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COMMENTARY

Time to integrate measurement uncertainty in method comparison and interpretation of hemostasis laboratory assays

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In the current issue of Research and Practice in Thrombosis and Haemostasis, Baker et al. [1] reported an important study for clinicians and laboratories managing patients with anti-Xa direct oral anticoagulants (DOACs) and requiring urgent procedures or surgery. They showed, using a limited number of apixaban (n = 70) and rivaroxaban (n = 59) samples from patients who participated in the PAUSE (Perioperative Anticoagulation Use for Surgery Evaluation) trial [2] that values obtained from 3 common instrument–reagent combinations: (a) Biophen reagents (Hyphen Biomed) with the BCS XP analyzer (Siemens Healthineers), (b) HemosIL reagents with the ACL TOP analyzer (Werfen), and (c) Stago reagents with the STA Compact MAX analyzer (Diagnostica Stago), correlate with each other (apixaban, r = 0.7271-0.9467, and rivaroxaban, r = 0.6531-0.9702). Although there are statistically significant differences between anti-Xa levels that resulted in discrepant classification across the 30 ng/mL threshold in about 8% of samples, the authors consider the differences as not clinically relevant.

While we tend to agree with the conclusion of the authors [3], we would like to stress that the results were predictable given the measurement uncertainty associated with any biomarker measurement in laboratory medicine and the differences in methodologies between the reagents (1- or 2-step, sample dilution, Xa origin and concentration, calibration [lack of a reference measurement system for anticoagulant drugs]) [4].

Therefore, we take the opportunity to summarize some key elements of the concept of measurement uncertainty in laboratory medicine as follows.

Diagnosis and monitoring of bleeding and thrombotic disorders depend on reliable hemostatic measurements of the components of the hemostatic system.

Levels of hemostasis factors vary between and within individuals as a result of genetic and environmental factors and analytical variation of the assays [5].

All laboratory measurements are accompanied by variability, including preanalytical variability, analytical variability (bias and imprecision), and intraindividual biological variation. Measurement uncertainty corresponds to the numerical range around the value returned by the test in which the true biological value lies. For assays

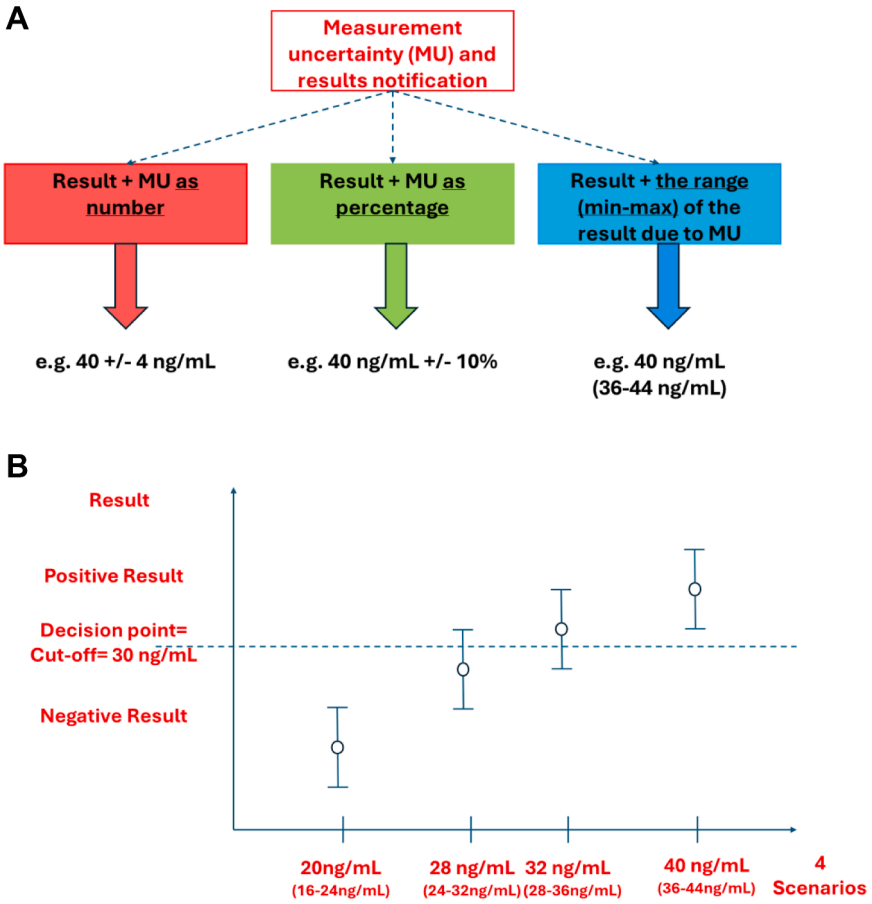

FIGURE The concept of measurement uncertainty. (A) Three different options to mention measurement uncertainty (MU) for apixaban level in medical reports. (B) MU for apixaban level is used to assess risk in relation to a decision threshold (30 ng/mL). The results are presented as the result + the range (min-max) of the result due to MU in 4 scenarios. The MU was provided in one author's lab for Liquid anti-Xa, STA R Max2, Diagnostica Stago.

for which aiding diagnosis is the intended use (those without clear medical decision points [cutoffs] such as levels of DOAC, von Willebrand factor, and coagulation factors, ie, fibrinogen [6]) or for monitoring disease and/or therapy (ie, A Disintegrin And Metalloprotease with ThromboSpondin type I repeats-13 [ADAMTS-13] activity), measurement uncertainty should be integrated into the conclusion of the medical report [7–9]. There are several methods to estimate measurement uncertainty, including one based on internal and external quality controls, which are thus easily available in each laboratory. For DOAC levels, which means, for example, with a measured level of 40 ng/mL, the true biological value lies between 30 and 50 ng/mL and should be interpreted accordingly (Figure).

Unfortunately, data in the literature are limited on measurement uncertainty and biological variation in the field of hemostasis [7,10], unlike clinical chemistry, despite an increase over the last 5 to 10 years, as such data is required for ISO15189 laboratory accreditation.

Research is therefore still needed to improve knowledge about measurement uncertainty and biological variation for their application in clinical practice. We recommend following the Biological Variation Data Critical Appraisal Checklist developed by the European Federation of clinical chemistry and Laboratory Medicine, which verifies whether publications have included all essential elements that may impact the veracity of associated biological variation estimates [11].

Finally, the authors compared the analytical performances of the 3 combinations against a fixed cutoff of 30 ng/mL. Even if this threshold is considered clinically relevant [2,12–15], it lacks validation in clinical trials, some of which are ongoing (for example, the Perioperative Anticoagulant Use for Surgery Evaluation [PAUSE] 2 pilot randomized trial [16]). The 30 ng/mL cutoff was originally proposed for dabigatran and rivaroxaban based on theoretical grounds: because plasma concentration decreases exponentially after time to peak drug concentration (T_{max}) discontinuing therapy is expected to lower the drug level to roughly 10% of the maximum concentration (C_{max}) value (theoretically 12.5%) after 3 elimination half-lives [17]. In addition, in a surgical setting, the perioperative bleeding risk appears to be more closely related to the value of minimum concentration (C_{min}) than to that of C_{max} [18]. However, as the residual DOAC level is only one of the elements determining the bleeding risk; a subanalysis of PAUSE found no significant association between preoperative DOAC levels (< 30 ng/mL, 30-50 ng/mL, or > 50 ng/mL) and perioperative major or nonmajor bleeding [19].

Moreover, these cutoffs do not reflect the different pharmacodynamic profile of DOACs. For example, thrombin generation assays have shown that a 30 ng/mL concentration produces different degrees of coagulation inhibition across the various DOACs [20].

Furthermore, Shaw and colleagues [21] have recently shown in a nested case-control study using samples from apixaban-/rivaroxaban-treated patients from the PAUSE perioperative study that low residual DOAC levels impact thrombin generation and suggest a causal contribution of thrombin generation parameters (lag time,

time-to-peak, peak, mean velocity rate index) and low DOAC levels toward bleeding. The association of low residual trough levels of anti-Xa direct anticoagulants with impaired thrombin generation was already reported [22].

In conclusion, one may wonder how such comparison studies of laboratory assays should be interpreted and used. Differences between assays are expected, but the reasons are difficult to elucidate experimentally. Measurement uncertainty is inherent to laboratory medicine and must be considered when interpreting the results, requiring laboratories to provide an estimate of the measurement uncertainty along with the result.

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AUTHOR CONTRIBUTIONS

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RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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