Reticuloendothelial System Activity and Organ Failure in Patients With Multiple Injuries

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Hypothesis: Reticuloendothelial system function is altered in patients with multiple trauma and organ failure.

Design: Prospective cohort study.

Setting: Surgical intensive care unit at a level I trauma center.

Patients: Patients with multiple blunt trauma and injury severity scores greater than 20, with no referrals.

Interventions: Every second day reticuloendothelial system (RES) clearance capacity and liver blood flow were determined by administering labeled human albumin. Liver function was measured by enzymatic decay of indocyanine green, and levels of plasma tumor necrosis factor α were evaluated.

Results: In nonsurviving patients with blunt trauma, RES function was altered and was associated with organ dysfunction and infectious complications. Of 61 patients, 42 survived and 19 did not. Sixteen patients (84%) died of multiple organ failure. Significantly elevated RES activity (colloid clearance rate) was present between day 5 and day 13 after trauma in nonsurvivors (0.86 ± 0.16 [mean ± SD] on day 7, P = .003) compared with survivors (0.48 ± 0.08 on day 7) and 20 healthy volunteers (0.47 ± 0.06); RES activity then decreased to subnormal levels in nonsurvivors. Tumor necrosis factor α plasma levels were elevated early after injury only in nonsurvivors (on day 1: nonsurvivors, 1.2 ± 0.4 ng/mL [mean ± SD]; survivors, 0.5 ± 0.2 ng/mL; P = .02). Indocyanine green half-life values increased late after trauma, indicating late organ failure (on day 19: nonsurvivors, 111 ± 29 minutes [mean ± SD]; survivors, 12 ± 4 minutes; P < .001).

Conclusions: Early after trauma, nonsurviving patients demonstrated increased proinflammatory cytokine levels, followed by a state of pathological hyperactivation of the reticuloendothelial system prior to death. These results indicate that the stationary host defense system is involved in the mechanisms causing organ failure after severe trauma.

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The pathogenesis of multiple organ failure (MOF) has been attributed to disturbances of the intestinal barrier and the systemic immunologic response. These mechanisms suggest that a loss of intestinal barrier function associated with increased intestinal permeability allows systemic invasion of intestinal pathogens. In turn, intestinal permeability damage, which has been demonstrated after trauma and burns, may pave the way for further translocation of intestinal bacteria and bacterial endotoxin. In combination with other stimuli, endotoxin is able to cause MOF in sheep. Some authors have attributed the development of organ failure to overwhelming invasion of bacteria and endotoxins, exceeding the capacity of bacterial clearance of the reticuloendothelial system (RES). Other authors have doubted the clinical significance of these findings, since clinical studies have failed to prove a relationship between intestinal bacteria and later organ failure or death. Moreover, bacterial translocation to mesenteric lymph nodes and to the portal system was documented in human organ donors, suggesting that bacterial translocation may occur despite an anatomically intact gastrointestinal tract. Irrespective of the controversial discussion about the clinical significance of the “gut-liver axis,” the changes in function of the RES in response to trauma and hemorrhagic shock have been well described. Altered RES function has also been reported following low-grade intravascular coagulation or ischemia reperfusion damage. In these studies, the stimulus caused impairment of
MATERIALS AND METHODS

The study was performed according to the international guidelines of the declaration of Helsinki. Informed consent was obtained from each patient’s closest relative prior to the start of the study. Patients with multiple injuries were prospectively studied based on the following inclusion criteria: an injury severity score of more than 20; an initial Glasgow Coma Scale score greater than 8; primary admission and intensive care at our institution; no death due to hemorrhagic shock; a survival rate greater than 14 days after trauma; no liver or bowel disease or abdominal surgery; and no hepatic or colorectal malignant neoplasms or alcoholism.

DEFINITIONS

Adult respiratory distress syndrome and sepsis were defined according to scoring systems. Severity of injury was categorized by the injury severity score (ISS) and the Glasgow Coma Scale score. Multiple organ failure was defined according to the criteria described by Knaus and Wagner. Changes of organ function during the course of intensive care were also documented according to a multiple organ dysfunction score.

All measurements were done every second day during intensive care therapy, starting on the day of injury. The study period encompassed the intensive care unit stay. In our institution, early intubation and prophylactic ventilation are routinely performed. Using standard laboratory tests, total serum bilirubin level, total protein level, and serum-platelet counts were determined, along with ventilatory parameters and gas analyses.

The patients were separated into 2 groups: survivors and nonsurvivors.

clearance functions and subsequent downregulation of RES capacity. Other studies have revealed that RES function, based on measurements in isolated Kupffer cells, responds to endotoxemia and tumor necrosis factor α (TNF-α) stimulation by an increase in oxygen radical production. These results suggest that changes in RES clearance capacity may not depend on alterations of the intestinal barrier.

Human data on the RES and its possible role in MOF are sparse. Therefore, our prospective clinical study compares the function of the RES in patients with multiple trauma who develop late organ failure and death with the function of the RES in patients who demonstrate an uneventful clinical course.

RESULTS

Between October 15, 1994, and April 15, 1997, consecutive patients who met the inclusion criteria were investigated. Of 68 patients, 4 died before 14 days after trauma and 3 died of hemorrhagic shock and were excluded. Sixty-one patients with multiple trauma were studied, with 42 survivors (29 men, 13 women) and 19 nonsurvivors (11 men, 8 women). Demographic data revealed no statistically significant difference in the severity of injury, the degree of head injury on the scene, and the duration of intubation and intensive care therapy. The amount of intravascular fluid received during rescue was significantly higher in nonsurvivors (Table 1).

Adult respiratory distress syndrome occurred in 6 survivors and 3 nonsurvivors. Renal failure requiring hemofiltration was found only in association with late organ failure. Hemofiltration was performed in 7 survivors and 3 nonsurvivors. There was a significantly higher rate of pulmonary infections among nonsurvivors. The other data associated with infectious complications revealed no differences between groups (Table 1). Table 2 demonstrates the course of the MOF score in patients who did and who did not survive.

Serum bilirubin levels were normal early after trauma and remained normal in survivors. Among nonsurvivors, a statistically significant increase followed until the end of the third week after trauma (P<.003 compared with survivors, P<.001 compared with baseline). The liver perfusion values demonstrated no statistically significant difference between patient groups during the entire study period (Table 3). The independent measure of intestinal blood flow by Doppler ultrasound revealed large SDs despite the fact that a mean of 3 measurements was calculated. Therefore, these data were not used and are not shown.

All coagulation values were significantly altered early after trauma in both groups of patients. Nonsurvivors...
calculated. Three points on the time-activity curve were investigated: the beginning of arterial liver perfusion, the beginning of portal venous perfusion, and the beginning of recirculation effects at the end of the first portal passage.

As an independent measure of perfusion, Doppler ultrasound examination of the hepatic vein was also performed (Sonoline AC, No. U3-040.201.01.01, Siemens Corp, Erlangen, Germany), using a 5-MHz device. Three sequential measurements were made and the mean of these measurements was calculated.

PROINFLAMMATORY CYTOKINE

Tumor necrosis factor α levels were determined using an enzyme-linked immunosorbent assay technique (polyclonal rabbit anti–human TNF-α coating, Polysan-TNF BPS 30; Biochrom KG, Berlin, Germany). Also, recombinant TNF-α (standard, 10 μg per vial [Bissendorf KG, Hannover, Germany]) and monoclonal mouse Fab anti-human TNF-α peroxidase 20 (No. 1198688; Boehringer Mannheim GmbH, Mannheim, Germany) was used.

INDOCYANINE GREEN TEST

Indocyanine green (ICG), an anionic water-soluble dye, was freshly dissolved prior to each measurement. Following initial blood sampling, the sterile preparation of ICG was injected intravenously at 0.5 mg/kg body weight (ICG Pulsion; Pulsion Medicine Systems KG, Munich, Germany). At 5, 10, 15, and 20 minutes, 5 mL of blood was withdrawn from the antecubital vein and the serum concentration was determined enzymatically at 800 nm (Beckmann DU-6 photometer; Beckmann GmbH, Hoelhof, Germany). Calibration was performed using control blood. The plasma disappearance rate was calculated by linear regression analysis of the plasma concentrations at 5, 10, and 15 minutes.

COAGULATION STUDIES

Platelet-poor plasma was prepared from 4.5 mL of blood mixed with 0.5 mL of 0.1-mol/L sodium citrate in a siliconized glass tube and centrifuged at 2600 rpm for 20 minutes.

Antithrombin III was measured by means of the Coamatic LR Antithrombin test kit (Cromogenix AB, Malmö, Sweden) using the CobasFara chemistry analyzer (Hoffmann–La Roche Ltd, Basel, Switzerland). This test is based on the blocking effect of the activity of factor Xa by the antithrombin-heparin complex. Samples from patients with multiple trauma were compared with samples from 20 healthy controls.

The activity of protein C was measured using the Berichrom kit (Behring Diagnostics, Frankfurt, Germany). A sodium citrate solution was carefully mixed at a 1:9 ratio with venous blood. Subsequently, the mixture was centrifuged at 3000 g for 10 minutes. Plasma samples were stored at −20°C; at this temperature the sample is stable for at least 1 month.

The measurement of D-dimer cross-linked fibrin degradation products was performed using a D-dimer enzyme-linked immunosorbent assay kit (American Diagnostica Inc, Greenwich, Conn).

STATISTICS

Paired and unpaired t tests were performed. Probabilities less than .05 were considered significant. Values are expressed as mean±SEM.

| Table 1. Demographic and Infection Rate Data for Study Participants* |
|-----------------|-----------------|-----------------|-----------------|
| **Nonsurvivors** | **Survivors**   | **Nonsurvivors** | **Survivors**   |
| No. of patients  | 19              | 42              | 30              |
| Age, y           | 39.2 ± 4.5      | 36.7 ± 4.9      | 39.8 ± 4.3      |
| Injury severity score | 32.8 ± 6.1 | 27.8 ± 6.9 | 30.8 ± 6.5 |
| Glasgow Coma Scale score | 12.3 ± 3.8 | 11.4 ± 5.7 | 12.0 ± 3.7 |
| Prehospital rescue time, min | 64 ± 48 | 59 ± 43 | 62 ± 50 |
| Prehospital intravenous fluids, mL† | 4620 ± 440 | 1770 ± 890 | 4000 ± 360 |
| Infections during intensive care unit stay, No. (%) | | | |
| Pneumonia‡ | 11 (58) | 8 (19) | 10 (50) |
| Sepsis | 2 (11) | 3 (7) | 3 (15) |
| Wound infection | 5 (26) | 7 (17) | 7 (35) |

*Values are expressed as mean ± SEM unless otherwise indicated.
†Marshall et al.23
‡P<.05.

Table 2. Clinical Course of Patients With Multiple Trauma as Determined by Multiple Organ Dysfunction Score ‡†

<table>
<thead>
<tr>
<th>Day</th>
<th>Nonsurvivors</th>
<th>Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.9 ± 4.1</td>
<td>4.3 ± 3.9</td>
</tr>
<tr>
<td>3</td>
<td>5.2 ± 3.0</td>
<td>4.9 ± 3.8</td>
</tr>
<tr>
<td>5</td>
<td>6.4 ± 3.8</td>
<td>6.5 ± 3.8</td>
</tr>
<tr>
<td>7</td>
<td>8.8 ± 6.3</td>
<td>8.2 ± 2.6</td>
</tr>
<tr>
<td>9</td>
<td>12.5 ± 7.9</td>
<td>7.3 ± 7.0</td>
</tr>
<tr>
<td>14</td>
<td>19.7 ± 8.9</td>
<td>5.9 ± 5.4</td>
</tr>
<tr>
<td>21</td>
<td>22.2 ± 8.6</td>
<td>5.5 ± 5.2</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SEM.
†Marshall et al.25
‡Multiple Organ Dysfunction Score

...demonstrated lower protein C values and higher levels of D-dimer cross-linked fibrin degradation products at days 1 and 3 than survivors (Table 4).

Figure 1 demonstrates changes in RES function. Early after trauma, data were normal. Among nonsurvivors, an increase developed within the first week after injury (day 7, 0.86±0.1; P=.003) compared with survivors (day 7, 0.48±0.08) and controls (0.47±0.06). During the third week, values among nonsurvivors decreased to subnormal levels.

The central venous levels of plasma TNF-α were significantly higher among nonsurvivors than among survivors until day 5 after injury (day 1: nonsurvivors, 1.2±0.4 ng/mL; survivors, 0.5±0.2 ng/mL; P=.02). Following the first week, no difference was found between nonsurvivors and survivors (Figure 2).
Indocyanine green values were normal in both groups early after trauma. No significant changes from baseline occurred in survivors during the entire course of intensive care. Nonsurvivors showed a significant increase compared with baseline and with survivors (Figure 3).

Table 3. Liver Excretory Function Based on Serum Bilirubin Levels

<table>
<thead>
<tr>
<th>Day</th>
<th>Nonsurvivors</th>
<th>Survivors</th>
<th>Nonsurvivors</th>
<th>Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin, µmol/L (mg/dL)</td>
<td>22.3 ± 11 (1.3 ± 0.6)</td>
<td>21 ± 6 (1.2 ± 0.4)</td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Liver Perfusion Constant</td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Parameters Indicative of Coagulatory Changes

<table>
<thead>
<tr>
<th>Day</th>
<th>Antithrombin III, %</th>
<th>Protein C, mg/L</th>
<th>D-Dimer, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsurvivors</td>
<td>Survivors</td>
<td>Nonsurvivors</td>
<td>Survivors</td>
</tr>
<tr>
<td>1</td>
<td>50 ± 21</td>
<td>61 ± 26</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>78 ± 30</td>
<td>83 ± 28</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>85 ± 28</td>
<td>91 ± 22</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>14</td>
<td>107 ± 37</td>
<td>130 ± 26</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td>21</td>
<td>95 ± 29</td>
<td>126 ± 22</td>
<td>3.8 ± 1.1</td>
</tr>
</tbody>
</table>

Table 3. Liver Excretory Function Based on Serum Bilirubin Levels

*Values are expressed as mean ± SEM.
†Calculated from the uptake of technetium Tc 99m decay.
‡P = .04.
§P = .007.
¶P = .003.

COMMENT

Death from MOF continues to represent an unsolved problem in critically ill patients, affecting 15% of patients admitted to intensive care units in the United States and Europe. In the present study, we attempted to investigate whether there is a difference in RES clearance capacity between patients who demonstrate an uneventful clinical course and patients who die late after severe trauma, generally from MOF.

To our knowledge, the only clinical study measuring RES function after blunt trauma involved patients with liver injuries. One severe problem with this study was the inability to perform RES measurements in the intensive care unit. Since a bedside system was not available, all patients had to be transferred to the department of nuclear medicine. This influenced the selection criteria; patients could only be investigated if their cardiorespiratory status was stable enough for transport. Moreover, patient transport immediately prior to investigation may have influenced the results obtained.

These difficulties were avoided in the present study. The method we used to evaluate RES function is reliable and has been used in previous experimental investigations. In a previous study undertaken at our hospital, this method was able to assess the alterations of RES function in liver transplant recipients. In our study, normal levels in the control group were reproducible and showed a mean RES function of 0.42 ± 0.06. Nevertheless, the presence of multiple sources of hemorrhage, as in patients with multiple trauma, may influence the results obtained. Our study considered the effects of hemorrhagic shock.

We assumed that both liver perfusion (K1) and RES function (K2) values are influenced by splanchnic perfusion. In contrast, other authors have argued that only the accumulation of tracer is influenced by the liver’s sinusoidal blood flow, whereas the elimination of tracer from the liver is much less altered in cases of decreased blood flow. It was unlikely that the effects of slowed splanchnic perfusion played a major role, because the time-activity curve was calculated over 15 minutes. Moreover, clinical studies have found no correlation between hepatic blood flow and the removal constant of radioactive tracer.

Nevertheless, we tried to account for the possible effects of impaired liver blood flow and performed an ICG test parallel to the measurements of RES function in all

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patients. The ICG test is a well-established method for evaluation of liver blood flow.\textsuperscript{32,33} Splanchnic perfusion can be quantified because ICG is removed from the blood solely by the liver and is excreted without biotransformation and uptake occurs through a flow-limited mechanism.\textsuperscript{34,35} The results of RES function measurements were deemed reliable only if the ICG test result was normal. In view of these prerequisites, we feel that it is justified to draw conclusions concerning RES function from the data obtained.

We measured several parameters indicative of post-traumatic coagulatory changes to account for impaired microvascular blood flow secondary to disseminated intravascular coagulation.

Antithrombin III is well known to decrease after trauma and hemorrhage.\textsuperscript{36} Protein C levels were also measured because protein C has a similar function to antithrombin III but is more sensitive because it has a shorter half-life (half-life of antithrombin III, 2-5 days; half-life of protein C, 4-6 hours).\textsuperscript{37} Moreover, the measurement of D-dimer cross-linked fibrin degradation products is a sensitive indicator of hypercoagulability in trauma patients.\textsuperscript{38} All data were corrected for hematocrit to exclude effects due to dilution secondary to therapy for shock. Observed decreases of the activity of various substrates are in fact due to their consumption during an increased coagulatory response. Our results demonstrate alterations of the coagulatory response early after injury that we attributed to hemorrhagic shock. All 3 parameters were abnormal early after trauma and normalized thereafter. Among nonsurviving trauma patients, the early state of hypercoagulability was worse at days 1 and 3. At time points when RES function was different between groups, the coagulatory response was comparable. Therefore, according to the coagulation studies, it is unlikely that the disseminated intravascular coagulation influenced the hepatic microvascular perfusion following the first 3 days after injury.

Our principal result is a biphasic response in RES activity in among nonsurviving patients. During the first week after injury, the RES function in nonsurvivors was significantly increased in comparison with survivors and with the control group. Later during the clinical course, nonsurvivors demonstrated a decrease in RES function to subnormal levels. Also, a higher incidence of pulmonary infections was found in among nonsurvivors. In 9 of the 11 nonsurviving patients with pneumonia, the onset of infection occurred between day 3 and day 10, suggesting that an impaired immune defense was present despite RES hyperactivation during this period. No changes of ICG levels occurred in either group during the first week after trauma. We conclude that during this period liver perfusion was not impaired, and that changes in RES function results were due to increased RES activity. The late decrease in RES function values may be related to cardiocirculatory changes or impairment of liver function, because serum bilirubin concentrations and ICG levels showed pathologic values following the second week after injury. The finding of increased RES function is in accordance with a previous animal study from our laboratory.\textsuperscript{39} Moreover, increased RES phagocytic capacity has also been observed in patients with peritonitis.\textsuperscript{39}

In contrast, other authors have reported a decreased phagocyte response following a variety of stimuli. Kaplan and Saba\textsuperscript{12,40} described transient RES dysfunction after intravascular coagulation, which was ex-
plained by decreased hepatic Kupffer cell clearance. It was argued that the stationary system can sufficiently explain these changes, since previous in vivo studies also have indicated depressed Kupffer cell function in response to traumatic hemorrhagic shock.\(^\text{12,14}\)

Several factors may influence RES function, including failure of opsonic activity, phagocytic cell dysfunction, and failure of the test substance to be delivered to the hepatic sinusoid area, where it would be cleared by Kupffer cells. The latter may be caused by sinusoidal blood flow secondary to splanchnic malperfusion, sinusoidal congestion, or disseminated coagulation.\(^\text{15}\) The studies describing suppression of RES function as a uniform reaction to a variety of insults\(^\text{12,14,40}\) have to be considered in view of these principles. Splanchnic vasoconstriction is known to occur despite normal systemic blood pressure. Impaired hepatic perfusion should be considered to have influenced RES function in these studies; however, the effect of splanchnic perfusion cannot be determined since no data were available in these investigations.\(^\text{12,14,40}\)

Trauma and hemorrhagic shock have been demonstrated to cause aggregation of polymorphonuclear leukocytes to liver endothelial cells, which may cause impairment of microvascular blood flow.\(^\text{41}\) Moreover, in a study investigating intravascular coagulation, impaired sinusoidal blood flow is likely to occur and influence the degree of impairment of RES function.\(^\text{15}\)

In our investigation, the opsonizing activity in the stationary defense system is not measurable, since liver biopsy is not acceptable in these patients for ethical reasons. In contrast, data on opsonizing activity are available for the circulating defense system (polymorphonuclear leukocytes [PMNLs]). In PMNLs isolated from central venous blood, the opsonizing capacity was not different on the day of injury regardless of whether organ failure developed.\(^\text{42}\) Further clinical studies showed a state of increased activation of PMNLs following severe trauma.\(^\text{43}\) In the present study, the early increase in RES function values among nonsurviving patients was most likely due to an increase in RES capacity, because the ICG test demonstrated comparable results in both groups. Disseminated intravascular coagulation as a cause of impaired intrahepatic blood flow was also considered, but did not play a role at time points when RES function was different between groups (Table 4).

As a further cause of changed RES function, the accumulation of activated PMNLs on liver endothelium must be considered. Hemorrhagic shock has been demonstrated to induce significant adhesion of PMNLs to hepatic endothelial cells.\(^\text{41}\) The associated impaired microvascular perfusion is known to develop prior to histologic evidence of hepatocellular injury.\(^\text{44}\) In our patients, liver perfusion was adequate during the course of intensive care; however, this may not have been the case during the resuscitation period. The amount of intravenous fluid administration during rescue was significantly higher among nonsurviving patients. Sufficient volume replacement obviously was performed, since there was no difference between groups in admission blood pressures, which adequately reflected the severity of hemorrhagic shock (data not shown).\(^\text{45}\) The assumption that initial hemorrhagic shock was different between groups is supported by the fact that there was a higher degree of activation of the inflammatory response (TNF-\(\alpha\)) in nonsurvivors. These results are consistent with previous reports.\(^\text{46}\) Production of TNF-\(\alpha\) is known to be inducible by endotoxin.\(^\text{47}\) It has been suggested that lipopolysaccharide activates gut-associated macrophages on translocation and causes further activation of Kupffer cells, which consequently produce more TNF-\(\alpha\).\(^\text{46}\) This effect would explain the clinical course of RES data in the present study. Moreover, a similar response has been confirmed in previous studies of blunt trauma in both patients and animals subjects.\(^\text{29,30}\)

Whether gut barrier failure leading to the passage of viable enteric bacteria and endotoxin is responsible for the altered RES function seen in our study cannot be determined on the basis of our results, because endotoxin levels and bacterial translocation were not determined. The activation of the RES measured in nonsurvivors, most of whom died of MOF, may constitute indirect evidence for the necessity to clear bacteria and endotoxins. As postulated above, the late decrease in hepatic function is likely to have influenced RES function, but a disturbance in the clearance capacity may have been present independent of perfusion.

No clinical study has proven that changes in the intestinal barrier cause MOF.\(^\text{5,10}\) Among other host defense mechanisms, a pathologic increase in the systemic posttraumatic inflammatory response has been documented\(^\text{48}\) that might involve the RES. Whether direct stimulation of RES function occurs or whether invading PMNLs following shock might affect RES function, as previously discussed,\(^\text{49}\) remains speculative.

Our results demonstrate that a sustained increase in the clearance capacity of the reticuloendothelial system occurred in patients who died after severe trauma, predominantly from late MOF. Despite this increased RES capacity, a higher pulmonary infection rate was found...
in nonsurvivors, suggesting impaired defense mechanisms. Reticuloendothelial system function does not depend on liver perfusion. Further studies may clarify whether these findings are a result of failure of the gut axis or whether hyperactivation of the RES is part of a generalized hyperinflammatory reaction.

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REFERENCES