The Influence of Fibronectin Administration on the Incidence of Sepsis and Septic Mortality in Severely Injured Patients

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Eighty-five trauma patients between the ages of 18 and 55, with American College of Surgeon's (ACOS) trauma scores greater than or equal to 7 were entered into a double-blind, randomized, placebo-controlled study to assess the efficacy of prophylactic fibronectin (Fn) administration on clinical course, sepsis development, and septic mortality. Patients were randomized on admission to receive purified human virus-inactivated Fn or placebo control (human serum albumin, HSA). Fn or HSA was administered on a daily basis if and when the patient was Fn deficient (less than 75% normal). When a Fn deficiency was not evident, the patient received saline. Seventy one patients developed Fn deficiencies during their initial clinical course: 36 received Fn, 35 received HSA. Fourteen patients did not develop a Fn deficiency after trauma and thus received only saline. Analysis of admission data demonstrated no significant differences between the three groups with respect to extent of injury (injury severity score, ACOS trauma score) or physiologic assessments of organ function (serum creatinine, bilirubin, lactic acid). On day 1 after trauma, Fn levels were shown to correlate with other plasma proteins and cellular components (range of r values, 0.24 to 0.75; all $p < 0.05$), but not with organ function parameters. Eighteen of 85 patients became septic as judged by clinical criteria. Ten of these patients had received Fn (10 of 36), five had received HSA (5 of 35), and three had received only saline (3 of 14) before the development of sepsis (differences not significant).

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This project was supported by a grant from the Department of the Navy, Naval Medical Research and Development Command.

Accepted for publication: April 13, 1989.

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When septic, nine of 17 patients developed Fn deficiencies. Six patients received Fn while septic, three received albumin, and eight received saline. Seven patients died: 5 of 6 Fn patients, 1 saline, and 1 HSA recipient. Our data suggest that exogenous Fn repletion in states of deficiency does not alter clinical course, the development of sepsis, or septic mortality.

WITHIN RECENT YEARS, fibronectin (Fn) has received considerable attention as a possible treatment adjunct in the care of critically ill patients.[1-3] This interest is based on Fn's known binding and functional properties.[4] Fn has been shown to be a major opsonin, important for the systemic clearance of denatured collagens and coagulation products by the reticuloendothelial system (RES).[5-7] The importance of the RES in host defense is well established. Treatment modalities that decrease RES function concomitantly decrease host resistance to shock and injury.[8,9] It has been suggested that the phagocytic dysfunction seen in trauma and septic patients is mediated, at least in part, by a plasma Fn deficiency.[10] As such, repletion of Fn would be expected to improve RES function, with demonstrable benefit to the critically ill patient.

To test this hypothesis in a clinical setting, cryoprecipitate (Cryo) was used as a source of Fn.[10-19] In the initial case reports of trauma and septic patients, Cryo infusion was associated with improvements in cardiopulmonary parameters, limb blood flow, limb oxygen consumption, and improved creatinine clearance.[10-13] A number of uncontrolled studies, each with limited numbers of patients, followed these initial reports, many with positive re-

sults.[14–16] These studies sparked an era of interest in the use of Fn and Fn-enriched concentrates in critical care patients. However, subsequent controlled studies have not supported these early observations.[1,17–19] The interpretation of positive and/or negative results of these studies must be made with caution. The attribution of effects seen with Cryo (positive or negative) to Fn is inappropriate because there are many other proteins present in Cryo in addition to Fn.[20,21] The Fn content of individual units of Cryo is extremely variable, reflecting the wide range of Fn levels in normal donors.[2,21–23] Finally, it has been shown recently that storage conditions (time and temperature) of Cryo are extremely important in the maintenance of Fn's opsonic activity.[24]

With the advent of purified preparations of Fn, controlled clinical studies of Fn repletion could be performed, however very few have appeared in the literature. In a study of surgical, trauma, and burn patients, Saba et al.[25] have shown that Fn antigenic and opsonic deficiencies can be reversed by the administration of purified Fn. Regrettably the clinical effects of such repletion were not addressed. Lundsgaard-Hansen and his colleagues[26] have studied the influence of Fn administration in patients with severe abdominal infections, many with organ failure on admission. Despite the poor entry status of some patients and the limited Fn administration (0.8 g/day for five days, regardless of the patient's Fn level), this study showed a trend toward prolonged survival in those patients receiving Fn ($0.1 > p > 0.05$). In contrast to the case reports showing beneficial effects of Cryo in similar patients, Lundsgaard-Hansen[26] found no statistically significant differences in ultimate clinical course, nor in the biochemical and laboratory parameters measured between the Fn and control groups. Statistical differences were only seen between survivors and patients who died.

Fn's capability to reverse existent organ failure may in fact be limited. In planning our double-blind controlled clinical trial, we chose a slightly different approach than Lundsgaard-Hansen and his colleagues: we did not want to treat sepsis with its concomitant organ failures per se, but to prevent its development. If sepsis did develop, we wanted to diminish its sequelae. Our study was designed to replete and maintain Fn levels within normal range in multiple system trauma patients. The goals of the study were to determine the effect of Fn repletion on clinical course, the incidence of sepsis, end organ effects of sepsis, and septic mortality rate.

Materials and Methods

Purified Fibronectin

The Fn preparation used in this study was prepared by the New York Blood Center according to published procedures.[27] The preparation is virus inactivated, and has been shown to retain Fn's biologic activities: gelatin binding, heparin binding, cell attachment and spreading, and opsonic activities remain intact.[27] The preparation is stable for at least 2 years when stored in a lyophilized state at 4 C. Lyophilized material was reconstituted according to the manufacturer's instructions before use.

Patients: Admission Criteria

Our study had four entry criteria: (1) Patients had to be 18 to 55 years old. (2) Admission to the Shock Trauma Unit of the Medical College of Georgia, Augusta Georgia must have been within 24 hours of injury. (3) The patient had to have suffered major trauma, excluding thermal and/or lethal neurologic injury, with an American College of Surgeon's injury severity score greater than or equal to 7. (4) Informed consent had to be obtained from the patient or the next of kin.

Protocol

Our study protocol was approved by the Human Assurance Committee of the Medical College of Georgia. The study can be broken into two phases: preseptic and septic. The first phase assesses the efficacy of prophylactic Fn administration to prevent the development of sepsis. The second phase determines the influence of Fn in modulating the consequences of sepsis and septic mortality.

Patients were randomized on admission to receive either purified Fn or placebo (human serum albumin). Previous pharmacokinetic studies in trauma patients established the relationship between Fn deficit and patient weight used to determine the amount of Fn needed to restore the patient's levels to normal.[28]

Our treatment protocol called for the administration of a 100 mL volume of a Fn or placebo solution on a daily basis if and when the patient's Fn levels fell below 75% of normal average (defined as a Fn deficiency) as assessed by a gelatin binding assay.[29] When a Fn deficit did not occur, patients were given an equal volume of saline. Once deemed Fn deficient, data analyses on that day and all subsequent days grouped the patient according to the Fn or albumin treatment received. If a patient became septic, he or she progressed to the second phase of the study designed to determine the effects of Fn administration on septic course and death.

With respect to data analysis, patients were considered to have failed treatment if and when they became septic. Data obtained during their septic episode(s) were not considered in the analyses that follow. Patients who were nonseptic while in the Shock Trauma Unit but who developed sepsis within 72 hours of discharge from the unit were deemed a treatment failure, and were included in all preseptic analyses. Those patients who died due to nonseptic causes within seven days or who were trans-

ferred from the Shock Trauma Unit within 72 hours of study entry were excluded from all data analyses.

The investigators responsible for patient clinical care, as well as all nursing staff, remained blinded throughout the course of the study.

Parameters Measured

On admission, a spectrum of physiologic and biochemical parameters were recorded. The patient's ACOS score, ISS score, type of trauma, resuscitation history (amounts of crystalloids, colloids, fresh frozen plasma, platelets, whole blood, and packed red blood cells), as well as biochemical and physiologic status were assessed. Parameters included hemoglobin, hematocrit, total lymphocyte count, white blood cell count, platelet count, a plasma protein profile: total protein, albumin, retinol binding protein, prealbumin, transferrin, and Fn, as well as assessments of physiologic status: Lactic acid, Creatinine, Bilirubin, SGOT, LDH, Alkaline Phosphatase, and CPK activity.

The above biochemical/laboratory parameters were assessed on a daily basis, with the addition of measurements of IgG, IgM, Antithrombin III, Complement component C3. Fn determinations were made on samples collected at 6:00 AM, and again before and after prophylactic infusion independent of which treatment the patient received that day. The physiologic profile was also expanded to include measurements of patient awareness (Glascow Coma scale), temperature, and respiratory parameters.

Once a patient became septic, additional physiologic parameters were measured. The changes in these parameters will be the subject of a separate report.

Statistical Analyses

The association of classification variables (*e.g.*, sepsis/nonsepsis, survivors/nonsurvivors) with treatment groups was analyzed using chi square analyses, or Fisher's Exact test, where appropriate. Differences in Fn and albumin administration distributions were also assessed using the Kolmogorov-Smirnov two-sample test. Differences in quantitative parameters were analyzed, where appropriate, by Student's t test, paired t test, or one-way analysis of variance using a Duncan range test to assess significance in parametric data. Nonparametric statistics (Mann Whitney U test and Kruskal-Wallis one-way analysis of variance) were used for ordinal data. Where averages are listed in tables and figures, they represent the arithmetic means ± standard error of the mean (SEM). Probabilities less than $p < 0.05$ are considered significant.

Results

During the period from January 1986 to February 1989, 355 patients were admitted to the Shock Trauma Unit of the Medical College of Georgia. Of those admitted, 95 patients met the entry criteria of our study. Ten of these patients were subsequently excluded from analysis because their stay in the unit was less than 72 hours (eight discharges and two deaths from injury within 72 hours). Thus 85 patients completed the study and are included in these analyses. Our patient population was predominantly male (73 of 85). The median age on admission was 30.7 years (range, 18 to 54 years). Nonpenetrating traumas constituted the majority of our cases (69 of 85). As required by our inclusion criteria, injuries were severe, encompassing more than one organ system: median ACOS trauma score was 12 (range, 8 to 22), while the median Injury Severity Score (ISS) was 34 (range, 16 to 75). Before sepsis or discharge, Fn deficiencies developed in 71 of these 85 patients. Thirty-six patients received Fn, and 35 received albumin. The remaining 14 patients received saline throughout the preseptic phase of our study.

Analysis of Admission Profile

On admission 62 of 85 patients showed a Fn deficiency (72.9%). Of the remaining 23 patients, another nine patients developed a Fn deficiency before discharge or the development of sepsis. Grouping the patients according to the treatment they received during this phase, we can compare their physiologic status on admission (Table 1). In general the groups are comparable (*i.e.*, no significant differences) with respect to age, height, weight, and degree of injury (ACOS and ISS scores are the same). Furthermore, cell counts (platelets, white blood cells, and total lymphocytes), physiologic organ assessments (lactic acid, creatinine, bilirubin, and enzymes SGOT, LDH, and CPK) show no significant differences between groups. Where differences do occur, they suggest that the patients who developed Fn deficiencies were resuscitated in a different manner than those patients who never developed deficiencies. The saline group (*i.e.*, patients who did not develop a Fn deficiency during phase I of the study) received the least crystalloids, the least red cell concentrates, and no fresh frozen plasma. As a result, they have significantly higher levels of hemoglobin and hematocrit than do the albumin or Fn groups, as well as higher levels of total protein, transferrin, and Fn. Although the resuscitation history of the groups differs, there is no statistically significant difference between these groups with respect to organ-related parameters.

Correlation Between Fibronectin Levels (6:00 AM Sample) on Day 1 and Other Measured Parameters

Fn levels obtained at 6:00 AM on day 1 represent the starting point for our repletion study. However before any repletion, Fn levels are highly correlated with other

TABLE 1. *Status of Patients on Admission*

	Fibronectin		Albumin		Saline	
	Mean	SEM	Mean	SEM	Mean	SEM
AGE	31.80	1.48	30.32	1.38	33.87	2.54
ISS	32.97	1.92	36.03	2.01	30.57	2.95
ACOS	12.58	0.54	12.77	0.51	11.50	0.89
HGT	174.46	1.43	173.37	1.84	174.36	2.51
WT	85.37	2.95	85.03	3.08	84.73	4.66
Resuscitation Fluids						
Crystalloids	15975.78	1377.34	12957.20	1136.84	10773.93	1288.55
Whole Blood	27.78	27.78	0.0	0.0	0.0	0.0
PRBC	3484.72	456.97	2050.86	241.79	1032.14	293.36
FFP	539.33	136.61	240.43	74.76	0.0	0.0
Albumin	290.28	86.09	175.71	66.50	0.0	0.0
Platelets	85.56	28.29	107.29	59.47	0.0	0.0
Patient's Admission Profile						
Hemoglobin	10.36	0.47	10.97	0.43	12.72	0.62
Hematocrit	30.69	1.36	32.69	1.21	37.59	1.72
WBC	17.53	1.62	18.07	1.57	14.82	1.61
Platelets	236.48	16.87	244.30	14.16	300.31	26.77
Lymphocytes	3551.58	440.77	3202.06	291.10	4033.50	494.87
Creatinine	1.32	0.05	1.37	0.07	1.23	0.07
Lactic Acid	7.49	0.96	6.88	1.00	6.51	1.31
Bilirubin	0.33	0.04	0.33	0.04	0.38	0.04
SGOT	212.31	39.15	259.54	69.81	217.50	50.46
LDH	556.22	63.78	613.60	75.44	580.50	79.86
Alk. phosph.	69.77	4.65	75.31	4.18	94.00	9.68
CPK	1558.39	522.90	2737.00	1792.46	1463.79	648.47
Total protein	4.97	0.26	5.22	0.18	6.18	0.32
Albumin	3.02	0.15	3.21	0.12	3.63	0.24
Ret. bind.	3.25	0.22	3.51	0.28	3.51	0.31
Prealbumin	20.28	1.15	22.67	1.44	23.45	1.88
Transferrin	203.06	9.12	211.54	10.14	254.07	20.53
Fibronectin	76.77	4.10	78.83	3.54	109.15	6.43

Shown for each group are the means ± SEM for each admission parameter.

measured parameters. Fn correlates with leukocyte and platelet counts ($p \leq 0.014$; r values = 0.24 and 0.61, respectively) but not with lymphocyte counts. Significant associations ($p \leq 0.01$) are also seen between Fn levels and levels of Antithrombin III (r value = 0.38), complement component C3 (r = 0.75), prealbumin (r = 0.35), albumin (r = 0.46), transferrin (r = 0.61), retinol binding protein (r = 0.40), IgG and IgM (r = 0.73 and 0.46, respectively), as well as total protein (r = 0.61). Of particular interest is the observation that Fn levels are *not* associated with measures of physiologic dysfunction on day 1. Fn levels do not correlate with temperature ($p = 0.148$), positive end expiratory pressure (PEEP) ($p = 0.152$), bilirubin ($p = 0.484$), serum creatinine ($p = 0.325$), or lactic acid ($p = 0.472$). For sake of comparison, mean Fn levels in all patients before repletion was 66.5% ± 1.9% normal pool, Antithrombin III levels were 64.2% ± 2.8 (% normal plasma), and C3 levels were 36.7 ± 2.1 mg/dL (normal range, 63 to 135). Each of these parameters is below normal range.

Analysis of Fn/Albumin Administration Profile

During the first phase of our study, there were 135 instances of Fn deficiencies in our patients (of 784 preseptic patient days). Nearly one half (62 of 135) of the Fn deficiencies occurred on admission, with 90.5% of deficiencies occurring within the first seven days of study. Fn patients received significantly fewer infusions than did albumin patients (51 *versus* 84, respectively). Figure 1 shows the distribution of the number of days of Fn deficit in both the albumin and Fn groups. One dose of Fn was sufficient in 75% of the Fn patients to elevate Fn levels to within normal range (more than 75% pool). In contrast, albumin patients, who received no exogenous Fn after initial resuscitation, had a significant prolongation of their Fn deficiency. Sixty-three per cent of the albumin patients had more than one day of Fn deficiency.

Relationship of Treatment to Development of Sepsis

Despite the significant differences in Fn deficiencies between these groups, we found no association between

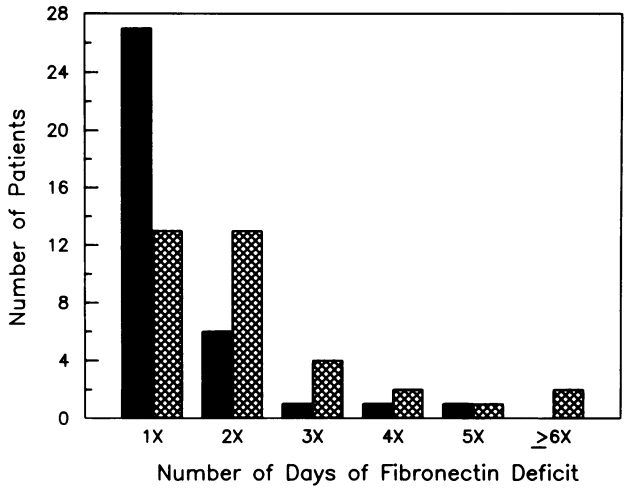

FIG. 1. Distribution of the number of days of Fn deficit in albumin (hatched bars) and Fn (solid bars) recipients. These distributions were shown to be statistically different from one another using the Kolmogorov two sample test (p = 0.006). Fn recipients had fewer deficiencies than Albumin recipients.

treatment group and the development of sepsis (chi square = 1.936; dof = 2; p = 0.38). Of the 85 patients studied, 18 became septic (21%) as assessed by clinical criteria. Blood cultures were positive in 12 of the patients (67%): 3 patients with gram-positive organisms, 8 with gram-negative organisms, and 1 with a mixed culture. The breakdown of the septic patients by group is shown in Table 2. Of the 36 patients treated prophylactically with Fn, 10 became septic (28%). Of the 14 patients who showed no Fn deficit (saline recipients), 3 became septic (21%); and finally, of the 35 patients who received albumin, 5 became septic (14%).

Not only was there no association between treatment and sepsis development, but there was also no association between sepsis development and the number of times the patient demonstrated a Fn deficit (Table 3). Finally the timing of the septic episode was also examined. The three saline recipients became septic 3, 6, and 30 days after trauma, respectively. Median onset of sepsis in Fn recipients was on day 9 after trauma (range, 4 to 21 days); albumin recipients had a median onset of day 4 (range, 4 to 13 days). Despite the differences in ranges, the time to sepsis development was not significantly different between the three groups (Kruskal-Wallis; p = 0.62).

Analysis of Daily Profiles

To assess the efficacy of Fn administration after trauma, we examined the daily profiles of each of the three treatment groups with respect to physiologic and laboratory parameters. The data displayed in Figures 2 to 8 are from days 1 through 7 after trauma. Our analyses were not continued beyond this point for two reasons: (1) there remained only 44 nonseptic patients in the unit beyond

TABLE 2. *Lack of Association Between Prophylactic Treatment and the Development of Sepsis (Chi Square Analysis, p =0.38)*

Recipient	Became Septic	Never Septic	Total
Fn recipients	10	26	36
Albumin recipients	5	30	35
Saline recipients	3	11	14
Total	18	67	85

day 7 after trauma (less than 50% of our initial patient population); and (2) more than 90% of the Fn deficits occurred within the first seven days, 74% within days 1 to 3. It was assumed that if Fn administration had an effect on physiologic parameters, this effect would be demonstrable within days of its infusion.

Examining the parameters measured on a daily basis, we found no significant differences in lactic acid, bilirubin, serum creatinine, temperature or PEEP between patients receiving Fn, albumin, or saline. Statistical differences were found, however, in the profiles of a number of plasma proteins (C3, IgG, IgM, transferrin, total protein). In each of these cases, the plasma protein profile for the saline patients was higher than those of patients with Fn deficiencies. After day 1, there were no statistically significant differences between the Fn and albumin groups with the exception of exogenously administered proteins (Fn and albumin) in which differences would be expected.

Figure 2 shows the daily 6:00 AM Fn levels of each patient group from days 1 to 7. On day 1 the Fn levels for patients without Fn deficiencies (*i.e.*, saline recipients) are significantly higher than for those patients with deficits. There is no significant difference between the patients who will receive albumin and Fn that day. Figure 3 shows the same groups after the administration of their respective infusates. On day 1, Fn recipients increased from 57.2% to 96.5% pool (paired t test, $p < 0.001$), whereas the albumin recipients spontaneously increased from 59.6% to 64.6% pool (paired t test, p = 0.003). In contrast, saline recipients remained fairly constant during the course of the day, decreasing only slightly from 88.5% to 85.0% pool (p = 0.244). Average morning Fn levels (Fig. 2) slowly rise in the patients treated with albumin (reaching normal

TABLE 3. *Lack of Association Between Development of Sepsis and the Number of Days of Fn Deficiency*

Septic State	Number of Days of Fn Deficiency*					
	0	1	2	3	4	≥5
Never septic	11	32	15	4	2	3
Became septic	3	8	4	1	1	1

* The distribution of days of Fn deficiency in patients who became septic and those who do not are *not* significantly different (Kolmogorov-Smirnov two-sample test: p = 0.963).

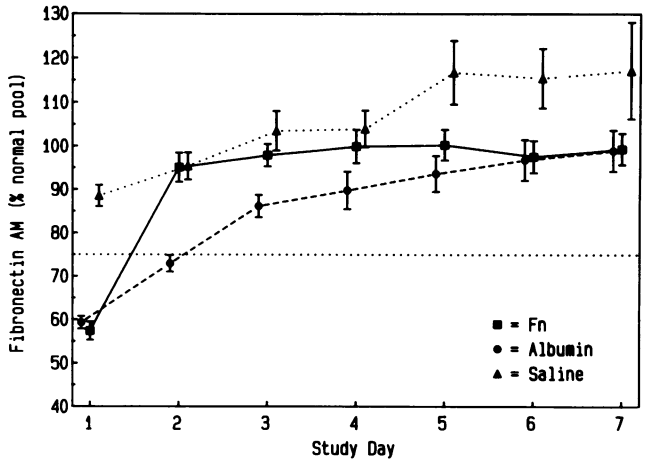

FIG. 2. Fibronectin profile. Fibronectin levels measured on samples drawn at 6:00 AM of study days 1 through 7. Data represent mean ± SEM. Fn recipients are designated by (■), albumin (●), and saline by (▲). The treatment threshold of 75% normal pool is shown as a dotted line. Significant differences are described in the text.

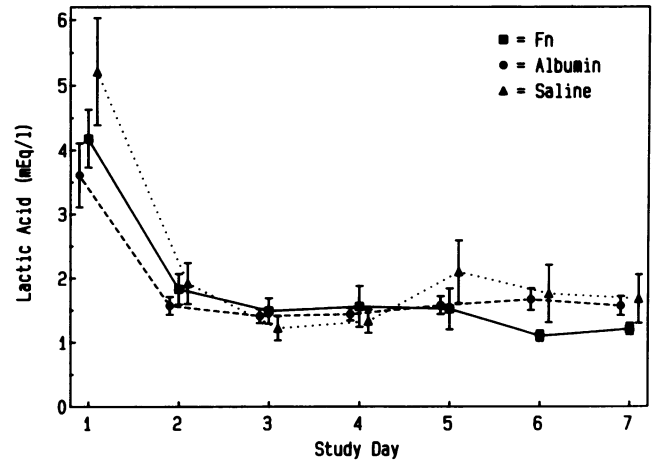

FIG. 4. Lactic acid profile. Maximum lactic acid levels in Fn, albumin, and saline recipients on days 1 to 7 of study. Data represent mean ± SEM. Fn recipients are designated by (■), albumin (●), and saline by (▲). No significant differences between groups were found during the 7-day period. Normal range for lactic acid is 0.5 to 2.2 mEq/l.

levels by the third post-trauma day), while they are maintained at a consistently high level in the Fn recipients.

Figures 4 to 6 represent the daily values of the physiologic parameters lactic acid, serum creatinine, and bilirubin in each of the three treatment groups. As assessed by these parameters, Fn administration does not significantly affect clinical course. Mean lactic acid levels (Fig. 4) decrease from their post-trauma high on day 1 to normal range (0.5 to 2.2 mEq/1) in all groups. No significant differences between these treatment groups are seen during the 7-day course. Figure 5 shows the daily profiles of serum creatinine. At no time point is there a significant difference between treatment groups. Only on days 1 to 3 do the groups approach the upper end of normal range of serum creatinine (1.5 mg/dL). Bilirubin, shown in Figure 6, shows a different temporal profile. Whereas the patients tended to decrease their serum creatinine levels after trauma, bilirubin levels increase in each of the treatment groups. Although the albumin recipients appear to have increases in their bilirubin levels earlier than the Fn or saline recipients, these differences are not statistically significant.

Figures 7 and 8 show the daily profiles of platelets and complement component C3, respectively. Platelet counts

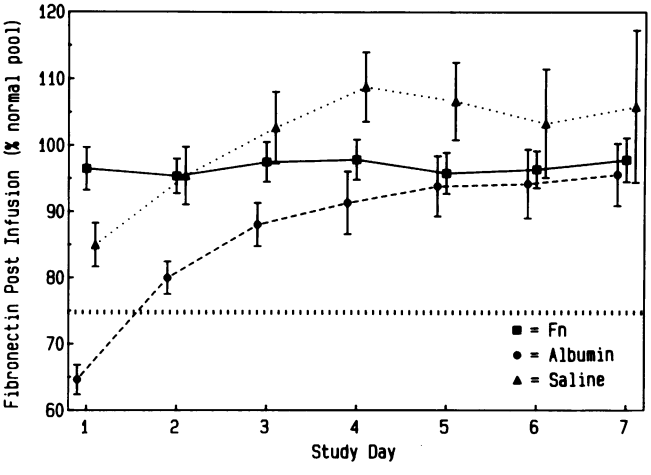

FIG. 3. Postinfusion fibronectin profile. Fibronectin levels measured on samples drawn one hour after the infusion of Fn, albumin, or saline in respective recipients on study days 1 through 7. Data represent mean ± SEM. Fn recipients are designated by (■), albumin (●), and saline by (▲). The treatment threshold of 75% normal pool is shown as a dotted line. Significant differences are described in the text.

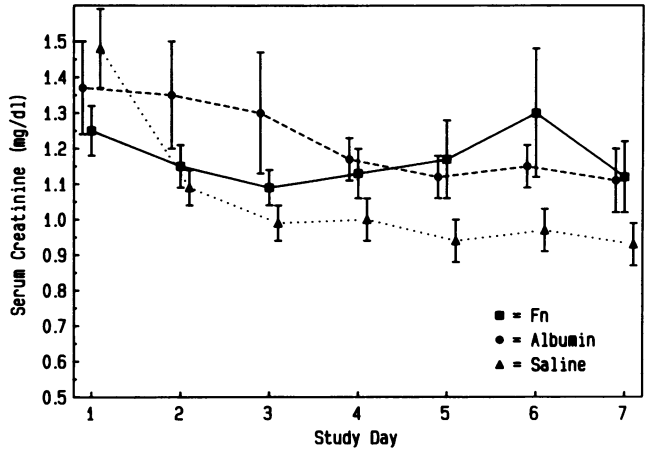

FIG. 5. Serum creatinine profile. Serum creatinine levels are shown in Fn, albumin, and saline recipients on days 1 to 7 of study. Data represent mean ± SEM. Fn recipients are designated by (■), albumin (●), and saline by (▲). No significant differences between groups were found during the 7-day period. Normal range for serum creatinine is 0.5 to 1.5 mg/dL.

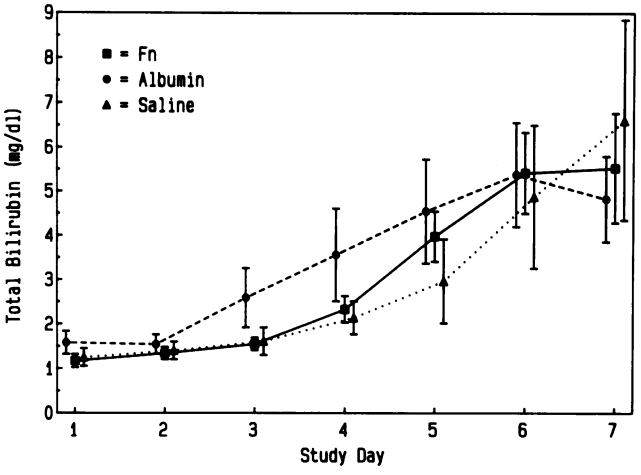

FIG. 6. Total bilirubin profile. Bilirubin levels are shown in Fn, albumin, and saline recipients on days 1 to 7 of study. Data represent mean ± SEM. Fn recipients are designated by (■), aLbumin (●), and saline by (▲). No significant differences between groups were found during the 7-day period. Normal range for bilirubin is 0.0 to 1.5 mg/dL.

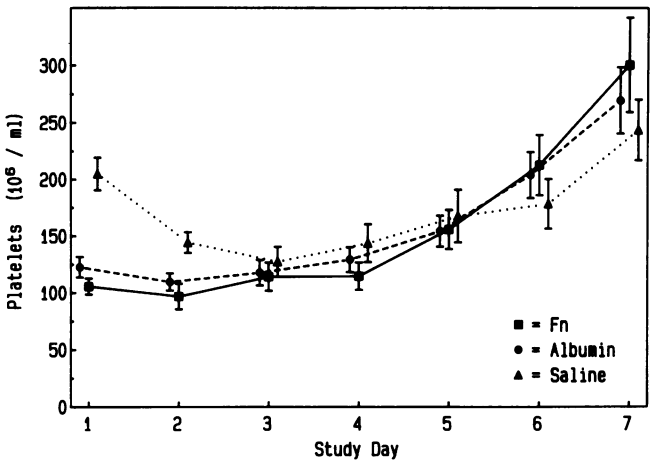

FIG. 7. Platelet profile. Platelet counts are shown in Fn, albumin, and saline recipients on days 1 to 7 of study. Data represent mean ± SEM. Fn recipients are designated by (■), albumin (●), and saline by (▲). Significant differences between groups are discussed in the text. Normal range of platelet counts is 150 to 400 × 10^6/mL.

in albumin and Fn recipients are virtually identical during the first seven days after trauma. These groups are statistically different from Saline recipients only on days 1 and 2 after trauma. C3 levels shown in Figure 8 show a near-parallel increase in all treatment groups. Patients without Fn deficiency (saline recipients) have significantly higher C3 levels after trauma than do those with deficiencies. Differences also exist between Fn and albumin recipients; the Fn group has the lowest C3 levels. By day 2 after trauma there are no significant differences between the three treatment groups. By day 4 after trauma the mean C3 levels of each group are within normal range.

Relationship of Treatment to Septic Mortality

Of the 18 septic patients, one patient had been discharged from the Shock Trauma Unit before developing sepsis and was thus lost to further study. The remaining 17 patients were entered into the second phase of our study. We initially rerandomized patients when they became septic, however we discontinued this practice after the 7th septic patient. Our septic rate and fairly small sample size precluded stratification of treatment before and after sepsis. For simplicity, patients remained randomized to the treatment regimen they had before sepsis.

Data analyses were performed on the basis of treatment modality while septic. Of the 17 septic patients (Table 4), only nine (53%) developed a Fn deficiency while septic. Of these nine patients, six received Fn and three received albumin while septic. The eight remaining patients did not develop Fn deficiencies while septic and thus received only saline infusions. Seven patients died as a result of their septic episode(s). Five of the six patients receiving Fn died (83%), one of three albumin recipients died (33%), and one of eight saline recipients died (13%). Mortality was clearly associated with the development of Fn deficiencies. Eighty-six per cent of the mortalities developed a Fn deficiency, whereas only 30% of the survivors developed such a deficiency (Fisher's exact test; p = 0.036). However Fn repletion during sepsis was ineffective in reducing mortality: patients receiving Fn had the highest lethality rate (chi square test = 7.2; dof = 2; p = 0.027).

Stability of Fn in Plasma Samples[30]

Plasma samples of patients from each treatment group both with and without sepsis were tested for the presence

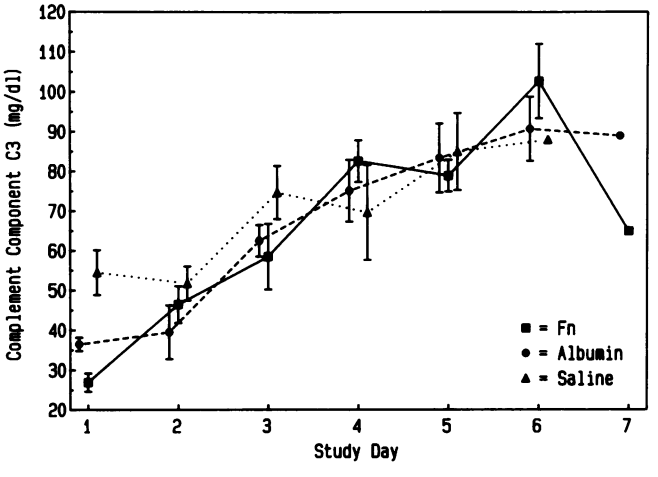

FIG. 8. Complement component C3 profile. C3 levels are shown in Fn, albumin, and saline recipients on days 1 to 7 of study. Data represent mean ± SEM. Fn recipients are designated by (■), albumin (●), and saline by (▲). Significant differences between groups are discussed in the text. Normal range of C3 is from 63 to 135 mg/dL.

TABLE 4. *Association Between Septic Death and Fn Deficiency**

Survival	Fn Deficiency No	Yes	Total
Patients survived	7	3	10
Patients died	1	6	7
Total	8	9	17

Association of mortality with treatment while septic†

Mortality with Sepsis	Survived	Died	Total
Fn recipients	1	5	6
Albumin recipients	2	1	3
Saline recipients	7	1	8
Total	10	7	17

* Fn deficiency during the septic clinical course was associated with lethality in our patients (Fisher's Exact probability = 0.0364).
† Chi square analysis, p = 0.027.

of Fn degradation products by immunoblotting following SDS-PAGE. Electroblotted proteins were probed with polyclonal antihuman Fn and with six monoclonal antibodies specific for Fn's various binding domains. No Fn degradation products were found in these samples, independent of treatment group and presence or absence of sepsis. The sensitivity of the immunogold/silver staining methods used permits detection of known fragments admixed with normal plasma at levels less than 10 ng. Fn degradation products in the plasma, if present, were undetectable using this technique.

Discussion

State of Patients on Admission / Day 1 After Trauma

The admission status of our patients clearly reflected their severity of injury. A Fn deficiency as defined in our study (Fn less than 75% normal pool) was found in 73% of the patients. Fn depletion after injury has been shown in many other studies, both clinical and experimental. Animal studies using a standardized, graded mechanical trauma demonstrated a proportionality between the severity of injury inflicted and the post-traumatic depression of Fn observed.[31,32] However, the Fn deficiency seen in our patients does not represent an isolated protein depletion; reductions in other plasma proteins (*e.g.*, C3, Antithrombin III, and transferrin) were also evident.[26,33] The association between Fn and other plasma proteins and cellular constituents of plasma are not unexpected. Other investigators, not interested specifically in Fn, have clearly established decreases in complement components and immunoglobulin levels after trauma.[34,35] Fn has been shown to correlate well with complement components and coagulation factors in patients having severe infections.[17,26,33]

Influence of Fn Repletion on Clinical Course

The Fn deficiency seen on admission is fairly short lived. Albumin patients, receiving no exogenous Fn, increased their Fn levels, as a group, to within normal range within three days of trauma. As would be expected, patients receiving exogenous Fn had a significantly shorter period of Fn deficit after trauma. Other plasma proteins showed similar normalization patterns (C3 (Fig. 8) and Antithrombin III, data not shown). Within the first seven days of trauma, there were no significant differences found in physiologic parameters (Figs. 4 to 6) between treatment groups. Where differences in biochemical or laboratory parameters occur, they primarily reflect differences between Fn-deficient (Fn and albumin recipients) and nondeficient patients (saline recipients). These differences tend to be short lived as well, disappearing within three to four days of injury. Despite our ability to abrogate Fn deficits by purified Fn infusion, Fn repletion does not alter the early preseptic clinical course of these patients.

Influence of Fn Repletion on the Development of Sepsis

Patients having a Fn deficit on admission were indistinguishable from nondeficit patients (saline recipients) with respect to organ damage and physiologic parameters (Table 1). This comparability permits us to address the importance of Fn deficit in the development of sepsis and if Fn repletion modulates septic incidence or course in these patients.

Sepsis developed in our patients between three and 30 days after trauma. Early sepsis (seven or less days after trauma) was found in each treatment group (2 of 3 Saline recipients, 5 of 10 Fn recipients and, 3 of 5 albumin recipients). These data would suggest that Fn levels *per se* are not indicative of imminent sepsis. The Fn levels of the saline recipients were more than 75% normal pool as were the five Fn recipients when these patients were classified as septic according to clinical criteria. In total, only five of 18 patients showed decreases in their Fn profile (within normal range or below) within days of their septic episode. These results do not support the observations of Lanser et al.[36] who reported a drop in Fn levels 24 to 48 hours before clinical assessment of sepsis in burn patients. Studies performed by Genestal[37] and Snyder[38] and their colleagues also failed to support Lanser's observations. They found that decreases in Fn levels coincided with the development of the clinical manifestations of sepsis.

In our patient population, sepsis rates were identical in patients with and without Fn deficiencies (Table 2). There was also no association between sepsis development and the number of times the patients demonstrated a Fn deficit (Table 3). Furthermore, exogenous administration of purified Fn had no effect on the development of sepsis.

Thus chronic Fn deficiency did not predispose a patient to sepsis, nor did Fn repletion and abrogation of deficiencies diminish the incidence of sepsis. Even more disappointing is the observation that Fn repletion during sepsis did not alter mortality rates in these patients (Table 4).

These findings do not support the early anecdotal reports of clinical benefits of Cryo infusion in sepsis.[10,12,14] They do, however, support the results of the recent controlled clinical trials[1,17–19] that used Cryo as the infusion media. It has recently been contended that the use of Cryo lacking Fn opsonic activity explains the "negative" results of these trials. However the present report also supports the results of Lundsgaard-Hansen and his colleagues[26] who used purified opsonically active Fn in patients with severe abdominal infections. He showed no definitive long-term effects of Fn on the clinical course in these patients, but a trend ($0.05 < p < 0.10$) toward prolonged survival.

Studies (either positive or negative) that used Cryo do not provide information on Fn's clinical efficacy. It is only through the use of purified and fully characterized Fn preparations that Fn's role in critically ill patients can be examined. The preparation used in this report[27] is virus inactivated, stable in its lyophilized form, and fully characterized according to its physical, opsonic, and other functional characteristics. In addition, fragmentation studies[30] indicated that Fn degradation products were not detected in patient plasma. Either degradation did not occur or the RES was capable of removing this material.

The design of our study is different than other studies appearing in the literature. Unlike Lundsgaard-Hansen's study,[26] we tried to maintain patient Fn levels within normal range for as long as the patient remained in the unit. We accomplished this aim by defining a level of Fn deficiency (less than 75% normal) and repleting patients with a dose of Fn calculated to return these patients to 100% normal. These two levels, intrinsic to the design of the study, deserve some scrutiny. These levels were chosen after consideration of Fn variability in the normal population (normal range of this assay is 65% to 135%), and the purported risks of elevating Fn levels above "normal" in critically ill patients. Aukberg and Kaplan[39] demonstrated decreased survival and increased pulmonary localization of a gelatin-coated colloidal emulsion in septic animals supplemented to elevated Fn levels. With a 75% treatment threshold, we may have been actually treating "Fn deficiencies" in patients who simply had naturally low normal Fn levels. If a patient's levels are naturally low, elevating these levels to supranormal may be contraindicated. Our concern in this area may have been misplaced because Fn levels in some patients in Lundsgaard-Hansen's study[26] were elevated to more than 200% normal without ill effect.

Taken together, our data do not demonstrate significant differences in the preseptic clinical course of patients receiving Fn as compared to albumin. Furthermore prophylactic repletion of Fn does not influence the development of sepsis or septic mortality. Our data do not support the prophylactic or therapeutic use of Fn in trauma patients.

Acknowledgments

The authors wish to acknowledge the participation and the tireless efforts of the nursing staff of the Trauma Unit.

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Discussion

Dr. Robert Zeppa (Miami, Florida): This elegant and detailed study, which has required an enormous volume of work, has clearly confirmed that fibronectin deficiencies as defined by the authors are closely correlated with other measured parameters as indicated in the text. To my mind, however, the most important correlation that has been reconfirmed in this study was that between fibronectin deficiency and death due to sepsis.

Unfortunately the ability to mitigate against death by the further provision of fibronectin seems not to alter the risk. This raises the question as to whether fibronectin deficiency may be merely a harbinger rather than a prime mediator. For example, does fibronectin deficiency signal the appearance of a subtle yet apparently global effect such as hepatocellular injury? It has recently been shown that death from sepsis is closely correlated with the failure to restore elevated bilirubin levels. In this study the authors report bilirubin levels only to day 7. Yet the fibronectin-treated patients demonstrated the onset of sepsis at day 9. Perhaps Dr. Mansberger would comment on this.

The definition of sepsis as applied to these patients needs to be exposed. What were the criteria so employed?

Dr. John A. Mannick (Boston, Massachusetts): I think this is a well-performed study of real clinical importance.

As most of you know, there has been considerable interest in the past 12 years or so in the observation that critically injured patients and particularly septic patients have fibronectin deficiency. Fibronectin has been shown to help the reticuloendothelial system clear the blood of particulate debris, although probably not bacteria. However it seemed logical to assume that a deficiency of something that helped the reticuloendothelial system would be bad for an injured patient.

It also seemed logical to assume that giving the patient back that something would be good, and as is the case often in such matters, the early uncontrolled studies of administering cryoprecipitate to these patients—cryoprecipitate contains fibronectin—had very encouraging results. Now Dr. Mansberger has done what I believe to be the definitive study. He has administered purified fibronectin to a series of patients in a prospective fashion after critical injury, and while some may criticize the study because he didn't administer the fibronectin or placebo to all such patients, that wasn't the study he wanted to do.

He assumed correctly that the patients who needed the fibronectin were those who had fibronectin deficiency and he gave them the fibronectin or placebo, and what he has shown, of course, is that fibronectin administration did not influence the clinical course of these patients, and it certainly didn't protect them from sepsis.

So I believe that we are forced to conclude now after Dr. Mansberger's very careful study that while fibronectin deficiency accompanies critical injury and sepsis, restoration of fibronectin levels sadly will not help our patients.

Dr. N. McSwain (New Orleans, Louisiana): Many of the drugs that we have used over the years on trauma patients have seemed to be effective but have not turned out to be. Some of them were ineffective because they are effective only when given before the trauma, such as steroids. Often we have a great delay from the time of the trauma until the time that we can give medications. Even in a very effective urban system, there may be seven to eight minutes of response time of the EMS service, ten minutes on the scene, and another five to ten minutes of transport time, which makes it 30 minutes after the time of the initial trauma until the medication or other treatment might be given. Dealing with a rural system such as they do in Georgia, there is an even greater delay time, as Dr. Mansberger identified, of up to 24 hours.

I would then ask Dr. Mansberger if the prehospital care providers had been allowed the opportunity to provide fibronectin shortly after the time of trauma (within ten minutes) and this was given on a randomized basis, do you think that this early application of the medication might have influenced the study?

Dr. James Peoples (Dayton, Ohio): I also believe that this is a very elegant and convincing study that indeed demonstrates that fibronectin probably does very little to restore the immune deficiency when given