Definition of the International Normalized Ratio (INR) and its consequences for the calibration procedure of thromboplastin preparations: a rebuttal

A.M.H.P. VAN DEN BESSELAAR, L. POLLER,* and A. TRIPODI†

*Haemostasis and Thrombosis Research Center, Department of Haematology, Leiden University Medical Center, Leiden, the Netherlands; *ECAA Central Facility, School of Biological Sciences, The University of Manchester, Manchester, UK; and †A Bianchi Bonomi Haemophilia and Thrombosis Center, IRCCS Maggiore Hospital, University of Milan, Milan, Italy*

Reliable determination of the International Normalized Ratio (INR) is mandatory for the control of oral anticoagulant therapy. Determination of the INR is based on a calibration model adopted by the WHO [1]. In a recent paper, Attermann argued that inaccuracy of the INR is due to faulty assumptions of the calibration model [2]. It should be realized that other factors are likely to influence INR reliability far more than faults with the established statistical method (Table 1). Here we would like to comment on Attermann's arguments.

In the WHO model, the international sensitivity index (ISI) plays a central role. The ISI of the first international reference preparation (IRP) 67/40 is 1.0 by definition. Attermann argued that the ISI of all other PT systems, including all secondary international standards, are not known but merely are estimated with inbuilt statistical error. In the WHO guidelines, INR is defined as follows: 'For a given plasma or whole blood specimen from a patient on long-term oral anticoagulant therapy, a value calculated from the prothrombin-time ratio using a prothrombin-time system with a known ISI according to the formula $INR = (PT/MNPT)^{ISI}$.' The word 'known' in this definition does not mean that there is no statistical uncertainty, but refers to the fact that the ISI estimate must be known in order to determine the INR. According to this definition, there is intrinsic uncertainty in the INR. INR therefore is not exact but an approximation that is sufficiently reliable in clinical terms. The above definition of INR is identical to the definition given by Kirkwood [3].

Attermann argued that the INR should be defined in a different way, namely as the PT ratio that would have been obtained if the same plasmas had been tested using the first

Correspondence: A. M. H. P. van den Besselaar, Haemostasis and Thrombosis Research Center, Department of Haematology, Leiden University Medical Center, Leiden, the Netherlands.
Tel.: +31 71 526 1894; fax: +31 71 526 6755; e-mail: a.m.h.p. van_den_besselaar@lumc.nl

IRP 67/40 with the manual tilt tube method. Attermann's alternative definition of INR cannot be used in daily practice because the first IRP 67/40 is no longer available. Furthermore, it should be realized that the first IRP 67/40 has never been used to find the optimal target intensities of anticoagulation in patients. Therapeutic ranges have been established by clinical trials using other thromboplastin reagents. These reagents were then linked to the INR scale by a series of ISI calibrations. The main purpose of the INR scale is to define therapeutic ranges. As the therapeutic ranges have been established with multiple reagents that are different from the first IRP 67/40, it is not appropriate to define the INR only in terms of the PT ratio that would have been obtained with the first IRP 67/40. IRP 67/40 had been established as a yardstick to compare the different reagents in terms of ISI which were used in clinical practice.

In the WHO calibration model it is assumed that the relation of normals follows the same relation as patients (i.e. coincident lines). In practice, this assumption is not always true. The WHO guidelines indicate that, if the deviation from the model is not greater than 10% in the INR range 2–4.5, the assignment of an ISI is acceptable. Multicenter studies have shown that the deviation from the model does not occur in all laboratories and is not the same in all laboratories. It seems that a deviation from the model depends on the local conditions or the person who performs the manual clotting time determinations. There is indication that the assumption of coincident lines does hold true for the present IRP in most of the calibrating laboratories [4,5]. Attermann's suggestion to describe all relationships between PT systems in terms of patients' clotting times only is therefore unwarranted and undesirable and would lead to other problems. The first problem is that all calibration relations must be recalculated from the present generation of reference thromboplastins back to IRP 67/40. This is not a simple linear chain of calibrations but shunts must be considered as well. For example, ISI calibration of rTF/95 has been the result of simultaneous comparisons with RBT/90, OBT/79, and BCT/253 [5]. If ISI calibration would be replaced by calibration based on blood from anticoagulated patients only, it is not clear how relations described by a slope and an

Table 1 Causes of erroneous INR

Preanalytical	1.	Sampling and blood collection problems
	2.	Evacuated tube effects
	3.	Sodium citrate concentration
	4.	Storage time
	5.	Storage temperature
	6.	Inadequate sample
Selection of patients and normals for ISI and MNPT determination	7.	Inadequate number of normals and patients
	8.	Unrepresentative selection of normals
	9.	Patients' test samples beyond treatment range
	10.	Poor distribution of patients' test samples across treatment range
	11.	Patients not yet stabilized on treatment
Analytical	12.	Incorrect choice of IRP
	13.	Between-operator variations in manual technique
	14.	Imprecision of PT determination
	15.	Instrument effects on PT and ISI
Statistical	16.	Non-use of geometric mean normal PT
	17.	Deviation from ISI calibration model
	18.	Non-use of orthogonal regression line

intercept for different pairs of thromboplastins can be combined. Furthermore, the imprecision of a slope based on patients' clotting times alone is much greater than the imprecision of a slope based on the clotting times of both patients and normals. The imprecision of the calculated INR probably would be much greater when it is based on patients-only relations rather than on combined patients plus normals relations. The establishment of standard thromboplastins for which the assumption of coincident lines does not hold, should be avoided, and this has been the practice in recent choice of new WHO successor IRP [5]. It should be acknowledged that in local calibration with lyophilized plasmas the rate of non-coincident lines is very high when the MNPT of fresh plasmas is combined with lyophilized abnormal plasmas [6]. This is a special case that can be explained by the different nature of the two types of plasmas and must not be generalized.

In the extended calibration model proposed by Attermann, a distinction is made between measurement errors and the 'linear error' or 'equation error' [7]. We agree that there are 'linear' errors that are due to interaction between the individual patient's factors and the PT system. We do not agree that 'underestimation of the linear error tends to result in an overestimation of the slope'. Overestimation of the slope by orthogonal regression would occur only if the equation error would be associated with the Y-measurement only. However, the equation error in relating prothrombin times measured

with two different systems cannot be associated with either one of the systems only. In other words, orthogonal regression seems to be the best model for estimating the relationship between the log (PT) determined with two systems with similar experimental error.

For the calibration of a secondary standard using individual fresh plasma or blood samples, it is recommended that patients' samples with INR values in the range 1.5–4.5 should be selected [1]. It is appropriate to exclude samples with INR outside the 1.5–4.5 range because these are likely derived from non-stabilized patients which would increase the imprecision of the ISI calibration. If the patients' samples are evaluated by INR calculated from measurements with the reference PT system on the vertical axis, samples with high INR tend to lie above the line and samples with low INR tend to lie below. Alternative procedures for selecting patient samples for ISI calibration should be explored in future studies.

Outlying data points are defined as points at a relatively large distance from the orthogonal regression line, e.g. at a distance greater than three standard deviations from the line. Some statisticians oppose the exclusion of outliers when there is no explanation for the outlier. We believe that gross outliers, even if there is no explanation available, should be deleted. Gross outliers may be caused by preanalytical or clerical errors and therefore bias the relationship between PT systems.

In summary, the different definition of INR proposed by Attermann leads to a different calibration model and different calibration equations. We have given arguments why the definition of INR stated in the WHO guidelines is more realistic and therefore should be maintained.

References

1 WHO Expert Committee on Biological Standardization. Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy. *WHO Tech Rep Series* 1999; **889**: 64–93.
2 Attermann J. Inaccuracy of international normalized ratio (INR) measurements and suggestions for improved WHO guidelines for calibration of reference preparations. A statistical study. *J Thromb Haemost* 2003; **1**: 537–44.
3 Kirkwood TBL. Calibration of reference thromboplastins and standardisation of the prothrombin time ratio. *Thromb Haemost* 1983; **49**: 238–44.
4 Hermans J, van den Besselaar AMHP, Loeliger EA, van der Velde EA. A collaborative calibration study of reference materials for thromboplastins. *Thromb Haemost* 1983; **50**: 712–7.
5 Tripodi A, Chantarangkul V, Negri B, Clerici M, Mannucci PM. International collaborative study for the calibration of a proposed reference preparation for thromboplastin, human recombinant, plain. *Thromb Haemost* 1998; **79**: 439–43.
6 Chantarangkul V, Tripodi A, Cesana BM, Mannucci PM. Calibration of local systems with lyophilized calibrant plasmas improves the inter-laboratory variability of the INR in the Italian external quality assessment scheme. *Thromb Haemost* 1999; **82**: 1621–6.
7 Carroll RJ, Rupert D. The use and misuse of orthogonal regression in linear errors-in-variables models. *Am Statist* 1996; **50**: 1–6.