Purified Fibronectin Administration to Patients with Severe Abdominal Infections

A Controlled Clinical Trial

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Subnormal plasma fibronectin (Fn) levels are found in patients with severe abdominal infections (SAI). The repletion of Fn has been postulated to have therapeutic benefit by virtue of its opsonic, reticuloendothelial system (RES) stimulating effects. A controlled, prospective trial of Fn administration was performed in patients with SAI to assess its use as an adjunct to standard procedures of intensive care. Thirty-three SAI patients were given daily doses of 0.8 g of purified Fn on days 1–5 following admission to the ICU, whereas 34 control patients received no Fn. All patients received the clinical care, antibiotics, and pharmacologic agents appropriate to their individual needs. The admission status and laboratory profiles of the two patient groups (+ and −Fn) were comparable on admission to the study. No side effects of the Fn preparation were observed. As judged by subgroup averages, the Fn replacement regimen was effective in elevating Fn levels to within normal range from day 2 onwards, as measured by immunological and functional assays. The estimated intravascular recovery of Fn averaged 82% in those patients who survived, yet only 52% in the nonsurvivors. Ultimate hospital mortality was 9/33 (27.3%) in the +Fn group *versus* 13/34 (38.2%) in the −Fn group ($p = 0.244$, Fisher's exact test). Although ultimate mortality was not significantly changed by the administration of Fn, the Fn treated patients appeared to survive longer than did the control patients. This trend was confirmed through the analysis of expected survival curves ($D = 3.12$, $0.1 > p > 0.05$). When compared to the survivors, the ultimate nonsurvivors entered the study with statistically higher group averages of bilirubin and creatinine concomitant with lower averages of Fn, antithrombin III, C4, C3, C3b-INH, and transferrin. These differences persisted throughout the 11-day monitoring period; differences between survivors and nonsurvivors with respect to platelets, plasminogen, B-1-H, alpha-2-macroglobulin, and prealbumin appeared during the same period. Dramatic differences between the +Fn and −Fn treatment groups were not seen. Other than Fn, the Fn recipients only developed higher levels of the acute phase reactants C4, C3b-INH, B-1-H and alpha-1-antitrypsin ($p < 0.05$) than did their non-Fn treated counterparts. In the present study, we again found a highly significant pattern of correlations between the absolute levels as well as the changes of Fn and other plasma proteins. Our results suggest that the behavior of Fn in patients with SAI is only one small part of a general plasma protein "depletion and recovery syndrome" that is associated with the clinical course of the disease. It is concluded that further controlled therapeutic trials of purified Fn alone or in combination with other depleted factors would be desirable.

PLASMA FIBRONECTIN (Fn) has been postulated to be an essential opsonic mediator of the clearance function of the reticuloendothelial system (RES).[1–3] As such, an acute depletion of Fn is thought to impair the RES defense potential as a result of "hypoopsonemia,"[4] whereas Fn repletion in states of Fn deficiency is proposed to normalize RES function, thereby having therapeutic value.[5–7] Animal studies documented a parallelism between RES function and plasma Fn levels following blunt tissue trauma.[1–3] However, a review of the recent literature[8] reveals that the parallelism between RES function and Fn levels is much less clearcut during thrombin-induced intravascular coagulation, episodes of acute inflammation, endotoxemia, and sepsis. In man, subnormal Fn levels are clearly associated with the triad of intravascular coagulation, organ failure, and sepsis.[9–22] However, such reductions in Fn levels are part of a broader pattern of acute plasma protein depletion including that of antithrombin III (AT3).[19]

To date, the majority of clinical studies of Fn repletion therapy in patients with sepsis and/or organ failure have used cryoprecipitate, a plasma fraction enriched with respect to Fn, albeit to a variable degree.[23] The large majority of these clinical studies were anecdotal and uncontrolled;

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successes[9,14,18,21,22,24,25] as well as failures were recorded.[12,23,26] Very recently, abstracts from two prospective, controlled clinical trials with cryoprecipitate have been reported. Brodin[27] saw no clinical effects of a cryoprecipitate regimen that maintained normal plasma Fn levels in acute myeloid leukemia patients as judged by the number of episodes wherein body temperatures exceeded 38 C. Similarly, Hesselvik and coworkers[28] concluded from a study of 28 patients in hyperdynamic septic shock secondary to pneumonia (N = 9) or intra-abdominal infection (N = 19) that cryoprecipitate administration (N = 14) had no demonstrable clinical effects as compared to stored plasma (N = 14), although a recalculation of their 2-week survival rates (12/14 *vs.* 7/14 patients) reveals a difference with p = 0.05. However, no data on the ultimate fate of these patients were given.

There is a clear need for controlled, prospective therapeutic studies utilizing purified Fn with known functional characteristics. In this paper, we present the results of such a study in patients with severe abdominal infections (SAI). It is realized that this is not the only and perhaps not the most promising group of critically ill patients showing a depletion of plasma Fn, however such patients were included in many of the anecdotal studies published thus far, including the pioneering papers by Saba and coworkers.[9,20–22]

Apart from our target parameter Fn and standard ICU monitoring parameters like body temperature, leukocytes, platelets, serum bilirubin, and creatinine, we measured a number of plasmatic coagulation parameters known to be depleted in septic states or endotoxemia, presumably as a result of activated intravascular coagulation and fibrinolysis: fibrinogen, Factor V, immunoreactive and functional antithrombin III (AT3), and plasminogen.[29–36]

Besides immunoglobulin G (IgG), the C4 and C3 components of complement, representing antibacterial defense mechanisms, were measured. An acute depletion of C3 has been described during life-threatening infections.[33,36,37] Since a "chaotic activation" of the complement cascade has furthermore been postulated to damage the vascular endothelium especially in the lungs during sepsis,[38,39] we also measured the complement system inhibitors C1-Inhibitor (C1-INH), C3b-Inhibitor (C3b-INH), and B-1-H.

Release of proteolytic enzymes, notably granulocyte elastase, and their neutralization by the body's major antiproteases alpha-1-antitrypsin (A1AT) and alpha-2-macroglobulin (A2MG), which are thereby depleted, have also been described in septic patients and experimental endotoxemia;[29,36,40] we therefore measured these antiproteases. Finally, we monitored transferrin and prealbumin because they are, in a general way, sensitive indicators of hepatic protein synthesis[41,42] and/or actual hepatocellular damage.[43]

Of the abovementioned proteins, fibrinogen, C4, C3, the complement inhibitors, and A1AT are "acute phase reactants" in man, *i.e.*, their serum levels increase during an acute inflammatory episode.[42,44,45] In such states, transferrin and prealbumin decrease, *i.e.*, they may be considered as "negative acute phase reactants,"[42,44] whereas antithrombin III (AT3)[46,47] and alpha-2-macroglobulin[42,44] are not changed by inflammation as such. Fn is synthesized by a wide variety of cells including hepatocytes and has been shown to behave as an acute phase reactant[48] in some experimental systems. Of the proteins that we measured, IgG is the only one that is not synthesized exclusively or predominantly by the liver, being a product of the plasma cells.[42,45,49]

The questions to be answered by our study included:

1. Does Fn administration as a supplement to established principles of intensive care influence mortality?
2. Does it influence the duration of ICU treatment and/or length of total hospitalization?
3. Does it influence the behavior of "non-Fn parameters," including the pattern of associations among various plasma proteins previously observed by our group[19] in septic patients?

Methods

Admission Criteria and Terms Used

Patients were aged 16 years and older of both sexes, having either a localized or diffuse severe abdominal infection (SAI) as a consequence of visceral perforation or primary surgery. Patients were referred to the septic ICU from within the University Hospital in Berne or other community hospitals. In addition to the duration of preadmission acute illness, preexisting cardiovascular, bronchopulmonary, hepatic, or renal diseases requiring treatment were also recorded.

Organ failures on admission were defined as follows:

Respiratory failure. The need for mechanical ventilation to maintain gas exchange, *i.e.*, $paO_2 < 60$ mmHg and $paCO_2 > 45$ mmHg on nasal oxygen; in intubated patients oxygenation index $paO_2/FiO_2 < 200$ or total compliance < 25 ml/cm H_2O.

Shock. Circulatory failure requiring dopamine > 20 mg/h (3.5 μg/kg/min) in addition to optimal volume support.

Liver failure. Serum bilirubin > 2.4 mg/dl (>40 μmol/l).

Renal failure. Serum creatinine > 2 mg/dl (>170 μmol/l) or twice the usual level in preexisting renal disease.

Additionally, gastrointestinal hemorrhage or platelet counts $< 60 \times 10^3/\mu l$ were also considered to be organ failures. For each organ failure present on admission, a

score of 1 was given, and the total "organ failure score" on admission was recorded.

The number of days spent within the ICU and the total number of consecutive days spent in the hospital following admission for SAI were recorded for each patient. Ultimate survival *versus* death was defined as a patient's discharge to home *versus* death in the hospital. In many patients, these events occurred in outlying hospitals to which the patient had been transferred for post-ICU care. Patient follow-up was by questionnaire or personal communication with the responsible physicians; information was obtained on all patients admitted to the study.

Design of Study

Patients meeting the admission criteria were allocated in an alternating fashion according to their time of entry into the ICU to receive either "conventional" intensive care conforming to established practice in the ICU (−Fn group) or "conventional" intensive care plus a daily dose of 0.8 g of immunoreactive Fn on day 1 (admission) through day 5 of the study (+Fn group). Each dose of purified Fn (details below) was dissolved in 40 ml of distilled water and infused over a 60-minute period. Patients received their first dose within 6 hours of admission. All subsequent doses were administered between 9 and 10 AM. Patients allocated to the +Fn group were given the full course of Fn supplementation (if surviving through day 5), irrespective of their initial and subsequent Fn levels. No further therapeutic protocol was specified. Quantitative data were collected from days 1 through 11 (monitoring period), after which time only survival or mortality was recorded. Since the evaluation of the therapeutic results was based exclusively on quantitative data, the study was not blinded nor did the −Fn group receive placebo infusions in lieu of Fn.

The design of the study as described was adopted with the specific intent of testing the therapeutic effects of Fn under conditions reflecting realistic and feasible ICU practice. This study was approved by the Medical Faculty Ethical Committee.

Isolation of Fibronectin by Precipitation[50]

Cryoprecipitate was washed with cold Tris buffer (20 mM Tris/HCl, pH 7.0), then dissolved in Tris/phosphate buffer (186 mM Tris, 250 mM NaH_2PO_4, pH 7.0). Contaminating proteins were precipitated by adding solid glycine to a final concentration of 1.5 M.[51] Fibronectin was precipitated with cold ethanol from the supernatant after dilution with water; this precipitate was dissolved in citrate buffer (28 mM Na_3citrate, 100 mM glycine, 1 IU/ml heparin, pH 7.25). Human albumin was added to the solution, which was then filter sterilized and lyophilized. When redissolved in 40 ml of sterile water, the final preparation contained about 18 g/l each of albumin and fibronectin and about 3 g/l of contaminating proteins (mostly fibrinogen). Western blots revealed only slight contamination with fibronectin split products. The preparation was biologically active in both a competitive gelatin binding assay[52] and a macrophage assay.[53] No side effects attributable to Fn administration were observed.

TABLE 1. *Reference Values for Laboratory Parameters Measured*

Sample	Parameter	Units	$\bar{x}$	$\bar{x} \pm 2$ SD
—	Leukocytes	1000/μl	—	3–10
—	Platelets	1000/μl	—	125–320
Serum	Bilirubin	mg/dl	1.52 (max)	—
Serum	Creatinine	mg/dl	1.3 (max)	—
EDTA pl	Fn-BMK	μg/ml	369	195–545
EDTA pl	Fn-GBA	% pool	101	67–135
Cit. pl	Fibrinogen	g/l	2.75	1.5–4.0
Cit. pl	AT3 imm	mg/dl	30.5	22–39
Cit. pl	AT3 funct	%	100	70–130
Cit. pl	Factor V	%	100	60–150
Serum	Plasminogen	g/l	0.154	0.081–0.228
Serum	IgG	g/l	10.82	7.41–15.69
Serum	C4	g/l	0.26	0.12–0.53
Serum	C3	g/l	1.38	0.89–2.09
EDTA pl	C1-INH	conc %	100	55–135
EDTA pl	C3b-INH	funct %	100	60–135
EDTA pl	B-1-H	conc %	100	60–140
Serum	A1AT	g/l	1.75	1.14–2.63
Serum	A2MG	g/l	2.04	1.10–3.58
Serum	Prealbumin	mg/dl	25.0	10–40
Serum	Transferrin	g/l	3.26	2.31–4.52

Parameters Recorded

Immunoreactive Fn in μg/ml[54] as well as gelatin-binding, functional Fn in per cent of a normal plasma pool[52] were measured on daily samples collected from days 1–11. Table 1 lists the parameters measured on samples obtained on days 1, 3, 5, 7, 9, and 11 of the study. In addition, this table provides the normal range of these parameters as measured in samples of EDTA or citrated plasma, native serum, or whole blood, as appropriate. The blood samples were obtained from a central venous line around 6 AM and, with few exceptions, centrifuged within 4 hours for 10 minutes at 400 × g.

Leukocytes and platelets in $10^3/\mu l$ as well as bilirubin and creatinine in mg/100 ml were measured by standard methods. Fibrinogen was measured according to Clauss,[55] immunoreactive AT3 (AT3 imm) = radial immunodiffusion with Behring Nor-Partigen® plates; functional heparin cofactor AT3 (AT3 funct) = chromogenic substrate Kabi S2238, endpoint method;[56] Factor V according to Koller;[55] plasminogen, IgG, C3, C4, A1AT, A2MG, and transferrin with the automatic Hyland laser nephelometer "Disk 120"® and the corresponding reagents; prealbumin = immunodiffusion with Behring M-Partigen® plates; complement inhibitors C1-INH conc %, C3b-INH funct

TABLE 2. *Characteristics of Patients Excluded from Statistical Analyses*

Patients Treated with Fn:

Patient 33, female, age 49, admitted with multiple intestinal perforations and diffuse peritonitis. Clinical course indicated complete recovery. Death on day 17 was due to cardiovascular collapse. Autopsy revealed massive, bilateral pulmonary embolism.

Patient 43, male, age 70, admitted with perforated appendix, diffuse peritonitis. During the course of study, he developed aspiration pneumonia, "burst abdomen." Death on day 43 was due to massive gastrointestinal hemorrhage. Autopsy revealed esophageal ulcer with adherent clot.

Patient 59, female, 58, long-term (2-year) hemodialysis patient. Admitted after 3 bouts of *Pseudomonas aeruginosa* peritonitis, with relapse. Recovery, discharge to dialysis unit on the fourth day postoperative. Death on day 31 was due to relapsing peritonitis, progressive coma. Autopsy revealed chronic renal disease and acute cerebral edema with uncal herniation.

Patients Treated without Fn:

Patient 60, male, age 51, admitted for an acute intestinal obstruction, fulminating septic shock in outlying hospital. On admission to the ICU, the patient was hypoxic, anuric, and unconscious. Persistent anuria continued during the course of treatment; patient lapsed into a neurologic, presumably posthypoxic coma and died on day 14. No autopsy was performed.

Patient 68, female, age 52, recurrent intestinal perforations, necrosis of ascending colon. Patient had hemicolectomy, favorable for 2 weeks then developed intestinal paralysis and signs of SAI. Sudden death on day 23. Autopsy revealed acute cerebral edema with uncal herniation, and massive stenosis of the left coronary artery.

%, and B-1-H conc % by radial immunodiffusion.[57] Antisera were obtained from Atlantic Antibodies (Scarborough, ME) and Miles Laboratories (Naperville, IL). The immunoreactive and functional AT3 values correlated with r = 0.951 (N = 284); thus only the functional values are given in detail.

Statistical Methods

The distributions of parameters (*e.g.*, organ failure scores on admission) in various groups were analyzed using Chi square analyses or Fisher's exact test. Differences of quantitative parameters (*e.g.*, Fn in μg/ml) between groups were analyzed, where appropriate, by the Mann-Whitney U-test or a one-way or a two-way analysis of variance (factors "survival" and "Fn" as well as their interaction). Relationships between parameters were examined, as appropriate, by the Spearman rank correlation coefficient r_s or the Pearson product-moment correlation coefficient r.[58,59] Survival analysis was performed according to the procedures of Peto et al.[60,61] The averages listed in Tables 1 and 4–9 are the arithmetic means $\bar{x} \pm$ SEM. Significance levels were read from standard tables[62] and all findings with $p < 0.05$ were considered as significant (symbols: NS = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

Results

Patient Characteristics

A total of 72 patients were assigned to receive either conventional intensive care alone (−Fn) or conventional intensive care supplemented with a daily dose of 0.8 g immunoreactive Fn on days 1–5 postadmission (+Fn). Three post-ICU deaths in the patient group receiving Fn and two in the control group were considered to be unrelated to the primary SAI and were excluded from all statistical analyses; the characteristics of these patients are summarized in Table 2. Our analyses were thus confined to the remaining 67 patients, 34 of whom received conventional care alone, while 33 patients were supplemented with Fn. All patients underwent counter-SAI surgery (designed to eradicate the focus) within 24 hours of admission to the study. Forty-five patients were ultimate survivors, as defined under Materials and Methods, whereas 22 (32.8%) died.

Retrospective analyses revealed a number of admission parameters that were associated with mortality. These parameters included the duration of preadmission illness ($p < 0.05$), admission from outside hospitals ($p < 0.01$), the presence of postoperative SAI ($p < 0.05$), as well as the number and type of organ failures (Table 3) present on admission.

TABLE 3. *Clinical Characteristics of Patients on Admission*†

Parameter	Survivors	Nonsurvivors	Significance	+Fn	−Fn	Significance
Total patients	45	22	—	33	34	—
Postoperative SAI	22	17	*	20	19	NS
Respiratory failure	4	10	***	6	8	NS
Shock	4	10	***	7	7	NS
Liver failure	9	11	*	11	9	NS
Renal failure	7	10	**	6	11	NS
Organ failure score	25	45	***	32	38	NS

† Clinical characteristics of patients on admission to the study that were associated with mortality, together with their representation in the recipients (+Fn) and nonrecipients (−Fn) of fibronectin.

TABLE 4. *Pharmacologic and Laboratory Parameters on Admission*†

Parameter	+Fn	−Fn		Significance	Survivors	Nonsurvivors		Significance
5% HSA	894 ± 258	868 ± 270		NS	511 ± 151	1636 ± 437		**
Dopamine	9.1 ± 2.5	9.8 ± 2.5		NS	4.0 ± 1.0	20.7 ± 4.0		***
Temp.	37.9 ± 0.2	38.2 ± 0.2		NS	38.0 ± 0.2	38.2 ± 0.2		NS
Leukocytes	12.0 ± 1.2	13.8 ± 1.7		NS	13.5 ± 1.1	11.7 ± 2.1		NS
Platelets	192 ± 15	220 ± 17		NS	216 ± 13	186 ± 22		NS
Bilirubin	2.47 ± 0.47	2.06 ± 0.36	↑↑	NS	1.74 ± 0.20	3.33 ± 0.74	↑↑	*
Creatinine	1.50 ± 0.25	1.90 ± 0.30	↑↑	NS	1.40 ± 0.20	2.30 ± 0.40	↑↑	***
Fn-BMK	170 ± 13	159 ± 14	↓↓	NS	177 ± 10	138 ± 18	↓↓	*
Fn-GBA	70.2 ± 4.1	61.0 ± 4.1	—↓	NS	67.6 ± 2.8	60.3 ± 7.1	—↓	NS
Fibrinogen	3.01 ± 0.14	3.09 ± 0.17		NS	3.16 ± 0.11	2.85 ± 0.23		NS
AT3 funct	60.4 ± 3.6	55.7 ± 3.8	↓↓	NS	62.8 ± 2.6	48.1 ± 5.4	↓↓	**
Factor V	66.6 ± 5.4	71.5 ± 3.2		NS	69.8 ± 3.0	67.7 ± 7.1		NS
Plasminogen	0.08 ± 0.01	0.07 ± 0.01	↓↓	NS	0.08 ± 0.01	0.07 ± 0.01	↓↓	NS
IgG	5.89 ± 0.37	6.24 ± 0.46	↓↓	NS	6.27 ± 0.28	5.70 ± 0.68	↓↓	NS
C4	0.18 ± 0.02	0.17 ± 0.02		NS	0.19 ± 0.01	0.15 ± 0.03		*
C3	0.92 ± 0.06	0.95 ± 0.07		NS	1.01 ± 0.05	0.79 ± 0.09	—↓	**
C1-INH	104 ± 7	112 ± 10		NS	111 ± 5	101 ± 15		NS
C3b-INH	82.6 ± 5.6	80.4 ± 10.2		NS	87.7 ± 5.8	69.1 ± 12.5		*
B-1-H	76.1 ± 7.0	73.9 ± 7.8		NS	77.1 ± 4.8	70.8 ± 12.4		NS
A1AT	2.29 ± 0.11	2.59 ± 0.16		NS	2.51 ± 0.12	2.31 ± 0.19		NS
A2MG	1.08 ± 0.09	1.08 ± 0.09	↓↓	NS	1.13 ± 0.08	0.98 ± 0.09	—↓	NS
Prealbumin	12.1 ± 0.9	9.5 ± 0.8	—↓	*	10.4 ± 0.7	11.4 ± 1.2		NS
Transferrin	1.04 ± 0.08	1.03 ± 0.09	↓↓	NS	1.16 ± 0.08	0.80 ± 0.07	↓↓	***

† Pharmacologic and laboratory parameters of patients on admission to the study, $\bar{x} \pm$ SEM. Grouping by +Fn *versus* −Fn and by ultimate survival *versus* mortality. ↓ = average below normal range, ↑ = above normal range (see Table 1).

None of these factors showed a significantly different representation in the +Fn and the −Fn group. Respiratory failure, shock, and the organ failure score on admission as well as referral from outside hospitals were also significantly associated with early mortality, *i.e.*, during the days 1–11 monitoring period ($p < 0.01$, $p < 0.001$, $p < 0.05$, and $p < 0.05$, respectively), whereas liver and renal failure on admission showed no such relationship. Interestingly, preexisting respiratory, cardiovascular, renal, and liver disease as well as the extent of SAI (localized *vs.* diffuse) on admission did not influence survival/mortality within the study period, nor was their representation different in the ±Fn groups. In addition, the sex and age distributions of the two treatment groups were comparable (14/33 and 15/34 female; average age of the two treatment groups: 57.0 ± 2.4 and 57.2 ± 2.7 years).

In addition to these classification variables, the admission status of the patients assigned to the +Fn and −Fn groups was comparable as assessed by analyses of pharmacologic requirements and laboratory parameters (Table 4). The quantities of 5% human serum albumin (HSA) and dopamine required on the day of admission were similar, as were the laboratory values of the major regulatory proteins. The only significant difference between the two treatment groups was for prealbumin: the −Fn recipients had lower levels ($p < 0.05$) than did the +Fn recipients. This parallelism between groups was not seen when the same data were analyzed in terms of survivors and nonsurvivors (Table 4). On the day of admission, the nonsurvivors required significantly more 5% HSA and dopamine than did the survivors ($p < 0.01$ and $p < 0.001$, respectively). Organ status as reflected by bilirubin and creatinine levels were significantly different in the survivors *versus* the nonsurvivors ($p < 0.05$ and $p < 0.001$); these figures are in agreement with the higher incidence of shock as well as renal and liver failures in the nonsurvivors (*cf.* Table 3). In addition, the laboratory findings of the survivors and nonsurvivors, as groups, indicate that the nonsurvivors entered the study in a measurably worse condition than did the survivors. Nonsurvivors demonstrated a more pronounced plasma protein depletion syndrome, as evidenced by significantly lower group averages of fibronectin, functional AT3, C4, C3, C3b-INH and transferrin than the survivors (Table 4). Prealbumin (the only protein showing differences in the + and −Fn groups) did not show a difference in the survival/mortality subgroup breakdown.

Since there were virtually no differences in admission status of the patients who received fibronectin and those who did not, the comparison between these groups with respect to their further course is appropriate.

Results of Treatment (Course of Patient Groups)

Effect of purified fibronectin administration on fibronectin levels. In the patients receiving Fn, we determined the plasma levels of Fn by immunological and functional assays before and 15 minutes after the end of a 1 hour

TABLE 5. *Pre- and Postinfusion Plasma Levels**

	Immunoreactive Fn Survivors			Immunoreactive Fn Nonsurvivors		
	Pre	Post	Increment	Pre	Post	Increment
Day 1	176 ± 15	414 ± 22	238 ± 15	152 ± 24	307 ± 34	147 ± 23
Day 2	289 ± 22	492 ± 27	204 ± 10	237 ± 43	406 ± 45	169 ± 30
Day 3	397 ± 26	594 ± 32	197 ± 10	302 ± 50	432 ± 51	130 ± 22
Day 4	480 ± 35	705 ± 45	225 ± 20	309 ± 47	428 ± 50	119 ± 10
Day 5	544 ± 40	786 ± 58	243 ± 33	314 ± 44	441 ± 51	132 ± 14
	Gelatin-binding Fn Survivors			Gelatin-binding Fn Nonsurvivors		
	Pre	Post	Increment	Pre	Post	Increment
Day 1	69 ± 4	103 ± 5	34 ± 3	74 ± 12	93 ± 10	19 ± 3
Day 2	91 ± 5	123 ± 5	32 ± 5	91 ± 14	110 ± 13	19 ± 2
Day 3	104 ± 5	131 ± 6	26 ± 3	103 ± 14	114 ± 12	12 ± 4
Day 4	120 ± 5	152 ± 8	32 ± 5	106 ± 10	116 ± 9	9 ± 7
Day 5	137 ± 8	170 ± 10	33 ± 5	107 ± 10	121 ± 11	13 ± 5

* Pre- and 15 minute post-infusion levels of immunoreactive Fn, in μg/ml, as well as gelatin-binding Fn, in % of a normal plasma pool, $\bar{x}$ ± SEM, in patients receiving 0.8 g Fn daily on days 1 through 5 of study. Also shown are the average incremental differences of paired samples. On days 1, 3, and 4, the increments in the survivors differ from those in the nonsurvivors with $p < 0.01$ for immunoreactive and $p < 0.05$ for gelatin-binding Fn. On day 5, $0.06 > p > 0.05$ in both cases (Mann-Whitney U test).

intravenous infusion of the 0.8 g dose. No side effects of Fn infusion were observed. Pre- and postinfusion plasma levels are shown in Table 5 as well as the average incremental difference of the paired samples. Clearly, the concentration increments produced in the nonsurvivors are smaller than those in the survivors as assessed by both immunoreactive and gelatin-binding assays. Interestingly, the concentration increments for each group are virtually constant with time, independent of the preinfusion level. As judged by these data, our Fn replacement regimen was effective in bringing our patient's Fn levels to within normal range by day 2.

Effect of treatment on survival/mortality. Twenty-two of our 67 patients ultimately died (32.8%); 9/33 patients (27.3%) in the +Fn group, 13/34 patients (38.2%) in the −Fn group. This difference is not significant ($p = 0.244$, Fisher's exact test). Death within the monitoring period (days 1–11) accounted for 2/9 (22%) +Fn deaths, while it accounted for 6/13 (46%) of the −Fn deaths ($p = 0.139$, not significant). The autopsy rates in the +Fn and −Fn groups were 5/9 (56%) and 6/13 (46%) deaths, respectively. In the +Fn group, deaths in autopsied patients occurred on days, 8, 9, 16, 21, and 60 following admission to the study. The autopsy findings suggested sepsis in 5/5, peritonitis was found in 5/5, and gram-negative bacteria were found in postmortem cardiac blood in 4/5 patients. One patient (death on day 21) presented an additional intra-abdominal abscess. In the −Fn patients, death occurred on days 3, 7, 9, 18, 19, and 38. Autopsy findings suggested sepsis in 4/6, peritonitis was found in 4/6, and gram-negative organisms as above were found in 4/6 patients. All six patients were "positive" with respect to at least one of the above findings. One patient (death on day 9) had a subphrenic abscess.

TABLE 6. *Ultimate Mortality**

Status on Admission	Ultimate Mortality/ Frequency +Fn Group	Ultimate Mortality/ Frequency −Fn Group	Significance
Respiratory failure	4/6	6/8	NS
Shock	5/7	5/7	NS
Liver failure	6/11	5/9	NS
Renal failure	4/6	6/11	NS
Postoperative SAI	7/20	10/19	NS

* Ultimate mortality in recipients and nonrecipients of Fn admitted with organ failures and postoperative SAI. Analysis of data by Fisher's exact test.

Patients admitted with organ failures and postoperative SAI were analyzed separately in terms of their survival or death under each treatment regimen. No significant differences in survival between the +Fn and −Fn groups were discernible (Table 6) by Fisher's exact test. Thus, supplemental Fn treatment did not seem to alter the mortality associated with these states on admission. Furthermore, Fn had no influence on the length of hospital stay or the number of days spent in the ICU. No significant differences in the distributions of these parameters were demonstrable ($0.5 > p > 0.3$, and $0.8 > p > 0.7$, Chi square analyses).

Although the absolute numbers of deaths in the +Fn and −Fn groups were not statistically different, the timing of these deaths suggested a trend toward prolonged survival in the +Fn group. This trend was confirmed by the use of survival analysis (which takes into account the time

of deaths as well as the number of patients remaining at risk[60,61]), yielding a D statistic of 3.12, $0.1 > p > 0.05$. This trend is most easily visualized in terms of the cumulative mortality distributions of the two treatment groups (Fig. 1).

Effect of treatment: pharmacologic and quantitative laboratory parameters. The presentation of our quantitative data will be limited to the absolute values of the pharmacologic and laboratory parameters for days 5 (end of Fn treatment) and 11 (end of monitoring period) grouped according to ±Fn treatment and secondarily according to survival/mortality. In addition, the changes in these parameters between admission (day 1) and days 5 and 11 are shown. Complete data from the other days of study will not be specified.

Data for days 5 and 11 subgrouped in terms of Fn treatment are summarized in Table 7, along with indications as to whether these values are below, within, or above normal range (Table 1). As seen from Table 7, apart from fibronectin (which was specifically substituted), there are no intergroup differences on day 5, whereas intergroup differences in C4, C3b-INH, B-1-H, and A1AT appear on day 11 ($p < 0.05$ for all). In each of these cases, the higher values were found in the +Fn group. All measured laboratory data were within or above normal range in both treatment groups with the exception of AT3 on day 5 and transferrin, which only reached approximately 50% of normal in both treatment groups by day 11.

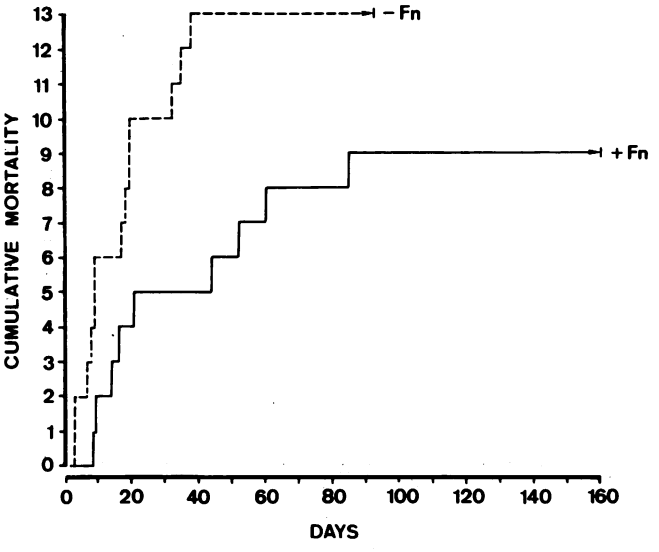

FIG. 1. Cumulative mortality in absolute number of deaths as a function of time in days following admission to the study. Survival analysis[60,61] yields $0.1 > p > 0.05$ for the difference between the treatment groups.

These data are in marked contrast to the pronounced differences seen when the same data are grouped according to survival/mortality (Table 8). When compared to the admission data in Table 4, improvement in virtually all parameters was demonstrable in the survivors, whereas the patients who later died, for the most part, did not improve. On day 5, the nonsurvivors remained below

TABLE 7. *Pharmacologic and Laboratory Data on Days 5 and 11*†

	Day 5			Day 11		
	+Fn	−Fn	Significance	+Fn	−Fn	Significance
5% HSA	182 ± 78	297 ± 105	NS	169 ± 78	180 ± 108	NS
Dopamine	7.0 ± 1.6	6.1 ± 1.9	NS	7.2 ± 2.3	5.4 ± 2.1	NS
Temp.	37.9 ± 0.1	38.0 ± 0.2	NS	37.7 ± 0.2	37.8 ± 0.2	NS
Leukocytes	11.9 ± 1.0	14.1 ± 1.7	NS	13.2 ± 0.9	12.2 ± 1.6	NS
Platelets	199 ± 19	218 ± 23	NS	344 ± 26	316 ± 24 ↑—	NS
Bilirubin	3.69 ± 0.80	2.09 ± 0.31 ↑↑	NS	5.04 ± 1.25	2.12 ± 0.60 ↑↑	NS
Creatinine	1.50 ± 0.28	1.78 ± 0.35 ↑↑	NS	2.30 ± 0.40	1.70 ± 0.30 ↑↑	NS
Fn-BMK	481 ± 36	245 ± 25	***	426 ± 30	219 ± 26	**
Fn-GBA	131 ± 7	81.1 ± 6.5	***	125 ± 6	90.2 ± 7.3	**
Fibrinogen	3.82 ± 0.16	3.81 ± 0.27	NS	3.98 ± 0.19	3.73 ± 0.21	NS
AT3 funct	64.1 ± 4.1	64.7 ± 4.2 ↓↓	NS	81.6 ± 4.0	81.2 ± 6.0	NS
Factor V	91.1 ± 5.3	96.4 ± 4.8	NS	102 ± 6	109 ± 7	NS
Plasminogen	0.11 ± 0.01	0.10 ± 0.01	NS	0.14 ± 0.01	0.14 ± 0.01	NS
IgG	7.52 ± 0.49	7.68 ± 0.58	NS	11.3 ± 0.6	10.6 ± 0.7	NS
C4	0.28 ± 0.03	0.22 ± 0.02	NS	0.36 ± 0.03	0.27 ± 0.03	*
C3	1.33 ± 0.10	1.23 ± 0.09	NS	1.77 ± 0.13	1.53 ± 0.12	NS
C1-INH	164 ± 10	152 ± 10 ↑↑	NS	172 ± 9	158 ± 10 ↑↑	NS
C3b-INH	124 ± 10	100 ± 10	NS	151 ± 10	116 ± 11 ↑—	*
B-1-H	108 ± 10	85.0 ± 7.5	NS	138 ± 9	111 ± 14	*
A1AT	3.19 ± 0.08	2.94 ± 0.13 ↑↑	NS	3.07 ± 0.13	2.77 ± 0.11 ↑↑	*
A2MG	1.28 ± 0.13	1.17 ± 0.10	NS	1.36 ± 0.10	1.56 ± 0.20	NS
Prealbumin	11.3 ± 0.9	11.2 ± 1.0	NS	18.8 ± 1.4	17.2 ± 2.0	NS
Transferrin	1.12 ± 0.10	1.06 ± 0.08 ↓↓	NS	1.58 ± 0.14	1.62 ± 0.22 ↓↓	NS

† Pharmacologic and laboratory data, $\bar{x} \pm$ SEM, on day 5 (end of Fn treatment) and day 11 (end of monitoring period) in recipients (+Fn) and nonrecipients (−Fn) of fibronectin. ↓ = average below normal range, ↑ = above normal range (Table 1).

TABLE 8. *Pharmacologic and Laboratory Data According to Survival and Death*†

Parameter	Day 5 Survivors	Day 5 Nonsurvivors		Day 5 Significance	Day 11 Survivors	Day 11 Nonsurvivors		Day 11 Significance
5% HSA	89 ± 37	575 ± 175		*	54 ± 29	536 ± 219		*
Dopamine	3.3 ± 1.0	13.8 ± 2.7		***	1.8 ± 1.1	19.6 ± 3.5		***
Temp.	37.7 ± 0.1	38.6 ± 0.2		***	37.5 ± 0.1	38.5 ± 0.2		***
Leukocytes	11.8 ± 0.8	15.6 ± 2.5		NS	12.8 ± 1.0	12.6 ± 1.8		NS
Platelets	234 ± 18	152 ± 23		**	376 ± 18	203 ± 19	↑—	***
Bilirubin	1.82 ± 0.32	5.34 ± 1.08	↑↑	***	1.81 ± 0.44	9.51 ± 2.13	↑↑	***
Creatinine	1.37 ± 0.24	2.23 ± 0.44	↑↑	**	1.40 ± 0.20	3.90 ± 0.50	↑↑	***
Fn-BMK	423 ± 32	235 ± 29		***	417 ± 23	223 ± 30		***
Fn-GBA	114 ± 7	82.3 ± 8.3		*	119 ± 6	83.8 ± 9.4		**
Fibrinogen	3.94 ± 0.20	3.54 ± 0.20		NS	3.94 ± 0.16	3.66 ± 0.31		NS
AT3 funct	71.2 ± 3.0	49.1 ± 5.3	—↓	***	90.9 ± 3.2	52.9 ± 4.6	—↓	***
Factor V	97.6 ± 4.2	85.0 ± 6.4		NS	107 ± 6	100 ± 9		NS
Plasminogen	0.12 ± 0.01	0.08 ± 0.01	—↓	**	0.15 ± 0.01	0.11 ± 0.02		**
IgG	7.86 ± 0.43	7.03 ± 0.75	—↓	NS	11.3 ± 0.5	10.0 ± 1.0		NS
C4	0.28 ± 0.02	0.18 ± 0.03		**	0.35 ± 0.02	0.23 ± 0.04		**
C3	1.40 ± 0.08	1.01 ± 0.10		*	1.83 ± 0.10	1.19 ± 0.12		**
C1-INH	164 ± 7	144 ± 14	↑↑	NS	174 ± 7	144 ± 13	↑↑	NS
C3b-INH	127 ± 8	78.8 ± 10.6		**	151 ± 7	94.4 ± 14.8	↑—	**
B-1-H	102 ± 7	83.9 ± 13.3		NS	137 ± 9	97.7 ± 14.6		*
A1AT	3.13 ± 0.09	2.93 ± 0.16	↑↑	NS	2.85 ± 0.10	3.14 ± 0.19	↑↑	NS
A2MG	1.33 ± 0.11	0.99 ± 0.08	—↓	NS	1.59 ± 0.13	1.06 ± 0.10	—↓	*
Prealbumin	11.9 ± 0.8	9.9 ± 1.2	—↓	NS	20.4 ± 1.4	12.0 ± 1.4		***
Transferrin	1.23 ± 0.08	0.78 ± 0.07	↓↓	***	1.87 ± 0.14	0.82 ± 0.10	↓↓	***

† Data as shown in Table 7, but subgrouped according to ultimate survival *versus* death. ↓ = average below normal range, ↑ = above normal range (Table 1).

normal range with respect to AT3, plasminogen, IgG, A2MG, prealbumin, and transferrin, whereas the survivors remained below range only with respect to transferrin. As time progressed, the differences between survivors and nonsurvivors became more numerous and more clear-cut. By day 11, subnormal levels of AT3, A2MG and transferrin remained in the nonsurvivors (53%, 52%, and 25% of normal, respectively), whereas the only parameter remaining below normal in the survivors was transferrin (57%).

Changes in individual parameters were also assessed as a function of time. Using each individual as his own control, we determined changes in the patient's parameters from days 1 to 5 and days 1 to 11. The results shown in Table 9 reflect the breakdown of the data in terms of Fn treatment as well as the breakdown for survivors and nonsurvivors.

Here, the patterns of changes clearly show that by far the most numerous effects are due not to Fn treatment but rather to the clinical course, *i.e.*, to the ultimate survival or mortality of the patient. Only a few changes occurring within the therapy phase (days 1 to 5) were associated with Fn treatment. Other than fibronectin itself, the +Fn treatment group displayed significantly greater increases in C1-INH, C3b-INH, and B-1-H (all at $p < 0.05$) and A1AT ($p < 0.001$) than did the nonrecipients. Over the entire monitoring period (days 1 to 11), C4 and A1AT were the only proteins demonstrating significant incremental differences between the Fn treatment groups (again the difference being larger in the +Fn group). Statistically, creatinine levels increased more in the +Fn treatment group than in the −Fn group ($p < 0.05$). This finding is put into clinical perspective when one considers the following: In the +Fn group, there were three ultimate nonsurvivors with creatinine increases > 3 mg/dl from days 1–11. One of these entered the study in shock and required dopamine > 20 mg/h throughout the monitoring period. The other two patients developed shock and required 30–50 mg/h of dopamine during days 7–11. Among the −Fn nonsurvivors, there was only one comparable patient. In both groups of survivors, creatinine showed no significant changes with time.

When the data were grouped according to survival/mortality, the ultimate survivors displayed significantly greater changes in nearly all of the plasma proteins from day 1 to 11 than did the nonsurvivors.

A more detailed ("two dimensional") breakdown of the patients yielded the four subgroups: plus Fn/Survivor (+Fn/S); plus Fn/Mortality (+Fn/M); −Fn/Survivor (−Fn/S); and −Fn/Mortality (−Fn/M). The time profiles of these four subgroups for the most informative parameters from day 1 (admission) through day 11 are shown in Figures 2–6. The day 1 data were analyzed by one-way ANOVA. For days 5 and 11, two-way ANOVAs were

TABLE 9. *Significance of Changes in Pharmacologic and Laboratory Parameters*†

Parameter	+Fn *versus* −Fn				Survivors *versus* Nonsurvivors			
	Day 1-5		Day 1-11		Day 1-5		Day 1-11	
5% HSA		NS		NS		NS		NS
Dopamine		NS		NS		NS		NS
Temperature		NS		NS	↓	*	↓	*
Leukocytes		NS		NS	↓	*		NS
Platelets		NS		NS	↑	*	↑	**
Bilirubin		NS		NS	↓	*	↓	***
Creatinine		NS	↑	*		NS	↓	***
Fn-BMK	↑	***	↑	**	↑	**	↑	***
Fn-GBA	↑	***	↑	*	↑	*	↑	*
Fibrinogen		NS		NS		NS		NS
AT3 funct		NS		NS	↑	*	↑	***
Factor V		NS		NS		NS		NS
Plasminogen		NS		NS	↑	**	↑	**
IgG		NS		NS		NS		NS
C4		NS	↑	*	↑	*	↑	*
C3		NS		NS	↑	*	↑	**
C1-INH	↑	*		NS		NS	↑	*
C3b-INH	↑	*		NS	↑	**	↑	**
B-1-H	↑	*		NS		NS	↑	**
A1AT	↑	***	↑	*		NS		NS
A2MG		NS		NS	↑	*	↑	*
Prealbumin		NS		NS	↑	*	↑	***
Transferrin		NS		NS		NS	↑	**

† Significance of *changes* in pharmacologic and laboratory parameters (analysis of paired samples) from day 1 (admission) to days 5 and 11, grouped according to +Fn *versus* −Fn and ultimate survivors *versus* mortalities. The arrows indicate the direction of changes in recipients as compared to nonrecipients of Fn, and in survivors as compared to mortalities.

used to assess the effects of the factors "survival" and "fibronectin" as well as their possible interactions. The significances are specified in the figure legends.

Figure 2 shows the Fn levels in the four subgroups as a function of time. The noteworthy points of this figure are: (1) On admission, all four subgroup averages were below normal range; (2) from day 3 onwards, all subgroup averages except the −Fn/M group had reentered the normal range; (3) the nonsurviving Fn recipients (+Fn/M) and the surviving nonrecipients (−Fn/S) did not differ significantly at any point in time. In addition, the +Fn/M group was back to normal range from day 2 onwards and stayed there throughout the monitoring period. With a normal range ($x \pm 2$ SD) of 67–135% of a plasma pool for the functional, gelatin-binding Fn assay, both groups of Fn recipients were within range from the outset and rose to levels exceeding 100%. As a group, the surviving nonrecipients of Fn reentered the normal range on day 3 (73.7 ± 4.7%), and the nonsurvivors on day 5 (69.7 ± 8.6).

The functional AT3 levels depicted in Figure 3 yield a clearcut distinction between the surviving and nonsurviving patients. The surviving patients are back within normal range on day 7, whereas the nonsurvivors remain depressed (approximately 50% of normal range). No differences are attributable to ±Fn treatment.

The same basic pattern is seen with transferrin (Fig. 4).

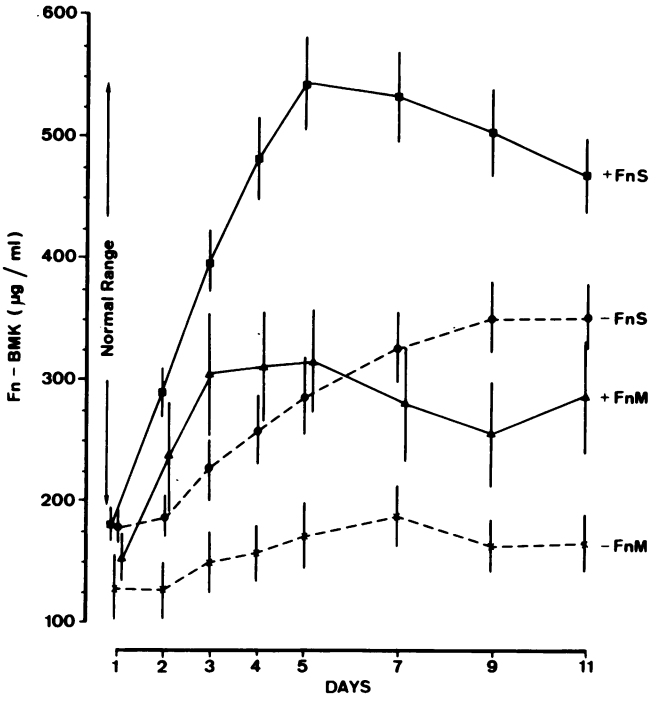

FIG. 2. Immunoreactive plasma Fn levels, in µg/ml, on days 1–11 of the study in the four treatment subgroups +Fn/Survivors (+Fn/S), −Fn/Survivors (−Fn/S), +Fn/Mortalities (+Fn/M), and −Fn/Mortalities (−Fn/M), $\bar{x} \pm$ SEM. On day 1, the difference between the subgroups is NS. On days 5 and 11, the differences S *versus* M as well as the +Fn *versus* −Fn groups are significant with $p < 0.01$; interaction between Fn and survival was not significant. Normal range = $\bar{x} \pm 2$ SD; *cf* Table 1.

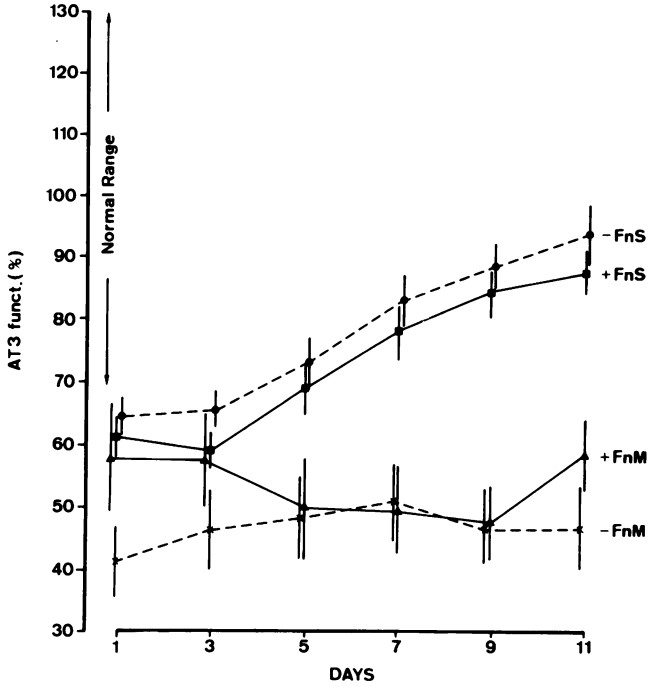

FIG. 3. Functional plasma antithrombin III levels ($\bar{x} \pm$ SEM) in percentage of normal (100%) on days 1–11 of study in the four treatment subgroups, together with normal range as in Figure 2. Differences between subgroups: day 1 $p < 0.05$; days 5 and 11 $p < 0.001$ for S and M, NS for +Fn *vs.* −Fn, interaction NS.

Here there is an even more clear-cut distinction between survivors and nonsurvivors, which is independent of Fn supplementation. The major difference between the AT3 curves and those of transferrin is that transferrin remains

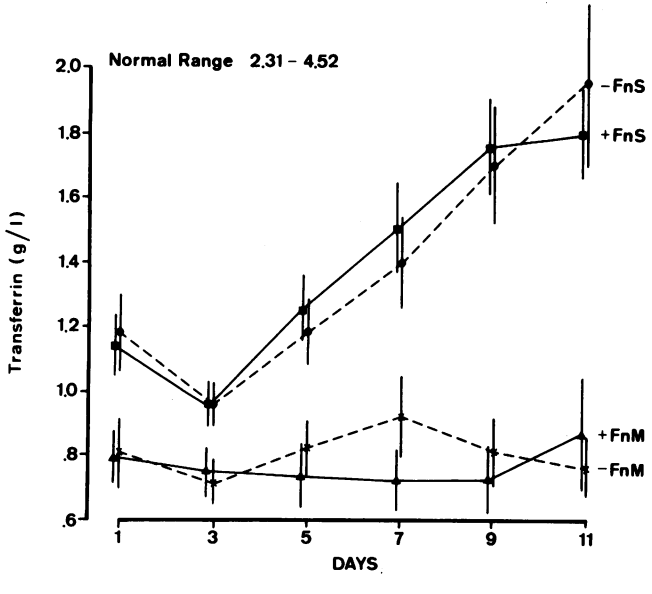

FIG. 4. Serum transferrin levels in g/l, $\bar{x} \pm$ SEM, in the four treatment subgroups as in Figures 2 and 3. The normal range of 2.31–4.52 g/l is above the values shown on the ordinate. Differences between subgroups: day 1 $p < 0.05$; days 5 and 11 $p < 0.001$ for S *vs.* M, NS for +Fn *vs.* − FN, interaction NS.

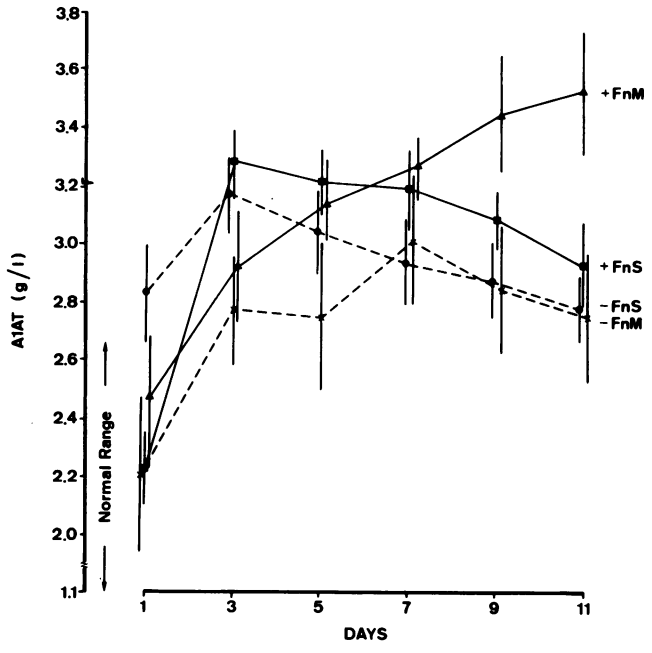

FIG. 5. Serum alpha-1-antitrypsin (alpha-1-proteinase inhibitor) levels in g/l, $\bar{x} \pm$ SEM, in the four treatment subgroups as in Figures 2–4. There are no significant differences among the four subgroups at any point in time.

substantially below normal range in all subgroups throughout the 11-day monitoring period. On day 11, the nonsurvivors remain about 25% of normal, whereas the survivors have reached 55% of normal.

The subgroup patterns for the classic acute phase protein alpha-1-antitrypsin (Alpha-1-proteinase inhibitor) are shown in Figure 5. Here three out of four subgroups are within range on admission; all four subgroups are and remain above normal range from day 3 onwards. These effects are independent of survival/mortality as well as Fn treatment. As judged by these subgroup averages, all patients in the present study had the synthetic capacity to mount an acute phase response to their infection.

Finally, the parallelism between the platelet counts and the patterns shown by transferrin and AT3 is striking. As seen in Figure 6 and although no single subgroup average was below normal range, the platelets remained essentially depressed in the nonsurvivors, but manifestly rose in the survivors. Again, there was no influence of Fn treatment.

Correlation Patterns

Our observations to this point suggested that the behavior of any individual protein was not an isolated occurrence but rather a part of a broader pattern of protein depletion and recovery. The pattern of correlations between Fn, other serum/plasma proteins, and platelets was determined for all complete patient data sets for days 1 through 11 (Fig. 7). This figure includes the data from the +Fn as well as the −Fn recipients.

As seen from this figure, immunoreactive plasma Fn is only one part of a nearly complete pattern of associations between the absolute values of the parameters measured. A more stringent analysis focusing on the changes of various parameters from days 1 to 11 showed that the changes of Fn levels correlated with those of platelets, functional AT3, C3, C1-INH, C3b-INH, B-1-H, transferrin, and prealbumin (all $p < 0.001$); fibrinogen, IgG, C4 (all $p < 0.01$); Factor V, plasminogen, and A2MG (all $p < 0.05$). An inverse correlation with the changes of bilirubin levels ($p < 0.05$) was also present.

Discussion

State of Patients on Admission

As in most clinical studies, the admission status of our patients was variable. As a group, the nonsurvivors were admitted to the study in a significantly worse clinical condition than were the survivors (Table 3). In agreement with other writers,[63] the prevalence of postoperative SAI and organ failures as well as the serum bilirubin and creatinine levels were higher, as were the requirements for 5% HSA and dopamine; these patients also had a more severe plasma protein depletion as witnessed by lower average levels of fibronectin, functional AT3, C4 and C3, C3b-inhibitor, and transferrin (Table 4). There were, however, no significant differences of these criteria in patients assigned to the +Fn and −Fn groups. The demonstration that the admission status of these two treatment groups was similar permitted the evaluation of fibronectin administration as an adjunct to established intensive care.

Results of Treatment

Survival. The early mortality—within the days 1–11 monitoring period—as well as ultimate mortality, *i.e.*, death in hospital *versus* discharge to home, were lower in the Fn recipients, but these differences were not significant ($p = 0.139, 0.244$, respectively). Survival analysis as used, *e.g.*, in leukemia treatment trials[60,61] confirmed the trend toward prolonged survival in the Fn treatment group ($0.1 > p > 0.05$). These data support the results of the controlled, prospectively randomized study reported by Hesselvik et al.,[28] who recorded a lower 2-week mortality in septic patients receiving cryoprecipitate (12/14 *vs.* 7/14 survivors, $p = 0.05$). However, no data on the long-term survival of these patients were given. Despite the apparent prolongation of survival in the Fn treatment group, autopsy findings in both groups of fatalities were virtually identical: peritonitis, sepsis, and positive blood culture of gram-negative bacteria. Thus, the administration of Fn did not lead to a markedly different presentation at autopsy, suggesting that its use did not fundamentally alter the disease process in nonsurvivors. Neither did it influence the survival of patients admitted with organ failures nor affect the duration of ICU treatment or total period of hospitalization.

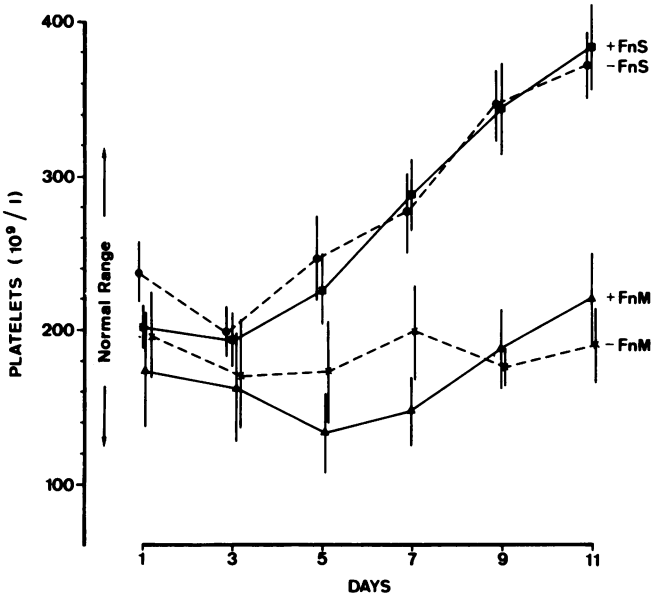

FIG. 6. Platelet counts in $10^3/\mu l$ ($10^9/l$), $\bar{x} \pm$ SEM, in the four treatment subgroups as in Figures 2–5. Differences between subgroups: day 1 NS, day 5 $p < 0.05$, and day 11 $p < 0.001$ for S *vs.* M; NS for +Fn *vs.* −Fn, interaction NS.

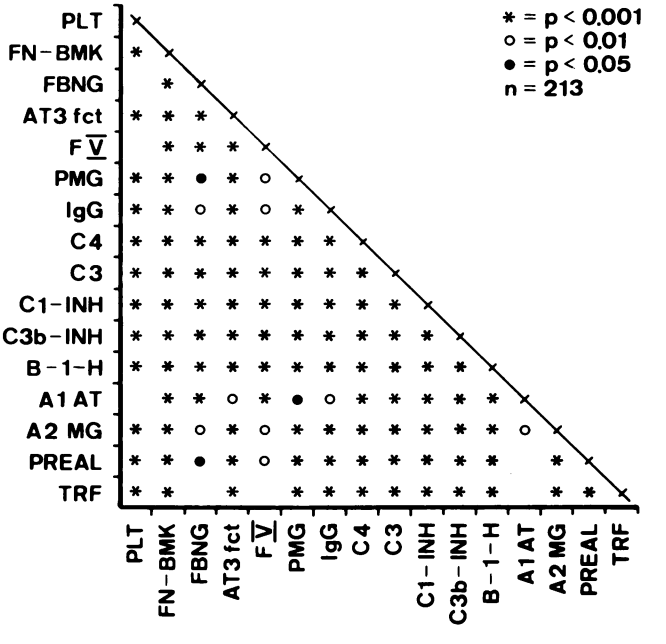

FIG. 7. Correlation matrix of the absolute values day 1 through 11 in the +Fn and −Fn groups. All correlations are positive, indicating parallel variations. The parameters are: PLT = platelets, Fn-BMK = immunoreactive Fn; FBNG = fibrinogen; AT3fct = functional antithrombin III; FV = coagulation factor V; PMG = plasminogen; IgG = Immunoglobulin G; C4, C3, C1-INH, C3b-INH, B-1-H are usd to represent the complement components and inhibitors; A1AT = alpha-1-antitrypsin; A2MG = alpha-2-macroglobulin; PREAL = prealbumin; and TRF = transferrin.

Quantitative pharmacologic and laboratory data. Our analyses of quantitative data utilized two major subdivisions, *i.e.,* into recipients/nonrecipients of purified Fn, and survivors/nonsurvivors. Our analyses focused on day 1 (admission), day 5 (end of Fn administration), and day 11 (end of monitoring period). For selected parameters, we show the group averages for each of the four subgroups +Fn/S, +Fn/M, −Fn/S, and −Fn/M as a function of time.

The subdivision of our patients into recipients and nonrecipients of Fn (Tables 7 and 9) yielded few differences. Other than fibronectin, no statistically significant differences between +Fn and −Fn treatment groups are seen in the absolute values (Table 7) of laboratory parameters on day 5 (the end of Fn administration). During the same period, analysis of paired samples (Table 9) revealed significantly greater increases of C1-INH, C3b-INH, B-1-H, and A1AT in the Fn treatment group compared to their nontreated counterparts. By day 11 (end of monitoring period), there were significant differences in absolute group averages (Table 7) of C4, C3b-INH, B-1-H, and A1AT in addition to Fn. Transferrin was the only parameter found to be below normal range; all other parameters were within or above normal range. When one examines the changes from day 1 to day 11 (Table 9), both C4 and A1AT show significantly greater increases in the Fn treatment group than in the control group.

The group differences seen with respect to A1AT are further documented by analysis of the subgroup values (Fig. 5). It is clear that all patient groups were capable of mounting an acute phase response, yet, for reasons unknown, this response was stronger in the patients receiving Fn (Table 9). By day 11, all subgroup averages were above normal range. The kinetic patterns of response are different: both groups of survivors had their peak A1AT response on day 3, whereas the nonsurvivors peaked on day 7 at the earliest. Although statistically different, it is not clear whether these findings are clinically significant.

Apart from A1AT, the analysis of pharmacologic and laboratory parameters broken down according to survival/mortality showed progressively greater differences between these groups with time. The generalized plasma protein depletion syndrome present on admission virtually disappeared in the survivors while vestiges remained in the ultimate nonsurvivors. On day 11, survivors remained below normal range only with respect to transferrin, whereas nonsurvivors remained below normal range with respect to functional AT3, A2M, and transferrin (Table 8). Although as groups, both survivors and nonsurvivors entered normal range for the majority of parameters, the paired differences (days 5–1 and 11–1) indicate a stronger response by the survivors (Table 9). These observations on survival/mortality responses agree with data published by other authors,[32,36,40] confirming our rationale for measuring these parameters. Absolute values of bilirubin and creatinine remain above normal range in both patient groups; however, these parameters decrease or remain constant in the survivors while they increase in the nonsurvivors (Table 8).

The four subgroup patterns in Figures 3, 4, and 6 show quite distinctly the similarities of the responses of the survivors and nonsurvivors independent of Fn administration with respect to AT3, transferrin, and platelets. Further comments on the four-subgroup pattern displayed in Figure 2 seem pertinent. The fact that the Fn levels of the surviving nonrecipients (−Fn/S) and the nonsurviving recipients (+Fn/M) do not differ significantly at any point in time is of major interest, inasmuch as it shows that on a group basis, the rapid "active" restoration of Fn levels to within normal range did not in and of itself prevent a fatal outcome, whereas the spontaneous recovery of Fn to the same levels was associated with survival. This observation appears to fit recent experimental studies[8] suggesting that the relationship between Fn levels and RES function during endotoxemia and sepsis is much less clear-cut than in the model of blunt tissue trauma[1–3] that furnished the rationale for earlier, uncontrolled therapeutic attempts. Thus, group averages of Fn levels as such did not, in our patients, give any reliable information as to survival or mortality. Our conclusion agrees with Brodin's observations[10] and confirms our earlier report[19] that Fn levels *per se* are a poor prognostic indicator of survival/mortality in septic patients.

The use of Fn levels as a prognostic/diagnostic indicator of a patient's condition is complicated by the variability of normal Fn levels. As shown in Table 1, the span of the normal reference range is 350 μg/ml, a value approximately equal to the average of 370 μg/ml. For this reason, the return of a patient to "within normal range" by no means ensures that he has come back to his individual (usually unknown) normal level. Furthermore, we do not know if such changes in Fn levels reflect alterations in RES function. The problems inherent in this situation are not easily solved, but important for future therapeutic trials of Fn.

Altogether, our data analysis suggests that in such trials, caution is appropriate when relating improvements of quantitative parameters to the administration or nonadministration of Fn; in our study, the predominating determinant of such improvements was ultimate survival *versus* nonsurvival of the patient irrespective of Fn administration.

Correlation patterns. The pattern of highly significant associations between the absolute levels of the various plasma proteins on days 1 through 11 in our patients (Fig. 7) reconfirms and extends our earlier observation[19] that the behavior of Fn in human patients with severe gram-negative infections is only one part of a broader plasma protein "depletion-and-recovery syndrome." This phe-

nomenon was not modified by the administration of Fn in our patients. In addition, the changes of Fn, AT3, C3, the complement inhibitors, transferrin and prealbumin as well as platelets show a highly significant parallelism when paired samples from individual patients were considered. Whereas the survivors recovered from their protein depletion, the multifactorial depression persisted in the ultimate fatalities. We therefore contend that instead of focusing on Fn alone in the manner that has so far prevailed on the experimental and clinical level,[8] it is essential to consider this broader perspective, and that a multifactorial therapeutic approach might more likely be effective if the deficits of pathogenic importance can be identified and, if possible, quantified.

Causes of observed plasma protein patterns. As in a previous study,[19] our present data suggest that a number of causes contributed to the plasma protein pattern that we observed. The fact that several proteins participating in intravascular consumption, such as Fn, AT3, plasminogen, C4, C3, the complement inhibitors, and A2MG, rose with time in the survivors while remaining depressed in the nonsurvivors, might reflect a more intense and protracted consumption in the ultimate fatalities. This hypothesis is supported by the strikingly parallel behavior of AT3 (Fig. 3) and the platelets (Fig. 6), as well as by the larger pre- to postinfusion concentration increments of Fn in the survivors as compared to the nonsurvivors (Table 5). An estimate of the intravascular recovery of Fn based on an assumed, average plasma volume of 3000 ml yields 82% recovery of the administered dose 15 minutes postinfusion in the survivors, as compared to 52% in the nonsurvivors. These figures agree remarkably well with the 30-minute postinfusion recoveries calculated by Blauhut et al.[64] for AT3 concentrates in patients without and with DIC (83% and 47%, respectively). Cembrowski and Mosher[65] have recently confirmed the close correlation between Fn, AT3, and plasminogen levels in patients with acquired consumptive coagulopathies.

On the other hand, the differences of the above-mentioned proteins between survivors and nonsurvivors, amplified by similar differences for prealbumin and transferrin—which do not participate in intravascular consumption, but reflect the intensity of hepatic protein synthesis[41–43]—might indicate a general preponderance of protein catabolism over synthesis, especially in the ultimate fatalities. Patients with SAI have resting metabolic expenditures 30–40% above predicted basal metabolism rates.[66] If present, however, such an imbalance was modified by an acute phase response, in that the two classical acute phase reactants of hepatic origin, fibrinogen and A1AT, were both in the high normal range or above and showed no differences between survivors and nonsurvivors at any point in time. In that context, it is of interest that the only "nonliver" protein that we measured, namely IgG, also showed no differences related to outcome, although it ran on a lower level than fibrinogen and A1AT. Finally, the behavior of IgG suggests that a capillary leak syndrome was not a major cause of the plasma protein pattern that we observed, inasmuch as the nonsurvivors required significantly larger volumes of 5% HSA throughout the 11-day monitoring period (Tables 4, 7, and 8), yet they did not show lower intravascular levels of the relatively diffusible IgG (molecular weight of 140,000 d).

Our data furnish no proof as to the relative importance of these different mechanisms, but they do show that the clinical situation is vastly more complex than the experimental setups that stimulated an interest in the therapeutic use of Fn.[1–3]

Conclusions

The 10% difference in ultimate mortality between the recipients and nonrecipients of Fn—9/33 patients (27.3%) *versus* 13/34 (38.2%)—recorded in our study was not statistically significant ($p = 0.244$, Fisher's exact test). Survival analysis as applied to cancer chemotherapy trials[60,61] suggested a trend toward prolonged survival in the +Fn group with $0.1 > p > 0.05$. Our laboratory data indicated a stronger acute phase response in the Fn recipients at the $p < 0.05$ level. In our opinion, these results in a limited number of SAI patients treated with one specific (yet perhaps not optimal) regimen with respect to purified Fn warrants further controlled trials of its therapeutic potential, alone or in combination with other "defensive" plasma proteins. Power analysis[67] reveals that 450 patients would have to be enrolled in each treatment group to be assured an 85% chance of observing a statistically significant decrease in mortality (38% to 28%) at the 95% confidence level, *i.e.*, a multicenter trial would be necessary to obtain a clear-cut answer within a reasonable period of time.

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