine the laboratory specific ISI value and the laboratory specific 100% value. The laboratory specific ISI can be calculated directly from the linear correlation between INR in seconds or by means of a graphical evaluation as given in Figure 3. For the graphical evaluation, a calibration curve is plotted; then a parallel line (the standardized straight line), is drawn through the point of intersection of the coordinates. The laboratory specific ISI value can then be read off on the ISI scale in the righthand margin.

For the PT normal plasma value the MNPT is widely used in accordance with ICTH recommendations.[17] The MNPT represents the geometric mean of the prothrombin times determined for 20 healthy persons. The relationship between the NPP and the MNPT is given by the *VDGH Reference Plasma,*[18] which has been calibrated against the MNPT in an international study by the IFCC.[19] For a lyophilized NPP, the deviation from the MNPT can be reported as Ratio A.

The use of calibrated plasmas and the different INR calculation models were assessed in a proficiency test by the *Deutsche Gesellschaft für Klinische Chemie*,

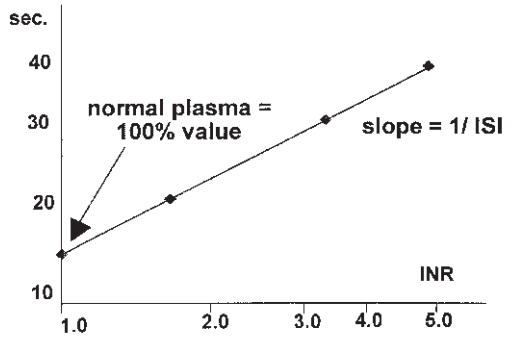

Figure 2. INR calibration curve: linear relationship between INR and PT (in seconds).

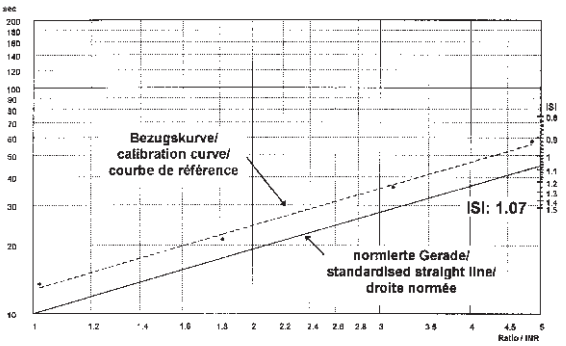

Figure 3. Graphical evaluation of laboratory-specific ISI value.

the *German Association of Clinical Chemistry* (DGKC).[20] The study was aimed at proving the reliability of the INR values by comparing identical AK plasmas in a proficiency test in Germany and in Austria, and at evaluating the application of calibrated plasmas as alternative INR calculation models.

In proficiency test 1/95 from the DGKC, five lyophilized plasma samples were used: one normal plasma and four AK plasmas within the INR range of 1.9 to 3.7. PT times were returned by 552 laboratories, 355 of which also reported the ISI values for the thromboplastin used (ISI pack insert). Table 1 shows the most widely used thromboplastins and instruments.

The INR values for all four AK plasmas were then calculated using the ISI pack insert and the statistical data are given in Table 2. An outlier elimination (according to the Öquasta 2-SD method, an iterative elimination of results outside the 2-SD range) was performed. The mean values are strongly affected by outlier elimination. The coefficient of variance (CV) is reduced from about 20% to 4-7%. The mean values are nearly identical in the proficiency test in Germany and in Austria.

Table 1. Reagents and instruments used by at least 10 laboratories in the DGKC Proficiency Test.

Code	*No. Labs*	*Reagent*	*Manufacturer*
R 1	20	Thromboplastin FS	Dade
R 2	54	Innovin	Dade
R 5	149	Thromborel S	Behring
R 7	15	Hepatoquick	Boehringer Mannheim
R 9	31	Neoplastin	Boehringer Mannheim
R12	33	PT-FIB Plus	Instrumentation Lab.
R16	10	Excel S	Organon
Code	*No. Labs*	*Instrument*	*Manufacturer*
I 1	131	KC 4/ KC 10 /KC 40	Amelung
I 2	14	Coag.Schnitger and Gross	Amelung
I 3	38	Electra 900 / 1000 / 1600	Dade
I 4	37	Fibrintimer	Behring
I 6	15	CL 4 / CL 8	Behnck-Elektronic
I 7	12	STA	Boehringer Mannheim
I 9	15	CA 1000, CA 5000	Sysmex / Toa
I11	45	ACL 100 / 200 / 300...	Instrumentation Lab.

Table 2. Statistical parameters of 4 AK-plasmas for all data and after outlier elimination

Plasma	*INR-Mean*	*CV*	*N*	*INR-Mean*	*CV*	*INR-Mean**
	all data		*after outlier elimination*			*Öquasta°*
A	1.93	16.4%	282	1.94	3.9%	1.99
B	3.61	23.2%	301	3.63	7.0%	3.67
C	2.90	20.2%	289	2.88	6.0%	2.96
D	3.17	21.0%	281	3.17	5.9%	3.15

**after outlier elimination. °Austrian Proficiency Test with about 200 participants (see ref. #10).*

Table 3. Data for plasma D with 3 different calculation models

Plasma	*INR-Mean*	*N*	*INR-Mean*	*CV*
	all data	*after outlier elimination*		
I	3.17	281	3.17	5.9%
II	3.31	320	3.24	3.3%
III	3.41	329	3.25	4.4%

In the next step, different INR calculation models were compared. Method I is the conventional INR calculation; for methods II and III, an INR curve was constructed using the pooled normal plasma and the AK plasmas A, C and D. Method II uses the laboratory specific ISI value and method III uses the direct conversion of prothrombin times to INR by means of the reference curve. The INR for plasma B was then calculated according to all three methods (results are given in Table 3). The INR mean values for all three calculation models remain within a narrow range, but using calibrated plasmas (methods II and III), less outliers were eliminated and the CVs obtained were smaller. These results show that problems inherent in INR determination can be reduced when calibrated AK plasmas are used for determining a laboratory-specific ISI value or when a laboratory-specific INR curve is applied.

The distribution patterns of INR values for all data and for the four most frequently used reagent/instrument combinations are given in Figure 4. The mean and median values of the total and individual collectives do not differ to a great extent; this shows that different thromboplastins and endpoint detection methods had no pronounced effect and that the variation of INR values can be reduced significantly when calibrated plasmas are used.

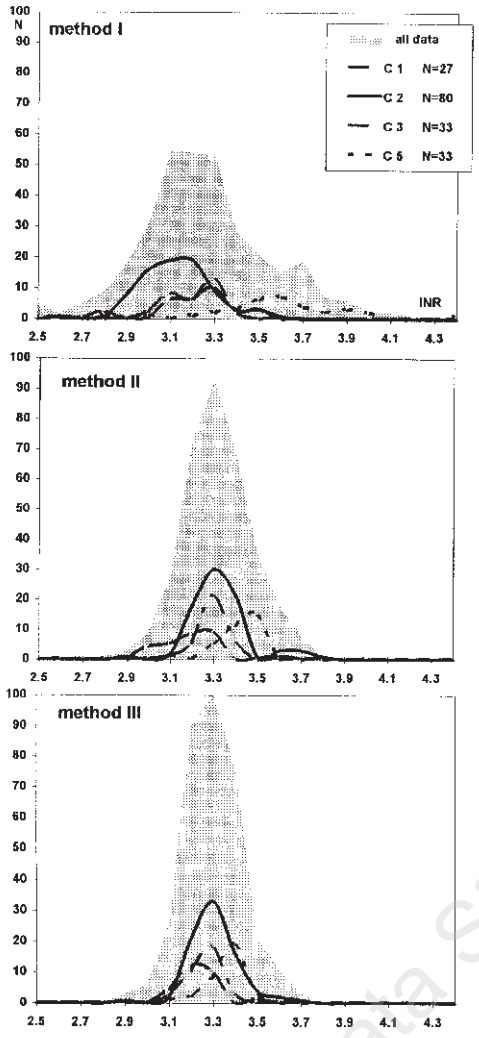

Figure 4. Distribution patterns of INR values for all data and for the four most frequently used reagent/instrument combinations.

Conclusions

Technical and methodological problems concerning the use of PT for monitoring oral anticoagulant treatment had already been discussed in the *First Winter Meeting on Basic, Laboratory and Clinical Aspects of Venous Thromboembolism*, held in Cortina d'Ampezzo, Italy, on March 9-12, 1994. The *Second Winter Meeting* provided the opportunity of defining some of these problems. We examined the use of plasmas from donors under oral anticoagulant treatment for the expression of INR values. Identical INR mean values obtained for AK plasmas in proficiency tests in Germany and Austria offer a novel tool for establishing a set of calibrated plasmas (*AK-Calibrant*). This considerably improves INR standardization and the comparability of results from different laboratories or from different instruments within one laboratory. This tool is therefore beneficial for patients under oral anticoagulant treatment.

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