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Full Length Article

Explaining and reducing the variation in inter-laboratory reported values for International Normalised Ratio

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ABSTRACT

Introduction: Monitoring of vitamin K antagonist (VKA) therapy is usually achieved using the International Normalised Ratio (INR). However, despite international standardisation, there remains considerable concern regarding ongoing high levels of inter-laboratory variation, as generated by different laboratories using the same homogeneous plasma sample. Notably, significant discrepancies continue to be evidenced in external quality assessment (EQA) environments, prompting additional investigations to determine causes and to identify potential inconsistencies of practice.

Materials and methods: Several investigations involving all 580 participants in the Haemostasis program of the RCPAQAP Haematology were undertaken from 2009 to 2016, gathering details of methodology, and comparative assessments of INR values differentially obtained directly from participants versus values calculated using raw data for PT, ISI and MNPT provided by the same participants.

Results: Up to 6% of laboratories reported substantially different INR results compared to results calculated using differentially provided ISI, MNPT and PT data in 6 out of 8 surveys in 2009, highlighting discrepancies in ISI and MNPT values reported vs used by laboratories. Subsequent highlighting of issues to laboratories led to significant improvements in later surveys, with <1% of laboratories yielding different values in 2012, 2013 and 2016.

Conclusions: Our study identified that pre- or post- analytical errors explained a large proportion of inter-laboratory variation in INR. These errors can lead to serious clinical consequences if such data discrepancies are applied to patients, with incorrectly reported INRs potentially leading to altered warfarin therapy. Further education in the importance of the INR process appears warranted.

1. Introduction

Patients are routinely placed on anticoagulant therapy for a variety of indications, including prevention and treatment of thrombosis (e.g., deep vein thrombosis (DVT) and pulmonary embolism (PE); [1]. Despite the introduction of the newer direct oral anticoagulants (DOACs), Vitamin K antagonist (VKA) therapy (e.g. Warfarin) remains a significant long term therapeutic intervention for many individuals [1–6]. Monitoring of VKA therapy is nowadays usually achieved using the International Normalised Ratio (INR), a test first introduced in 1983 [7,8] to provide a process for the standardisation of the prothrombin time (PT) across different laboratories [2–6]. Historically, this was because of the very high variation of PT values across laboratories using different reagents and instruments, and the dangers to patients recognised by both under and over anticoagulation, based on inappropriate adjustments to VKA dosages as a result of the reported PTs [9]. The INR represents a 'simple' calculation, based on the patient's PT, 'adjusted' by means of 'correction factors' that reflect the different sensitivities of reagents/instruments to coagulation factors affected by VKAs. The two additional components involved are the International Sensitivity Index (ISI) and the Mean Normal Prothrombin Time (MNPT); thus, the INR = $(PT/MNPT)^{ISI}$ [6]. For good laboratory practice, laboratories are encouraged to verify each new lot number of thromboplastin reagent before use with their instrumentation in order to provide accurate INRs for patient management [10]. Such verification may also be encouraged by local regulations and accreditation processes.

Simplistically, the patient PT is now usually derived from an automated instrument using commercial reagents and thus represents an analytical event. With modern instrumentation and commercial reagents, intra-laboratory variation in PT for the same instrument/reagent is expected to be low, and generally <5%. The ISI is usually provided by

Abbreviations: PT, prothrombin time; INR, International Normalised Ratio; ISI, International Sensitivity Index; MNPT, Mean Normal Prothrombin Time; VKA, vitamin K antagonists; RCPAQAP, Royal College of Pathologists Quality Assurance Program.

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manufacturers of the reagent for select instruments (usually those also provided by the same manufacturer). The MNPT usually needs to be estimated by the laboratory. Expert guidance for generation and/or verification ISI and MNPT is available, and can involve different processes, some of which are onerous and not generally employed [3,6,10,11]. However generated and/or verified, both ISI and MNPT can be considered 'extra-analytical' variables that will influence the accuracy of any INR generated by a laboratory for similar 'analytical' PT values.

Pre-analytical issues are now generally known to comprise a large proportion of errors in haemostasis testing, although to our knowledge, this has not been considered substantially within the context of ISI and MNPT, and thus INR resulting. Most known pre-analytical issues arise due to sample collection (e.g., incorrect sample collected, such as wrong patient, tube or anticoagulant), transport (e.g., poor storage temperature) and processing (e.g., inappropriate centrifugation, storage temperature and time to perform the test) [12,13]. However, another important potential source of pre-analytical error for the INR is the incorrect assignment of the ISI and MNPT, due to incorrect values being determined or otherwise used by the laboratory. In addition, post-analytical errors may also be possible in this context, whereby the laboratory may report INRs using incorrect ISI and/or MNPT values (perhaps older values related to previous reagent lots, or simple typographical error).

Thus, although the introduction of the INR has certainly reduced between laboratory assay reporting, or differences in test results [9], permitting patients to have tests performed by different laboratories with better standardisation and greater assurance about the accuracy of anticoagulant monitoring, evidence remains that there exists ongoing higher than expected variation in inter-laboratory INR reporting, as generated using the same homogeneous plasma sample [14,15]. These differences in test results are still considered by many as being largely attributed to the many different reagent/instrument combinations available (i.e., analytical issues) [16,17]. However, the INR process theoretically takes these variations into consideration via the ISI and MNPT, and thus other factors must contribute to the high variation. In the current report, we highlight that a large proportion of current inter-laboratory variation in INR reporting is due to non-analytic issues, and namely the use of incorrect ISI and MNPT values in the calculation of the INR, which would arise either as a pre-analytical or post-analytical issue.

2. Materials and methods

The current report has utilised two separate but linked processes to identify potential sources of error related to INR reporting by laboratories. The first is data from our standard external quality assessment (EQA) process, employing homogeneous samples, thus alleviating the possibility of the more 'usual' pre-analytical events (collection and processing problems). The second process employed Questionnaires of laboratory practice, using numerical data from the same laboratories, to permit us to evaluate for potential non-concordance of data and thus non-analytical causes of error.

2.1. Questionnaires of practice

During the period of 2009 to 2016, a questionnaire was sent in four separate years (i.e., 2009, 2012, 2013 and 2016) to all 580 participants enrolled in the Haemostasis program of the Royal College of Pathologists of Australasia (RCPA) Quality Assurance Program (QAP) Haematology. The same questionnaire was included on all 8 separate occasions covering each of the individual ($n = 8$) haemostasis surveys ($n = 16$ samples) performed in 2009, and then subsequently repeated once each year in 2012, 2013 and 2016. One difference in the questionnaires between study periods, however, was that those in 2102 and 2013 included the INR formula ($INR = [PT/MNPT]^{ISI}$) for the laboratory to manually calculate their own INR, whereas the 2009 and 2016 surveys did not (i.e., laboratories were expected to know how the INR was calculated from the composite of the patient's PT, and the reagent/instrument related ISI and MNPT values). This was to determine if inclusion of this formula could identify improved performance by laboratories. Also, in the intervening period between 2010 and 2016, the RCPAQAP Haematology provided participants with information and educational material regarding the INR system. This was to determine if such education could also lead to improved performance by laboratories.

At its simplest, the questionnaire aimed to assess current methodologies in use by individual laboratories for performing the INR and its subsequent calculation. The questionnaires requested specific methodology details (including reagent-type, lot number of reagent and instrument used), as well as identification of the laboratory's current ISI and MNPT values at the time of each survey. Other details requested included how laboratories assigned or calculated their own ISI and MNPT values. Incomplete questionnaires, particularly those that did not provide ISI and MNPT values in use, were not included in any subsequent survey analysis. All methodology details and results were entered in an Excel spread sheet. Data was also analysed by Graphpad Prism and *t*-tests performed to highlight any significant differences in INR results.

2.2. Test samples

Samples provided for the haemostasis surveys are prepared as 1.0 mL lyophilized plasma samples, currently by a commercial manufacturer. Homogeneity and stability studies are performed prior to dispatch to ensure the integrity of the samples during postage. EQA participants receive 8 surveys per year, which include 2 samples per dispatch (for total of 16 samples per year). INRs for the generated plasma samples cover a wide range from 1.0 to ~4.5, thus including the therapeutic range for anticoagulant therapy, as well as 'under' and 'over' anti-coagulation. We have recently reported findings from our EQA program [14], which indicated inter-laboratory co-efficient of variation of 6–14%. This inter-laboratory co-efficient of variation was similar to that identified by another EQA provider [15]. In the current report, the intention was to identify what proportion of such variation could be attributed to non-analytical issues.

Only routine laboratory INR testing results from Haemostasis survey samples were included in this study; no point of care testing data is included.

2.3. Discrepancy in findings between participant-submitted INR result and RCPAQAP calculated INR

As indicated above, in this study, we compared two INR values obtained from the same laboratory for each survey where a questionnaire was included. One INR value was that which the laboratory provided directly to the RCPAQAP as part of their participation in an EQA process ('Submitted INR'). A second INR was generated from the data the same laboratory provided for PT, ISI and MNPT values, as separately submitted on the returned questionnaire forms ('Calculated INR'). Data as returned by participants on these questionnaires was electronically entered by RCPAQAP staff. We then compared the 'Submitted INR' values to the 'Calculated INR' values to identify any discrepancies in the two INR values. The premise here is that both INR values should be 'identical', since they both derived from the same laboratories for the same survey test results. However, some minor variation may occur due to truncation or rounding of numerical values used in the INR calculation (i.e., via instrument or laboratory information system for 'Submitted INR' vs spreadsheet calculation for 'Calculated INR'). Thus, for this report, a numerically 'significant' difference in INRs was arbitrarily considered to have occurred when the two INR values differed by ≥0.2 INR units. This value was chosen to ensure that we were not unfairly over-selecting errors due to potential differences in INR based on rounding effects. For example, the participant provided INR would have been calculated on instruments or in laboratory information systems using the