PAPER

# Inflammatory Response in Peritoneal Exudate and Plasma of Patients Undergoing Planned Relaparotomy for Severe Secondary Peritonitis

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**Objective:** To study the pattern of intraperitoneal cytokine release in secondary peritonitis and its correlation with plasma levels and prognosis.

**Design:** Noncomparative descriptive case series.

**Setting:** Department of surgery in a university hospital.

**Patients:** Seventeen consecutive patients undergoing planned relaparotomy for severe intra-abdominal infection (Acute Physiological and Chronic Health Evaluation [APACHE II] score >10; mean score, 17.5).

**Interventions:** The following were measured at the first and last serial operations in the peritoneal exudate and plasma: endotoxin, tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), elastase, and neopterin.

**Main Outcome Measures:** Survival and death.

**Results:** Six patients died. Peritoneal endotoxin levels were significantly higher than in the plasma and were significantly higher in the nonsurvivors. Plasma TNF-α, IL-6, elastase, and neopterin levels remained elevated in the nonsurvivors prior to death. Levels of TNF-α, IL-6, elastase, and endotoxin were 19, 993, 239, and 7 times higher, respectively, in the peritoneal exudate than in plasma, all significant differences. Elastase and TNF-α levels decreased in survivors during the operative treatment but remained elevated in the nonsurvivors.

**Conclusions:** Secondary peritonitis is associated with a significant cytokine-mediated inflammatory response that is compartmentalized in the peritoneal cavity and indicates an adverse prognosis. Levels of cytokines in the exudate of peritonitis may be used to better stratify the severity of peritonitis and, in future, to guide local therapy.

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THE MORTALITY rate of severe bacterial peritonitis remains high despite the maximal available therapy.[1] The systemic manifestations of peritonitis are mediated by a cascade of cytokines produced by macrophages and other host cells in response to the by-products of bacterial destruction (ie, endotoxin).[2] Death is inevitable when an exaggerated cytokine release leads to a continuous "mediator disease," causing a generalized autodestructive inflammatory response that is resistant to all therapeutic options.[3]

An immense body of data has increased our understanding of the biologic cascades that produce the systemic inflammatory response and septic shock.[2,4] Improved knowledge of the concentrations of endotoxin and cytokines during sepsis from various causes has led to consideration of using endotoxin and cytokine levels in the prediction of outcome and thus clinical decision making.[5]

Much less is known, however, about the dynamics of peritoneal cytokines and their role in secondary bacterial peritonitis. Studies in models of experimental peritonitis[6-9] and analysis of infected ascitic fluid in spontaneous bacterial peritonitis[10,11] or infected dialysate in patients undergoing continuous ambulatory peritoneal dialysis[12-14] showed that cytokines are released intraperitoneally and that the magnitude of the phenomenon is directly proportional to the mortality rate (**Table 1**).

Several authors measured circulatory cytokine levels in secondary bacterial peritonitis in humans[15-18] and suggested that high tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) levels,[16] in particular IL-6 levels,[15,17] are cor-

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## DISCUSSION

**Robert E. Condon, MD, Milwaukee, Wis:** The first has to do with symptoms and signs. I didn't see a recording of whether or not your patients exhibited a limp. A limp is a very good marker for mischief in and around the hip, and most of the patients who develop a primary iliopsoas abscess will come limping into the emergency room. I was just wondering if you had had an opportunity to record whether or not they had this particular physical sign and could share that information with us.

Similarly, the classic psoas sign elicited by hyperextension of the hip is perhaps overtouted in cases of appendicitis but is really useful with a psoas abscess. I am curious about in how many patients, whose abscess was not so far advanced that they lay in bed with a flexion deformity, attempted extension of the hip elicited a complaint of increased pain.

And then let me turn to the "gold standard" diagnostic modality of CT, which certainly is as useful in this context as it is with abscesses in the abdomen generally. But there are alternative technologies, particularly ultrasound, that might have a useful role to play here. Ultrasound is readily available, should be available in the emergency room, is easy to use at the bedside, is noninvasive, and is a lot less expensive as a screening examination than is a CT examination. Did you and your colleagues have ultrasound available, did you use it in investigating patients with a suspected psoas abscess, and, if so, what was your experience?

Finally, a question not seeking facts but your opinion. The primary psoas abscess presumably is the result of hematogenous dissemination of bacteria from some point source elsewhere in the body. Do you have any thoughts about why this hematogenously disseminated infection localizes in the psoas rather than in some other body site, such as bone, which it seems to do preferentially in children under other circumstances, or in the spleen or elsewhere? Is this just a random process, or are there some features about the psoas that make it particularly liable to become the seat of an abscess?

**J. Wesley Alexander, MD, Cincinnati, Ohio:** Of historical interest, the founding president of the SIS, Dr Altemeier, and I wrote a paper about 30 years ago on retroperitoneal abscess, and included in those were a number of psoas abscesses. One curious thing we found was that most of the psoas abscesses that we identified at that time, which reflected some 30 years before, were largely hematogenous in origin, such as the ones you have suggested here for the primary psoas abscesses. We also identified several other retroperitoneal abscesses that had involved the psoas muscle in one way or another, as you have suggested by your secondary psoas abscess, but we had at that time classified them as retroperitoneal abscesses.

I suppose it doesn't really make too much difference how they were classified except to identify the underlying etiology, because if they come from gastrointestinal structures or the urinary tract, they are certainly treated differently from a primary abscess. Would you comment upon why you classified these as psoas abscesses rather than retroperitoneal abscesses with the idea in mind that the workup certainly may be different?

**Maria D. Allo, MD, San Jose, Calif:** We've recently had a patient with a primary psoas abscess who presented with bacteremia and, in fact, had *Staphylococcus aureus*, growing in both wrists, both knees and a hip. In a vein similar to what Dr Condon suggests, how many, if any, of the patients that you saw with primary psoas abscesses had other concomitant sites of *Staphylococcus aureus*.

**Dr Santaella:** In reference to Dr Condon's first few questions, the presence of a limp or psoas sign, unfortunately, a retrospective experiment is always subject to the observations of those people who initially see the patient, and, unfortunately, a psoas sign, although a very classic presentation for an iliopsoas abscess, was not very commonly noted on admission criteria or throughout the hospital stay, as well as a limp.

And as far as diagnostic CT vs ultrasound, part of our evaluation was based on a CT, and we did not compare CT vs ultrasound. Although some series in the literature suggest that ultrasound is a good initial screen, it may not be sensitive enough to pick up iliopsoas abscess based on CT's superior imaging of fascial planes. Therefore, it has been recommended in several articles that I have reviewed recently that CT scan is possibly a much better exam, being more sensitive.

As far as hematogenous dissemination, I believe that other structures are certainly at risk for a hematogenous dissemination of infection, certainly endocarditis being one that we are very familiar with. I believe that iliopsoas abscess of the iliopsoas muscle may be especially at risk because of its rich vascular supply around and adjacent to the muscle, but whether it is at particular greater risk than other structures that are more commonly found, I wouldn't be able to comment on that fact. As far as Dr Alexander's comment, I am familiar with Dr Altemeier's paper. As far as the distinction between retroperitoneal and iliopsoas compartments, certainly I believe that the iliopsoas compartment and that area would be a subset of retroperitoneal abscesses. We restricted our focus to those CT diagnoses which were restricted around and adjacent to, involving the iliopsoas muscle, and therefore did not look at all retroperitoneal abscesses in general, and I can't provide a comparison between those two.

As far as Dr Allo's question, her experience with *Staphylococcus aureus* infection in primary abscesses, our primary abscess patients did not have other sites of *Staphylococcus aureus* infection, although certainly with risk factors and with the introduction of *Staphylococcus aureus* in the bloodstream, I wouldn't find that that would necessarily be uncommon.

## PATIENTS AND METHODS

### PATIENTS

Seventeen patients with advanced intra-abdominal infection (Acute Physiology and Chronic Health Evaluation II [APACHE II] score >10) were entered into this study. All underwent planned relaparotomy over a period of 18 months. Four patients without peritonitis after colonic operations served as controls. Ten patients were male and seven female; mean age was 59 years (range, 35 to 81 years).

All patients suffered from diffuse secondary peritonitis (defined as peritonitis originating from a spontaneous or postoperative defect in an abdominal viscus). The origin of infection was postoperative anastomotic dehiscence (six patients), perforation caused by diverticular or malignant colonic disease (five patients), upper gastrointestinal tract perforations (four patients), and infected pancreatic necrosis (two patients).

The severity of acute illness was measured using the APACHE II scoring system.[19]

Planned relaparotomies were executed according to the staged abdominal repair (STAR) technique, as previously described.[20] Briefly, at the first (index) procedure for peritonitis, the commitment was made to perform laparotomies at 24-hour intervals until the abdomen was macroscopically clean. Between relaparotomies, the abdominal-wall defect was bridged with a temporary abdominal closure device. The mean number of reoperations was 3.3 per patient (range, one to eight).

Plasma and peritoneal exudate samples were collected during the serial relaparotomies. The following samples were then analyzed: (1) from the first serial operation in all patients, (2) from the last operation in the series (during which the abdomen was formally closed) in the survivors, and (3) from the last reoperation in the series prior to death in the nonsurvivors. As control measurements, plasma levels of the above variables were obtained from four patients immediately following an elective colonic resection.

### MEASUREMENTS

The blood samples were collected in heparinized tubes, centrifuged within 30 minutes after collection for 10 minutes at 200g and 4°C, and stored at −30°C for further processing.

Endotoxin concentrations were determined by the commercially available *Limulus* amebocyte lysate test (Kabi-test, Kabivitrum, Kabi Pharmacia, Erlangen, Germany); elastase concentrations by enzyme immunoassay (E Merck, Darmstadt, Germany); and neopterin concentrations by radioimmunoassay (Henning, Berlin, Germany). We measured IL-6, IL-1, and TNF-α concentrations with commercially available enzyme-linked immunosorbent assay test kits (R&D Systems, Minneapolis, Minn).

Statistical analysis was performed using the Instat 2 program (GraphPad, San Diego, Calif) or the STATA program (Stata, Santa Barbara, Calif). Results were expressed as mean±SEM and were compared by the Mann-Whitney test; $P<.05$ was considered significant.

related with poor prognosis. Others claimed, however, that the patient group with lethal outcome was characterized by significantly lower levels of TNF-α and IL-6.[18]

To the best of our knowledge, the patterns of cytokine release have not been hitherto examined in the peritoneal cavity of patients suffering from secondary bacterial peritonitis. The aim of this prospective clinical study was to measure levels of cytokines in the peritoneal exudate of patients undergoing serial, planned relaparotomy for severe intra-abdominal infections, correlating the dynamics of cytokines with plasma levels and outcome.

## RESULTS

Six patients (35%) died. The mean APACHE II score was 17.5; it was 14.9 in the survivors and 21.8 in the nonsurvivors.

### PLASMA SAMPLES

Endotoxin levels were significantly higher in the nonsurvivors prior to death than in the survivors (**Table 2**). Elastase levels were elevated at the initial operation. At the last operation, elastase levels were higher in the nonsurvivors than in the survivors ($P=.09$). A similar pattern was observed for neopterin levels; they were elevated early in both groups and were significantly higher at the last operation in the nonsurvivors than in the survivors. Levels of TNF-α were significantly higher in the nonsurvivors prior to death than in the survivors. Levels of IL-6 were higher in nonsurvivors than in survivors at the initial operation, but the difference was not significant ($P=.09$); the difference, however, was significant at the last procedure. Levels of IL-1 were undetectable.

### PERITONEAL EXUDATE SAMPLES

Average levels of endotoxin, TNF-α, elastase, and IL-6 were 7-fold, 19-fold, 239-fold, and 993-fold higher, respectively, in the peritoneal exudate than in the plasma (**Table 3**). Levels of TNF-α decreased significantly in survivors during the operative treatment but remained elevated in the nonsurvivors. Levels of IL-1, which were constantly elevated, did not differ between the survivors and nonsurvivors. Endotoxin levels remained elevated in the nonsurvivors prior to death, although the difference between survivors and nonsurvivors was not significant ($P=.07$). Elastase concentrations in the survivors decreased significantly during the study,

**Table 1. Cytokines in Peritoneal Fluid: Review of the Literature***

| Source, y | Model | Changes in Cytokines | Message |
|---|---|---|---|
| | | **Experimental Peritonitis** | |
| Bagby et al,[6] 1991 | IP *Escherichia coli* | IP TNF ↑ within 2 h; plasma TNF ↑ but much less than after IV *E coli* injection; pretreatment with anti-TNF IgG led to ↓ mortality rate following the IV challenge but was ineffective in peritonitis | Significant difference in the role of TNF in IV model of sepsis and bacterial peritonitis |
| Astiz et al,[7] 1994 | Cecal ligation and puncture | IP TNF and IL-1 ↑ (↑ mortality rate); lower IP TNF and IL-1 in a group pretreated with IP induction of endotoxin tolerance | IP cytokine levels correlate with outcome |
| McMasters and Cheadle,[8] 1993 | Cecal ligation and puncture | ↑ levels of TNF and IL-1 in peritoneal macrophages; ↓ levels of Ia subunit in peritoneal macrophages | An early shift in peritoneal macrophage function from antigen recognition to cytokine production |
| Zanetti et al,[9] 1992 | IP *E coli* | IP TNF 50- to 100-fold lower than plasma TNF after IV *E coli*; anti–TNF antibodies reduced serum IL-1 and IL-6 and mortality rate after IV *E coli* but not after *E coli* peritonitis, in which IP IL-1 and IL-6 remained ↑ | Local peritoneal cytokines do not diffuse readily into the systemic circulation; thus, some cytokines in peritonitis are produced systemically; TNF may be less important in lethal peritonitis than in lethal bacteremia |
| | | **Spontaneous Bacterial Peritonitis in Liver Cirrhosis** | |
| Propst et al,[10] 1993 | ... | IP levels of IL-6, TNF, neopterin, and GCSF ↑; levels of IL-2, IL-1, and interferon gamma were normal | IP IL-6, TNF, and neopterin levels significantly correlated with outcome |
| Zeni et al,[11] 1993 | ... | IP TNF and IL-6 ↑↑; serum TNF and IL-6 slightly ↑; IP levels ↓ during antibiotic therapy | IP TNF and IL-6 useful markers for the diagnosis and monitoring of this condition |
| | | **Peritonitis in Chronic Ambulatory Peritoneal Dialysis** | |
| Nakahama et al,[12] 1992 | ... | IL-6 detectable in peritoneal dialysate of 3/21 patients with peritonitis; IL-6 ↑↑ in 2 patients with bacterial peritonitis, returning to normal as peritonitis subsided | IP IL-6 marker of bacterial peritonitis |
| Zemel et al,[13] 1994 | ... | IP IL-6 and IL-8 ↑ in peritonitis in parallel and following the IP TNF ↑; levels return to normal during recovery; IP IL-8 corresponded to the number of leukocytes in the dialysate | IL-8 involved in the recruitment of neutrophils into the dialysate in peritonitis |
| Zemel et al,[14] 1993 | ... | IP IL-6 and TNF ↑ in peritonitis, with peak values on day 1; IL-6 levels > TNF levels; TNF peak very early and of short duration; changes in peritoneal permeability related to IP IL-6 and TNF levels | Local cytokines involved in increased peritoneal permeability in peritonitis |

**IP indicates intraperitoneal; TNF, tumor necrosis factor; ↑, elevated; IV, intravenous; IL, interleukin; ↓, decreased; GCSF, granulocyte colony-stimulating factor; and ↑↑, markedly elevated.*

while in the nonsurvivors they remained elevated. Levels of IL-6 decreased in both the survivors and the nonsurvivors.

## COMMENT

A few clinical studies have assessed levels of plasma cytokines together with circulating endotoxin in secondary intra-abdominal infection. Patel et al[17] detected no significant differences in endotoxin concentration between survivors and nonsurvivors; the levels decreased with time in all patients. This was not confirmed by our study, in which endotoxin levels remained elevated during the course of disease and were higher in the nonsurvivors than in the survivors. The present study reproduces the results of other studies that demonstrated elevated levels of TNF-α and IL-6,[15-17] the latter well correlated with APACHE II scores.[15,17] In the present study, both TNF-α and IL-6 levels were significantly higher in the nonsurvivors before they died than in the survivors. Conversely, Hamilton et al[18] reported decreasing levels of TNF-α and IL-6 prior to death, suggesting that this reflects an "anergic immune status."

Trauma of any sort to the peritoneal cavity, including that resulting from bacterial infection, is responsible for a local acute-phase reaction involving the release of certain cytokines[21] that are synthesized by peritoneal mesothelial cells and macrophages.[8,22-25] Studies in experimental peritonitis[6-9] and in patients with spontaneous (primary) bacterial peritonitis[10,11] or peritonitis

**Table 2. Plasma Levels of Endotoxin and Cytokines**

| | Endotoxin, U/mL | Elastase, pg/mL | Neopterin, μg/mL | Tumor Necrosis Factor, pg/mL | Interleukin-6, pg/mL |
|---|---|---|---|---|---|
| First operation | | | | | |
| Survivors | 2.6±1.5 | 104±24 | 7±3 | 5±0.3 | 433±170 |
| Died | 3.0±1.5 | 175±35 | 10±5 | 9±2.4 | 2630±108* |
| Last operation | | | | | |
| Survivors | 1.2±0.4 | 58±10 | 9±4 | 5±0.6 | 14.5±2 |
| Died | 3.4±0.6 | 104±17* | 90±52† | 10±2.4† | 1017±620† |
| Control‡ | 1.5 | 27±8 | 1 | 2±2.8 | 0 |

*P=.09 compared with survivors.
†P<.05 compared with survivors.
‡Immediately after elective colonic resection.

**Table 3. Peritoneal Exudate Levels of Endotoxin and Cytokines**

| | Endotoxin, U/mL | Elastase, pg/mL | Tumor Necrosis Factor, pg/mL | Interleukin-1, pg/mL | Interleukin-6, pg/mL |
|---|---|---|---|---|---|
| First operation | | | | | |
| Survivors | 17±6 | 11 194±1608 | 52±19 | 800±301 | 33 838±8457 |
| Died | 25±9 | 9993±3335 | 406±270 | 4982±3613 | 15 458±3342 |
| Last operation | | | | | |
| Survivors | 9±7 | 2213±1093* | 16±7* | 580±410 | 41 995±11 785 |
| Died | 22±6† | 2063±782 | 161±147 | 669±377 | 22 048±7021 |

*P<.05 compared with survivors in the first operation.
†P=.07 compared with survivors in the last operation.

complicating chronic ambulatory peritoneal dialysis[12-14] shed some light on the pattern and role of intraperitoneal cytokine release (Table 1).

The peritoneal proinflammatory cascade may not be different from the better-described systemic one.[26] The initiation of peritonitis results in the local release of TNF-α and IL-1, which in turn stimulate the release of secondary mediators, such as IL-6 and IL-8.[6-14] Others have reported elevation in peritoneal levels of neopterin similar to the findings of the present study,[10] and in the present study levels of peritoneal elastase were significantly elevated.

Typically, after the injection of an identical intravenous and intraperitoneal bacterial inoculum, levels of TNF-α and IL-1 inoculum were much lower in peritoneal exudate than in plasma.[6,9] Peritoneal levels of TNF-α and IL-6 during spontaneous bacterial peritonitis, however, were very high, while plasma levels measured simultaneously were just above normal.[11] In addition, in the present study, peritoneal levels of endotoxin, TNF-α, IL-1, IL-6, and elastase were many times higher than simultaneously measured plasma levels. These marked differences between plasma and peritoneal levels suggest that plasma cytokines do not equilibrate readily between the peritoneal space and blood and that peritoneal cytokines do not diffuse easily into the systemic circulation.[8] Moreover, these differences indicate that bacterial peritonitis induces a compartmentalized inflammatory process (peritoneal macrophages may have different endotoxin tolerance than macrophages elsewhere, or peritoneal clearance may be less effective than systemic clearance); plasma levels of cytokines in peritonitis are produced systemically or may represent a systemic spillover.

Studies in systemic sepsis suggest that some (magnitude as yet undefined) release of cytokines is beneficial to the patient or animal, but excessive concentrations are detrimental.[27,28] The same is probably true for the peritoneal cavity, where elevated levels of cytokines are associated with adverse outcome, but a certain amount of cytokines may play a beneficial role in supporting local host defense mechanisms.[29] Thus, for example, local action of TNF-α and IL-6 would induce peritoneal inflammation and hyperpermeability[14] but at the same time would recruit neutrophils into the peritoneum (IL-8)[13] to stimulate phagocytosis of bacteria and debris[30] and to induce the production of plasminogen activator inhibitor by mesothelial cells, thus promoting the formation of infection-localizing fibrin adhesions.[31] That cytokines are locally beneficial in early peritonitis was suggested by studies that demonstrated that anti–TNF-α antibodies provided protection in intravenous infection models but were not effective when administered intraperitoneally in experimental peritonitis.[6,9] Moreover, anti–TNF-α antibodies given intraperitoneally at the time of cecal ligation and puncture increased mortality, a trend that was reversed by the administration of TNF-α.[30]

We can only speculate about the significance of the results of this study. That the plasma and peritoneal endotoxin levels remained elevated in the nonsurvivors prior to death (albeit nonsignificantly) and decreased in the survivors may reflect damage to the intestinal barrier,[32] allowing the translocation of luminal endotoxin into the

peritoneal cavity. On the other hand, it may indicate failure to surgically eliminate the source of infection or failure of antibiotic therapy.

Elevated plasma neopterin levels, which were higher in the nonsurvivors at their last operation than in the survivors, indicate enhanced activation of macrophages. Elevated plasma neopterin levels could also indicate macrophage apoptosis or programmed cell death.[33] Increased plasma neopterin concentrations were found in a variety of infectious, malignant, and autoimmune conditions,[34] and elevated plasma neopterin levels predicted adverse outcome in patients with sepsis in the intensive care unit.[35] Elastase is produced by polymorphonuclear cells and is thus a marker of their activation and degranulation, as demonstrated in patients with sepsis[36] or following trauma.[37] In the present study, elastase levels remained elevated in the plasma of nonsurvivors prior to death while levels decreased in the peritoneal exudate of survivors, indicating ongoing activation of polymorphonuclear cells in the former group.

Increased plasma levels of TNF-α, IL-6, elastase, and neopterin, taken together with the failure of peritoneal levels of TNF-α, IL-1, and elastase to decrease in nonsurvivors during the last operation, suggest continuous, nonbeneficial, local, and systemic inflammatory activity in these patients during the repeated laparotomies and until death. To what degree reoperative trauma activates or escalates the ongoing inflammatory response remains to be established.[38-40]

What are the practical implications of our results? The evidence that levels of cytokines in the peritoneal fluid during peritonitis are much higher than in the systemic circulation and that increased concentrations of cytokines signify adverse prognosis suggests that peritoneal cytokine measurement can be used to better stratify the severity of the acute illness. The use of cytokine serum concentrations in a cytokine scoring system[26] to predict outcome in sepsis[5] has been frustrated by the fact that circulatory concentrations of free bioactive cytokines may be negligible, yet significant amounts of cytokines may be present at the tissue level (ie, in the peritoneal cavity).[5] Local estimation of cytokine levels may better reflect the severity of an initially local process (ie, peritonitis).

Measurements of intraperitoneal cytokines could represent an important research tool in the efforts to improve the management of peritonitis. For example, should antibiotic therapy be started only after the evacuation of pus from the abdomen, to prevent antibiotic-induced endotoxemia?[41] Intraoperative estimation of peritoneal levels of endotoxin, TNF-α, and other cytokines before or after the administration of antibiotics may shed new light on this controversy. In the future, rapid intraoperative peritoneal cytokine assays may guide specific intraperitoneal therapy (ie, local anti–TNF-α antibodies).

If the intraperitoneal cytokines are advantageous to the normal peritoneal immune response, the notion of, "Don't block local cytokines: remove excess cytokines from the systemic circulation,"[42] may be true. In certain concentrations and at certain times, however, intraperitoneally compartmentalized cytokines probably cause persistent end-organ damage[5] or may spill over to produce adversely high levels in the systemic circulation. That the elimination of excessive amounts of intraperitoneal cytokines may be beneficial is supported by decreased mortality of patients who were treated by staged abdominal repair (STAR), an operative strategy in which the abdominal cavity is purged every 24 hours.[43]

We conclude that secondary peritonitis is associated with a significant cytokine release that is compartmentalized in the peritoneal cavity. The magnitude of this cytokine release reflected the severity of the process and prognosis. The systemic and local inflammatory response persisted during relaparotomy in the nonsurvivors. More experimental and clinical studies are required to distinguish the local, intraperitoneal, beneficial, and adverse effects of cytokines, including magnitude and timing, before any therapeutic implications can be defined.

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## DISCUSSION

**Eugen H. K. J. Faist, MD, Munich, Germany:** Being a follower of planned relaparotomy, as well as having invested a substantial amount of time in my career with proinflammatory responses, the title of your presentation, which promised to link the treatment of peritonitis by programmed daily lavage with new insights into the biochemistry of inflammation, evoked a great deal of curiosity in me.

Your idea of measuring proinflammatory cytokines as well as elastase, endotoxin, and neopterin in plasma and peritoneal exudate is a great design. All we have learned from your experiments was that patients—and this is no surprise—dying of peritonitis have hyperinflammation. The patients who survive peritonitis and have their abdomen closed have less inflammation, so I do believe that there are no surprises and no new data derived from your study. I'm really wondering why you did not exploit your basic design and compare patients on a day-to-day basis for the benefit of programmed relaparotomy.

I further challenge your idea that by evaluating the inflammatory mediators from the peritoneal exudate you might gain information with respect to prognosis. I also do not believe that it is very valuable to compare, for example, endotoxin levels in the peritoneal cavity vs the plasma. I would thus like to ask you to do the study again, to reevaluate it in terms of daily scrutiny, and I do believe that this might then give us interesting data.

**Carl J. Hauser, MD, Jackson, Miss:** Was the fluid examined truly exudate, or was it a lavage of the peritoneal cavity? Using peritoneal lavage creates significant problems with respect to standardizing the dilution of proteins in any peritoneal fluid or on peritoneal surfaces. I would like to focus for a moment on the elastase levels. These were decreased late in both survivors and nonsurvivors. Do you think this reflects an appropriate decrease in polymorphonuclear leukocyte activity in survivors owing to decreased inflammation, and perhaps polymorphonuclear leukocyte failure in the nonsurvivors?

**Lyle L. Moldawer, PhD, Gainesville, Fla:** Is it interleukin-1$\alpha$ or interleukin-1$\beta$ that you are measuring? Secondly, tell us about the TNF-$\alpha$ enzyme-linked immunosorbent assay. If you are looking at differences in concentration between 2 and 4 pg/mL, is that within the sensitivity of the assay? What is the frequency of patients in whom you could detect positive levels of TNF? What percentage of plasma samples were detectable?

**J. Wesley Alexander, MD, Cincinnati, Ohio:** Dr Gennari in our laboratory recently showed that the administration of anti–IL-6 antibody to mice in a translocation model significantly improved survival, even when the drug was given during the postburn period. In your studies, the IL-6 in the exudate was basically the same between survivors and nonsurvivors. However, in the plasma, levels were strikingly different, both at the initial operation and at the last operation. Where does this IL-6 come from, and was there an initial prognostic value of plasma IL-6? Finally, what do you think the role of IL-6 is in the mortality of these patients?

**Wolfgang Ertel, MD, Zurich, Switzerland:** I assume that the pH varies in the exudates dependent on the severity and stage of peritonitis. Do the differences in pH influence your enzyme-linked immunosorbent assays, thus explaining some of the differences in cytokine measurements which you have described?

**Cora K. Ogle, PhD, Cincinnati:** Where do you think the peritoneal cytokines are coming from? Do you think they are coming from the gut? Do you think the endotoxin in the peritoneal cavity is stimulating the intestinal cells from a site in which one normally doesn't see endotoxin? I wonder if the enterocytes are making neopterin. It might be a way to differentiate them, what cells are actually making some of the cytokines, because as you heard this morning, we have reported from some of our other work that enterocytes can produce inflammatory cytokines.

**Basil A. Pruitt, MD, San Antonio, Tex:** I wonder how you corrected the neopterin values for renal function. We have found that neopterin is very sensitive to creatinine clearance changes. Consequently, impaired renal function might account for the rise in neopterin in the nonsurvivors. Also, for the plasma levels of other mediators, how did you account for changes in pool size in these dying patients who received a variable amount of intravenous fluids?

**Dr Holzheimer:** It is true that we have presented here only the data of the beginning and the end, but I can assure you that we have done the daily measurements of the cytokines at least as long as the patients were in the lavage program. We also did it prelavage, when we opened the abdomen again, and when we finished the lavage, and we could see a clear reduction of the cytokines after lavage.

Despite the fact that it is certainly known that plasma cytokines are elevated in secondary peritonitis, there are some benefits of this study. One is that we have shown that peritoneal TNF is decreased in survivors already at the index operation. This also corresponds to measurement of TNF in the plasma. Of course, we were not able to detect TNF-α in each patient at every time point, but it is certainly true that we could measure or detect TNF-α in the exudate, so TNF measurement is much more reliable in the exudate.

Concerning prognosis, it was said that IL-6, for example, or other cytokines may be able to indicate a bad prognosis. This we could not demonstrate in our study. There was a trend toward an increased IL-6 production in nonsurvivors at the first index operation, so I would be very cautious in saying that in plasma a cytokine could indicate prognosis. This may be different in the exudate.

To measure endotoxin in exudate and plasma, Dr Faist said that it is not right to compare that. Well, we did it in order to demonstrate whether lavage was able to down-regulate and to clear the abdomen of endotoxin.

Then there was a question about the technique of measurement. In the first operation, of course, we measured the exudate and not the lavage fluid. In regard to renal function, the question from Dr Pruitt, I have to admit that the cytokine levels demonstrated here were not corrected for renal function. We have measured IL-1β in this assay.

In regard to the question of Dr Alexander on the role of IL-6, we have demonstrated that, curiously, in the nonsurvivors, toward the end of the operation, there was a trend that there is less IL-6 available in the exudate. I have no idea at the moment what this means in the exudate, if it is good or bad.

There was a question of Dr Ertel on pH differences. We have not measured pH in the exudate. I am aware, and there were studies done in Wurzburg some years ago, that pH is important and that it influences, for example, also the antibiotic efficacy.

And the question from Dr Ogle: where do we think the peritoneal cytokines come from? There is clearly a bacterial translocation. We have also demonstrated this in another study, where we measured endotoxin from aortic aneurysm repair, and we could demonstrate, after clamping of the aorta, an endotoxin release. The cytokine production is mainly from peritoneal phagocytes in the peritoneal exudate, and it is not clear whether this is transmitted into the plasma. We have not done any other study to demonstrate if there is a link between the cytokines in the peritoneal exudate and in the plasma.

*Surgical Anatomy*

Because the *Pectoral Muscles* belong to the anterior wall of the axilla, they must be supplied by anterior cords (ie, lateral and medial cords). The branch from the lateral cord is the *lateral pectoral nerve* (lateral anterior thoracic n.). The branch from the medial cord, the *medial pectoral nerve* (medial anterior thoracic n.), pierces the Pectoralis Minor and supplies it and the lower half of the Pectoralis Major.

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