Elevated Levels of Angiopoietin-2 as a Biomarker for Respiratory Failure After Cardiac Surgery

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Objectives: Angiopoietin-1 and angiopoietin-2 are important factors in regulating endothelial vascular permeability. This study evaluated perioperative changes in serum levels of angiopoietin-1 and -2 in patients undergoing cardiac surgery.

Design: Measurement of serum levels of angiopoietin-1 and angiopoietin-2 in samples collected during a previously conducted prospective, multicenter, observational study.

Setting: Three university hospitals.

Participants: Eighty-four adult patients undergoing cardiac surgery.

Intervention: Serum levels of angiopoietins were measured at baseline, immediately after surgery, and the day after surgery (POD-1).

Measurements and Main Results: Serum levels of angiopoietin-2 were elevated by POD-1 (median 3.3 ng/mL, interquartile range [IQR] 2.5-4.6 ng/mL) compared with baseline (median 1.6 ng/mL, IQR 1.3-2.1 ng/mL, $p < 0.0001$), and angiopoietin-1 levels were decreased immediately after surgery (baseline median 23.2 ng/mL, IQR 10.2-32.8 ng/mL; postoperative median 8.0 ng/mL, IQR 1.5-13.2 ng/mL, $p < 0.0001$). Angiopoietin-2 levels on POD-1 in patients undergoing off-pump coronary artery bypass grafting were significantly lower than those in patients undergoing aortic surgery ($p = 0.0009$) and valve surgery ($p = 0.008$). Angiopoietin-2 levels on POD-1 had a predictive performance of the area under the curve (AUC) of the receiver operating characteristic curve 0.74 for mechanical ventilation >3 days. Angiopoietin-1 levels and the angiopoietin-2/angiopoietin-1 ratio showed lower predictive performance (AUC values 0.58 and 0.68, respectively).

Conclusions: Angiopoietin-2 serum levels were elevated after cardiac surgery. Elevated angiopoietin-2 had a good predictive performance for respiratory failure after cardiac surgery, perhaps reflecting the severity of lung dysfunction related to postoperative increases in vascular permeability.

KEY WORDS: biomarker, lung injury, cardiac surgery, angiopoietin-1, angiopoietin-2, Tie-2 Receptors, vascular permeability

SURGICALLY INDUCED systemic inflammation often results in elevated vascular permeability after cardiac surgery. Subsequent tissue edema and organ dysfunction are associated with prolonged stays in the intensive care unit (ICU). Recent studies have shown that reactions regulated by the Tie-2 receptor and angiopoietins are thought to be an important regulatory mechanism for endothelial vascular permeability.[1,2] In the steady state, angiopoietin-1, released from vascular pericytes, binds to Tie-2 receptors and promotes junctional integrity by regulating the accumulation of adhesion proteins; most importantly, vascular endothelial cadherin at endothelial cell-to-cell junctions.[2] However, once systemic inflammation occurs, angiopoietin-2 is released from the Weibel-Palade bodies in endothelial cells, which competitively binds to Tie-2 receptors, resulting in an increase in endothelial permeability. In this context, previous studies have shown that angiopoietin-2 is a biomarker for the severity of sepsis[3–5] and acute lung injury.[6–8] Therefore, the authors hypothesized that surgically induced systemic inflammation would result in an elevation of serum angiopoietin-2 and a decline in levels of angiopoietin-1 and that the magnitude of these changes would be associated with lung dysfunction following adult cardiac surgery.

The objectives of this study were to determine perioperative changes in serum levels of angiopoietin-1 and angiopoietin-2 in adult patients undergoing cardiac surgery and to assess whether those changes were associated with systemic inflammation after the use of cardiopulmonary bypass (CPB). The authors also studied the predictive performance of angiopoietin-1 and angiopoietin-2 for postoperative respiratory failure. They studied clinical data and serum samples from a previous multicenter-observational study that was designed to evaluate the performance of the soluble isoform of the receptor for advanced glycation end-products (sRAGE) as a biomarker for postoperative respiratory failure.[9] The primary endpoint of the original study was to investigate the relationship between sRAGE levels and duration of mechanical ventilation. For this purpose, the study population was designed to eliminate factors related to prolonged mechanical ventilation other than lung dysfunction, as described in the Methods section. These characteristics are suitable for studying the relationship between the levels of angiopoietins and lung dysfunction.

METHODS

Clinical data and biologic samples for the current study were obtained from patients enrolled in a multicenter observational study to evaluate perioperative serum levels of the soluble isoform of the receptor for advanced glycation end-products (sRAGE) as a biomarker for respiratory failure after cardiac surgery.[9] The original study is registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (ID: UMIN000010674; www.umin.ac.jp/ctr/index/htm). A total of 87 patients undergoing cardiac surgery

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were enrolled in the original study. Serum sRAGE levels were found to be elevated immediately after surgery, and the range of elevation was associated with respiratory failure morbidity after cardiac surgery. The details and results have been published previously in full.[9] Three university hospitals participated in this study, and approval for the protocol was obtained from each institutional review board for clinical studies. Patients undergoing cardiac surgery in each of the 3 institutions were included. Preoperative exclusion criteria were (1) age <20 years, (2) history of myocardial infarction within 30 days before the day of surgery, (3) preoperative diagnosis of pneumothorax, (4) diagnosis of chronic obstructive pulmonary disease, (5) neuromuscular disease, (6) morbid obesity (body mass index >40 kg/m^2), and (7) systemic connective tissue disease. Postoperative exclusion criteria were (1) severe neurologic complications requiring mechanical ventilation, (2) the need for an extracorporeal circuit because of cardiac failure, and (3) patients whose pulmonary artery occlusion pressure was >18 mmHg in the first postoperative week. Each patient provided written informed consent.

Anesthetic management was conducted by the anesthesiologists in charge of each case. Anesthesia was induced by intravenous administration of midazolam (0.05-0.1 mg/kg) and fentanyl (2-6 μg/kg), and tracheal intubation was facilitated with vecuronium (0.2 mg/kg). Anesthesia was maintained with propofol (5-6 mg/kg/h) or sevoflurane (1%-2.5%), supplemented with continuous infusion of fentanyl (2-4 μg/kg/h) and vecuronium (2 mg/h). In patients undergoing surgery using cardiopulmonary bypass, methylprednisolone (1 g) was administered before the start of cardiopulmonary bypass. In some patients managed with deep hypothermia, the dose of methylprednisolone was increased (by 1.5 g in 3 patients and 2 g in 4 patients). Management of the pulmonary artery catheter was based on the decision of the attending physicians.

Postoperative mechanical ventilation in the ICU began with synchronized intermittent mandatory ventilation with pressure support (PS) (5-10 cm H_2O) with the same tidal volume setting used in the operating room. The authors aimed to decrease the frequency of mandatory ventilation to the range necessary to maintain minimal minute volume (>30 mL/kg of body weight) then decreased the PS level if needed. Positive end-expiratory pressure (PEEP) and F_IO_2 were set according to the patient's oxygenation level (PaO_2/F_IO_2). First, F_IO_2 was reduced stepwise to 0.4 then PEEP was decreased stepwise in maximal increments of 3 cm H_2O to the final target value of 5 cm H_2O. A weaning trial was attempted at least 3 times a day, and the result was judged successful when a patient passed the following tests successfully: (1) frequency of spontaneous breathing (f_{spont}) <25 breaths/min, (2) maintenance of arterial partial pressure of carbon dioxide ($PaCO_2$) between 35 and 50 mmHg, (3) $SpO_2 > 90\%$, (4) patient remained clinically stable, and (5), in cases without mandatory ventilation, tidal volume >5 mL/kg of predicted body weight and rapid shallow breathing index (calculated as f_{spont}/tidal volume [L]) <100 within the allowed pressure support range. Predicted body weight (PBW) was calculated from the following equation: Male PBW (kg) $= 50 + 0.91$ (height in cm-152.4), and female PBW (kg) $= 45.5 + 0.91$ (height in cm-152.4). During the weaning process, a change to controlled ventilation was conducted in cases of induction of general anesthesia, eg, for an invasive procedure or surgery.

Chest radiographs were taken during the first admission to the ICU (POD 0), POD 1, 3, and 7, and scored by the number of quadrants of alveolar consolidation.[10] Chest radiographs were quantified by an attending physician blinded to the values of the biomarkers. The worst score among the 4 chest radiographs was recorded.

Because of limited availability of serum from three patients, 84 patients from the original clinical study were included in the current study. The authors measured angiopoietin-1 and angiopoietin-2 in serum samples collected at the following 3 time-points: (1) immediately after the induction of general anesthesia (baseline), (2) at admission to the ICU immediately after surgery (post-op), and (3) the day after surgery (POD-1). Commercially available enzyme-linked immunosorbent assay kits were used to measure serum angiopoietin levels (Quantikine DANG10 for angiopoietin-1 and Quantikine DANG20 for angiopoietin-2, R&D Systems, Minneapolis, MN). Data for postoperative serum interleukin (IL)-8 and N-terminal-pro brain natriuretic peptide (NT-proBNP) were obtained for the same sample of 84 patients from the results of the original study.

All statistical analyses were performed using STATA/IC software (version 11, StataCorp, College Station, TX). For 2-group comparisons, the authors used the Mann-Whitney U-test. For intergroup comparisons, the authors used the chi-square test for categoric data and the Kruskal-Wallis test for numeric data with non-normal distribution. In the latter case, post-hoc multiple comparisons were analyzed with Scheffe's test or Steel's test as appropriate. To find clinical variables with significant association with angiopoietin-2 levels on POD-1, multiple regression analysis was performed. Clinical data consisted of patients' demographic data (age, sex, body weight, and smoking history), past history of diabetes mellitus, laboratory data on POD-1 (white blood cell count, hemoglobin, platelet count, albumin, creatinine, PaO_2/F_IO_2, and Acute Physiology and Chronic Health Evaluation II [APACHE-II] score), and variables related to the surgery (type of surgery, intraoperative packed red cell transfusion, duration of surgery and duration of CPB). Furthermore, the association between the duration of postoperative mechanical ventilation and clinical variables including serum levels of angiopoietin-2 was tested using multiple regression analysis. Because the results of this multiple regression analysis showed that the duration of postoperative mechanical ventilation significantly correlated with age, serum albumin, APACHE-II score, and angiopoietin-2 levels, the authors analyzed the association between angiopoietin-2 levels and morbidity of prolonged ventilatory support (>3 days) by multiple logistic regression analyses using age, serum albumin level, and APACHE II score as possible confounding factors. In these multivariable tests, backward elimination was performed at $p > 0.20$. The authors used Spearman's test for correlation analyses, with the correlation graded depending on the r_s value as follows: $0.7 > r_s > 0.4$ as moderate correlation and $0.4 > r_s > 0.2$ as weak correlation. To evaluate the predictive performance for prolonged mechanical ventilation ($>$ 3 days), non-parametric receiver operating characteristic (ROC) curves were computed. The area under the curve (AUC) of the ROC curve was calculated with 95% confidence intervals (CIs), and chi-square tests were performed for comparisons. The optimal cut-off point was determined by calculating Youden's index.[11,12] Statistical significance was defined as $p < 0.05$.

RESULTS

Baseline demographic data and clinical variables are summarized in Table 1. Twenty patients underwent aortic surgery, 33 patients underwent valve surgery, and 31 patients underwent off-pump coronary artery bypass grafting (OPCAB). The characteristics of the study cohort were approximately the same as those of the original study.[9] The duration of surgery was significantly longer in the aortic surgery group ($p = 0.012$ *v* the valve surgery group; $p < 0.0001$, *v* the OPCAB group), whereas there were no significant differences in the CPB time between the aortic surgery group and the valve surgery group. Intraoperative median tidal volume was 9.2 mL/kg (interquartile range [IQR] 8.1-10.2 mL/kg), and there were no significant differences among the 3 surgical groups. Although intraoperative positive end-expiratory pressure levels were slightly higher in the aortic surgery group, there were no significant differences in peak inspiratory pressure among the 3 surgical