

Effects of Decreased Preoperative Endotoxin Core Antibody Levels on Long-term Mortality After Coronary Artery Bypass Graft Surgery

Eugene W. Moretti, MD, MHSc; Mark F. Newman, MD; Lawrence H. Muhlbaier, PhD; David Whellan, MD, MHSc; Rebecca P. Petersen, MD, MSc; Daniel Rossignol, PhD; Charles B. McCants, Jr, BS; Barbara Phillips-Bute, PhD; Elliott Bennett-Guerrero, MD

Hypothesis: Decreased preoperative levels of antiendotoxin core antibody (EndoCAb) in patients undergoing cardiac surgery with cardiopulmonary bypass are associated with increased long-term mortality.

Design: Observational study.

Setting: Academic medical center.

Patients: A total of 474 patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass.

Interventions: Preoperative serum IgM EndoCAb levels were determined, and established preoperative risk factors were assessed. Patients were assigned a risk score using a validated method.

Main Outcome Measures: The primary end point was mortality. Statistical analysis used the Cox proportional hazards regression model with log EndoCAb as the predictor

of interest and Parsonnet additive risk score as a covariate. Kaplan-Meier survival curves were generated to visually compare groups with high vs low EndoCAb levels.

Results: Forty-six deaths occurred in 5 years. Annual follow-up rates during the 5 years were 100%, 94%, 93%, 98%, and 98% for the 1-, 2-, 3-, 4-, and 5-year periods, respectively. Parsonnet additive risk score (hazard ratio, 1.07; 95% confidence interval [CI], 1.04-1.11; $P < .001$) and log EndoCAb (hazard ratio, 0.73; 95% CI, 0.53-0.99; $P = .04$) were independent predictors of long-term mortality in the final model. Kaplan-Meier analysis revealed that the preoperative EndoCAb level was significantly associated with mortality up to 5 years ($P = .01$ by log-rank test).

Conclusion: Lower preoperative serum EndoCAb level is a significant predictor of long-term mortality independent of other known risk factors.

Arch Surg. 2006;141:637-641

Author Affiliations:

Departments of Anesthesiology (Drs Moretti, Newman, Phillips-Bute, and Bennett-Guerrero), Biostatistics and Bioinformatics (Dr Muhlbaier), and Surgery (Drs Muhlbaier and Petersen) and Duke Clinical Research Institute (Drs Newman, Muhlbaier, Whellan, and Bennett-Guerrero and Mr McCants), Duke University Medical Center, Durham, NC; Eisai Global Clinical Research Inc, Ridgefield Park, NJ (Dr Rossignol); and Division of Cardiology, Department of Medicine, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pa (Dr Whellan).

EVERY YEAR, MORE THAN 800 000 coronary artery bypass graft (CABG) procedures are performed worldwide.¹ Complications remain common after cardiac surgery, and much of this morbidity may be due to an exaggerated pro-inflammatory response known to occur in this setting.²⁻⁴ Endotoxin is believed to be a major stimulus for development of the inflammatory response.^{5,6} Some of its effects include myocardial dysfunction, increased tissue oxygen demand, complement activation, contact activation, coagulopathy, and microvascular thrombosis with subsequent organ failure.⁷⁻⁹

Endogenous endotoxin immunity (antibodies to various serotypes of endotoxin) is conferred early in fetal life from maternal transfer, and it is enhanced by subsequent exposure throughout an individual's life.¹⁰ In contrast to the variety of serotype epitopes present in endotoxins, the inner core region of the endotoxin mol-

ecule is highly conserved across the entire spectrum of gram-negative organisms.^{11,12} An assay for antiendotoxin core antibody

See Invited Critique at end of article

(EndoCAb) has been used in numerous clinical studies.¹³⁻¹⁹ Most patients undergoing cardiac surgery involving cardiopulmonary bypass (CPB) are exposed to endotoxin²⁰; therefore, it is attractive to speculate that endotoxin may be partially responsible for the exaggerated systemic inflammatory response that is commonly observed.

A previous study¹⁴ demonstrated an association between preoperative endotoxin immune status and short-term outcome after cardiac surgery. However, the relationship between preoperative endotoxin immune status and long-term survival is unknown. Therefore, we decided to study the association of preoperative EndoCAb level and long-term outcome in a large cohort of cardiac surgical patients.

After institutional review board approval and written informed consent, we prospectively followed 475 patients at Duke University Medical Center who underwent isolated CABG surgery with CPB between October 1, 1997, and March 12, 2001. Unrelated data regarding endotoxin immunity and cognitive decline were collected from many of these patients and have been reported elsewhere.¹⁵

PATIENT MANAGEMENT

General anesthesia was induced and maintained with infusions of midazolam hydrochloride and fentanyl citrate and supplemented with 0.5% to 1% isoflurane. Pancuronium chloride was used for neuromuscular blockade. Patients underwent nonpulsatile hypothermic (30°C–32°C) CPB, with a membrane oxygenator. Porcine heparin sodium, 300 U/kg, was administered and supplemented as necessary to maintain an activated clotting time of 450 seconds during CPB. After termination of CPB, heparin was neutralized with protamine sulfate. Patients were managed postoperatively in the cardiothoracic intensive care unit based on a standard “care pathway” whereby patients without significant complications were discharged from the hospital on postoperative day 4 or 5.

IgM EndoCab LEVEL DETERMINATION

Immediately before the induction of general anesthesia, blood samples were obtained through a radial arterial catheter. Samples were collected in additive-free glass tubes, centrifuged at 2000g, and stored at –70°C until assayed. Dr Robin Barclay, Scotland, provided endotoxin-coated enzyme-linked immunosorbent assay plates and standard serum containing 165 median units (MU) of IgM EndoCab. Validated enzyme-linked immunosorbent assay conditions have been previously described^{16,17} and included the use of horseradish peroxidase–conjugated anti-IgM antibody (A-6907; Sigma-Aldrich Corp, St Louis, Mo) and tetramethylbenzidine (T-0440; Sigma-Aldrich Corp), with the exception that the phosphate-buffered saline wash buffer contained 0.3% Triton X-100 and sample incubation was performed at 37°C for 60 minutes. The conjugate incubation and substrate incubation were performed at room temperature for 30 minutes. All serum standards and samples were diluted ($\geq 1:100$) with enzyme-linked immunosorbent assay dilution buffer (1% bovine serum albumin, 2.5% adult bovine serum, 0.1% Triton X-100, and 0.1% sodium azide in phosphate-buffered saline). The IgM EndoCab levels were measured, as in previous publications,^{13–17} because this class of antibody remains intravascular, and levels are unaltered by fluid shifts between the intravascular and extravascular compartments.

PATIENT CHARACTERISTICS AND RISK SCORING

Demographic and clinical information were collected on all patients. Similar to a previous study,¹⁴ each patient was assigned a mortality risk score using the Parsonnet additive risk score.²¹ This is a scoring system, developed based on 3500 cardiac surgery cases and validated on an additional 1300, that generates a score between 0 and 148 based on 19 factors known to increase mortality after cardiac surgery. This score was used in the Cox proportional hazards regression model to adjust for the effects of individual patient comorbidities. Definitions of diabetes mellitus, angina, congestive heart failure, hypertension, and left ventricular ejection fraction were as previously reported.¹⁴ Data regarding CPB, aortic cross-clamp, anesthesia, and surgical durations

were collected from the patient’s intraoperative anesthetic record. Chronic obstructive pulmonary disease was defined as being present based on patient history, physical examination findings, and medication regimen. Significant obesity was defined as a body mass index (calculated as weight in kilograms divided by the square of height in meters) of 35 or greater. Hyperlipidemia was considered to be present based on the patient’s lipid profile and medication regimen. Patients who were currently smoking at least a half pack per day or who had stopped smoking for less than 2 years were considered to have a positive smoking history. A family history of coronary artery disease was present if it was documented in the patient’s medical record or patient history or if there was a first-degree relative who had received a diagnosis based on documented evidence of significant coronary disease. Renal insufficiency was determined to be present if a patient’s preoperative serum creatinine level exceeded 3.0 mg/dL (265 μ mol/L). Liver disease was considered to be present if the patient had a history of any of the following: cirrhosis, chronic active hepatitis, primary biliary cirrhosis, ascites, esophageal varices, portal hypertension, or hepatic encephalopathy.

OUTCOME

The primary outcome variable was mortality assessed for 5 years. Assessment of this outcome was facilitated by the fact that all the patients undergoing cardiac surgery at Duke University Medical Center, including those in this study, are followed by the Duke Database for Cardiovascular Disease. Outcome data from this database have been used in numerous previously published clinical studies.^{22–25} The Duke Database for Cardiovascular Disease has supported the prospective entry of clinical care and outcome data since 1971. Outcome data are obtained for the determination of clinical efficacy and quality assurance in all patients undergoing CABG surgery. The quality of these data are verified by features designed to reduce transcription errors (coded data-entry algorithms, error limit checking, dual entry, etc). Data quality has been further ensured by having senior clinicians review and then sign the reports, which thus provides a review of the data files stored in the database. Death and date of death were determined by contact with the next of kin, contact with the referring physician, or a match in the National Death Index and were confirmed by hospital records or death certificates.

STATISTICAL ANALYSIS

Group differences for continuous variables were assessed using the 2-tailed *t* test. The Fisher exact test (2-sided probability) assessed group differences for categorical variables. The main outcome of time to mortality was assessed using the Cox proportional hazards regression model with log EndoCab as the primary predictor of interest and Parsonnet additive risk score as a covariate, with censoring occurring at the date of last follow-up. Because EndoCab level is positively skewed, a logarithmic transformation was performed to achieve a linear fit. Model assumptions were checked in the data. Hazard ratios and 95% confidence intervals (CIs) are reported.

We also examined age, height, weight, body surface area, history of congestive heart failure, and preoperative hematocrit level as potentially important covariates in the Cox proportional hazards regression model. Because of the limitations of the sample size, these variables were investigated one at a time in a model containing log EndoCab and Parsonnet additive risk score as predictors.

For the purposes of displaying Kaplan-Meier survival curves illustrating time to death, IgM EndoCab level was dichotomized at 80 MU/mL. This characterization of EndoCab levels has been shown to be a clinically significant threshold based on previous studies.^{14–16} Cumulative event plots according to

Table 1. Patient Demographics and Baseline Characteristics

Characteristic	EndoCAb <80 MU/mL (n = 272)	EndoCAb ≥80 MU/mL (n = 202)	P Value
Age, mean ± SD, y	64 ± 11	60 ± 12	<.001
Male sex, %	68	40	.10
White race, %	68	68	.99
Height, mean ± SD, cm	172 ± 10	170 ± 11	.04
Weight, mean ± SD, kg	86 ± 19	83 ± 17	.09
BSA, mean ± SD, m ²	1.9 ± 0.2	1.9 ± 0.2	.05
Obese (BMI ≥35), %	13	13	.99
History of CHF, %	11	20	.008
Unstable angina, %	62	54	.11
Previous MI, %	24	21	.37
Ejection fraction	0.54	0.53	.12
Diabetes mellitus, %	32	31	.92
Liver disease, %	<1	<1	.99
Renal insufficiency, preoperative creatinine ≥3.0 mg/dL (≥265.2 μmol/L), %	2	2	.99
History of hypertension, %	61	59	.74
COPD, %	7	9	.22
Smoker, %	36	39	.70
Parsonnet additive risk score, mean ± SD	9 ± 8	9 ± 7	.24

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); BSA, body surface area; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; EndoCAb, antiendotoxin core antibody; MI, myocardial infarction; MU, median units.

EndoCAb level were compared using the log-rank test. For all outcomes and comparisons, *P* values were 2-sided, and *P* < .05 was considered statistically significant. All statistical analyses were performed using statistical software packages (SAS version 8.2; SAS Institute Inc, Cary, NC, and STATA version 7.0; STATA Technology Corp, College Station, Tex).

RESULTS

Between October 1, 1997, and March 12, 2001, 474 patients were enrolled. Baseline (**Table 1**), clinical (**Table 2**), and intraoperative (**Table 3**) characteristics of the overall study population are given for the dichotomized groups. There were 46 deaths in the study population. Mean follow-up was 3.7 years (1361 days), with the longest follow-up being 6.25 years. The last patient was enrolled on March 12, 2001. Rates of follow-up per year during the 5 years were 100% (474/474), 94% (432/457), 93% (416/446), 98% (386/395), and 99% (261/264) for the 1-, 2-, 3-, 4-, and 5-year periods, respectively.

The Cox proportional hazards regression model demonstrated that log IgM EndoCAb level was a significant predictor of hazard even after adjusting for Parsonnet additive risk score (hazard ratio, 0.73; 95% CI, 0.53-0.99; *P* = .04). Parsonnet additive risk score was also significant in this model (hazard ratio, 1.07; 95% CI, 1.04-1.11; *P* < .001). No other variable was a significant predictor of mortality after adjusting for Parsonnet additive risk score.

Mean Parsonnet additive risk scores between the groups with high vs low EndoCAb levels were not significantly different (*P* = .24). The EndoCAb concentra-

Table 2. Preoperative Medications and Laboratory Values

Medication	EndoCAb <80 MU/mL (n = 272)	EndoCAb ≥80 MU/mL (n = 202)	P Value
β-Blocker treatment, % of patients	60	59	.92
Calcium channel blocker treatment, % of patients	10	8	.51
Aspirin, % of patients	70	73	.68
Intravenous nitroglycerin, % of patients	13	13	.89
EndoCAb, median (25%-75%), MU/mL	41 (25-57)	139 (99-266)	.001*
Preoperative hematocrit, mean ± SD, %	0.40 ± 0.5	0.39 ± 0.7	.05
Preoperative creatinine, mean ± SD, mg/dL	1.0 ± 0.2	1.0 ± 0.4	.84
Preoperative potassium, mean ± SD, mEq/L	4.2 ± 0.4	4.1 ± 0.4	.07

Abbreviations: EndoCAb, antiendotoxin core antibody; MU, median units. SI conversion factor: To convert creatinine to micromoles per liter, multiply by 88.4.

*This *P* value was determined using the *t* test performed on the log-transformed variable.

Table 3. Intraoperative Characteristics

Characteristic	EndoCAb <80 MU/mL (n = 272)	EndoCAb ≥80 MU/mL (n = 202)	P Value
DBGAs, mean ± SD, No.	3.0 ± 0.8	3.0 ± 0.9	.82
IMA DBGAs, % ≥1	53	51	.85
Bypass duration, mean ± SD, min	108 ± 55	113 ± 91	.53
Aortic cross-clamp duration, mean ± SD, min	66 ± 34	69 ± 36	.36
Anesthesia duration, mean ± SD, min	323 ± 81	320 ± 88	.66
Surgical duration, mean ± SD, min	265 ± 66	258 ± 79	.33

Abbreviations: DBGAs, distal bypass graft anastomoses; EndoCAb, antiendotoxin core antibody; IMA, internal mammary artery; MU, median units.

tions and preoperative Parsonnet additive risk score were not highly correlated (Spearman correlation coefficient, -0.079). Eleven deaths (5.4%) occurred in the group with high EndoCAb levels, and 35 deaths (12.8%) occurred in the group with low EndoCAb levels. Kaplan-Meier 5-year survival curves illustrate that survival was lower for the group with low EndoCAb levels (*P* = .01 by log-rank test) (**Figure 1**).

COMMENT

This is the first study, to our knowledge, to evaluate the association between preoperative antiendotoxin immune status and long-term survival in patients after cardiac surgery. The data demonstrate that decreased IgM EndoCAb levels are associated with decreased long-term survival up to 5 years after CABG surgery. This association persists even

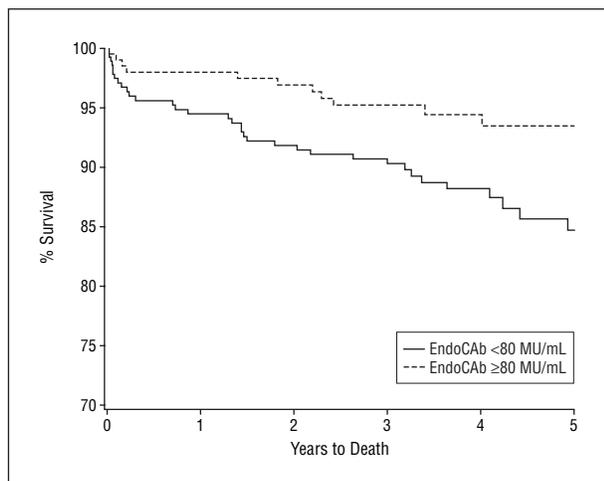


Figure 1. Kaplan-Meier estimates of the probability of 5-year survival based on high vs low antiendotoxin core antibody (EndoCAB) levels. $P = .01$ using the log-rank test.

when risk-adjusted statistical analysis is applied, using the Cox proportional hazards regression model, controlling for Parsonnet additive risk score.

Endotoxin is now widely accepted as a major stimulus for development of the systemic inflammatory response syndrome.⁵ Intravenous administration of endotoxin causes cytokine release, as evidenced by an increase in tumor necrosis factor levels and, within 3 hours, by elevations in interleukin (IL) 1, IL-6, and IL-8 levels.²⁶⁻²⁸ Exposure to endotoxin is also associated with complement, plasminogen, and neutrophil activation; hypercoagulability; and synthesis of bradykinin.²⁹ Endotoxin exposure is common during cardiac surgery and most likely results from hypoperfusion of the gut mucosa, resulting in leakage of endotoxin across the gut mucosal barrier. Depletion of EndoCAB occurs intraoperatively through its consumption during endotoxemia, adherence to CPB tubing, and decreased production.³⁰ Rothenburger et al³⁰ studied 100 patients who underwent CABG with CPB and demonstrated that lower preoperative EndoCAB levels are associated with a greater rise in endotoxin and IL-8 release. In a smaller study involving 26 patients, Mythen et al¹⁸ revealed that higher EndoCAB levels are associated with less endotoxin-induced contact activation and neutrophil degranulation. Finally, in a study of 29 medical intensive care patients diagnosed as having sepsis syndrome, low EndoCAB levels were associated with increased mortality.³¹ In contrast to all previous studies,^{13,14,18,30,31} which focused only on short-term postoperative end points, the present study is the first to assess the association of preoperative endotoxin immune status with long-term mortality.

The potential mechanisms by which patients with lower preoperative endotoxin immunity have increased risk of long-term mortality are unclear. Speculative mechanisms include the following. (1) Greater endotoxin immunity seems to be associated with less injury in the perioperative period,^{13,14,30,31} and this may translate into long-term outcome differences through some other mechanisms. This has been demonstrated in other settings. For example, Mangano et al³² showed that patients randomized to only 7 days of β -adrenergic blockade (vs placebo) exhibited a lower mor-

tality up to 2 years after surgery. These data and others³²⁻³⁴ suggest that subtle injury in the perioperative period may have an effect on long-term outcome. (2) Patients with low EndoCAB levels may have a genetic predisposition to unfavorable outcomes. Our use of risk adjustment using a validated risk score minimizes, but does not rule out, this possibility. (3) Patients with low preoperative endotoxin immunity may be more likely to have a low level of immunity to endotoxin several years later, which might predispose them to greater risk during a subsequent insult.

As implied previously herein, a limitation of any observational study such as this one is that low antiendotoxin immunity may not be causally related to adverse outcome but merely a marker for sicker patients at higher operative risk. We addressed this possibility by using a validated preoperative risk scoring system to quantify the degree of risk. Although Parsonnet additive risk score is a statistically significant predictor of mortality, the association of low EndoCAB levels with decreased long-term survival is independent of Parsonnet score, suggesting that preoperative health status is not a significant confounder. However, without mechanistic inference it is difficult to know why patients with decreased EndoCAB levels have higher long-term mortality.

The theory that endotoxemia is an important cause of postoperative morbidity is subject to certain criticisms. One relates to the low incidence of culture-positive bacteremia in surgical patients and intensive care unit patients.^{6,35-37} Endotoxemia clearly exists in these patients and does so in the setting of negative blood cultures.³⁸⁻⁴¹ The routine administration of antibiotics for surgical prophylaxis would be expected to kill or prevent the growth of susceptible gram-negative bacteria and could conceivably elevate endotoxin serum concentrations through increased shedding of endotoxin.⁴² Because of the intermittent nature of endotoxemia, studies attempting to detect endotoxemia probably underestimate its incidence. Another criticism resides in the failure of 2 anti-lipid A monoclonal antibodies (HA-1A; Centocor, Malvern, Pa, and E5; Xoma, Berkeley, Calif) to improve outcome on an intention-to-treat basis in intensive care unit patients with established sepsis.^{43,44} The anti-lipid A monoclonal antibodies failed to bind to endotoxin with high affinity⁴⁵ and, in fact, are likely to bind to epitopes on lipid A not present on endotoxin,⁴⁶ helping explain their lack of demonstrable efficacy.

These previous monoclonal antibody studies are not relevant to the present study. They were tested in patients with established sepsis and organ failure, which is in an entirely different setting than in elective surgical patients, who are more likely to benefit from prophylaxis with other endotoxin-related strategies. This is borne out by a resurgence in the development of antiendotoxin strategies that may benefit the high-risk surgical patient.⁴⁷⁻⁵³

This study is the first, to our knowledge, to demonstrate that decreased preoperative EndoCAB levels are associated with increased long-term mortality in cardiac surgery patients. Results from well-designed randomized studies in which endotoxin is selectively neutralized are necessary to confirm the clinical relevance of these findings. Several antiendotoxin agents are currently in development that may benefit cardiac surgery patients if given prophylactically.⁴⁷⁻⁵³

Accepted for Publication: June 1, 2005.

Correspondence: Eugene W. Moretti, MD, MHSc, Department of Anesthesiology, Duke University Medical Center, Durham, NC 27710 (moret002@mc.duke.edu).

Funding/Support: Assays were funded by Eisai Global Clinical Research Inc and were performed by Zep-tometrix. All other work was funded by Duke University.

Role of the Sponsor: Eisai Global Clinical Research Inc did not influence the design or conduct of this study; the collection, analysis, or interpretation of the data; or the preparation, review, or approval of the manuscript. These functions were entirely the purview of the authors.

REFERENCES

1. American Heart Association. Heart disease and stroke statistics: 2005 update. <http://www.americanheart.org>. Accessed December 31, 2003.
2. Hammermeister KE, Burchfiel C, Johnson R, Grover FL. Identification of patients at greatest risk for developing major complications at cardiac surgery. *Circulation*. 1990;82(suppl 5):IV380-IV389.
3. Goris RJ, te Boekhorst TP, Nuytinck JK, Gimbrenre JS. Multiple-organ failure: generalized autodestructive inflammation? *Arch Surg*. 1985;120:1109-1115.
4. Bone RC, Balk RA, Cerra FB, et al; ACCP/SCCM Consensus Conference Committee, American College of Chest Physicians/Society of Critical Care Medicine. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest*. 1992;101:1644-1655.
5. Danner RL, Elin RJ, Hosseini JM, Wesley RA, Reilly JM, Parillo JE. Endotoxemia in human septic shock. *Chest*. 1991;99:169-175.
6. Rush BF Jr, Sori AJ, Murphy TF, Smith S, Flanagan JJ Jr, Machiedo GW. Endotoxemia and bacteremia during hemorrhagic shock: the link between trauma and sepsis? *Ann Surg*. 1988;207:549-554.
7. Suffredini AF, Fromm RE, Parker MM, et al. The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med*. 1989;321:280-287.
8. Bjork J, Hugli TE, Smedegard G. Microvascular effects of anaphylatoxins C3a and C5a. *J Immunol*. 1985;134:1115-1119.
9. Morrison DC, Cochrane CG. Direct evidence for Hageman factor (factor XII) activation by bacterial lipopolysaccharides (endotoxins). *J Exp Med*. 1974;140:797-811.
10. Barclay GR. *Bacterial Endotoxins: Lipopolysaccharides From Genes to Therapy*. New York, NY: John Wiley & Sons; 1995.
11. Poxton IR. Antibodies to lipopolysaccharide. *J Immunol Methods*. 1995;186:1-15.
12. Baumgartner JD. Immunotherapy with antibodies to core lipopolysaccharide: a critical appraisal. *Infect Dis Clin North Am*. 1991;5:915-927.
13. Bennett-Guerrero E, Panah MH, Barclay GR, et al. Decreased endotoxin immunity is associated with greater mortality and/or prolonged hospitalization after surgery. *Anesthesiology*. 2001;94:992-998.
14. Bennett-Guerrero E, Ayuso L, Hamilton-Davies C, et al. Relationship of preoperative antiendotoxin core antibodies and adverse outcomes following cardiac surgery. *JAMA*. 1997;277:646-650.
15. Mathew JP, Grocott HP, Phillips-Bute B, et al. Lower endotoxin immunity predicts increased cognitive dysfunction in elderly patients after cardiac surgery. *Stroke*. 2003;34:508-513.
16. Hamilton-Davies C, Barclay GR, Cardigan RA, et al. Relationship between preoperative endotoxin immune status, gut perfusion, and outcome from cardiac valve replacement surgery. *Chest*. 1997;112:1189-1196.
17. Bennett-Guerrero E, Barclay GR, Youssef ME, et al. Exposure to bacteroides fragilis endotoxin during cardiac surgery. *Anesth Analg*. 2000;90:819-823.
18. Mythen MG, Barclay GR, Purdy G, et al. The role of endotoxin immunity, neutrophil degranulation and contact activation in the pathogenesis of post-operative organ dysfunction. *Blood Coagul Fibrinolysis*. 1993;4:999-1005.
19. Windsor JA, Fearon KC, Ross JA, et al. Role of serum endotoxin and antiendotoxin core antibody levels in predicting the development of multiple organ failure in acute pancreatitis. *Br J Surg*. 1993;80:1042-1046.
20. Andersen LW, Baek L, Degn H. Presence of circulating endotoxin during cardiac operations. *J Thorac Cardiovasc Surg*. 1987;93:115-119.
21. Parsonnet V, Dean D, Bernstein AD. A method of uniform stratification of risk for evaluating the results of surgery in acquired adult heart disease. *Circulation*. 1989;79:113-112.
22. Califf RM, Harrell FE Jr, Lee KL, et al. The evolution of medical and surgical therapy for coronary artery disease: a 15-year perspective. *JAMA*. 1989;261:2077-2086.
23. Jones RH, Kesler K, Phillips HR III, et al. Long-term survival benefits of coronary artery bypass grafting and percutaneous transluminal angioplasty in patients with coronary artery disease. *J Thorac Cardiovasc Surg*. 1996;111:1013-1025.
24. Mark DB, Nelson CL, Califf RM, et al. Continuing evolution of therapy for coronary artery disease: initial results from the era of coronary angioplasty. *Circulation*. 1994;89:2015-2025.
25. Califf RM, DeLong ER, Ostbye T, et al. Underuse of aspirin in a referral population with documented coronary artery disease. *Am J Cardiol*. 2002;89:653-661.
26. Luca R, Lijnen HR, Suffredini AF, et al. Increased angiotensin levels in bronchoalveolar lavage fluids from ARDS patients and from human volunteers after lung instillation of endotoxin. *Thromb Haemost*. 2002;87:966-971.
27. O'Grady NP, Preas HL, Pugin J, et al. Local inflammatory responses following bronchial endotoxin instillation in humans. *Am J Respir Crit Care Med*. 2001;163:1591-1598.
28. Suffredini AF, Fromm RE, Parker MM, et al. The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med*. 1989;321:280-287.
29. Martich GD, Boujoukos AJ, Suffredini AF. Response of man to endotoxin. *Immunobiology*. 1993;187:403-416.
30. Rothenburger M, Soeparwata R, Deng MC, et al. The impact of anti-endotoxin core antibodies on endotoxin and cytokine release and ventilation time after cardiac surgery. *J Am Coll Cardiol*. 2001;38:124-130.
31. Strutz F, Heller G, Krasemann K, Krone B, Muller GA. Relationship of antibodies to endotoxin core to mortality in medical patients with sepsis syndrome. *Intensive Care Med*. 1999;25:435-444.
32. Mangano DT, Layug EL, Wallace A, Tateo I; Multicenter Study of Perioperative Ischemia Research Group. Effect of atenolol on mortality and cardiovascular morbidity after noncardiac surgery. *N Engl J Med*. 1996;335:1713-1720.
33. Poldermans D, Boersma E, Bax JJ, et al; Dutch Echocardiographic Cardiac Risk Evaluation Applying Stress Echocardiography Study Group. The effect of bisoprolol on perioperative mortality and myocardial infarction in high-risk patients undergoing vascular surgery. *N Engl J Med*. 1999;341:1789-1794.
34. Landesberg G, Shatz V, Akopnik I, et al. Association of cardiac troponin, CK-MB, and postoperative myocardial ischemia with long-term survival after major vascular surgery. *J Am Coll Cardiol*. 2003;42:1547-1554.
35. DeCamp MM, Demling RH. Posttraumatic multisystem organ failure. *JAMA*. 1988;260:530-534.
36. Moore FA, Moore EE, Poggetti R, et al. Gut bacterial translocation via the portal vein: a clinical perspective with major torso trauma. *J Trauma*. 1991;31:629-636.
37. Ford EG, Baisden CE, Matteson ML, Picone AL. Sepsis after coronary bypass grafting: evidence for loss of the gut mucosal barrier. *Ann Thorac Surg*. 1991;52:514-517.
38. Buller HR, ten Cate JW, Sturk A, Thomas LL. Validity of the endotoxin assay in post surgical patients. *Prog Clin Biol Res*. 1985;189:405-417.
39. Munster AM, Smith-Meek M, Dickerson C, Winchurch RA. Translocation: incidental phenomenon or true pathology? *Ann Surg*. 1993;218:321-326.
40. Bion JF, Badger I, Crosby HA, et al. Selective decontamination of the digestive tract reduces gram-negative pulmonary colonization but not systemic endotoxemia in patients undergoing elective liver transplantation. *Crit Care Med*. 1994;22:40-49.
41. Deitch EA, Morrison J, Berg R, Specian RD. Effect of hemorrhagic shock on bacterial translocation, intestinal morphology, and intestinal permeability in conventional and antibiotic-decontaminated rats. *Crit Care Med*. 1990;18:529-536.
42. Giamarellou-Bourbouli E, Perdios J, Lelekis M, Economou E, Tsuroulas P, Giamarellou H. Impact of cefuroxime administration on endotoxin (LPS) and tumour necrosis factor- α (TNF α) blood levels in patients suffering from acute pyelonephritis: a preliminary report. *Int J Antimicrob Agents*. 1999;11:115-119.
43. Ziegler EJ, Fisher CJ Jr, Sprung CL, et al; HA-1A Sepsis Study Group. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin: a randomized, double-blind, placebo-controlled trial. *N Engl J Med*. 1991;324:429-436.
44. Greenman RL, Schein RM, Martin MA, et al; XOMA Sepsis Study Group. A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of gram-negative sepsis. *JAMA*. 1991;266:1097-1102.
45. Warren HS, Amato SF, Fitting C, et al. Assessment of ability of murine and human anti-lipid A monoclonal antibodies to bind and neutralize lipopolysaccharide. *J Exp Med*. 1993;177:89-97.
46. Brade L, Engel R, Christ WJ, Rietschel ET. A nonsubstituted primary hydroxyl group in position 6' of free lipid A is required for binding of lipid A monoclonal antibodies. *Infect Immun*. 1997;65:3961-3965.
47. Mullarkey M, Rose JR, Bristol J, et al. Inhibition of endotoxin response by e5564, a novel Toll-like receptor 4-directed endotoxin antagonist. *J Pharmacol Exp Ther*. 2003;304:1093-1102.
48. Lynn M, Rossignol DP, Wheeler JL, et al. Blocking of responses to endotoxin by E5564 in healthy volunteers with experimental endotoxemia. *J Infect Dis*. 2003;187:631-639.
49. Rossignol DP, Lynn M. Antagonism of in vivo and ex vivo response to endotoxin by E5564, a synthetic lipid A analogue. *J Endotoxin Res*. 2002;8:483-488.
50. Gordon BR, Parker TS, Levine DM, et al. Safety and pharmacokinetics of an endotoxin-binding phospholipid emulsion. *Ann Pharmacother*. 2003;37:943-950.
51. Woo YJ, Taylor MD, Cohen JE, et al. Ethyl pyruvate preserves cardiac function and attenuates oxidative injury after prolonged myocardial ischemia. *J Thorac Cardiovasc Surg*. 2004;127:1262-1269.
52. Sappington PL, Fink ME, Yang R, Delude RL, Fink MP. Ethyl pyruvate provides durable protection against inflammation-induced intestinal epithelial barrier dysfunction. *Shock*. 2003;20:521-528.
53. Rossignol DP, Wasan KM, Choo E, et al. Safety, pharmacokinetics, pharmacodynamics, and plasma lipoprotein distribution of eritoran (E5564) during continuous intravenous infusion into healthy volunteers. *Antimicrob Agents Chemother*. 2004;48:3233-3240.