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Use of EDTA samples for prothrombin time measurement in patients receiving oral anticoagulants

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Background and Objectives. Oral anticoagulant therapy is commonly called for in health care. Hitherto sampling for prothrombin time (PT) has been measured on blood collected into a coagulation tube and diluted in citrate solution. Blood samples anticoagulated with EDTA are used for hematologic tests and the same sample could also be available for PT.

Design and Methods. We studied 107 patients on oral anticoagulant therapy. Samples were taken by from both coagulation tubes (citrate) and EDTA tubes. The PT from both samples was measured with an ACL 7000 analyzer and reported in seconds and as an international normalized ratio (INR). The regression equation between citrate and EDTA samples was calculated in both units. We studied the clinical significance of INR results from both sample types and compared the effect of different combined thromboplastin reagents on the correlation equation between citrate and EDTA samples.

Results. The regression equation for PT by Owren's PT reagent from citrate (y) and EDTA ($\times$) plasma was $y = 1.11\times - 0.24$ INR, $R^2 = 0.99$. We observed no clinically significant difference between INR results from citrate and EDTA samples using the regression equation for INR calculation from EDTA samples. ISI depends on sample type (dilution, anticoagulant) and the difference is 0.117, 10%. We calculated ISI for EDTA samples and no clinically significant difference was seen between citrate and EDTA INR results.

Interpretation and Conclusions. A good correlation was observed between INR results with citrate and EDTA samples from patients receiving oral anticoagulants using Owren's PT reagent with the same citrate calibration. Using the regression equation (INR or sec) for analysis of INR results from EDTA samples is clinically acceptable and offers the possibility of using EDTA samples for PT measurement with citrate calibrators. Reagent international sensitivity index (ISI) values for citrate and EDTA samples differ from each other. ISI determination for EDTA samples requires mathematical calculation of EDTA ISI as in the present study or EDTA-based ISI calibrators. The regression equation for INR from citrate and EDTA samples depended on the reagent used, not only on sample dilution or anticoagulant.

Key words: anticoagulants, EDTA, coagulation, INR, oral anticoagulant therapy.

Prothrombin time (PT) is the most commonly used coagulation test and has diverse clinical applications. The most marked expansion in its use is monitoring oral anticoagulant therapy. The need for tests is constantly increasing as the population ages and more coronary artery diseases are encountered. PT tests are needed at regular intervals because the therapeutic range is narrow and patients, mostly old, are in greater danger of bleeding. Over-medication adds to this danger and under-medication entails the possibility of thrombosis.

The PT test is most commonly applied using the *Quick method*, based on the technique described by Quick in 1935.[1,2] The *Owren method*[3] (combined thromboplastin reagent) was subsequently developed as a modification of the P & P[4] method and to overcome the drawbacks of the Quick method. Both methods are accepted for anticoagulant therapy control.

The International Normalized Ratio (INR) and the World Health Organization (WHO) recommendations using reference thromboplastins should harmonize PT results both in practice and in the literature.[5,6] The use of INR results is designed to eliminate dependence on the sensitivity of the

reagents, rendering recommendations for care uniform. The use of INR is not without difficulties and much remains to be done to achieve uniformity of results. Reagents and instruments do not behave in the same way.[7-11] The studies in question were done using the Quick method. The Owren and Quick methods do not agree particularly well without international sensitivity index (ISI) correction because of theoretical, methodologic and preanalytic differences.[12] Regardless of the reagent, instrument or method used, the results should be the same when using the INR system.

PT tests are usually performed on blood collected with citrate and this is usable only for coagulation measurements (9 parts blood and one part of citrate solution, 0.109 mol/L, 3.2%, or 0.129 mol/L, 3.8%).[10] Because EDTA is the anticoagulant for hematologic estimations, the use of EDTA plasma might also prove convenient for PT measurement. In one previous study good correlation was found between citrate and EDTA samples using the Owren method (Nycotest PT) with citrate plasma calibrators.[13] The EDTA sample is stable in a glass vacuum tube at room temperature for PT measurement within 6h.[14]

The aim of this study was to establish the clinical agreement of INR and results in seconds in EDTA and citrate samples from the same patients receiving oral anticoagulant therapy for PT measurement with combined thromboplastin reagent (Owren's PT) using citrate-based calibrators (only for INR) and a correlation equation to calculate INR and results in seconds for EDTA samples.

We attempted to ascertain the similarity of ISI values in the measurement of citrate and EDTA samples, because no manufactured EDTA-based ISI calibrators are available for PT. One hundred per cent normal pooled plasma (citrate and EDTA) from the same healthy people was used for PT normal sample estimation in seconds to eliminate differences in sampling, e.g. citrate dilution and anticoagulant.

The aim was to investigate the dependence of the correlation equation between INR results with citrate and EDTA samples on a combined thromboplastin reagent.

Materials and Methods

Patients and blood sampling

All procedures were approved by our institution's ethics committee in accordance with the Helsinki Declaration of 1975. We studied paired samples from 107 patient receiving oral anticoagulant therapy, chosen without conscious bias from hospital and health-center patients. PT was measured from both the coagulation tube (citrate) and the EDTA tube, and reported in seconds and as an INR. Measurement commenced within 2 hours of blood collection.

The citrate coagulation tube (Greiner Labortechnik GmbH, Vacuette cat. #454322, 9NC) contained 0.2 mL 0.109 mol/L (3.2%) citrate solution and a blood volume of 1.8 mL. The EDTA sample tube (Becton Dickinson, Vacutainer Cat. #367652) contained 0.072 mL K3EDTA, 75 g/L. Three milliliters of blood was drawn, thus providing 1.5 mg EDTA/mL blood. The final total volume was 2.34% EDTA solution and 97.66% blood.

The sample needle (Becton Dickinson, Precision Glide, Cat. #360213) was 0.8×38 mm.

Sample tubes were centrifuged at 1,560 g for 10 min. at 20°C to separate plasma.

Instruments and reagents

The ACL 7000 (Instrumentation Laboratory, IL, USA) is a fully automatic micro-centrifugal analyzer. We measured PT with an ACL analyser with a coagulation reagent (Owren's PT, cat. #GHI 131-10, ISI 1.22, rabbit brain thromboplastin) and $CaCl_2$ 25 mmoL/L (cat. #GHI 155) and a diluent, Owren's buffer (cat. #GHI 150) from the Global Hemostasis Institute (GHI), Sweden and a 10 uL sample, 50 uL diluent and 140 uL reagent. We used IL Test Reference (cat. #97569-00) as an instrument internal standard.

ISI calibration for GHI reagent was done by ISI calibrators (Etaloquick cat. #00496, Diagnostica Stago, France).

The ACL was calibrated with calibration plasma (cat. #08467300, IL) for the correlation study, n=4 at every point: 100%, 20.0 sec, CV= 0.00; 50%, 27.6 sec, CV= 0.00; 25% 38.8 sec, CV= 0.00, R2=0.994. The ACL was calibrated with normal citrate and normal EDTA plasma pooled from the same eight healthy people for the ISI (EDTA) study; 100% normal citrate plasma calibration was used for citrate PT and 100% EDTA plasma calibration for EDTA PT. ACL calibration is needed for percentages and INR units. ACL PT calibration data, normal citrate plasma, n=4 at every point: 100%, 19.0 sec, CV= 0.00; 50%, 25.8 sec, CV= 0.00; 25% 35.1 sec, CV= 0.33, R^2=0.990 and normal EDTA plasma, n=4 at every point: 100%, 19.9 sec, CV=0.58; 50%, 25.3 sec, CV= 0.45; 25% 33.8 sec, CV=0.59, R^2=0.996.

Within the same run (n=6) the CVs were: Owren's PT 0.4% (mean 21.2 s), 0.9 % (mean 35.3 s).

The CV of runs from day to day (n=14) was: Owren's PT 1.7% (mean 20.3 s).

Methods and statistics

In the correlation study we used Microsoft Excel software to calculate the linear fitness for INR and results in seconds with citrate and EDTA samples from the same patients using citrate-based calibrators (only for INR). Using a correlation equation ($y = 1.11\times - 0.24$ INR, $R^2 = 0.99$; $y = 1.10\times - 3.88$ sec, $R^2 = 0.99$) we changed EDTA results to citrate-result level (sec and INR) and compared sec and INR results for clinical acceptance[15] of both sample types.

The 100% normal pooled plasma (citrate and EDTA) from the same healthy people was used for PT calibration to study the effect of the anticoagulant and sample dilution used on the ISI. We calculated INR results from seconds using the formula:

$$INR = (sample_{sec}/normal_{sec})^{ISI}$$

For the calculation of ISI for EDTA samples, we used Microsoft Excel power function fitness between INR (citrate samples, ISI 1.216) and rate ($sample_{sec}/normal_{sec}$, EDTA samples).

To calculate results we used the Microsoft Excel 5.0 program to obtain the correlation functions and INR results and the Bland & Altmann method to compare clinical differences between results.[15]

Results

PT results with citrate samples were between 0.83 INR (17.2 sec) and 5.48 INR (81.0 sec). The regression equations for PT estimations with Owren's PT from citrate plasma (y) and EDTA plasma (×) were:

$y = 1.11\times - 0.24$ INR, $R^2 = 0.99$ (Figure 1)
$y = 1.10\times - 3.88$ sec, $R^2 = 0.99$

INR results from citrate and EDTA samples were not significantly different[15] using citrate calibrators and the correlation equation (INR) for PT measurement in EDTA plasma (mean difference 0.00 INR, 2 SD limits –0.14 to 0.14 INR, Figure 2). Only 4 of the107 results were out of range. The statistical t-test gave a *p* value of 1.0 and no significant difference.

Using the correlation equation for seconds ($y = 1.10\times - 3.88$ sec, $R^2 = 0.99$) to change EDTA seconds to citrate seconds had no clinical difference (mean difference 0.11 sec, 2 SD limits –1.79 to 2.01 sec; 4 of 107 out of range).

Using the 100% normal pooled plasma (citrate and EDTA) from the same healthy people for calibration we observed a power function correlation ($y = 0.98\times^{1.333}$, $R^2 = 1.00$) between citrate INR (y) and EDTA rates (×) ($sample_{sec}/normal_{sec}$). We noted a difference in ISI for citrate and EDTA samples. ISI for citrate samples was 1.216 and that for EDTA samples 1.333, a difference of 0.117 (10%). Calibrating measurements for PT (EDTA) using ISI 1.33 ($0.98\times^{1.333} \sim 1.0\times^{1.333}$) we compared the clinical significance of results between citrate and EDTA samples (mean difference -0.06 INR, 2 SD limits –0.20 to 0.08 INR). There were no clinical or statistically significant differences in INR results from citrate and EDTA samples ($p = 0.65$). Four of the 107 results were out of range.

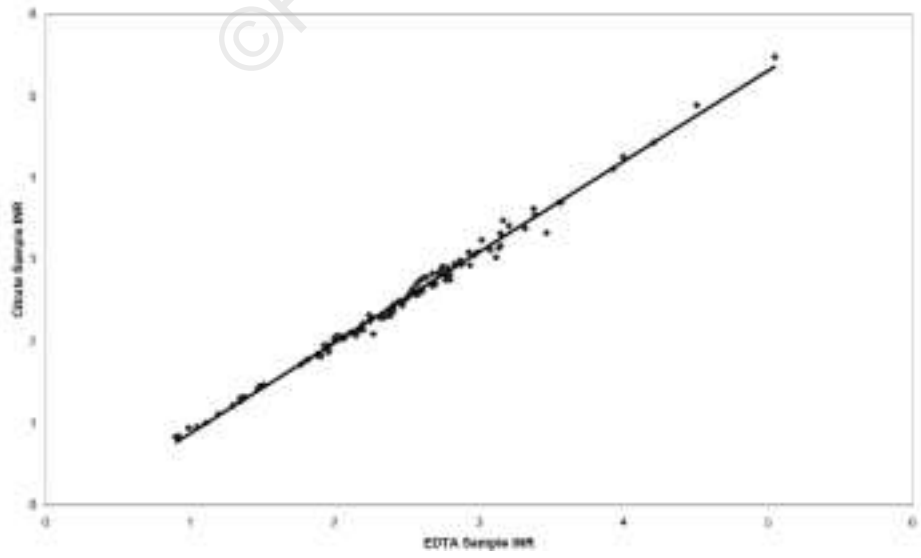

Figure 1. Correlation study of citrate and EDTA samples for PT using citrate calibrators, $y = 1.11\times - 0.24$, $R^2 = 0.99$.

Discussion

The strong correlation observed shows the possibility of using EDTA samples from patients receiving oral anticoagulants for the PT test and control of therapy. We found four different possibilities of using EDTA samples for PT measurement: 1) using a correlation equation in seconds ($y = 1.10\times - 3.88$ sec, $R^2 = 0.99$) and changing seconds in EDTA plasma to seconds in citrate plasma; 2) using a correlation equation in INR units ($y = 1.11\times - 0.24$ INR, $R^2 = 0.99$) and changing INR results in EDTA plasma to INR results in citrate plasma; 3) using normal EDTA plasma (100% = 1.0 INR) to estimate normal seconds for EDTA samples and calculating ISI (unlinear power function) for EDTA samples as in this study using, for example, 20 points; 4) preparing EDTA-based ISI calibrators for ISI calibration.

In this study we observed clinically acceptable INR results for EDTA samples using methods 1, 2 and 3. In cases 1 and 2 we need only a few points to estimate a linear regression equation and can use citrate calibrators. Data calculation is needed for instrumentation or automatic data handling.

Two differences noted between measurements with the same reagent were: the anticoagulant itself and the dilution of the sample with anticoagulant. The difference between results depends mainly on sample dilution by citrate solution (1:10), while the liquid EDTA dilutes the sample minimally (2.34%).

Using $INR = (sample_{sec}/normal_{sec})^{ISI}$ we need calibration for normal plasma (100%) measurement in seconds and for ISI estimation, which is related to reagent sensitivity. Ideally the calibrators for normal plasma and ISI calibration are sampled with the same anticoagulant and the same dilution as patient samples. The difference between normal citrate (19.0 sec) and EDTA (19.9 sec) plasma is small (5%). When PT is calibrated with normal EDTA plasma, differences in dilution and anticoagulant are eliminated. According to the findings here the ISI is not exactly the same for citrate (1.216) and EDTA (1.333) samples. The method used is a handy means of determining the EDTA ISI for a reagent.

In this study we used tubes in which EDTA was in liquid form and the dilution was 2.34%. Calibrators must have the same dilution as the samples. All ISI calibrators available from manufacturers are citrate-based and diluted with citrate 1:10. The same applies to the Etaloquick, Diagnostica Stago used in this study. We assume that the reason why the ISI is different for calculating citrate and EDTA INR results lies in the citrate dilution in the ISI calibrators.

In a previous study in which Nycotest PT reagent was used, the correlation equation between citrate (y) and EDTA (×) samples was different: $y = 1.20\times - 0.162$ INR, $R^2 = 0.99$.[13] Although there is a high correlation between reagents Nycotest PT (×), Owren's PT (y), INR, $y = 1.08\times - 0.15$ ($R^2 = 0.99$),[12] they may behave separately in correlation between citrate and EDTA samples. We may draw the conclusion that the correlation equation between citrate and EDTA samples is also dependent on the

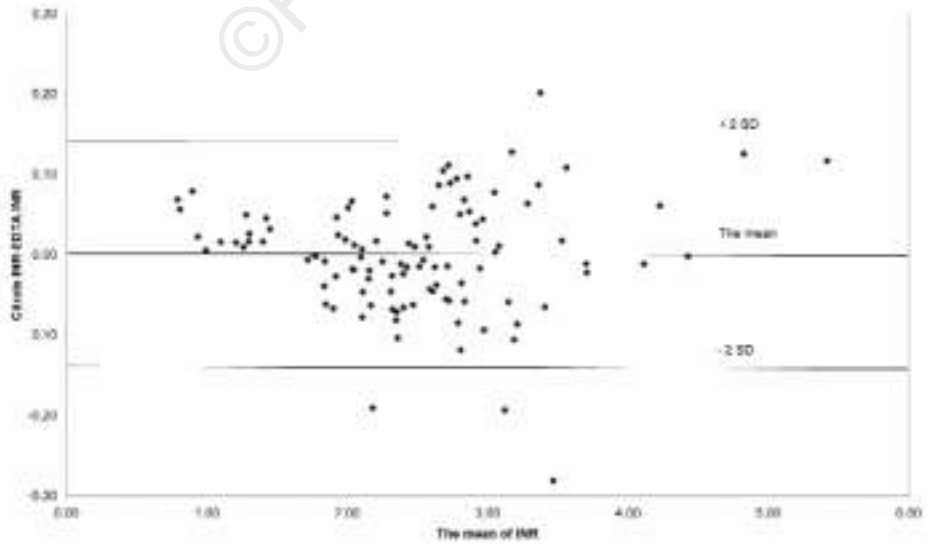

Figure 2. Difference between citrate and EDTA INR results.

reagent and is not the same for different reagents.

There is no calibration for results in seconds; the equation for Owren's PT is, y = 1.10× –3.88 sec, R^2=0.99. In a previous study with Nycotest PT reagent the correlation equation in seconds was: y = 1.17× – 2.37, R^2 = 1.00.[13]

It would be an advantage to be able to use an EDTA tube for only coagulation assays and for hematologic and coagulation assays. After hematologic estimations have been made, the sample could be centrifuged to obtain plasma for PT measurement, but would still be subsequently available for hematologic estimations. This procedure has many advantages: only one type of vacuum tube is needed; one sample tube affords new possibilities for development in automation (hematology + coagulation), sampling is faster in one tube, and material and waste costs are lower, thus saving natural materials. The citrate dilution is a source of error if the dilution rate is not appropriate, for example in an under-filled vacuum tube.

Correlation functions and a mathematical equation are now available for analyzing PT in EDTA plasma with Owren's PT and Nycotest PT, but the use of EDTA-based normal plasma and ISI calibrators would improve calibration for the normal situation and facilitate the use of EDTA samples for PT measurement.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Armando d'Angelo, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Prof. D'Angelo and the Editors. Manuscript received May 15, 2000; accepted June 27, 2001.

Potential implications for clinical practice

Our study shows that it is possible to use an EDTA tube for both coagulation and hematologic assays, saving time and resources.

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