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ORIGINAL ARTICLE

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Evaluation of a new thromboplastin reagent STA-NeoPTimal on a STA R Max analyzer for the measurement of prothrombin time, international normalized ratio and extrinsic factor levels

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STA®—Neoplastine® CI+, STA®—Neoplastine® R and STA®—NeoPTimal were provided by Stago Diagnostica.

Abstract

Introduction: We aimed at evaluating the performance of a new prothrombin time (PT) reagent (STA-NeoPTimal) with two other PT reagents (STA-Neoplastine R and STA-Neoplastine CI Plus) and the reference PT reagent used in our laboratory (ReadiPlasTin).

Methods: Evaluation consisted in intra- and interassay precision assessment, determination of sensitivity to unfractionated heparin (UFH) or enoxaparin in spiked samples and to direct oral anticoagulants (DOACs) in patients (n = 43). Method comparison of the 4 PT reagents, factor II, V, VII and X assays was tested on normal (n = 20) and abnormal samples: VKA (n = 47), preoperative (n = 23), liver failure (n = 12) and burned patients (n = 37).

Results: Analytical performance met manufacturers' criteria for all reagents. All PT reagents gave correlation coefficients >0.8 and even >0.9 in many situations. In some VKA samples, differences ≥ 0.5 INR units were found in samples within and above therapeutic ranges. For burned patients, PT correlations were good but with some minimal bias (<5.0%) while factor assays gave very consistent results (R > .8 and mainly >0.9). As expected, poor responsiveness of the PT to DOAC concentrations was observed with all four assays.

Conclusion: The STA-NeoPTimal showed comparable performance to ReadiPlasTin, making it suitable for VKA control, detection of factors II, V, VII, X deficiency and assessment of liver disease coagulopathy. However, for patients receiving VKA, some significant differences were observed. We confirmed the inability of the PT assay to detect residual DOAC concentrations. Finally, burned patients results showed that recombinant thromboplastins were less sensitive to factor deficiencies in comparison to extraction thromboplastins.

KEYWORDS

apixaban, burned, preoperative, rivaroxaban, thromboplastin

1 | INTRODUCTION

The prothrombin time (PT) is widely used to monitor vitamin K antagonists (VKA) and screen for defects of the extrinsic coagulation pathway.

In addition, in the absence of specific assays and even if PT is not reliable to assess direct oral anticoagulants (DOAC) concentration, PT is sometimes used to estimate presence DOACs. Among the DOACs, rivaroxaban shows the strongest effect on PT, followed by edoxaban and then apixaban, although different PT reagents show variations in their sensitivities toward these drugs.[1]

Despite the fact that International Normalized Ratio (INR) was introduced to reduce the interlaboratory variability, this variability is still clinically significant (>10%).[2] This latter is explained by the variations between instruments and by reagents composition: recombinant human thromboplastins and tissue-extract thromboplastins do not have the same sensitivity to Vitamin K-dependent factors and factor V deficiencies [2,3] due to differences in phospholipid composition[4] and presence of traces of factor VIIa.[5]

The reagent STA-NeoPTimal is a new rabbit brain extraction thromboplastin with International Sensitivity Index (ISI) close to 1.0, recently launched by Diagnostica Stago.

The aim of this study was to compare the performance of this new reagent with three other PT reagents: ReadiPlasTin (Werfen), STA-Neoplastine R and STA-Neoplastine CI+ (Diagnostica Stago) on a STA R Max2 analyzer (Diagnostica Stago). ReadiPlasTin was considered as the reference reagent as it was routinely used in our laboratory at the beginning of the study.

2 | MATERIALS AND METHODS

2.1 | Study population

Plasma samples were obtained from patients addressed to the laboratory of the CHU UCL Namur site Godinne, Yvoir, Belgium. Residual anonymized samples were collected after completion of the routine testing. Samples from the DOAC population and the burned population were provided by the laboratory plasma banks (CHU UCL Namur (Namur) and Queen Astrid military hospital (Brussels)).

This study was approved by the Ethical Committee of the CHU UCL Namur (site Godinne) (Number: BE039201940772).

A total of 164 plasmas from 149 patients were enrolled in the study: 23 patients were addressed to the lab for preoperative blood tests, 47 patients were treated with VKA, 45 patients were treated with DOACs, 12 patients had liver failure and 37 plasmas came from 22 burned patients. Twelve patients were severely burned patients (second and third-degree burns with a total burn surface area from 0.5% to 50%). Two of them had severe dermatological disorders (Fournier gangrene and cutaneous sarcoidosis). Eight samples were available from a patient that had a total burn surface area of 50%.

The liver failure population was defined by aspartate aminotransferase/alanine aminotransferase levels >2 times the upper limit of normal.

To evaluate the sensitivity of the thromboplastins to heparin, pooled normal plasma was supplemented with different concentrations of unfractionated heparin (UFH, Heparin Leo 5000 UI/mL) or low molecular weight heparin (LMWH, enoxaparine (Clexane) 10 000 UI/mL). Heparin was diluted in saline prior to spiking the diluted heparin in plasma (1/20 final dilution in plasma). Final concentrations obtained were 0-0.52-0.87-1.16-1.31 for UFH and 0-0.45-0.97-1.5 and 1.81 for LMWH.

Lyophilized quality controls (STA-System Control N and P from Diagnostica Stago) were used for intrarun and inter-run precision assessment, according to ISO 5725 and SH-GTA-04 recommendations. Intra-run precision assessment was performed by running the 2 levels of quality controls 20 times during 1 day. Inter-run precision assessment was performed by running the 2 levels of quality controls on 20 different nonconsecutive days.

2.2 | Sample analysis

Blood samples were collected in Vacuette® tubes (Greiner, Austria) containing 1 volume of 3.2% citrate per 9 volumes of blood, which were then centrifuged at 1500 g for 15 minutes to separate plasma. Fresh plasma samples were used for the study tests except for the DOAC and burned populations for which frozen plasma samples stored at −80°C were used.

All the samples testing were processed on STAR Max 2 coagulation analyzer (Diagnostica Stago).

For all samples, PT was expressed in seconds, % and INR. Depending on the population, one or the other unit could be used.[6] When necessary, PT ratio was calculated (patient clotting time divided by the Mean Normal Prothrombin Time (MNPT)). Extrinsic factors levels were expressed in %.

2.3 | Reagents

Four commercially thromboplastin reagents were compared: HemosIL ReadiPlasTin (Instrument Laboratory Company), STA®—Neoplastine® CI+, STA®—Neoplastine® R and STA®—NeoPTimal (Diagnostica Stago) for PT/INR and extrinsic factor (factor II, V, VII, X) levels measurements.

HemosIL ReadiPlasTin and STA®—Neoplastine® R are prepared from human recombinant tissue factor. STA®—Neoplastine® CI+ and STA®—NeoPTimal are prepared from fresh rabbit cerebral tissues.

For factor assays, all the thromboplastins were tested using the same deficient plasma for each extrinsic factor (STA-Deficient from Diagnostica Stago) except for factor II/STA-Neoplastine R for which an immunodepleted factor is necessary (STA-Immunodef II). The STA-Unicalibrator (Diagnostica Stago) was used as calibrator.

The INR for Stago thromboplastins were reported using the ISI from the manufacturer. Stago determines the ISI of each of its thromboplastins on at least 120 fresh samples (with a ratio of 1:3 between blood samples from healthy subjects and blood samples from patients who have been on oral anticoagulants for at least 6 weeks with INR values in the range 1.5-4.5) in comparison to its Internal MasterLot. This Internal MasterLot is calibrated against the international standard: RBT/ 16 for STA-NeoPTimal and STA-Neoplastine CI Plus, and *r*TF/09 for STA-Neoplastine R.

In addition, the Relative Uncertainty of the ISI was calculated in accordance with:

- ISO 5725-2," Accuracy (trueness and precision) of measurement methods and results"- Part 2: "Basic method for the determination of repeatability and reproducibility of a standard measurement method", - GUM (NF ENV 13005) "Guide to the expression of Uncertainty in Measurement". The uncertainties include the contribution of Internal Master Lot and intermediate precision of the method. The uncertainty on ISI for STA-NeoPTimal is ±5.4% which is also acceptable. For that reason, Stago does not recommend their users to redetermine their ISI locally.

For ReadiPlasTin, we have determined ISI of ReadiPlasTin on the STAR Max 2 by processing 20 normal controls, and 60 aVK plasmas with INR between 1.5 and 4.5 on STAR Max 2 and an ACL TOP instrument and defining the ISI as the multiplication product of the ReadiPlastin ACL TOP ISI by the slope of the bilogarithmic orthogonal regression line of clotting times. The system ReadiPlasTin/ACL TOP was therefore considered as secondary reference material since the ISI of this reagent was confirmed by the use of HemosIL® INR Validate on the ACL TOP instrument.

For PT% results, calibrations provided by the manufacturer were used, after adjusting it to the locally determined MnPT.

An ecarin-based chromogenic assay, STA®—ECAII (Diagnostica Stago) combined to dedicated STA®—Dabigatran calibrator was used for the determination of dabigatran levels. DOAC-calibrated chromogenic assays, STA®—Liquid Anti-Xa (Diagnostica Stago) combined to dedicated STA®—Apixaban, STA®—Rivaroxaban, and STA®—Edoxaban calibrators, were used to determine the levels of apixaban, rivaroxaban, and edoxaban.

2.4 | Statistical analysis

The MnPT for each of the thromboplastins was determined from 20 healthy subjects using the geometric mean. The PT% calibration curve was adjusted with the locally determined MnPT for each thromboplastin through manual entry of the obtained data onto the STA R Max^2 software. Intra-assay and interassay precision was assessed through mean, standard deviation, and coefficient of variation computations.

The prespecified within run and between run precision acceptance criteria were set at 3% and 5%, respectively, for PT (%) assessment, and 8% and 15%, respectively, for extrinsic factor levels assessment, according to the "Groupe d'Aide à l'Accréditation des Laboratoires» (unpublished data). For PT (sec) and INR assessment, the acceptance criteria were set at 3.6% and 5.0%, respectively, according to the "Groupe d'étude sur l'hémostase et la thrombose" criteria (https://site.geht.org/), a group of the French Society of Hematology (SFH).

Sensitivity to UFH and LMWH was determined through calculation of relative differences of PT results (seconds) between the pooled normal plasma spiked with heparin and the unspiked pooled normal plasma. The difference was arbitrarily considered as significant if it exceeded 10% (equal to twice the interassay reproducibility (5%)). In addition, the reference change value (RCV) was calculated as follows: $RCV = 2^{1/2} \times Z \times (CVa^2 + CVi^2)^{1/2}$, where Z is 1.96 (representing the probability of 95%), CVa the analytical coefficient of variation (reproducibility) and CVi the intraindividual coefficient of variation which was set at 4%, according to the Westgard database.[7] The RCV was used to confirm the cutoff of PT ratio (ie, 1.2).

Method comparisons were performed using Passing-Bablok regressions and Bland and Altman differences plot according to CLSI EP09-A3 guidelines.[8] All comparisons were done taking ReadiPlasTin (our reference reagent) as the reference method. A P-value < .05 on Spearman's rank test correlation coefficient of those regressions was considered as statistically significant.

The method comparison was done on:

1. PT (%) results from preoperative patients, liver failure patients, and burned patients.
2. INR results from VKA patients.
3. Extrinsic factor levels for VKA patients and burned patients.

In addition, to check the ISI assignment of the thromboplastins, we compared the average relative difference in the INR range: 2-4.5 (26 samples). A difference of 10% is considered as a critical difference according to the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (SSC/ISTH).[9]

For patients on DOAC treatment, all reagents were compared on PT ratios, first considering each DOAC molecule separately and then considering all DOACs together for plasma concentrations below 50 ng/mL. A thromboplastin reagent was considered to be sensitive to DOAC if it exceeded 1.2 PT ratio or the maximal PT ratio value obtained among the 20 healthy subjects included for MnPT assessment, for a DOAC concentration of 30 ng/mL.

Results obtained on samples from burned patients were also analyzed to assess thromboplastins sensitivity to depressed factor levels using the commonly accepted threshold of PT = 75%. This PT = 75% is usually accepted as the PT% corresponding to the 1.2 PT ratio, and this was verified for STA-NeoPTimal in this study, considering the calibration curve adjusted for the local MnPT. We used the factor II, V, VII and X levels from these burned patients to estimate how rapidly PT results were decreasing (ie, a PT% below 75% for all the factors) for each PT reagent, by plotting the factor level against PT%.

Finally, overall comparability of PT% and factor levels results with the different reagents was determined on the whole samples

of burned patients data set using a Wilcoxon paired test with a *P*-value < .05 as threshold for statistically significant difference.

3 | RESULTS

3.1 | Analytical performance of STA-NeoPTimal for PT and factor assays

For PT, CVs for within run precision were 0.9% for the normal level and 1.5% for the pathological level. The CVs for between run precision were 2.3% for the normal level and 4.5% for the pathological level.

The RCV for PT (STA-NeoPTimal) was therefore: $2^{1/2} \times 1.96 \times ((4.5)^2 + (4)^2)^{1/2}$:16.7%.

For factor assays, the CVs for within run precision (normal level) ranged from 1.4% (Factor V) to 3.4% (Factor X). The CVs for within run precision (pathological level) ranged from 0.9% (Factor VII) to 3.1% (Factor II).

The CVs for between run precision (normal level) ranged from 4.1% (Factor X) and 12.0% (Factor VII). The CVs for between run precision (pathological level) ranged from 2.7% (Factor II) to 13.1% (Factor VII).

3.2 | Sensitivity to heparins

All the four reagents were insensitive to LMWH levels of up to 1.8IU/ml. At 1.8IU/ml of LMWH, the relative differences were 6.8%, 5.5%, 5.1, and 2.2% for STA-NeoPTimal, Readiplastin, STA-Neoplastine CI+, and STA-Neoplastine R, respectively.

STA-Neoplastine CI Plus and STA-Neoplastine R were also insensitive to UFH up to 1.5IU/ml of UFH.

STA-NeoPTimal and ReadiPlasTin showed interference at 1.5IU/ml of UFH, where the relative differences were 11.5% and 10.4%, respectively, whereas for STA-Neoplastine CI+ and STA-Neoplastine R, it was 8.8% and 1.5%, respectively.

3.3 | Reference range

We did a verification of the reference ranges. Reference ranges in seconds (min-max; n = 20 fresh samples per assays) were 11.0-13.1 (ReadiPlasTin), 12.5-14.4 (STA-NeoPTimal), 12.5-14.2 (STA-Neoplastine R) and 12.1-14.7 (STA-Neoplastine CI+). Reference ranges in % on the same samples were 81-108 (ReadiPlasTin), 82-111 (STA-NeoPTimal), 91-113 (STA-Neoplastine R) and 83-114 (STA-Neoplastine CI+). The PT (%) corresponding to a PT ratio of 1.2 was 73 (ReadiPlasTin), 75 (STA-NeoPTimal), 74 (STA-Neoplastine R), and 68 (STA-Neoplastine CI+). A comparison with the reference ranges established by the manufacturers is mentioned in Table S1.

3.4 | Method comparison

3.4.1 | Prothrombin time

Comparability was assessed on 23 preoperative patients (PT(%)), 12 patients with hepatic failure (PT(%)) and 45 patients receiving VKA (INR).

Preoperative patients and patients with hepatic failure (PT(%))

All the reagents demonstrated good correlation with ReadiPlasTin (STA-Neoplastine R and STA-Neoplastine CI+: *R* = .93) except STA-NeoPTimal (*R* = .89) (Figure 1).

For preoperative patients, all the reagents were in agreement for the samples with a PT ratio > 1.2 (n = 3) and <1.2 (n = 19). For PT ratio = 1.2, the results were lower or higher than the lower limit of the normal range, depending on the reagent (n = 1).

For liver failure patients, all the reagents were in agreement for the samples with a PT ratio > 1.2 (n = 6) and <1.2 (n = 6) except for one patient (Ratio 1.13 with ReadiPT, 1.19 with STA-Neoplastine R, 1.23 with STA-Neoplastine CI+: and 1.34 and STA-NeoPTimal).

Patients receiving vitamin K antagonists (INR)

All the reagents demonstrated good correlation with ReadiPlasTin (STA-NeoPTimal *R* = .98, STA-Neoplastine R, *R* = 1.00 and STA-Neoplastine CI+ *R* = .99) (Figure 2). However, there are significant differences (INR differences higher than 0.5) between methods for samples within and above therapeutic ranges. ReadiPlasTin appeared to give higher INR values than STA-Neoplastine CI+ and STA-NeoPTimal. For STA-NeoPTimal, a significant difference was observed in 13 samples. Six (out of 26) of these samples were from patients with an INR between 2 and 4.5, while 7 (out of 9) of these samples were from patients with an INR above 4.5. For STA-Neoplastine CI+, a difference of more than 0.5INR units was observed in 14 samples. Six (out of 26) of these samples were from patients with an INR between 2 and 4.5 while 8 (out of 9) of these samples were from patients with an INR above 4.5. The significant differences were observed in the same samples (for STA-Neoplastine CI+ and STA-NeoPTimal) for 11 samples.

The average relative difference in the INR range: 2-4.5 was calculated as follows (Figure 2):

- STA-Neoplastine R vs ReadiPlasTin: 3.2%
- STA-Neoplastine CI Plus vs ReadiPlasTin: −11.2%
- STA-NeoPTimal vs ReadiPlastin: −10.3%
- STA-NeoPTimal vs STA-Neoplastine CI Plus: −0.8%

One sample explains that the average relative difference of the STA-Neoplastin CI Plus and STA-NeoPTimal in comparison to ReadiPlasTin was higher than 10% (INR ReadiPlasTin 4.43; STA-NeoPTimal 3.32; STA-Neoplastine R. 4.75; STA-Neoplastine CI Plus 3.22).

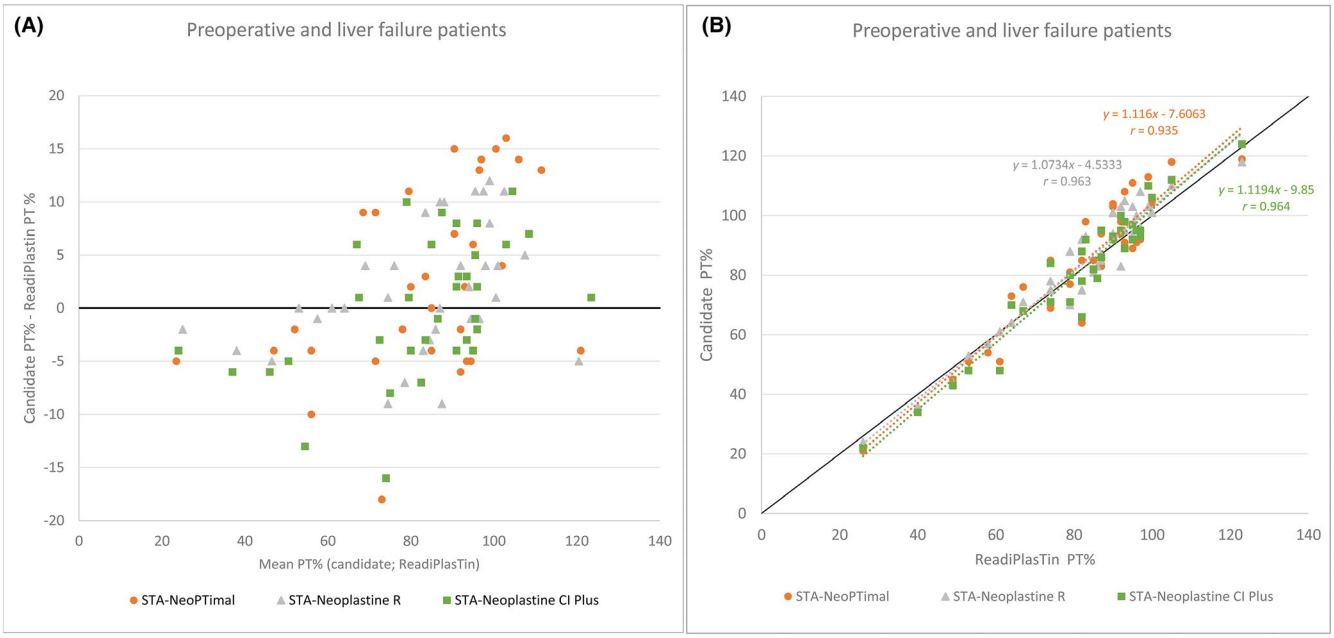


FIGURE 1 Comparison of PT(%) between STA-NeoPTimal (orange), STA-Neoplastine R (gray), STA-Neoplastine CI Plus (green) with the reference PT reagent used in our laboratory (ReadiPlasTin), on preoperative and liver failure patients. A, Bland and Altman representation. B, Regression plot

3.4.2 | Factor assays

VKA patients

STA-NeoPTimal, STA-Neoplastine R and STA-Neoplastine CI + showed excellent correlations in comparison to ReadiPlasTin for factor II, V, VII and X assays (R > .9), except for factor V assay (R: STA-Neoplastine R = .88, STA-Neoplastine CI+ R = .86).

Burned patients

Figure 3 shows the results of factor II, V, VII and X assays using STA-NeoPTimal (orange), STA-Neoplastine R (gray) or STA-Neoplastine CI Plus (green) in comparison to our reference PT reagent (ReadiPlasTin).

Factor II and factor V are significantly lower with extraction thromboplastins (STA-NeoPTimal and STA-Neoplastine CI+) compared with the recombinant ones (ReadiPlasTin and STA-Neoplastine R). For Factor VII, only determinations with STA-Neoplastine CI+ are higher than with ReadiPlasTin. For Factor X, only determinations with STA-NeoPTimal are lower than with ReadiPlasTin.

3.5 | Sensitivity of the PT to isolated or multiple factor deficiency (ies)

The highest concentration of a given factor able to produce a PT below 75% was different according to the PT reagent. As illustrated in Figure 4, it seems that recombinant thromboplastins (open symbols) are less sensitive to factor deficiencies in comparison to extraction thromboplastins (closed symbols). Three patients showed coagulopathy with factor deficiency(ies). One patient showed factor VII deficiency and two patients showed multiple deficiencies.

Figure 4 compares the sensitivity of STA-NeoPTimal (orange), STA-Neoplastine R (gray), STA-Neoplastine CI Plus (green) and ReadiPlasTin (blue) to factor II levels in 8 samples from the patient with 50% of burned body surface and Fournier gangrene.

STA-NeoPTimal and STA-Neoplastine CI Plus (extraction thromboplastins) were more sensitive than STA-Neoplastine R and ReadiPlasTin (recombinant thromboplastins) to detect a factor II deficiency.

3.6 | Sensitivity of the PT to direct oral anticoagulants

Poor responsiveness of the PT to DOAC concentrations was observed. Whatever the thromboplastin, samples with a normal PT (≥75% or ratio ≤1.2) were observed with DOAC concentrations > 30 ng/mL. Prothrombin time was not affected with DOAC plasma concentrations up to 44 ng/mL of rivaroxaban, 152 ng/mL of dabigatran and 414 ng/mL of apixaban. For DOAC concentrations < 50 ng/mL (n = 18), PT values were within normal values (≥75% or ratio ≤ 1.2) except for 4 samples. Three samples with rivaroxaban < 50 ng/mL had prolonged PT (ReadiPlasTin: 3, STA-NeoPTimal: 1, STA-Neoplastine CI+:2, STA-Neoplastine R: 2). One sample with dabigatran had prolonged PT with all the reagents except STA-NeoPTimal) (Figure 5).

4 | DISCUSSION

Our study showed excellent analytical performance of the STA-NeoPTimal, on a STA R Max2 analyzer. Overall, for PT, the CVs for within run precision were ≤1.5% and those for reproducibility were ≤4.5%.

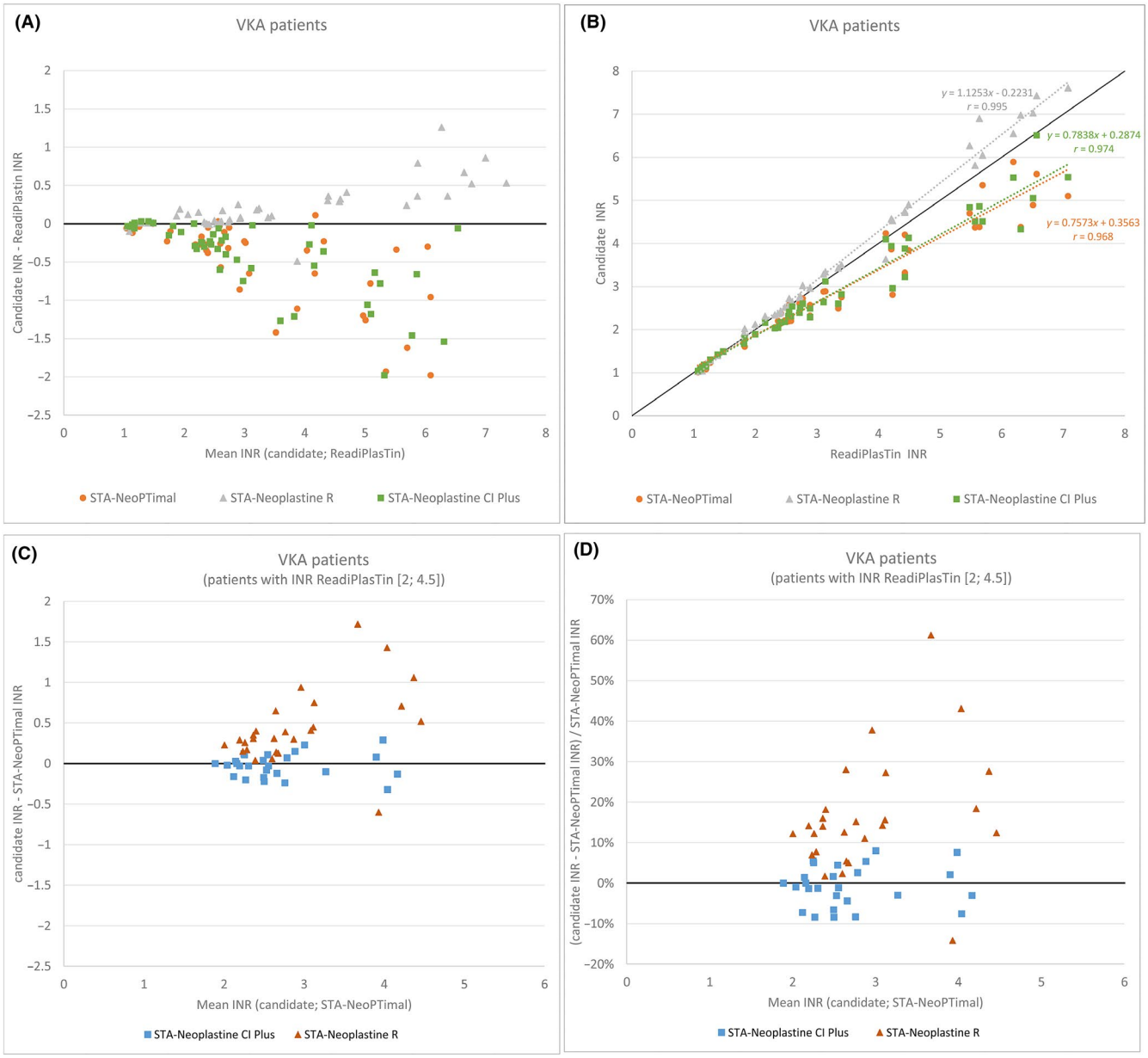


FIGURE 2 Comparison of INR between STA-NeoPTimal (orange), STA-Neoplastine R (gray), STA-Neoplastine CI Plus (green) with the reference PT reagent used in our laboratory (ReadiPlasTin), in VKA patients (A and B), and comparison of STA-Neoplastine R (brown) and STA-Neoplastine CI Plus (blue) with STA-NeoPTimal (C and D). (A) Bland and Altman representation. (B) Regression plot (C) Bland and Altman representation in absolute and (D) relative

For factor assays, the CVs for within run precision were below 3.4%, whereas the ones for between run precision were below 13.1% (Factor VII). The relatively high CV for between run precision for VII assessment was probably not due to the reagent instability. The reagent vials were renewed each 24h which was lower than stability after opening claimed by the manufacturers in the products instructions for use (48h for STA-NeoPTimal, STA-Neoplastine CI Plus, ReadiPlasTin and 5 days for STA-Neoplastine R).

By using the concept of RCV (which combines the analytical and intraindividual variabilities), we confirmed that a PT ratio of 1.2 could be used as the upper limit of the normal range.

Only STA-NeoPTimal and ReadiPlasTin showed some interference with samples with spiked with UFH at 1.5 IU/mL. This is in agreement with the manufacturers specifications (LMWH: 1.5 IU/mL, UFH: 1IU/ml for STA-NeoPTimal and STA-Neoplastine CI+, LMWH: 1.5 IU/mL, UFH: 2 IU/mL for STA-Neoplastine R, LMWH: 1.4 IU/mL, UFH: 1 IU/mL for ReadiPlasTin). There is no standardization in the protocol used to study the heparin sensitivity (type of plasmas (units or pooled), type of centrifugation (simple vs double), delay between spiking and assay, ratio between heparin volume and sample volume…). In addition, we have arbitrarily considered as significant a difference of 10% between PT in the sample spiked with heparin with the sample spiked with vehicle. Alternatives are to use a cutoff of 5%[10] or the Reference

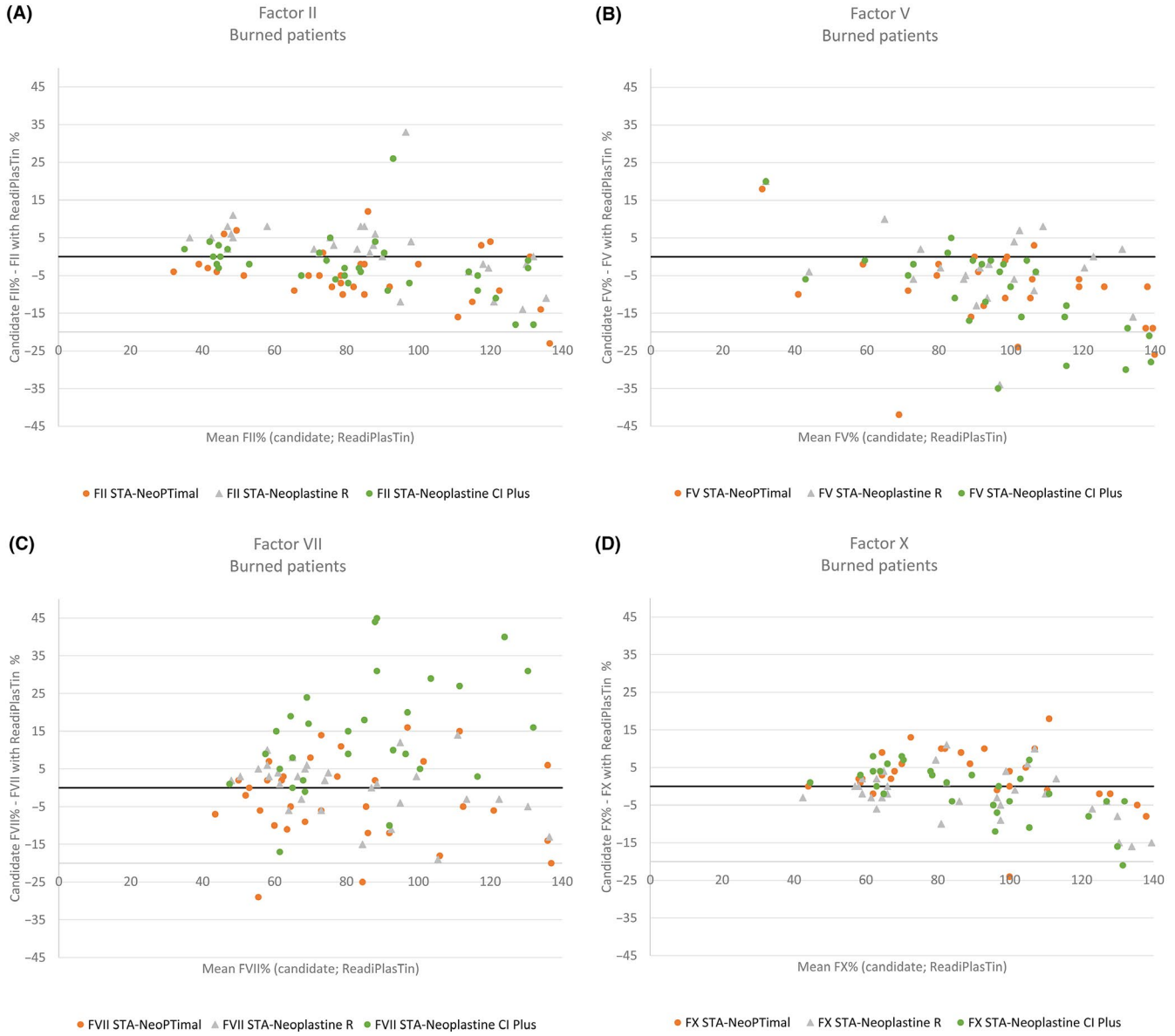


FIGURE 3 Comparison of factor II, V, VII and X between STA-NeoPTimal (orange), STA-Neoplastine R (gray), STA-Neoplastine CI Plus (green) with the local PT reagent (ReadiPlasTin), in burned patients using Bland and Altman representation

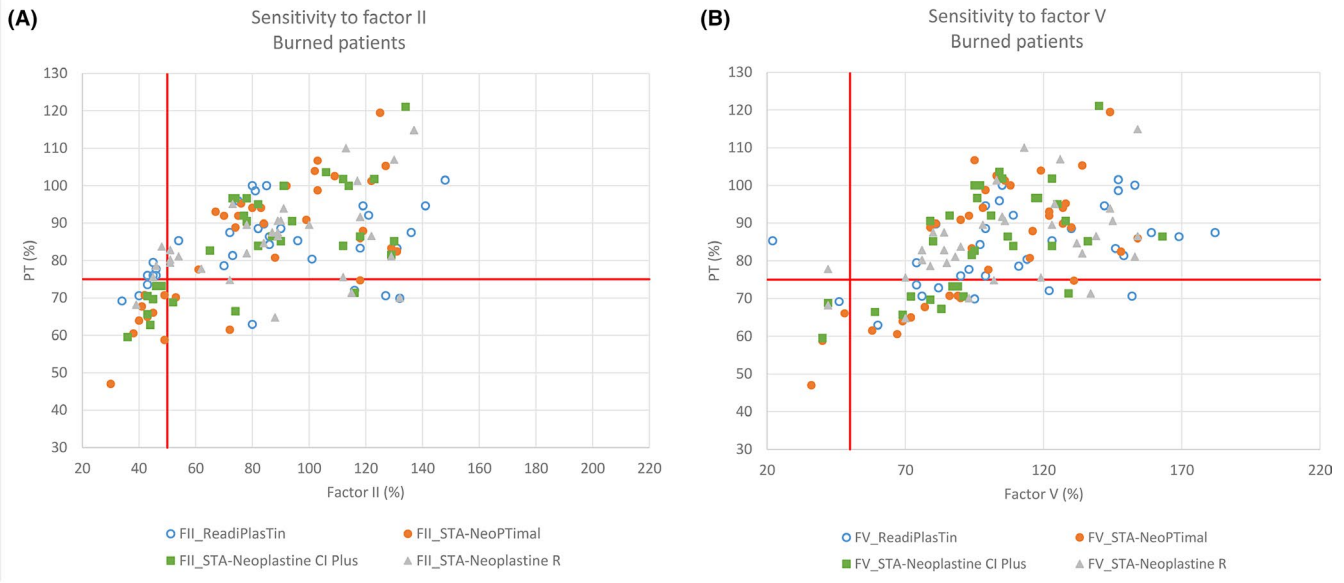
Change Value.7,11 These would have led to a different interpretation. Interestingly, thromboplastins from the same manufacturer did not show the same heparin sensitivity, suggesting a different composition and/or amount of the heparin neutralizer in the reagent.

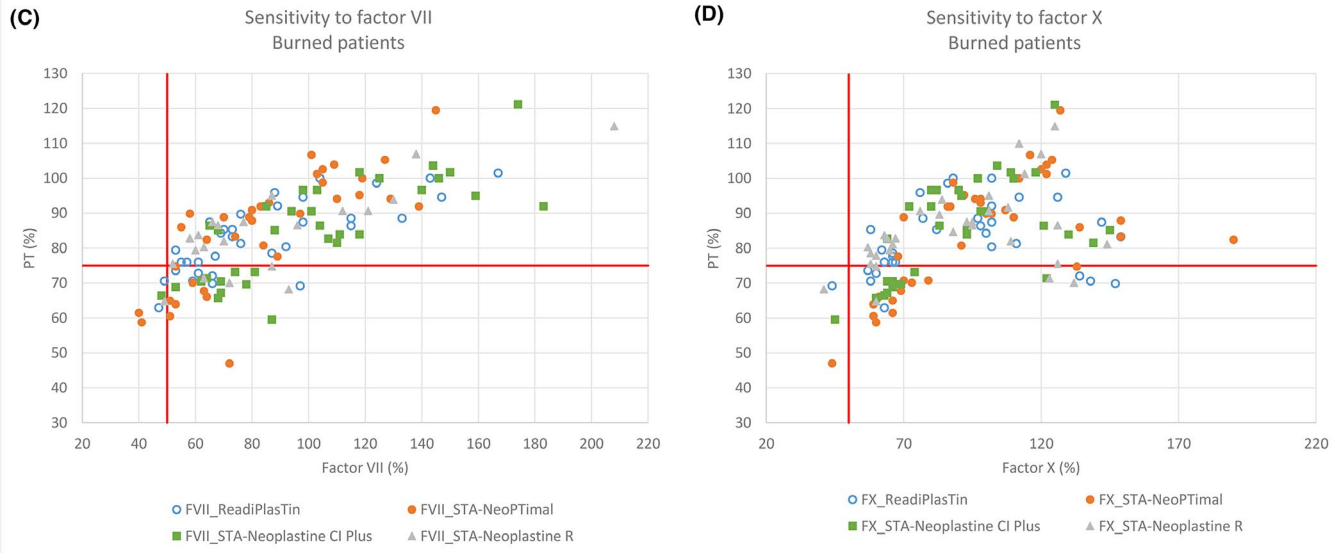
Our study confirms inter-reagent variability in PT, which has not been totally removed by the introduction of the INR.[2,3,12]

The mean relative difference for plasmas with an INR between 2 and 4.5 was only −0.8% between STA-Neoplastine CI Plus and STA-NeoPTimal but up to 16.4% when comparing STA-Neoplastine R and STA-NeoPTimal.

Thus, this higher discrepancy are not explained by an error in ISI assignment of the reagents. There are still significant differences (difference of more than 0.5INR units) between methods for samples within the therapeutic and mostly above the therapeutic ranges. The differences could be attributed to the method of preparation of the thromboplastin (extraction vs recombinant), the origin of the thromboplastins (rabbit vs human), the existence of two international calibrators (one for rabbit thromboplastins and one for human thromboplastins) and the difference in the phospholipid and VIIa composition of the reagents.[4,5,13] Addition of VIIa to thromboplastin reagents prepared using recombinant human tissue factor, decreased thromboplastin sensitivity to plasma FVII but increased sensitivity to plasma levels of FII, FV and FX.[5]

The differences in INR could lead to an impact on clinical management of patient receiving VKA. It does not make sense to change therapeutic range. However, these differences justify the use of a therapeutic target (eg, INR 2.5) instead of a therapeutic range (eg, INR 2.0-3.0) to optimize the management of VKA anticoagulant therapy.[14]


(A)
Sensitivity to factor II
Burned patients
PT (%)
Factor II (%)
FII_ReadiPlasTin
FII_STA-NeoPTimal
FII_STA-Neoplastine CI Plus
FII_STA-Neoplastine R
(B)
Sensitivity to factor V
Burned patients
PT (%)
Factor V (%)
FV_ReadiPlasTin
FV_STA-NeoPTimal
FV_STA-Neoplastine CI Plus
FV_STA-Neoplastine R


(C)
Sensitivity to factor VII
Burned patients
PT (%)
Factor VII (%)
FVII_ReadiPlasTin
FVII_STA-NeoPTimal
FVII_STA-Neoplastine CI Plus
FVII_STA-Neoplastine R
(D)
Sensitivity to factor X
Burned patients
PT (%)
Factor X (%)
FX_ReadiPlasTin
FX_STA-NeoPTimal
FX_STA-Neoplastine CI Plus
FX_STA-Neoplastine R

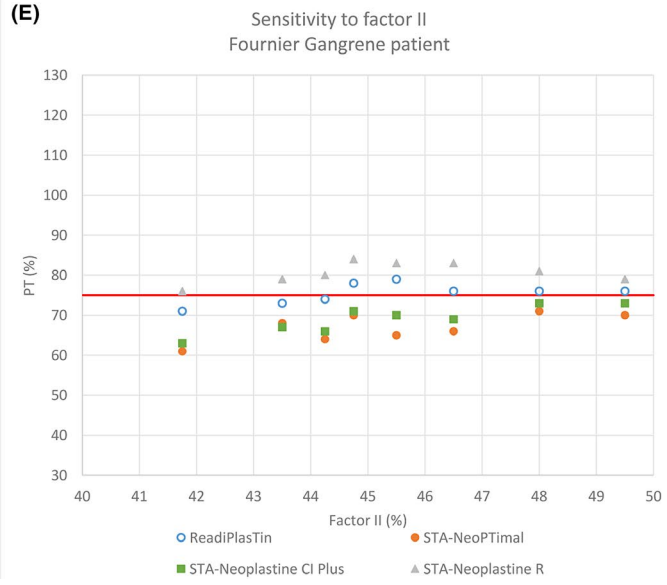

(E)
Sensitivity to factor II
Fournier Gangrene patient
PT (%)
Factor II (%)
ReadiPlasTin
STA-NeoPTimal
STA-Neoplastine CI Plus
STA-Neoplastine R

FIGURE 4 Sensitivity of PT to factor II (a), V (b), VII(c) and X(d) levels in burned patients using the four thromboplastins: STA-NeoPTimal (orange), STA-Neoplastine R (gray), STA-Neoplastine CI Plus (green) and ReadiPlasTin (blue). The sensitivity for factor II, V, VII, X deficiencies was determined by plotting the factor level against PT% to determine the highest concentration of a given factor to produce a prolongation of the PT above the upper limit of the normal reference range (ie, a PT% below 75% for all the factors). In graph e), the sensitivity of the four thromboplastins (STA-NeoPTimal (orange), STA-Neoplastine R (gray), STA-Neoplastine CI Plus (green) and ReadiPlasTin (blue)) was compared to mean of the factor II levels (provided by the 4 reagents) in 8 samples from the burned patient with Fournier gangrene)

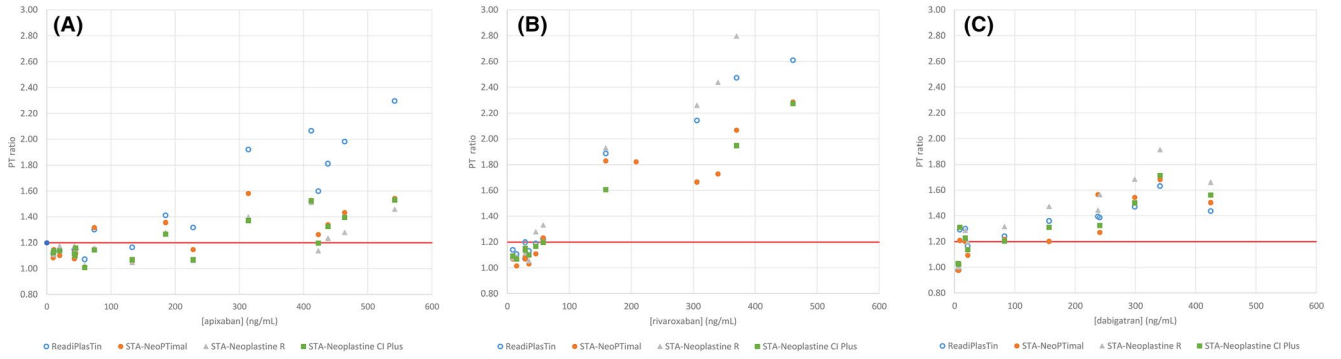


FIGURE 5 Sensitivity of thromboplastins to apixaban (A), rivaroxaban (B) and dabigatran (C) as assessed through PT ratio. The sensitivity to apixaban, rivaroxaban and dabigatran was determined by plotting the apixaban, rivaroxaban and dabigatran concentration against PT ratio to determine the lowest concentration of a given DOAC to produce a prolongation of the PT ratio above the upper limit of the normal reference range (ie, a PT ratio above 1.2). Note: One sample was excluded to keep the scale up to 3

In addition it is important to recognize the thromboplastin behavior over the upper limit of the therapeutic range. In external quality controls surveys it is really clear that INRs obtained with different thromboplastins used with instrument specific ISI from manufacturers present huge variations in samples with INR > 4.5.

Moreover, the bleeding risk increased exponentially with an INR higher than 4.5.[15] The differences in INR should thus lead to discussions with hematologists and physicians that take care of patients to define which is the better value to be considered as a critical value according to the performance of the type of thromboplastin. (Critical value: laboratory test result significantly lying outside the normal (reference) range and necessitating immediate reporting to safeguard patient health).[16]

In a NASCOLA survey, the median critical value for INR was 5 with an upper interquartile of 6.[17] The differences in INR could also impact scoring systems using INR: MELD score, Maddey score….[18,19] It should be noted that INR was not designed to be used in patients with liver disease because the ISI calibration is performed by using samples of VKA patients that only have functional deficiency of vitamin K-dependent factors but normal factor V. Differences between different thromboplastins with different ISI have been recognized as great limitations of the MELD to organ allocations for many years.[20,21]

The INR results of STA-NeoPTimal and STA-Neoplastine CI+ are lower than the results of STA-Neoplastine R and ReadiPlasTin. The impact of the thromboplastin origin is well described.[3] The similar performances of STA-NeoPTimal and STA-Neoplastine CI+ are observed despite their different ISI (ie, target ISI STA-NeoPTimal: 0.9-1.0, ISI STA-Neoplastine CI+: 1.1-1.3). This is in favor of a more important impact of the type of thromboplastin (origin and/or method of preparation) than the ISI on the performance of a thromboplastin reagent in the therapeutic and above therapeutic ranges. The burned patients did not show any typical hemostatic profile.[22,23] The PT allows identification of burned patients with acquired extrinsic factor deficiencies.

A difference in the performance of extraction thromboplastins versus recombinant thromboplastins was also observed in burned patients. Extraction thromboplastins were more sensitive to detect a factor II deficiency with the PT. However, the number of burned patients with coagulopathy was too low to draw conclusions. In addition, the sensitivity of thromboplastin to factor deficiency should be calculated using H47A2 CLSI guideline or using samples from patients with congenital isolated factor deficiencies.[24,25]

To the best of our knowledge, this study was the first comparing the performances of thromboplastins to detect coagulopathy in burned patients. There was no impact of the origin of thromboplastin on the results of factor assays due to dilution of the plasma sample. It was previously shown that recombinant thromboplastins are more sensitive than tissue-extract thromboplastin to detect factor VII deficiency.[26,27] The method of preparation was shown to be more important the origin of thromboplastin as Thromborel S (a tissue-extract human thromboplastins) was less sensitive than recombinant thromboplastin to factor VII deficiency. In the present study, this was confirmed with STA-Neoplastine CI Plus. However, we have to acknowledge that the objective of using burned patients with coagulopathy (isolate or multiple factors deficiencies) was not to assess the sensitivity to factor deficiency but to analyze the ability of each of the thromboplastin reagent to detect this(ese) deficiency(ies).

Finally, we confirmed the poor responsiveness of the four PT to DOAC concentrations, confirming that PT should not be used for the estimation of DOAC concentrations.[1,28] As with the three other thromboplastins, rivaroxaban showed also the strongest effect on PT using the STA-NeoPTimal, followed by edoxaban (data not shown) and then apixaban.

In conclusion, the STA-NeoPTimal showed comparable performance relative to ReadiPlasTin, STA-Neoplastine CI Plus and STA-Neoplastine R. It is suitable for VKA control, detection of factors II, V, VII and X deficiency, and assessment of liver disease coagulopathy. However, for plasmas from patients under VKA, the significant method differences for samples within and above therapeutic INR ranges claims for good interaction between laboratory and clinicians to establish new cutoffs. Furthermore, this highlights the importance for patients on VKA to have their follow-up in PT measurements in the same laboratory, which one should in return warn the clinicians if any methodological change would affect results interpretation. Poor responsiveness of the PT to DOAC concentrations was expected and confirmed, meaning that a normal PT(%) or PT (ratio) cannot exclude relevant residual DOAC concentrations.

CONFLICTS OF INTEREST

François Mullier reports institutional fees from Stago, Werfen, Nodia, Sysmex and Bayer. He also reports speaker fees from Boehringer Ingelheim, Bayer Healthcare, Bristol-Myers Squibb-Pfizer, Werfen, Aspen, Sysmex and Stago, all outside the submitted work. Sarah Lessire reports speaker fees from Stago, outside the submitted work.

AUTHOR CONTRIBUTIONS

F.Mullier, B.Chatelain, S.Lessire, H.Jacqmin designed the study. MS Paridaens, L.Miller, S.Lessire participated to the patients's recruitment. J.Baudar, M.Guldenpfennig et C.Devroye performed the experiments. F.Mullier, J.Evrard, B.Chatelain, and H.Jacqmin analyzed the results. F.Mullier wrote the first draft of the manuscript. All the authors revised the intellectual content of the manuscript and approved its final version. We would like to thank Audrey Carlo and Celine Ayel for their assistance in the setting of this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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