Effect of factor V Leiden polymorphism in severe sepsis and on treatment with recombinant human activated protein C

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Objective: **Coagulation activation is part of the acute innate host response to infection that, when uncontrolled, may contribute to organ dysfunction and death. Activated protein C limits excessive coagulation activation by inactivating factors Va and VIIIa. The factor V Leiden mutation (R506Q), a prothrombotic gene polymorphism, disrupts the activity of this natural anticoagulant by rendering factor Va partially resistant to inactivation by activated protein C. Previous findings in the mouse factor V Leiden endotoxemia model and in patients with severe sepsis suggest that factor V Leiden constitutes a rare example of a balanced gene polymorphism that may provide a survival advantage for heterozygous carriers with severe sepsis. We sought to confirm that carriers of this prothrombotic factor V Leiden mutation do not have an increased risk of developing severe sepsis and that carriers with severe sepsis derive similar treatment benefit from recombinant human activated protein C (drotrecogin alfa [activated]) as non–factor V Leiden carriers.**

Design: **Prospective collection of factor V Leiden status from two clinical studies of severe sepsis (PROWESS and ENHANCE).**

Setting: **A total of 447 clinical sites across 25 countries.**

Patients: **A total of 3,894 adult patients with severe sepsis.**

Intervention: **Either 24 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ drotrecogin alfa (activated) (n = 3063) or placebo (n = 800) for 96 hrs or no exposure to the study drug (n = 31).**

Main Results: **The effect of the factor V Leiden carrier status in severe sepsis in the PROWESS study has been previously reported. The combined data on factor V Leiden status from 3,894 adult patients with severe sepsis from the PROWESS and ENHANCE (a single-arm, open-label study of drotrecogin alfa [activated]) studies are reported here. At study entry, 3.9% of patients (150/3894) presenting with severe sepsis were heterozygous carriers. No homozygous factor V Leiden carriers were identified. The proportion of factor V Leiden carriers in patients with severe sepsis differs slightly from that predicted (allelic frequency of 2.5%) by the Hardy–Weinberg equation for the general white population ($p = .05$). There was no significant difference in baseline disease severity (Acute Physiology and Chronic Health Evaluation II score or number of organ dysfunctions) between heterozygous carriers and non-Leiden carriers. There was no significant difference in serious bleeding or thrombotic event rates with drotrecogin alfa (activated) treatment between heterozygous carriers and non-Leiden carriers. The 28-day mortality rates for heterozygous carriers and non-Leiden carriers with drotrecogin alfa (activated) treatment were 20.3% and 24.9%, respectively (risk ratio, 0.82; 95% confidence interval, 0.57–1.17).**

Conclusions: **Compared with non-Leiden carriers, factor V Leiden heterozygous carriers may have a slightly decreased risk of developing severe sepsis from infection, do not seem to have increased mortality in severe sepsis, and derive similar benefit and risk profiles from drotrecogin alfa (activated) treatment. Therefore, factor V Leiden carriers should not be excluded from this new sepsis therapy. (Crit Care Med 2004; 32[Suppl.]:S239–S246)**

Key Words: activated protein C; factor V Leiden mutation; recombinant human activated protein C; severe sepsis; single-nucleotide polymorphism

The protein C (PC) pathway limits excessive activation of blood coagulation and protects against inadvertent formation of blood clots by inactivating factors Va and VIIIa. The single-nucleotide polymorphism factor V Leiden (R506Q) (1) disrupts the activity of this natural anticoagulant pathway by rendering factor V partially resistant to inactivation by activated PC (APC). Despite increasing the risk of thrombosis in both heterozygous ($VL^{+/-}$) and homozygous ($VL^{+/+}$) carriers of the mutation (1), the factor V Leiden allele is prevalent among whites and in the population in the Indian subcontinent, with a prevalence of between 4% and 6% (2–4). This raises the intriguing question of whether the factor V Leiden mutation might confer a survival advantage exerting a positive selection pressure (5, 6), for example, by reducing blood loss during childbirth or by enhancing—via some unknown mechanism—embryo implantation (7–10).

Coagulation activation and inflammation are both part of the acute, innate, nonspecific host response to infection, which can lead to multiple organ dysfunction and death (11). Animal studies have shown that natural anticoagulants, in particular the components of the PC pathway (e.g., thrombomodulin and PC/APC), can reduce mortality associated with septic shock (12–14). Infusion of a low concentration of the coagulation protease thrombin in dogs has been shown to significantly protect the animals from endotoxin-induced mortality (15). The infused thrombin is likely to result in augmented activation of endogenous PC by the thrombomodulin–thrombin complex, thus potentially producing the same protective effect as is observed on administration of exogenous APC. A subsequent large clinical trial (PROWESS) demonstrated that recombinant human APC

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Presented, in part, at the Margaux Conference on Critical Illness, Atlanta, GA, December 9–11, 2003.

DOI: 10.1097/01.CCM.0000126122.34119.D1

(drotrecogin alfa [activated]) treatment reduced relative all-cause 28-day mortality by 19% in patients with severe sepsis compared with placebo-treated patients (16). Taken together, these data suggest that mutations resulting in enhanced thrombin formation, such as the factor V Leiden mutation, might have a similar beneficial effect on survival in acute sepsis by enhancing the formation of thrombin and by concomitantly augmenting the activation of PC. On the other hand, as severe sepsis is the consequence of exaggerated systemic host coagulation (increased thrombin generation) and inflammation in response to infection, the presence of the prothrombotic factor V Leiden mutation, together with a loss of intact thrombomodulin due to endothelial damage in severe sepsis (17), might result in higher mortality and morbidity.

A combination of clinical data on factor V Leiden carrier status obtained from the PROWESS study and animal data from a factor V Leiden mouse endotoxemia model (18, 19) suggest that: a) factor V Leiden confers a survival advantage in severe sepsis and constitutes a rare example of a balanced gene polymorphism, b) optimum generation of thrombin may be protective in the setting of sepsis, c) factor V Leiden carriers are not at an increased risk of developing severe sepsis from infection, and d) heterozygous factor V Leiden patients ($VL^{+/-}$) derive similar treatment benefit from drotrecogin alfa (activated) as non-Leiden carriers ($VL^{-/-}$).

In this study, we sought to confirm the previously reported clinical results of factor V Leiden status in severe sepsis from PROWESS (18) by using the factor V Leiden status data from a recently completed single-arm, open-label, phase IIIB study of drotrecogin alfa (activated) in severe sepsis.

MATERIALS AND METHODS

PROWESS was a randomized, double-blind, placebo-controlled, phase III trial evaluating the efficacy of drotrecogin alfa (activated) in severe sepsis. Details of the trial design, including inclusion and exclusion criteria, have been reported previously (16). Patients were enrolled between June 1998 and August 2000. The ENHANCE study was a single-arm, open-label, phase IIIB study of severe sepsis and drotrecogin alfa (activated). Patients were enrolled between March 2001 and January 2003. The inclusion and exclusion criteria of the ENHANCE study were the same as for the PROWESS study. The primary end point for both studies was 28-day all-cause mortality (efficacy) and safety. PROWESS patients were enrolled from 164 hospitals (across 11 countries) and ENHANCE patients from 361 hospitals (across 25 countries) after approval from ethics review boards. The informed consent obtained from all adult (>18 yrs) patients in both studies included permission for a blood sample for the determination of the factor V Leiden single-nucleotide polymorphism, the only genetic marker determination used in these two studies.

Factor V Leiden status was evaluated by polymerase chain reaction determination and has been described in detail previously (18). The determination of baseline values of prothrombin time, activated partial thromboplastin time, platelet counts, and PC levels were determined with methods previously described (18).

Both trials included intent-to-treat analysis. The PROWESS study enrolled 1,690 adult patients, randomized 1:1 to a 96-hr infusion of drotrecogin alfa (activated) at a rate of 24 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ (n = 850) or placebo (n = 840). The ENHANCE study enrolled 2,378 adult patients, all of whom received drotrecogin alfa (activated) at a rate of 24 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ for 96 hrs. The factor V Leiden status of 205 patients (PROWESS, 89; 116, ENHANCE) was unavailable due to errors in sampling or sample handling or because their samples failed to amplify during the polymerase chain reaction determination. In addition, the factor V Leiden status of 31 patients (PROWESS, 16; ENHANCE, 15) who were not exposed to the study drug or placebo (i.e., who were not in the intent-to-treat analysis) was also determined. Data from these 31 patients are included in only one of the analyses in this study (Table 1).

Baseline comparisons used chi-square tests for categorical and Wilcoxon's rank-sum tests for continuous variables. Chi-square tests were also used to determine the goodness of fit of the Hardy–Weinberg equilibrium assumptions. Statistical significance was assessed using a type I error rate of 5%. As

Table 1. Prevalence of factor V Leiden mutation in patients with severe sepsis in the PROWESS and ENHANCE studies, categorized by country

	No. of Factor V Leiden Carriers/No. of ITT Patients		
Country	PROWESS (%)	ENHANCE (%)	PROWESS + ENHANCE (%)
Australia	6/129 (4.7)	2/50 (4.0)	8/179 (4.5)
Belgium	8/143 (5.6)	3/83 (3.6)	11/226 (4.9)
Brazil	0/6 (0)	3/30 (10)	3/36 (8.3)
Canada	6/209 (2.9)	16/280 (5.7)	22/489 (4.5)
France	3/69 (4.3)	2/225 (.9)	5/294 (1.7)
Germany	5/67 (7.5)	19/218 (8.7)	24/285 (8.4)
Netherlands	4/76 (5.3)	3/63 (4.8)	7/139 (5.0)
New Zealand	1/68 (1.5)	—	1/68 (1.5)
South Africa	0/39 (0)	1/20 (5.0)	1/59 (1.7)
Spain	3/129 (2.3)	4/181 (2.2)	7/310 (2.3)
United States	29/666 (4.4)	6/256 (2.3)	35/922 (3.8)
Argentina	—	1/23 (4.3)	1/23 (4.3)
Austria	—	2/31 (6.5)	2/31 (6.5)
Denmark	—	1/18 (5.6)	1/18 (5.6)
Finland	—	0/25 (0)	0/25 (0)
Ireland	—	0/7 (0)	0/7 (0)
Israel	—	0/20 (0)	0/20 (0)
Italy	—	7/192 (3.6)	7/192 (3.6)
Luxembourg	—	0/1 (0)	0/1 (0)
Mexico	—	0/9 (0)	0/9 (0)
Norway	—	1/27 (3.7)	1/27 (3.7)
Portugal	—	0/19 (0)	0/19 (0)
Puerto Rico	—	0/1 (0)	0/1 (0)
Sweden	—	2/23 (8.7)	2/23 (8.7)
Switzerland	—	2/53 (3.8)	2/53 (3.8)
United Kingdom	—	10/407 (2.5)	10/407 (2.5)
Patients enrolled but not exposed to the study drug/placebo[a]	0/16 (0)	0/15 (0)	0/31 (0)
Incidence rate	65/1617 (4.0)	85/2277 (3.7)	150/3894 (3.9)
95% CI	3.1, 5.1	3.0, 4.6	3.3, 4.5

ITT, intent to treat; —, Data not available; CI, confidence interval.

[a]Not included in the intent-to-treat analysis in the PROWESS or ENHANCE studies; patients in the denominator are the total number of non-ITT patients for whom factor V Leiden status data was available.

Table 2*A*. Baseline characteristics of patients with severe sepsis in the PROWESS and ENHANCE studies, categorized by factor V Leiden mutation status[a]

Baseline Characteristics	PROWESS		ENHANCE		
	$VL^{+/-}$ Patients (n = 65)	$VL^{-/-}$ Patients (n = 1536)	$VL^{+/-}$ Patients (n = 85)	$VL^{-/-}$ Patients (n = 2177)	p^b
Demographics					
Age, yrs	63.2 ± 14.9	60.5 ± 16.8	55.6 ± 17.9	59.1 ± 17.0	.56
Male sex, %	58.5	57.0	50.6	58.3	.36
White skin color, %	93.9	81.6	94.1	90.6	.01
Previous or preexisting conditions, %					
Cancer	24.6	17.6	11.8	14.8	.88
Congestive cardiomyopathy	6.2	7.6	3.5	3.8	.93
COPD	29.2	24.0	16.5	15.0	.47
Diabetes	21.5	21.2	20.0	20.0	.22
Hypertension	32.3	36.9	31.8	35.7	.19
Liver disease	0.0	2.5	0.0	3.2	.06
Myocardial infarction	12.3	13.5	7.1	9.6	.44
Pancreatitis	3.1	3.8	8.2	3.4	.15
Recent trauma	1.5	4.2	1.2	3.4	.25
Recent surgical history	36.9	29.6	40.0	37.7	.50
Indicator of disease severity					
APACHE II score	24.9 ± 8.1	24.8 ± 7.7	22.0 ± 7.2	22.0 ± 7.4	.93
Shock, %	69.2	71.2	69.4	75.9	.21
SOFA score (cardiovascular)	2.3 ± 1.5	2.7 ± 1.5	3.1 ± 1.3	3.0 ± 1.4	.22
Use of any vasopressor, %	46.2	63.0	75.3	73.6	.09
Ventilation, %	76.9	75.5	95.3	81.5	.01
No. of dysfunctional organs, %					.70
1	21.5	24.7	12.9	15.8	
2	32.3	32.4	31.8	29.7	
≥3	46.2	42.9	55.3	54.6	
Time from first sepsis-induced organ failure to initiation of study drug, %					.69
≤24 hrs	92.3	89.1	47.1	48.2	
>24 hrs	7.7	10.9	52.9	51.8	
Site of infection, %[c]					.44
Intraabdominal	24.6	20.2	25.9	24.7	
Lung	55.4	54.1	40.0	47.4	
Other	13.8	15.5	27.0	19.5	
Urinary tract	6.2	10.2	7.1	8.4	
Infection types, %[d]					.81
Pure Gram positive	27.7	25.5	29.4	26.6	
Pure Gram negative	23.1	22.3	25.9	22.9	
Mixed Gram	23.1	14.6	7.1	13.5	
Unconfirmed	26.1	37.6	37.6	37.0	

$VL^{+/-}$, heterozygous factor V Leiden carriers; $VL^{-/-}$, patients who did not carry factor V Leiden; COPD, chronic obstructive pulmonary disease; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sepsis-related Organ Failure Assessment.

[a]This table only includes patients in the intent-to-treat analysis. Some of the PROWESS data were reported previously (1). Numbers indicate the mean ± SD. Not all percentages total 100 due to rounding; [b]*p* values from comparisons of all $VL^{+/-}$ patients with all $VL^{-/-}$ patients are pooled from both studies using Wilcoxon's rank-sum test for continuous variables and the chi-square test for categorical variables; [c]The site of infection was presumed on the basis of clinical findings; [d]Patients may have had more than one organism cultured. Classification of infection type is based on adjudication from the clinical evaluation committee for PROWESS. Data from ENHANCE are based on the results reported by the investigators.

recommended by the CONSORT guidelines (20) for subgroup analyses, we reported the relative risk point estimates and the 95% confidence intervals for the complementary subgroups. SAS for Windows version 8.2 (SAS Institute, Cary, NC) was used for these analyses.

RESULTS

Prevalence of Factor V Leiden Carriers in Patients with Severe Sepsis. Factor V Leiden carrier status was available for a total of 3,894 patients from the PROWESS and ENHANCE studies (Table 1). Among these, there were 150 heterozygous factor V Leiden carriers (carrier frequency, 3.9%; allelic frequency, 1.9%). No homozygous factor V Leiden carriers were identified. Of the 150 factor V Leiden carriers, nine were nonwhites (three Hispanic, one African American, one western Asian, and four "other"). The mean allelic frequencies for factor V Leiden in the European population and in whites in the United States are estimated to be 2.7% and 3.0%, respectively (2). Only white patients with severe sepsis were analyzed for the Hardy–Weinberg calculation to evaluate whether there is a selection pressure on the factor V Leiden mutation in severe sepsis. Using a conservative allelic frequency of 2.5%, the proportion of white factor V Leiden carriers in the PROWESS and ENHANCE studies was slightly, but significantly, lower than predicted with the Hardy–Weinberg equation ($VL^{+/+}$, 0 vs. 2; $VL^{+/-}$, 141 vs. 165; $VL^{-/-}$, 3250 vs. 3224; $p = .05$).

The prevalence of heterozygous factor V Leiden carriers was similar in the PROWESS and ENHANCE studies. Ten of the 25 countries enrolled a total of 100 or more patients into each of the two clinical studies. The prevalence of heterozy-

Table 2*B*. Baseline characteristics of patients with severe sepsis in the PROWESS and ENHANCE studies, categorized by factor V Leiden mutation status[a]

		PROWESS				ENHANCE				
		$VL^{+/-}$ Patients (n = 65)		$VL^{-/-}$ Patients (n = 1536)		$VL^{+/-}$ Patients (n = 85)		$VL^{-/-}$ Patients (n = 2177)		
Biomarkers	Normal Range	n	Median (Range)	n	Median (Range)	n	Median (Range)	n	Median (Range)	p^b
Platelet count, $\times 10^9$/L	140–400	59	172 (117–244)	1300	183 (119–253)	85	150 (108–235)	2162	159 (94–240)	.72
PT, secs	10.6–14.5	62	18.2 (15.9–21.4)	1420	18.7 (16.5–21.9)	43	17.0 (15.0–25.9)	1178	16.3 (14.0–20.1)	.44
APTT, secs	21–39	61	42.0 (36.1–49.0)	1425	42.4 (36.4–50.4)	83	41.0 (34.3–51.0)	2070	40.0 (33.0–50.0)	.97
Protein C, %	81–173	63	49 (35–65)	1435	48 (31–66)	73	42 (22–62)	1783	45 (30–64)	.48

$VL^{+/-}$ patients, heterozygous factor V Leiden carriers; $VL^{-/-}$, patients who did not carry factor V Leiden; PT, prothrombin time; APTT, activated partial thromboplastin time.

[a]This table includes only patients in the intent-to-treat analysis. Some of the PROWESS data were reported previously (1); [b]*p* values are for comparisons of all $VL^{+/-}$ patients with all $VL^{-/-}$ patients pooled from both studies using Wilcoxon's rank-sum test.

gous factor V Leiden carriers among these ten countries ranged from a low of 1.7% (France) to a high of 8.5% (Germany). The United States enrolled the highest total number of patients into the studies and also enrolled the largest number of heterozygous factor V Leiden carriers (n = 35).

Baseline Characteristics. The baseline characteristics of patients from both studies were recorded, and the data from pooled heterozygous factor V Leiden carriers and non-Leiden carriers were compared (Table 2). Patient data were analyzed on an intent-to-treat basis. There was no difference in preexisting conditions other than the $VL^{+/-}$ patients having a lower frequency of liver disease. There was no difference in Acute Physiology and Chronic Health Evaluation (APACHE) II score or number of organ dysfunctions at study entry between factor V Leiden carriers and non-Leiden carriers. There was a trend for $VL^{+/-}$ patients to have less cardiovascular dysfunction (less shock, less vasopressor therapy, or a lower cardiovascular Sepsis-related Organ Failure Assessment score) at study entry than non-Leiden carriers. There was a higher proportion of $VL^{+/-}$ patients than $VL^{-/-}$ patients on ventilators at study entry, as evidenced mainly by data from the ENHANCE study. Factor V Leiden status had no effect on the site or type of infection or on markers of coagulopathy at baseline.

The PROWESS and ENHANCE study protocols had the same window of 48 hrs between patients meeting the entry criterion of the development of the first sepsis-associated organ dysfunction and first administration of the study drug. In the PROWESS study, >90% of patients had <24 hrs of sepsis-associated organ dysfunction at study entry before study drug/placebo infusion. On the other hand, in the ENHANCE study, only about half of the patients had <24 hrs of organ dysfunction before study entry, whereas the rest had more. However, there was no difference between $VL^{+/-}$ and $VL^{-/-}$ patients in time from organ dysfunction to start of study drug infusion.

Effect of Drotrecogin Alfa (Activated) Treatment on Mortality, Serious Bleeding, and Thrombotic Events by Leiden Carrier Status. The drotrecogin alfa (activated) treatment effect according to factor V Leiden status was reported previously for the PROWESS patients (18). Because the ENHANCE study was a single-arm study and all patients were treated with drotrecogin alfa (activated), there are no new data for placebo-treated patients to add in this article. All of the new analyses are primarily between drotrecogin alfa (activated)–treated pooled $VL^{+/-}$ and $VL^{-/-}$ patients from the PROWESS and ENHANCE studies.

The 28-day all-cause mortality data for the PROWESS patients, ENHANCE patients, and the combined PROWESS and ENHANCE patients are shown in Table 3 by Leiden carrier status. The mortality data were also analyzed by time from first sepsis-associated organ failure to start of study drug infusion for patients enrolled in the United States (the highest enroller of patients in both studies). The combined PROWESS and ENHANCE data show that heterozygous factor V Leiden carriers tended to have lower 28-day mortality rates than non-Leiden carriers when looking at all intent-to-treat patients, drotrecogin alfa (activated)–treated patients, patients enrolled in the United States alone, and time from first organ failure to the start of study drug infusion. Due to the small sample size of the heterozygous factor V Leiden carriers, the 95% confidence intervals are relatively wide.

There was a trend (although it was not significant) toward lower rates of serious bleeding events in heterozygous factor V Leiden carriers vs. non-Leiden carriers (Table 4) both during the drotrecogin alfa (activated) infusion period and during the 28-day study period.

There were two serious thrombotic events reported for the 150 heterozygous factor V Leiden patients (2/150, 1.3%) during the 28-day study period. This is similar to the respective serious thrombotic event rates of 3% and 2% for placebo- and drotrecogin alfa (activated)–treated patients reported for the PROWESS trial (16).

DISCUSSION

Genetic association studies can be powerful tools for providing a better understanding of disease pathogenesis. Pharmacogenomic studies are important in providing treatment risk/benefit information for carriers of genetic variants. In the PROWESS trial, the factor V Leiden mutation was studied for possible genetic association with susceptibility and outcome in sepsis and for its pharmacogenomic effect on drotrecogin alfa (activated) treatment in severe sepsis (18). Indeed, in this study (18), factor V Leiden heterozygous carriers with severe sepsis were found to derive similar survival benefit from drotrecogin alfa (activated) as non-Leiden carriers and thus should not be excluded from this new therapy. Some

Table 3. All-cause mortality at 28 days in the PROWESS and ENHANCE studies, categorized by factor V Leiden mutation status[a]

Study	All-Cause Mortality at 28 Days No. of Deaths/Total Patient No. (%)		Relative Risk (95% CI)
	$VL^{+/-}$ Patients	$VL^{-/-}$ Patients	
PROWESS			
All patients	9/65 (13.9)	428/1536 (27.9)	0.50 (0.27, 0.92)
Placebo	5/32 (15.6)	238/768 (31.0)	0.50 (0.22, 1.14)
Time from first organ failure to start of placebo infusion			
<24 hrs	4/30 (13.3)	214/688 (31.1)	0.43 (0.17, 1.07)
≥24 hrs	1/2 (50.0)	24/80 (30.0)	1.67 (0.40, 6.94)
Drotrecogin alfa (activated)	4/33 (12.1)	190/768 (24.7)	0.49 (0.19, 1.24)
Time from first organ failure to start of drotrecogin alfa (activated) infusion			
<24 hrs	4/30 (13.3)	168/681 (24.7)	0.54 (0.22, 1.36)
≥24 hrs	0/3 (0)	22/87 (25.3)	—
ENHANCE			
All patients (all drotrecogin alfa [activated])	20/85 (23.5)	543/2174 (25.0)	0.94 (0.64, 1.39)
Patients from the United States only	1/6 (16.7)	66/251 (26.3)	0.63 (0.10, 3.84)
Patients from countries common to PROWESS	15/59 (25.4)	306/1346 (22.7)	1.12 (0.71, 1.75)
Time from first organ failure to start of drotrecogin alfa (activated) infusion			
<24 hrs	12/40 (30.0)	233/1045 (22.3)	1.35 (0.83, 2.19)
≥24 hrs	8/45 (17.8)	310/1128 (27.5)	0.65 (0.34, 1.22)
PROWESS + ENHANCE			
All patients	29/150 (19.3)	971/3710 (26.2)	0.74 (0.53, 1.03)
Placebo	5/32 (15.6)	238/768 (31.0)	0.50 (0.22, 1.14)
Drotrecogin alfa (activated)	24/118 (20.3)	733/2942 (24.9)	0.82 (0.57, 1.17)
Patients from the United States only			
Drotrecogin alfa (activated)	4/20 (20)	142/568 (25)	0.80 (0.33, 1.94)
Time from first organ failure to start of drotrecogin alfa (activated) infusion			
<24 hrs	16/70 (22.9)	401/1726 (23.2)	0.98 (0.63, 1.53)
≥24 hrs	8/48 (16.7)	332/1215 (27.3)	0.61 (0.32, 1.16)

$VL^{+/-}$ patients, heterozygous factor V Leiden carriers; $VL^{-/-}$ patients, patients who did not carry factor V Leiden; CI, confidence interval; —, not applicable.

[a]Only patients in the intent-to-treat analysis are included in this table. Some of the PROWESS data were reported previously (18).

of the observations, obtained from data from a small number of heterozygous factor V Leiden patients with severe sepsis, were augmented by animal data from the factor V Leiden mouse endotoxemia model (18). Recent comprehensive reviews (21–23) of >600 genetic association studies of 166 genetic variants pointed out that only a handful of results were able to be consistently replicated. This may be particularly problematic for genetic association studies in complex diseases. Some of the potential reasons for nonreproducibility of results include inadequate sample size and the effect of other genetic or environmental factors on clinical outcome. Heeding the recommendations from these comprehensive reviews (21–23) of genetic association studies, we have re-estimated the confidence interval of the advantage of factor V Leiden heterozygosity in severe sepsis using the additional data available from the recently completed ENHANCE study. However, because ENHANCE was a single-arm study in which all patients were treated with drotrecogin alfa (activated), the additional data do not provide any further assessment of the survival advantage of the factor V Leiden mutation alone in severe sepsis. We hope that the intensive review of more factor V Leiden patient data from PROWESS and ENHANCE in this article will facilitate meta-analyses of future clinical data sets on the factor V Leiden polymorphism in sepsis.

Both the PROWESS and ENHANCE study protocols had the same inclusion and exclusion criteria. PROWESS was a two-arm study with 1:1 randomization to placebo or drotrecogin alfa (activated), whereas ENHANCE was a single-arm study in which all patients were treated with drotrecogin alfa (activated). There were two main variations between PROWESS and ENHANCE that may potentially have influenced the size of the effect of the factor V Leiden mutation in severe sepsis: a) patients were enrolled from 25 countries in ENHANCE vs. 11 in PROWESS, and standard clinical practices may vary between countries; b) whereas about 90% of the PROWESS patients were enrolled within 24 hrs of the first sepsis-associated organ dysfunction, approximately 50% of the ENHANCE patients were enrolled >24 hrs after the first sepsis-associated organ dysfunction. A temporal aspect of the protective effect of genetic polymorphisms has previously been reported elsewhere (24).

Heterozygous Factor V Leiden Carriers May Have a Lower Risk of Developing Severe Sepsis from Infection. Recent data from a factor V Leiden mouse endotoxemia model suggest that the prothrombotic genetic polymorphism confers a survival advantage in LD_{50} endotoxin challenge (18) and that this single-point mutation in factor V constitutes a rare example of a balanced gene polymorphism, in which heterozygous carriers have an advantage over the homozygous carriers of either allele. Furthermore, $VL^{+/-}$ mice demonstrate earlier increased thrombin generation, with resultant earlier increased generation of endogenous APC, after challenge with a high dose of endotoxin than $VL^{-/-}$ mice. Earlier increases in endogenous APC and thrombin and a possibly improved APC/thrombin ratio in the $VL^{+/-}$ mice during endotoxemia may provide the survival advantage. If the protective effect of the factor V Leiden mutation were to extend to infection in humans, then one might speculate that factor V Leiden carriers would be at a lower risk of developing severe sepsis from infection.

Data from previous studies of the PROWESS patients suggest that factor V Leiden carriers are not at greater risk of

Table 4. Serious bleeding events in patients in the PROWESS and ENHANCE studies, categorized by factor V Leiden mutation status[a]

Study	No. of Patients with Events/Total Patient No. (%)		Relative Risk (95% CI)
	$VL^{+/-}$ Patients	$VL^{-/-}$ Patients	
Serious bleeding events during infusion period (4 days + 1 day postinfusion)			
PROWESS			
Placebo	0/32 (0)	8/768 (1.0)	—
Drotrecogin alfa (activated)	0/33 (0)	18/768 (2.3)	—
ENHANCE			
Drotrecogin alfa (activated)	3/85 (3.5)	84/2177 (3.9)	0.91 (0.30, 2.83)
PROWESS + ENHANCE			
Drotrecogin alfa (activated)	3/118 (2.5)	102/2945 (3.5)	0.73 (0.24, 2.28)
Serious bleeding events at 28 days			
PROWESS			
Placebo	0/32 (0)	15/768 (2.0)	—
Drotrecogin alfa (activated)	0/33 (0)	28/768 (3.6)	—
ENHANCE			
Drotrecogin alfa (activated)	4/85 (4.7)	143/2177 (6.6)	0.72 (0.27, 1.89)
PROWESS + ENHANCE			
Drotrecogin alfa (activated)	4/118 (3.4)	171/2945 (5.8)	0.58 (0.22, 1.55)

$VL^{+/-}$ patients, heterozygous factor V Leiden carriers; $VL^{-/-}$ patients, patients who did not carry factor V Leiden; CI, confidence interval; —, not applicable.

[a]Only patients in the intent-to-treat analysis are included in this table. Some of the PROWESS data were reported previously (1).

developing severe sepsis from infection than non-Leiden carriers (18). Combining the factor V Leiden carrier status databases from the PROWESS and ENHANCE studies offers a larger sample size to further examine the question of susceptibility. The frequency of heterozygous factor V Leiden status in patients with severe sepsis seems to be lower than the mean frequency of the mutation in the general populations of the countries from which the patients were enrolled (3.9% vs. 5–6%). The majority of the patients (54%) in the two studies were enrolled from North America, the UK, and Germany, where the general population allelic frequencies for the factor V Leiden mutation are reported to be 3.0% (for whites), 3.4%, and 3.6%, respectively (2). Using only data from white patients in our study and a conservative allelic frequency of 2.5% for factor V Leiden, we find that, as described in "RESULTS," the proportion of factor V Leiden carriers with severe sepsis is slightly, but significantly, lower than that predicted by the Hardy–Weinberg equilibrium equation.

Because patients with severe sepsis are older (in this study, the median age was 63 yrs) than the general population, it may be more appropriate to compare the prevalence of the factor V Leiden mutation in our study with the general elderly white population in case factor V Leiden carriers have a shorter life span. Data from five published studies (25–29) indicate that heterozygosity for the factor V Leiden mutation does not seem to have an effect on longevity and has a similar frequency in the elderly population as it does in the general population. The data were pooled from these five studies, and the overall prevalence of heterozygous factor V Leiden carriers in the normal elderly population was found to be 5.5% (140/2,537). The 2,537 elderly subjects were ≥65 yrs old (534 were centenarians) and were sampled from five different countries (France, Italy, United States, Sweden, and the Netherlands).

The data from our study suggest that heterozygous factor V Leiden carriers may have a lower risk of developing severe sepsis from infection than non-Leiden carriers. However, we cannot rule out possible selection bias due to the inclusion and exclusion criteria of the PROWESS and ENHANCE studies. Future epidemiologic studies in the general population (for example, using the ninth revision of the International Classification of Diseases code for severe sepsis) will be needed to confirm our results.

No homozygous factor V Leiden carriers were identified among the 3,894 patients with severe sepsis in our study and, interestingly, neither were any identified among the pooled 2,537 normal elderly subjects from the five previous studies (25–29). The frequency of homozygous factor V Leiden in severe sepsis and in normal elderly subjects is below the estimated frequency (0.06% to 0.25%) of homozygous factor V Leiden carriers in the general white population (2, 30). A recent large screening study in Germany reported the frequency of homozygous factor V Leiden carriers to be 0.13% (116/8,5304) in neonates (31), confirming that there is no apparent selection bias against homozygosity of this mutation for fetal loss. The reason for not having a single homozygous factor V Leiden carrier in our study may either be chance alone, because of the low frequency of the homozygous state, or a result of the homozygous state having a possible negative effect on longevity.

Results from the factor V Leiden mouse endotoxemia study suggest that homozygous factor V Leiden mice have a similar mortality rate to non-Leiden mice (18). Because of the low frequency of human homozygous factor V Leiden carriers, it may not be possible to test whether they are more susceptible to developing severe sepsis from infections and whether they have worse outcomes from severe sepsis than non-Leiden carriers. Future studies of factor V Leiden mice with various pathogens may help answer questions about homozygosity for the factor V Leiden mutation that may not be feasible in human studies.

Factor V Leiden Heterozygosity Does Not Significantly Affect Baseline Characteristics. The factor V Leiden prothrombotic polymorphism does not seem to affect the severity of severe sepsis when compared with non-Leiden carriers, as has been evidenced by baseline APACHE II score, number of organ dysfunctions, and levels of laboratory markers of coagulopathy. As observed previously (18), $VL^{+/-}$ carriers had a tendency to enter the studies with less cardiovascular dys-

Compared with non-Leiden carriers, factor V Leiden heterozygous carriers may have a slightly decreased risk of developing severe sepsis from infection, do not seem to have increased mortality in severe sepsis, and derive similar benefit and risk profiles from drotrecogin alfa (activated) treatment.

function than $VL^{-/-}$ carriers. If the protective effect of the factor V Leiden mutation acts by attenuating a drop in blood pressure as a result of early elevation of endogenous thrombin and APC levels—as suggested by the factor V Leiden mouse endotoxemia model—then the protective effect of endogenous APC may be due to mechanisms beyond its antithrombotic activity alone. There was no significant difference in preexisting conditions between $VL^{+/-}$ and $VL^{-/-}$ patients, with the possible exception of liver disease. Large studies would be required to determine whether this mutation truly has a protective effect on liver disease.

Heterozygous Factor V Leiden and Non-Leiden Carriers with Severe Sepsis Derive Similar Risks and Benefits from Drotrecogin Alfa (Activated) Treatment. There is no evidence from the combined PROWESS and ENHANCE data that carriers of the factor V Leiden mutation are at any increased risk of serious thrombotic events during their episode of severe sepsis compared with non-Leiden carriers, with or without drotrecogin alfa (activated) treatment. Recent analyses of serious thromboembolic events in patients with severe sepsis indicate that these events are predominately arterial events, such as myocardial infarction and ischemic stroke, and not venous events (32). The fact that the factor V Leiden mutation has only been associated with an increased risk of deep vein thrombosis and not arterial thrombosis (33, 34) is consistent with the data in this report that, compared with non-Leiden carriers, heterozygous factor V Leiden carriers do not have an increased risk of serious thromboembolic complications from severe sepsis.

With drotrecogin alfa (activated) treatment, there is a consistent trend across both the PROWESS and ENHANCE studies for heterozygous factor V Leiden carriers with severe sepsis to be at lower risk for serious bleeding than non-Leiden carriers. Because of the low event rates, this trend did not reach significance. This lower risk of bleeding with drotrecogin alfa (activated) treatment is consistent with previous reports that this prothrombotic polymorphism provides the advantage of reduced blood loss during childbirth and in surgery (7, 10).

Data from the ENHANCE study show the same trend as the PROWESS data in terms of the survival advantage gained from heterozygous factor V Leiden. By pooling all drotrecogin alfa (activated)–treated patients from both studies and comparing the mortality in severe sepsis of $VL^{-/-}$ and $VL^{+/-}$ carriers, the relative risk works out as 0.82 (95% confidence interval, 0.57–1.17) in favor of the $VL^{+/-}$ carriers. These data are from a large sample size of $VL^{+/-}$ carriers, and the estimate of the protective effect of the factor V Leiden mutation is probably more reflective of the true value than the data from the PROWESS study alone (relative risk, 0.49; 95% confidence interval, 0.19–1.24).

CONCLUSIONS

Factor V Leiden carrier status data were pooled from the PROWESS and ENHANCE studies. Compared with non-Leiden carriers, factor V Leiden heterozygous carriers may have a slightly decreased risk of developing severe sepsis from infections because the frequency differs slightly, but significantly, from the Hardy–Weinberg equilibrium prediction ($p = .05$). At baseline, the disease severity (according to clinical measures and laboratory markers of coagulopathy) of heterozygous factor V Leiden carriers was similar to, and not more than, that observed in non-Leiden carriers. Also, patients heterozygous for the factor V Leiden mutation did not experience more serious thromboembolic events than non-Leiden carriers during the 28-day study period. Factor V Leiden carriers had less serious bleeding events than non-Leiden carriers with drotrecogin alfa (activated) treatment, although this was not significant because of the low event rate. With study drug treatment, the 28-day mortality rates were 20.3% and 24.9% for $VL^{+/-}$ and $VL^{-/-}$ carriers, respectively (relative risk, 0.82; 95% confidence interval, 0.57–1.17). Therefore, factor V Leiden heterozygous carriers with severe sepsis derived benefits similar to those of non-Leiden carriers from drotrecogin alfa (activated) treatment.

ACKNOWLEDGMENTS

We thank Ms. Chuyun Huang for statistical support, Dr. Sandy Kirkwood for consultation concerning Hardy–Weinberg equilibrium, and Dr. John Brandt for review of the manuscript.

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