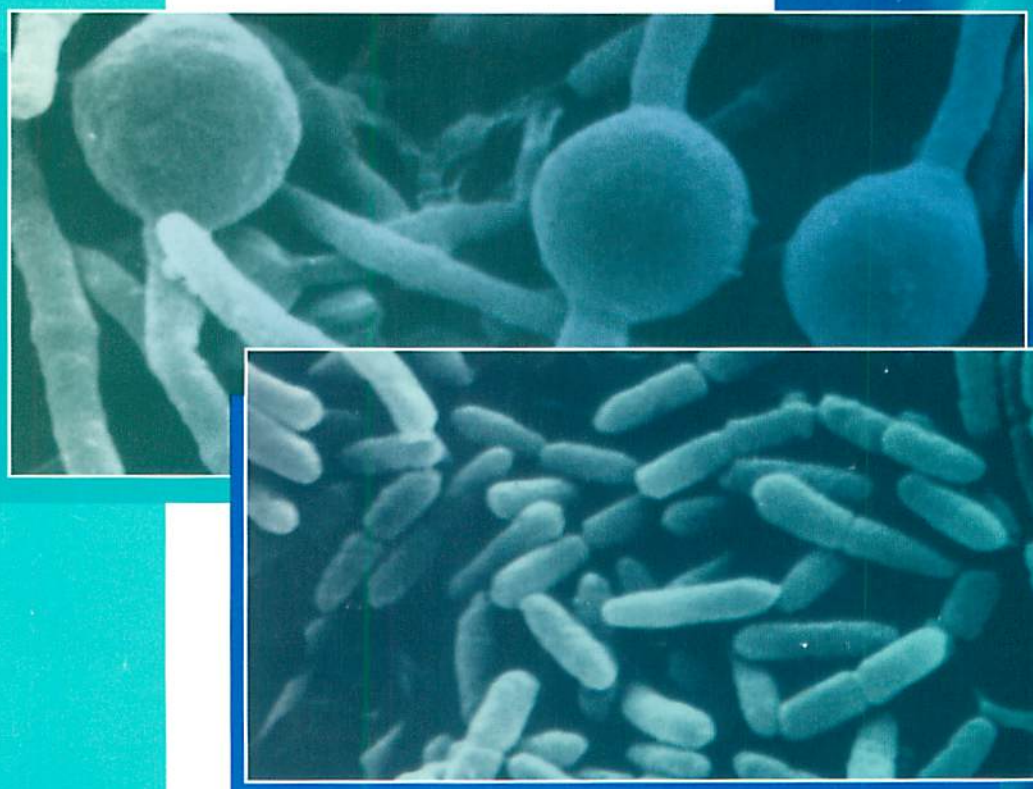
Presented at the

4th International Congress on the Immune Consequences of Trauma, Shock, and Sepsis

Munich, Germany — March 6, 1997

Proceedings

of the Symposium ***Antibiotic-Induced Endotoxin Release – Potential Contribution to the Pathophysiology of Sepsis***



Rene Gordon Holzheimer, MD, PhD, Ulrich Ebentreich, MD, Thomas Maseizik, MD, Wolf Gunther Steinmetz, MD

Chirurgische Universitätsklinik Würzburg, Würzburg, Germany

Clinical Significance of Antibiotic-Induced Endotoxin Release in Surgical Patients

Abstract

In vitro and animal studies have demonstrated the different endotoxin release after penicillin-binding protein (PBP)-2-specific antibiotics (e.g., imipenem) and PBP-3-specific antibiotics (e.g., cephalosporins). Clinical reports are still missing, with the exception of a few reports on the antibiotic-induced endotoxin release in urosepsis and meningitis. However, recent studies in animals and humans have indicated a difference in the pathomechanism of systemic sepsis (e.g., urosepsis, adult respiratory distress syndrome) and intra-abdominal infection. The immune system in patients with intra-abdominal sepsis may be harmed by minor amounts of endotoxin. In surgical intensive care and intra-abdominal infections, beta-lactam antibiotics are widely used, and the possibility of antibiotic-induced endotoxin release may be of clinical interest. We have investigated the antibiotic-induced endotoxin release after the administration of ceftriaxone, cefotaxime, ciprofloxacin, and imipenem. After imipenem administration, we did not observe any limulus amoebocyte lysate (LAL) activity; cefotaxime and ceftriaxone, however, were associated with endotoxin release. There was also a difference in interleukin (IL)-6 levels visible. Clinical acute physiology parameters showed no difference.

In general, the pathogenesis of intra-abdominal infection is complex. Therefore it may be difficult to correlate clinical outcome with endotoxin release. It should be mentioned that we have new methods of testing and monitoring available that should be used in further studies to evaluate the effect of antibiotics on endotoxin release.

Introduction

Sepsis and intra-abdominal infections continue to be a challenge in hospitals despite intensive care treatment and potent antibiotics. Sepsis has a large impact on the socioeconomic system in Europe and the United States. Every year 500,000 patients will suffer from sepsis in the United States, and similar numbers are expected for Europe. Of these, 175,000 patients will die from sepsis.[1] Overall, sepsis mortality is 35 percent; however, in surgical patients, the mortality may be even higher, ranging from 40 to 70 percent.[2] The mortality rate in surgical patients has not changed within the past decades, despite the introduction of powerful antibiotics (e.g., beta-lactam antibiotics).[3] For surgeons, the surgical therapy is still the mainstay for peritonitis treatment. Antibiotics play a role of adjuvant therapy. It is true that, without focus elimination, the mortality rate in surgical patients is even higher (80 to 100 percent). Kirschner was the first surgeon in 1926 to demonstrate that surgical therapy with focus elimination, debridement, and intraperitoneal lavage can lower mortality to 70 to 80 percent without any antibiotic given.[4] Nowadays, we know that the immune system in surgical patients is in a critical balance,[5] and outcome may be mainly determined by the patient's own immune response.[6] We have demonstrated that in patients with elective aortic aneurysm repair, the immune system is challenged by minor amounts

of endotoxin released after cross-clamping of the aorta.[7] This has led to the conclusion that in patients with severe peritonitis and an immune system challenged repeatedly by infection and operation, only minor amounts of endotoxin may be relevant to tip the balance to deterioration. This may be caused by antibiotic-induced endotoxin release.

A report by Jackson and Kropp in 1992 has revealed that there may be different endotoxin release after different antibiotics. In penicillin-binding protein (PBP)-2-specific antibiotics, less endotoxin was detected *in vitro* than after PBP-3-specific antibiotics (e.g., cephalosporins).[8] This was supported by other investigators who found similar results *in vitro*.[9-22] Also, several studies performed in different animal models support the notion that PBP-2-specific antibiotics release less endotoxin.[23-28]

With regard to surgical patients, several questions need to addressed:

1. Is endotoxin of clinical importance?
2. Is there a difference between intra-abdominal infection (surgery) and systemic sepsis (e.g., adult respiratory distress syndrome [ARDS]), and what does this mean for studies and treatment of patients?
3. Is the pathogen relevant to antibiotic-induced endotoxin release in clinical circumstances?
4. Can we observe clinical changes in patients with regard to different endotoxin release, and what would be the clinical parameters to study?
5. How can we improve our study setup to be able to see the differences?
6. What is the conclusion for clinical therapy?

Clinical Relevance of Endotoxin

In several animal studies, it was demonstrated that endotoxin is one of the most important trigger substances for the inflammatory response in sepsis.[29-31] It activates macrophages and polymorphonuclear cells to release pro-inflammatory cytokines.[32,33] These inflammatory cytokines are responsible for some clinical symptoms (e.g., fever, hypotension)[34] in septic patients. It was concluded that blocking either endotoxin or tumor necrosis factor (TNF) may be beneficial for the clinical follow-up and outcome.[35] However, most of the clinical studies failed to improve survival.[36,37] Endotoxin, which is released from the cell wall of disintegrating gram-negative pathogens, may not be present, because there are not always gram-negative pathogens present. The incidence of gram-negative bacteremia in patients with sepsis syndrome in the intensive care unit (ICU) who are receiving antibiotics may be so low that endotoxin may not be relevant.[38] In surgical patients with intra-abdominal infections, pathogens are invading the peritoneal cavity and have been associated with the severity of disease. However, in a recent study by Schöffel et al,[39] microorganisms and antibiotic treatment in intra-abdominal infections were not considered to be major determinants of the clinical course of peritonitis. The failure to increase survival with anti-endotoxin antibodies is often used as an argument against the clinical significance of endotoxin. Patients treated with E5, a monoclonal antibody against endotoxin structures, did not show a difference in 30-day mortality or a difference in mortality with gram-negative sepsis and organ failure.[40] However, in this study, there was evidence that E5 positively influenced the resolution of organ failure or prevented ARDS and central nervous system (CNS) dysfunction. So, organ dysfunction or organ failure may be associated with endotoxin release. Several studies were performed to investigate a correlation of endotoxin with clinical parameters or outcome. Berger reported a correlation of endotoxemia with pulmonary and infectious complications in surgical patients.[41] He further evaluated endotoxin levels in patients with urinary tract infection and could demonstrate that endotoxin determination in urine may be a sensitive method for the detection of bacterial contamination.[42] This is supported by Schöffel's study, in which the presence of intra-abdominal pathogens was associated with high local and systemic levels of endotoxin. Cytokines are released after intravenous (IV) endotoxin administration in healthy volunteers.[43] In patients with hematological malignancies who received cytotoxic medication, interleukin-6 (IL-6), phospholipase A2 (PLA 2), and C reactive protein (CRP) were valuable tools for the detection of sepsis.[44]

For the critical evaluation of endotoxin in clinical sepsis, the technique and method of endotoxin determination is a crucial step. Most of the commercially available tests function very well in plasma free solutions and give reproducible results in these circumstances. However, in the meantime, we know that there are several compounds in the blood (e.g., plasma proteins, lipids, bacterial permeability-inducing [BPI] factor) that may interfere with the limulus amoebocyte lysate (LAL) reaction. We also do not know if endotoxin is active below the detection limit of our assays. So, negative endotoxin results do not rule out endotoxin in the blood.[45] To optimize the endotoxin determination, we are using a kinetic LAL assay with internal standard that can take the interference of plasma proteins with the LAL into account.[46] The use of unheated plasma (with plasma proteins) allows us to measure how much added endotoxin in known concentrations can be neutralized by the blood's own neutralizing compounds (e.g., plasma proteins, lipids) (endotoxin neutralizing capacity).[47] Other methods are indirect methods to verify endotoxin in the plasma. One deals with endotoxin core antibodies;[48] others use TNF-alpha as a method to describe effects of endotoxin.[49] This is based on the assumption that endotoxin is responsible for TNF-alpha release. IL-6 has been evaluated in clinical sepsis and peritonitis.[50-53] Outcome and complications have been correlated with IL-6 levels. In principle, no test method is perfect or can measure the mediators or endotoxin without any interference. Clinically, several methods should be used to evaluate antibiotic-induced endotoxin release. They should be reproducible, fast, and easy to handle. A system to measure IL-6 within 70 minutes is available now and has been tested by us.[54]

Differences in Intra-Abdominal Sepsis and Systemic Sepsis

The pathogenesis of sepsis is complex. Several cell and immune compartments become activated and may influence one another. The idea of having many septic patients in a study may be appealing with regard to time and costs. However, recent clinical trials in which the inclusion criteria did not discriminate between different expressions of sepsis were hampered by the fact that intra-abdominal sepsis and systemic sepsis may not follow the same rules.[55] Several studies helped us to understand this difference. The concept that TNF-alpha is detrimental and blocking TNF-alpha is good was first challenged by Echtenacher, who nicely demonstrated in a cecal ligation puncture (CLP) model that the administration of anti-TNF-alpha antibodies increased mortality, while the addition of TNF-alpha reversed the trend.[56] In another animal study, systemic sepsis and intra-abdominal sepsis were compared. Systemic pretreatment with anti-TNF-alpha Ab decreased mortality following IV challenge with *Escherichia coli*, but was ineffective in intra-abdominal sepsis.[57] The notion that intra-abdominal sepsis is different from systemic sepsis is further supported by a study in which TNF, IL-1 beta, and IL-6 were increased less than they were after systemic lipopolysacccaride (LPS) injection. Treatment resulted in different outcomes, depending on the type of infection. Pretreatment with dexamethasone, ibuprofen, and L-arginine led to a reduced survival; antibiotics and pentoxifylline improved survival in mice in which CLP was performed. LPS mortality was reduced with chlorpromazine and dexamethasone.[58] There is evidence that endotoxin or LPS leads to a variable challenge of the immune system, according to the type of sepsis. Our own clinical investigations demonstrated that in peritonitis, TNF, IL-1, and IL-6 were higher in peritoneal exudate than in plasma. While plasma TNF, IL-6, elastase, and neopterin remained elevated in non-survivors, peritoneal TNF and elastase decreased in survivors.[59] The pathomechanism in clinical peritonitis is not fully understood and deserves further attention. This also may explain why several studies have failed to demonstrate an effectiveness of compounds against sepsis.

Relevance of Pathogens in Peritonitis/Intra-Abdominal Infection and Their Relationship to Antibiotic-Induced Endotoxin Release

Pathogens get access to the peritoneal cavity mostly after perforation of the intestine or an infection of intra-abdominal organs. In general, these are normal pathogens of the gastrointestinal flora. The most often isolated pathogens are listed in Table I.[60] In cases of immunosuppres-

Pathogens	Peritonitis (%)	Intra-abdominal abcess (%)	Immuno-compromised patients (%)
E. coli	61	68	53
Streptococci	28	47	43
Klebsiella, Enterobacter	26	15	43
Proteus	23	25	11
Pseudomonas	8	6	19
Staphylococci	8		15
Serratia marcesens			17
Eubacteria	25	6	
Clostridia	18	35	9
Bacteroides	15	59	23
Fusobacteria	9	27	
Candida	2		21
Others			4

Table I: Pathogens in intra-abdominal infections. (Modified according to Wang, 1997, and Hau, 1979.)

sion, there can be alterations in the quantity of pathogens isolated. The pathogens (e.g., *Pseudomonas*, *Serratia*, and *Candida*) may be more often isolated.[61]

We have demonstrated differences in pathogen distribution with regard to pathogens in nosocomial wound infections and all nosocomial infections. In wound infections, including deep wound infections such as peritonitis, *E. coli*, *Staphylococcus aureus*, and enterococci were the dominant pathogens, whereas in overall distribution *E. coli*, *Candida*, enterococci, and coagulase-negative staphylococci were the prevalent pathogens. The rate of isolation of pathogens can also be attributed to the ICU patients or patients in the general surgery ward. *E. coli*, *S. aureus*, and enterococci were observed in isolates from patients in the general ward; *Candida*, coagulase-negative Staphylococci, and enterococci were found in isolates from patients in the ICU. Different operations show different distributions of isolates; pancreatic operations are prone to have infections with coagulase-negative staphylococci, *Candida*, and *Pseudomonas*.[62]

The *in vitro* studies on antibiotic-induced endotoxin release investigated mainly the effect of selected pathogens on antibiotic-induced endotoxin release. Several *in vitro* studies have revealed that endotoxin release after antibiotic administration may also be influenced by the type of pathogen used in the model (Table II). Induction of LPS in *P. aeruginosa* cultures suggested that ceftazidime-induced filamentation released larger quantities of bioreactive endotoxin than did non-filamentous fast-lyzing imipenem.[63] Total endotoxin levels increased after single treatment with cefuroxime or aztreonam, whereas ceftazidime, tobramycin, or a combination of tobramycin with cefuroxime released less endotoxin. The increase in free endotoxin was higher than that in total endotoxin.[64] In whole blood assays, endotoxin was higher when cells were treated with ceftazidime or ciprofloxacin than when imipenem or gentamicin was used.[65] Crosby reported that cefotaxime, ciprofloxacin, and piperacillin caused significant endotoxin release *in vitro* in cultures of *Enterobacter cloacae* and *E. coli*. Little endotoxin was released when bacteria were exposed to tobramycin.[66]

Pathogens	Antibiotics	Authors
E. coli, Salmonella	E5 mAb, amoxicillin, gentamicin	Seelen et al, 1995
E. coli	Ceftazidime, ciprofloxacin, imipenem, gentamicin, polymyxin B, rBPI-21	Prins et al, 1995
E. coli	BPI, antibacterial 15-kDa protein isoforms (p15s), defensins	Levy et al, 1995
E. cloacae, E. coli	Cefotaxime, ciprofloxacin, piperacillin	Crosby et al, 1994
E. coli	Ceftazidime, imipenem	Bucklin et al, 1994
Salmonella minnesota	Teicoplanin	Foca et al, 1993
E. coli	Mab 8G9, polymyxin B	Burd et al, 1993
E. coli	Cefuroxime, ceftazidime, aztreonam, imipenem, taurolidine	Dofferhoff et al, 1993
E. coli, K. pneumoniae, E. cloacae, P. aeruginosa, S. aureus	Aztreonam, imipenem, quinolones	Eng et al, 1993
Haemophilis influenzae type b	Ceftriaxone, imipenem, polymyxin B	Arditi et al, 1993
E. coli	Gentamicin, amoxicillin, ciprofloxacin	Van den Berg et al, 1992
Haemophilus influenzae	Ampicillin, cefotaxime, amikacin	Bingen et al, 1992
P. aeruginosa	Imipenem, ceftazidime	Jackson and Kropp, 1992
E. coli	Imipenem, tobramycin, ceftazidime, cefuroxime, aztreonam, chloramphenicol	Dofferhoff et al, 1991
E. coli	Amikacin, ciprofloxacin, ceftazidime, cefotaxime, aztreonam, imipenem	Simon et al, 1991

Table II: **In vitro *studies of antibiotic-induced endotoxin/cytokine release in pathogens.***

It was also demonstrated that with regard to the pathogen, antibiotic-induced endotoxin release may be different within the same antibiotic. In *E. coli*, ceftazidime released more endotoxin than imipenem; however, in *P. aeruginosa*, endotoxin release was equal.[67] It is obvious that the studies are important to detect mechanisms of antibiotic-induced endotoxin release; for clinical purposes, the effect of polymicrobial infections should be investigated. In established peritonitis, only a few species remain. These infections are almost always polymicrobial, containing a mixture of aerobic and anaerobic bacteria.[68-70] Also, the results of Schöffel et al suggesting that pathogens and respective antibiotic treatment may not influence the outcome in peritonitis deserve further attention.[39]

Alteration of Clinical Parameters After Antibiotic-Induced Endotoxin Release

In many *in vitro* and animal studies, the effects of antibiotic-induced endotoxin release have been demonstrated. In selected patient groups (e.g., those with urosepsis, meningitis), a different endotoxin release after administration of PBP-2-specific and PBP-3-specific antibiotics was also observed.[71-74] In surgical patients, intra-abdominal

Antibiotic	0	60	120	180	240
Imipenem	0	0	0	0	0
Cefotaxime	2	4	4	5*	5*
Ciprofloxacin	1	1	1	1	2
Ceftriaxone	2	5*	3	4	5*

*Endotoxin positive results

Table III. Antibiotic-induced endotoxin release in surgical patients.

infections remain a serious challenge, and antibiotics play an important role in the treatment strategy. Can we demonstrate the same effects seen *in vitro* in animal experiments and in surgical infections? What endpoints would be reliable to indicate the effect of antibiotic-induced endotoxin release?

At the present time, there are only a few studies investigating the antibiotic-induced endotoxin release in surgical patients.[75,76] In a retrospective analysis of data from a study with interferon (IFN)-γ, Mock and co-workers found evidence that antibiotics known to have a greater release of endotoxin were associated with higher TNF-α levels and a higher mortality in septic trauma patients. However, because no endotoxin was determined, it may be difficult to correlate the antibiotic treatment to endotoxin release. In our own study, we observed significantly more endo-

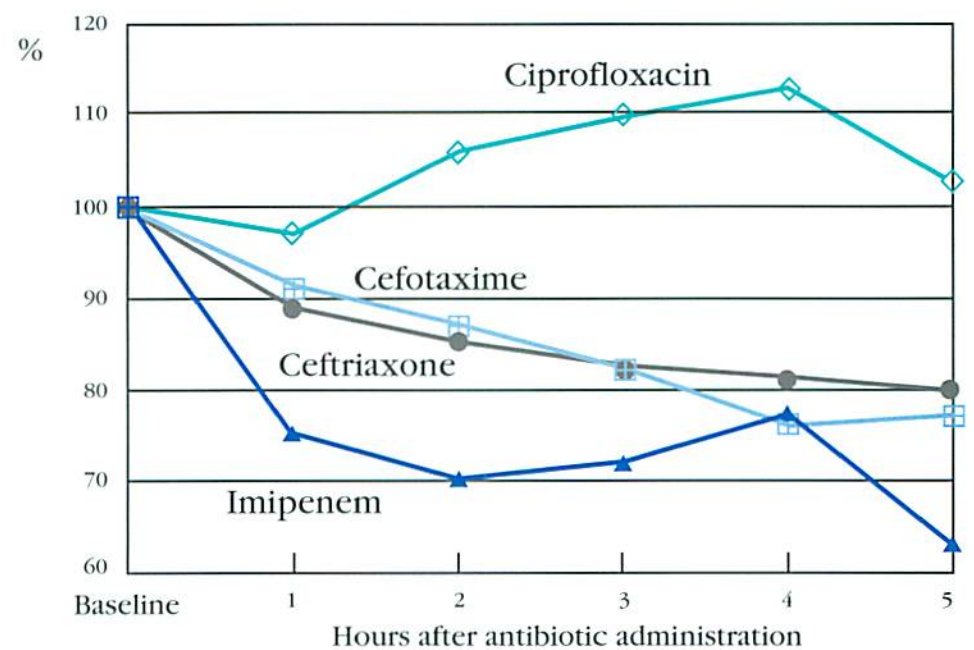


Figure 1. IL-6 levels after antibiotic administration: A diagram with baseline level of 100%.

toxin-positive results after PBP-3-specific antibiotics than after imipenem. In the group of PBP-3-specific antibiotics, there may be a difference in the kinetics of endotoxin release, with ceftriaxone showing a faster release than cefotaxime (Table III).

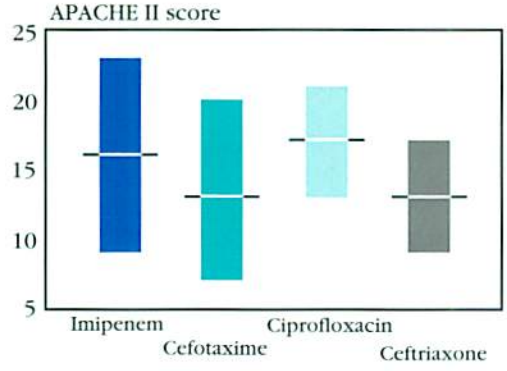


Figure 2. Antibiotic-induced endotoxin release in surgical patients: APACHE II scores.

To quantify the amount of circulating endotoxin released following administration may not be accurate in clinical circumstances. Currently, it is not known which form of endotoxin (free or neutralized, protein-bound or bacterial-bound) may activate immunocompetent cells.[77] Brandenburg et al conclude that the basic determinant for endotoxicity is the conformation of the lipid A moiety, whether in its free form or as a constituent of LPS. A prerequisite for the biological activity is the conical molecular shape that may trigger the cell activation.[78]

Endotoxins derived from different bacterial strains may vary in their ability to activate the Limulus assay.[79] Measurable levels of endotoxin activity were greater with ceftazidime than with imipenem after treatment with *E. coli* and *P. aeruginosa* strains, but not *Klebsiella pneumoniae*. We could not attribute the LAL activation to a single strain in our study. As is the case with most intra-abdominal infections, most of the isolates (70%) were polymicrobial, and the distribution of pathogens was similar.

Endotoxin is known to cause pro-inflammatory cytokine release. IL-6 has been intensively studied in patients (e.g., trauma, sepsis, elective surgery, peritonitis). There is a growing body of evidence that IL-6 may reflect the severity of disease.[80,81] In our study, we found evidence that imipenem administration, which was not associated with LAL activation, was followed by a remarkable IL-6 decrease (Figure 1). PBP-3-specific antibiotics had a less prominent decrease of IL-6, and after ciprofloxacin treatment a temporary increase in IL-6 was observed. It is known that antibiotics have immunomodulating properties,[82] and macrophage activation with IL-6 release may be "side effect" of an antibiotic.

Clinical outcome (e.g., survival) is certainly an accurate end point. However, in most recent sepsis trials, this end point was not influenced by the treatment.[83-85] The clinical course of sepsis is very complex, and intensive care treatment may have a confounding effect on outcome. Certainly the mortality rate for sepsis, on an average 35 percent, is not high enough to allow significant differences in a small study population.

What remain are factors of morbidity (e.g., temperature, blood pressure, leukocytes, heart rate, and scores consisting of acute physiology parameters [APACHE II, III score]).[86-88] The APACHE II score for the four patient groups did not differ — all patients were in a similar critical situation (Fig. 2). Temperature and blood pressure did not reveal changes with different antibiotic administration (Figs. 3 and 4).

The problem in all clinical studies is to decide what assay to use, to find the best time point in the clinical course where changes in clinical parameters may be visible, and to find what clinical

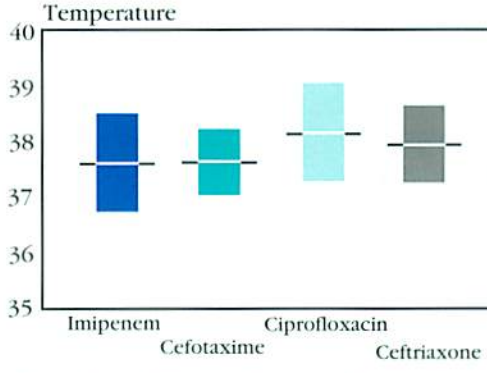


Figure 3. Antibiotic-induced endotoxin release in surgical patients: Temperature.

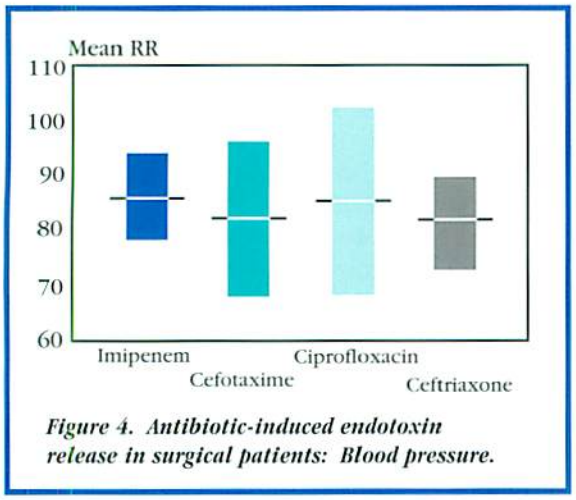


Figure 4. Antibiotic-induced endotoxin release in surgical patients: Blood pressure.

parameters may be reliable to detect effects of endotoxin release. Time series analysis techniques may help to overcome these difficulties and should be introduced in clinical studies.[89] An automated system that handles data from a Cobas TM analyzer may automatically analyze routine laboratory and clinical parameters, calculate scores (e.g.,APACHE II score), and analyze various proteases (proenzymes, enzyme activators, enzyme cofactors, and inhibitors).[90] Much clinical evidence has accumulated that analyses of various proteases can provide indicators and prognostic tools for severely ill patients.[91] The proenzyme functional inhibition index may contribute information on the severity of illness.[92] It became rather obvious that with a single assay, no one can evaluate the immune mechanisms in the septic patient. However, the combination of time series analysis of routine laboratory and clinical data, the proteases, together with endotoxin, endotoxin neutralizing index, and IL-6 may allow a more accurate evaluation of antibiotic-induced endotoxin release.

In summary, there is evidence that endotoxin is a major trigger for the inflammatory response in sepsis and trauma, which makes antibiotic-induced endotoxin release a possible candidate for risk factor in intensive care treatment. However, the pathogenesis of sepsis and peritonitis is very complex, and therefore it is a difficult task to correlate outcome or morbidity with antibiotic-induced endotoxin release. Other confounding factors are pharmacodynamics of antibiotics, the sensitivity of pathogens, and the test methods available for clinical research and clinical studies. The time course of different events during intensive care treatment has to be observed closer and with regard to organ dysfunction. The methods available can improve the evaluation of antibiotics and their potential for endotoxin release.

References

1. Stone R: Search for sepsis drugs goes on despite past failures. *Science 264*:365-367, 1994

2. Wittmann DH et al: Staged abdominal repair compares favorably with conventional operative therapy for intra-abdominal infections when adjusting for prognostic factors with a logistic model. *Theor Surg 9*:201-207, 1994

3. Wittmann DH, Schein M, Condon RE: Management of secondary peritonitis. *Ann Surg 224*:10-18, 1996

4. Kirschner M: Die Behandlung der akuten freien Bauchfellentzündung. *Langenbecks Arch Klin Chir 142*:253-311, 1926

5. Holzheimer RG et al: Clinical and basic science aspects of immune pathogenesis of sepsis and peritonitis. *Med Microbiology Letters 5*:386-393, 1996

6. Michie HR, Wilmore DW: Sepsis, signals, and surgical sequelae (a hypothesis). *Arch Surg 125*: 531-536, 1990

7. Holzheimer RG et al: Aortic aneurysm repair may cause bacterial translocation with concomitant endotoxin release into the systemic circulation and activation of the inflammatory cascade. *J Endotoxin Res 1*:31, 1994

8. Jackson JJ, Kropp H: Beta-lactam antibiotic induced release of free endotoxin: In vitro comparison of penicillin-binding protein (PBP) 2-specific imipenem and PBP 3-specific ceftazidime. *J Infect Dis 165*:1033-1041, 1992

9. Seelen MA et al: The anti-lipid A monoclonal antibody E5 binds to rough gram-negative bacteria, fixes C3, and facilitates binding of bacterial immune complexes to both erythrocytes and monocytes. *Immunology 84*:653-661, 1995

10. Levy O et al: Antibacterial proteins of granulocytes differ in interaction with endotoxin. Comparison of bactericidal/permeability-increasing protein, p15s, and defensins. *J Immunol 154*: 5403-5410, 1995

11. Crosby et al: Antibiotic-induced release of endotoxin from bacteria in vitro. *J Med Microbiol 40*:23-30, 1994

12. Bucklin SE et al: Differential antibiotic-induced release of endotoxin from gram-negative bacteria. *Eur J Clin Microbiol Infect Dis 13 (suppl 1)*: 3-51, 1994

13. Foca A, Matera G, Berlinghieri MC: Inhibition of endotoxin-induced interleukin 8 release by teicoplanin in human whole blood. *Eur J Clin Microbiol Infect Dis 12*:940-944, 1993

14. Burd RS et al: Anti-endotoxin monoclonal antibodies inhibit secretion of tumor necrosis factor-alpha by two distinct mechanisms. *Ann Surg 218(3)*:250-259, 1993

15. Dofferhoff AS et al: The release of endotoxin from antibiotic-treated Escherichia coli and the production of tumor necrosis factor by human monocytes [see Comments]. *J Antimicrob Chemother 31(3)*:373-384, 1993

16. Eng RH et al: Effect of antibiotics on endotoxin release from gram-negative bacteria. *Diagn Microbiol Infect Dis 16(3)*:185-189, 1993

17. Arditi M, Kabat W, Yogev R: Antibiotic-induced bacterial killing stimulates tumor necrosis factor-alpha release in whole blood. *J Infect Dis 167(1)*:240-244, 1993

18. Van Den Berg et al: Delayed antibiotic-induced lysis of Escherichia coli in vitro is correlated with enhancement of LPS release. *Scand J Infect Dis 24(5)*: 619-627, 1992

19. Bingen E et al: Bactericidal activity of beta-lactams and amikacin against Haemophilus influenzae: Effect on endotoxin release [see Comments]. *J Antimicrob Chemother 30(2)*: 165-172, 1992

20. Jackson JJ, Kropp H: Beta-lactam antibiotic-induced release of free endotoxin: In vitro comparison of penicillin-binding protein (PBP) 2-specific imipenem and PBP 3-specific ceftazidime [see Comments]. *J Infect Dis 165(6)*: 1033-1041, 1992

21. Dofferhoff AS et al: Effects of different types and combinations of antimicrobial agents on endotoxin release from gram-negative bacteria: An in-vitro and in-vivo study. *Scand J Infect Dis 23(6)*:745-754, 1991

22. Simon DM, Koenig G, Trenholme GM: Differences in release of tumor necrosis factor from THP-1 cells stimulated by filtrates of antibiotic-killed Escherichia coli. *J Infect Dis 164(4)*: 800-802, 1991

23. Morrison DC et al: Contribution of soluble endotoxin released from Gram-negative bacteria by antibiotics to the pathogenesis of experimental sepsis in mice. *J Endotoxin Res 3*:237-243, 1996

24. Bucklin SE, Morrison DC: Differences in therapeutic efficacy among cell wall active antibiotics in a mouse model of Gram-negative sepsis. *J Infect Dis 172*:1519-1527, 1995

25. Opal SM et al: The in vivo significance of antibiotic-induced endotoxin release in experimental Gram-negative sepsis. *J Endotoxin Res 3*: 245-252, 1996

26. Cohen J, McConnell JS: Release of endotoxin from bacteria exposed to ciprofloxacin and its prevention with polymyxin B. *Eur J Clin Microbiol 5*:13-17, 1986

27. Artenstein AW, Cross AS: Inhibition of endotoxin reactivity by aminoglycosides. *J Antimicrob Chemother 24*:826-828, 1989

28. Healy DP et al: Influence of drug class and dose size on antibiotic-induced endotoxin/IL-6 release and impact on efficacy of anti-endotoxin antibody. *J Endotoxin Res 3*:219-227, 1996

29. Casey LC, Balk RA, Bone RC: Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome [see Comments]. *Ann Intern Med 119(8)*: 771-778, 1993

30. Bellomo R: The cytokine network in the critically ill. *Anaesth Intensive Care 20(3)*:288-302, 1992

31. Goris RJ: Mediators of multiple organ failure. *Intensive Care Med 16 (suppl 3)*:S192-S196, 1990

32. Van der Poll T et al: Tumor necrosis factor is involved in the appearance of interleukin-1 receptor antagonist in endotoxemia. *J Infect Dis 169(3)*: 665-667, 1994

33. Leeson MC, Fujihara Y, Morrison DC: Evidence for lipopolysaccharide as the predominant proinflammatory mediator in supernatants of antibiotic-treated bacteria. *Infect Immun 62(11)*:4975-4980, 1994

34. Bone RC et al: Definition for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest 101*:1644-1655, 1992

35. Wewers MD et al: Tumor necrosis factor infusions in humans prime neutrophils for hypochlorous acid production. *Am J Physiol 259(4 Pt 1)*: L276-L282, 1990

36. Cohen J, Carlet J, and the INTERSEPT Study Group: Intersept: An international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-α in patients with sepsis. *Crit Care Med 24*:1431-1440, 1996

37. Morrison DC: Assessment of antibiotic-mediated endotoxin release. *J Endotoxin Res 3*:275-279, 1996

38. Willatts SM, Speller DC, Winter RJ: Incidence of gram-negative bacteremia in sepsis syndrome. Implications for immunotherapy. *Anaesthesia 49*:751-754, 1994

39. Schöffel U et al: Intraperitoneal microorganisms and the severity of peritonitis. *Eur J Surg 161*:501-508, 199

40. Bone RC et al: A second large controlled clinical study of E5, a monoclonal antibody to endotoxin: Results of a prospective, multicenter, randomized controlled trial. The E5 Sepsis Study Group. *Crit Care Med 23(6)*:994-1006, 1995

41. Berger D et al: Incidence and pathophysiological relevance of postoperative endotoxemia. *FEMS Immunol Med Microbiol 11*:285-290, 1995

42. Berger D et al: Evaluation of endotoxinuria for diagnosis of urinary tract infection after major surgical procedures. *Clin Chim Acta 244*:155-161, 1996

43. Michie HR, Manogue KR, Spriggs DR: Detection of circulating tumor necrosis factor after endotoxin administration. *N Engl J Med 318*:1481-1486, 1988

44. Rintala E et al: Endotoxin, interleukin-6 and phospholipase A2 as markers of sepsis in patients with hematological malignancies. *Scand J Infect Dis 27*:39-43, 1995

45. Urbaschek B et al: Protective effects and role of endotoxin in experimental septicemia. *Circ Shock 14*:209-222, 1984

46. Urbaschek R, Becker KP: Endotoxinnachweis im Plasma: Spezifität und Aussagekraft für Entwicklung und Prognose einer Sepsis. *Infusinsther Transfusionsmed 20 (suppl 1)*: 16-20, 1993

47. Nishida J et al: Ethanol exacerbates hepatic microvascular dysfunction, endotoxemia, and lethality in septic mice. *Shock 1(6)*:413-418, 1994

48. Goldie AS et al: Natural cytokine antagonists and endogenous antiendotoxin core antibodies in sepsis syndrome. The Sepsis Intervention Group. *JAMA 274(2)*:172-177, 1995

49. Prins JM et al: Release of tumor necrosis factor alpha and interleukin 6 during antibiotic killing of *Escherichia coli* in whole blood: Influence of antibiotic class, antibiotic concentration and presence of septic serum. *Infect Immun 63*:2236-2242, 1995

50. Patel RT et al: Interleukin 6 is a prognostic indicator of outcome in severe intra-abdominal sepsis. *Br J Surg 81(9)*:1306-1308, 1994

51. De Bont ES et al: Diagnostic value of plasma levels of tumor necrosis factor alpha (TNF alpha) and interleukin-6 (IL-6) in newborns with sepsis. *Acta Paediatr 83(7)*: 696-699, 1994

52. Fugger R et al: Perioperative TNF alpha and IL-6 concentrations correlate with septic state, organ function, and APACHE II scores in intra-abdominal infection. *Eur J Surg 159(10)*: 525-529, 1993

53. Holzheimer RG, Schein M, Wittmann DH: Inflammatory response in peritoneal exudate and plasma of patients undergoing planned relaparotomy for severe peritonitis. *Arch Surg 130*:1314-1320, 1995

54. Holzheimer RG et al: Clinical routine determination as part of a European Research Network. *Shock 7*:121, 1997

55. Holzheimer RG et al: Clinical and basic science aspects of immune pathogenesis of sepsis and peritonitis. *Med Microbiology Letters 5*:386–393, 1996

56. Echtenacher B et al: Requirements of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. *J Immunol 145*:3762-3766, 1990

57. Bagby GJ et al: Divergent efficacy of antibody to tumor necrosis factor alpha in intravascular and peritonitis models of sepsis. *J Infect Dis 163*:83-88, 1991

58. Villa P et al: Pattern of cytokines and pharmacomodulation in sepsis induced by cecal ligation and puncture compared with that induced by endotoxin. *Clin Diagn Lab Immunol 2*:549-553, 1995

59. Holzheimer RG, Schein M, Wittmann DH: Inflammatory response in peritoneal exudate and plasma of patients undergoing planned relaparotomy for severe secondary peritonitis. *Arch Surg 130*:1314-1320, 1995

60. Hau T, Ahrenholz DH, Simmons RL: Secondary bacterial peritonitis: The biological basis of treatment. *Curr Probl Surg 16*:1, 1979

61. Hau T, Mozes MF, Jonasson O: Peritonitis nach Nierentransplantation. *Langenbecks Arch Chir 353*:269, 1981

62. Holzheimer RG et al: Nosocomial infections in general surgery: Surveillance report from a German university clinic. *Infection 18*:219-225, 1990

63. Jackson JJ, Kropp H: Beta lactam antibiotic-induced release of free endotoxin: In vitro comparison of penicillin-binding protein (PBP) 2-specific imipenem and PBP 3-specific ceftazidime [see Comments]. *J Infect Dis 165(6)*:1033-1041, 1992

64. Dofferhoff AS et al: Effects of different types and combinations of antimicrobial agents on endotoxin release from gram-negative bacteria: An in-vitro and in-vivo study. *Scand J Infect Dis 23(6)*:745-754, 1991

65. Prins JM et al: Release of tumor necrosis factor alpha and interleukin 6 during antibiotic killing of *Escherichia coli* in whole blood: Influence of antibiotic class, antibiotic concentration, and presence of septic serum. *Infect Immun 63*:2236-2242, 1995

66. Crosby HA et al: Antibiotic induced release of endotoxin from bacteria in vitro. *J Med Microbiol 40*:23-30, 1994

67. Hurley JC: Antibiotic-induced release of endotoxin. A therapeutic paradox. *Drug Safety 12(3): 183-195*, 1995

68. Stone HH, Kolb LD, Geheber CE: Incidence and significance of intra-abdominal infections. *Ann Surg 181*:705, 1975

69. Lorber B, Swenson RM: The bacteriology of intraabdominal infections. *Surg Clin North Am 55*:1349, 1975

70. Rotstein O: Peritonitis and intra-abdominal abscesses. In: *Surgical Infections — Diagnosis and Treatment*. (Meakins JL, ed.). Scientific American Medicine Inc. New York, New York, USA, 1994, pp 329-351

71. Prins JM et al: Antibiotic-induced endotoxin release in patients with gram-negative urosepsis: A double-blind study comparing imipenem and ceftazidime. *J Infect Dis 172(3)*:886-891, 1995

72. Arditi M, Ables L, Yogev R: Cerebrospinal fluid endotoxin levels in children with H. influenzae meningitis before and after iv ceftriaxone. *J Infect Dis 160*:1005-1011, 1990

73. Hurley JC et al: Antibiotic-induced release of endotoxin in chronically bacteriuric patients. *Antimicrob Agents Chemother 35*:2388-2394, 1991

74. Prins JM: Antibiotic induced release of endotoxin — clinical data and human studies. *J Endotoxin Res 3*:269-273, 1996

75. Mock CN et al: Clinical significance of antibiotic endotoxin releasing properties in trauma patients. *Arch Surg 130*:1234-1240, 1995

76. Holzheimer RG et al: Different endotoxin release and IL-6 plasma levels after antibiotic administration in surgical intensive care patients. *J Endotoxin Res 3*:261-267, 1996

77. Morrison DC: Assessment of antibiotic mediated endotoxin release. *J Endotoxin Res 3*:275-279, 1996

78. Brandenburg K et al: Conformation of lipid A, the endotoxic center of bacterial lipopolysaccharide. *J Endotoxin Res 3*:173-178, 1996

79. Opal SM et al: The in vivo significance of antibiotic-induced endotoxin release in experimental Gram-negative sepsis. *J Endotoxin Res 3*:245-252, 1996

80. Biffl WL et al: Interleukin 6 in the injured patient — Marker of injury or mediator of inflammation? *Ann Surg 224*:647-664, 1996

81. Tang GJ et al: Perioperative plasma concentrations of tumor necrosis factor-alpha and interleukin-6 in infected patients. *Crit Care Med 24*:423-428, 1996

82. Wittmann DH: Immunological consequences of antibiotic therapy. In: *Immunology and Its Impact on Infections in Surgery*. (Engemann R, Holzheimer RG, Thiede A, eds.). Springer, New York, 1995, pp 217-223

83. Astiz ME et al: Pretreatment of normal humans with monophosphoryl lipid A induces tolerance to endotoxin: A prospective, double-blind, randomized, controlled trial. *Crit Care Med 23(1)*:9-17, 1995

84. Bone RC et al: A second large controlled clinical study of E5, a monoclonal antibody to endotoxin: Results of a prospective, multicenter, randomized, controlled trial. The E5 Sepsis Study Group [see Comments]. *Crit Care Med 23(6)*:994-1006, 1995

85. Greenman RL et al: A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of gram-negative sepsis. The XOMA Sepsis Study Group [see Comments]. *JAMA 266(8)*:1097-1102, 1991

86. Rogy MA et al: Correlation between Acute Physiology and Chronic Health Evaluation (APACHE) III score and immunological parameters in critically ill patients with sepsis. *Br J Surg 83*:396-400, 1996

87. Holzheimer RG, Haupt W, Thiede A: The challenge of postoperative infections — Does the surgeon make a difference? *Infect Control Hosp Epidemiol* 1997, in press

88. Haupt W et al: The association of low preoperative serum albumin concentrations with an acute phase response. *J Trauma* 1997, in press

89. Imhof M, Bauer M: Time series analysis in critical care monitoring. *New Horizons 4*: 519-531, 1996

90. Aasen AO et al: Consequences of trauma on circulating cells and the plasma cascade systems. In: *Trauma Care — An Update*. (Risberg B, ed.). Pharmacia & Upjohn, Stockholm, Sweden, 1996, pp 118-128

91. Hesselvik JF et al: Coagulation, fibrinolysis, and kallikrein systems in sepsis: Relation to outcome. *Crit Care Med 17*:724-733, 1989

92. Aasen AO: The proenzyme functional inhibition index. A new parameter for the evaluation of severely injured and septic patients. *Acta Chir Scand (suppl) 522*:211-233, 1985