ORIGINAL ARTICLE

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

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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.

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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 molecule is highly conserved across the entire spectrum of gram-negative organisms.[11,12] An assay for antiendotoxin core antibody (EndoCAb) has been used in numerous clinical studies.[13-19] Most patients undergoing cardiac surgery involving cardiopulmonary bypass (CPB) are exposed to endotoxin[20]; therefore, it is attractive to speculate that endotoxin may be partially responsible for the exaggerated systemic inflammatory response that is commonly observed.

See Invited Critique at end of article

A previous study[14] 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.

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METHODS

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 (≥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 µmol/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.

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
DBGA, mean ± SD, No.	3.0 ± 0.8	3.0 ± 0.9	.82
IMA DBGA, % ≥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: DBGA, distal bypass graft anastomoses; EndoCab, antiendotoxin core antibody; IMA, internal mammary artery; 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 concentrations 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

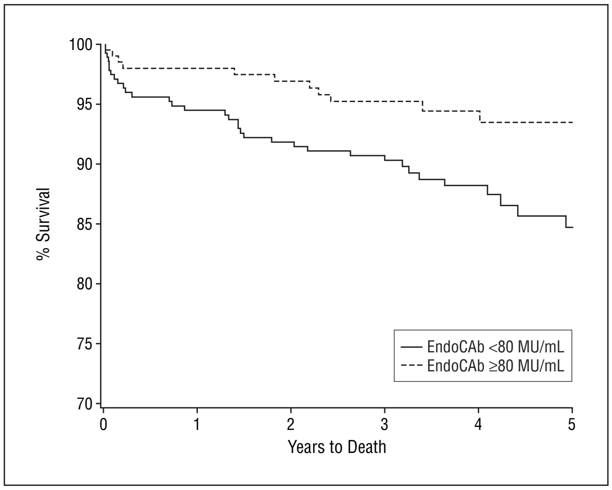

Figure 1. Kaplan-Meier estimates of the probability of 5-year survival based on high vs low antiendotoxin core antibody (EndoCAb) levels. $P = .01$ using the log-rank test.

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 al[30] 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 al[18] 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 al[32] showed that patients randomized to only 7 days of β-adrenergic blockade (vs placebo) exhibited a lower mortality up to 2 years after surgery. These data and others[32-34] 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 affinity[45] 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]

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