Comparison of Two Different Fibrinogen Concentrates in an in vitro Model of Dilutional Coagulopathy

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Keywords

Coagulation disorders · Blood coagulation tests · Colloids · Fibrinogen · Thromboelastometry

Abstract

Introduction: Fibrinogen concentrates are widely used to restore clot stability in situations of bleeding. Fibrinogen preparations are produced using different production methods, resulting in different compounds. Thus, different preparations might have a distinct impact on blood coagulation. We tested the effect of fibrinogen concentrates Haemocomplettan[®] (CSL Behring, Marburg, Germany) and fibryga[®] (Octapharma GmbH, Langenfeld, Germany) on the impairments induced by 60% dilutional coagulopathy in vitro. **Materials and Methods:** The influence of the fibrinogen concentrates fibryga[®] and Haemocomplettan[®] on colloid (gelatine, hydroxyethyl starch [HES], albumin)-induced or crystalloid (Ringer's acetate)-induced dilutional coagulopathy was analysed using rotational thromboelastometry (ROTEM[®]) and standard laboratory tests. The following experimental conditions were analysed in vitro: whole blood, 60% dilution (40% blood and 60% diluent) ± 50 or 100 mg/kg⁻¹ fibryga[®] or Haemocomplettan[®], respectively. **Results:** Dilution with either diluent resulted in prolonged clotting time (CT) in an extrinsic activated test (CT_{EXTEM}) and decreased maximum clot firmness (MCF_{FIBTEM}) as expressed, e.g., by gelatine: (59.5 s [62/54.8] vs. 95 s [102.8/86.8]; $p <$

0.001 and 14 mm [16/10.5] vs. 3 mm [4–3]; $p < 0.001$). Substitution after 60% dilution with HES resulted in no difference between the preparations, except for shorter thrombin time with fibryga[®] (14 s [15/14] vs. 18 s [18.8/17]; $p = 0.0093$; low dose). CT_{EXTEM} was higher with Haemocomplettan[®] in a gelatine-induced dilution (51 s [54.5/47.5] vs. 63 s [71/60.3]; $p = 0.0202$; low dose) whereas thrombin time was lower with fibryga[®] (19.5 s [20.8/19] vs. 27 s [29/25.3]; $p = 0.0017$). In dilution with albumin, differences in CT_{EXTEM} (69 s [76.5/66] vs. 56 s [57/53.3]; $p = 0.0114$; low dose) and thrombin time (18 s [18/17] vs. 24.5 s [25.8/24]; $p = 0.0202$; low dose) were seen. In dilution with crystalloid solution, again differences in CT_{EXTEM} (53.5 s [57.8/53] vs. 45 s [47/43]; $p = 0.035$; low dose) and thrombin time (17 s [17/16] vs. 23.5 s [24/23]; $p = 0.0014$; low dose) were seen. Fibrinogen levels were more increased by high-dose substitution of both preparations. **Conclusion:** Based on this data it can be stated that both fibryga[®] and Haemocomplettan[®] had the same performance in our in vitro model except for CT_{EXTEM} and thrombin time.

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Introduction

Severe haemorrhage results in reduced blood volume as well as loss of erythrocytes and coagulation factors. The loss of blood volume is primarily treated with infusion solutions (e.g., crystalloids and colloids) or packed red

cells. Furthermore, dilutional coagulopathy is composed of both, consumption and loss of coagulation factors, which is substituted using fresh frozen plasma and coagulation factor preparations. In this regard, fibrinogen is of particular interest as it is one of the first coagulation factors reaching critical low plasma concentrations during bleeding [1–3]. Fibrinogen concentrate substitution, traditionally administered in hereditary hypofibrinogenemia, is also becoming more common as an early treatment option in acquired deficiency.

Previous studies have shown the *in vitro* and *in vivo* effects of crystalloid and colloid infusion on thromboelastometry (e.g., ROTEM®)-based blood coagulation measurements, focussing on maximum clot firmness (MCF) both in FIBTEM® (MCF_{FIBTEM}) (extrinsically activated viscoelastic assay with blocked platelet function to evaluate fibrinogen-dependent clot stability) and EXTEM® (MCF_{EXTEM}) (extrinsically activated viscoelastic assay to evaluate the extrinsic coagulation pathway) tests and clotting time (CT) in EXTEM® (CT_{EXTEM}) [4–9]. Furthermore, it has been shown that fibrinogen administration can partially reverse dilutional coagulopathy [4–6]. Therefore, we chose a similar way in an *in vitro* model of dilutional coagulopathy to test the performance of a new fibrinogen concentrate compared to an established product.

Clinicians might emphasize that one fibrinogen preparation equals another. However, there are differences in production techniques, quality controls, as well as rules and regulations. In detail, it is not the actual amount of fibrinogen that is measured and added to every single vial; in contrast, total protein including a variety of further coagulation factors is used. Thus, different preparations can differ by 20% regarding fibrinogen and even more for other clotting proteins. Thus, there can be relevant differences between 2 distinct preparations of fibrinogen. This might impact on the effect in dilutional coagulopathy, that is, following administration of crystalloids or colloids, and has been investigated in this study.

We tested the hypothesis that the new fibrinogen concentrate fibryga® (Octapharma GmbH, Langenfeld, Germany) is equally effective in reversing an *in vitro* 60% dilutional coagulopathy induced by a crystalloid and various colloids compared to the established fibrinogen concentrate Haemocomplettan® P (CSL Behring, Marburg, Germany).

Materials and Methods

Healthy volunteers were recruited by bulletins at our institution and enrolled after written informed consent. Twelve healthy individuals, without coagulation disorders or intake of anticoagulants, were included in this prospective trial and volunteer characteristics were documented. Venous blood samples (S-Monovette®

Table 1. Characteristics of healthy volunteers

Gender (male/female), <i>n</i> (%)	6/6 (50/50)
Age, years	38.3±8.9
Body mass index, kg m ⁻²	23.7±3.0
Creatinine serum concentration, mg/dL	0.94±0.09
Albumin serum concentration, g/dL	4.5±0.3
Red blood cell count, 10 ¹² /L	4.9±0.5
Haemoglobin, g/dL	14.4±1.3
Haematocrit, %	0.43±0.04
White blood cell count, 10 ⁹ /L	5.2±0.6
Platelets, 10 ⁹ /L	243±45
INR, median (Q1–Q3)	1 (1–1)
PTT, s	22.7±2.8
Fibrinogen (Clauss method), mg/dL	277±62
Thrombin time, s	17.9±1.4
Medication intake (yes/no), <i>n</i> (%)	4/8 (33/67)

Values are mean ± SD unless otherwise indicated. INR, international normalized ratio; PTT, partial thromboplastin time. * Medication intake: pantoprazol (*n* = 1), L-thyroxin (*n* = 1), Thyronajod (*n* = 1), contraceptive pill (*n* = 1).

Citrate 3.2%; Sarstedt, Nümbrecht, Germany) were withdrawn twice within 4 h and processed within 2 h.

Blood coagulation was assessed by both thromboelastometry (ROTEM®) and standard laboratory tests at baseline (undiluted citrated blood), following a 60% dilution with colloids (hydroxyethyl starch [HES], gelatine, or albumin) or a balanced crystalloid solution (60% diluent + 40% blood), and following dilution plus either of 2 fibrinogen dosages of both concentrates (80 and 160 mg/dL⁻¹).

Sample Preparation

The influence of 3 colloids – HES 6% 130/0.4 (Voluven®; Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany), succinylated gelatine 4% (Gelafluidin® 4%; Braun Melsungen AG, Melsungen, Germany), and human serum albumin (Albunorm® 5%; Octapharma GmbH) and balanced Ringer's acetate (Jonosteril®; Fresenius Kabi Deutschland GmbH) – on blood coagulation (with and without fibrinogen substitution) was assessed. Diluents were added to citrated tubes prior to all experiments to equalize citrate concentrations in blood and dilutional agents. Dilutions were done by mixing 680 µL of citrated whole blood and 1,020 µL of citrated diluent in 2-mL micro tubes (Sarstedt®). fibryga® (Octapharma GmbH) and Haemocomplettan® P (CSL Behring) were dissolved in water for injection (Aqua ad iniectionem; Berlin Chemie AG, Berlin, Germany) to a final concentration of 20 mg/mL⁻¹ based on the labelled quantity of 1 g per vial. A new 1-g fibrinogen concentrate vial from the same production lot was used every day. Fibrinogen was filtered following the manufacturer's instructions using a 17-µm filter (PALL Medical®, Port Washington, USA) to ensure particle free solutions.

Six different experimental conditions were analysed per diluent (24 conditions in total): undiluted blood, 60% dilution (40% blood and 60% diluent) ± low-dose and high-dose fibryga® or Haemocomplettan® P. For low-dose fibrinogen substitution, 68 µL (1.36 mg) of fibrinogen concentrate was added to 1,700 µL of citrated blood (0.8 mg/mL⁻¹ or 80 mg/dL⁻¹). This corresponds to a clinical dose of 50 mg/kg⁻¹ (or 4 g per 80 kg body weight). For high-dose

Table 2. Dilution with hydroxyethyl starch

Hydroxyethyl starch	Undiluted blood	60% dilution	<i>p</i>	fibrinogen low dose	<i>p</i>	Haemo-complettan low dose	<i>p</i>	fibrinogen high dose	<i>p</i>	Haemo-complettan high dose	<i>p</i>
CT _{EXTEM} , s	59.5 (58.25/65.25)	154 (134.8/166)	<0.0001*	100 (78.5/118.3)	0.0293* 0.0592 [#]	137 (113.8/143.5)	0.0001* >0.9999 [#]	69.5 (60.3/76.8)	>0.9999* <0.0001 [#]	76.5 (57/91.3)	>0.9999* <0.0001 [#]
MCF _{EXTEM} , mm	59 (57/65.75)	31 (28.3/37.8)	<0.0001*	39 (37/43.8)	0.0017* 0.1148 [#]	38 (33.75/42)	<0.0001* >0.9999 [#]	46.5 (43/48.8)	>0.9999* <0.0001 [#]	42 (37.3/44.8)	0.0419* 0.0062 [#]
MCF _{FIBTEM} , mm	13 (11/18.5)	0 (0/0)	<0.0001*	3 (3/4)	0.0009* 0.3289 [#]	2.5 (0/3.75)	<0.0001* >0.9999 [#]	6 (5/6.75)	>0.9999* <0.0001 [#]	4 (4/5)	0.0592* 0.0093 [#]
Fibrinogen _{plasma} , mg/dL	253.5 (226.3/322.3)	121 (95.5/17.8)	0.0017*	219.5 (201.5/237)	0.4962* >0.9999 [#]	225.5 (208.5/237)	>0.9999* 0.2853 [#]	408 (373/430.8)	>0.9999* <0.0001 [#]	425.5 (390.8/4.413)	0.2129* <0.0001 [#]
aPTT, s	28 (26.25/29.75)	78.5 (67/91.8)	<0.0001*	74 (63.3/85.8)	0.0062* 0.1832 [#]	73.5 (64/84.5)	0.0167* 0.0829 [#]	73.5 (60.3/84.3)	0.0202* 0.0702 [#]	73 (64.3/85.8)	0.0017* 0.4338 [#]
INR	1 (1/1)	1.9 (1.8/2.1)	<0.0001*	1.75 (1.6/1.88)	0.0977* 0.0062 [#]	1.8 (1.6/1.88)	0.0202* 0.0350 [#]	1.75 (1.6/1.9)	0.0419* 0.0167 [#]	1.8 (1.63/1.98)	<0.0001* >0.9999 [#]
Thrombin time, s	17 (17/18.8)	27 (22.5/30.8)	0.4338*	14 (14/15)	0.0293* <0.0001 [#]	18 (17/18.8)	>0.9999* 0.9336 [#]	12 (12/12)	0.0001* <0.0001 [#]	16 (15/16)	0.8270* 0.0009 [#]

Data are presented as median (Q1/Q3). * Comparison to undiluted blood. [#] Comparison to diluted blood.

fibrinogen substitution, 136 µL (2.72 mg) of fibrinogen concentrate was added to 1,700 µL of citrated blood (1.6 mg/mL⁻¹ or 160 mg/dL⁻¹). This corresponds to a clinical dose of 100 mg/kg⁻¹ (or 8 g per 80 kg body weight). All samples were stored at 37 °C in a digital block heater (Dry Block Heater 3; IKA®, Staufen, Germany) until ROTEM® analyses were started, as described in previous studies.

ROTEM®

Thromboelastometric tests (EXTEM® and FIBTEM®) were performed for each sample using ROTEM® delta analysers (TEM Innovations, Munich, Germany). Tests were performed according to manufacturer's instructions and terminated after 75 min [4, 10]. For statistical analysis, we focussed on MCF measured by EXTEM® (MCF_{EXTEM}) and FIBTEM® (MCF_{FIBTEM}) and CT (time from starting the test until a clot firmness amplitude of 2 mm has been achieved) by EXTEM® (CT_{EXTEM}). If the sample did not clot at all, CT was set to the maximum run time of 75 min (4,500 s).

EXTEM® uses tissue factor as activator and represents the extrinsic pathway of coagulation. FIBTEM® contains cytochalasin D to block platelet function in addition to tissue factor. Accordingly, MCF_{FIBTEM}® is based on fibrin polymerization, only. ROTEM® analyses were performed on 3 ROTEM® delta analysers, with different experimental conditions alternatively performed in a standardized fashion on all analysers. ROTEM® analyses were performed within 2 h after blood sampling.

Standard Laboratory Tests

Standard coagulation tests including international normalized ratio (INR; Thromborel S), thrombin time (TT; Berichrom Thrombinreagenz), and activated partial thromboplastin time (aPTT; Actin FSL; all from Siemens Healthcare GmbH, Erlangen, Germany) and blood counts were performed. Furthermore, fibrinogen plasma concentration as measured by the Clauss method was assessed (optical measurement, Multifibren U; Siemens Healthcare GmbH). All standard laboratory tests were performed by the Ludwig Maximilian University Institute for Laboratory Medicine, according to

institutional standards. Tests were performed on BCS XP (Siemens Healthcare GmbH).

Statistical Analysis

Data are presented as median with interquartile range unless otherwise indicated. Statistical analysis was performed using IBM® SPSS® Statistics version 24 (Armonk, NY, USA) and Graph Pad® Prism 7 (La Jolla, CA, USA). Statistical differences between groups were analysed using the Friedman test and Dunn's multiple comparison test. Correlation between MCF_{FIBTEM} and fibrinogen plasma concentration (Clauss method) was done by Spearman correlation. The alpha error was adjusted for multiple testing ($p = 0.05/n$). The sample size was determined using G*Power 3.1 (Dusseldorf, Germany) and an a priori power analysis ($\alpha = 0.05$; $d = 0.8$; $1 - \beta = 0.7$) revealed 12 individuals per group.

Results

We assessed the performance of the 2 fibrinogen preparations fibrinogen® and Haemocomplettan® P based on an in vitro dilutional coagulopathy model. All blood samples of the 12 healthy volunteers were taken into account.

Volunteer Characteristics

Detailed volunteer characteristics are displayed in Table 1. None of them had known coagulation disorders or intake of anticoagulants.

Sixty Percent Dilution with HES 6% 130/0.4

After dilution with 60% HES all ROTEM parameters as well as standard laboratory parameters except for thrombin time deteriorated significantly (Table 2). Sub-

Table 3. Dilution with gelatine

Gelatine	Undiluted blood	60% dilution	<i>p</i>	fibryga low dose	<i>p</i>	Haemocomplettan low dose	<i>p</i>	fibryga high dose	<i>p</i>	Haemocomplettan high dose	<i>p</i>
CT _{EXT^{EM}} , s	59.5 (54.75/62)	95 (86.75/102.8)	0.0499*	63 (60.3/71)	>0.9999* 0.6439 [#]	51 (47.5/54.5)	0.3289* <0.0001 [#]	57.5 (55.3/62.5)	>0.9999* 0.0244 [#]	42.5 (41/44.8)	0.0033* <0.0001 [#]
MCF _{EXT^{EM}} , mm	60 (56.25/66)	36.5 (33.25/41.75)	<0.0001*	45 (43.3/49)	0.0001* 0.4338 [#]	45.5 (42.25/49)	<0.0001* 0.9336 [#]	49 (47.5/53)	0.2129* 0.0004 [#]	50 (47.5/53.8)	>0.9999* <0.0001 [#]
MCF _{FIB^{TEM}} , mm	14 (10.5/16)	3 (3/4)	<0.0001*	8 (6.5/9)	0.0001* >0.9999 [#]	9 (8/9)	0.0167* 0.1148 [#]	10 (10/12)	>0.9999* 0.0006 [#]	14 (13/15)	>0.9999* <0.0001 [#]
Fibrinogen _{plasma} , mg/dL	257.5 (220.5/339.8)	76 (66.25/95.75)	<0.0001*	179.5 (155.8/191.8)	0.0202* 0.2853 [#]	153.5 (122.5/171.8)	0.0017* >0.9999 [#]	274 (246/314)	>0.9999* <0.0001 [#]	224.5 (209.3/241.8)	>0.9999* 0.0004 [#]
aPTT, s	28 (25.25/30)	88.5 (74.75/108)	<0.0001*	81 (70/97)	0.0093* 0.0829 [#]	79.5 (66.5/100.3)	0.3289* 0.0014 [#]	81.5 (68.3/)	0.0062* 0.1148 [#]	83.5 (72/100.3)	0.0001* >0.9999 [#]
INR	1 (0.925/1)	2.15 (1.925/2.275)	<0.0001*	1.75 (1.6/1.9)	0.1345* 0.0009 [#]	1.8 (1.7/2)	0.0007* 0.1572 [#]	1.75 (1.63/1.9)	0.0977* 0.0014 [#]	1.85 (1.63/1.9)	0.0017* 0.0829 [#]
Thrombin time, s	17.5 (17/18)	35 (30.5/38.75)	<0.0001*	19.5 (19/20.8)	>0.9999* 0.0011 [#]	27 (25.25/29)	0.0007* >0.9999 [#]	16 (16/16.8)	>0.9999* <0.0001 [#]	24 (24/25)	0.0829* 0.1148 [#]

Data are presented as median (Q1/Q3). * Comparison to undiluted blood. [#] Comparison to diluted blood.

Table 4. Dilution with albumin

Albumin	Undiluted blood	60% dilution	<i>p</i>	fibryga low dose	<i>p</i>	Haemocomplettan low dose	<i>p</i>	fibryga high dose	<i>p</i>	Haemocomplettan high dose	<i>p</i>
CT _{EXT^{EM}} , s	57.5 (54.25/61)	89 (81/94.75)	0.0022*	69 (66/75.5)	0.4338* >0.9999 [#]	56 (53.25/57)	>0.9999* <0.0001 [#]	61 (56.3/68.3)	>0.9999* 0.0499 [#]	51 (48.3/53)	0.1148* <0.0001 [#]
MCF _{EXT^{EM}} , mm	60 (56/66.5)	41 (37/41)	<0.0001*	49 (46.3/53.8)	0.0004* 0.2129 [#]	48 (46.25/52)	<0.0001* >0.9999 [#]	53 (50.5/47)	0.5660* <0.0001 [#]	52.5 (50.5/57.75)	0.7307* <0.0001 [#]
MCF _{FIB^{TEM}} , mm	14 (11/20)	3.5 (3/5)	<0.0001*	8.5 (8/10.75)	0.0051* >0.9999 [#]	10 (9/11)	0.1832* 0.1572 [#]	14 (13/15)	>0.9999* <0.0001 [#]	18 (16/19.75)	>0.9999* <0.0001 [#]
Fibrinogen _{plasma} , mg/dL	243.5 (218.8/336.8)	70.5 (62.5/92.25)	<0.0001*	163 (150.3/187.3)	0.0419* 0.1148 [#]	133.5 (112.8/145)	0.0003* >0.9999 [#]	246 (236.3/267)	>0.9999* <0.0001 [#]	214.3 (211/221)	>0.9999* 0.0004 [#]
aPTT, s	27.5 (25.25/30)	87.5 (76.3/105.5)	<0.0001*	83 (66/99.75)	0.0022* 0.8270 [#]	79 (67/97.25)	0.3289* 0.0093 [#]	84 (68.75/104)	<0.0001* >0.9999 [#]	81 (64.5/102.3)	0.0167* 0.2129 [#]
INR	1 (1/1)	2.2 (2.03/2.48)	<0.0001*	1.8 (1.7/2.08)	0.5660* 0.0003 [#]	1.95 (1.83/2.1)	0.0011* 0.2468 [#]	1.95 (1.8/2.1)	0.0977* 0.0041 [#]	2 (1.8/2.2)	0.0001* 0.8270 [#]
Thrombin time, s	17 (17/18)	30.5 (27/33)	<0.0001*	18 (17/18)	>0.9999* 0.0002 [#]	24.5 (24/25.75)	0.0062* >0.9999 [#]	15 (15/15)	0.9336* <0.0001 [#]	22.5 (22/23)	0.4338* 0.1345 [#]

Data are presented as median (Q1/Q3). * Comparison to undiluted blood. [#] comparison to diluted blood.

stitution with low-dose fibryga[®] and low-dose Haemocomplettan[®] did not improve ROTEM parameters and standard laboratory parameters to values before dilution. Comparison of both preparations showed no significant difference (Table 2). Substitution with high-dose fibryga[®] and high-dose Haemocomplettan[®] improved ROTEM and standard laboratory parameters significantly compared to diluted blood. However, parameters did not reach undiluted blood values (Table 2). Comparison between both preparations showed no difference. The comparison between low-dose and high-dose fibryga[®] showed no differences except for the fibrinogen plasma concentration (219.5 vs. 408 mg/dL⁻¹; *p* = 0.0017). Comparable results were observed with Haemocomplettan[®] (225.5 vs. 425.5 mg/dL⁻¹; *p* = 0.0011).

Of note, MCF_{FIB^{TEM}} and fibrinogen plasma concentration did not correlate (*r* = 0.2423; *p* = 0.4697).

Sixty Percent Dilution with Gelatine 4%

After dilution with 60% gelatine all ROTEM parameters as well as standard laboratory parameters deteriorated significantly (Table 3). Substitution with low-dose fibryga[®] and low-dose Haemocomplettan[®] improved the ROTEM parameters but not to values before dilution (Table 3). Comparison of both low-dose preparations showed no significant difference except for CT_{EXT^{EM}} (63 vs. 51 s; *p* = 0.0202). Substitution with high-dose fibryga[®] and Haemocomplettan[®] corrected the ROTEM parameters to values of undiluted blood. Haemocomplettan[®] overcorrected the CT_{EXT^{EM}} compared to undiluted blood (59.5 vs. 42.5 s; *p* = 0.0033; Table 3). Standard laboratory tests were still significantly different to values of undiluted blood after high-dose substitution of both preparations except for thrombin time after fibryga[®] (17.5 vs. 16 s; *p* > 0.999). Comparison between both high-dose preparations showed no differences except for the aforemen-

Table 5. Dilution with balanced crystalloid

Balanced crystalloid	Undiluted blood	60% dilution	<i>p</i>	fibrinogen low dose	<i>p</i>	Haemocomplettan low dose	<i>p</i>	fibrinogen high dose	<i>p</i>	Haemocomplettan high dose	<i>p</i>
CT _{EXTEM} , s	58.5 (56.3/63.8)	65 (60.3/69.8)	>0.9999*	53.5 (53/57.8)	>0.9999* 0.1345 [#]	45 (43/47)	<0.0001* <0.0001 [#]	52.5 (48.5/56.8)	0.3289* 0.0167 [#]	45.5 (41/46.75)	<0.0001* <0.0001 [#]
MCF _{EXTEM} , mm	60.5 (56/64.8)	41 (37.3/45.8)	<0.0001*	50 (48/53)	0.0001* 0.5660 [#]	50 (46.5/53.8)	<0.0001* 0.7307 [#]	54.5 (51.5/57.75)	0.2468* 0.0004 [#]	56 (52.25/59.5)	>0.9999* <0.0001 [#]
MCF _{FIBTEM} , mm	14.5 (10.3/16)	5.5 (4/6)	0.4338*	13 (11.3/14.8)	>0.9999* 0.9336 [#]	17 (14.3/18.8)	0.7307* 0.0007 [#]	19.5 (18/21.75)	0.0350* <0.0001 [#]	25.5 (23.25/29.25)	0.0001* <0.0001 [#]
Fibrinogen _{plasma} , mg/dL	270.5 (212/329.5)	62 (48.5/81.3)	<0.0001*	164 (144/182.3)	0.0093* 0.1148 [#]	112 (109/135.3)	<0.0001* >0.9999 [#]	246 (235.8/267.8)	>0.9999* <0.0001 [#]	216 (198.3/228.8)	0.4962* 0.0011 [#]
aPTT, s	28 (25.3/31.5)	79.5 (71.5/94)	<0.0001*	77 (60.8/94.3)	0.0027* >0.9999 [#]	71 (61/93)	0.0977* 0.1148 [#]	74.5 (60.8/98.8)	0.0001* >0.9999 [#]	73.5 (63.5/93.5)	0.0062* 0.9336 [#]
INR	1 (1/1)	2.1 (1.93/2.28)	<0.0001*	1.75 (1.7/1.9)	0.3783* 0.0004 [#]	1.85 (1.73/2)	0.0006* 0.3289 [#]	1.75 (1.63/1.9)	0.3783* 0.0004 [#]	1.9 (1.73/2)	<0.0001* >0.9999 [#]
Thrombin time, s	17.5 (17/18)	28 (25.3/30.8)	0.0011*	17 (16/17)	>0.9999* <0.0001 [#]	23.5 (23/24)	0.0499* >0.9999 [#]	14 (14/14)	0.1832* <0.0001 [#]	22 (21/22.8)	>0.9999* 0.2129 [#]

Data are presented as median (Q1/Q3). * Comparison to undiluted blood. [#] Comparison to diluted blood.

tioned CT_{EXTEM} (57.5 vs. 42.5 s; $p = 0.0076$) and TT (16 vs. 24 s; $p = 0.0017$). Comparing low-dose and high-dose fibrinogen[®] showed no differences except for fibrinogen plasma concentrations. Comparing low-dose and high-dose Haemocomplettan[®] showed differences in MCF_{FIBTEM} (9 vs. 14 mm; $p = 0.0244$) and MCF_{EXTEM} (45.5 vs. 50 mm; $p = 0.0167$).

A correlation of MCF_{FIBTEM} and fibrinogen plasma concentration was observed; however, it was not statistically significant ($r = 0.5682$; $p = 0.0520$).

Sixty Percent Dilution with Human Albumin 5%

After dilution with 60% human albumin all ROTEM parameters as well as standard laboratory parameters deteriorated significantly (Table 4). Substitution with low-dose fibrinogen[®] and Haemocomplettan[®] improved all of the parameters, but most were lower than before dilution. In detail, Haemocomplettan[®] only improved CT_{EXTEM} (57.5 vs. 56.0 s; $p > 0.999$) and MCF_{FIBTEM} (14 vs. 10 mm; $p = 0.1832$) to values before dilution (Table 4), and fibrinogen[®] corrected TT to values of undiluted blood (17 vs. 18 s; $p > 0.999$). Thus, comparing low-dose fibrinogen[®] and Haemocomplettan[®] showed differences in CT_{EXTEM} (69 vs. 56 s; $p = 0.0114$) and TT (18 vs. 24.5 s; $p = 0.0202$). Comparison of high-dose fibrinogen[®] and Haemocomplettan[®] corrected all parameters to values of undiluted blood except for INR (Table 4), and comparison showed differences between both preparations for CT_{EXTEM} (61 vs. 51 s; $p = 0.0062$) and TT (15 vs. 22.5 s; $p = 0.0011$).

Comparison of low-dose and high-dose fibrinogen[®] presented significant differences for MCF_{FIBTEM} (8.5 vs. 14 mm; $p = 0.0244$) and fibrinogen plasma concentrations (163 vs. 246 mg/dL⁻¹; $p = 0.0093$). Comparing low-dose and high-dose Haemocomplettan[®] showed the same results but also a difference in MCF_{EXTEM} (48 vs. 52.5 mm; $p = 0.0244$).

After dilution with human albumin, MCF_{FIBTEM} and fibrinogen plasma concentration showed a strong correlation ($r = 0.87$; $p = 0.0005$).

Sixty Percent Dilution with Balanced Ringer's Acetate

After dilution with 60% crystalloid solution only MCF_{EXTEM}, fibrinogen plasma concentration, aPTT, INR, and TT deteriorated significantly (Table 5). Substitution of low-dose fibrinogen[®] already corrected CT_{EXTEM}, MCF_{FIBTEM}, and INR to values of undiluted blood (Table 5). Haemocomplettan[®] corrected MCF_{FIBTEM} and aPTT and overcorrected CT_{EXTEM}. Comparison of both preparations showed significant differences in CT_{EXTEM} (53.5 vs. 45.5 s; $p = 0.035$) and TT (17 vs. 23.5 s; $p = 0.0014$). Substitution of high-dose fibrinogen[®] and Haemocomplettan[®] corrected CT_{EXTEM} (again outperformed correction with Haemocomplettan[®]), MCF_{EXTEM}, fibrinogen plasma concentration, and TT (Table 5). Comparison of both preparations showed differences in TT (14 vs. 22 s; $p = 0.0009$). Comparing low-dose and high-dose fibrinogen[®] showed differences for MCF_{FIBTEM} (13 vs. 19.5 mm; $p = 0.0114$) and fibrinogen plasma concentrations (164 vs. 246 mg/dL⁻¹; $p = 0.0202$). Comparison of the 2 Haemocomplettan[®] preparations showed differences in MCF_{EXTEM} (50 vs. 56 mm; $p = 0.0202$).

MCF_{FIBTEM} and fibrinogen plasma concentration showed a strong correlation ($r = 0.89667$; $p = 0.0002$).

Discussion/Conclusion

In this study we show, that the new fibrinogen concentrate fibrinogen[®] and the established product Haemocomplettan[®] had comparable effects in our in vitro model of dilutional coagulopathy. However, minor differences in

thrombin time and CT_{EXTEM} between both preparations were seen. fibryga[®] had a stronger impact on thrombin time compared to Haemocomplettan[®], that is, due to factor XIII-related improvement in fibrin polymerization. Additionally, Haemocomplettan[®] had a more pronounced effect on shortening of CT_{EXTEM} which might be due to increased factor VIII concentration compared to fibryga[®].

Coagulopathy in severely injured patients with high blood loss is characterized by dilution, consumption of coagulation factors, and critically low fibrinogen plasma concentrations. As fibrinogen is often one of the first coagulation factors to reach critically low plasma concentrations, early administration of fibrinogen and tranexamic acid in cases of heavy trauma and blood loss is being proposed [11, 12]. Thus, the European guidelines recommend fibrinogen administration if plasma concentrations fall below concentrations of 150–200 mg/dL⁻¹ [13]. Usually between 50 and 100 mg/kg⁻¹ body weight of fibrinogen are administered in severe bleeding and relevant blood loss [14, 15]. Thus, we analysed both 50 and 100 mg/kg⁻¹ fibrinogen administration to simulate low-dose and high-dose fibrinogen substitution of both preparations and its performance. We used 4 common diluents to investigate the performance of the 2 preparations. The influence and impact on coagulation of those diluents are well known and therefore eligible for our model [9, 16–21].

Our results with the crystalloid model, showing similar impact of fibryga[®] and Haemocomplettan[®], are in accordance with a previously published analysis of Haas et al. [18]. This group also compared the performance of the 2 fibrinogen preparations as it was done in this study. In contrast to our study this group used a model with crystalloid only. Therefore, this is the first study to analyse the performance of fibryga[®] and Haemocomplettan[®] under different conditions. Haas et al. [18] used different lots and compared the amount of fibrinogen to calculate a factor to adjust the samples. The group could demonstrate a distinctive increase of MCF_{EXTEM} after substitution with fibryga[®]. This effect could not be seen with our data. Besides, they did not evaluate the dynamics of clot formation as we did with CT_{EXTEM} and did not use standard laboratory tests. In a prior study comparing both preparations in congenital fibrinogen deficiency, no differences in MCF_{FIBTEM} and no differences in complication rate were found [22].

An increase in MCF_{EXTEM} and MCF_{FIBTEM} after fibrinogen replacement was seen in the presence of all diluents, which is in line with the results of prior studies evaluating the effect of fibrinogen substitution on these parameters [4, 6]. They postulate that the sole administration of fibrinogen particularly influences clot stability and less the dynamics of clot formation. Only high-dose fibrinogen

substitution slightly influenced clot formation dynamics [6]. In the present study, the dynamics of both clot formation and clot stability were influenced. In particular, Haemocomplettan[®] had an impact on the dynamics of clot formation, as seen by improvement of the CT_{EXTEM} . Effects on clot dynamics is possibly caused by slight differences of co-factors (e.g., FVIII) within the preparation of the fibrinogen product.

As expected, HES had a profound effect on ROTEM[®] parameters, which were only partly restored even by high-dose fibryga[®] and Haemocomplettan[®] administration [9, 18]. This confirms the data of Schlimp et al. [8]. Fibrinogen plasma concentrations assessed using the Clauss method (functional assay with optical measurement of the clot formation time in the laboratory) differed from ROTEM[®] results, with no correlation between MCF_{FIBTEM} and fibrinogen plasma concentration which is a well-known effect. It can be explained by methodological issues [23]. Fibrinogen plasma concentration using the Clauss method is assessed photometrically and can be disturbed by HES [23–26]. Thus, fibrinogen plasma concentration is overestimated, and low fibrinogen plasma concentrations can be mimicked when HES is present in the probe, which leads to bad correlations of those optical tests with other tests [23–26]. The impact of the other diluents was in accordance with prior publications and showed deteriorated coagulation parameters as expected [9, 17, 18, 21].

The effects seen in ROTEM[®] analyses were compared (not correlated) to the changes in standard laboratory coagulation tests. INR and aPTT did not change significantly after fibrinogen substitution, whereas TT increased especially after administration of fibryga[®]. TT was significantly shorter in contrast to supplementation with Haemocomplettan[®]. The reason for this effect is still not completely clear. One explanation can be the effect due to FXIII-related improvement in fibrin polymerization as described in the manufacturer's information. Some of our observed differences might be explained by differences in the exact dosage of fibrinogen despite the labelled and standardized dosage of the factor concentrates: using fibrinogen concentrates, clinicians might think that 1 g of fibrinogen concentrate contains exactly 1 g of fibrinogen. Due to rules and regulations, quality must ensure that a fibrinogen concentrate contains at least the indicated amount of fibrinogen. However, some range – both below and above the target value – is allowed by the “National Federal Institute for Drugs and Medical Devices” and other public health authorities. This explains differences in the actual amount of fibrinogen in a specific product. Comparing fibryga[®] and Haemocomplettan[®] P, it turns out that in the specific lot used for our study, fibryga[®] contained 27.3% less fibrinogen than Haemocomplettan[®] P measured by the Clauss method (information is

part of the quality data provided by the manufacturer). Immunological measurement of fibrinogen showed only 4.3% difference between the preparations. Both lots were in the allowed range. This can serve as an explanation for the few but not significant differences in MCF_{EXTEM} and MCF_{FIBTEM} and the significant improvement of CT_{EXTEM} after Haemocomplettan[®]. The observed slightly higher impact of fibryga[®] when compared to Haemocomplettan[®] on thrombin time, despite no differences in other standard laboratory values, nonetheless remains difficult to understand. However, it should be taken into account that despite the similar active compound of fibrinogen the preparations may vary slightly in composition due to differences in production. Therefore, a specific effect of one distinct constituent potentially interfering with single laboratory tests cannot be excluded completely.

Our study has limitations. We simulated dilutional coagulopathy in vitro and the results are based on standardized conditions, which would vary in vivo, for example, in the operating room. In detail, sample temperature, citrate concentrations, time until test initiation, and so on, which influence results were all standardized throughout this study and, thus, did not mimic the in vivo situation. The influence of those cannot be seen with standardized coagulation tests (standard laboratory as well as thromboelastometry) because both are performed, for example, at 37 °C and following recalcification and can differ, therefore, from a real situation. Furthermore, dilutional coagulopathy in vivo is a complex setting and influenced by additional factors like endothelial integrity, glycocalyx shedding, endogenous heparinoids, temperature, platelet function, and so on, none of which has been analysed in our study [27–30]. The standard laboratory tests used do not reflect an in vivo clotting situation, that is, fibrinogen analysed by the Clauss method is influenced by colloids. Thus, results might not be completely comparable to the thromboelastometric assays used. This is of particular interest, as thromboelastometric assays better represent whole blood coagulation.

Dilution by a single preparation is not common and does not reflect the real situation in the operating room. However, it can be used as a model to evaluate the performance of different fibrinogen preparations with standardized conditions.

In conclusion, both fibryga[®] and Haemocomplettan[®] P, as commercially available fibrinogen concentrates, had the same performance in an in vitro model of dilutional coagulopathy. As expected, higher doses of 100 mg/kg⁻¹ of both fibrinogen concentrates proved to be more effective than 50 mg/kg⁻¹ in this model, which should be evaluated in clinical, randomized studies. Minor differences between the 2 concentrates were observed in some of the ROTEM[®] variables depending on the different diluents. Minor differences could also be observed in the standard

laboratory tests with a slightly higher impact of unknown significance of fibryga[®] on thrombin time compared to Haemocomplettan[®].

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Statement of Ethics

This in vitro study was approved by the Ludwig Maximilian University Ethics Committee (No. 17-525) and performed in accordance with the Declaration of Helsinki. Healthy volunteers were recruited by bulletins at our institution and enrolled after written informed consent.

Disclosure Statement

S.T.S. received lecture fees from CSL Behring. The other authors have no conflicts of interest to declare.

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Author Contributions

P.G.: study design, data collection, statistical analysis, and manuscript writing. T.W.: data collection, statistical analysis, and manuscript writing. T.K., P.M., M.M., and A.B.: statistical analysis and manuscript writing; K.G.: statistical analysis, interpretation of data and manuscript writing; M.R.: interpretation of data and manuscript writing; S.T.S.: study design, data collection, statistical analysis, interpretation of data, and manuscript writing.

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