

Elevated D-dimers in attacks of hereditary angioedema are not associated with increased thrombotic risk

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To cite this article: Reshef A, Zanichelli A, Longhurst H, Relan A, Hack CE. Elevated D-dimers in attacks of hereditary angioedema are not associated with increased thrombotic risk. *Allergy* 2015; **70**: 506–513.

Keywords

C1 esterase inhibitor; D-dimer; fibrinolysis; hereditary angioedema; thromboembolism.

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Accepted for publication 25 January 2015

DOI:10.1111/all.12587

Edited by: Hans-Uwe Simon

Abstract

Background: Recommended management of attacks of hereditary angioedema (HAE) due to C1 esterase inhibitor (C1-INH) deficiency (C1-INH-HAE) includes therapy with exogenous C1INH. Thrombotic/thromboembolic events (TEE) have been reported with plasma-derived C1INH, but so far none with recombinant human C1INH (rhC1INH). This phase III, randomized, placebo (saline)-controlled study evaluated the safety of rhC1INH 50 IU/kg for the treatment of acute attacks in 74 patients with C1-INH-HAE.

Methods: Monitoring for TEE and assessment of risk of deep vein thrombosis (DVT) by the Wells prediction rule were performed, and levels of fibrin degradation products (plasma D-dimers) were assessed before study drug administration (baseline), 2 h, and 7 days posttreatment.

Results: Plasma D-dimer levels were elevated in 80% of the patients (median [25th–75th percentiles]: 2149 [480–5105] µg/l; normal ≤250 µg/l) and were higher in patients with submucosal (abdominal, oropharyngeal–laryngeal) attacks (3095 [890–10000] µg/l; *n* = 29) compared with subcutaneous (peripheral, facial) attacks (960 [450–4060] µg/l; *n* = 35). Median plasma D-dimer levels were comparable across treatment groups at baseline (1874 [475–4568] µg/l rhC1INH; 2259 [586–7533] µg/l saline) and 2 h postinfusion (2389 [760–4974] µg/l rhC1INH; 2550 [310–8410] µg/l saline); median plasma D-dimer levels were decreased by Day 7 in both groups (425 [232–3240] µg/l rhC1INH; 418 [246–2318] µg/l saline). No increased risk of DVT was identified, nor any TEE reported in rhC1INH treated or controls.

Conclusion: Elevated plasma D-dimer levels were associated with acute C1-INH-HAE attacks, particularly with submucosal involvement. However, rhC1INH therapy was not associated with thrombotic events.

Activation of the contact–kinin pathway in patients with hereditary angioedema (HAE) due to C1 esterase inhibitor (C1INH) deficiency (C1-INH-HAE) results in bradykinin generation, vascular leakage, accumulation of watery fluid in the tissues, and edema formation (1). Additionally, when Factor XII (FXII) becomes activated (FXIIa) *in vitro*, particularly in the absence of its main serine-protease inhibitor-C1INH, FXIIa, and kallikrein may activate plasminogen, resulting in plasmin formation. Plasmin is the principal enzyme that degrades stable fibrin lattices (2). Moreover, plasmin facilitates the cleavage of high molecular weight

kininogen by kallikrein, thus increasing bradykinin generation and edema formation (3). Plasminogen activation by FXIIa may be due to the high homology of FXII and tissue plasminogen activator (tPA), a major fibrinolysis initiator (4), and to similarity in their catalytic sites (both are inhibited by corn trypsin inhibitor—CTI) (5). In addition, endothelial cell activation has been shown to be associated with elevated FXII-dependent fibrinolytic activity (6) and plasminogen activation is triggered by bradykinin-stimulated release of tPA from the surface of endothelial cells (7). Indeed, high levels of procoagulant and fibrinolytic activity have been

demonstrated in patients with C1-INH-HAE during acute attacks and remissions (8–11). In clinical practice, elevated plasma D-dimers are considered biomarkers of extensive thrombosis but are also elevated in certain nonpathologic conditions (12).

Despite evidence of extensive activation of both coagulation–contact and fibrinolytic systems, relatively low rates of spontaneous thromboembolic events (TEEs) have been reported in patients with C1-INH-HAE. However, recent reports of TEE during treatment with commercially available human plasma-derived C1 inhibitor (pdC1INH) products have raised concerns. Up to ten confirmed and seven possible cases of TEE have been associated with the use of pdC1INH (Cinryze®) in both controlled clinical trials and postmarketing studies; of which, five were considered serious (13). Similarly, TEEs have been associated with recommended and off-label high doses of another pdC1INH (Berinert®) (14). Animal studies have also supported a potential risk of thrombosis from intravenous administration of pdC1INH products (15).

Recombinant C1INH (rhC1INH, conestat alfa, Ruconest®) is a novel product homologous to human C1INH and produced in transgenic rabbits (16). To date, no TEE has been observed following repeated treatments with rhC1INH in 250 patients with over 1000 administrations (17–21). Furthermore, animal studies have corroborated lack of thrombotic risk, including a no-observed-adverse-effect level of 2000 IU/kg/day, in a 14-day repeated dose toxicity study in cynomolgus monkeys (22).

Here, we present results of a clinical study where plasma D-dimers were measured in patients with C1-INH-HAE during acute attacks and after treatment with rhC1INH, and evaluate their utility as a biomarker of fibrinolytic activity and potential probe of disease activity.

Materials and methods

Patients and study design

Seventy-five patients participated in a randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of rhC1INH compared with saline, for the treatment of acute angioedema attacks in patients with C1-INH-HAE (<https://www.clinicaltrials.gov/ct2/results?term=NCT01188564&Search=Search;identifier=NCT01188564>).

Patients (age ≥ 13 years), with a laboratory-confirmed diagnosis of C1-INH-HAE, were randomized (3 : 2) to receive an intravenous injection of rhC1INH (50 IU/kg for patients < 84 kg, or 4200 IU for patients ≥ 84 kg) (17) or saline. One patient randomized to the rhC1INH group was withdrawn at the discretion of the investigator, did not receive study medication, and has not been included in the current analysis (safety population, $N = 74$). Thirteen patients in the saline treatment group received rhC1INH as rescue medication for acute attacks. For all analyses, these patients are included in the saline summaries up until the time they received rescue medication, and are included in the rhC1INH summaries afterward.

C1-INH-HAE patients with peripheral (extremities), abdominal, facial, or oropharyngeal–laryngeal attacks were eligible for rhC1INH treatment if the onset of attack was < 5 h prior to presentation to the clinic. Overall severity of the attack was rated by the patient to be ≥ 50 mm on a Visual Analog Scale (VAS, markings made on a 0- to 100-mm horizontal line represent the severity/intensity of each item) (17). For patients with multiple eligible attack locations, the primary attack location was defined as the location with the highest VAS score at baseline. All patients provided written informed consent. The study was approved by the local institutional review board at each site.

Thrombotic risk assessments

All randomized patients were clinically monitored for TEE including deep vein thrombosis (DVT) and pulmonary embolism (PE). The risk of DVT was also assessed using the Wells prediction rule (23). Patients with an increase in D-dimer levels were to be clinically evaluated for the possible development of TEE, including ultrasound examination as indicated.

Plasma sample collection

For the determination of D-dimer levels in the plasma, citrated blood samples were collected at baseline (i.e., prior to intravenous injection of study medication or placebo), at 2 h, and at Day 7 (after the attack resolved) following intravenous injection of study medication or placebo.

Plasma D-dimer measurement

This was a multicenter study where separated plasma samples were sent immediately to local laboratories for measurement of plasma D-dimer levels, according to standard protocols. D-dimer levels were measured by two latex-based turbidimetric immunoassays: HemosIL D-Dimer HS (Instrumentation Labs, Bedford MA, USA) and Innovance D-Dimer (Siemens AG, Erlangen, Germany). Results in FEU (fibrinogen equivalent units) were converted to DDU (D-Dimer units). Values of ≤ 250 $\mu\text{g/l}$ were considered normal in the final analysis.

Statistics

Data were analyzed using SAS software version 9.3 (SAS Institute Inc. Cary, North Carolina, USA). All data were summarized by descriptive statistics using the safety population. Descriptive statistics for continuous variables include the mean, standard deviation, median, interquartile range (25th and 75th percentiles), and range (minimum and maximum values); categorical variables were presented as counts (n) and percentages (%). Plasma D-dimer levels were summarized and presented by time point (baseline, 2 h, and Day 7 posttreatment) and treatment group, based on anatomical location (submucosal vs subcutaneous) and baseline severity (moderate: VAS between 50 and 75 mm; severe ≥ 75 mm) at the primary attack location. The Wilcoxon rank sum test was used to compare medians for plasma D-dimer

levels in patients presenting with submucosal vs subcutaneous attacks.

Results

Patient demographics

Seventy-four patients presenting with eligible acute attacks were randomized and received either 50 IU/kg rhC1INH ($N = 43$) or saline ($N = 31$). Patient disposition, key demographics, and HAE attack frequency and severity of the eligible attack were similar between groups (Table 1). Attack severity at baseline, as rated by the patients using a 100-mm VAS scale, was similar in both groups (group means: 73.5 mm [rhC1INH] vs 77.3 mm [saline]). The most common primary attack locations were peripheral and abdominal and were similar in the rhC1INH and the saline groups (peripheral: 44% rhC1INH and 45% saline; abdominal: 37% rhC1INH and 39% saline).

Thrombotic or thromboembolic adverse events

There were no reports of thrombotic or thromboembolic adverse events in patients treated with rhC1INH or placebo.

Risk of deep vein thrombosis

Wells scores were available for 39 of 43 rhC1INH-treated patients and 30 of 31 saline-treated patients. None of the patients were identified as having an increased risk of DVT

based on these scores (23). Ultrasounds performed on two patients (one rhC1INH and one saline) were normal in both abdomen and lower extremities with no evidence of DVT.

Plasma D-dimer levels

Median plasma D-dimer levels were elevated in the patients at baseline (2149 [IQR: 480–5105] $\mu\text{g/l}$, normal range ≤ 250 $\mu\text{g/l}$) (Table 2), with 51 of 64 patients (79.7%) having levels above normal. D-dimer levels continued to increase in all patients 2 h after treatment with either rhC1INH or saline, to a median level of 2469 (643–5827) $\mu\text{g/l}$. By Day 7 posttreatment, D-dimer levels in both treatment groups regressed toward near-normal levels. Median plasma D-dimer levels were not statistically different between the groups at 2 h ($P = 0.8706$) and Day 7 ($P = 0.9753$) after treatment with either rhC1INH or saline (Fig. 1), suggesting that treatment with rhC1INH did not influence plasma D-dimer production in patients with C1-INH-HAE.

There were 15 submucosal (abdominal, oropharyngeal–laryngeal) attacks and 21 subcutaneous (facial, peripheral) attacks treated with rhC1INH; 14 submucosal and 14 subcutaneous attacks were treated with saline (Table 3 and Fig. 2). Median plasma D-dimer levels were at least threefold higher at baseline ($P = 0.0274$) and 2 h posttreatment ($P = 0.0126$) in patients with submucosal attacks compared to patients with subcutaneous attacks.

Overall, median baseline plasma D-dimer levels were similar in patients with moderate (1674 [593–5241] $\mu\text{g/l}$) and severe (2320 [260–5550] $\mu\text{g/l}$) attacks (Table 4). Of note,

Table 1 Patient demographics and baseline characteristics for safety population

	rhC1INH ($N = 43$)	Saline ($N = 31$)
Female (%)	65	61
Caucasian (%)	95	97
Age at screening (years)		
Mean (SD)	39.1 (12.63)	41.4 (15.38)
Range	17–67	18–69
HAE attacks/year		
Mean (SD)	25 (23.9)	31 (27.2)
Range	0–143	3–111
Use of prophylactic maintenance therapy (n [%])	21 [49]	15 [48]
Primary attack location (n [%])*		
Peripheral	19 [44]	14 [45]
Abdominal	16 [37]	12 [39]
Facial	6 [14]	2 [6]
Oropharyngeal–laryngeal	2 [5]	3 [10]
Overall severity VAS score at baseline for primary attack location (mm)*		
Mean (SD)	73.5 (14.13)	77.3 (12.61)
Range	50–100	49–100

HAE, hereditary angioedema; rhC1INH, recombinant human C1 esterase inhibitor; VAS, Visual Analog Scale.

*For patients with >1 eligible attack location, the primary attack location was defined as the eligible location with the highest overall severity VAS score at baseline.

Table 2 D-dimer levels over time in safety population

Time point	D-dimer levels (µg/l)	Percentage of patients with D-dimer levels >250 µg/l*
Baseline		
Mean (SD)	4211 (5622)	79.7
Median (25th–75th percentile)	2149 (480–5105)	
Range	6–24634	
N	64	
2 h after treatment†		
Mean (SD)	4421 (5740)	83.8
Median (25th–75th percentile)	2469 (643–5827)	
Range	9–5827	
N	68	
Day 7 after treatment†		
Mean (SD)	1842 (2867)	73.4
Median (25th–75th percentile)	425 (241–3240)	
Range	1–14250	
N	64	

*Normal D-dimer levels are ≤ 250 µg/l.

†Thirteen patients who originally received saline solution subsequently received rhC1INH as rescue medication. These patients are included in the saline solution summaries up until the time they received rescue medication, and included in the rhC1INH summaries afterward.

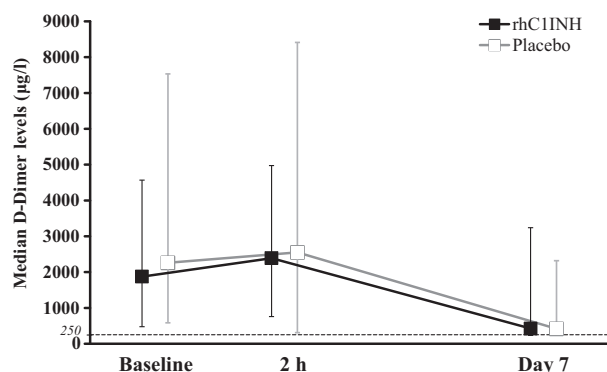


Figure 1 D-dimer (median, 25th–75th percentile) levels over time in HAE patients treated with rhC1INH or saline. rhC1INH, recombinant human C1 esterase inhibitor.

severe attacks treated with rhC1INH did tend to have lower plasma D-dimer values (280 [109–925] µg/l) by Day 7 than those treated with saline (560 [273–4056] µg/l; $P = 0.1323$, not significant).

Sixty-four patients reported single-site attacks and ten reported multiple-affected-site attacks. At baseline and at 2 h, median plasma D-dimer levels were higher in patients with multiple affected locations than those in patients with single locations. By Day 7, D-dimer levels had returned to near-normal for both groups (Fig. 3).

Discussion

In the absence of normal inhibition by C1INH, kinin–contact pathway activation is accelerated, resulting in bradykinin

generation and tissue edema (1). Additionally, increased fibrinolytic activity has been demonstrated in patients during HAE attacks and even in remissions (8–11, 24, 25). Moreover, activation of coagulation may give rise to prothrombin fragments F1 + 2, Factor VIIa, and thrombin, with similar activation observed during HAE attacks (10, 11). In a recent study by van Geffen et al. (25), activated plasma and ‘nonactivated’ plasma (taken during attacks and remissions) were studied in patients with C1-INH-HAE. Interestingly, F1 + 2, thrombin, and fibrinolysis markers (PAP, D-dimers) were significantly elevated during attacks as compared to remissions and healthy controls. Despite this, spontaneous TEE has been rarely reported in patients with HAE, implying that fibrin degradation occurs in the absence of increased thrombotic risk (11, 24).

The therapeutic use of pdC1INH is generally considered safe, although concerns have been recently raised regarding the risk of TEE for other C1INHs (13, 14). Some events were possibly related to very high off-label doses of pdC1INH (i.e., in capillary leak syndrome), but others occurred during therapy for HAE with recommended doses (13). Therefore, thrombogenicity might have been related to the C1INH protein itself, any impurities in the preparation, its production process, batch-to-batch heterogeneity, delivery systems, or patient factors. For example, human plasma products such as therapeutic immunoglobulins may carry prothrombotic risk, as increased amidolytic activity of kallikrein and FXIa contaminants has been recently demonstrated by Etscheid et al. (26). In view of these reports and the lifelong need for large amounts of pdC1INH for both on-demand and prophylactic purposes, potential risks should be reassessed.

As recombinant C1INH (Ruconest[®], rhC1INH) is the newest on the market, its potential thrombotic risk was

Table 3 D-dimer levels in HAE patients with submucosal vs subcutaneous locations of the eligible attack

Time point/anatomical location*	rhC1INH (N = 43)	Saline (N = 31)	Total (N = 74)
Baseline, µg/l			
Submucosal†	3095 (250–8676)	3055 (1700–11350)	3095 (890–10000)
Subcutaneous‡	1000 (500–4060)	899 (260–3800)	960 (450–4060)
P-value	0.2050	0.1029	0.0274
2 h, µg/l			
Submucosal	4100 (1030–7731)	5470 (2550–12500)	4100 (1030–12140)
Subcutaneous	1080 (730–4260)	835 (310–2200)	1070 (600–4100)
P-value	0.1771	0.0308	0.0126
Day 7, µg/l			
Submucosal	768 (266–4250)	418 (245–2614)	454 (266–4250)
Subcutaneous	376 (150–1400)	453 (246–2318)	376 (168–1400)
P-value	0.1958	0.9581	0.2699

HAE, hereditary angioedema; rhC1INH, recombinant human C1 esterase inhibitor.

Values are presented as median (25th–75th percentiles).

*Anatomical location represents the primary attack location (see Methods for description).

†Submucosal = oropharyngeal–laryngeal, abdominal. No urogenital attacks were reported.

‡Subcutaneous = peripheral, facial.

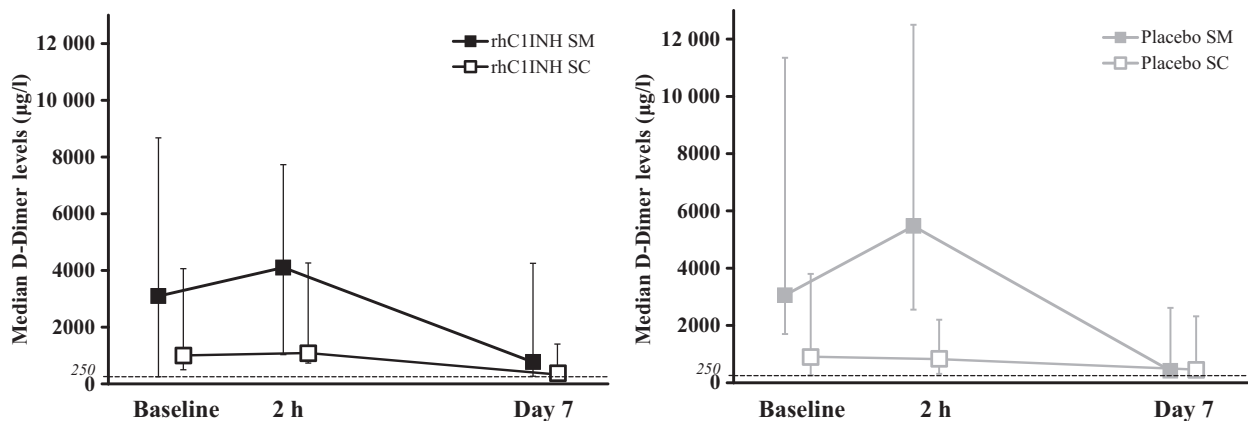


Figure 2 D-dimer levels over time in HAE patients with submucosal vs subcutaneous locations of the eligible attack. rhC1INH, recombinant human C1 esterase inhibitor; SC, subcutaneous; SM, submucosal.

recently investigated by Relan et al. (27). This study demonstrated that although coagulation and fibrinolysis pathways were indeed activated during acute attacks, treatment with rhC1INH had little or no effect on other coagulation markers, suggesting that it does not exert prothrombotic activity *in vivo* (16, 22).

The present study focused on plasma D-dimers because these are considered a reliable and sensitive index of thrombosis *in vivo* and indicative of a dynamic process of thrombus formation and lysis (12). In clinical practice, D-dimers are elevated in various thrombotic conditions, including venous thromboembolism, disseminated intravascular coagulation, and cerebrovascular accidents (28). However, D-dimers have also been shown to be elevated in conditions without clinical evidence for thrombosis (29–31).

Despite such limitations, plasma D-dimers are regarded as useful laboratory markers for the diagnosis of thrombotic conditions (12). Our study corroborates other studies, which have suggested that, despite elevated plasma D-dimers at baseline and during attacks, C1-INH-HAE patients are not at increased thrombotic risk (24, 25, 27). This seemingly paradoxical situation, where activation of the contact coagulation (i.e., via Factor XI), kinin pathway, and fibrinolytic systems does not lead to increased thrombosis, is reconciled by the observation that while FXII-driven fibrin formation is important for pathologic thrombus formation, it has no important function in fibrin formation during normal hemostasis (32). In this context, it is of interest that bradykinin B2 receptor-deficient mice (a rodent model of HAE) are protected from thrombosis

Table 4 D-dimer levels by severity at the primary attack location

	<i>n</i>	Moderate (≥50 mm, <75 mm)*	<i>n</i>	Severe (≥75 mm)*
Baseline, µg/l	24	1674 (593–5241)	39	2320 (260–5550)
2 h, µg/l	27	2000 (656–5884)	40	2678 (615–5840)
Day 7, µg/l	26	1025 (382–3770)	37	300 (150–1250)

VAS, Visual Analog Scale.

Values are presented as number of patients who had D-dimer levels at each time point and as median (25th–75th percentiles).

*Severity is based on the overall VAS score at each visit at the primary attack location.

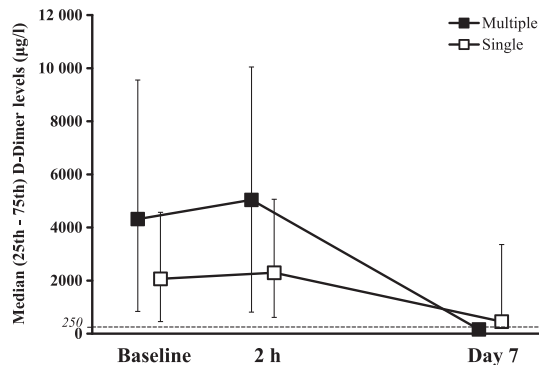


Figure 3 D-dimer levels over time in patients with multiple vs single affected locations.

(33). Furthermore, there is recent evidence for a dichotomy between bradykinin formation by activated contact system and lack of procoagulant activity in both human and rodent systems (34). Maas et al. have shown that the activation of FXII by misfolded proteins or heparin initiates contact system activation without triggering the intrinsic (FXIIa/FXIa dependent) coagulation pathway, while activation by naturally occurring polyanions (i.e., polyphosphates released by microorganisms or platelets) triggers fibrin formation in a FXII-dependent manner (32, 34). We propose that in patients with C1-INH-HAE, subclinical formation of fibrin degradation products (i.e., D-dimers) may reflect a disturbed balance between coagulation and fibrinolysis. Recently, Koning et al. (11) have shown that there is no preferential activation of the contact over the intrinsic coagulation system in C1-INH-HAE and proposed that the absence of thrombotic complications is due to other regulatory mechanisms controlling the coagulation and fibrinolysis cascades (11).

Our results do show higher plasma D-dimer levels in patients with C1-INH-HAE during attacks, as compared to remission, that is, at 7 days after the attack, which may imply that this parameter might be suitable as a biomarker of disease activity, so much desired in this intermittent disease. Such changes were especially prominent in submucosal-type attacks (abdominal, oropharyngeal–laryngeal) as compared with skin involvement, which may indicate an

organ-specific association. The contact system can assemble on endothelial surfaces, where C1INH is less capable of inactivating FXII and kallikrein, which may enhance local contact system activity (34). It seems plausible that the extent of endothelial involvement or vascular permeability changes (35) may have contributed to the differences observed in this study, as more extensive attacks at multiple locations were also associated with higher D-dimer levels.

Evidently, the administration of rhC1INH does not reduce elevated D-dimer levels (which remained high in the saline-treated cohort as well), suggesting that the major source of ongoing coagulation in C1-INH-HAE is unrelated to activation of the intrinsic pathway, and thrombus formation does not necessarily follow after Factor XII activation (32, 34). This indicates that the major source of plasmin activity responsible for D-dimer formation in C1-INH-HAE is unrelated to the Factor XII-dependent intrinsic pathway, which is in line with *in vivo* studies demonstrating that the large majority of plasminogen activation is attributable to tPA and uPA (6). The source of increased plasma D-dimers in patients with C1-INH-HAE is presently unknown. We can only speculate that local fibrin lattice formation and stabilization may be related to several factors, such as extensive endothelial hyperpermeability, vascular leakage, extravascular tissue factor, or a systemic inflammatory process.

We recognize that the main limitation of this study is that it represents the results of a single treatment, whereas in real-life situation, patients with C1-INH-HAE undergo repeated treatments with multiple doses of C1INH.

In conclusion, based on the analyses of this cohort, as well as other RCTs and clinical experience (17–21), we suggest that rhC1INH does not carry an increased prothrombotic risk. Moreover, this study indicates that increased plasma D-dimer levels are strongly associated with acute C1-INH-HAE attacks. The value of measuring plasma D-dimers in the decision-making process of C1-INH-HAE treatment needs to be evaluated by RCTs specifically designed for this purpose.

Acknowledgments

The authors acknowledge Reshma Shringarpure, PhD, formerly of Santarus, for writing portions of the manuscript.

Pharming provided the data and statistical analyses. Santarus/Salix assisted with the editing and submission.

Author contributions

A. Reshef contributed data for interpretation, contributed to the interpretation of the data, and wrote the original manuscript and further versions. A. Zanichelli and H. Longhurst contributed to the clinical study and to the interpretation of the data. A. Relan contributed to the design of the trial and to the interpretation of the data. C. E. Hack contributed to the interpretation of the data. All authors contributed to the drafting or revision of the manuscript and provided final approval of the manuscript for submission.

Funding

Supported by Pharming Technologies BV, Leiden, The Netherlands, and Santarus, Inc., a wholly owned subsidiary of Salix Pharmaceuticals, Inc.

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Conflicts of interest

A. Reshef has received research funding, travel grants, and speaker honorariums from the following companies: Dyax, CSL Behring, Shire, Pharming, Cephalon, and Teva, and worked as an advisor and speaker for Shire HGT. A. Zanichelli has received funding to attend conferences and other educational events, acted as medical advisor or speaker and participated in clinical trials with the following companies: CSL Behring, Shire, Sobi, and ViroPharma. H. Longhurst has received funding to attend conferences and other educational events, acted as medical advisor or speaker, received donations to her departmental fund, received financial and other assistance with patient care projects, and/or participated in clinical trials with the following companies: Biocryst, CSL Behring, SOBI Biovitrum, Shire, Dyax, and ViroPharma. A. Relan is an employee and shareholder of Pharming Technologies BV. C. E. Hack has received consultancy fees from Pharming Technologies in the past.

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