Određivanje koncentracije D-dimera različitim kvantativnim testovima – vježba harmonizacije

Determination of D-dimer by different quantitative assays – A harmonization exercise

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Sažetak

Uvod: Koncentracija D-dimera izmjerena različitim testovima pokazuje značajnu varijabilnost zbog nedostatka standardizacije. Opisujemo harmonizaciju koju smo proveli kako bi pokušali smanjiti tu varijabilnost koristeći uzorke plazme s visokom koncentracijom D-dimera.

Materijali i metode: Sudjelovalo je pet laboratorija s nekoliko različitih kvantitativnih D-dimer testova: Vidas D-dimer Exclusion (proizvođača Biomerieux), Auto-Dimer (Biopool), D-dimer Plus, Acute Care D-dimer (oba Dade Behring) i Hemosil D-Dimer (Instrumentation Laboratory). Za harmonizaciju je pripremljen set od šest plazmi: smjesa (engl. *pool*) normalnih plazmi i uzoraka A-E, pripremljenih miješanjem normalne plazme s rastućim udjelom (1, 2, 3, 8 i 14,7) smjese plazme bolesnika s visokom koncentracijom D-dimera. Za validaciju se koristilo 15 uzoraka plazme od bolesnika s venskom tromboembolijom.

Rezultati: Izračunat je referentni regresijski pravac kroz srednje vrijednosti koncentracije D-dimera u uzorcima A-E te je korišten za harmoniziranje rezultata 15 validiranih uzoraka. Nakon harmonizacije su se koeficijenti varijacije znatno poboljšali s 89% na 19% (srednje vrijednosti) za validirane uzorke s visokom koncentracijom D-dimera. Za validirane uzorke s niskom koncentracijom D-dimera (normalna ili približno granična) koeficijenti varijacije su se čak povisili sa srednje vrijednosti od 86% prije harmonizacije na srednju vrijednost od 224% nakon harmonizacije.

Zaključak: Proces harmonizacije znatno je smanjio varijabilnost između različitih testova kod uzoraka s visokom koncentracijom D-dimera. Međutim kod uzoraka s koncentracijom D-dimera oko granične, što je kritično za kliničku primjenu, nismo primjetili poboljšanje.

Ključne riječi: D-dimer, harmonizacija, validacija

Abstract

Background: D-dimer determined by different assays shows considerable variability due to lack of standardization. We describe a harmonization exercise performed in an attempt to diminish this variability using plasma samples with high D-dimer concentration.

Materials and methods: Five laboratories participated with several different quantitative D-dimer assays: Vidas D-dimer Exclusion (Biomerieux), Auto-Dimer (Biopool), D-dimer Plus, Acute Care D-dimer (both Dade Behring) and Hemosil D-Dimer (Instrumentation Laboratory). For harmonization a set of six plasmas was prepared: normal pooled plasma and samples A-E, prepared by mixing normal pooled plasma with increasing parts (1, 2, 3, 8 and 14.7) of patient pooled plasma with a high D-dimer concentration. For validation 15 plasma samples from patients with venous thromboembolism were utilized.

Results: A reference regression line through the mean values of D-dimer in samples A-E was calculated and used to harmonize the results of the 15 validation samples. After harmonization the coefficients of variation improved considerably from 89% to 19% (mean values) for validation samples with high D-dimer concentration. For validation samples with low D-dimer concentration (normal or around cut-off) coefficients of variation even increased from a mean value of 86% before harmonization to a mean value of 224% after harmonization.

Conclusions: The harmonization procedure considerably decreased variability between different D-dimer assays in samples with a high D-dimer concentration. However, for samples with D-dimer concentration around cut-off, which is critical for clinical application, no improvement was observed.

Key words: D-dimer, harmonization, validation

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Uvod

Koncept mjerenja koncentracije D-dimera kao indikatora aktivacije koagulacije *in vivo* predstavljen je prije više od 20 godina (1,2). Danas su mjerenja koncentracije D-dimera postala bitan element u dijagnostici duboke venske tromboze i plućne embolije (3-6).

Primjećeno je da rezultati različitih brojčanih testova za određivanje D-dimera značajno variraju (7). Uglavnom su tome uzrok heterogenost fibrinskih razgradnih produkata u uzorcima bolesnika (8), kao i specifičnost različitih antitijela korištenih u tim testovima (9). Testovi za određivanje D-dimera pokazuju različitu reaktivnost prema različitim vrstama fibrinskih derivata, kao što su npr. visoko- i nisko molekularni fibrinski derivati, ili unakrsnu reaktivnost s nepoprečno povezanim fibrinogenskim i fibrinskim razgradnim produktima. Nedostatak standardizacije i različiti tipovi kalibratora koje koriste proizvođači dodatni su čimbenici koji utječu na rezultate testova (10).

U pokušaju standardiziranja testova za određivanje D-dimera koristili su se različiti standardi kao npr. pročišćeni D-dimer, lizat pune krvi i x-oligomeri, ali neuspješno (11,12). Stoga se čini da je druga mogućnost standardizacije harmonizacija testova za D-dimere za koju se kao referentna priprema koristi smjesa (engl. *pool*) plazme bolesnika s visokom koncentracijom D-dimera (13).

Ovaj se pristup harmonizaciji rezultata kvantitativnih testova za D-dimere koristeći smjesu plazme bolesnika s visokom koncentracijom D-dimera (14) koristio u Sveučilišnom kliničkom centru Ljubljana, gdje se u nekoliko laboratorija različitih klinika provodi određivanje D-dimera. Pretpostavljalo se da će postupak harmonizacije povećati usporedivost rezultata D-dimera dobivenih u različitim laboratorijima istog medicinskog instituta.

Materijali i metode

Priprema plazme

Za pripremu smjese normalne plazme krv je bila sakupljena od 25 naizgled zdravih dobrovoljaca, uglavnom osoblja laboratorija i studenata. Izvađena krv iz kubitalne vene stavljena je u vakutejnere od 5 mL koji su sadržavali 0,5 mL natrijevog citrata koncentracije 0,109 mol/L (Becton Dickinson, Vacutainer System Europe, Heidelberg, Njemačka), dobro promješana s antikoagulansom, pohranjena odmah u ledenu vodu i centrifugirana 30 minuta na +4 °C i 2000 x g unutar 4 sata od vađenja krvi. Plazma je bila izvađena, pomiješana, alikvotirana, zamrznuta u tekućem dušiku i pohranjena na -70 °C do analize. Za pripremu harmoniziranih uzoraka pohranjeni su uzorci plazme 25 bolesnika s venskom tromboembolijom i visokom koncentracijom D-dimera (znatno iznad granične vrijednosti) bili odmrznuti i pomiješani (smjesa plazme bolesnika). U tim se uzorcima koncentracija D-dimera odredila Auto-Di-

Introduction

The concept of D-dimer measurement as an indicator of *in vivo* coagulation activation has been introduced more than two decades ago (1,2). Today, measurement of D-dimer has become an essential element in the diagnostics of deep vein thrombosis and pulmonary embolism (3-6).

It has been observed that the numerical assay results of different D-dimer assays vary considerably (7). This is mainly caused by the heterogeneity of fibrin degradation products in patient samples (8), as well as the specificity of the different antibodies used in these assays (9). D-dimer assays show different reactivity to different kinds of fibrin derivatives, such as high or low molecular weight fibrin derivatives, or cross-reactivity with non-cross-linked fibrinogen- and fibrin degradation products. Lack of standardization and different types of calibrators used by the manufacturers are additional issues that affect the assay results (10).

In an attempt to standardize D-dimer assays various standards such as purified D-dimer, whole blood lysate and X-oligomers have been used, but failed (11,12). Therefore, harmonization of D-dimer assays by using patient pooled plasma with a high D-dimer concentration as a reference preparation seems to be another option (13).

This approach to harmonization of quantitative D-dimer results by the use of patient pooled plasma with a high D-dimer value (14) was used at the University Medical Centre Ljubljana, where D-dimer assays are performed in several laboratories affiliated to different clinical departments. It was presumed that the harmonization procedure would increase the comparability of D-dimer results obtained in different laboratories of the same medical institution.

Materials and Methods

Plasma preparation

For preparation of normal pooled plasma, blood was collected from 25 apparently healthy volunteers, mainly laboratory staff and students. Blood from the cubital vein was collected into 5 mL vacuum tubes containing 0.5 mL of 0.109 mol/L sodium citrate (Becton Dickinson, Vacutainer System Europe, Heidelberg, Germany), thoroughly mixed with the anticoagulant, placed immediately in ice water and centrifuged at 4 °C and 2000 x g for 30 minutes within 4 hours of venepuncture. Plasma was removed, pooled, aliquotted, frozen in liquid nitrogen and stored at -70 °C until analysis. For preparation of harmonization samples, stored plasma samples from 25 patients with venous thromboembolic disease and high D-dimer concentration (well above the cut-off level) were thawed and pooled (patient pooled plasma). In these samples D-dimer was determined by Auto-Dimer (Biopool Trinity

merom (Biopool Trinity Biotech, Umea, Švedska). Set od pet harmoniziranih uzoraka plazme s rastućom koncentracijom D-dimera pripremljen je miješanjem smjese normalne plazme s rastućim udjelom (1, 2, 3, 8 i 14,7) smjese plazme bolesnika (harmonizirani uzorci A-E). Uzorci su alikvotirani i zamrznuti u tekućem dušiku. Miješanje 6,35 mL smjese normalne plazme s 0,15 mL smjese plazme bolesnika (uzorak A) je određeno kao jedan udio. Za validaciju postupka harmonizacije korišteno je 15 pohranjenih uzoraka plazme (različite od one korištene za harmonizirane uzorke) s različitom koncentracijom D-dimera od bolesnika s venskom tromboembolijom (validirani uzorci V01-V15). Zamrznuti alikvoti smjese normalne plazme, harmonizirani uzorci A-E i validirani uzorci V01–V15 dopremljeni su laboratorojima koji su sudjelovali u istraživanju na Sveučilišnom kliničkom centru Ljubljana. Nakon odmrzavanja uzorci su se analizirali u roku od dva sata. Svi su uzorci plazme bolesnika korišteni za harmonizaciju i validaciju dobiveni na isti način kao što je opisano za smjesu normalne plazme.

Metode

Pet je laboratorija Sveučilišnog kliničkog centra Ljubljana sudjelovalo u istraživanju. Pet različitih kvantitativnih testova za određivanje D-dimera koristilo se rutinski ili samo za ovo istraživanje: Vidas D-dimer Exclusion on a Vidas analyser (oba od proizvođača: BioMerieux, Durham, North Carolina, SAD, laboratorij 1), Auto-Dimer (Biopool Trinity Biotech, Umea, Švedska, laboratorij 1) i D-dimer Plus (Dade Behring, Marburg/Lahn, Njemačka) na Behring Coagulation Timer (BCT, Dade Behring, Marburg/Lahn, Njemačka, laboratorij 1 i laboratorij 4) i na Behring Coagulation System (BCS, Dade Behring, Marburg/Lahn, Njemačka, laboratorij 2), Acute Care™ D-Dimer na Stratus CS (oba od proizvođača: Dade Behring, Marburg/Lahn, Njemački, laboratorij 1 and laboratorij 3), Hemosil D-dimer test na ACL 7000 (laboratorij 4) i na ACL 9000 (svi od proizvođača: Instrumentation Laboratory, Warrington, Ujedinjeno Kraljevstvo, laboratorij 5).

Postupak harmonizacije i validacije te drugi izračuni

Svi su laboratoriji tri puta testrali smjesu normalne plazme i harmonizirane uzorke A-E. Izračunate su srednje vrijednosti svakog testa posebno (Tablica 1.) i korigirane za udio D-dimera u smjesi normalne plazme. Korištena je regresijska analiza kako bi se izračunali regresijski pravci svakog testa i opći referentni regresijski pravac kroz medijane svakog testa te cjelokupni medijan posebno za svaki uzorak (Tablica 2.).

Validirani uzorci V01-V15 testirani su dva puta i rezultati su iskazani kao srednje vrijednosti. Rezultati su harmonizirani kao što je prethodno opisano (14) prema nagibu (a) i odsječku na osi y (b) regresijskog pravca za svaki test (m)

Biotech, Umea, Sweden). A set of five harmonization plasma samples with increasing concentrations of D-dimer were prepared by mixing normal pooled plasma with increasing parts (1, 2, 3, 8 and 14.7) of patient pooled plasma (harmonization samples A-E), aliquotted and frozen in liquid nitrogen. Mixing of 6.35 mL of normal pooled plasma with 0.15 mL of patient pooled plasma (sample A) was stated as one part. For validation of the harmonization procedure, 15 stored plasma samples (others than those as for harmonization samples) with a different concentration of D-dimer from patients with venous thromboembolism (validation samples V01-V15) were utilized. Frozen aliquots of normal pooled plasma, harmonization samples A-E and validation samples V01-V15 were transported to participating laboratories of the University Medical Centre Ljubljana. After thawing, samples were analysed within two hours. All patient plasma samples used for harmonization and validation were obtained according to the same protocol as described for normal pooled plasma.

Methods

Five laboratories from the University Medical Centre Ljubljana participated in the study. Five different quantitative D-dimer assays were used as routinely or for this exercise only: Vidas D-dimer Exclusion on a Vidas analyser (both bioMerieux, Durham, North Carolina, USA, Laboratory 1), Auto-Dimer (Biopool Trinity Biotech, Umea, Sweden, Laboratory 1) and D-dimer Plus (Dade Behring, Marburg/Lahn, Germany) on a Behring Coagulation Timer (BCT, Dade Behring, Marburg/Lahn, Germany, Laboratory 1 and Laboratory 4) and on a Behring Coagulation System (BCS, Dade Behring, Marburg/Lahn, Germany, Laboratory 2), Acute Care™ D-Dimer on a Stratus CS (both Dade Behring, Marburg/Lahn, Germany, Laboratory 1 and Laboratory 3), Hemosil D-dimer test on an ACL 7000 (Laboratory 4) and an ACL 9000 (all Instrumentation Laboratory, Warrington, United Kingdom, Laboratory 5).

Harmonization and validation procedure and other calculations

All laboratories tested normal pooled plasma and harmonization samples A-E in triplicate. Assay-specific mean values were calculated (Table 1) and corrected for the contribution of the D-dimer in normal pool plasma. Regresion analysis was utilized to calculate assay-specific regression lines and an overall reference regression line through assay-specific median values and overall median value for each sample, respectively (Table 2).

Validation samples V01-V15 were tested in duplicate and reported as means. Results were harmonized as described previously (14) according to the slope (a) and intercept (b) of the assay-specific regression line (m) and referen-

Tablica 1. Koncentracija D-dimera pojedinog testa (μg/L) u smjesi normalne plazme (NPP) i kod harmoniziranih uzoraka A–E.

Table 1. Assay specific mean D-dimer levels (μg/L) in normal pooled plasma (NPP) and in harmonization samples A–E.

		Harmonization Sample				
Method	**NPP**	**A**	**B**	**C**	**D**	**E**
Parts of patient pooled plasma in NPP	0	1	2	3	8	14.7
Vidas D-dimer Exclusion	298	394	1204	1159	2529	4280
Auto-Dimer	75	160	352	406	854	1464
D-Dimer Plus	140	39	80	104	248	471
Acute Care D-dimer	176	756	1463	1781	3825	7405
Hemosil D-dimer	185	121	444	337	782	1777
Overall median value	**176**	**160**	**444**	**406**	**854**	**1777**

Tablica 2. Nagib, odsječak na osi y i koeficijenti linearne regresije kroz vrijednosti pojedinačnog testa te sveukupni medijan.

Table 2. The slope, intercept and regression coefficients of the linear regression analysis through the assay-specific values and the overall reference median line.

Method	**Slope μg/L**	**Intercept μg/L**	**Regression coefficient**
Vidas D-dimer Exclusion	266.9	381.2	0.992
Auto-Dimer	91.7	120.7	0.997
D-Dimer Plus	31.0	10.4	0.999
Acute Care D-dimer	472.0	336.6	0.998
Hemosil D-dimer	112.4	47.6	0.980
Overall reference median line	**89.8**	**132.8**	**0.990**

i referentnog regresijskog pravca cijelokupnog medijana (h) prema formuli:

$$DD_h = a_h \times \left(\frac{DD_m - b_m}{a_m} \right) - b_h$$

Npr. harmonizirana je vrijednost uzorka V08 određena Vidas D-dimer Exclusion testom (Tabelica 3.) izračunata kao:

$$2037\,\mu g/L = 89{,}8 \times \left(\frac{6831\,\mu g/L - 381{,}2}{266{,}9} \right) - 132{,}8$$

gdje je 6831 μg/L bio sirovi rezultat za uzorak V08 dobiven testom Vidas D-dimer Exclusion, a 2037 μg/L je izračunato kao harmonizirani rezultat.
Varijabilnost rezultata izražena je u postotku kao koeficijent varijacije. Za sva se računanja koristio Excel 97 (Microsoft Corporation, Orlando, Florida, SAD).

ce regression line of the overall median values (h) according to the formula:

$$DD_h = a_h \times \left(\frac{DD_m - b_m}{a_m} \right) - b_h$$

For example, the harmonized value of sample V08 determined with Vidas D-dimer Exclusion (Table 3) was calculated as:

$$2037\,\mu g/L = 89.8 \times \left(\frac{6831\,\mu g/L - 381.2}{266.9} \right) - 132.8$$

where 6831 μg/L was the raw result for sample V08 obtained with Vidas D-dimer Exclusion and 2037 μg/L was calculated as the harmonized result.
The variability of the results was expressed in percent as the coefficient of variation. Excel 97 (Microsoft Corporation, Orlando, Florida, USA) was used for all calculations.

Rezultati

Tablica 1 pokazuje dobivenu srednju vrijednost koncentracije D-dimera (μg/L) kod harmoniziranih uzoraka A-E za svaki test. Vrijednosti smjese normalne plazme dobivene svakim testom oduzete od sirovih podataka vrijednosti uzoraka A-E također su prikazane u Tablici 1. U Tablici 2 prikazani su nagib, odsječak na osi y i koeficijenti regresije linearne regresijske analize kroz vrijednosti svakog testa te cjelokupni medijan.

Najviša koncentracija D-dimera registrirana je s testom Acute Care™ D-Dimer na instrumentu Stratus CS, dok su približno 18-26 puta niže vrijednosti koncentracije bile zabilježene testom D-dimer Plus na instrumentu BCT ili BCS, a ostale vrijednosti koncentracije D-dimera ostale su između.

Kao što je očekivano, rezultati mjerenja koncentracije D-dimera u validacijskim uzorcima V01-V15 različitim testovima pokazali su značajnu varijabilnost prije harmonizacije. Općenito, harmonizacija je rezultirala značajno smanjenom varijabilnosti koncentracije D-dimera u validaciji uzoraka plazme V01-V15. Za sve se validacijske uzorke srednja vrijednost koeficijenta varijacije snizila s 89% prije harmonizacije na 60% nakon harmonizacije. Veće sniženje varijacije s 89% na 19% (oba se postotka odnose na srednju vrijednost) zabilježeno je za validacijske uzorke V04-V15 s visokom koncentracijom D-dimera. S druge strane, za tri validacijska uzorka V01-V03 s normalnom koncentracijom D-dimera i onom koja se kreće oko graničnih vrijednosti, koeficijenti varijacije čak su porasli sa srednje vrijednosti od 86% prije harmonizacije na srednju vrijednost od 224% nakon harmonizacije (Tablica 3.).

Rasprava

Ovo je ispitivanje pokazalo da je harmonizacija rezultata kvantitativnih testova za određivanje D-dimera bila moguća sa smjesom plazme bolesnika, no bila je ograničena na visoke koncentracije D-dimera. U području koncentracije oko granične vrijednosti harmonizacija nije smanjila varijabilnost rezultata.

Inicijativa za harmonizacijom potaknuta je činjenicom da se nekoliko testova za određivanje D-dimera u istom medicinskom institutu. Budući da rezultati dobiveni tim testovima nisu usporedivi, liječenje bolesnika neće biti optimalno, ukoliko liječnici nisu svjesni odstupanja između različitih testova i time različite granične vrijednosti njihovih rezultata. Štoviše, razmjena rezultata za D-dimere između bolnica često je otežana. Preporuča se da se zbog razlike u monoklonalnim antitijelima, tehnologijama testova i kalibraciji, rezultati različitih testova za određivanje D-dimera ne koriste u kliničkim istraživanjima te da se, ne samo u znanstvenim publikacijama, već i u kliničkoj rutini, jasno navodi test koji je korišten kako bi se omogućila odgovarajuća interpretacija izmjerenih koncentracija D-dimera (10).

Results

Mean D-dimer concentrations (μg/L) in harmonization samples A-E for each assay obtained are shown in Table 1. Values of normal pool plasma obtained by each assay, which were deducted from raw values of samples A-E are also shown in Table 1. In Table 2 the slope, intercept and regression coefficients of the linear regression analysis through the assay specific values and the overall median line are shown.

The highest D-dimer values were observed with Acute Care™ D-Dimer on Stratus CS, while approximately 18-26 times lower values were determined with D-dimer Plus on a BCT or BCS, with other D-dimer concentrations in between.

As expected, results of D-dimer measurements in validation samples V01-V15 with different assays showed considerable variability before harmonization. In general, harmonization resulted in significantly reduced variability in D-dimer concentration in validation plasma samples V01-V15. For all validation samples the mean coefficient of variation decreased from 89% before harmonization to 60% after harmonization. A greater reduction in variability from 89% to 19% (both mean values) was observed for validation samples V04-V15 with high D-dimer concentrations. On the other hand, for 3 validation samples V01-V03 with normal and D-dimer concentrations around cut-off the coefficients of variation even increased from a mean value of 86% before harmonization to mean value of 224% after harmonization (Table 3).

Discussion

The present study showed, that harmonization of results of quantitative D-dimer assays with patient pooled plasma was possible, but was limited to high D-dimer values. In the range of D-dimer around the cut-off, harmonization did not decrease the variability of the results.

The initiative for harmonization was provoked by the fact that in the same medical institution several D-dimer assays are used. Since the results obtained with these assays are not comparable, management of patients might not be optimal if physicians are not aware of the differences between different assays and therefore the different cut-off values. Moreover, exchange of D-dimer results between different hospitals is hampered. It is recommended that due to differences in monoclonal antibodies, assay technologies, and calibration, the results of different D-dimer assays should not be used in clinical studies, and the assay used should be clearly stated not only in scientific publications but also in clinical routine, to allow appropriate interpretation of D-dimer concentration measured (10).

Tablica 3. Koncentracija D-dimera (μg/L) kod validacijskih uzoraka V01–V15 dobivena različitim testovima prije i poslije harmonizacije. Koeficijenti varijacije (CV) prikazani su za svaki validacijski uzorak.

Table 3. D-dimer levels (μg/L) in validation samples V01-V15 obtained by different assays before and after harmonization. Coefficients of variation (CV) are shown for each validation sample.

Before harmonization															
Validation sample Method	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14	V15
Vidas D-dimer Exclusion	152	321	628	1861	2799	4237	3901	6831	5502	6509	8253	8846	8304	6745	10155
Auto-Dimer	6	62	135	1082	1104	2185	2102	2846	2754	2649	3553	2900	2652	2816	3838
D-Dimer Plus	122	184	145	268	365	679	474	737	677	800	937	845	781	799	1075
Acute Care D-dimer	70	161	580	2772	4253	7526	7571	9387	11797	10252	14904	15074	15632	13229	18279
Hemosil D-dimer	0	9	124	341	821	1798	1718	2440	2049	2269	3416	3159	3286	2514	4008
CV (%)	**96.9**	**81.9**	**80.0**	**83.9**	**86.7**	**82.1**	**87.5**	**79.8**	**96.9**	**85.6**	**89.0**	**94.1**	**97.8**	**95.4**	**92.3**
After harmonization															
Validation sample Method	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14	V15
Vidas D-dimer Exclusion	0	0	0	365	681	1165	1052	2037	1590	1929	2516	2716	2533	2009	3156
Auto-Dimer	0	0	0	808	830	1889	1808	2536	2446	2343	3229	2589	2346	2507	3508
D-Dimer Plus	194	373	261	617	898	1808	1213	1974	1800	2157	2555	2287	2101	2154	2953
Acute Care D-dimer	0	0	0	331	613	1235	1244	1590	2048	1754	2640	2672	2778	2321	3282
Hemosil D-dimer	0	0	0	102	486	1267	1203	1780	1467	1643	2560	2355	2456	1839	3034
CV (%)	**223.6**	**223.6**	**223.6**	**61.5**	**23.7**	**23.5**	**22.4**	**17.9**	**20.9**	**14.6**	**11.1**	**7.6**	**10.2**	**12.0**	**6.9**

Budući da antigen D-dimera nije homogeni analit, već predstavlja koglomerat fibrinskih derivata različitih veličina i struktura molekula (15), standardizacija testova za određivanje D-dimera je teška (13,16,17). Trenutno se mjerenja D-dimera zasnivaju na referentnim krivuljama svakog pojedinačnog testa, a kalibracija se odabire od svakog pojedinačnog proizvođača. Brojčani se rezultati izražavaju kao koncentracija D-dimera, koja se zasniva na kalibraciji s pročišćenim D-dimerom, ili u jedinicama FEU (engl. *fibrinogen-equivalent units*) koje se zasnivaju na broju pročišćenog fibrinogena korištenog za pripremu poprečno povezanog fibrinskog ugruška, kojega tada plazmin razlaže te se koristi se kao kalibrator. Treća vrsta kalibratora koji koriste neki proizvođači zasniva se na smjesi plazme bolesnika s povišenom koncentracijom D-dimera (10). Budući da su različiti pristupi kalibraciji doveli do promjenjivih rezultata koncentracije D-dimera, konsenzusom je odlučeno da je potreban univerzalni kalibrator. Taj bi kalibrator trebao odražavati heterogenost spojeva koji sadržavaju antigen D-dimera nađen u kliničkim uzorcima krvi, što predstavlja cilj koji još nije postignut (18).

Drugi pristup usporedivim vrijednostima koncentracije D-dimera dobivenih različitim testovima jest harmoni-

Since D-dimer antigen is not a homogeneous analyte, but represents a conglomerate of fibrin derivatives of different molecular sizes and structures (15), standardization of D-dimer assays is difficult (13,16,17). Currently, results of D-dimer measurements are based on assay-specific reference curves, the calibrators being selected by the respective manufacturer. Numerical results are reported as D-dimer concentration, based on calibration with purified D-dimer, or as fibrinogen-equivalent units (FEU), based on the amount of purified fibrinogen used for the preparation of a cross-linked fibrin clot, which is then degraded by plasmin and used as a calibrator. The third kind of calibrators used by some manufacturers are based on pooled plasma from patients with elevated D-dimer concentration (10). Since the different approaches to calibration lead to variable results of D-dimer concentration, a consensus was reached that a common calibrator is needed. This calibrator should reflect the heterogeneity of D-dimer antigen-containing compounds found in clinical blood samples, a goal that has not yet been achieved (18).

Another approach to comparable D-dimer values obtained with different assays is harmonization by the use of

zacija korištenjem smjese plazme bolesnika (13,17). Harmonizacija se zasniva na matematičkoj transformaciji regresijskih pravaca koji prolaze kroz vrijednosti svakog pojedinačnog testa provedenog na setu uzoraka plazmi s različitom koncentracijom D-dimera u referentni regresijski pravac koji prolazi kroz cjelokupnu srednju vrijednosti svih testova koji se harmoniziraju (14,19). Prednost ovog pristupa je da će se u smjesi plazme bolesnika pojaviti sve različite vrste razgradnih produkata fibrina, a ograničenje je da bi se rezultati pojedinačnih bolesnika ili specifičnih grupa bolesnika (npr. bolesnici s dubokom venskom trombozom ili bolesnici s diseminiranom intravaskularnom koagulacijom) mogli ponašati različito. Budući da ne postoji referentni test za određivanje D-dimera, kao nezavisna varijabla koristi se udio smjese plazme bolesnika koja se dodaje smjesi normalne plazme. Vrijednosti pojedinačnih testova korištene u ovom modelu harmonizacije odražavaju odgovor različitih testova na vrstu D-dimera korištenu u smjesi plazme bolesnika. Ako u ovoj harmonizaciji sudjeluje velik broj laboratorija, tada procijenjene i prihvaćene vrijednosti pojedinačnog testa postaju pouzdane aproksimacije takozvanih „analitički točnih vrijednosti" za te uzorke.

U ovom je istraživanju izvedena harmonizacija rezultata različitih testova za određivanje D-dimera kao što je objavljeno (14). U usporedbi s jednom objavljenom harmonizacijom (14) koja je dovela do smanjenja varijabilnosti s oko 75% na 5,5%, u našem je istraživanju smanjenje bilo puno manje (s oko 89% na oko 60%) za sve validirane uzorke, no bilo je znatno bolje za uzorke s visokom koncentracijom D-dimera (V04-V15) (sniženje s oko 89% na 19%). Za uzorke s koncentracijom D-dimera oko i ispod granične vrijednosti (V01-V03), koeficijent varijacije čak je porastao nakon harmonizacije (s oko 86% na oko 224%). U spomenutom, objavljenom istraživanju (14) sudjelovalo je više od 500 laboratorija i korišteno je više od 20 različitih testova. Iako je u postavljanju modela harmonizacije upotrebljeno samo sedam najčešće korištenih testova, broj rezultata bio je bitno viši u usporedbi s našim istraživanjem u kojem je sudjelovalo samo pet laboratorija. Iako se postupak harmonizacije proveden u našem istraživanju ne bi mogao primijeniti u izvještavanju o rezultatima iz raznih laboratorija, on je predstavio prikaz (ne)kompatibilnosti rezultata D-dimer testova koje koristi naš medicinski institut.

Zaključak

Zaključno, usvojeni postupak harmonizacije značajno je poboljšao usporedivost različitih testova za određivanje D-dimera kod uzoraka s visokom koncentracijom D-dimera. Međutim, kod uzoraka s normalnom koncentracijom D-dimera i onom blizu granične vrijednosti nije primjećeno smanjenje varijabilnosti rezultata. Tome je vjerojatno

patient pooled plasma (13,17). Harmonization is based on mathematical transformation of regression lines through the assay-specific values of a set of plasma samples with different D-dimer concentration to a reference regression line through the overall mean values of all the D-dimer assays being harmonized (14,19). The advantage of this approach is that all the different kinds of fibrin degradation products will be present in the patient pooled plasma, and a limitation is that an individual patient or specific patient groups (e.g. patients with deep vein thrombosis or patients with disseminated intravascular coagulation) may behave differently. Because there is no reference assay for D-dimer available, the amount of patient pooled plasma added to normal pooled plasma is used as an independent variable. Assay-specific values used in this harmonization model reflect the response of the different assays to the kind of D-dimer in the patient pooled plasma used. If a large number of laboratories are participating in the harmonization, then the estimated assay-specific consensus values are a reliable approximation to the so-called "analytical true values" for these samples.

In the present study harmonization of test results of different D-dimer assays was performed as published (14). Compared to the harmonization procedure published (14), which lead to a reduction of the variability from about 75% to 5.5%, in our study reduction of variability was much smaller (from about 89% to about 60%) for all validation samples, but was considerably better for samples with high D-dimer concentration (V04-V15) (reduction from about 89% to 19%). For samples with D-dimer concentration around and below the cut-off (V01-V03), the coefficient of variation even increased after harmonization (from about 86% to about 224%). In the published study (14) over 500 laboratories participated using in a total of 20 different assays. Although only the seven most frequently used assays were used in the set-up of the harmonization model, the number of results was considerably greater compared to our study in which only five laboratories participated. Although the harmonization procedure performed in our study could not be adopted to report results from different laboratories, it represented an exercise on the (non)comparability of the D-dimer assays results used in our medical institution.

Conclusion

To conclude, the harmonization procedure adopted considerably improved comparability between different D-dimer assays in samples with high D-dimer concentration. However, for samples with normal D-dimer and D-dimer close to the cut-off value no improvement in variability was observed. This could partly be attributed to the small number of participating laboratories, but also to the small number of harmonization samples in the nor-

djelomično pridonio mali broj laboratorija koji su sudjelovali, ali i mali broj harmoniziranih uzoraka u rasponu od normalnih do oko graničnih vrijednosti koncentracije D-dimera. Budući da su vrijednosti koncentracije D-dimera oko granične ključne za isključivanje dijagnoze duboke venske tromboze i plućne embolije ovaj postupak harmonizacije nije mogao biti usvojen u izvještavanju o rezultatima iz raznih laboratorija.

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mal and around the cut-off ranges. Since D-dimer values around the cut-off are critical for exclusion of deep vein thrombosis or pulmonary embolism, this harmonization procedure could not be adopted to report results from different laboratories.

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