

Blood and urinary cytokine balance and renal outcomes at cardiac surgery

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Abstract

BACKGROUND

Increased perioperative pro-inflammatory biomarkers, renal hypoperfusion and ischemia reperfusion injury (IRI) heighten cardiac surgery acute kidney injury (CS-AKI) risk. Increased urinary anti-inflammatory cytokines attenuate risk. We evaluated whether blood and urinary anti-inflammatory biomarkers, when expressed as ratios with biomarkers of inflammation, hypoperfusion and IRI are increased in CS-AKI patients.

METHODS

Preoperative and 24-hour postoperative blood and urinary pro-inflammatory and anti-inflammatory cytokines, blood VEGF and H-FABP (hypoperfusion biomarkers), and MK, a biomarker for IRI, were measured in 401 cardiac surgery patients. Pre- and postoperative concentrations of biomarkers and selected ratios thereof, were compared between non-CS-AKI and CS-AKI patients.

RESULTS

Compared with non-CS-AKI, blood pro-inflammatory (pre- and post-op TNF α , IP-10, IL-12p40, MIP-1 α , NGAL; pre-op IL-6; post-op IL-8, MK) and anti-inflammatory (pre- and post-op sTNFsr1, sTNFsr2, IL-1RA) biomarkers together with urinary pro-inflammatory (pre- and post-op uIL-12p40; post-op uIP-10, uNGAL) and anti-inflammatory (pre- and post-op usTNFsr1, usTNFsr2, uIL-1RA) biomarkers, were significantly higher in CS-AKI patients. Urinary anti-inflammatory biomarkers, when expressed as ratios with biomarkers of inflammation (blood and urine), hypoperfusion (blood H-FABP and VEGF) and IRI (blood MK) were decreased in CS-AKI. In contrast, blood anti-inflammatory biomarkers expressed as similar ratios with blood biomarkers were increased in CS-AKI.

CONCLUSIONS

The urinary anti-inflammatory response may protect against the injurious effects of perioperative inflammation, hypoperfusion and IRI. These finding may have clinical utility in bioprediction and earlier diagnosis of CS-AKI and informing future therapeutic strategies for CS-AKI patients.

1. Introduction

Three major causes of CS-AKI

Cardiac surgery acute kidney injury (CS-AKI) postoperatively involves three major processes. Firstly, hypoperfusion impairs perioperative renal oxygen delivery (1) reflected in increased heart-type fatty acid-

binding protein (H-FABP) (2) and vascular endothelial growth factor (VEGF) (3). Secondly, ischemia reperfusion injury (IRI) accentuates intrarenal inflammatory processes (5), (reflected in increased midkine (MK) (4)). Of note, MK deficient mice have been shown to have resistance to renal IRI (6). Thirdly, additional pro-inflammatory injury may arise from filtered reno-toxic pro-inflammatory biomarkers generated by the surgical trauma and the cardiopulmonary bypass (CPB) process (7). This is indirectly reflected in increased anti-inflammatory plasma tumor necrosis factor soluble receptor 1 (pTNFsr1) and plasma tumor necrosis factor soluble receptor 2 (pTNFsr2) (8) which may be considered as surrogate biomarkers for the underlying blood pro-inflammatory response (9). Accordingly, in preliminary analysis of serum samples of 401 patients undergoing elective cardiac surgery, logistic regression analyses confirmed that biomarkers predictive of postoperative renal dysfunction represented the three underlying processes: first hypoperfusion (H-FABP), second IRI (MK) and third pro-inflammation; indirectly reflected by the compensatory much larger increases in anti-inflammatory serum tumor necrosis factor soluble receptor 1 (sTNFsr1) and serum tumor necrosis factor soluble receptor 2 (sTNFsr2) (9).

Mechanisms protecting against CS-AKI

In contrast to the three major injurious processes identified in blood samples, a possible protective role against CS-AKI has been attributed to an intra renal anti-inflammatory cytokine response at cardiac surgery, characterized by perioperative increases in urinary tumor necrosis factor soluble receptor 2 (uTNFsr2) and urinary interleukin-1 receptor antagonist (uIL-1RA) (7, 10–12). The urinary anti-inflammatory uTNFsr2 response precedes and greatly exceeds the corresponding plasma anti-inflammatory perioperative response (10). Moreover, the molecular weights of uIL-1RA, urinary tumor necrosis factor soluble receptor 1 (uTNFsr1) and uTNFsr2, are greater than 20 kDa and therefore, their glomerular filtration is lower than their smaller pro-inflammatory counterparts (13). These considerations suggest urinary anti-inflammatory biomarkers are mainly generated within the kidney with a smaller additional top up contribution from glomerular filtration (10, 11). Therefore, plasma levels of anti-inflammatory pTNFsr1, pTNFsr2 and plasma interleukin-1 receptor antagonist (pIL-1RA) are constitutively higher than the normal detectable levels of proinflammatory tumor necrosis factor α (TNF α) and interleukin-1 α (IL-1 α) which are more readily filtered. Previously McBride et al. (7) demonstrated that post-operative CS-AKI patients had lower uIL-1RA and uTNFsr2 responses than non-CS-AKI patients prompting suggestions of an inadequate urinary anti-inflammatory response heightening vulnerability to CS-AKI. However, the study did not evaluate how urinary anti-inflammatory/pro-inflammatory balance affected CS-AKI development as key filtered pro-inflammatory biomarkers (interleukin-1 β (IL-1 β) and TNF α) involved in tubular injury *in vitro* (14) were not meaningfully detectable in normal urine (15). This is because they are thought to be absorbed by the tubules (16). Urinary pro-inflammatory biomarkers are now measurable e.g. interferon gamma-induced protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1 δ (MIP-1 δ) in diabetic nephropathy patients (15); neutrophil gelatinase-associated lipocalin (NGAL), macrophage inflammatory protein-1 α (MIP-1 α) and IP-10 in CS-AKI patients (1, 3, 17); MCP-1 in mice with AKI (18) and interleukin-12 subunit p40 (IL-12p40) in type 1 diabetes (19). The renal impact of disordered urinary anti- and pro-inflammatory balance is currently unknown.

Although both hypoperfusion and ensuing IRI are directly injurious to the kidneys (6), their ability to indirectly injure kidneys through augmenting the pro-inflammatory response (20, 21) raises the question whether the urinary anti-inflammatory response confers some protection against collateral pro-inflammatory damage indirectly effected by hypoperfusion and IRI. In particular it is unknown how changes in ratios of biomarkers of urinary anti-inflammatory biomarkers/hypoperfusion and urinary anti-inflammatory/IRI biomarkers affect CS-AKI development.

Study objective

De novo measurements of blood pro-inflammatory biomarkers and urinary pro- and anti-inflammatory biomarkers were analyzed together with the previously reported data on serum biomarkers of inflammation, hypoperfusion and IRI (9) to determine the following hypothesis.

Detailed hypothesis

We investigated the primary hypothesis that CS-AKI development involves (I-a) higher blood pro-inflammatory responses in parallel with (I-b) lower blood anti-inflammatory responses. Furthermore, (I-c) the ratio of blood anti-inflammatory/pro-inflammatory biomarkers would be lower in CS-AKI than non-CS-AKI.

The secondary hypothesis postulates that CS-AKI patients (II-a) exhibit higher urinary pro-inflammatory responses in parallel with (II-b) lower urinary anti-inflammatory responses. Furthermore, the ratio of urinary anti-inflammatory/pro-inflammatory biomarkers (II-c) and ratio of urinary anti-inflammatory/blood pro-inflammatory biomarkers (II-d) would be lower in CS-AKI than non-CS-AKI patients.

Since hypoperfusion is an important contributing factor to CS-AKI, H-FABP and VEGF would be measured in all cases. It was anticipated that the insult of hypoperfusion would be mitigated in those patients who managed to mount a satisfactory urinary anti-inflammatory response. Accordingly, our tertiary hypothesis is that (III-a) pre- and postoperative H-FABP and VEGF would be higher in CS-AKI patients and (III-b) the ratio of urinary anti-inflammatory biomarkers/serum hypoperfusion biomarkers (VEGF and H-FABP) would be lower in the CS-AKI patients.

Finally, since IRI is an important mechanism in CS-AKI we hypothesized that MK in blood (IV-a) and urine (IV-b) will be higher in CS-AKI patients and the ratios of blood (IV-c) and urinary (IV-d) anti-inflammatory biomarkers/serum MK will be lower in CS-AKI patients.

2. Materials And Methods

Details of patient recruitment, demographics, inclusion and exclusion criteria, anesthetic technique, sample collection and processing, laboratory analysis of biomarkers have been described previously (9). The study complied with the Declaration of Helsinki, was approved by the Office for Research Ethics Committee Northern Ireland, the Royal Victoria Hospital Research Office Research Governance Committee

and written informed consent was obtained from all participating patients. The study complied with Standards for Reporting Diagnostic Accuracy (STARD) guidelines.

In addition to serum and plasma, urine samples were also collected from all patients. Urine samples were aliquoted and frozen at -80 °C. Patient samples were analyzed using Randox cytokine arrays and run on an Evidence Investigator, as per manufacturer's instructions (Randox Laboratories Ltd, Crumlin, Northern Ireland).

Briefly, 401 consecutive patients (n = 401) undergoing elective cardiac surgery were included in the study. Patients were recruited within the Cardiac Surgical Unit of the Royal Victoria Hospital Belfast, Northern Ireland. Local ethical committee and institutional approvals were received and written informed patient consent was obtained. Exclusion criteria included preoperative dialysis-dependent renal failure or significant renal disease (estimated glomerular filtration rate eGFR < 40) and diabetes mellitus. Patients on preoperative angiotensin conversion enzyme (ACE) inhibitor therapy were not excluded. The anesthetic technique was based on the use of propofol and fentanyl. Isoflurane was used in most patients either as an adjunct anesthetic agent or to control blood pressure. Pancuronium provided muscle relaxation. Postoperative analgesia was with morphine infusion.

Definitions of renal dysfunction used in the study

Using the risk, injury, failure, loss, end-stage (RIFLE) Criteria of Renal Dysfunction (22, 23) CS-AKI was defined as in our earlier study (7) as a drop from baseline eGFR of greater than 25% (as calculated by the method of modification of diet in renal disease (MDRD) (24, 25) occurring within the first 24 and 48 postoperative hours (early renal dysfunction), on the fifth postoperative day (late renal dysfunction) or at any time throughout the 5-day postoperative period (early and late combined).

Cytokine analysis

Cytokines that were studied in blood and urine are described in the Supplemental.

Statistical Analysis

Statistical analysis was performed using SPSS v25. Mann Whitney U test was used to identify significant biomarkers. Biomarkers with a $p < 0.05$ were considered significant. Cytokine ratios were calculated and ratios with the highest predictive ability to identify patients at risk of developing CS-AKI Any Day, pre, and post cardiac surgery, were investigated. Backward Wald and Forced Entry logistic regression, was used to identify the ratio combinations with the highest predictive ability to identify CS-AKI patients.

3. Results

Patient demographics

Full details of demographic data, distribution of surgical procedures, postoperative management and major outcomes have been previously described (9). Patient characteristics are described in Table 1.

Results for CS-AKI in comparison with non-CS-AKI patients

Hypothesis Ia (blood pro-inflammatory biomarkers)

Compared with non-CS-AKI patients, blood pro-inflammatory biomarkers were increased both pre- and postoperatively in patients with CS-AKI identified at Any Day (Table 2) and Days 1, 2, 5 (Supplemental Table 1, 3, 5, respectively).

Hypothesis Ib (blood anti-inflammatory biomarkers)

Pre- and postoperative sTNFsr1, sTNFsr2 and sIL-1ra were lower in non-CS-AKI patients than CS-AKI identified at Any Day (Table 2), Days 1 and 2 (Supplemental Table 1 and 3). Some cytokines were increased at Day 5 (Supplemental Table 5).

Hypothesis Ic (ratios of blood anti-inflammatory/blood pro-inflammatory biomarkers)

Ratios were higher in both pre- and post-operative CS-AKI patients compared with non-CS-AKI patients identified at Days 1, 2, 5 and Any Day (Supplemental Table 7, 13, 19, 25, respectively).

Hypothesis II-a (urinary pro-inflammatory biomarkers)

Pre- and postoperative uIL-12p40; postoperative uIP-10 and uNGAL were higher in Any Day CS-AKI patients (Table 3). Several cytokines were raised in Days 1, 2 and 5 CS-AKI patients (Supplemental Table 2, 4, 6, respectively).

Hypothesis II-b (urinary anti-inflammatory biomarkers)

Pre- and postoperative uTNFsr1, uTNFsr2 and postoperative uIL-1RA were significantly elevated in Any Day CS-AKI patients (Table 3). On Day 1, only postoperative uTNFsr2 was significantly increased in CS-AKI patients (Supplemental Table 2) whereas on Day 2 preoperative uTNFsr1 was increased (Supplemental Table 4). At Day 5 there was no significant differences in the level of urinary anti-inflammatory cytokines between non-CS-AKI and CS-AKI patients.

Hypothesis II-c (Ratios of urinary anti-inflammatory/urinary pro-inflammatory biomarkers)

Several postoperative ratios were significantly lower in the Any Day, Days 1, 2 and 5 CS-AKI patients (Supplemental Table 8, 14, 20 and 26, respectively).

Hypothesis II-d (Ratios of urinary anti-inflammatory/blood pro-inflammatory biomarkers)

Pre- and postoperative uIL-1RA/pIL-6, uIL-1RA/sIL-12p40, uIL-1RA/pMIP-1 α , uIL-1RA/pNGAL and eleven other postoperative ratios were significantly lower in Any Day CS-AKI patients (Supplemental Table 27).

On Day 1, preoperatively only one ratio uIL-1RA/ pIL-6 was significantly lower in CS-AKI patients whereas postoperatively eight ratios were significantly lower (Supplemental Table 9).

Hypothesis III-a (Hypoperfusion biomarkers - VEGF and H-FABP)

Preoperative VEGF and postoperative H-FABP were significantly elevated on Days 1, 2, 5 and Any Day CS-AKI patients (Supplemental Tables 10, 16, 22, 28, respectively). Preoperative H-FABP was significantly increased on Day 1, 2 and Any Day in CS-AKI patients (Supplemental Tables 10, 16 and 28, respectively).

Hypothesis III-b (Ratios of urinary anti-inflammatory/blood hypoperfusion biomarkers)

Preoperative uIL-1RA/pVEGF was significantly lower only in Day 5 in CS-AKI patients (Supplemental Table 23). Postoperative uTNFsr1/sH-FABP and uTNFsr2/sH-FABP were significantly lower in Day 1, 2, 5 and Any Day in CS-AKI patients (Supplemental Tables 11, 17, 23, 29, respectively). Pre- and postoperative uIL-1RA/sH-FABP was significantly lower in Day 2 and Any Day CS-AKI patients (Supplemental Tables 17 and 29, respectively).

Hypothesis IV-a, -b (Serum and urinary MK elevated post-operatively in CS-AKI patients)

Serum (but not urinary) MK post-operatively was elevated in Days 1, 2 and 5 in CS-AKI patients.

Hypothesis IV-c (Ratios of blood anti-inflammatory/sMK)

Pre-operatively the ratio of sTNFsr2 ratio with sMK was higher in anytime CS-AKI group).

Hypothesis IV- d (Ratios of urinary anti-inflammatory/sMK)

Postoperatively ratios of uIL-1RA, uTNFsr1 and uTNFsr2 with sMK were lower in Days 1, 2 and 5 in CS-AKI patients. A summary of the hypotheses results is presented in Table 4.

Logistic regression models

Preoperatively the ratio of uIL-1RA/pIL-6 had the highest predictive ability (area under receiver operating characteristic (AUROC) 0.616) to identify postoperative CS-AKI (Table 5). However, postoperatively the combination of ratios uIL-1RA/sIL-12p40 + uTNFsr2/sH-FABP + uIL-1RA/sMK (Any Day) had the highest predictive ability to identify patients at risk of developing CS-AKI with AUROC 0.824 (Table 5, Figure 1A and 1B).

4. Discussion

General findings

We previously reported that blood mediators of pro-inflammation, hypoperfusion and ischaemia reperfusion injury can predict AKI in cardiac surgery (9) and major orthopaedic surgery (26). Reasoning that the anti-inflammatory response in blood and urine would protect against direct and indirect renal

injurious effects of proinflammation, hypoperfusion and IR, we investigated the hypotheses that in cardiac surgery those patients who develop CS-AKI would, in comparison with normal renal function patients, have lower ratios of biomarkers of A) anti-inflammation in blood and urine /pro-inflammation in blood and urine (hypothesis I-c, II-c, II-d); B) anti-inflammation in urine/hypoperfusion markers in blood (as detected by HFABP and VEGF in blood) (III-b) and C) anti-inflammation in blood and urine/IRI (as detected by MK in blood) (hypothesis IV-c and IV-d).

In CS-AKI patients, although the blood pro-inflammatory markers were higher than non CS-AKI patients (I-a), the corresponding blood anti-inflammatory response was even greater (I-b and I-c), such that the ratio of anti-inflammation/pro-inflammation in blood was higher in CS-AKI than non CSAKI (I-c). Similarly, the renal injurious effects of the greater blood based IRI responses in the CS-AKI patients (IV-a) were not effectively countermanded by the even greater blood anti-inflammatory responses which accompanied them (IV-c). It seems that whereas the blood anti-inflammatory response is commensurate with and even more than the blood pro-inflammatory response which drives it (I-c), this blood anti-inflammatory response appears to lack adequate renal protective efficacy. This may be because the blood anti-inflammatory biomarkers are larger (>20kDa) than many of the smaller blood pro-inflammatory biomarkers whose effects they limit and thus are unable to be filtered to the same extent. This means that whereas much of the body is shielded from effects of excessive pro-inflammation in blood by an accompanying blood anti-inflammatory response, the glomerular filtrate and tubular cells do not have this same level of protection (13). Accordingly, the ratios of blood anti-inflammation/blood pro-inflammation and blood anti-inflammation/sMK are elevated in CS-AKI compared with non-CS-AKI, to the extent that they are predictive of CS-AKI. The elevated ratios reflect a greater renal injurious underlying pro-inflammatory response. Although this pro-inflammatory response drives the compensatory blood anti-inflammatory response, its renal injurious effects are not constrained by the latter due to these smaller pro-inflammatory biomarkers being more readily able to escape from the blood to the glomerular filtrate. Our results suggest that the blood anti-inflammatory response doesn't adequately protect against the renal injurious effects of pro-inflammation and IRI. Since ischemia reperfusion is normally preceded by a period of hypoperfusion and blood antiinflammation/sMK was observed to be higher in CS-AKI, then we suggest that that the blood anti-inflammatory response is also unlikely to protect against hypoperfusion associated CS-AKI.

In contrast to blood, urinary ratios of biomarkers of 1) anti-inflammation/pro-inflammation, 2) anti-inflammation/hypoperfusion and 3) anti-inflammation/IR were lower in urine in the CS-AKI compared with non-CS-AKI. This suggests, that in contrast to the blood anti-inflammatory response which does not appear to confer adequate renal protection, the urinary anti-inflammatory response is effective against mechanisms of renal injury arising from proinflammation, hypoperfusion and IRI. The renal anti-inflammatory response, as detected by increased perioperative uTNFsr1 and 2 and uIL1Ra, is believed to be largely an intrarenal response, since it develops in the urine before significant increases of these anti-inflammatory biomarkers in the blood (10), and at much higher concentrations (10). Presence of an adequate urinary anti-inflammatory response seems to mitigate against the renal injurious effects of

proinflammation, hypoperfusion and IRI. This may have implications for understanding CS-AKI pathogenesis and its management both preventative and reactive.

Detailed remarks:

Result (I-a), confirms existing evidence of the perioperative pro-inflammatory response being injurious to kidneys (10). However, our demonstration of significantly elevated concentrations of pre-operative blood pro-inflammatory mediators in those who later developed CS-AKI suggests that pre-operative drivers of pro-inflammation are important in subsequent pathogenesis of CS-AKI.

Similarly, consistent with elevated pro-inflammation preoperatively in blood of patients who developed CS-AKI, there was also elevated urinary pro-inflammatory biomarkers (e.g. pre- and postoperative uIL-12p40; postoperative uNGAL, uIP-10).

In contrast with the secondary hypothesis (II-b), which was based on the demonstration of a significantly reduced urinary anti-inflammatory response in CS-AKI patients (7), patients who developed CS-AKI in the present study had higher pre- and postoperative urinary concentrations of anti-inflammatory biomarkers (pre- and postoperative uTNFsr1, uTNFsr2; postoperative uIL-1RA).

Since our previous study (7), clinical practice has changed in the ICU at the Royal Victoria Hospital Belfast where routine use of noradrenaline has increased providing consistently higher perioperative blood pressures particularly during CPB. In the 2013 study (7) only 10.6% of all patients were on noradrenaline perioperatively compared to 60% in the current study. In neither study was there a difference in noradrenaline use between CS-AKI and non-CS-AKI groups. It could be argued that the increased noradrenaline use, may increase perioperative blood pressure and reduce perioperative hypotensive episodes sufficiently to lead to somewhat enhanced filtration of the relatively difficult to filter, large molecular weight blood anti-inflammatory biomarkers (TNFsr1, TNFsr2, IL-1RA). However, less noradrenaline usage (7), may have resulted in lower mean arterial pressures perioperatively. This would cause reduced filtration of the perioperatively increased blood anti-inflammatory biomarkers, resulting in changes in the magnitude of urinary anti-inflammatory biomarkers, since the latter are largely intrarenally generated, without a significant top up from filtered blood biomarkers.

In the present study, blood anti-inflammatory biomarkers are of higher concentration in CS-AKI than non-CS-AKI patients. Any improvement in filtration of these blood cytokines due to greater noradrenaline usage, could lead to an augmentation of the intrarenally-generated anti-inflammatory response. Since blood anti-inflammatory biomarkers are higher in CS-AKI than non-CS-AKI, this could explain the increased urinary concentrations of these biomarkers in the CS-AKI (finding II-b). Furthermore, any increase in urinary anti-inflammation is likely to be accompanied by an even greater increase in filtration of blood pro-inflammatory biomarkers, although this is difficult to measure directly. This may explain the increased urinary anti-inflammatory biomarkers in CS-AKI, and yet despite this, such increases seemed inadequate to confer renal protective advantage. The proportion of filtered anti-inflammatory biomarkers was not commensurate with the filtered pro-inflammatory biomarkers as indirectly measured by the latter

in the urine. However, in patients who can develop an adequate intrarenally-generated anti-inflammatory response, renal protection against pro-inflammatory biomarkers is conferred.

This leads to the key finding in the present study, namely confirmation of the secondary hypotheses II-c and II-d, that patients who developed CS-AKI had lower ratios of urinary anti-/pro-inflammatory biomarkers (II-c) and urinary anti-/blood pro-inflammatory biomarkers (II-d).

Findings II-a and II-b suggest that although the urinary pro- (II-a) and anti-inflammatory (II-b) responses were greater in CS-AKI than non-CS-AKI patients, in contrast to blood, urinary anti-/pro-inflammatory ratios (II-c) and urinary anti-/blood pro-inflammatory ratios (II-d) were consistently lower (II-c and II-d) in CS-AKI than non-CS-AKI patients. This suggests that in CS-AKI patients, while there is a measure of compensatory anti-inflammatory activity in urine (II-b), in contrast to blood (I-b), this intrarenal anti-inflammatory response is of inadequate magnitude (II-c and II-d) to protect against inflammatory-mediated CS-AKI. This work suggests, for the first time, that a compromised intrarenal anti-inflammatory response constitutes an important and until now, undescribed mechanism involved in the pathogenesis of CS-AKI.

We hypothesized that an anti-inflammatory response in blood and urine adequately protected against a hypoperfusion insult indicated by increased blood VEGF and H-FABP. Blood VEGF preoperatively and blood H-FABP pre- and postoperatively were higher in CS-AKI than non-CS-AKI patients (III-a). However, this risk can be reduced where there are compensatory increases in pre- and postoperative uIL-1RA and postoperative uTNFsr1 and uTNFsr2.

MK is a biomarker of IRI associated with CS-AKI (9). Since pro-inflammatory cytokine generation is an important aspect of IRI (20) we evaluated if the ratio of urinary anti-inflammatory cytokine/serum MK was lower in CS-AKI patients. In general, the magnitude of change in concentrations of blood and urinary anti-inflammatory biomarkers is greater than corresponding changes in blood and urinary pro-inflammatory biomarkers which are usually at lower concentrations. For example, concentrations of blood TNF α and IL-1 β and changes in their level are small in comparison with serum TNFsr1, TNFsr2 and IL-1RA. Moreover, filtered TNF α and IL-1 β are normally undetectable in the urine (as they are absorbed and destroyed by the tubules) (16). Therefore, surrogates for urinary TNF α were used, namely uIP-10 and uIL-12p40 whose presence in urine may reflect TNF α activity more proximally in the filtrate. However, if it is assumed that the sTNFsr1 and sTNFsr2 constitute an adequate compensatory response to transient, small increases in blood TNF α , and serum IL-1RA represents the response to blood IL-1 β , then sTNFsr1 and sTNFsr2 could be taken as surrogates for filtered TNF α , whereas IL-1RA is a surrogate for filtered IL-1 β . Therefore, the ratios of sTNFsr1 or sTNFsr2 or sIL-1RA/ uTNFsr1 or uTNFsr2 or uIL-1RA were investigated as an extrapolation of II-d. This is effectively a ratio of filtered blood pro-inflammatory biomarker/urinary anti-inflammatory biomarker, if blood TNFsr1 and TNFsr2 are surrogates for filtered blood TNF α and blood IL-1RA a surrogate for filtered IL-1 β . Postoperative ratios sTNFsr1/uTNFsr2, sTNFsr2/uTNFsr2, sIL-1RA/uTNFsr2, sTNFsr1/uTNFsr1, sTNFsr2/uTNFsr1, sIL-1RA/uTNFsr1 were all

significantly higher in Day 5 CS-AKI patients. The data suggests that the urinary anti-inflammatory response is protective against filtered pro-inflammatory biomarkers (Figure 2).

Clinical application

When the above blood and urinary ratios were subjected to Backward Wald and Forced Entry logistic regression, preoperative Any Day ratio uIL-1RA/pIL-6 had the highest predictive ability to identify postoperative CS-AKI with AUROC 0.616. However, postoperatively the combination of Any Day ratios uIL-1RA/sIL-12p40 + uTNFsr2/sH-FABP + uIL-1RA/sMK had the highest predictive ability to identify patients at risk of developing CS-AKI with AUROC 0.824 (Table 5, Figure 1a and 1b). The results illustrate the imbalance between postoperative urinary anti-inflammation and three factors: (i) blood pro-inflammation (uIL-1RA/sIL-12p40), (ii) hypoperfusion (uTNFsr2/sH-FABP) and (iii) IRI (uIL-1RA/sMK). This model reflects the underlying concept of urinary anti-inflammatory response protecting against the three insults of perioperative pro-inflammation, hypoperfusion and IRI. This information can be used to inform clinical management in the areas of treatment choices and planning intraoperative management.

Treatment choices and planning

Increasing use of minimally invasive techniques (e.g. mini-sternotomy, robotic surgery), allow a reduced perioperative pro-inflammatory response as compared with traditional cardiac surgery. These options may be more attractive in patients where preoperatively an inadequate urinary anti-inflammatory response has been identified. Furthermore, since this response renders the patient more vulnerable to hypoperfusion, a treatment option involving temporary permissive hypoperfusion such as off pump coronary artery bypass surgery would not be advisable.

Intraoperative management

More studies are needed to confirm if managing patients perioperatively on higher than normal perfusion pressures improves the urinary anti-inflammatory response. However, it could be argued that preoperative identification of patients exhibiting inadequate urinary anti-inflammatory levels and a resulting reduction in protection in subsequent hypoperfusion, could result in these vulnerable patients being managed at supra-normal perioperative blood pressures to avoid hypoperfusion (even though this may make the surgical field more challenging for the operator).

Post-operative care

A post-operatively identified unfavorable urinary anti-inflammatory response may be taken as a contraindication to non-steroidal anti-inflammatory analgesics.

In the long term, these findings may help identify at risk patient populations who could be enrolled in interventional trials. One concept in need of testing is the effectiveness of deploying strategies to precondition the anti-inflammatory response of patients found preoperatively to have deficient anti-inflammatory protection.

Abbreviations

CS-AKI – cardiac surgery acute kidney injury

H-FABP – heart-type fatty acid-binding protein

VEGF – vascular endothelial growth factor

IRI – ischemia reperfusion injury

MK – midkine

pTNFsr1 – plasma tumor necrosis factor soluble receptor 1

pTNFsr2 – plasma tumor necrosis factor soluble receptor 2

sTNFsr1 – serum tumor necrosis factor soluble receptor 1

sTNFsr2 – serum tumor necrosis factor soluble receptor 2

uTNFsr2 – urinary tumor necrosis factor soluble receptor 2

uIL-1RA – urinary interleukin-1 receptor antagonist

uTNFsr1 – urinary tumor necrosis factor soluble receptor 1

pIL-1RA – plasma interleukin-1 receptor antagonist

TNF α – tumor necrosis factor α

IL-1 α – interleukin-1 α

IL-1 β – interleukin-1 β

IP-10 (CXCL10) – interferon gamma - induced protein-10 (C-X-C motif chemokine 10)

MCP-1 (CCL2) – monocyte chemotactic protein-1 (chemokine (C-C motif) ligand 2)

MIP-1d – macrophage inflammatory protein-1d

NGAL – neutrophil gelatinase-associated lipocalin

MIP-1a (CCL3) – macrophage inflammatory protein-1a (chemokine (C-C motif) ligand 3)

IL-12p40 – interleukin-12 subunit p40

eGFR – estimated glomerular filtration rate

ACE – angiotensin converting enzyme

RIFLE – risk, injury, failure, loss, end-stage

MDRD – modification of diet in renal disease

AUROC – area under receiver operating characteristic

IL-6 – interleukin-6

IL-8 (CXCL8) – interleukin-8 (chemokine (C-X-C motif) ligand 8)

TLR – toll-like receptors

ICU – intensive care unit

UTI – urinary tract infection

MIP-2 – macrophage inflammatory protein-2

IL-12p35 – interleukin-12 subunit p35

IL-12p70 (IL-12) – interleukin-12 subunit p70

IL-23 – interleukin-23

CPB – cardiopulmonary bypass

CK-MB – creatine kinase-muscle/brain

AHF – acute heart failure

HIF-1 α – hypoxia-inducible factor-1 α

Pre-op – preoperative

Post-op – postoperative

Declarations

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Authors' contributions

W.McB., M.J.K., G.McL., M.W.R. and J.V.L. made substantial contributions to conception and design, analysis and interpretation of data, revising the manuscript and gave final approval of the version to be published. Furthermore, G.McL. and, to a lesser extent, J.J. was responsible for data acquisition. A.D. and J.W. have made substantial contributions to analysis and interpretation of data and manuscript revision. P.F. provided conceptual support and contributed to manuscript revision.

Additional Information

Supplemental Information accompanies this paper.

Competing Interests: M.J.K., A.D., J.W., J.V.L., and M.W.R. are employees of Randox Laboratories Ltd but hold no shares in the Company. P.F. is the Managing Director and owner of Randox, a privately-owned Company. A patent has been submitted by Randox to protect the biomarkers identified from this work.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Figure 2 was created by Randox Laboratories Ltd.'s design department.

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Tables

Table 1. Clinical characteristics of the study patients

Clinical characteristics	non-CS-AKI (n=273)	CS-AKI (n=71)	p value
Age (years)	65.4 ± 11.6	68.6 ± 10.7	0.020
Gender (male)	192/273 (70.3%)	50/71 (70.4%)	0.988
Weight (kg)	80.9 ± 17.5	84.8 ± 16.6	0.061
Height (cm)	167.8 ± 11.4	165.1 ± 14.0	0.082
BMI (kg/m ²)	28.9 ± 10.2	31.0 ± 6.0	0.001
Diabetes	29/268 (10.8%)	16/68 (23.5%)	0.006

CS-AKI – cardiac surgery acute kidney injury, n – number of patients, BMI – body mass index

Table 2. Blood pro-inflammatory and anti-inflammatory cytokines

Blood cytokines	Pre-op or post-op	Any day				p value
		non-CS-AKI		CS-AKI		
		n	median	n	median	
Pro-inflammatory cytokines						
sIP-10	pre-op	260	113.10	64	133.83	0.012
sIP-10	post-op	254	101.82	65	125.87	0.046
sIL-12p40	pre-op	260	368.87	64	569.18	<0.001
sIL-12p40	post-op	254	262.82	65	442.36	<0.001
sMK	post-op	212	979.28	58	2365.00	<0.001
pIL-6	pre-op	254	2.17	64	3.03	0.001
pIL-8	post-op	259	8.54	65	11.82	0.005
pMIP-1a	pre-op	251	3.53	64	4.52	0.001
pMIP-1a	post-op	256	4.33	65	6.63	<0.001
pMCP-1	pre-op	254	129.00	64	143.00	0.008
pMCP-1	post-op	259	194.00	65	248.00	0.001
pNGAL	pre-op	255	572.89	64	672.05	0.001
pNGAL	post op	260	948.07	65	1439.01	<0.001
pTNFa	pre-op	254	2.05	64	2.50	<0.001
pTNFa	post-op	259	2.33	65	3.11	<0.001
Anti-inflammatory cytokines						
sTNFsr1	pre-op	260	0.33	64	0.52	<0.001
sTNFsr1	post-op	254	0.68	65	1.04	<0.001
sTNFsr2	pre-op	260	0.35	64	0.57	<0.001
sTNFsr2	post-op	254	0.71	65	1.25	<0.001
sIL-1RA	pre-op	260	60.80	64	81.99	0.005
sIL-1RA	post-op	254	406.48	65	739.25	<0.001
pIL-10	post-op	259	2.27	65	3.27	0.009

pre-op – preoperative, post-op – postoperative, CS-AKI – cardiac surgery acute kidney injury, n – number of patients, sIP-10 – serum interferon gamma - induced protein-10, sIL-12p40 – serum interleukin-12

subunit p40, sMK – serum midkine, pIL-6 – plasma interleukin-6, pIL-8 – plasma interleukin-8, pMIP-1a – plasma macrophage inflammatory protein-1a, pMCP-1 – plasma monocyte chemotactic protein-1, pNGAL – plasma neutrophil gelatinase-associated lipocalin, pTNF α – plasma tumor necrosis factor α , sTNFsr1 – serum tumor necrosis factor soluble receptor 1, sTNFsr2 – serum tumor necrosis factor soluble receptor 2, sIL-1RA – serum interleukin-1 receptor antagonist, pIL-10 – plasma interleukin-10

Table 3. Urinary pro-inflammatory and anti-inflammatory cytokines

Urinary cytokines	Pre-op or post-op	Any day				p value
		non-CS-AKI		CS-AKI		
	n	median	n	median		
Pro-inflammatory cytokines						
uIP-10	post-op	256	12.68	65	22.97	<0.001
uIL-12p40	pre-op	258	0.94	65	2.53	0.035
uIL-12p40	post-op	256	2.87	65	4.47	<0.001
uNGAL	post-op	247	135.09	64	234.44	<0.001
Anti-inflammatory cytokines						
uTNFsr1	pre-op	257	0.53	65	0.70	0.044
uTNFsr1	post-op	254	6.66	65	7.70	0.020
uTNFsr2	pre-op	257	0.83	65	1.26	0.020
uTNFsr2	post-op	255	8.08	65	9.56	0.018
uIL-1RA	post-op	247	8616.30	64	13274.55	0.024

pre-op – preoperative, post-op – postoperative, CS-AKI – cardiac surgery acute kidney injury, n – number of patients, uIP-10 – urinary interferon gamma - induced protein-10, uIL-12p40 – urinary interleukin-12 subunit p40, uNGAL – urinary neutrophil gelatinase-associated lipocalin, uTNFsr1 – urinary tumor necrosis factor soluble receptor 1, uTNFsr2 – urinary tumor necrosis factor soluble receptor 2, uIL-1RA – urinary interleukin-1 receptor antagonist

Table 4 Summary of hypotheses results

Hypothesis: In CS-AKI:	Result	Conclusion
Blood pro-inflammatory biomarkers are elevated	Increased in CS-AKI	Hypothesis I-a confirmed
Blood anti-inflammatory biomarkers are lower	Increased in CS-AKI	Hypothesis I-b disproved
Blood anti-inflammatory/blood pro-inflammatory biomarkers are lower	Increased in CS-AKI	Hypothesis I-c disproved
Urinary pro-inflammatory biomarkers are elevated	Increased in CS-AKI	Hypothesis II-a confirmed
Urinary anti-inflammatory biomarkers are lower	Increased in CS-AKI	Hypothesis II-b disproved
Urinary anti-inflammation/urinary pro-inflammation are lower	Decreased in CS-AKI	Hypothesis II-c confirmed
Urinary anti-inflammation/blood pro-inflammation are lower	Decreased in CS-AKI	Hypothesis II-d confirmed
Pre- and postoperative blood H-FABP and VEGF are elevated	Increased in CS-AKI	Hypothesis III-a confirmed
Urinary anti-inflammation/blood hypoperfusion are lower	Decreased in CS-AKI	Hypothesis III-b confirmed
Blood MK is elevated	Increased in CS-AKI	Hypothesis IV-a confirmed
Urine MK is elevated	Decreased in CS-AKI	Hypothesis IV-b disproved
Blood anti-inflammation/ sMK is lower	Increased in CS-AKI	Hypothesis IV-c disproved
Urinary anti-inflammation/sMK is lower	Decreased in CSAKI	Hypothesis IV-d confirmed

Our main findings were, that blood anti-inflammation did not protect against CS-AKI whereas in the urine it did.

Table 5. Cytokine ratios with the highest predictive ability to identify patients at risk of developing CS-AKI

Any Day					
	Biomarker(s)	AUROC	CI	Sensitivity	Specificity
Preoperative	uIL-1RA/pIL-6	0.616	0.538-0.694	67.2%	52.4%
Postoperative	uIL-1RA/sIL-12p40 + uTNFsr2/sH-FABP + uIL-1RA/sMK	0.824	0.770-0.879	82.8%	67.8%
	uIL-1RA/sIL-12p40 + uTNFsr2/sH-FABP	0.775	0.715-0.835	72.3%	67.1%
	uIL-1RA/sIL-12p40 + uIL-1RA/sMK	0.784	0.720-0.848	74.1%	69.1%
	uTNFsr2/sH-FABP + uIL-1RA/sMK	0.771	0.709-0.832	74.1%	68.6%
	uIL-1RA/sIL-12p40	0.725	0.658-0.792	72.3%	63.9%
	uTNFsr2/sH-FABP	0.685	0.617-0.753	73.8%	56.6%
	uIL-1RA/sMK	0.704	0.632-0.776	74.1%	55.3%

CS-AKI - cardiac surgery acute kidney injury, AUROC – area under receiver operating characteristic, CI – confidence interval, uIL-1RA – urinary interleukin-1 receptor antagonist, pIL-6 – plasma interleukin-6, sIL-12p40 – serum interleukin-12 subunit p40, uTNFsr2 – urinary tumor necrosis factor soluble receptor 2, sH-FABP – serum heart-type fatty acid-binding protein, sMK – serum midkine

Figures

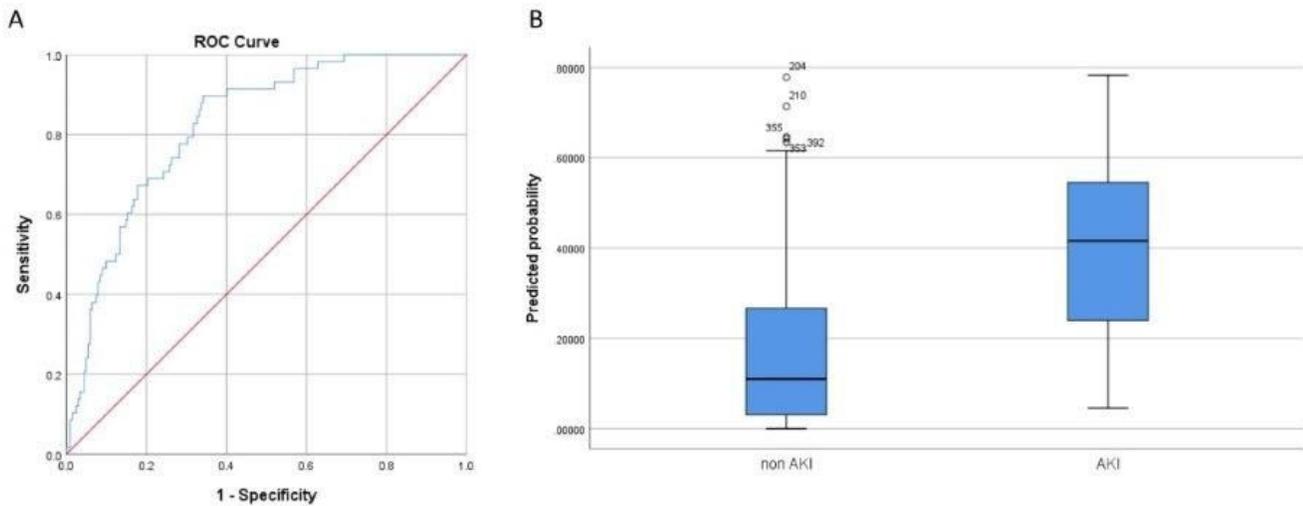


Figure 1

The model with the highest predictive ability to identify patients at risk of developing CS-AKI Any Day postoperatively was uIL-1RA/sIL-12p40 + uTNFsr2/sH-FABP + uIL-1RA/sMK (A) Postoperative model of biomarkers used to distinguish between non-CS-AKI and CS-AKI patients (AUROC 0.824). (B) Predicted probability of the model to identify non-CS-AKI and CS-AKI patients.

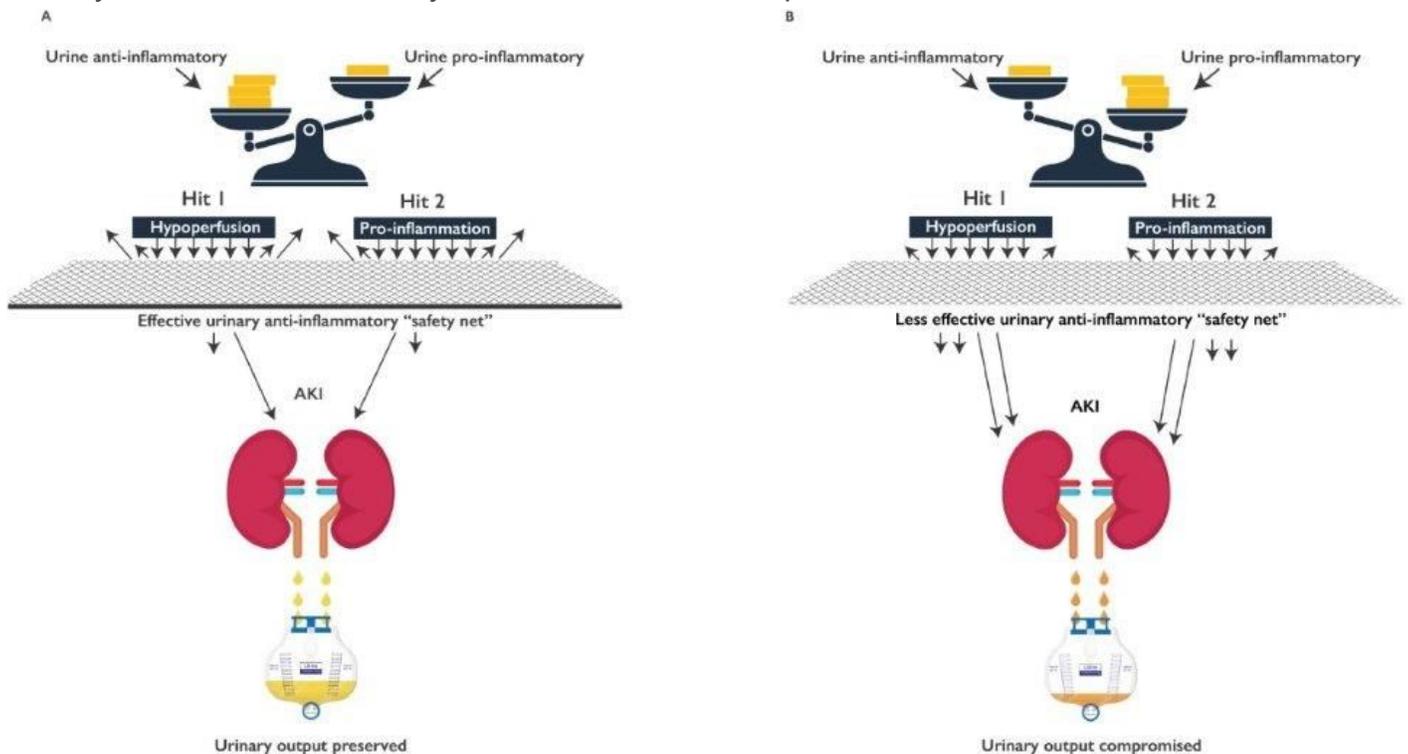


Figure 2

Heightened urinary anti-inflammatory response protects against renal injury caused by pro-inflammation and hypoperfusion. (A) When urinary anti-inflammatory response is proportionally greater than urinary pro-inflammatory response, the balance of urinary anti-/pro-inflammatory biomarkers is in favour of anti-inflammation. (B) However, when the response is smaller, there is less protection against pro-inflammation and hypoperfusion, the balance is less favourable with respect to anti-inflammation.

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