JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2017, 68, 4, 637-645
www.jpp.krakow.pl

L.J. KRZYCH, P.F. CZEMPIK

ASSOCIATION BETWEEN STANDARD LABORATORY AND FUNCTIONAL TESTS OF COAGULATION IN DILUTIONAL COAGULOPATHY: AN *IN VITRO* STUDY

Department of Anaesthesiology and Intensive Care, School of Medicine in Katowice,
Medical University of Silesia, Katowice, Poland

Standard laboratory tests (SLTs) of coagulation are in common use in clinical practice. We aimed to determine the association between SLTs and functional tests of coagulation in blood samples diluted with balanced crystalloid and colloid solutions in an *ex vivo* setting. The study group comprised 32 healthy young male volunteers. Whole blood samples were diluted at a 4:1 ratio with balanced crystalloid (Plasmalyte®) and two balanced colloids, 6% hydroxyethyl starch 130/0.4 (Volulyte®) and succinylated gelatin (Geloplasma®). SLTs included aPTT (activated partial thromboplastin time), PT (prothrombin time), fibrinogen concentration (FIB), D-dimers and number of platelets (PLT). Platelet aggregation was determined using multiple electrode aggregometry (MEA) with TRAP (thrombin receptor activating protein-6) as an assay activator. Coagulation and fibrinolysis were assessed functionally using rotational thromboelastometry (ROTEM). We found correlation between aPTT and INTEM (i.e. intrinsic coagulation pathway screening test) clotting time ($R = 0.38$ to 0.77; $P < 0.05$) for both undiluted and diluted samples. FIB and PLT were shown to be correlated with alpha angle in both INTEM and EXTEM (i.e. extrinsic coagulation pathway screening test) (FIB: $R = 0.38$ to 0.69; $P < 0.05$; PLT: 0.41 to 0.56; $P < 0.05$) again for both undiluted and diluted samples. FIB and PLT were associated with clot formation time in both INTEM and EXTEM (FIB: $R = –0.44$ to $–0.70$; $P < 0.05$; PLT: $–0.36$ to $–0.58$; $P < 0.05$). MEA results shown no correlation with ROTEM findings. There was also no correlation between number of platelets and their function as determined by MEA. Fibrinogen concentration correlated positively with fibrinogen function as determined by FIBTEM (i.e. fibrinogen deficiency/dysfunction screening test) maximum clot firmness ($R = 0.49$ to 0.73; $P < 0.05$). ROTEM results were predominantly associated with fibrinogen concentration and number of platelets. When there is no access to functional tests, concentration of fibrinogen is the most reliable test of coagulation, also in the context of fluid-induced coagulopathy.

Key words: *standard coagulation tests, functional coagulation tests, multiple electrode aggregometry, rotational thromboelastometry*

INTRODUCTION

Standard laboratory tests (SLTs) of coagulation enable us to diagnose coagulation factors deficiency but on most occasions remain blind to functional abnormalities or antibodies against coagulation factors (1). Although SLTs such as activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen concentration (FIB) and number of platelets (PLT) are frequently used to monitor coagulation status in massive bleeding (2), they seem to be inaccurate in the setting of ongoing haemorrhage, especially when hypothermia and fluid resuscitation are involved (3, 4). Rotational thromboelastometry/thromboelastography (ROTEM/TEG) and platelet aggregometry are becoming gold standards in evaluation of coagulation disorders in both bleeding and non-bleeding patients (5). ROTEM/TEG appears to be promising in clinical practice. This method of functional assessment of coagulation and fibrinolysis is characterised by shorter turn-around times, what matters in timely management of coagulopathy. Available data suggest that transfusions of blood products and mortality are decreased when decisions are based on viscoelastic tests rather than on clinical judgement or SLTs alone (6). Cost-effectiveness studies also seem to favour ROTEM/TEG (6). Despite the above-mentioned superiority of functional assessment, SLTs are still in common clinical use. A few factors are responsible: financial constraints, limited access to ROTEM/TEG equipment, and expertise.

Although the association between standard and functional parameters of coagulation has been investigated (7-12), there are still some inconsistent findings as to which ROTEM parameters correlate best with which SLTs (13). Therefore we aimed to investigate this association in an *in vitro* setting of dilutional coagulopathy induced by balanced solutions of one crystalloid and two colloids. We hypothesized that in patients undergoing moderate degree blood dilution with commonly used balanced intravenous solutions, more accessible and less expensive SLTs might be used interchangeably with more informative but also more expensive functional tests of coagulation.

MATERIAL AND METHODS

The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB1/158/15/16). Written informed consent was obtained from 32 American Society of Anesthesiologists (ASA) physical status class I male volunteers, aged 21 – 35 (29 ± 4) years, weighting 59 – 103 (81.2 ± 9.8) kg, with no history of coagulopathy. Females were excluded due to additional blood loss during menstruation and proven impact of hormonal variations on coagulation (14). Additionally women have lower blood volume and haemoglobin level. No drugs were allowed for one week and no alcohol or strenuous physical exercise for one day prior blood sampling.

Sampling

Venous blood samples were drawn *via* a 16 or 18G indwelling cannula from an antecubital vein of a non-dominant arm. Only minimal stasis was allowed to reduce the risk of pre-analytical error (15). First portion of blood (2 mL) was discarded and four blood samples were drawn: baseline (12.5 mL) and one blood sample (10 mL) for mixing with each study solution. Samples were collected in citrated blood tubes (S-Monovette, Sarstedt, Germany) for complete blood count (CBC) and rotational thromboelastometry, and hirudin blood tubes (S-Monovette, Sarstedt, Germany) for platelet aggregometry analysis. Hirudin test tubes were used specifically as alternative anticoagulants have been shown to bias the results of multiple electrode aggregometry (MEA) (16). After collection samples were immediately processed using point-of-care ROTEM machine or sent to the central hospital laboratory for analysis. Samples were delivered to the laboratory by the personnel because pneumatic tube transport systems have been shown to affect determinations made with MEA (17).

Haemodilution

The solutions used in the study included balanced crystalloid (Plasmalyte®, Baxter) (PL), and two balanced colloid solutions, namely 6% hydroxyethyl starch 130/0.4 (Volulyte®, Fresenius Kabi) (VL) and succinylated gelatin (Geloplasma®, Fresenius Kabi) (GEL). Due to growing number of observational studies and randomised controlled trials regarding Plasmalyte® (18-20), we chose this type of balanced crystalloid to make our observations more in line with current practice. Whole blood was diluted at a 4:1 ratio with the study solutions to make an end-concentration of 20 vol.% of each solution, equivalent to an infusion of about 1 L into a 70 kg person. Taking into account mean weight of volunteers (81.2 ± 9.8 kg), the amount of study solution administered in this recreated scenario averaged 15 mL kg^{-1}.

Standard laboratory tests

SLTs performed included: aPTT, PT, FIB (optical Clauss method), D-dimers, PLT. The reference values were 25 – 37s, 10.4 – 13s, 10 – 400 mg dL^{-1}, 0 – 0.05 µg ml^{-1} and 150000 – 350000 $µl^{-1}$ respectively.

Aggregometry

MEA was performed by Multiplate analyser (Roche Diagnostics GmbH, Germany). Thrombin receptor activating peptide-6 (TRAP-6) was used as a platelet activator. Results were given in aggregation units (AU*min). The reference value provided by the laboratory was 941 – 1563 AU*min.

Rotational thromboelastometry

ROTEM coagulation analysis was carried out using a four-channel ROTEM delta analyser (Tem Innovations GmbH, Munich, Germany) and assays were allowed to run for 30 min. In order to minimize a pre-analytical error, blood samples were processed immediately after withdrawal. It has been shown that coagulation test results may change during citrate storage as coagulation might continue to occur (15). We performed the following ROTEM assays: INTEM, EXTEM, FIBTEM. All ROTEM assays were performed by the same investigator according to the manufacturer's instructions. The samples were recalcified with 0.2 M $CaCl_2$ using an electronic pipette system. INTEM, an intrinsic coagulation assay, was initiated by addition of ellagic acid. EXTEM, an extrinsic coagulation assay, was performed by addition of tissue factor. In FIBTEM, screening test for fibrinogen deficiency/dysfunction, we used cytochalasin D as a platelet inhibitor. Parameters measured included: clotting time (CT), clot formation time (CFT), clot formation ratio (CFR), alpha angle (AA), amplitude at different time points (minutes) (A5, A10, A15, A20), maximum clot firmness (MCF). CT is a time from the start of measurement till formation of 20 mm clot. MCF is the maximal firmness of the clot and is influenced by fibrin, platelets and factor XIII concentration. Using the MCF value we calculated maximum clot elasticity (MCE) for EXTEM and FIBTEM assays using the following formula: MCE = 100 × MCF/100 – MCF. Assessment of platelet contribution to clot strength was measured according to formula $\Delta MCE = MCE_{EXTEM} - MCE_{FIBTEM}$. All dilutions were performed by the same investigator. The investigator was not blinded to a test solution used.

Statistical analysis

The statistical analysis was performed using the licensed MedCalc statistical software (version 16.1 2016, MedCalc Software bvba, Belgium). The quantitative variables were presented as a median (interquartile range, IQR) or a mean (± standard deviation, S.D.). The association between SLTs and individual functional tests of coagulation for undiluted and diluted samples was assessed in univariate analysis using Spearman rank coefficient of correlation (R) and was verified by multiple regression. Post-hoc comparisons were performed with the use of Scheffe test or the Conover post-hoc analysis, when appropriate. The level of statistical significance was set at $P < 0.05$.

RESULTS

All fluids caused similar degree of haemodilution, as assessed by the haematocrit levels (*Fig. 1*). Haematocrit dropped statistically significantly from a median of 44.4% to 35.7%, 35.2% and 35.2% for PL, VL and GEL, respectively ($P < 0.05$ for all univariate comparisons).

There was a moderate to strong positive correlation between aPTT and INTEM CT for both undiluted ($R = 0.38$; $P < 0.05$) and diluted samples ($R = 0.64$ to 0.77; $P < 0.05$) (*Table 1*). Those between-method associations were confirmed by multiple regression analyses (*Table 2*). We also found a few individual associations between CT and: PLT ($R = 0.37$ for GEL and $R = 0.52$ for PL), FIB ($R = –0.64$ for VL), and PT ($R = –0.38$ for VL and $R = –0.57$ for GEL) (*Table 1*).

FIB positively correlated with AA, both in INTEM and EXTEM, for both undiluted ($R = 0.36$ and $R = 0.56$, both $P < 0.05$) and diluted samples ($R = 0.46$ to 0.69; $P < 0.05$) (*Table 3*). PLT also positively significantly correlated with AA, both in

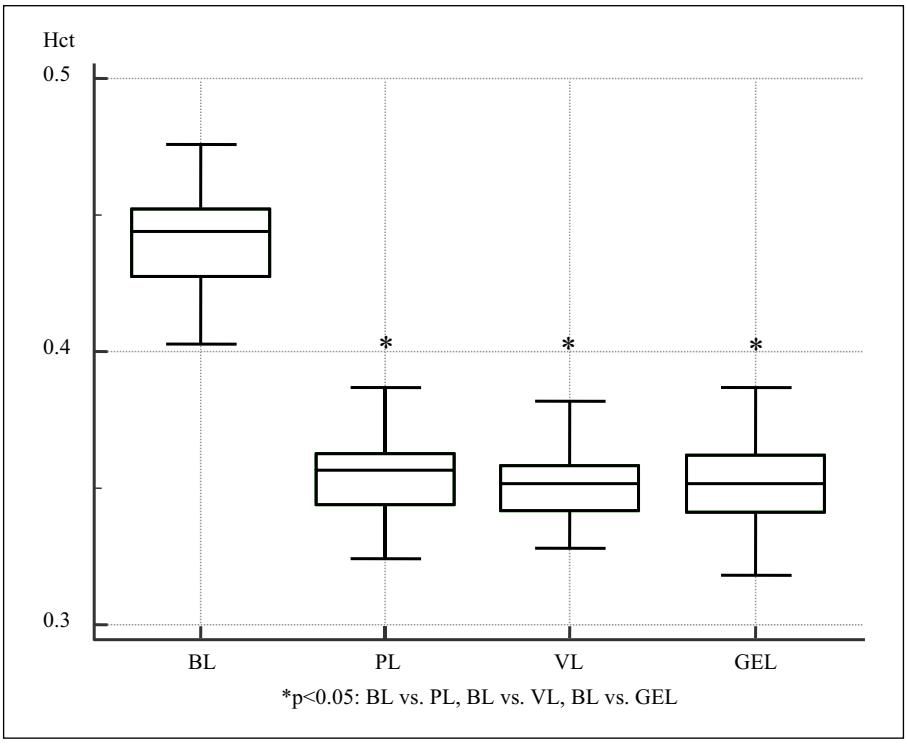

Fig. 1. Impact of study solutions on haematocrit level.
Values given are median (bar), interquartile range (box) and range (whiskers). Post-hoc comparisons assessed by Scheffe test. BL, undiluted sample; GEL, Geloplasma®; Hct, haematocrit level; PL, Plasmalyte®,; VL, Volulyte®.

Table 1. Correlation between standard tests of coagulation and coagulation time in INTEM and EXTEM.

Test solution	INTEM clotting time			
	Fibrinogen	aPTT	Platelets	MEA
Baseline (Undiluted)	0.09	0.38*	0.09	–0.32
Plasmalyte®	–0.13	0.64*	0.52*	0.11
Volulyte®	–0.15	0.76*	0.14	–0.25
Geloplasma®	–0.003	0.77*	0.37*	0.002
	EXTEM clotting time			
	Fibrinogen	PT	Platelets	MEA
Baseline (Undiluted)	0.13	–0.30	–0.25	0.15
Plasmalyte®	–0.23	–0.31	0.13	–0.07
Volulyte®	–0.64*	–0.38*	–0.14	0.17
Geloplasma®	–0.29	–0.57*	0.17	0.03

Values given are Spearman rank correlation coefficients. *P < 0.05; aPTT, activated partial thrombin time; MEA, multiple electrode aggregometry; PT, prothrombin time.

INTEM and EXTEM, for both undiluted (R = 0.56 and R = 0.43) and diluted samples (R = 0.37 to 0.51). Those associations were also confirmed by regression analyses (*Table 4*). FIB and PLT negatively and significantly correlated with CFT, both in INTEM and EXTEM, for both undiluted (FIB: R = –0.44 and R = –0.62 / PLT: R = –0.58 and R = –0.48) and diluted samples (FIB: R = –0.48 to –0.70 / PLT: R = –0.36 to –0.51) (*Table 5*). Regression analyses verified those findings (*Table 6*).

By univariate analyses there were multiple statistically significant correlations between FIB, PLT, D-dimers and A10, both in INTEM and EXTEM, for both undiluted (FIB: R = 0.62 and R = 0.74 / PLT: R = 0.51 and R = 0.43 / D-dimers: R = –0.74 and R = –0.78) and diluted samples (FIB: R = 0.51 to 0.74 / PLT: R =0.37 to 0.46 / D-dimers: R = –0.52 to –0.56) (*Table* 7). Most of them were not confirmed by multiple analyses (*Table 8*).

Although all correlations between FIB and MCF were statistically significant (R = 0.49 to 0.70) only two of them remained of statistical importance in regression analyses (for VL and GEL) (*Table 9* and *10*).

MEA measurements did not correspond with ROTEM findings. There was also no correlation between number of platelets and their function as determined by MEA TRAP (*Table 11*) as well as between MEA TRAP and ROTEM MCE (*Table 12*). Fibrinogen concentration positively statistically significantly correlated with fibrinogen function as determined by FIBTEM MCF in all samples (R = 0.64 to 0.79, P < 0.05) (*Table 13*).

Additionally, we did not find any correlation between D-dimers and maximum lysis (ML) of the clot in FIBTEM assay (*Table 14*).

Table 2. Multiple regression analysis for association between standard laboratory tests of clotting and coagulation time in INTEM and EXTEM.

Test solution	INTEM clotting time			
	Fibrinogen	aPTT	Platelets	MEA
Baseline (Undiluted)	0.13 ± 0.21	2.83 ± 1.70	–0.008 ± 0.18	–0.02 ± 0.03
Plasmalyte®	0.09 ± 0.13	4.35 ± 1.07*	0.43 ± 0.17*	0.03 ± 0.02
Volulyte®	0.03 ± 0.11	5.79 ± 1.12*	–0.09 ± 0.12	0.05 ± 0.02
Geloplasma®	0.09 ± 0.11	5.06 ± 0.84*	0.007 ± 0.13	–0.008 ± 0.02
	EXTEM clotting time			
	Fibrinogen	PT	Platelets	MEA
Baseline (Undiluted)	0.06 ± 0.04	–0.27 ± 0.13*	–0.05 ± 0.03	0.01 ± 0.01*
Plasmalyte®	–0.04 ± 0.03	–0.31 ± 0.18	0.01 ± 0.04	0.01 ± 0.00
Volulyte®	–0.14 ± 0.04*	–0.20 ± 0.14	–0.004 ± 0.03	0.01 ± 0.00
Geloplasma®	0.003 ± 0.03	–0.62 ± 0.15*	0.04 ± 0.03	0.004 ± 0.005

Values given are coefficients of regression ± its standard errors by least squares multiple regression analysis. *P < 0.05; aPTT, activated partial thrombin time; MEA, multiple electrode aggregometry; PT, prothrombin time.

Table 3. Correlation between standard tests of coagulation and alpha angle INTEM and EXTEM.

Test solution	INTEM alpha angle		
	Fibrinogen	Platelets	MEA
Baseline (Undiluted)	0.38*	0.56*	–0.13
Plasmalyte®	0.46*	0.18	0.14
Volulyte®	0.69*	0.49*	0.16
Geloplasma®	0.54*	0.41*	0.09
	EXTEM alpha angle		
	Fibrinogen	Platelets	MEA
Baseline (Undiluted)	0.56*	0.43*	–0.09
Plasmalyte®	0.53*	0.40*	0.27
Volulyte®	0.63*	0.51*	0.16
Geloplasma®	0.48*	0.37*	–0.02

Values given are Spearman rank correlation coefficients; *P < 0.05; MEA, multiple electrode aggregometry.

Table 4. Multiple regression analysis for association between standard laboratory tests of coagulation and alpha angle in INTEM and EXTEM.

Test solution	INTEM alpha angle		
	Fibrinogen	Platelets	MEA
Baseline (Undiluted)	0.02 ± 0.01	0.04 ± 0.01*	–0.002 ± 0.002
Plasmalyte®	0.03 ± 0.01*	0.03 ± 0.02	–0.0008 ± 0.002
Volulyte®	0.08 ± 0.02*	0.04 ± 0.02*	–0.001 ± 0.003
Geloplasma®	0.05 ± 0.01*	0.04 ± 0.01*	0.003 ± 0.002
	EXTEM alpha angle		
	Fibrinogen	Platelets	MEA
Baseline (Undiluted)	0.05 ± 0.02*	0.03 ± 0.01*	–0.0007 ± 0.003
Plasmalyte®	0.04 ± 0.01*	0.05 ± 0.01*	–0.00 ± 0.00
Volulyte®	0.07 ± 0.01*	0.03 ± 0.01*	0.00 ± 0.00
Geloplasma®	0.04 ± 0.01*	0.04 ± 0.01*	0.00 ± 0.00

Values given are coefficients of regression ± its standard errors by least squares multiple regression analysis; *P < 0.05; MEA, multiple electrode aggregometry.

Table 5. Correlation between standard tests of coagulation and clot formation time in INTEM and EXTEM.

Test solution	INTEM clot formation time		
	Fibrinogen	Platelets	MEA
Baseline (Undiluted)	–0.44*	–0.58*	0.13
Plasmalyte®	–0.48*	–0.23	–0.14
Volulyte®	–0.70*	–0.48*	–0.13
Geloplasma®	–0.57*	–0.36*	–0.08
	EXTEM clot formation time		
	Fibrinogen	Platelets	MEA
Baseline (Undiluted)	–0.62*	–0.48*	0.08
Plasmalyte®	–0.55*	–0.39*	–0.22
Volulyte®	–0.66*	–0.51*	–0.16
Geloplasma®	–0.50*	–0.38*	0.02

Values given are Spearman rank correlation coefficients; *P < 0.05; MEA, multiple electrode aggregometry.

Table 6. Multiple regression analysis for association between standard laboratory tests of coagulation and clot formation time in INTEM and EXTEM.

Test solution	INTEM clot formation time		
	Fibrinogen	Platelets	MEA
Baseline (Undiluted)	–0.15 ± 0.08	–0.21 ± 0.07*	0.01 ± 0.01
Plasmalyte®	–0.21 ± 0.07*	–0.19 ± 0.09*	0.00 ± 0.01
Voluluye®	–0.55 ± 0.09*	–0.26 ± 0.10*	0.01 ± 0.02
Geloplasma®	–0.32 ± 0.08*	–0.27 ± 0.09*	–0.01 ± 0.01
	EXTEM clot formation time		
	Fibrinogen	Platelets	MEA
Baseline (Undiluted)	–0.28 ± 0.07*	–0.18 ± 0.06*	0.00 ± 0.01
Plasmalyte®	–0.24 ± 0.06*	–0.26 ± 0.07*	0.00 ± 0.01
Volulyte®	–0.41 ± 0.09*	–0.21 ± 0.09*	0.00 ± 0.02
Geloplasma®	–0.27 ± 0.08*	–0.24 ± 0.09*	–0.00 ± 0.01

Values given are coefficients of regression ± its standard errors by least squares multiple regression analysis; *P < 0.05; MEA, multiple electrode aggregometry.

Table 7. Correlation between standard tests of coagulation and amplitude in 10 min in INTEM and EXTEM.

Test solution	INTEM amplitude in 10 min			
	Fibrinogen	Platelets	MEA	D-dimers
Baseline (Undiluted)	0.62*	0.51*	–0.13	–0.74*
Plasmalyte®	0.51*	0.37*	0.30	–0.50
Volulyte®	0.70*	0.43*	0.09	–0.52*
Geloplasma®	0.54*	0.30	0.20	–0.14
	EXTEM amplitude in 10 min			
	Fibrinogen	Platelets	MEA	D-dimers
Baseline (Undiluted)	0.74*	0.43*	–0.05	–0.78*
Plasmalyte®	0.52*	0.43*	0.34	–0.56*
Volulyte®	0.74*	0.46*	0.08	–0.54*
Geloplasma®	0.55*	0.29	0.05	–0.19

Values given are Spearman rank correlation coefficients; *P < 0.05; MEA, multiple electrode aggregometry.

Table 8. Multiple regression analysis for association between standard laboratory tests of coagulation and amplitude in 10 min in INTEM and EXTEM.

Test solution	INTEM amplitude in 10 min			
	Fibrinogen	Platelets	MEA	D-dimers
Baseline (Undiluted)	0.06 ± 0.05	0.04 ± 0.02	0.00 ± 0.01	1.87 ± 15.80
Plasmalyte®	0.03 ± 0.02	0.06 ± 0.03	0.00 ± 0.00	–0.24 ± 2.31
Volulyte®	0.05 ± 0.02*	0.06 ± 0.02*	0.00 ± 0.00	1.51 ± 2.23
Geloplasma®	0.04 ± 0.03	0.02 ± 0.03	0.00 ± 0.00	–1.76 ± 3.66
	EXTEM amplitude in 10 min			
	Fibrinogen	Platelets	MEA	D-dimers
Baseline (Undiluted)	0.11 ± 0.07	0.03 ± 0.03	0.00 ± 0.01	–4.65 ± 22.46
Plasmalyte®	0.03 ± 0.02	0.07 ± 0.02*	0.00 ± 0.00	–0.47 ± 1.96
Volulyte®	0.08 ± 0.03*	0.05 ± 0.02	0.00 ± 0.00	1.05 ± 2.87
Geloplasma®	0.07 ± 0.02*	0.04 ± 0.03	0.00 ± 0.00	0.01 ± 3.44

Values given are coefficients of regression ± its standard errors by least squares multiple regression analysis; *P < 0.05; MEA, multiple electrode aggregometry.

Table 9. Correlation between standard tests of coagulation and maximum clot firmness in INTEM and EXTEM.

Test solution	INTEM maximum clot firmness			
	Fibrinogen	Platelets	MEA	D-dimers
Baseline (Undiluted)	0.60*	0.32	–0.19	–0.76*
Plasmalyte®	0.56*	0.27	0.29	–0.46
Volulyte®	0.65*	0.37*	0.18	–0.48
Geloplasma®	0.59*	0.18	0.20	–0.09
	EXTEM maximum clot firmness			
	Fibrinogen	Platelets	MEA	D-dimers
Baseline (Undiluted)	0.70*	0.33	–0.15	–0.83*
Plasmalyte®	0.49*	0.36*	0.36*	–0.59
Volulyte®	0.73*	0.41*	0.187	–0.47
Geloplasma®	0.63*	0.21	0.11	–0.20

Values given are Spearman rank correlation coefficients; *P < 0.05; MEA, multiple electrode aggregometry.

Table 10. Multiple regression analysis for association between standard laboratory tests of coagulation and maximum clot firmness in INTEM and EXTEM.

Test solution	INTEM maximum clot firmness			
	Fibrinogen	Platelets	MEA	D-dimers
Baseline (Undiluted)	0.03 ± 0.08	0.00 ± 0.04	–0.00 ± 0.01	–18.81 ± 28.73
Plasmalyte®	0.03 ± 0.03	0.07 ± 0.04	0.00 ± 0.00	0.02 ± 3.43
Volulyte®	0.04 ± 0.02	0.05 ± 0.02*	0.01 ± 0.00	2.54 ± 2.48
Geloplasma®	0.05 ± 0.03	0.03 ± 0.03	0.00 ± 0.00	0.92 ± 4.14
	EXTEM maximum clot firmness			
	Fibrinogen	Platelets	MEA	D-dimers
Baseline (Undiluted)	0.07 ± 0.08	–0.00 ± 0.04	–0.00 ± 0.01	–23.20 ± 28.73
Plasmalyte®	0.02 ± 0.02	0.06 ± 0.03	0.00 ± 0.00	–0.43 ± 2.70
Volulyte®	0.07 ± 0.02*	0.05 ± 0.02*	0.01 ± 0.00	2.34 ± 2.45
Geloplasma®	0.06 ± 0.02*	0.04 ± 0.03	0.00 ± 0.00	1.13 ± 3.38

Values given are coefficients of regression ± its standard errors by least squares multiple regression analysis; *P < 0.05; MEA, multiple electrode aggregometry.

Table 11. Correlation between number of platelets and function of platelets as evidenced by multiple electrode aggregometry.

Test solution		Baseline (Undiluted)	Plasmalyte®	Volulyte®	Geloplasma ®
		Number of platelets			
Baseline (Undiluted)	MEA TRAP	0.44	0.03	0.05	0.18
Plasmalyte®		0.21	0.26	0.31	0.19
Volulyte®		0.19	0.20	0.29	–0.03
Geloplasma®		0.09	0.16	0.17	0.16

Values given are Spearman rank correlation coefficients; All P > 0.05 (non-significant); MEA, multiple electrode aggregometry, TRAP, thrombin receptor activating protein-6.

Table 12. Correlation between function of platelets as evidenced by multiple electrode aggregometry TRAP and maximum clot elasticity as evidenced by ROTEM.

Test solution		Baseline (Undiluted)	Plasmalyte®	Volulyte®	Geloplasma®
		Maximum clot elasticity			
Baseline (Undiluted)	MEA TRAP	0.05	–0.03	–0.13	–0.13
Plasmalyte®		0.28	0.38*	0.28	0.36*
Volulyte®		0.22	0.28	0.18	0.24
Geloplasma®		0.34	0.23	0.07	0.13

Values given are Spearman rank correlation coefficients; *P < 0.05; MEA, multiple electrode aggregometry; TRAP, thrombin receptor activating protein-6.

DISCUSSION

In this experimental *in vitro* study we performed comprehensive analyses of relationships between commonly used standard plasma based laboratory tests and whole blood ROTEM and MEA functional parameters of coagulation for undiluted blood samples and samples mixed with study solutions. We failed to prove that standard tests are equally useful as functional tests of coagulation in patients undergoing moderate blood dilution with balanced intravenous solutions. However, fibrinogen concentration may serve as the best indicator of coagulation abnormalities: fibrinogen concentration obtained using both the Claus method and the ROTEM FIBTEM test would produce similar results.

We also confirmed that AA and CFT were influenced by number of platelets. From a practical point of view, the strength of our study is to show that platelet number and fibrinogen concentration constitute good alternatives to ROTEM tests in diagnosing coagulopathy.

Functional assessment of coagulation is based on viscoelastic tests performed on a whole blood sample, usually as point-of-care thromboelastometry or thromboelastography. The turnaround time for SLTs is significantly longer compared to the former tests, in certain environments exceeding one hour (21) or even reaching 108 min (22), whereas for functional tests first results are obtained after approximately 10 min after blood withdrawal (23). Whole blood functional tests of coagulation give insight into the clot formation phase and beyond, which is dependent on coagulation factors, platelets and a variety of substances affecting their function (24), local pH, temperature, concentration of ionic calcium, whereas standard tests measure coagulation solely up to the moment of clot formation (25). The viscoelastic tests provide comprehensive information on activation, propagation and resolution of the clot, important issues in management of acute traumatic coagulopathy (26).

Our findings are in line with previous studies. Rotational thromboelastometry has diagnostic accuracy of at least 70% in diagnosing thrombocytopenia and fibrinogen deficiency (27). Fast assessment of clot amplitude, obtainable as soon as after 5 min, may be powerful in this context (28). As showed by Vucelic *et al*., FIBTEM-MCF might help diagnose fibrinogen deficiency or derangements of fibrin polymerization (29), and there might be poor correlation between other standard and functional parameters of coagulation. Hagemo *et al*. showed good sensitivity of fibrinogen concentration as determined by Clauss method in diagnosing acute traumatic coagulopathy and risk of massive bleeding (sensitivity of 84.2% for fibrinogen concentration threshold of 1.48 g/L), performing comparable with EXTEM amplitude after 5 min (A5) (sensitivity of 72.7% for A5 ≤ 40mm) and FIBTEM maximum clot firmness (MCF) (sensitivity of 77.5% for MCF ≤ 7mm), the most representative ROTEM parameters of acute traumatic coagulopathy (30). In cardiac surgery it was confirmed that FIBTEM-derived measures correlated with fibrinogen concentration (31, 32). Apart from fibrinogen concentration also other SLTs, namely International Normalized Ratio (INR), may be used to stratify the risk of massive transfusion, defined by the authors as a need to transfuse at least two red blood cells packages over 24 hours (23).

Additionally, we showed differences between test solutions, as far as correlations between SLTs and functional parameters of coagulation are concerned. The positive correlation coefficient between aPTT and INTEM CT as well as between fibrinogen concentration and INTEM AA was stronger for synthetic colloids compared to balanced crystalloid. The negative correlation between fibrinogen concentration and INTEM CFT was stronger for synthetic colloids compared to balanced crystalloid.

Study limitations

Several limitations should be taken into account in data interpretation. Firstly, in this *ex vivo* setting we were unable to assess real impact of fluids on individual haemostasis, which may significantly differ between patients, is dynamic and multifactorial in nature. Hemostasis in general might be affected by a plethora of factors, both acquired and genetic (33). Secondly, thrombin generation during the clot initiation phase remain directly related to fibrin formation and activation of platelets, however in dilutional coagulopathy it may differ between individuals (34). Formation of thrombus is dependent on balance between prothrombotic factors and fibrinolysis (35). Therefore, our *in vitro* results should be interpreted with caution and an *in vivo* assessment should be performed to verify this interesting effect. Additionally, the dose of solution we used (15 mL/kg) might have been insufficient be insufficient to detect differences that we used between solutions. Noteworthy, changes in haematocrit may affect the correlation between plasma FIB and FIBTEM MCF results (36, 37). We did not assessed the effect of 0.9% NaCl on coagulation, which is the most commonly used solution and may differ both qualitatively and quantitatively (37). Finally, we recruited only men as study subjects, what might have implications when trying to apply the results to female population.

ROTEM results are influenced mainly by fibrinogen concentration and platelet count. When there is no access to functional tests (ROTEM/TEG), concentration of fibrinogen is the most reliable test of coagulation, also in the context of fluid-induced coagulopathy. From the practical point of view, the strength of our study is to show that fibrinogen concentration and number of platelets have maintained its diagnostic role in the setting of dilutional coagulopathy.

Acknowledgements: The study was supported by the 2015 annual grant of the Polish Cardiac Society funded by the Adamed corporation (granted to LJK).

Conflict of interests: None declared.

REFERENCES

1. Harris NS, Winter WE, Ledford-Kraemer MR. Hemostasis: A review & methods of assessment in the clinical laboratory. In: Contemporary Practice in Clinical Chemistry, Clarke W (ed). Washington DC. AACC Press, 2011, pp. 265-283.
2. Spahn DR, Bouillon B, Cerny V, *et al*. Management of bleeding and coagulopathy following major trauma: an updated European guideline. *Crit Care* 2013; 17: R76.
3. Martini WZ, Cortez DS, Dubick MA, Park MS, Holcomb JB. Thrombelastography is better than PT, aPTT, and activated clotting time in detecting clinically relevant clotting abnormalities after hypothermia, hemorrhagic shock and resuscitation in pigs. *J Trauma* 2008; 65: 535-543.
4. Spahn DR, Rossaint R. Coagulopathy and blood component transfusion in trauma. *Br J Anaesth* 2005; 95: 130-139.
5. Kozek-Langenecker SA, Afshari A, Albaladejo P, *et al*. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology. *Eur J Anaesthesiol* 2013; 30: 270-382.4d5b.
6. Shen L, Tabaie S, Ivascu N. Viscoelastic testing inside and beyond the operating room. *J Thorac Dis* 2017; 9 (Suppl. 4): S299-S308.
7. Fluger I, Maderova K, Simek M, Hajek R, Zapletalova J, Lonsky V. Comparison of functional fibrinogen assessment using thromboelastography with the standard von Clauss method. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2012; 156: 260-261.
8. Karlsson O, Jeppsson A, Hellgren M. Major obstetric haemorrhage: monitoring with thromboelastography, laboratory analyses or both? *Int J Obstet Anesth* 2014; 23: 10-17.
9. Karlsson O, Sporrong T, Hillarp A, Jeppsson A, Hellgren M. Prospective longitudinal study of thromboelastography and standard hemostatic laboratory tests in healthy women during normal pregnancy. *Anesth Analg* 2012; 115: 890-898.
10. Thomas O, Rein H, Strandberg K, Schott U. Coagulative safety of epidural catheters after major upper gastrointestinal surgery: advanced and routine coagulation analysis in 38 patients. *Perioper Med* (*Lond*) 2016; 5: 28.
11. Kander T, Larsson A, Taune V, Schott U, Tynngard N. Assessment of haemostasis in disseminated intravascular coagulation by use of point-of-care assays and routine coagulation tests, in critically ill patients; a prospective observational study. *PLoS One* 2016; 11: e0151202. doi: 10.1371/journal.pone.0151202
12. Larsson A, Tynngard N, Kander T, Bonnevier J, Schott U. Comparison of point-of-care hemostatic assays, routine coagulation tests, and outcome scores in critically ill patients. *J Crit Care* 2015; 30: 1032-1038.
13. Adler M, Ivic S, Bodmer NS, *et al*. Thromboelastometry and thrombelastography analysis under normal physiological conditions - systematic review. *Transfus Med Hemother* 2017; 44: 78-83.
14. Gee AC, Sawai RS, Differding J, Muller P, Underwood S, Schreiber MA. The influence of sex hormones on coagulation and inflammation in the trauma patient. *Shock* 2008; 29: 334-341.
15. Lima-Oliveira G, Lippi G, Salvagno GL, *et al*. Venous stasis and whole blood platelet aggregometry: a question of data reliability and patient safety. *Blood Coagul Fibrinolysis* 2015; 26: 665-668.
16. Karlsson M, Roman-Emanuel C, Thimour-Bergstrom L, Hakimi CS, Jeppsson A. Sampling conditions influence multiple electrode platelet aggregometry in cardiac surgery patients. *Scand Cardiovasc J* 2013; 47: 98-103.
17. Thalen S, Forsling I, Eintrei J, Soderblom L, Antovic JP. Pneumatic tube transport affects platelet function measured by multiplate electrode aggregometry. *Thromb Res* 2013; 132: 77-80.
18. Shaw AD, Bagshaw SM, Goldstein SL, *et al*. Major complications, mortality, and resource utilization after open abdominal surgery: 0.9% saline compared to Plasma-Lyte. *Ann Surg* 2012; 255: 821-829.
19. Young P, Bailey M, Beasley R, *et al*. Effect of a buffered crystalloid solution vs saline on acute kidney injury among patients in the intensive care unit: the SPLIT randomized clinical trial. *JAMA* 2015; 314: 1701-1710.
20. Yunos NM, Bellomo R, Hegarty C, Story D, Ho L, Bailey M. Association between a chloride-liberal vs chloride-restrictive intravenous fluid administration strategy and kidney injury in critically ill adults. *JAMA* 2012; 308: 1566-1572.
21. Toulon P, Ozier Y, Ankri A, Fleron MH, Leroux G, Samama CM. Point-of-care versus central laboratory coagulation testing during haemorrhagic surgery. A multicenter study. *Thromb Haemost* 2009, 101: 394-401.
22. Davenport R, Manson J, De'Ath H, *et al*. Functional definition and characterization of acute traumatic coagulopathy. *Crit Care Med* 2011; 39: 2652-2658.
23. Schochl H, Cotton B, Inaba K, *et al*. FIBTEM provides early prediction of massive transfusion in trauma. *Crit Care* 2011; 15: R265. doi: 10.1186/cc10539

24. Olas B, Kontek B, Szczesna M, Grabarczyk L, Stochmal A, Zuchowski J. Inhibition of blood platelet adhesion by phenolics' rich fraction of Hippophae rhamnoides L. fruits. *J Physiol Pharmacol* 2017; 68: 223-229.
25. Hans GA, Besser MW. The place of viscoelastic testing in clinical practice. *Br J Haematol* 2016; 173: 37-48.
26. Kashuk JL, Moore EE, Sawyer M, *et al*. Primary fibrinolysis is integral in the pathogenesis of the acute coagulopathy of trauma. *Ann Surg* 2010, 252: 434-442.
27. Roullet S, Pillot J, Freyburger G, *et al*. Rotation thromboelastometry detects thrombocytopenia and hypofibrinogenaemia during orthotopic liver transplantation. *Br J Anaesth* 2010; 104: 422-428.
28. Song JG, Jeong SM, Jun IG, Lee HM, Hwang GS. Five-minute parameter of thromboelastometry is sufficient to detect thrombocytopenia and hypofibrinogenaemia in patients undergoing liver transplantation. *Br J Anaesth* 2014; 112: 290-297.
29. Vucelic D, Jesic R, Jovicic S, *et al*. Comparison of standard fibrinogen measurement methods with fibrin clot firmness assessed by thromboelastometry in patients with cirrhosis. *Thromb Res* 2015; 135: 1124-1130.
30. Hagemo JS, Christiaans SC, Stanworth SJ, *et al*. Detection of acute traumatic coagulopathy and massive transfusion requirements by means of rotational thromboelastometry: an international prospective validation study. *Crit Care* 2015; 19: 97. doi: 10.1186/s13054-015-0823-y.
31. Pekelharing J, Furck A, Banya W, Macrae D, Davidson SJ. Comparison between thromboelastography and conventional coagulation tests after cardiopulmonary bypass surgery in the paediatric intensive care unit. *Int J Lab Hematol* 2014; 36: 465-471.
32. Mace H, Lightfoot N, McCluskey S, *et al*. Validity of thromboelastometry for rapid assessment of fibrinogen levels in heparinized samples during cardiac surgery: a retrospective, single-center, observational study. *J Cardiothorac Vasc Anesth* 2016; 30: 90-95.
33. Wypasek E, Mazur P, Bochenek M, *et al*. Factors influencing quality of anticoagulation control and warfarin dosage in patients after aortic valve replacement within the 3 months of follow up. *J Physiol Pharmacol* 2016; 67: 385-393.
34. Mitrophanov AY, Rosendaal FR, Reifman J. Computational analysis of intersubject variability and thrombin generation in dilutional coagulopathy. *Transfusion* 2012; 52: 2475-2486.
35. Siennicka A, Drozdzynska M, Chelstowski K, Cnotliwy M, Jastrzebska M. Haemostatic factors and intraluminal thrombus thickness in abdominal aortic aneurysm. Is secondary fibrinolysis relevant? *J Physiol Pharmacol* 2013; 64: 321-330.
36. Ogawa S, Szlam F, Bolliger D, Nishimura T, Chen EP, Tanaka KA. The impact of hematocrit on fibrin clot formation assessed by rotational thromboelastometry. *Anesth Analg* 2012; 115: 16-21.
37. Langer T, Santini A, Scotti E, Van Regenmortel N, Malbrain ML, Caironi P. Intravenous balanced solutions: from physiology to clinical evidence. *Anaesthesiol Intensive Ther* 2015; 47 (Spec No): s78-88. doi: 10.5603/AIT.a2015.0079.

Received: February 7, 2017
Accepted: July 26, 2017

Author's address: Lukasz J. Krzych, Department of Anaesthesiology and Intensive Care, Medical University of Silesia, 14 Medykow Street, 40-752 Katowice, Poland
E-mail: lkrzych@sum.edu.pl