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Unified calibration of D-dimer can improve the uniformity of different detection systems

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ABSTRACT

Background: D-dimer at a low level is important evidence for excluding the onset and progression of thrombosis. It is readily detectable and yields rapid results, although significant variability exists among different detection systems. Our study aims to enhance the consistency across various detection systems.

Methods: Twelve detection systems were included in our study. We sought to address this inconsistency by using various calibrators (two supplied by manufacturers and two comprising pooled human plasma diluted with different diluents) to standardize D-dimer measurements. We categorized the data into three groups according to D-dimer concentration levels: low (≤0.5 mg/L), medium (>0.5 mg/L - <3 mg/L), and high (≥3 mg/L). We then analyzed the data focusing on range, consistency, comparability, negative coincidence rate, and false negative rate.

Results: Calibrating with pooled human plasma led to narrower result ranges in the low and medium groups ($P < 0.05$). In the low group, consistency improved from weak to strong (ICC 0.4–0.7, P<0.05), while it remained excellent in the other groups and overall (ICC>0.75, P<0.05). The percentage of pairwise comparability increased in both the low and high groups. Additionally, there was an increase in the negative coincidence rate.

Conclusion: These findings demonstrate that uniform calibration of D-dimer can significantly enhance the consistency of results across different detection systems.

1. Introduction

D-dimer, a soluble fibrin degradation product in plasma, is hydrolyzed by plasmin and signifies the concurrent activation of coagulation and fibrinolysis [1–3]. The formation of D-dimer begins with the conversion of fibrinogen to fibrin monomers, catalyzed by thrombin. These monomers crosslink to form a soluble network, which then transforms into stable, insoluble clots due to factor XIIIa. Subsequent fibrinolytic activity, involving plasmin, degrades these clots. The resulting fibrin degradation products (FDP) eventually yield the D-dimer/fragment E complex (DD/E), characterized by two covalently-bound D-domains [4]. Known to increase

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in conditions such as inflammation, tumor, pregnancy, and liver or kidney disease, D-dimer is a vital biomarker for the diagnosis [3,5] and monitoring of thrombosis [6–8], and also an important monitoring indicator in the course of thrombus treatment and recurrence risk assessment [9].

Despite its significance, standardization of D-dimer assays remains challenging due to multiple factors: the use of various detection methods including ELISA, immunofluorescence quantitative methods, immunoturbidimetry, and chemiluminescence immunoassay; the employment of numerous antibodies targeting different D-dimer fragments; and the diverse reporting units used, such as fibrinogen equivalent units (FEU) and D-dimer units (DDU), with measurements in ng/mL, mg/mL, mg/L, or many other different units [10–12], there are already recommended conversion correlation coefficients for FEU and DDU [13], but not perfect.

The International Society on Thrombosis and Haemostasis Scientific Standardization Committee (ISTH-SSC) has long advocated for the standardization of D-dimer assays. Efforts in Europe have included multi-center collaborations to investigate discrepancies in test results from various brands and methodologies, using different antibodies [14–17]. Attempts at standardization have involved developing a reference regression line to minimize assay variability [15,16], creating a universal antibody for immunoturbidimetric detection [17,18], and preparing calibration samples with high-concentration patient plasma suitable for most assays [19]. However, each method had its limitations, and a definitive standardized approach for D-dimer remains unestablished. The plasma calibration method refers to be the most reliable, but the analyzers used in previous studies were relatively consistent [16], although ISTH SCC seems to have started processing human plasma, their research was only based on five analyzers [19].

In this study, we enlisted 12 brands of analyzers (with a total of 13 reagents), all using FEU as the reporting unit. We evaluated the comparability of D-dimer detection results across different analyzer brands, aiming to identify an effective method for achieving D-dimer standardization.

2. Materials and methods

2.1. Patients

Blood samples were collected from over 330 patients presenting diverse clinical conditions (including trauma, bone fracture, hypertension, burns, deep vein thrombosis, etc.) at Beijing Jishuitan Hospital between July 2022 and February 2023.

2.2. Sample Collection and Handling

Each blood sample (3 mL) was drawn into sodium citrate (0.109 m, 3.2%) Vacutainer tubes (Becton Dickenson, Franklin Lakes, NJ, USA).

2.3. Detection system

12 D-dimer analyzers from various brands, along with 13 different reagents reporting in FEU, were utilized. The analyzers were labeled A to L, comprising one chemiluminescence immunoassay and 11 immunoturbidimetric methods (Supplementary table). Analyzer D was further divided into D1 and D2 due to its two types of reagents (Table 1).

2.4. Groups

The study was conducted in two phases: initially, each analyzer was calibrated with its specific calibration product before sample testing. Subsequently, the analyzers were uniformly calibrated with standard calibrators and then used for sample testing. Four additional calibrators were used apart from the matching calibrators: calibrator 1 for the chemiluminescence analyzer, calibrator 2 for an immunoturbidimetric analyzer, and calibrator 3 and 4, which were human plasma pooled and diluted with the matching diluent

Table 1
Information of analyzers.

labeled letters	analyzers	reagents
A	TESMI i100 , Tellgen , China	D-Dimer (CP023434)
B	MDC 3500 , BSBE , China	D-Dimer reagent kit (MDD105 M)
C	CS 5100 , Sysmex , Japan	INNOVANCE® D-Dimer
D1/D2	SF 8200 , SUCCEEDER , China	D1:D-Dimer (SK3005); D2:D-Dimer (DD8)
E	MC 550 , PushKang , China	D-Dimer Assay Kit (HE50016)
F	Cobas t 511 , Roche , Switzerland	Tina-quant D-Dimer Gen.2
G	BCA 3000 , DIRUI , China	D-Dimer (3009918)
H	H 2600 , Maccura , China	D-Dimer (BL2102008)
I	Syscan 300 , Autobio , China	D-Dimer (Ci-SSDD-24)
J	GW 3000 , ACCURDX , China	D-Dimer Assay Kit (Z2010602A)
K	CX 9010 , Mindray , China	Latex D-Dimer Assay Kit
L	ACL TOP 500 , Werfen , USA	HemosIL D-Dimer HS 500

The detection method of instrument A is chemiluminescence immunoassay, and B-L is immunoturbidimetric. The reporting unit for all analyzers is FEU.

provided by manufacturer A and double distilled water respectively and assigned values using the chemiluminescence method. Thirty samples were tested in each round.

Based on the median results, the data were categorized into three groups according to D-dimer concentration: low (≤0.5 mg/L), medium (>0.5 mg/L - <3 mg/L), and high (≥3 mg/L).

2.5. Statistical analysis

Unpaired t-tests were applied to assess the range of detection results from various measurements. Consistency was evaluated using the Intraclass Correlation Coefficient (ICC): an ICC <0.4 indicates poor consistency, while an ICC >0.75 signifies good consistency. The Chi-square test was used to determine the statistical significance of changes in the negative coincidence rate and false negative rate. These analyses were conducted using SPSS. Graphs and Friedman's rank test were created using GraphPad Prism 10.

3. Results

3.1. Proximity of testing the same samples

Fig. 1 displays the test results from various measurements. Regardless of the group, it appears that calibrating with calibrator 3 and 4 resulted in more centralized results, whereas results calibrated with calibrator 1 and 2 more dispersed.

Data from different groups were collected and analyzed for the rang of results from various measurements testing the same samples. This range reflects the proximity of results when detecting a unified sample: the closer the results, the smaller the range. Table 2 demonstrates that in the low and medium groups, data from tests using the matching calibrator or calibrators 1 and 2 were dispersed, whereas results calibrated with calibrators 3 and 4 were relatively centralized. In the high group, data from tests calibrated with calibrators 1, 2, and 3 were dispersed, the difference between data calibrated with the matching calibrators and calibrator 4 were not significant ($P < 0.05$).

From Fig. 1 and Tables 2 and it is evident that calibrators 3 and 4 brought the results of different measurements testing the same

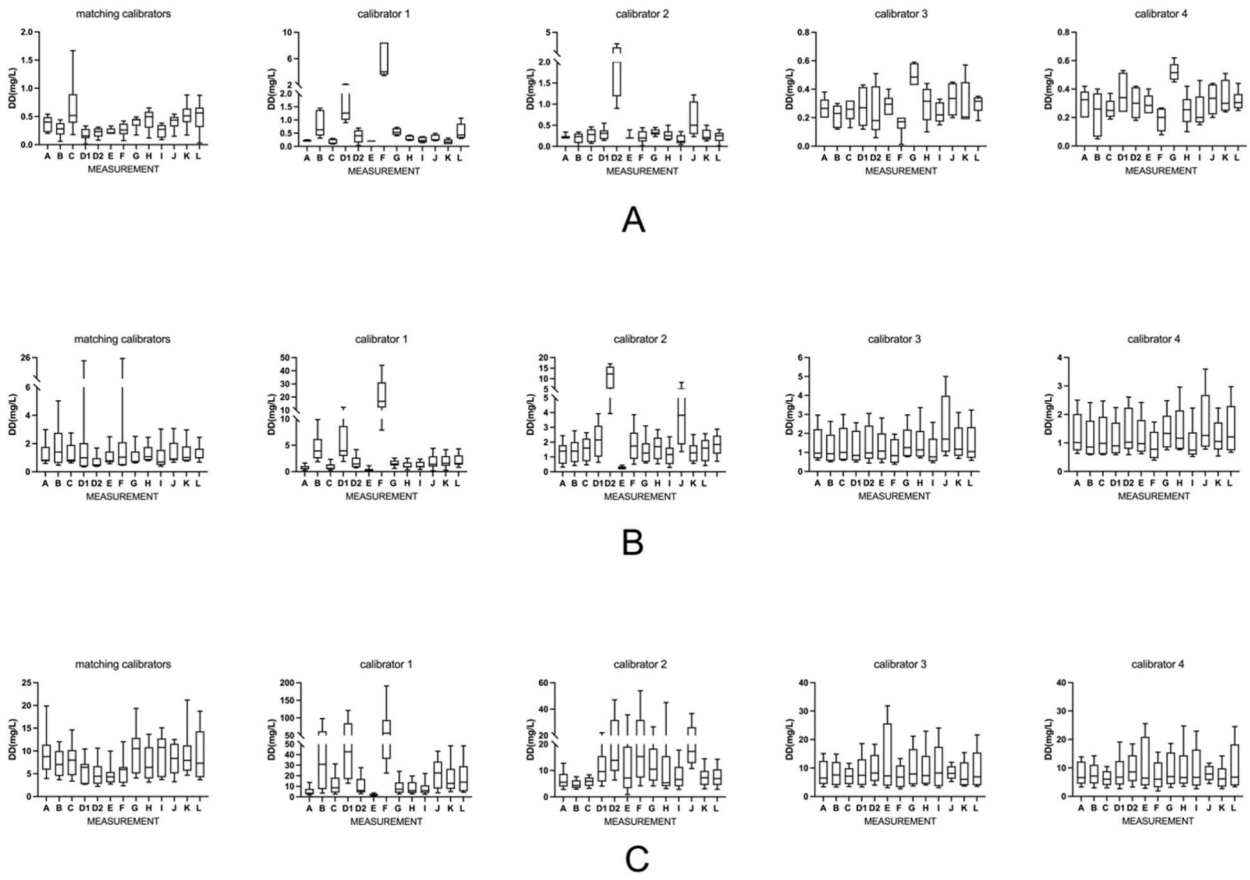

Fig. 1. D-dimer concentration in samples. The x-axis indicated the calibration and the y-axis indicated D-dimer concentration. (A) D-dimer concentration in the low group. (B) D-dimer concentration in the medium group. (C) D-dimer concentration in the high group.

Table 2
Range in each group.

	matching calibrators	calibrator 1	calibrator 2	calibrator 3	calibrator 4
low group	0.2–1.6	3.4–8.3	0.9–3.2	0.3–0.5*	0.3–0.4*
medium group	0.5–24.6	7.7–43.6	3.7–16.6	0.4–3.0*	0.3–1.9*
high group	2.5–15.9	41.7–189.6	12.6–46.4	1.7–24.4	2.6–18.2

The range of results from different measurements testing the same sample were analyzed through unpaired *t*-test, *P<0.05.

samples closer together, particularly calibrator 4.

3.2. Consistency analysis

The ICC was employed to assess data consistency. Post-calibration with calibrator 3/4, the ICC in the low group increased from 0.32 to 0.48/0.64 shifting consistency from weak to moderate/strong. In the medium group, ICC increased from 0.08 to 0.93/0.93, indicating a change from weak to excellent consistency. In the high group and overall, the ICC demonstrated excellent consistency. However, this improvement was not observed after calibration with calibrator 1 or 2 (Table 3).

No overall improvement in comparability was observed using Friedman analysis after calibration. However, calibration with calibrator 3 and 4 resulted in increased pairwise comparability in both the low and high groups (Fig. 2). Specifically, in the low group, comparability rose from 64.1% to 94.9%/92.3% (Fig. 2A), and in the high group, it increased from 71.8% to 89.7%/85.9% (Fig. 2C), for calibrator 3 and 4. No improvement was noted in the medium group (Fig. 2B). Similarly to the consistency analysis, no enhancement was detected following calibration with calibrators 2 or 3 (Fig. 2). Table 4 list the specific values.

3.3. Negative coincidence rate and false negative rate

The negative coincidence rate, analyzed using the median of 0.5 mg/L as a cutoff value, was initially 79.5%. After calibration with calibrator 1, it decreased to 67.7%, but increased to 85.5% with calibrator 2. Notably, calibration with Calibrators 3 and 4 significantly raised the rate to 93.6% and 92.3% respectively (Table 5).

The false negative rate, which was 13.2% using matching calibrators, decreased to 3.5% and 2.3% after calibrating with calibrator 3/4 (P<0.05, Table 6). However, no significant change was observed after calibrating with calibrator 1 or 2.

4. Discussion

Thrombosis is a leading cause of mortality in various diseases, including cancer, surgery, and cardiovascular disorders. Clinicians have consistently focused on reducing or preventing thrombosis during disease progression or post-surgery. In this context, D-dimer serves as a critical marker for evaluating treatment efficacy [20–22]. D-dimer, a small protein molecule, results from the hydrolysis of fibrin by plasmin and comprises fragments of varying sizes. The diversity of these fragments means that antibodies from different reagent manufacturers vary. Consequently, D-dimer concentrations detected by different analyzers differ, a fact corroborated by the uncalibrated sample results in our experiment. Contrary to previous studies [15,23], we chose to forego regression curve methods in favor of calibration.

As noted in introduction, different reagents identify various D-dimer fragments, and this also applies to their respective matching calibrators. Typically, commercial reagents are monoclonal antibodies targeting specific fragments and may be influenced by certain heterophilic antibodies. Although superior reagents might use double antibodies, their scope remains limited. Thus, after calibrating with two commercial calibrators provided by different manufacturers, only a slight improvement in pairwise pairing was noted in the low and medium concentration groups. However, our alternative approach of calibrating with human plasma yielded encouraging results: the results of different analyzers testing the same sample were more aligned within each group, as evidenced by the ranges; the consistency of results improved markedly, as demonstrated through ICC statistical methods, changing from “weak” to “strong” or “excellent”; and the percentage of pairwise comparability increased significantly in the low and medium groups.

In our study, we employed various calibrators to adjust measurements and analyzed a substantial volume of test results. Our comprehensive analysis, considering range, consistency, and comparability, revealed that calibrating measurements with non-specifically processed human plasma offered the most promising approach to achieving D-dimer standardization. Importantly, given D-dimer’s significance, a result below the cutoff value nearly excludes thrombosis. Thus, the enhanced negative coincidence rate

Table 3
ICC analysis result (P<0.05).

	matching calibrators	calibrator 1	calibrator 2	calibrator 3	calibrator 4
low group	0.32	0.16	0.34	0.48	0.64
medium group	0.08	0.21	0.45	0.93	0.93
high group	0.83	0.26	0.38	0.78	0.81
total	0.78	0.37	0.58	0.87	0.89

ICC was used with the following interpretation: <0.4: weak; from 0.4 to 0.6: moderate; from 0.6 to 0.75: strong; from 0.75 to 1.0: excellent.

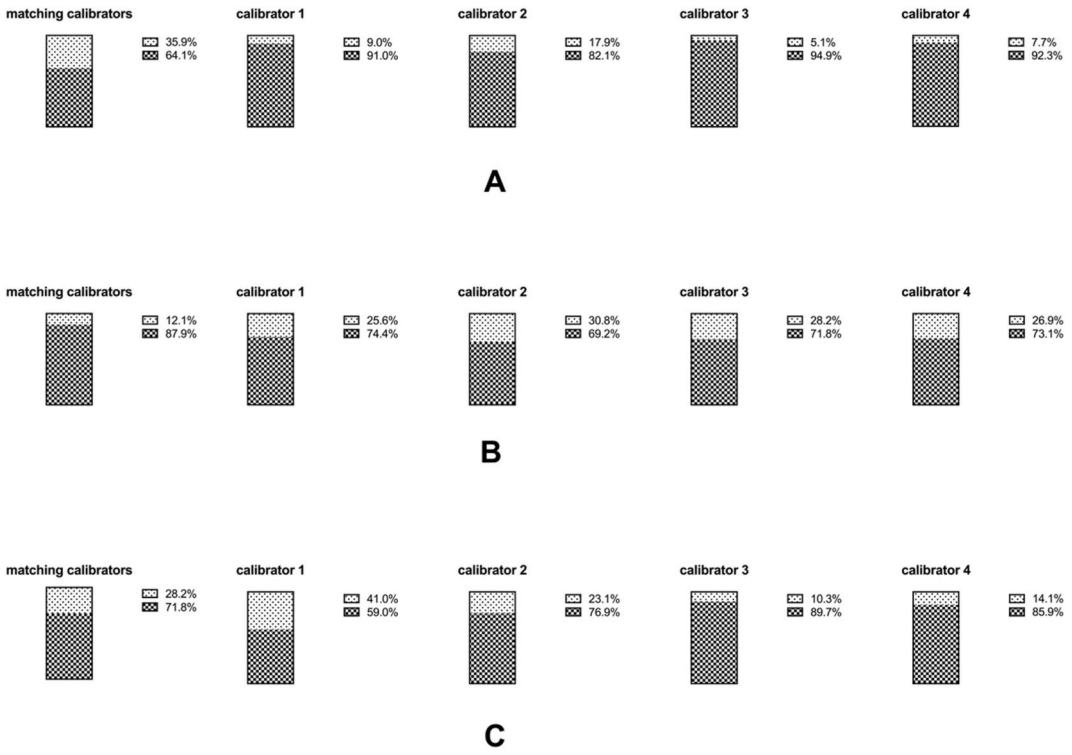

Fig. 2. Pairwise comparison between groups. The dark part of column indicated pairwise comparability and the blank part indicated incomparable. The A, B, C represented the low, medium and high groups respectively. Comparability refers to the lack of statistical significance in the differences in detection results between various analyzers within the same group.

Table 4
Pairwise comparison.

	matching calibrators	calibrator 1	calibrator 2	calibrator 3	calibrator 4
low group	64.1%	91.0%*	82.1%*	94.9%*	92.3%*
medium group	87.9%	74.4%*	69.2%*	71.8%*	73.1%*
high group	71.8%	59.0%	76.9%	89.7%*	85.9%*

The percentage of pairwise comparability were compared between matching calibrators and every calibration through chi-square, this table is the specific data from Fig. 2. *P<0.05.

Table 5
Negative coincidence rate.

	matching calibrators	calibrator 1	calibrator 2	calibrator 3	calibrator 4
rate	79.49%	67.69%	85.47%	93.59%*	92.31%*

The negative coincidence rate were compared between matching calibrators and every calibration through chi-square, we use a value less than the cutoff value of 0.5 mg/L to determine it as negative*P<0.05.

Table 6
False negative rate.

	matching calibrators	calibrator 1	calibrator 2	calibrator 3	calibrator 4
rate	13.19%	15.38%	12.59%	3.5%*	2.31%*

The false negative rate were compared between matching calibrators and every calibration through chi-square, *P<0.05.

in our study provides stronger evidence for the absence of thrombotic formations in clinical settings, reducing the need for patients to undergo excessive examinations. Additionally, the decrease in false negatives significantly lowers the risk of misdiagnosis in clinical practice. Unlike previous research, our method eschewed the need for complex regression curves, opting instead for straightforward

calibration by pooled human plasma, which satisfies and surpasses the condition of "the calibrator for D-dimer assays should contain an array of D-dimer-containing fibrin compounds with a composition similar to that of the clinical samples to be analyzed" [7]. Moreover, our study included not only the commonly used European and American instruments but also several Asian brands, reflecting diverse technological levels and methodologies. This inclusivity demonstrates that our approach is broadly applicable across various detection methods and brands, offering a more universal solution to the challenge of D-dimer standardization, and this aligns with the work of ISTH SSC [19].

5. Conclusion

We identified that calibrating with human plasma can improve the consistency and comparability of different systems on detecting D-dimer, and the detection systems recruited in our project were far exceed some previous studies. Meanwhile, we confirmed that calibrating with human plasma calibration can improve the negative conformity rate of various systems. Based on the important role of D-dimer in excluding thrombosis and as an important biomarker for thrombosis development, the present study certainly can provide more reliable diagnostic and therapeutic evidence for clinical practice.

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CRediT authorship contribution statement

Kun Wang: Writing – review & editing, Writing – original draft, Methodology. **Xinwei Zang:** Software, Data curation. **Wenjie Zhang:** Software, Data curation. **Xiangyu Cao:** Software, Data curation. **Huiru Zhao:** Writing – review & editing. **Chunyan Li:** Writing – review & editing. **Cuiying Liang:** Software, Data curation. **Jun Wu:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00413.

References

[1] S.S. Adam, N.S. Key, C.S. Greenberg, D-dimer antigen: current concepts and future prospects, Blood 113 (13) (2009) 2878–2887.
[2] L.A. Linkins, S. Takach Lapner, Review of D-dimer testing: good, Bad, and Ugly, Int J Lab Hematol 39 (Suppl 1) (2017) 98–103.
[3] R.S. Riley, et al., Widely used types and clinical applications of D-dimer assay, Lab. Med. 47 (2) (2016) 90–102.
[4] J. Favresse, et al., D-dimer: Preanalytical, analytical, postanalytical variables, and clinical applications, Crit. Rev. Clin. Lab Sci. 55 (8) (2018) 548–577.
[5] I. Gutierrez Garcia, et al., D-dimer during pregnancy: establishing trimester-specific reference intervals, Scand. J. Clin. Lab. Invest. 78 (6) (2018) 439–442.
[6] T. Ghys, et al., Diagnostic accuracy of the Triage D-dimer test for exclusion of venous thromboembolism in outpatients, Thromb. Res. 121 (6) (2008) 735–741.
[7] C.E. Dempfle, D-dimer: standardization versus harmonization, Thromb. Haemostasis 95 (3) (2006) 399–400.
[8] J.J. Mager, et al., The early course of D-dimer concentration following pulmonary artery embolisation, Thromb. Haemostasis 86 (6) (2001) 1578–1579.
[9] G. Palareti, et al., Risk of venous thromboembolism recurrence_ high negative predictive value of D-dimer performed after oral anticoagulation is stopped, Thromb. Haemostasis 87 (1) (2002) 7–12.
[10] G. Lippi, et al., International survey on D-dimer test reporting: a call for standardization, Semin. Thromb. Hemost. 41 (3) (2015) 287–293.
[11] C. Longstaff, et al., Harmonisation of D-dimer - a call for action, Thromb. Res. 137 (2016) 219–220.
[12] G. Reber, P. de Moerloose, D-dimer assays for the exclusion of venous thromboembolism, Semin. Thromb. Hemost. 26 (6) (2000) 619–624.
[13] B. Edlund, T.K. Nilsson, A proposed stoichiometrical calibration procedure to achieve transferability of D-dimer measurements and to characterize the performance of different methods, Clin. Biochem. 39 (2) (2006) 137–142.
[14] A. E, U. G, Pooled patient samples as reference material for D-dimer, Thromb. Res. 80 (1995) 85–88.
[15] P. Meijer, et al., A model for the harmonisation of test results of different quantitative D-dimer methods, Thromb. Haemostasis 95 (3) (2006) 567–572.
[16] I. Jennings, et al., Laboratory D-dimer measurement: improved agreement between methods through calibration, Thromb. Haemostasis 98 (5) (2007) 1127–1135.
[17] H.M. Hamer, et al., Diagnostic accuracy of four different D-dimer assays: a post-hoc analysis of the YEARS study, Thromb. Res. 201 (2021) 18–22.
[18] B. Torok-Nagy, J. Antal, B. Denes, Generation and characterization of D-dimer specific monoclonal antibodies for use in latex agglutination test, PLoS One 14 (2) (2019) e0212104.
[19] S. Bevan, C. Longstaff, Is it possible to make a common reference standard for D-dimer measurements? Communication from the ISTH SSC Subcommittee on Fibrinolysis, J Thromb Haemost 20 (2) (2022) 498–507.

[20] R. Ding, et al., Machine Learning-based Screening of risk factors and Prediction of deep vein thrombosis and pulmonary Embolism after Hip Arthroplasty, Clin. Appl. Thromb. Hemost. 29 (2023) 10760296231186145.
[21] P.C. Kruger, et al., Deep vein thrombosis: update on diagnosis and management, Med. J. Aust. 210 (11) (2019) 516–524.
[22] J.S. Cui, et al., D-dimer as a biomarker for acute aortic dissection: a systematic review and meta-analysis, Medicine (Baltim.) 94 (4) (2015) e471.
[23] L. Garcia de Guadiana-Romualdo, et al., Harmonized D-dimer levels upon admission for prognosis of COVID-19 severity: results from a Spanish multicenter registry (BIOCOVID-Spain study), J. Thromb. Thrombolysis 53 (1) (2022) 103–112.