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Exploration of disease mechanism in acute kidney injury using a multiplex bead array assay: a nested case–control pilot study

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Abstract

Background: Acute kidney injury (AKI) following cardiac surgery with cardiopulmonary bypass (CPB) causes increased morbidity and mortality.

Objective: To evaluate the plasma profile of biomarkers potentially involved in AKI development following CPB.

Methods: In a nested case–control study, plasma levels of 27 biomarkers in 11 AKI cases were compared with 25 controls.

Results: Pre-CPB, plasma levels of epidermal growth factor and macrophage inflammatory protein-1β, 2 h following CPB, soluble vascular cell adhesion molecule-1 (sVCAM-1), fractalkine and macrophage inflammatory protein-1α, and at later time points, sVCAM-1 and interleukin-6 were associated with AKI.

Conclusion: Biomarkers associated with AKI following CPB may merit further study.

Keywords: *Cardiac surgery; cardiopulmonary bypass; acute renal failure; pathophysiology; biomarker*

Introduction

Acute kidney injury (AKI) is a serious complication of cardiac surgery with cardiopulmonary bypass (CPB). It occurs in 5–20% of patients undergoing this procedure (Lassnigg et al. 2004, Liangos et al. 2005), and is associated with excessive in-hospital mortality (Lassnigg et al. 2004, Chertow et al. 1998). The extent of the host inflammatory response elicited by CPB, partly driven by circulating leukocytes as a result of contact activation by the extracorporeal circuit, is an important contributor to the development of AKI (Kuijpers et al. 1992, Elgebaly et al. 1994, Cremer et al. 1996, Wan et al. 1997). In addition, further disease mechanisms have been implicated, including ischaemia reperfusion, thrombosis and exposure to endotoxin (Laffey et al. 2002, Wan et al. 1997).

The evaluation of multiple AKI-related biomarkers, if geared toward gaining insight into disease mechanisms in both early and late stages of AKI, may potentially add important information to this field of study. In contrast, research efforts on biomarkers in human AKI have so far primarily focused on the evaluation of a few biomarkers for early detection of AKI in a clinical setting, i.e. prior to a rise in serum creatinine (Parikh et al. 2006, Haase et al. 2009, Liangos et al. 2009a, b). The application of multiplex assays that employ low-volume biological samples for the simultaneous measurement of multiple markers is a novel technology that can be applied to the study of human disease (Skogstrand et al. 2005, Ray et al.

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2005, Kofoed et al. 2006). In this pilot nested case–control study, we applied the multiplex bead array assay platform to characterize the profile of 27 circulating proteins of interest that might be incriminated in the development of AKI following CPB.

Patients and methods

Study design and setting

This was a nested case–control study to an ongoing, large, prospective cohort study of patients undergoing on-pump cardiac surgery, conducted at two tertiary care hospitals, Tufts Medical Center (Boston, MA, USA) and St Elizabeth's Medical Center (Boston, MA, USA). This parent study was designed to evaluate the relationship between single-nucleotide polymorphisms and AKI in the setting of CPB (Liangos et al. 2007). Pregnant women, patients with pre-existing AKI, end-stage renal disease on maintenance dialysis, solid organ or bone marrow transplant recipients, and those undergoing 'off-pump' or 'minimally invasive' coronary artery bypass grafting were excluded.

All consecutive patients over 18 years of age scheduled for on-pump cardiac surgery (elective, urgent or emergent coronary artery bypass grafting, valve surgery or both), who were enrolled into the principal cohort between January 2004 and October 2007, were eligible for selection into the nested case–control study.

We selected 11 patients with AKI as defined by an increase in serum creatinine by ≥50% within 72 h following CPB, to constitute our case group. This definition was based on stage 1 of the original RIFLE classification of AKI (Bellomo et al. 2002, Mehta & Chertow 2003), which has been widely used in studies of AKI following cardiac surgery (Kuitunen et al. 2006, Mishra et al. 2005, Parikh et al. 2006). Using a nearest-neighbour matching technique (MatchIt package, R software; Ho et al. 2007), we also selected 25 AKI-free patients as defined by a perioperative serum creatinine fluctuation of less than 0.2 mg dl^{-1}, to constitute our control group. The nearest-neighbour matching technique selects the best control match for each case by using the logit distance measure. This method represents a propensity score matching using a logistic regression model for predicting the case status and includes age, sex, preoperative left ventricular ejection fraction, surgery type and CPB perfusion time as covariates in the model. We did not perform an exact or coerced matching on any of the above covariates.

Written informed consent was obtained from all study participants, and the institutional review boards of the two participating centres approved the study protocol.

Data collection

Medical records of study participants were reviewed prospectively to retrieve preoperative variables including baseline demographic characteristics and coexisting conditions. Intraoperative variables included surgery type, aortic cross-clamp time and CPB perfusion time; postoperative variables included serial serum creatinine values, the Acute Physiology and Chronic Health Evaluation (APACHE) II score (Knaus et al. 1985) calculated on postoperative day 1, total mechanical ventilation time and length of stay in the intensive care unit.

Blood sampling

EDTA-anticoagulated blood was collected at enrolment (prior to CPB), and 2, 24 and 48 h after the discontinuation of CPB. Samples were kept on ice and processed within 30 min of collection. For plasma separation, samples were centrifuged for 10 min at 3000 rpm, followed by an additional 10 min at 13000 rpm after transfer of the supernatant, in order to remove platelets. Plasma samples were then aliquoted and stored at –80°C until assayed.

Multiplex bead array assay

We selected 27 gene products of interest representing 11 cytokines, six chemokines, three adhesion molecules, four growth factors, two pro-oxidant and extracellular matrix enzymes and one fibrinolysis inhibitor (Appendix 1). These biomarkers were included based on a literature review, suggesting a potential role in the pathogenesis of AKI. Simultaneous quantification of these 27 proteins in the plasma was performed on the Luminex platform using two customized commercially available multiplex immunoassay panels (Linco Inc., St Louis, MO, USA). In brief, 25 µl of standard, quality control or plasma sample were added to each well of a 96-well plate with 25 µl of the bead solution. The plate was sealed, covered with aluminum foil, and incubated overnight for 16–18 h with agitation on a plate shaker at 4°C. The plate was then washed twice with 200 µl per well of wash buffer, removing buffer by vacuum filtration between each wash. Following the addition of 25 µl of a detection antibody cocktail into each well, the plate was incubated at room temperature for 1.5 h. Twenty-five microlitres of a streptavidin–phycoerythrin solution was then added to each well, and the plate was incubated at room temperature for 30 min, and then analysed on the Luminex 100 IS analyser (Luminex, Inc., Austin, TX, USA). The data output was saved and evaluated as median fluorescence intensity using the Luminex 100 IS curve-fitting software version 2.3. A five-parameter weighting logistic method was used. The biomarkers were tested individually and in

combination to ensure that there was no cross-reactivity between individual markers. All measurements were performed in duplicate and by a blinded investigator.

Statistical analyses

Continuous variables are presented as means (± SD) or medians (with the interquartile range), and categorical variables as numbers and percentages. Continuous variables were compared with the use of the Friedman test (for repeated measures over time) and the Wilcoxon rank sum test (at each time point), and categorical variables with the use of the Fisher's exact test. Receiver-operating characteristic (ROC) curves were constructed to explore the association of each biomarker with the development of AKI at the specific time points. The 95% confidence interval (CI) for each area-under-the-ROC curve (AUC) was calculated. The comparison of the AUCs was performed according to the method by DeLong et al. (1988). We used different AUC cut-off values to assess the robustness of the association, suggesting possible involvement in the disease mechanism (AUC 0.50–0.69, 0.70–0.89 and ≥0.90 for mild, moderate and strong association, respectively). All hypothesis testing was two-tailed, and a p-value of less than 0.05 was used to indicate statistical significance. All statistical analyses were performed using SAS (SAS Institute, Cary, NC, USA) version 9.1.

Table 1. Characteristics of the nested case–control study groups

	Control group ($n=25$)	AKI group ($n=11$)	p-Value
Preoperative variables			
Age (years)	70 ± 12	74 ± 9	0.40
Serum creatinine (mg dl^{-1})	1.1 (0.3)	1.1 (0.2)	0.95
Serum urea nitrogen (mg dl^{-1})	19 (8)	17 (5)	0.70
eGFR (ml min^{-1} × 1.73 m^2)	67 (18)	65 (8)	0.62
Women (%)	24	27	0.83
Diabetes mellitus (%)	17	27	0.47
Hypertension (%)	71	73	0.91
History of stroke (%)	4	18	0.17
Peripheral vascular disease (%)	13	27	0.28
Left ventricular ejection fraction (%)	53 ± 14	48 ± 17	0.36
Use of radiocontrast agents (%)	56	64	0.67
Procedure status (%)			
Elective	29	36	0.74
Urgent	67	64	
Emergent	4	0	
Valvular surgery (%)	67	91	0.36
Intraoperative variables			
Aortic cross clamp time (min)	92 ± 33	99 ± 34	0.58
CPB perfusion time (min)	127 ± 37	143 ± 53	0.31
Postoperative variables			
Day 1 APACHE II score	9 ± 2	16 ± 5	0.002
Total mechanical ventilation time (h)	12 (9–16)	23 (18–65)	0.002
Length of stay in the intensive care unit (days)	3 (2–3)	4 (3–6)	0.01

Data presented as mean ± SD, median (interquartile range) or percentage. Comparisons were performed by the Fisher's exact test and Wilcoxon test. AKI, acute kidney injury; eGFR, estimated glomerular filtration rate according to MDRD formula (Levey et al. 1999); CPB, cardiopulmonary bypass; APACHE, Acute Physiology and Chronic Health Evaluation.

Results

Characteristics of the AKI and control group

Table 1 displays the characteristics of the AKI and control groups. In brief, the two groups were well matched with respect to the matching variables namely age, sex, preoperative left ventricular ejection fraction, surgery type and CPB perfusion time. The other pre- and intraoperative variables were also not significantly different between the two groups.

Figure 1 displays the perioperative kinetics of serum creatinine, reflecting excellent separation between the AKI and control group. Postoperatively, the AKI group had a significantly higher APACHE II score, prolonged total mechanical ventilation time, and consequently prolonged stay in the intensive care unit (Table 1).

Effect of CPB on plasma biomarker profiles

As shown in Table 2, in response to CPB, there was a significant increase in plasma level of several cytokines (e.g.

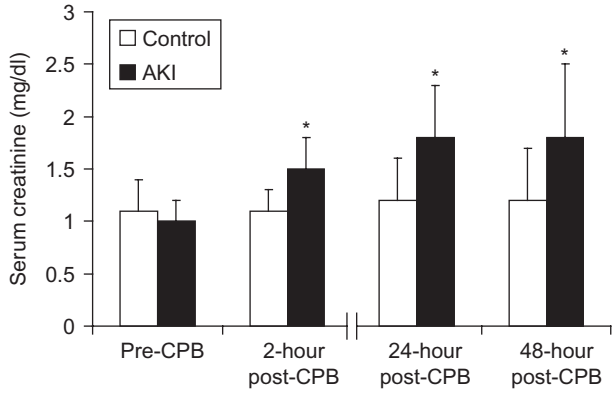

Figure 1. Perioperative profiles of serum creatinine following cardiac surgery in the acute kidney injury (AKI) ($n=11$) and control groups ($n=25$). The data are presented as mean values, and the error bars represent standard deviation. CPB, cardiopulmonary bypass; *p <0.02 vs. control group.

Table 2. Selected, time-dependent, acute kidney injury-independent plasma biomarker response.

	Pre-CPB	2-h post-CPB	24-h post-CPB	48-h post-CPB	*p*-Value
Cytokines					
IL-1α (pg ml^{-1})	3.2 (3.2, 10.9)	3.2 (3.2, 4.5)	14.3 (3.2, 97.2)	11.6 (3.2, 1032.7)	0.006
IL-1Ra (pg ml^{-1})	112.4 (38.5, 322.0)	824.3 (67.1, 5149.1)	202.9 (45.2, 703.4)	204.8 (72.5, 560.2)	<0.0001
IL-4 (pg ml^{-1})	3.2 (3.2, 3.2)	3.2 (3.2, 5.3)	53.8 (3.2, 322.1)	36.7 (3.2, 931.5)	0.02
IL-6 (pg ml^{-1})	4.2 (3.2, 20.3)	68.9 (34.1, 208.6)	216.9 (95.1, 353.0)	159.6 (115.1, 318.2)	<0.0001
IL-10 (pg ml^{-1})	6.6 (3.2, 27.5)	193.3 (44.2, 412.6)	70.7 (25.0, 177.3)	45.4 (11.3, 164.1)	<0.0001
Chemokines					
IL-8 (pg ml^{-1})	3.2 (3.2, 12.8)	14.5 (3.2, 49.0)	21.0 (5.7, 57.7)	21.7 (4.7, 49.7)	<0.0001
MCP-1 (pg ml^{-1})	52.7 (11.3, 117.6)	163.1 (78.7, 329.6)	133.8 (78.5, 178.4)	197.1 (112.3, 255.1)	<0.0001
MIP-1α (pg ml^{-1})	3.2 (3.2, 4.7)	3.8 (3.2, 7.2)	3.3 (3.2, 10.3)	5.5 (3.2, 13.3)	0.002
MIP-1β (pg ml^{-1})	3.2 (3.2, 32.2)	50.6 (11.4, 142.9)	3.2 (3.2, 20.9)	4.7 (3.2, 59.8)	0.001
IP-10 (pg ml^{-1})	235.4 (57.0, 776.2)	421.1 (235.6, 937.2)	243.3 (126.2, 425.8)	244.4 (165.7, 596.7)	0.01
Fractalkine (pg ml^{-1})	9.5 (3.2, 30.1)	3.2 (3.2, 13.5)	17.3, (3.2, 43.8)	14.3 (3.2, 70.8)	0.001
Adhesion molecules					
sE-selectin (ng ml^{-1})	5.5 (4.2, 6.9)	3.7 (2.4, 5.8)	5.9 (4.7, 9.8)	10.1 (6.9, 14.3)	<0.0001
sICAM-1 (ng ml^{-1})	68.5 (26.1, 143.6)	41.8 (31.0, 96.9)	94.8 (32.9, 196.9)	189.1, (76.1, 254.5)	<0.0001
sVCAM-1 (ng ml^{-1})	134.7 (77.8, 177.4)	133.2 (103.7, 198.3)	209.8 (169.5, 251.0)	248.5 (198.8, 272.3)	<0.0001
Growth factors					
G-CSF (pg ml^{-1})	3.2 (3.2, 3.2)	31.4 (3.2, 224.8)	178.3 (30.4, 551.6)	57.6 (14.8, 194.0)	<0.0001
Other markers					
MPO (ng ml^{-1})	1.7 (0.5, 2.9)	3.89 (2.9, 7.3)	2.15 (1.5, 5.5)	3.40 (2.1, 7.7)	0.005
MMP-9 (ng ml^{-1})	1.0 (0.4, 1.9)	4.1 (1.6, 7.9)	2.0 (0.8, 3.2)	1.4 (0.9, 2.3)	<0.0001
PAI-1 (ng ml^{-1})	1.9 (0.9, 4.0)	3.8 (2.1, 9.3)	5.4 (2.9, 16.1)	4.6 (2.6, 7.6)	0.0004

Data presented as median (25th, 75th percentile); p-value by the Friedman test. See Appendix for abbreviations.

interleukin (IL)-1α, IL-4, IL-6 and IL-10), chemokines (e.g. IL-8, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α and MIP-1β), soluble adhesion molecules (e.g. sE-selectin, intercellular adhesion molecule (sICAM)-1 and vascular cell adhesion molecule (sVCAM-1)), and granulocyte colony-stimulating factor (G-CSF), myeloperoxidase (MPO), matrix metalloproteinase (MMP)-9 and plasminogen activator inhibitor (PAI)-1 (p <0.05 by the Friedman test), while other markers, such as granulocyte/macrophage colony-stimulating factor (GM-CSF), transforming growth factor (TGF)-α and IL-2, showed no apparent changes in plasma level in response to CPB (data not shown). Three distinct patterns of response to CPB could be identified with a subset of biomarkers peaking at 2 h (IL-1Ra, IL-10, MPO, MIP-1β, interferon (IFN)-γ-inducible protein, (IP)-10, and MMP-9), at 24 h (IL-6, G-CSF, MCP-1 and PAI-1), and at 48 h (IL-1α, IL-4, IL-8, MIP-1α, fractalkine, sE-selectin, sICAM-1 and sVCAM-1).

Perioperative plasma biomarker profiles in the AKI and control group

Figure 2 displays the perioperative profiles of the plasma biomarkers that achieved significant or near significant fluctuations over the four time points, between the AKI and control group. In brief, prior to surgery, epidermal growth factor (EGF) and MIP-1β levels were significantly higher (p <0.05) in the AKI group compared with the control group, with a non-significant trend toward lower G-CSF levels (p = 0.07). Early after exposure to CPB, at the 2-h time point, sVCAM-1, fractalkine and MIP-1α levels were significantly higher (p < 0.05) in the AKI group compared with the control group, with a non-significant trend toward lower G-CSF (p = 0.09) and higher MPO (p = 0.09) levels. At the later, 24-h and 48-h post-CPB time points, sVCAM-1 and IL-12 levels were significantly lower (p <0.05) in the AKI group compared with the control group, with a non-significant trend toward higher MIP-1β (p = 0.08) and lower IL-6 (p = 0.08) levels.

Plasma biomarker diagnostic performance for AKI

Results of the ROC analysis over the four time points are summarized in Figure 3 for eight selected biomarkers. In brief, prior to surgery, among all 27 measured biomarkers, only plasma EGF (AUC 0.708; 95% CI 0.508–0.907; p = 0.04), MIP-1β (AUC 0.739; 95% CI 0.557–0.921; p = 0.01) and G-CSF (AUC 0.652; 95% CI 0.556–0.748; p = 0.002) predicted the development of AKI. At the 2-h post-CPB time point, four of the 27 biomarkers were associated with the development of AKI, namely sVCAM-1 (AUC 0.731; 95% CI 0.557–0.906; p = 0.01), fractalkine (AUC 0.727; 95% CI 0.546–0.908; p = 0.01), MPO (AUC 0.699; 95% CI 0.497–0.901; p = 0.05) and G-CSF (AUC 0.652; 95% CI 0.556–0.748; p = 0.002). At the 24-h post-CPB

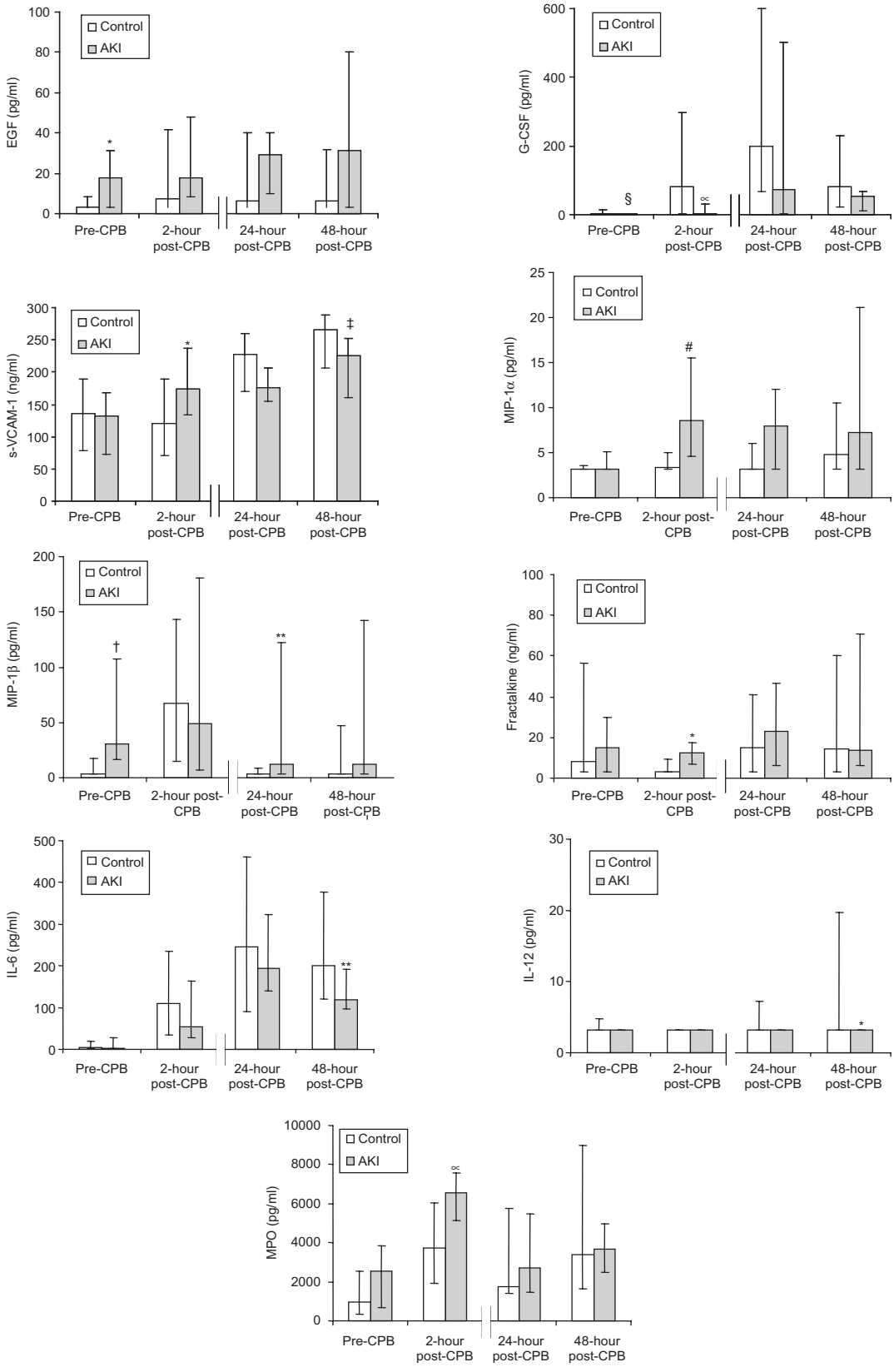

Figure 2. Perioperative profiles of selected plasma biomarkers following cardiac surgery in the acute kidney injury (AKI) ($n=11$) and control groups ($n=25$). The data are presented as median values, and the error bars represent 25th and 75th percentile values; †$p=0.02$, ‡$p=0.03$, *$p=0.04$, §$p=0.07$, **$p=0.08$, $p=0.09$ vs control group.

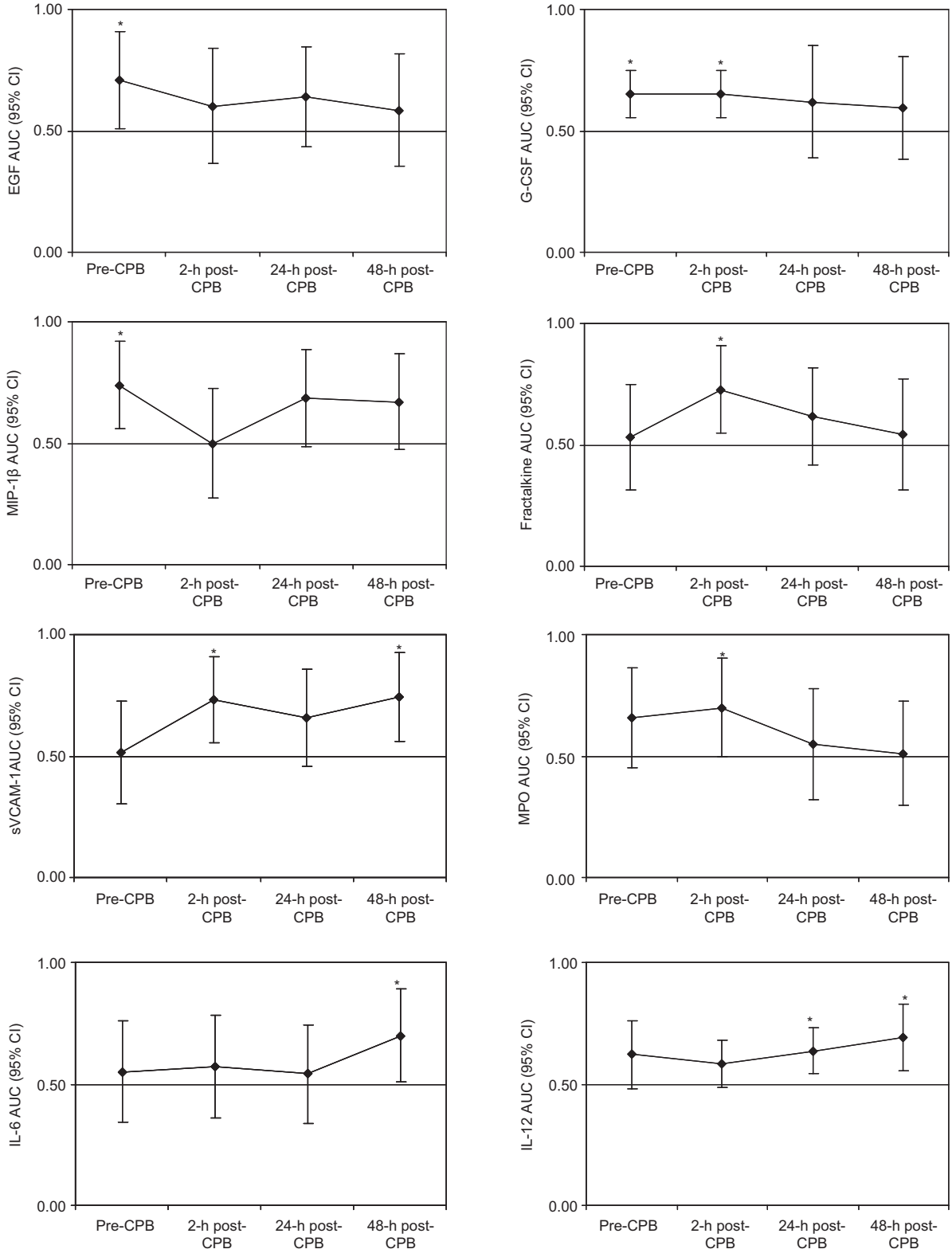

Figure 3. Diagnostic accuracy of selected plasma biomarkers according to the selected perioperative time points following cardiac surgery. The area under the receiver-operating-characteristic curve (AUC) is shown (with 95% confidence interval) for the eight biomarkers measured on plasma samples obtained prior to surgery, 2, 24, and 48 h after discontinuation of cardiopulmonary bypass (CPB), for the detection of acute kidney injury. *$p = 0.04$; †$p = 0.01$; ‡$p = 0.002$; **$p = 0.05$.

time point, only IL-12 (AUC 0.636; 95% CI 0.541–0.732; $p = 0.01$) was associated with development of AKI. At the late 48-h post-CPB time point, three markers maintained an association with development of AKI: sVCAM-1 (AUC 0.742; 95% CI 0.558–0.926; $p = 0.01$), IL-6 (AUC 0.700; 95% CI 0.507–0.893; $p = 0.04$) and IL-12 (AUC 0.693; 95% CI 0.556–0.829; $p = 0.01$).

Discussion

The measurement of blood and urine biomarkers is becoming increasingly important in the study of disease mechanisms and for the early detection of kidney disease. As a result, there is a growing demand for rapid, precise and cost-effective measurement of such analytes in both clinical and research laboratory settings (Elshal & McCoy 2006). Although the enzyme-linked immunosorbent assay has traditionally been considered the standard for quantitative analysis of biomarkers, this technique is not well suited for high-throughput measurement of multiple analytes. The multiplex bead array assay allows for a quantitative measurement of multiple analytes simultaneously in small volumes of biological fluids, using an automated platform.

In the present pilot nested case–control study, we explored the feasibility of measuring multiple proteins in serial plasma samples obtained from patients undergoing cardiac surgery with CPB, using the Luminex platform. Our goal was to identify biomarkers of interest that are potentially linked to the development of AKI, in an effort to elucidate disease mechanisms and provide directions for future research.

The hypotheses to be tested were that several pathophysiological pathways might be involved in mediating kidney injury following CPB, of which most can be classified as: (1) mediators of inflammation, including cytokines (IL-1α, IL-1β, IL-1Ra, IL-2, IL-4, IL-6, TNF-α, IFN-γ, IL-12, IL-10), chemokines (IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, fractalkine) and adhesion molecules (sE-selectin, sICAM-1, sVCAM-1); (2) oxidative stress mediators (MPO); and (3) growth factors (G-CSF, GM-CSF, EGF, vascular endothelial growth factor (VEGF)). We also included the protease MMP-9 and the thrombogenic factor PAI-1 in our exploratory analyses. We found that 16 of 27 gene products were upregulated in response to CPB, with several-fold increases in circulating levels of MIP-1α, and, as early as 2 h following CPB. Some biomarkers displayed a sustained increase following CPB (IL-6, IL-8, MCP-1, MPO, PAI-1 and G-CSF), whereas others displayed only a transient increase (IL-10, MIP-1β, MMP-9 and IP-10). Other markers displayed a significant but delayed response including IL-1α, IL-4, sE-selectin and sICAM-1. Our findings confirm some of the markers previously shown to be upregulated in response to CPB (Wan et al. 1997, Tomic et al. 2005, Laffey et al. 2002, Meldrum & Donnahoo 1999). One may speculate that an early and sustained response pattern of a marker indicates a stronger association with CPB-induced tissue injury and therefore a more important pathophysiological role in this setting. On the other hand, other CPB-independent injurious stimuli in the postoperative setting, such as reduced cardiac output (Gillies et al. 2005, Giannessi et al. 2007) and surgical wound repair processes (Henry & Garner 2003), may also play a causative role in the delayed/sustained compared with the early and transient patterns of response.

Our nested case–control study designed to match the AKI and control groups to important characteristics including CPB perfusion time (an important predictor of AKI), allowed us to decipher more clearly the differential regulation of the selected biomarkers in AKI. We observed differential expression of MIP-1β, EGF and G-CSF prior to surgery, sVCAM-1, fractalkine, MIP-1α, G-CSF and MPO 2-h post-CPB, and IL-6, IL-12, MIP-1β and sVCAM-1 at the later time points.

MIP-1α and MIP-1β are chemokines that are secreted by monocytes and lymphocytes including T cells (Irving et al. 1990). MIP-1β is a key trigger of macrophage migration, acting through the chemokine (C-C motif) receptor 5 (CCR5) (Cheung et al. 2009) and inducing downstream signalling pathways, resulting in increased generation of reactive oxygen species (Tatara et al. 2009). MIP-1β has been implicated in the pathogenesis of acute renal allograft rejection (Fischereder 2007) and progression of chronic kidney disease (Galkina & Ley 2006). It is conceivable that the pre-existing elevation of MIP-1β found in our study might be indicative of macrophage activation and enhanced migration prior to cardiac surgery, which in turn, could predispose to a proinflammatory surge in target organs in response to the CPB stimulus. In this context, our results might indicate that preoperative immune activation, perhaps induced by cardiac ischaemia and as evidenced by elevated plasma MIP-1β levels, may place patients at risk for developing AKI if subjected to cardiac surgery with CPB and may therefore serve as a preoperative renal risk marker.

Endothelial injury is an important pathophysiological feature of ischaemic AKI, particularly in the extension phase (Molitoris & Sutton 2004). Fractalkine expression on injured endothelium functions both as a potent chemoattractant and as an adhesion molecule for the recruitment and migration of fractalkine receptor-expressing circulating inflammatory cells into sites of inflammation (Beck et al. 2003, Imai et al. 1997). A soluble form of this chemokine is a potent chemoattractant of T cells and monocytes. Increased fractalkine expression and macrophage infiltration have been observed in cisplatin-induced AKI (Lu et al. 2008). In ischaemic AKI, there is upregulation of fractalkine specifically in kidney blood

vessels, and fractalkine receptor inhibition is protective and is associated with reduced macrophage infiltration in the kidney (Oh et al. 2008). Fractalkine has been implicated in the pathogenesis of thrombotic microangiopathies (Ramos et al. 2007, Zanchi et al. 2008). We observed a significant increase in soluble fractalkine levels 2 h after CPB in the AKI group compared with the control group, and in the ROC analysis, this biomarker achieved an AUC greater than 0.700, for the prediction of AKI, suggesting potential involvement of this chemokine in the disease mechanism. By contrast, other circulating markers of endothelial damage we tested were not differentially expressed between the AKI and control subjects.

Prior studies of patients undergoing on-pump cardiac surgery have shown an association of circulating sICAM-1 levels with pulmonary dysfunction (Gorlach et al. 2003), and of sE-selectin, P-selectin and sICAM-1 levels with prolonged postoperative requirement for vasopressor use (Wei et al. 2003), and postoperative development of sepsis (Paret et al. 2000). Although we found a uniform increase in plasma sICAM-1 and sE-selectin in response to CPB, these markers did not discriminate between the AKI and control groups. Similarly to E-selectin, VCAM-1 is also expressed on the surface of endothelial cells in response to inflammatory cytokines and facilitates leukocyte recruitment to sites of inflammation (Kluger 2004). Consistent with prior observations (Andresen et al. 2002), in our study, sVCAM-1 levels also rose following CPB, but were differentially expressed displaying a biphasic response between the AKI and control group. Indeed, sVCAM-1 was significantly higher at 2 h but significantly lower at 48 h post-CPB in the AKI versus the control group. Such a biphasic endothelial response to inflammatory stimuli has previously been described (Kim et al. 2004, Ricard et al. 1997, Ho et al. 2009).

Finally, we also found that plasma G-CSF and EGF levels were differentially expressed between the AKI and control groups. Indeed, G-CSF levels were lower in patients with AKI prior to and 2-h after CPB. This finding is in keeping with the hypothesis that haematopoietic stem cells may play a role in repair processes following AKI and may hasten recovery or abrogate the extension phase (Lin et al. 2003). However, this stands in contrast to a recent animal study, which found that granulocytosis induced by the exogenous administration of G-CSF, as opposed to spontaneous, physiological G-CSF fluctuations, worsened ischaemia reperfusion injury (Togel et al. 2004). Surprisingly in our study, EGF, a growth factor involved in reparative processes and known to accelerate restoration of kidney function in experimental models of AKI (Hammerman 1998), was elevated prior to surgery in the AKI group.

Our study has several strengths. We explored the disease mechanism in a human model of AKI where the timed insult to the kidney was clearly defined, i.e. the CPB stimulus. Furthermore, in our nested case–control study design, using a nearest-neighbour matching technique, we were able to match for important variables that are known to predict AKI including age, preoperative left ventricular ejection fraction, surgery type and CPB perfusion time. This allowed us to minimize the potentially confounding effect of these variables on the study of disease mechanisms using blood as a sentinel organ. In addition, we used a high-throughput customized platform that allowed us to measure simultaneously 27 biomarkers of interest over several time points, using low-volume plasma samples. These biomarkers were carefully selected based on their potential involvement in mechanisms of disease relevant to AKI. There are however, some important limitations to consider. We studied a relatively small sample of patients and performed multiple testing, and our observed associations between select biomarkers and AKI cannot prove causality. Further, our ROC analysis indicated rather mild to moderate as opposed to strong associations between these biomarkers and AKI. In addition, our AKI definition was based on incremental changes in serum creatinine. Although this creatinine-based definition has been well linked to adverse clinical outcomes (Uchino et al. 2006), it may not fully capture more subtle structural kidney damage that might have occurred in both the AKI and control groups. Our findings however, support a potential role for chemokines in the development of AKI following cardiac surgery, particularly fractalkine and MIP-1β. Further studies are needed to confirm and extend our preliminary findings.

In conclusion, in the present nested case–control study, we explored mechanisms of disease in AKI following on-pump cardiac surgery, using a multiplex bead array assay with blood as a sentinel organ. Although several inflammatory, leukocyte and oxidative stress markers, as well as growth factors were affected by CPB, only a few were associated with AKI following CPB, and might be of higher importance in the pathophysiology or for the prediction of this complex disorder, and merit further study.

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Appendix 1. Selected 27 plasma biomarkers

Cytokines
Tumor necrosis factor-α (TNF-α)
Interleukin-1α (IL-1α)
Interleukin-1β (IL-1β)
Interleukin-1Ra (IL-1Ra)
Interleukin-2 (IL-2)
Interleukin-4 (IL-4)
Interleukin-6 (IL-6)
Interleukin-10 (IL-10)
Interleukin-12 (IL-12)
Interferon-γ (IFN-γ)
Transforming growth factor-α (TGF-α)
Chemokines
Interleukin-8 (IL-8)
Monocyte chemoattractant protein-1 (MCP-1)
Macrophage inflammatory protein-1α (MIP-1α)
Macrophage inflammatory protein-1β (MIP-1β)
Induced protein-10 (IP-10)Fractalkine
Adhesion molecules
Soluble E-selectin (sE-selectin)
Soluble inter-cellular adhesion molecule-1 (sICAM-1)
Soluble vascular cell adhesion molecule-1 (sVCAM-1)
Growth factors
Granulocyte colony-stimulating factor (G-CSF)
Granulocyte macrophage colony-stimulating factor (GM-CSF)
Epidermal growth factor (EGF)
Vascular endothelial growth factor (VEGF)
Pro-oxidant enzymes
Myeloperoxidase (MPO)
Extracellular matrix enzymes
Matrix metalloproteinase-9 (MMP-9)
Fibrinolysis inhibitor
Plasminogen activator inhibitor-1 (PAI-1)