

Treatment effects of drotrecogin alfa (activated) in patients with severe sepsis with or without overt disseminated intravascular coagulation¹

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To cite this article: Dhainaut J-F, Yan SB, Joyce DE, Pettilä V, Basson B, Brandt JT, Sundin DP, Levi M. Treatment effects of drotrecogin alfa (activated) in patients with severe sepsis with or without overt disseminated intravascular coagulation. *J Thromb Haemost* 2004; 2: 1924–33.

Summary. Disseminated intravascular coagulation (DIC) is a serious condition associated with sepsis. Clinical management of DIC is hampered by lack of clear diagnostic criteria. The International Society on Thrombosis and Haemostasis (ISTH) has proposed a diagnostic scoring algorithm for overt DIC based on routine laboratory tests. The objective was to assess a modified version of the ISTH scoring system and determine the effect of drotrecogin alfa (activated) (DrotAA, recombinant human activated protein C) on patients with DIC. The large database from the PROWESS clinical trial in severe sepsis was retrospectively used to assess a modified ISTH scoring system. Baseline characteristics and treatment effects of DrotAA were evaluated. At baseline, 29% (454/1568) of patients had overt DIC. Overt DIC was a strong predictor of mortality, independent of APACHE II score and age. Placebo-treated patients with overt DIC had higher mortality than patients without (43 vs. 27%). DrotAA-treated patients with overt DIC had a trend towards greater relative risk reduction in mortality than patients

without (29 vs. 18%, $P = 0.261$) but both groups had greater relative risk reduction than placebo-treated patients. Serious bleeding rates during DrotAA infusion in patients with and without overt DIC were slightly increased ($P = 0.498$), compared with placebo, while clinically overt thrombotic events during the 28-day period were slightly reduced ($P = 0.144$). Modified ISTH overt DIC scoring may be useful as an independent assessment for identifying severe sepsis patients at high risk of death with a favorable risk/benefit profile for DrotAA treatment. Patients without overt DIC also received significant treatment benefit.

Keywords: disseminated intravascular coagulation, drotrecogin alfa (activated), severe sepsis.

¹Conflict of Interest/Financial Disclosure: Eli Lilly and Co. provided funding for this study. Jean-Francois Dhainaut was an investigator in the PROWESS study and is a consultant for Eli Lilly and Co. Ville Pettilä has participated as an investigator in Eli Lilly and Co.-sponsored clinical trials. Marcel Levi has participated as an investigator in Eli Lilly and Co.-sponsored clinical trials and has been an invited speaker at conferences supported by Eli Lilly and Co. S. Betty Yan, David E. Joyce, Bruce Basson, John T. Brandt and David P. Sundin are employees and stockholders of Eli Lilly and Co.

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Received 6 November 2003, accepted 30 June 2004

Introduction

Disseminated intravascular coagulation (DIC) is a complex disorder characterized by activation of coagulation and fibrinolytic pathways, consumption of coagulation factors and depletion of coagulation regulatory proteins [1]. DIC is widely recognized as one of the most common conditions associated with sepsis [1]. Although physicians have recognized DIC for more than a century, diagnosis of DIC still varies dramatically, with no clear consensus definition. Indeed, the clinical picture of DIC may vary depending on the clinical setting. Surgeons may consider DIC primarily a bleeding disorder, while many critical care specialists and hematologists consider DIC primarily a thrombotic disorder. In fact, DIC, which complicates a wide range of severe systemic diseases, is both a bleeding and a thrombotic disorder of small and mid-sized vessels [1]. It is important to note that DIC in surgery or major trauma may indeed have a different phenotype (i.e. more bleeding) due to disrupted blood vessels, massive transfusion, hypothermia and/or acidosis.

The clinical features of DIC include microvascular thrombotic complications that may hamper adequate blood supply to organs. In conjunction with hemodynamic and metabolic derangements seen in sepsis, DIC may contribute to development of the multiple organ dysfunction syndrome [1]. The simultaneous consumption and subsequent depletion of platelets and coagulation proteins due to ongoing activation of the coagulation system may also induce severe bleeding complications, which are uncommon in sepsis [2].

Data from many small clinical studies, using various definitions and scoring methods for DIC, have suggested that DIC might be an important predictor of mortality in patients with sepsis [1,3,4]. Recently, the DIC subcommittee of the International Society on Thrombosis and Haemostasis (ISTH) proposed a definition of DIC, accompanied by a cumulative scoring system for clinically overt DIC [5]. The proposal suggests patients should have an underlying medical condition known to be associated with DIC and uses a scoring system that assigns points to results from routine laboratory tests. In this system, a score of 5 or greater would meet the definition of overt DIC. Until prospectively validated, the proposed scoring system is based on retrospective analysis of cohorts of patients with DIC.

In a large (1690 patients) phase 3 clinical trial (PROWESS), drotrecogin alfa (activated), a recombinant version of human activated protein C, demonstrated a significant improvement in the survival of patients with severe sepsis [6]. The PROWESS trial was a multicenter, placebo-controlled, and randomised study. Patients were enrolled using guidance from the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) consensus definition of severe sepsis [7]. Fifteen systemic biomarkers of coagulopathy, inflammation, and endothelial dysfunction were prospectively defined and collected as part of the PROWESS study [8]. Compared with placebo, drotrecogin alfa (activated) was shown to significantly reduce D-dimer levels and other markers of coagulopathy in patients with severe sepsis [6,8]. The biomarker data from this large trial provided a unique opportunity to analyze and characterize DIC and the treatment response in patients with severe sepsis by DIC status. Furthermore, it provided an opportunity to assess the clinical utility of the proposed ISTH scoring system.

As fibrinogen levels were not collected in the PROWESS study, a modified ISTH definition was used for this report. A previous phase 2 clinical trial in patients with severe sepsis with drotrecogin alfa (activated) showed that only 2.9% of patients had decreased fibrinogen levels ($< 2 \text{ g L}^{-1}$, the lower limit of normal) [9]. In fact, a majority of patients had elevated plasma levels of fibrinogen ($> 4 \text{ g L}^{-1}$, the upper limit of normal), presumably due to the acute phase response of fibrinogen in sepsis. None of the patients with severe sepsis with decreased fibrinogen levels in this study had fibrinogen levels below 1 g L^{-1} ([9], and data on file at Eli Lilly & Co.). In the published ISTH scoring system for the diagnosis of DIC a value of one point is assigned for fibrinogen values $< 1 \text{ g L}^{-1}$. Therefore, patients with severe sepsis who would get an

additional overt DIC score point due to fibrinogen levels below 1 g L^{-1} are uncommon.

The objectives of this study were: (i) to estimate the frequency of overt DIC in patients with severe sepsis, using a modified ISTH scoring system; (ii) to use the estimates to compare baseline characteristics of patients with severe sepsis, with or without overt DIC; (iii) to estimate and evaluate overt DIC status as a predictor of 28-day all-cause mortality in patients with severe sepsis; and (iv) to estimate and evaluate the treatment effects of drotrecogin alfa (activated), including efficacy and safety, by overt DIC status.

Methods

Analyses in this study used data collected as part of the randomized, double blind, placebo-controlled, phase 3 clinical trial, PROWESS, which evaluated the efficacy and safety of drotrecogin alfa (activated) (Xigris[®], Eli Lilly and Company, Indianapolis, IN, USA) in patients with severe sepsis [6]. Inclusion criteria for the trial included: a known or suspected infection based on clinical assessment, three or more signs of systemic inflammation, and one or more sepsis-associated acute organ dysfunction [7]. Patients at risk of life-threatening bleeding or platelet counts below $30 \times 10^9 \text{ L}^{-1}$ were excluded. Details on other inclusion/exclusion criteria have been described previously [6]. A total of 1690 patients participated in the study, 850 in the drotrecogin alfa (activated) group and 840 in the placebo group. Using the scoring system proposed by the ISTH [5], PROWESS patients were assessed at study entry (baseline, prior to study drug administration) and over the next 28 study days to determine whether they had developed overt DIC. Blood samples were analyzed for a panel of prospectively defined coagulation markers including platelet count, prothrombin time (PT), and D-dimer, which were used to assign points toward the ISTH overt DIC score.

Definition of serious bleeding and serious thrombotic events

As described previously [6], serious bleeding events were defined as: any intracranial hemorrhage; any life-threatening bleeding event; any event that required 3 U or more of packed red blood cell transfusion per day for two consecutive days; any bleeding event classified as serious for other reasons by the investigator, or any bleeding event that met the regulatory definition of serious.

In PROWESS, serious thrombotic events were recorded based on clinical assessment by the investigators; objective confirmation was not required and routine screening for asymptomatic thrombosis was not a part of this protocol in severely ill patients. The definition of serious thrombotic events included any of the following: peripheral arterial thrombosis or embolus, cerebral arterial thrombosis, myocardial infarct, cerebral infarct, thrombotic cerebrovascular accident, pulmonary thromboembolism, and deep venous thrombosis.

Laboratory methods

Blood samples were drawn preinfusion and on study days 1–7, 14, and 28. Citrated plasma and serum samples were stored at -70°C until analyzed by the central laboratory. PT, activated partial thromboplastin time (APTT), D-dimer, protein C, protein S, and antithrombin (AT) levels were obtained at each time point. Central laboratory platelet counts were determined from EDTA-anticoagulated blood samples obtained prior to study drug infusion and on study days 4 and 6. Local hospital laboratory platelet counts were collected daily for each patient at study entry through day 7, and on days 14 and 28. Serum for interleukin 6 (IL-6) determinations was obtained preinfusion and on study days 1–7. The last 403 consecutive patients enrolled in the trial were analyzed for the following additional biomarkers: prothrombin fragment F1.2 (F1.2); thrombin-antithrombin complex (TAT); plasminogen activator inhibitor-1 (PAI-1); thrombin activatable-fibrinolysis inhibitor (TAFI); α_2 -antiplasmin (α_2 -AP); plasminogen; and soluble thrombomodulin (sTM) using citrated plasma collected prior to study drug infusion and on study days 1, 2, 4, and 5. These 403 patients were enrolled following the single PROWESS study amendment that included the collection of additional blood samples. Of these 403 patients, 197 patients came from the placebo group and 206 from the drotrecogin alfa (activated) group.

Coagulation and chromogenic assays were performed on either STA or STA Compact analyzers (Diagnostica Stago Inc., Asnieres, France). APTT (STA-APTT), PT (STA-Neoplastine CI plus), protein C (Staclot Protein C), and protein S (Staclot Protein S) were all measured with coagulation-based activity assays. Quantitative D-dimer levels were measured with the STA Liatest D-DI immunoassay. AT (Stachrom ATIII), α_2 -AP (Stachrom Antiplasmin), plasminogen (Stachrom Plasminogen), and PAI-1 (Stachrom PAI) activity levels were determined with chromogenic assays. TAFI (ELISA, Enzyme Research Laboratories, South Bend, IN, USA), sTM (Asserachrom Thrombomodulin, Diagnostica Stago), F1.2 (Behringer Diagnostics, Westwood, MA, USA), TAT (Behringer Diagnostics), and interleukin (IL)-6 (Quantikine Human IL-6 HS kit, R & D Systems, Minneapolis, MN, USA) antigen levels were measured by enzyme-linked immunoassay. Platelet counts were performed using flow-cytometric methodology.

Determination of overt DIC status using the modified ISTH definition

Because all PROWESS patients had severe sepsis, a disorder known to be associated with DIC, the *conditio sine qua non* was met for the use of the ISTH overt DIC scoring algorithm [5]. The global coagulation test score portion of the algorithm used for this study is listed in Table 1. Fibrinogen could not be used in the scoring system for the present study, as fibrinogen was not measured in the PROWESS study. With the exception of platelet counts, central laboratory values were used for scoring.

Table 1 Global coagulation test score for overt DIC using the modified (no fibrinogen levels) ISTH scoring system

| Global coagulation tests | Score |
|---|----------|
| Platelets counts (10^9 L^{-1}) | |
| < 50 | 2 |
| < 100 | 1 |
| ≥ 100 | 0 |
| D-Dimer levels ($\mu\text{g mL}^{-1}$) | |
| > 4 | 3 |
| > 0.39 | 2 |
| ≤ 0.39 (upper limit of normal) | 0 |
| Prothrombin time (s) | |
| > 20.5 | 2 |
| > 17.5 | 1 |
| ≤ 17.5 (14.5 as upper limit of normal) | 0 |
| Overt DIC status requires total score | ≥ 5 |

Local laboratory platelet counts were used for scoring because no clinically significant difference was found between local and central platelet counts and daily values were available for local but not central platelet counts. Patients with two or more of the global coagulation test values listed in Table 1 missing at baseline were classified as ‘unknown’ DIC status because only a maximal score of 3 could have been obtained for the remaining parameter.

D-Dimer levels were used for the global coagulation test category of ‘elevated fibrin-related marker’. The ISTH overt DIC definition proposed a score of 2 for a moderate increase and a score of 3 for a strong increase in the fibrin-related marker test, without providing a numerical cut-off for these points [5]. Cut-off values for D-dimer in Table 1 reflect the original intent of the ISTH DIC subcommittee to have a relatively low threshold for ‘moderate increases’ in the fibrin-related marker and a relatively high threshold value for ‘strong increases’ in the fibrin-related marker.

Statistics

χ^2 and Wilcoxon rank-sum tests were used to compare the distributions of baseline characteristics between patient groups with or without overt DIC. To evaluate whether overt DIC score at baseline was a predictor of 28-day mortality independent of age and APACHE II score, a logistic regression model was fit to 28-day all cause mortality in placebo patients ($N = 768$) using age, APACHE II score, and ISTH overt DIC points as continuous predictors.

As recommended by the Consolidated Standards of Reporting Trials (CONSORT) guidelines [10], we assessed potential treatment-by-subgroup interactions using the Breslow-Day test for homogeneity of odds ratios across strata. The odds ratio scale is the most generally accepted scale to perform interaction analyses across subgroups [11]. No statistical adjustments were made for the multiplicity of subgroup analyses presented. Treatment effects evaluated were 28-day mortality, serious bleeding, and thrombotic events.

Overt DIC status was evaluated on days 0–7, 14, and 28. Patients were classified as overt DIC, without overt DIC, unknown DIC status, or dead. Patients discharged from the hospital inevitably ended up in the unknown category since they were no longer available for laboratory evaluation. We compared the proportion of overt DIC between placebo and drotrecogin alfa (activated) treated patients using χ^2 tests for surviving patients with no more than 1 missing ISTH global coagulation measurement listed in Table 1, and tested for a treatment by time interaction in a generalized linear model (i.e. repeated measures; logit link).

Results

Overt DIC at baseline

At the time of study entry, 29% (454/1568) of patients with sufficient data for classification had overt DIC (Table 2). Of the 454 patients with overt DIC, 70.3% (319/454) had a total ISTH overt DIC score of 5. Of the 1114 patients without overt DIC, 34.9% (389/1114) and 35.4% (394/1114), respectively, had total ISTH overt DIC points of 3 and 4 (Table 2). Overt DIC status at baseline could not be determined in 7.2% of patients (122/1690) as a result of two or more missing data points necessary to complete the overt DIC score (Table 1). These patients were classified as the 'unknown' DIC group (Table 2) and were not included in further analyses.

Baseline characteristics were compared between patients with or without overt DIC (Tables 3–6). In terms of demographics, patients with overt DIC were more likely to be non-Caucasian and from surgical ICUs, while they were less likely to have underlying comorbidities (hypertension, COPD, and cardiomyopathy) than patients without overt DIC (Table 3). Patients at study entry with overt DIC had worse disease severity than patients without overt DIC as assessed by APACHE II scores, number of organ failures, and SOFA scores (Table 4). There were no clinically important differences observed in baseline disease severity between placebo-treated and drotrecogin alfa (activated)-treated patients by baseline overt DIC status (Table 4).

By definition, patients with overt DIC demonstrated worse coagulopathy at baseline than patients without overt DIC. This was reflected by lower platelet counts, higher levels of D-dimer, more prolonged PT and APTT values, and decreased levels of key regulatory factors (AT, protein C, and protein S) in patients with overt DIC than in patients without, at baseline (Table 5). Patients with overt DIC also had more thrombin

generation (higher levels of F1.2 and TAT), less fibrinolytic potential (higher levels of PAI-1), lower levels of plasminogen and TAFI, worse systemic inflammation (elevated IL-6 levels) and more endothelial injury (higher levels of sTM) than patients without overt DIC (Table 6).

Overt DIC as predictor of 28-day mortality in placebo-treated patients

In the placebo group, 28-day mortality for patients presenting with overt DIC at study entry was higher (43.0%, 95/221) than for patients presenting without overt DIC (27.1%, 148/547) (Table 7). When overt DIC points (0–7) were plotted against 28-day mortality for all placebo-treated patients ($N = 768$), there appeared to be a notable increase in mortality between a score of 3 and 4, from less than 25% to more than 30% (Fig. 1). Mortality rates continued to increase with increasing overt DIC points.

A logistic regression model confirmed that overt DIC score was an important predictor of the outcome of severe sepsis. The logistic regression model was fit to 28-day all-cause mortality

Table 3 Baseline demographics of PROWESS patients, with or without overt DIC

| Baseline characteristics | DIC status | | P-values |
|---|-------------------------------------|---------------------------------|----------|
| | Without overt DIC ($N = 1114$) | With overt DIC ($N = 454$) | |
| Mean age (years) | 60.8 ± 16.6* | 60.2 ± 17.6* | 0.706 |
| Gender (% male) | 58.2 | 54.2 | 0.148 |
| Ethnicity (% Caucasian) | 84 | 77 | < 0.001 |
| Location prior to study | | | 0.494 |
| % from home | 79.5 | 81.1 | |
| % not from home† | 20.5 | 18.9 | |
| Type of intensive care unit | | | < 0.001 |
| % surgical | 24 | 35 | |
| % medical | 76 | 65 | |
| Underlying comorbidities | | | |
| % hypertension | 39.0 | 30.4 | 0.001 |
| % chronic obstructive pulmonary disease | 27.5 | 16.5 | < 0.001 |
| % diabetes | 20.8 | 22.9 | 0.362 |
| % malignancies | 17.7 | 19.8 | 0.320 |
| % history of myocardial infarction | 14.5 | 11.7 | 0.134 |
| % cardiomyopathy | 9.1 | 4.1 | 0.001 |

*Values are mean ± standard deviation. †Skilled nursing home and other acute care hospitals prior to the investigative site hospital.

Table 2 Baseline overt DIC status of patients in PROWESS at study entry

| | Total number of patients | | | | | | | | Total ISTH overt DIC points |
|----------------------------------|--------------------------|----|-----|-----|-----|-----|-----|----|-----------------------------|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Patients with overt DIC | – | – | – | – | – | 319 | 109 | 26 | 454 |
| Patients without overt DIC | 3 | 4 | 324 | 389 | 394 | – | – | – | 1114 |
| Patients with unknown DIC status | 94 | 18 | 10 | – | – | – | – | – | 122 |

Table 4 Baseline disease severity of PROWESS patients, with or without overt DIC

| Disease severity (mean \pm SD*) | DIC status | | P-values |
|------------------------------------|------------------------------|--------------------------|----------|
| | Without overt DIC (N = 1114) | With overt DIC (N = 454) | |
| | Specific measure | | |
| Mean APACHE II* score | | | |
| All patients | 24.0 \pm 7.5 | 26.6 \pm 8.0 | |
| Placebo group | 24.3 \pm 7.7 | 26.5 \pm 8.2 | < 0.001† |
| Drotrecogin alfa (activated) group | 23.8 \pm 7.3 | 26.7 \pm 7.8 | |
| Mean number of organ failures‡ | | | |
| All patients | 2.2 \pm 1.0 | 2.9 \pm 1.2 | < 0.001† |
| Placebo group | 2.2 \pm 1.0 | 2.9 \pm 1.2 | |
| Drotrecogin alfa (activated) group | 2.2 \pm 1.0 | 3.0 \pm 1.2 | |
| Mean SOFA* scores | | | |
| Cardiovascular | 2.5 \pm 1.5 | 3.1 \pm 1.3 | < 0.001 |
| Respiratory | 2.7 \pm 1.0 | 2.7 \pm 1.1 | 0.691 |
| Renal | 1.0 \pm 1.1 | 1.5 \pm 1.2 | < 0.001 |
| Hepatic | 0.5 \pm 0.8 | 0.9 \pm 0.9 | < 0.001 |

*SD, standard deviation; APACHE II, Acute Physiology, Age, and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment. †P-values are comparisons between all patients with or without overt DIC. ‡One of the five organ dysfunctions defined in the study protocol was hematologic dysfunction. It was defined as platelet counts below $80 \times 10^9 \text{ L}^{-1}$ or 50% decrease from the highest values in the preceding 3 days.

Table 5 Baseline levels of circulating biomarkers in PROWESS patients, with or without overt DIC

| Biomarker | Biomarker levels (median, 95% CI)* | | P-values |
|---|------------------------------------|--------------------------|----------|
| | DIC status | | |
| | Without overt DIC (N = 1114) | With overt DIC (N = 454) | |
| Central lab platelet counts (10^9 L^{-1}) | 198 (192–205) | 109 (101–125) | < 0.001 |
| Local lab platelet counts (10^9 L^{-1}) | 192 (185–199) | 98 (92–110) | < 0.001 |
| D-Dimer ($\mu\text{g mL}^{-1}$) | 3.2 (3.0–3.4) | 7.9 (7.3–8.9) | < 0.001 |
| PT (s) | 17.4 (17.1–17.6) | 22.8 (22.3–23.5) | < 0.001 |
| APTT (s) | 40.1 (39.4–40.8) | 49.0 (47.2–50.3) | < 0.001 |
| AT levels (%) | 65 (63–67) | 46 (44–48) | < 0.001 |
| Protein C levels (%) | 54 (53–56) | 33 (31–35) | < 0.001 |
| Protein S levels (%) | 45 (39–47) | 29 (27–30) | < 0.001 |
| Interleukin-6 (pg mL^{-1}) | 338 (292–395) | 1051 (891–1446) | < 0.001 |

95% confidence interval (CI) for the median.

using age, APACHE II score, and overt DIC points as continuous predictors. Using placebo patients (N = 768) in this model, overt DIC points were found to be a significant predictor of 28-day mortality, after adjusting for age and

Table 6 Baseline levels of circulating biomarkers in a subset* of PROWESS patients, with or without overt DIC

| Biomarker | Biomarker levels (median, 95% CI)† | | P-values |
|-------------------------------------|------------------------------------|-------------------------|----------|
| | DIC status | | |
| | Without overt DIC (N = 298) | With overt DIC (N = 85) | |
| F1.2 (nmol L^{-1}) | 1.65 (1.47–1.79) | 2.12 (1.71–2.46) | 0.028 |
| TAT ($\mu\text{g L}^{-1}$) | 10.0 (9.3–11.0) | 14.9 (11.5–18.3) | < 0.001 |
| TAFI ($\mu\text{g mL}^{-1}$) | 4.9 (4.6–5.5) | 3.6 (3.0–4.2) | < 0.001 |
| α_2 -Antiplasmin levels (%) | 103 (98–107) | 81 (76–86) | < 0.001 |
| PAI-1 levels (AU mL^{-1}) | 32 (27–35) | 66 (41–87) | < 0.001 |
| Plasminogen levels (%) | 64 (61–68) | 50 (44–54) | < 0.001 |
| sTM (ng mL^{-1}) | 72 (65–78) | 80 (70–119) | 0.029 |

These biomarkers were measured for the last 403 consecutive patients in the PROWESS study, 20 patients in this group were assigned the 'Unknown' designation (see Methods and Fig. 2). †95% confidence interval (CI) for the median.

APACHE II score (odds ratios = 1.29, 1.07, and 1.03 for a 1 unit change in overt DIC points, APACHE II score, and age, respectively, $P < 0.001$ for all; that is, for each additional overt DIC point, APACHE II scale point, and year of age, a patient's odds of death increase by 29, 7, and 3%, respectively).

An alternative, less sophisticated approach to appreciate the independent predictive nature of overt DIC status was to consider the degree of overlap with more clinical assessments of disease severity. If overt DIC status were not an independent predictor of disease severity (e.g. APACHE II score or number of organ failures), PROWESS patients with overt DIC at baseline (29%, 454/1568) would be expected to be greatly concentrated in patients with higher disease severity. At study entry in the PROWESS trial, the mean APACHE II score for all patients was 24.8%, and 75% of the patients had two or more organ failures [6]. However, analysis of patients with higher disease severity showed that only 34.7% (264/761) of patients with APACHE II ≥ 25 and 33.2% (395/1191) of patients with two or more organ dysfunctions had overt DIC at baseline. A significant number of patients assessed clinically as having lower disease severity at baseline were found to have overt DIC: 23.5% (190/807) of patients with APACHE II scores < 25 and 15.6% (59/377) of patients with one organ dysfunction met the ISTH criteria for overt DIC.

Treatment effects of drotrecogin alfa (activated) by baseline overt DIC status

As shown in Table 4, there was no clinically important imbalance in baseline disease severity as assessed by APACHE II scores and number of organ failures between placebo-treated and drotrecogin alfa (activated)-treated patients classified by baseline overt DIC status. With drotrecogin alfa (activated) treatment, there was an absolute reduction in 28-day mortality

Table 7 Effect of drotrecogin alfa (activated) treatment on mortality by baseline overt DIC status

| Baseline overt DIC status | Drotrecogin alfa (activated) | | Placebo | | Relative risk (95% CI) |
|----------------------------|------------------------------|-------------------|--------------------|-------------------|------------------------|
| | Number of patients | Percent mortality | Number of patients | Percent mortality | |
| Without (<i>N</i> = 1114) | 567 | 22.1 | 547 | 27.1 | 0.81 (0.66–1.00) |
| With (<i>N</i> = 454) | 233 | 30.5 | 221 | 43.0 | 0.71 (0.55–0.91) |

Breslow-Day test of homogeneity of odds ratio between patients with or without overt DIC by treatment *P*-value = 0.261.

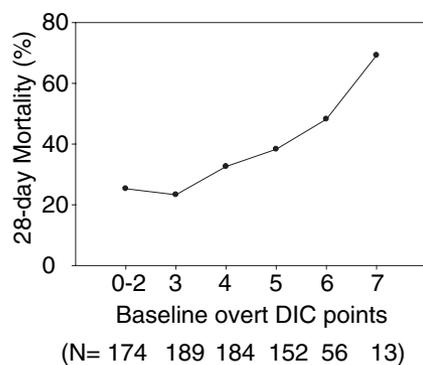


Fig. 1. Relationship of baseline overt DIC status and 28-day mortality in placebo-treated patients. The overt DIC score of placebo-treated patients (*N* = 768) was determined at baseline using the modified ISTH definition. Mortality rates for these groups of patients, as defined by overt DIC points, were determined over the 28-day study period. Numbers in parentheses refer to the total number of patients with that specific overt DIC score. Patients with 0–2 points were grouped together due to very small numbers of patients (i.e. < 5) with scores of less than 2.

of 12.5 and 5.0% in patients with or without overt DIC, respectively, when compared with placebo treatment (see Table 7). The relative risk reduction in mortality for patients either with or without overt DIC was 29.1 and 18.5%, respectively. These results were consistent with the increase in survival observed in the overall PROWESS trial population [6]. Although, the absolute reduction in mortality with drotrecogin alfa (activated) compared with placebo was slightly greater in patients with overt DIC than without, the Breslow-Day interaction test was not significant (*P* = 0.261).

When the treatment effect in lower risk patients was analyzed, mortality rates of patients with baseline APACHE II scores < 25 treated with drotrecogin alfa (activated) were 24.0% (23/96) and 17% (53/312) for patients with and without overt DIC, respectively. Mortality rates for the same group of patients treated with placebo were 24.5% (23/94) and 18.4% (54/312) for patients with and without overt DIC, respectively. Similar results were observed in the patients with more than two organ dysfunctions at baseline by overt DIC status. The drotrecogin alfa (activated) treatment group had mortality rates of 24.1% (7/29) and 18.2% (30/165) for patients with and without overt DIC, respectively. The placebo treatment group had mortality rates of 26.7% (8/30) and 20.9% (32/153) for patients with and without overt DIC, respectively.

The ability of drotrecogin alfa (activated) treatment to resolve overt DIC was assessed by comparing the frequency of overt DIC in surviving patients of the two treatment groups over the 28-day study period (Fig. 2). Following initiation of study drug, fewer patients treated with drotrecogin alfa (activated) had overt DIC than placebo treated patients. This difference reached statistical significance on days 6 and 14 (*P* = 0.037 and 0.047, respectively). In addition, generalized linear modeling for overt DIC with day, therapy, baseline overt DIC, and day by therapy interaction as covariates showed there was a significant interaction between therapy and day (overall *P*-value = 0.048).

Serious bleeding rates were analyzed for the study drug infusion period (4 days of study drug infusion plus 1 postinfusion day) and for the entire 28-day study period (Table 8). During the study drug infusion period, in patients without overt DIC at baseline, drotrecogin alfa (activated) treatment was associated with a 1.9% serious bleeding rate compared with a rate of 1.1% in placebo-treated patients (Table 8). In patients

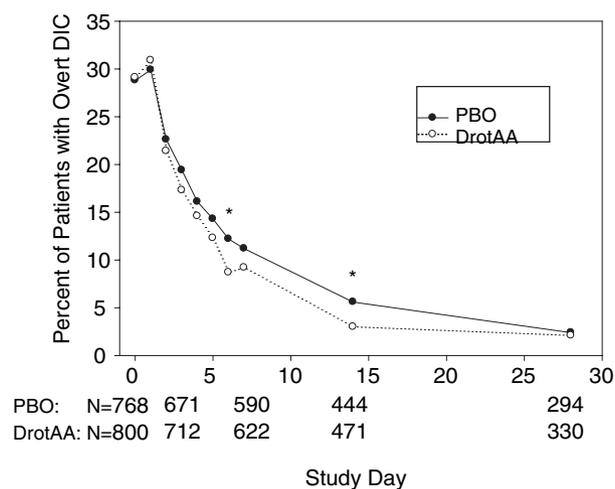


Fig. 2. Resolution of Overt DIC in survivors over time, by treatment category (drotrecogin alfa (activated) vs. placebo). Patients were assessed for overt DIC on days 0–7, 14, and 28 using the ISTH definition. Patients discharged before 28 days were assigned to the ‘unknown’ category, as they could no longer be measured. Comparisons of the proportion of patients with overt DIC at the indicated time points were made using χ^2 tests for surviving on measurable patients with no more than one missing value (**P* < 0.05). The bold numbers at the bottom of the graph refer to the total number of patients remaining in the group at the indicated time (i.e. 0, 4, 14, and 28 days). [PBO, placebo; DrotAA, drotrecogin alfa (activated)].

Table 8 Effect of drotrecogin alfa (activated) treatment on the risk of serious bleeding and thrombotic events by baseline overt DIC status

| Baseline overt DIC status | Drotrecogin alfa (activated) | | Placebo | | Relative risk (95% CI) |
|--|------------------------------|--|--------------------|--|------------------------|
| | Number of patients | % of patients with serious bleeding events | Number of patients | % of patients with serious bleeding events | |
| Serious bleeding events during study drug infusion period (study days 1–5) | | | | | |
| Without (<i>N</i> = 1114) | 567 | 1.94 | 547 | 1.10 | 1.77 (0.66–4.75) |
| With (<i>N</i> = 454) | 233 | 3.00 | 221 | 0.90 | 3.32 (0.70–15.81) |
| Serious bleeding events during 28-day study period | | | | | |
| Without (<i>N</i> = 1114) | 567 | 3.00 | 547 | 1.83 | 1.64 (0.76–3.55) |
| With (<i>N</i> = 454) | 233 | 4.72 | 221 | 2.71 | 1.74 (0.65–4.62) |
| Serious thrombotic events during 28-day study period | | | | | |
| Without (<i>N</i> = 1114) | 567 | 2.65 | 547 | 2.93 | 0.90 (0.45–1.81) |
| With (<i>N</i> = 454) | 233 | 0.43 | 221 | 2.26 | 0.19 (0.02–1.61) |

Breslow-Day test of homogeneity of odds ratio with or without overt DIC by treatment: $P = 0.498$ (days 1–5) for serious bleeding events; $P = 0.918$ (28 days) serious bleeding events; $P = 0.144$ (28 days) serious thrombotic events.

with overt DIC at baseline, the serious bleeding rate during the infusion period was 3.0% in drotrecogin alfa activated patients compared with 0.9% in placebo patients (Table 8). The odds of serious bleeding were not significantly different between patients with or without overt DIC during the infusion period of drotrecogin alfa (activated) (Breslow-Day interaction test, $P = 0.498$). Over the entire 28-day period, drotrecogin alfa (activated) treatment was associated with a 3.0% serious bleeding rate compared with a 1.8% rate in placebo-treated patients without overt DIC (Table 8). In patients with overt DIC, the serious bleeding rate over the 28-day period was 4.7% in drotrecogin alfa (activated)-treated patients compared with 2.7% in placebo-treated patients (Table 8). As during the study drug infusion period, the odds of serious bleeding were not different between patients with or without overt DIC over the 28-day study period by treatment (Breslow-Day interaction, $P = 0.918$). The 28-day serious bleeding rates for PROWESS were 3.5% for drotrecogin alfa (activated)-treated patients and 2.0% for placebo-treated patients [6].

Serious thrombotic events, as reported by the investigators, were analyzed only for the 28-day period (Table 8). In patients without overt DIC at baseline, serious thromboembolic events occurred in 2.7% of patients in the drotrecogin alfa (activated)-treated group compared with 2.9% of the placebo treated patients (Table 8). In patients with overt DIC at baseline, the serious thrombotic event rate was 0.4% with drotrecogin alfa (activated) treatment compared with a 2.3% event rate in placebo treated patients (Table 8). The odds of serious clinically overt thrombotic events were not significantly different between patients either with or without overt DIC by treatment over the 28-day study period (Breslow-Day interaction test, $P = 0.144$).

Discussion

DIC is commonly associated with sepsis and derangement of coagulation in patients with sepsis may contribute to organ failure and mortality. Evidence suggests amelioration of DIC is associated with an improvement of organ function and in

animal experiments may improve survival [12,13]. Histological studies show that fibrin deposition and microvascular thrombosis is associated with organ failure, while clinical studies indicate that DIC may be an important predictor of mortality [3, 4, 14].

Because clinical management of DIC may be hampered by the lack of clear diagnostic criteria for this syndrome, the ISTH has proposed a simple and easily applicable diagnostic scoring algorithm, based on routine laboratory parameters [5]. The proposed scoring system is based on retrospective analysis of cohorts of patients with DIC until prospective studies can be performed. PROWESS, a large clinical trial of patients defined as having severe sepsis [7] where the intervention [i.e. administration of drotrecogin alfa (activated)] was in part aimed at reducing coagulopathy, provided a unique and valuable opportunity to evaluate the ISTH overt DIC score in a large cohort of patients.

Using a slightly modified ISTH overt DIC scoring system, 29% of patients with severe sepsis were classified as having overt DIC at study entry into the PROWESS trial. The incidence of overt DIC in patients with severe sepsis enrolled in PROWESS was very consistent with the previously estimated rate of 30% for this condition [1]. The presence of overt DIC was also associated with but independent of other clinical assessments of disease severity such as a higher APACHE II score, more organ failure, and a higher SOFA score.

Placebo-treated patients with overt DIC had a much higher 28-day mortality compared with placebo-treated patients without overt DIC. Interestingly, there seemed to be a linear relationship between the number of points on the overt DIC score and 28-day mortality in patients with severe sepsis. Thus, the ISTH scoring system successfully identified a subpopulation at higher risk of death. Moreover, consistent with previous studies [3,4,14], overt DIC status, as defined by the ISTH, was also found to be a strong and independent predictor of mortality in this study.

A substantial number of patients with APACHE II scores less than 25 or with only one organ dysfunction (23.5 and

15.6%, respectively) had overt DIC at baseline. This suggested overt DIC, a potential sign of advancing disease, was present in patients who were clinically assessed as having lower disease severity and thus, by inference, a lower risk of death. In this context, the observation that the presence of overt DIC was associated with increased mortality, independent of APACHE II score, is especially notable. Relevant to this independence, overt DIC patients with baseline APACHE II scores < 25 or with less than two organ dysfunctions (i.e. low risk of death patients) had higher observed mortalities than the same group of patients without overt DIC. Those patients with overt DIC had observed mortalities of 24–27%, while those without overt DIC had mortalities of 17–21%. In addition, patients in this low risk of death group (i.e. APACHE II < 25 and less than two organ dysfunctions) that were treated with drotrecogin alfa (activated) tended to have lower mortality than placebo-treated patients, regardless of overt DIC status. Thus the presence of overt DIC, while independently affecting overall mortality in both drug- and placebo-treated patients, did not appear to affect the relationship between disease severity and treatment efficacy. However, these types of analyses clearly need to be treated with caution, as they are retrospective subgroup of a subgroup analyses. Nevertheless, in general, these data support the hypothesis that rather than being another consequence of severe sepsis, DIC may indeed contribute to the pathogenesis of organ failure and subsequent mortality in patients with severe sepsis. Further prospective studies are clearly needed to evaluate to what degree addition of the ISTH DIC-score may enhance the predictive power of APACHE II and SOFA, regarding hospital mortality.

In PROWESS, administration of drotrecogin alfa (activated) in randomized and treated patients resulted in an absolute reduction in mortality of 6.1% (relative risk reduction 19.4%) [6]. In the present analysis, the subgroup of patients diagnosed with overt DIC at baseline that were treated with drotrecogin alfa (activated) had an absolute reduction in mortality from 43 to 30.5% (i.e. 12.5%), which is a relative risk reduction of 29.1%. Hence, the presence of overt DIC, defined by a simple scoring system, identified another subgroup that is at high risk of death and receives substantial benefit from treatment with drotrecogin alfa (activated). This is consistent with the general trend that substantial benefit can be derived with drotrecogin alfa (activated) treatment in patients with greater disease severity and a high risk of death from severe sepsis. However, patients not classified as having overt DIC also received significant treatment benefit in 28-day mortality reduction from drotrecogin alfa (activated) treatment (absolute risk reduction: 5%; relative risk reduction: 18.5%).

A similar result was observed in a previous study in which there was a partial analysis of these data using different criteria [15]. In this publication, patients with two or more missing DIC laboratory values were classified as non-overt DIC patients, and not as 'unknown'. Therefore, the total number of patients used in the denominator to calculate the percentage mortality for overt DIC patients was 1690, 122 more than the current study (Table 2). Upon reviewing the clinical data of these 'unknown'

patients, some of them had purpura fulminans. In this report, the 122 patients are more appropriately reported as 'unknown'. In addition, the previous publication [15] used rounded PT cut-off values (0.5 s higher than the actual values used for this manuscript, see Table 1 for overt DIC scoring of > 21 s = 2; > 18 s = 1; ≤ 18 s = 0. As a result, there were more patients that met the definition of overt DIC in this manuscript than in the previous report (454 vs. 378). Lastly, in spite of the differences in criteria used in defining overt DIC patients, the relative risk of 28-day mortality (0.69) and serious bleeding events (1.70) previously reported [15] for overt DIC patients treated with drotrecogin alfa (activated), compared with placebo, are similar to what are reported here in Tables 4 and 5.

Although drotrecogin alfa (activated) has multiple activities, including anticoagulant and inflammation-modulating properties, at present it is not known to what extent each of these properties contribute to its beneficial effect. Because patients with overt DIC appeared to have enhanced benefit from administration of drotrecogin alfa (activated) compared with patients without overt DIC, it might be hypothesized that the effect of drotrecogin alfa (activated) on the coagulation system is indeed an important feature of this treatment. This view is supported by the observation in the present study that resolution of overt DIC in surviving patients proceeded more rapidly in patients treated with drotrecogin alfa (activated) than in placebo-treated patients (Fig. 2).

As suggested in a previous study, additional activities of activated protein C [endogenous and drotrecogin alfa (activated)], other than its anticoagulant activity, may also contribute to its beneficial effect on survival in severe sepsis [16]. There was no difference in the proportion of patients with baseline overt DIC when comparing heterozygous carriers of factor (F)V Leiden (a prothrombotic genetic polymorphism) to non-FV Leiden carriers in the PROWESS study [16]. Animal model and clinical findings from this recent publication suggest that heterozygous FV Leiden status does not worsen the coagulopathy in severe sepsis, rather it appears to provide a survival benefit [16].

A casual interpretation of this previous study [16] might suggest the anticoagulant activity of drotrecogin alfa (activated) is not important for its treatment benefit in reducing mortality in severe sepsis. However, heterozygous FV Leiden carriers are only partially resistant to the anticoagulant activity of activated protein C, as they still have half of their 'normal' FV, and their FVIIIa remains a target for the anticoagulant activity of activated protein C. Indeed, homozygous FV Leiden mice, with greater resistance to the anticoagulant activity of activated protein C, had higher mortality than heterozygous FV Leiden mice, suggesting that further loss of the anticoagulant activity of activated protein C is associated with increased mortality. Taken together, the data from this manuscript and the previous study [16] suggest that improvement in survival with drotrecogin alfa (activated) treatment may depend on the plurality of its biological activities, including both its anticoagulant/antithrombotic and inflammation-modulating activities.

The clinical presentation of DIC is characterized by microvascular thrombosis, which is thought to contribute to organ failure. Simultaneously, massive consumption of coagulation factors and platelets may also increase the risk of bleeding. Although serious bleeding in patients with sepsis and DIC is relatively uncommon, this complication may dominate the clinical picture and become a direct cause of death in affected patients [1]. Indeed, the present analysis showed a 0.9% absolute increase in incidence of major bleeding events in placebo-treated patients with overt DIC compared with placebo-treated patients without DIC during the 28-day study period (bleeding in placebo-treated patients without overt DIC minus bleeding in placebo-treated patients with overt DIC).

Because drotrecogin alfa (activated) is an antithrombotic protein, it has the potential to increase the risk of bleeding. However, treatment with drotrecogin alfa (activated) had only a modest effect on the incidence of serious bleeding events. The absolute increase in the incidence of serious bleeding during infusion of drotrecogin alfa (activated) was 2.1% in patients with overt DIC and 0.8% in patients without DIC [bleeding in drotrecogin alfa (activated)-treated minus bleeding in placebo-treated patients]. Thus, the absolute risk of serious bleeding appeared to remain relatively low for drotrecogin alfa (activated)-treated patients. It should be mentioned, however, that serious bleeding risk may have been influenced by the fact that patients known to be at high risk of bleeding were excluded from the PROWESS study [e.g. platelet counts less than $30 \times 10^9 \text{ L}^{-1}$ at baseline [17]].

On the other hand, patients who had severe thrombocytopenia were also at higher risk of death and derived substantial benefit from treatment with drotrecogin alfa (activated) [15]. Given that patients with overt DIC usually have lower platelet counts, the present analysis suggests the presence of overt DIC represents a situation in which substantial benefit from administration of drotrecogin alfa (activated) can be expected. In addition, it might be speculated that even though treatment with drotrecogin alfa (activated) may modestly increase bleeding in overt DIC patients or in patients with severe thrombocytopenia, this increase in bleeding risk may be more than offset by the benefit of treatment in terms of improved survival. This hypothesis needs confirmation in further prospective clinical studies.

Consistent with its potential to increase the risk of bleeding, drotrecogin alfa (activated) may also have potential for reducing thrombotic complications in DIC. In the drotrecogin alfa (activated) treated group, though not reaching statistical significance, the absolute reduction in the thrombotic event rate was greater in patients with overt DIC than without overt DIC. In view of these results, the risk (increased bleeding) and benefit (improved survival and decreased thrombotic events) of this therapy should be considered when treating patients who have increased disease severity and risk of death from severe sepsis. In this context, it should be noted that the incidence of thrombosis was determined by clinical findings in the PROWESS study. The lack of sensitivity and specificity in clinical diagnosis of venous thromboembolism has been well

established and is acknowledged. As a result, it is possible the true rate of thromboembolism was higher than the rate reported here.

There are several potential limitations to this study. It is important to note the large number of exclusion criteria in the PROWESS trial. Our estimation of the incidence of overt DIC is an extrapolation from a select population of severe sepsis patients. A further limitation is that 122 PROWESS patients (7.2%) did not have sufficient laboratory data available to calculate the modified overt DIC score. Also, fibrinogen was not measured in any of the patients and patients at high risk of bleeding or with platelet counts less than $30 \times 10^9 \text{ L}^{-1}$ were excluded from the PROWESS study. All three factors may have caused an underestimation of the incidence of overt DIC in severe sepsis.

As mentioned above, a previous clinical trial in patients with severe sepsis showed that a small minority of patients (2.9%) had decreased fibrinogen levels ($< 2 \text{ g L}^{-1}$, the lower limit of normal) [9] while a majority of patients actually had elevated plasma levels of fibrinogen ($> 4 \text{ g L}^{-1}$, the upper limit of normal). None of the patients with severe sepsis with decreased fibrinogen levels in this study had fibrinogen levels below 1 g L^{-1} ([9], and data on file at Eli Lilly & Co.). Because patients with severe sepsis who would get an additional overt DIC score point due to fibrinogen levels below 1 g L^{-1} are uncommon, it was felt that not measuring fibrinogen levels would cause little or no underestimation of overt DIC in severe sepsis. Other studies have also indicated that fibrinogen levels are not a useful parameter to assess the degree of DIC in severe sepsis [2,18].

A last limitation to point out is that the present results and conclusions are based on a retrospective analysis of subgroups within a large trial. Although most of the data are based on laboratory parameters at study entry (i.e. before administration of study drug) and concern objective parameters that cannot be confounded, this type of analysis should always be interpreted with care.

In conclusion, almost 30% of PROWESS patients had overt DIC at study entry using a modification of the proposed ISTH overt DIC scoring method. The presence of overt DIC was an important predictor of mortality, independent of APACHE II score and age, and an almost linear relationship existed between the overt DIC score and 28-day mortality. The 28-day mortality was much higher in placebo-treated patients with overt DIC than without at study entry (43 vs. 27%). Patients both with and without overt DIC demonstrated improvement in survival with drotrecogin alfa (activated) treatment compared with placebo-treated patients; this was consistent with the overall PROWESS population. Patients with overt DIC at baseline had a 29% relative risk reduction of mortality with drotrecogin alfa (activated) treatment, similar to the subgroup of patients with APACHE II score ≥ 25 [15]. This benefit was not statistically different from the 18% relative risk reduction of mortality received in drotrecogin alfa (activated) treated patients without overt DIC (Breslow-Day interaction, $P = 0.261$). The risk of serious bleeding due to administration of

drotrecogin alfa (activated) in patients with overt DIC was slightly increased (Breslow–day interaction, $P = 0.918$), while the rate of thrombotic events was slightly reduced (i.e. benefit, Breslow–day interaction, $P = 0.144$) compared with placebo-treated overt DIC patients.

Taken together, data from this study suggest the simple diagnostic algorithm of this modified ISTH overt DIC scoring system may be useful as an independent assessment of the risk of death from severe sepsis. Though prospective clinical studies should be conducted to confirm the utility of the modified ISTH algorithm in diagnosis of overt DIC in patients with severe sepsis, patients identified using this system appear to have a favorable risk (increased serious bleeding)/benefit (improved survival and decreased serious thrombosis) treatment effect from drotrecogin alfa (activated).

Acknowledgements

We would like to acknowledge the contributions of Samiha Sarwat for her statistical support in the development and completion of this manuscript.

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