Decreased Endotoxin Immunity Is Associated with Greater Mortality and/or Prolonged Hospitalization after Surgery

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Background: Patients undergoing noncardiac surgery often develop postoperative morbidity, potentially attributable to endotoxemia and the systemic inflammatory response syndrome. Endogenous antibodies to endotoxin may confer protection from endotoxin-mediated toxicity. The authors sought to determine the association of preoperative antiendotoxin immunity and death or prolonged hospitalization in a broad population of general surgical patients undergoing major surgery.

Methods: To test the hypothesis that low preoperative serum antiendotoxin core antibody (EndoCAb) concentration is an independent predictor of adverse outcome after general surgery, 1,056 patients undergoing routine noncardiac surgery were enrolled into a prospective, blinded, cohort study. Immunoglobulin M EndoCAb, immunoglobulin G EndoCAb, total immunoglobulin M, and immunoglobulin G concentrations were measured in serum obtained preoperatively. A physiologic risk score using the established POSSUM criteria was assigned preoperatively to each patient. The primary predefined composite end point (postoperative complication) was either in-hospital death or postoperative length of stay greater than 10 days. Multivariate logistic regression was used to test the study hypothesis.

Results: Overall, postoperative complication occurred in 234 of the 1,056 patients (22.1%). Lower immunoglobulin M Endo-CAb concentration ($P = 0.006$) predicted increased risk of postoperative complication independent of POSSUM physiologic risk score ($P < 0.001$). In contrast, total immunoglobulin M and total immunoglobulin G concentrations did not predict adverse outcome. Complications involved multiple organ systems and were generally unrelated to the type or site of surgery, consistent with the systemic inflammatory response syndrome.

Conclusions: Adverse outcome after routine noncardiac surgery is common and is predicted in part by low concentrations of EndoCAb. The authors' findings suggest that endotoxemia may be a cause of postoperative morbidity after routine noncardiac surgery.

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MORE than 40 million surgical procedures are performed in the United States and Europe annually. It is estimated that several million of these procedures represent moderate- to high-risk surgery.[1,2] Although the mortality rate for noncardiac surgery is low, postoperative complications remain common and include infection, gastrointestinal dysfunction, acute lung injury, confusion, and renal dysfunction.[3,4] These complications cause suffering, prolong hospitalization, and consume vital healthcare resources.[2]

A significant proportion of postoperative morbidity may be the manifestation of an exaggerated systemic proinflammatory response observed in many patients after illness or injury.[3] Endotoxin, the lipopolysaccharide component of gram-negative bacteria, may be an important trigger of the systemic inflammatory response syndrome in this setting.[3] Endotoxin is toxic to humans in nanogram quantities[5,6] and is found in large quantities in the human gastrointestinal tract. Surgical patients may be exposed to endotoxin through leakage *via* an impaired gut barrier[7–18] and as a result of manipulation of the bowel during intestinal surgery.[11]

The serum from nearly all mature humans contains endogenous serum antibodies to endotoxin,[19] *i.e.*, antibodies that have arisen in the absence of deliberate immunization with endotoxin antigen. The concentration of these antibodies varies by up to several hundred-fold[19] and appears to be unrelated to an individual's overall health status.[20] It is unknown if antibodies to endotoxin confer protection from endotoxin-mediated toxicity in a broad population of general surgical patients undergoing major surgery. We prospectively tested the hypothesis that low concentrations of preoperative endogenous antibodies to the highly conserved core region of endotoxin are associated with greater mortality and/or prolonged hospitalization after routine noncardiac surgery.

Material and Methods

Selection Criteria

After obtaining institutional review board approval, patients undergoing major, elective, noncardiac surgery at The Mount Sinai Hospital were enrolled in a prospective blinded observational study. The Mount Sinai Hos-

pital Institutional Review Board (New York, NY) did not require patient consent for this study given its observational nature. Patients undergoing the following elective surgical procedures were enrolled: major orthopedic (*e.g.*, revision hip arthroplasty, fusion–instrumentation of multiple lumbar or thoracic vertebrae); major general (*e.g.*, any laparotomy expected to exceed 2-h duration, including partial hepatectomy, pancreatic surgery, colon surgery); major urologic (*e.g.*, radical cystectomy, radical nephrectomy); major vascular (*e.g.*, abdominal aortic aneurysm repair); and major gynecologic (*e.g.*, cancer debulking procedure, abdominal hysterectomy with oophorectomy). These procedures were selected for several reasons: (1) they are routinely performed surgeries; (2) they represent a diverse group of procedure types; and (3) a previous study performed at Duke University Medical Center found these procedures to be associated with prolonged hospitalization and postoperative complications.[4] Patients undergoing emergency surgery were excluded from this study. Although every effort was made to enroll the patients in as consecutive a fashion as possible, this was not always possible because of logistical reasons.

Protocol

Study patients received routine anesthetic care and surgical management, including the routine use of prophylactic antibiotics. Each patient was managed postoperatively according to standard institutional surgical "care maps"; only patients undergoing surgical procedures enabling them to be targeted for discharge from the hospital before the 10th postoperative day were enrolled.

Assays for the determination of immunoglobulin M antiendotoxin core antibody (EndoCAb), immunoglobulin G EndoCAb, total immunoglobulin M, and total immunoglobulin G concentrations were conducted on residual serum that was no longer needed by the Mount Sinai Hospital clinical laboratory. All of these serum samples were obtained preoperatively (within 72 h before surgery) for the determination of routine chemistry tests. Blood samples were collected from patients by venipuncture into nonadditive glass tubes, centrifuged, and serum stored at $-70°C$ until assayed according to the institution's standard operating procedure. Immunoglobulin M and immunoglobulin G EndoCAb concentrations were measured using an enzyme-linked immunosorbent assay described previously.[19,21] In brief, incomplete core, rough, mutant lipopolysaccharide from each of four species of gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, and *Salmonella typhimurium*) were complexed with polymyxin B and coated on polystyrene microplates. Immunoglobulin G EndoCAb and immunoglobulin M EndoCAb concentrations were determined with alkaline phosphatase–conjugated antibodies specific for human

γ-globulin γ or μ heavy chains (Zymed Laboratories, Cambridge BioScience, Cambridge, United Kingdom). Test sera were compared in enzyme-linked immunosorbent assay to a reference serum calibrated in EndoCAb median units, where 100 is the median value for 1,000 healthy adults' immunoglobulin G or immunoglobulin M, respectively.[19] Serum was tested for total immunoglobulin G and immunoglobulin M concentrations by laser nephelometry.[22] All laboratory measurements were performed on coded samples so as to blind the investigators to the patients' identity and outcome.

Risk Assessment

Demographic information was collected and a preoperative physiologic risk score for mortality and morbidity was assigned according to the established POSSUM criteria,[23] which has been recognized as being the most appropriate available score for assessing risk in noncardiac surgical patients.[24]

The POSSUM physiologic score includes the following 12 preoperative factors: age, signs of symptoms of heart failure, abnormal electrocardiogram, respiratory function, blood pressure, heart rate, mental status, hemoglobin concentration, leukocyte count, blood urea concentration, blood sodium concentration, and blood potassium concentration. A point value of 1, 2, 4, or 8 is assigned for 10 of the 12 factors depending on the severity of the abnormality, *e.g.*, 1 point for no dyspnea and 8 points for dyspnea at rest, 1 point for normal potassium concentration and 8 points for a potassium concentration greater than or equal to 6, 1 point for normal heart rate and 8 points for a heart rate greater than or equal to 121 beats per minute. POSSUM physiologic scores can range between 12 and 88. The preoperative physiologic score predicts the risk of morbidity and mortality for noncardiac surgical patients.[23] This score was used in the multivariate logistic regression model to adjust for the effect of patient comorbidities. Use of this risk score obviated the need to adjust for the effect of individual patient comorbidities.

Outcomes

There is no established definition that encompasses all systemic inflammation–related morbidity in postoperative (non–intensive care unit) patients. End points for single-organ system failure are not applicable to studies such as this one. We prospectively defined adverse postoperative outcome as either in-hospital death or a postoperative length of stay greater than 10 days. This endpoint is identical to that used in a similar study involving more than 300 cardiac surgical patients.[20] Postoperative length of stay was defined as the number of days from the day of operation (day 0) to hospital discharge. A 10-day cutoff was selected *a priori* for several reasons. First, at our hospital and in many others, care maps target discharge of the patient from the hospital well

before the 10th postoperative day, with most patients being discharged within 1 week of surgery. In fact, the 25th percentile for postoperative length of stay was 6 days in this cohort; hence, a 10-day cutoff represented a significant prolongation (> 4 days) in the length of stay, not a trivial prolongation of 1 or 2 days that could be a result of "soft factors" such as physician preference or lack of transportation. Second, a retrospective analysis of patients undergoing noncardiac surgery at our institution demonstrated that the majority of patients with a length of stay of greater than 10 days suffered morbidity that could be consistent with an endotoxin-mediated proinflammatory response. It has been shown that the overwhelming majority of patients with prolonged hospitalization after cardiac and noncardiac surgery have morbidity.[4,20] It is important to emphasize that complications potentially related to excessive systemic inflammation are not limited to organ failure observed in patients in the intensive care unit, but include less severe forms of morbidity that nevertheless prolong hospitalization.

Characterization of Postoperative Morbidity

To characterize postoperative complications in patients with prolonged hospitalization (for descriptive purposes only), patients were evaluated on postoperative day 11 using a postoperative morbidity survey that has been previously described in a prospective study of 438 patients undergoing routine noncardiac surgery.[4] The criteria in this survey identify both severe complications (*e.g.*, pulmonary failure–acute respiratory distress syndrome) as well as more subtle morbidity that could delay discharge from the hospital (*e.g.*, moderate pulmonary dysfunction necessitating supplemental oxygen therapy). Existing morbidity scoring systems such as APACHE and SAPS were not used in this study because they are geared to the intensive care unit setting and thus have limited value in most postoperative patients.[25] All outcome measurements were performed by investigators blinded to the patients' laboratory data.

Statistical Analysis

The objective of this study was to test the specific hypothesis that low preoperative serum EndoCAb concentrations are associated with an increased likelihood of postoperative mortality or prolonged hospitalization after controlling for the POSSUM physiologic risk score and total immunoglobulin concentrations. Data from a previous study of 301 patients in a different population showed an incidence of 10% for the primary end point (mortality or prolonged hospitalization).[20] We estimated that for this study, a sample size of more than 1,000 would generate at least 100 events and allow for a stable logistic regression model to test the hypothesis of the study. The required sample size for the current study was estimated to be larger than our previous study of

Table 1. Perioperative Patient Characteristics

Number of patients	1,056
Age (yr)	59 (44–72)
Gender (% female)	49
Race (% W/B/H/A/O)	70/9/9/3/8
Weight (kg)	70 (60–80)
Preoperative serum creatinine (μM)	88.4 (70.7–106.1)
Operative duration (min)	220 (159–285)
Intraoperative fluid (l)	3.5 (2.4–5.0)
Temperature at the end of surgery (°C)	36 (35.4–36.5)
Postoperative complication (%)	22.1
In-hospital death (%)	2.1
Postoperative hospital stay (days)	8 (6–10)

Median and interquartile range where appropriate. Postoperative complication defined as in-hospital death or hospital stay longer than 10 days after surgery.

W = white; B = black; H = Hispanic; A = Asian; O = other or unable to define.

cardiac surgical patients because of the greater heterogeneity of procedures in this study.

The relation between each antibody, as well as preoperative POSSUM risk score, and chance of postoperative complication was explored initially with a series of contingency tables. The variables were then entered in a stepwise multiple logistic regression analysis; those with evidence of a linear relation were entered as continuous variables, and the others were dichotomized at the median. The procedure was set to enter and retain variables with a *P* value of 0.10 or less.

The associations between pairs of continuous variables (*e.g.*, immunoglobulin M EndoCAb and POSSUM risk score) were estimated by the Spearman rank correlation coefficient. All statistical calculations and analyses were conducted using the SAS software system (SAS Institute Inc., Cary, NC).

Results

A total of 1,056 patients were enrolled in the study. Perioperative characteristics (table 1) are presented for the study population. Of note, the median operative duration exceeded 3.5 h, which is consistent with our desire to enroll patients undergoing moderate- and high-risk surgical procedures. The mortality rate was 2.1% (n = 22). The causes of death were as follows: multiple system organ failure (n = 15), pulmonary failure (n = 4), bowel necrosis (n = 1), pulmonary embolus (n = 1), and myocardial infarction (n = 1). Two hundred thirty-four of the 1,056 patients (22.1%) fulfilled the prospectively defined composite outcome. For descriptive purposes only, the incidences of complications on postoperative day 11 are shown in table 2.

Immunoglobulin M EndoCAb concentrations (median units) ranged from 9 to 4,946, with a median of 139. Immunoglobulin G EndoCAb concentrations (median units) ranged from 9 to 4,638, with a median of 249. POSSUM preoperative physiologic risk scores ranged

Table 2. Postoperative Morbidity

Morbidity Type	% with Morbidity Type on Postoperative Day 11
Pulmonary	27 (21–33)
Infectious	52 (45–59)
Renal	20 (14–26)
Gastrointestinal	42 (35–49)
Cardiovascular	14 (9–19)
Neurologic	10 (6–14)
Wound complication	12 (7–17)
Hematologic	7 (3–11)
Pain	17 (12–22)

Results are expressed as % (95% confidence interval). The incidences of morbidity types do not add up to 100% because many patients had more than one type of complication.

from 12 to 42, with a median of 18. Median immunoglobulin M EndoCAb concentrations were 99 and 150 in patients with and without postoperative morbidity, respectively. Median immunoglobulin G EndoCAb concentrations were 232 and 252 in patients with and without postoperative morbidity, respectively.

Immunoglobulin M and immunoglobulin G EndoCAb concentrations were not highly correlated (Spearman correlation coefficient, 0.374), nor were EndoCAb concentrations and the physiologic preoperative POSSUM scores (immunoglobulin M Spearman correlation coefficient, -0.200; immunoglobulin G Spearman correlation coefficient, -0.011). Poor to marginal Spearman rank correlations were found between EndoCAb concentrations and their respective total concentrations (immunoglobulin G, 0.328; immunoglobulin M, 0.653).

Figure 1 presents the percentage of patients having a postoperative complication in approximate quartiles of immunoglobulin M EndoCAb concentration. Immunoglobulin G EndoCAb, total immunoglobulin M, and total immunoglobulin G showed no evidence of association
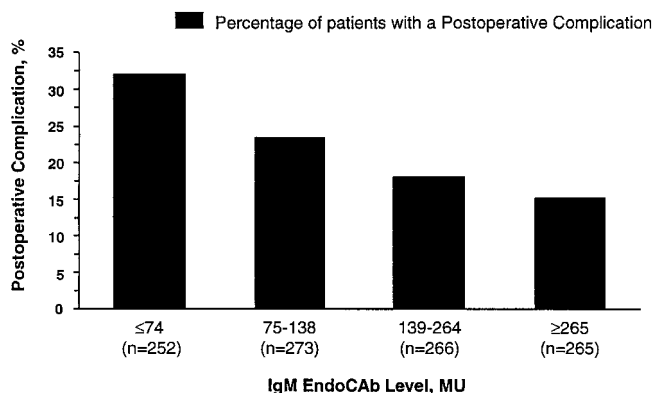

Fig. 1. Observed relation between serum preoperative immunoglobulin M (IgM) antiendotoxin core antibody (EndoCAb) concentration and risk of postoperative complication. The figure presents the percentage of patients (solid black bars) having a postoperative complication (mortality or prolonged hospitalization) in approximate quartiles of immunoglobulin M EndoCAb concentration. Multiple logistic regression was used to test this relation while controlling for preoperative risk factors (see Results).

with postoperative complication on either the univariate or the multivariate analyses. Immunoglobulin M EndoCAb concentration and POSSUM physiologic risk score were each significantly associated with a poor outcome (table 3) and, in the multiple logistic regression analysis, proved to be independent predictors. Lower immunoglobulin M EndoCAb concentration predicted increased risk of postoperative complication, independent of the effect of POSSUM physiologic risk score ($P = 0.048$, odds ratio = 0.925, 95% confidence interval, 0.856–0.999 for each 100-point increase in immunoglobulin M EndoCAb concentration). POSSUM physiologic risk score was also significant in this model ($P < 0.001$, odds ratio = 1.088, 95% confidence interval, 1.062–1.114).

Discussion

We have completed a large prospective study evaluating the association between preoperative antiendotoxin immune status and death or prolonged hospitalization in 1,056 patients undergoing routine noncardiac surgery. Low preoperative serum immunoglobulin M EndoCAb concentration is associated with adverse outcome after routine noncardiac surgery and may account in part for variability in outcome observed among patients with similar degrees of preoperative risk.

Vital healthcare resources are used to care for patients with postoperative morbidity. Endotoxemia has been reported to occur during major surgery[8–15] and may initiate proinflammatory cascades and cause organ dysfunction.[3,5,6] The serum from mature humans contains endogenous antibodies to endotoxin, presumably elicited in response to intermittent exposure to intestinal endotoxin.[19] Although serotype-specific antibodies are only protective against endotoxin from the homologous serotype,[26] antibodies directed against the core structure of endotoxin should theoretically be cross-reactive against most enteric lipopolysaccharides.[27,28] Therefore, in this study we used an assay that measures the serum concentration of EndoCAb.[19,21] The serum concentration of these antibodies has been previously measured in more than 1,000 human volunteer blood donors and demonstrate marked variability, with some subjects having several hundred-fold higher concentrations than others.[19]

Antiendotoxin core antibody concentrations have been measured previously in patients in the intensive care unit with established sepsis syndrome[29,30] and in cardiac surgical patients.[20,31,32] Results from studies involving these specialized patient groups cannot be generalized to the large number of patients who undergo noncardiac surgery for several reasons. Patients with sepsis syndrome are often admitted to an intensive care unit and enrolled into clinical studies hours or days after the inciting illness or injury.[29,30] Thus, in these studies, it

Table 3. Univariate Associations between Study Variables and Postoperative Complication

	Median (Interquartile Range)	P	OR	95% CI
IgM EndoCAb (MU)	139 (76–266)	0.006	0.890	0.820–0.966
IgG EndoCAb (MU)	249 (152–421)	NS		
Total IgM (g/dl)	1.3 (0.8–1.9)	NS		
Total IgG (g/dl)	11.3 (8.9–14.4)	NS		
POSSUM Physiological Risk Score	18 (15–23)	< 0.001	1.092	1.066–1.118

EndoCAb = antiendotoxin-core antibody, which historically is expressed in median units (MU; see Methods for more detail). Not significant (NS) = P > 0.1. P = probability of no association between variable and postoperative complication from the univariate logistic regression (see text for results from multivariable logistic regression). For immunoglobulin (Ig)M EndoCAb, the odds ratio (OR) and 95% confidence interval (CI) show the effect of a 100-point increase. For POSSUM Physiological Risk Score, the OR and 95% CI show the effect of a 1-unit increase.

is unclear whether antiendotoxin antibody concentrations are a predictor of disease *versus* a marker for patients who have sustained a greater insult. Studies involving cardiac surgery patients enrolled patients undergoing cardiopulmonary bypass, which has been shown to have marked effects on the immunologic and hematologic systems and may result in organ dysfunction in the absence of endotoxemia. Furthermore, in cardiac surgical patients, cardiopulmonary bypass can have deleterious effects on splanchnic perfusion and hence cause leakage of endotoxin.[3] This mechanism cannot be relevant to patients undergoing general surgery because they are not subjected to extracorporeal circulation.

In contrast to intensive care unit and cardiac surgical patients, little is known about the role of preoperative antiendotoxin immune status in general surgical patients. In a study of 86 women undergoing major gynecologic surgery, conducted move than a decade ago, the presence of preoperative antiendotoxin antibodies was associated with decreased postoperative infections.[33] However, this study did not quantitate antiendotoxin antibodies, measure total immunoglobulins, assign risk scores to patients preoperatively, or comment on postoperative length of stay or other complications.

We prospectively studied the association of preoperative antiendotoxin immune status and death or prolonged hospitalization in 1,056 patients undergoing routine noncardiac surgery. Several conclusions can be drawn from our study. First, low preoperative immunoglobulin M EndoCAb concentration predicted morbidity after noncardiac surgery independent of several known preoperative risk factors and overall humoral immunity. Our findings, in conjunction with similar results described in previous work involving cardiac surgical patients, suggest that endotoxemia may be an important cause of postoperative morbidity in a broad range of surgical procedures and does not appear to be limited to patients undergoing cardiopulmonary bypass.

Second, preoperative immunoglobulin M and immunoglobulin G EndoCAb concentrations were higher in our study involving patients in the United States compared with a series of 1,000 healthy Scottish volunteers.[32] This variation in endogenous immunity may reflect genetic

differences, environmental factors (*e.g.*, diet, which can alter intestinal flora), and/or a greater degree of exposure to endotoxin in patients with medical problems requiring surgery. This issue does not impact on our results because we did not analyze the data with regard to "normal" values. Differences of these concentrations in varying populations warrant further study.

A potential limitation of our study relates to the selection of a primary end point, a challenge to any investigator interested in studying postoperative morbidity attributable to excessive systemic inflammation. Mortality may be an appropriate end point in sepsis studies involving critically ill patients given the high incidence of death and the overwhelming magnitude of systemic inflammation in these patients. Surgical patients, however, appear to develop a less severe form of systemic inflammation that rarely results in death but nevertheless causes morbidity and prolonged hospitalization. There are no validated postoperative morbidity scores that can be easily used in large prospective studies such as ours. Existing morbidity scoring systems such as APACHE were not used in this study because they are geared to the intensive care unit setting, generally rely on laboratory tests that are not routinely available in postoperative patients, and thus have limited value in patients after routine elective surgery.[25] The morbidity observed in patients with prolonged hospitalization in our study involved organ systems unrelated to the type or site of surgery. Infectious, pulmonary, and renal complications were observed frequently. Although our study was not designed to prove a causal relation, the pattern of complications observed is consistent with an exaggerated systemic inflammatory response, perhaps partly as a result of exposure to endotoxin.

A major limitation of any observational study such as ours is that low antiendotoxin immunity may not be a cause of poor outcome but merely a marker for sicker patients with higher operative risk and poorer general immunity. Our study minimized this possibility by using an established preoperative risk scoring system to quantify degree of risk, and we also measured and accounted for total immunoglobulin concentrations. Preoperative antiendotoxin antibody concentrations and preoperative risk score and total immunoglobulin concentrations

were not well correlated, which is consistent with the results of the multivariate analysis in which immunoglobulin M EndoCAb concentration was an independent predictor of postoperative morbidity. Results from controlled studies in which endotoxin is selectively neutralized will be necessary to confirm the clinical relevance of endotoxemia. Several antiendotoxin-related agents are currently under development that may potentially benefit surgical patients if given prophylactically.[34–39]

There are two commonly stated criticisms of the theory that endotoxemia is an important cause of postoperative morbidity. One criticism relates to the low incidence of culture-proven bacteremia in surgical patients. However, endotoxemia is clearly prevalent in these patients and usually exists in the setting of negative blood cultures.[12] In fact, the routine administration of prophylactic antibiotics to surgical patients would be expected to kill or prevent the growth of susceptible gram-negative bacteria and might actually elevate endotoxin blood concentrations through increased shedding of endotoxin.[40] Studies attempting to detect endotoxemia probably underestimate its incidence given the intermittent nature of endotoxemia. Another criticism stems from the failure of two anti–lipid A monoclonal antibodies (HA-1A, Centocor, Malvern, PA; and E5, Xoma, Berkeley, CA) to improve outcome on an intention-to-treat basis in intensive care unit patients with established sepsis.[41,42] These monoclonal antibodies, however, were tested in patients with established sepsis and organ failure, which is an entirely different setting than elective surgical patients who are more likely to benefit from prophylaxis with other endotoxin-related strategies. Moreover, these anti–lipid A monoclonal antibodies do not bind to endotoxin with high affinity,[43] which may also explain their lack of demonstrable efficacy.

In summary, low preoperative endogenous antiendotoxin antibody concentrations were associated with death and/or prolonged hospitalization after routine noncardiac surgery independent of the POSSUM physiologic risk score and overall humoral immunity. Our findings suggest that endotoxemia may be a cause of postoperative morbidity after routine noncardiac surgery.

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