Stratifying risk of acute kidney injury in pre and post cardiac surgery patients using a novel biomarker-based algorithm and clinical risk score

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Acute kidney injury (AKI) following cardiac surgery significantly increases morbidity and mortality risks. Improving existing clinical methods of identifying patients at risk of perioperative AKI may advance management and treatment options. This study investigated whether a combination of biomarkers and clinical factors pre and post cardiac surgery could stratify patients at risk of developing AKI. Patients (n = 401) consecutively scheduled for elective cardiac surgery were prospectively studied. Clinical data was recorded and blood samples were tested for 31 biomarkers. Areas under receiver operating characteristic (AUROCs) were generated for biomarkers pre and postoperatively to stratify patients at risk of AKI. Preoperatively sTNFR1 had the highest predictive ability to identify risk of developing AKI postoperatively (AUROC 0.748). Postoperatively a combination of H-FABP, midkine and sTNFR2 had the highest predictive ability to identify AKI risk (AUROC 0.836). Preoperative clinical risk factors included patient age, body mass index and diabetes. Perioperative factors included cardio pulmonary bypass, cross-clamp and operation times, intra-aortic balloon pump, blood products and resternotomy. Combining biomarker risk score (BRS) with clinical risk score (CRS) enabled pre and postoperative assignment of patients to AKI risk categories. Combining BRS with CRS will allow better management of cardiac patients at risk of developing AKI.

Acute kidney injury (AKI) is a major complication following cardiac surgery that can affect the function of multiple organs including the brain, lungs and gut. Acute kidney injury increases the risk of death resulting in major use of hospital resources and elevated costs1. Acute kidney injury occurs in almost 30% of patients following cardiac surgery2 and 50% in high risk patients i.e. diabetics3,4. In the UK, approximately 100,000 deaths per year are linked to AKI. Acute kidney injury costs the NHS between £434 and £620 million per year6.

Several diagnostic criteria have been used for diagnosis of AKI including the risk, injury, failure, loss, end-stage renal disease (RIFLE) criteria in 20044; AKI Network (AKIN) modified RIFLE criteria in 20077; 2012 Kidney Disease: Improving Global Outcomes (KDIGO) criteria8, which combines RIFLE and AKIN criteria. The KDIGO criteria rely on changes in serum creatinine (SCr) levels and urine output, which is not ideal. Oliguria frequently occurs following cardiac surgery. Sometimes it precedes increased SCr due to renal injury, but often is a physiological response to hypovolemia or hypotension. Thus, the specificity of urine output as a criterion for AKI is low, and if used alone could misclassify non AKI as AKI patients8. Moreover, at cardiac surgery, increases in SCr above preoperative baseline due to AKI may take several hours to develop due to the inevitable haemodilution

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Effect of cardiopulmonary bypass. Accordingly, increases in SCr if used as a sole criterion for AKI could delay the time to AKI diagnosis and its potential management.

Existing strategies to prevent or reduce risk of AKI at cardiac surgery include (among others) maintaining a higher haematocrit perioperatively and supra normal blood pressure. However, the use of blood transfusion is costly and not risk free. Furthermore, maintaining a supra normal blood pressure perioperatively during cardiac surgery may heighten the risk of postoperative bleeding at the operative site with its attending complications. Accordingly, the anaesthesiologist must evaluate the risk/benefit balance of such reno-protective interventions for each patient. An ability to accurately stratify patients into AKI risk categories perioperatively would greatly assist in this decision and allow consideration of preventative strategies. However, in addition to predicting risk of AKI, earlier diagnosis might allow interventions such as earlier renal replacement therapy. In this context, some biomarkers have been evaluated to consider their utility in allowing preoperative prediction or perioperative diagnosis of AKI at cardiac surgery. Some of these studies were limited by small sample numbers and/or reduced areas under receiver operating characteristic (AUROC)11–14. McBride et al.15 have shown in elective cardiac surgery patients that IL-10 in postoperative plasma collected 2 hours after surgery and urinary transforming growth factor beta (TGFβ-1), collected 24 hours after surgery, were significantly higher in patients who developed early renal dysfunction. Furthermore, urinary IL-1RA and sTNFR2 were significantly lower 24 hours postoperatively in late renal dysfunction patients.

Due to the different processes involved and the dynamic nature of AKI, it is unlikely that one biomarker will predict or diagnose AKI across a wide range of clinical conditions and quantify its severity. Therefore, the aim of this study was to investigate whether a combination of biomarkers and clinical factors could be used to stratify the risk of a patient developing AKI pre and post cardiac surgery earlier than routinely used clinical methods. The biomarkers selected for this study were likely to represent key pathways in AKI pathogenesis namely, inflammation, hypoperfusion and reperfusion (see Table 1).

Materials and Methods

Study population. Cardiac patients who were consecutively scheduled for elective cardiac surgery within the Cardiac Surgical Unit of the Royal Victoria Hospital, Belfast, UK between May 2012 and August 2013, were recruited into the study. Patients were excluded if they were <18 years of age, had preoperative or pretrauma dialysis-dependent renal failure or known significant renal disease. In addition, emergency surgery patients and patients with active malignancy, active endocarditis, sepsis, septic or cardiogenic shock, or had pre-operative haemodynamic instability prior to entrance into the study (known estimated glomerular filtration rate (eGFR < 30)) were excluded. 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) guidelines16. Of the n = 401 patients recruited to the study, pre and postoperative samples were available from 344/401 (85.8%). Patient samples were not available from 57/401 (14.2%) and these patients were excluded from the study (Fig. 1).

Clinical data collection. Clinical data was recorded for each patient from medical records that included baseline demographic characteristics, comorbidity data and current medications. Creatinine levels in patients pre and post surgery were measured by the hospital laboratory and were used to calculate eGFR through the Modification of Diet in Renal Disease (MDRD) study equation formula17. Surgical interventions included: administration of inotropes (e.g. dopamine, adrenaline, milrinone), surgical methods (e.g. valve surgery), cardio pulmonary bypass (CPB) and aortic clamping. Moreover, the length of patient admission (days) in the intensive care unit (ICU) and high dependency unit (HDU), as well as administration of interventions during and postoperatively (e.g. platelets), were also recorded. Clinical and demographic data for each patient were recorded on a case report form and stored on a database.

Sampling and laboratory methods. Patient blood samples (10 ml) were collected preoperatively and on day 1 postoperatively. The preoperative blood sample was collected following routine arterial line insertion prior to induction of anesthesia. Day 1 (within 24 hours) postoperative blood sample (10 ml) was collected from the same line. Patient blood samples were centrifuged, serum/plasma was aliquoted within 30 minutes of collection and stored at −80°C. Patient blood samples were analysed by Randox Clinical Laboratory Services (RCLS) (Antrim, UK) on cytokine arrays (Randox Laboratories Ltd, Crumlin, UK) for the following proteins: Cytokine I Array: Interleukin(IL)-1α, -1β, -2, -4, -6, -8, -10, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), tumour necrosis factor alpha (TNFα), interferon gamma (IFNγ), and monocyte chemoattractant protein 1 (MCP-1); Cytokine II Array: insulin growth like factor 1 (IGF-1), Eotaxin, interleukin-1 receptor antagonist (IL-1Ra), platelet-derived growth factor beta homodimer (PDGF-BB), interferon gamma induced protein 10 (IP-10), interleukin 12 subunit p40 (IL-12p40); and Cytokine IV Array: soluble interleukin 2 receptor alpha (sIL2Rα), soluble interleukin 6 receptor (sIL6R), soluble tumour necrosis factor receptor 1 (sTNFR1), soluble tumour necrosis factor receptor 2 (sTNFR2) and matrix metallopeptidase 9 (MMP9); using an Evidence Investigator analyser according to manufacturer's instructions (Randox, Crumlin, UK). Neutrophil gelatinase-associated lipocalin (NGAL), C-reactive protein (CRP), D-Dimer, neuron-specific enolase (NSE), sTNFR1 were measured using Cerebral II Array (Randox, Crumlin, UK). Heart-type fatty-acid binding protein (H-FABP) was measured using an H-FABP IT assay (Randox, Crumlin, UK) on the RX Imola analyser (Randox, Crumlin, UK). Midkine (MK) was measured using a commercial ELISA according to manufacturer's instructions (CellMID, Sydney, Australia). Serum creatinine was measured in the Kelvin Laboratory (Royal Victoria Hospital, Belfast) on the Cobas 8000c701 (Roche Diagnostics, Basel, Switzerland).
MCP1 25.5 pg/ml; EGF 2.5 pg/ml; IL-10 1.1 pg/ml; IL-1 0.09 ng/ml; sTNFR2 0.2 ng/ml; MMP9 3.03 ng/ml; CRP 0.67 mg/l; D-Dimer 2.1 ng/ml; NSE 0.26, NGAL 17.8 ng/ml; IP-10 7.81 pg/ml, IL-12p40 7.81 pg/ml; Cytokine IV - sIL2A 0.12 ng/ml; sIL6R 0.62 ng/ml; sTNFR1 0.24 ng/ml; MK 8.0 pg/ml; H-FABP 2.94 ng/ml and SCr 5 μmol/L. Biomarker values below the limit of detection (LOD) were recorded as 90% of LOD.

Outcome definitions. The development of AKI was defined as an eGFR drop of ≥25% from preoperative baseline on any of the recorded postoperative sampling days (days 1, 2 or 5) or at any time postoperatively.
Statistical analyses. Statistical analyses were performed using SPSS v25. A Mann-Whitney U Test was used to identify significant biomarkers. Biomarkers with a p < 0.05 were considered significant. The ability of these biomarkers to predict AKI was further investigated using logistic regression (Backward Wald and Forced Entry). Areas Under the Receiver Operator Curve were generated pre and postoperatively for biomarker-based algorithms to provide a measure of how well the biomarker models distinguished between the two diagnostic groups (AKI vs. non AKI). The best combinations of biomarkers (with the greatest AUROC, sensitivity and specificity) were chosen to stratify patients at potential risk of developing AKI.

Results

A summary of baseline and clinical characteristics of the patients involved in the study are described in Table 2. Estimated GFR was recorded on days 1, 2 and 5 following surgery. To increase the number of patients in each cohort, the analyses are based upon an ‘AKI any-day’ definition (i.e. development of AKI on days 1, 2 and 5). Patients were included in this category if their eGFR dropped ≥25% from baseline, following cardiac surgery.

Preoperative biomarkers. Preoperatively sTNFR1 or sTNFR2 had the highest predictive ability to identify patients at risk of developing AKI (Table 3) (sTNFR1 sensitivity 70.3%; specificity 68.5%; AUROC 0.748 (CI 0.684–0.812)) (Fig. 2a,b).

Postoperative biomarkers. Postoperatively a combination of H-FABP, MK and sTNFR1 or sTNFR2 had the highest predictive ability to identify patients at risk of developing AKI (Table 3) (H-FABP + MK + sTNFR2 sensitivity 75.9%; specificity 69.1%; AUROC 0.836 (CI 0.785–0.888)) (Fig. 3a,b).

Clinical risk score (CRS). The main clinical factors identified for patients at potential risk for the development of AKI pre and postoperatively are described in Tables 4 and 5, respectively. Patients who have a cumulative score of 0, no risk. Patients who score >1 e.g. a 65-year-old patient with a BMI of 26 and diabetes would have a cumulative score of 2.5 (highest risk).

Biomarker risk score (BRS). Biomarker combination algorithm(s) can be applied clinically to provide a patient risk score for developing AKI. Patients with a score equal to/or greater than the value of the set point (cut-off) would be categorised positive (AKI); whereas patients below the cut-off would be categorised negative (non AKI) (Table 6).

Positive BRS is associated with higher risk for development of AKI, e.g. patients with negative BRS and high CRS are at lower risk of developing AKI (category 2) while patients with a positive BRS and low CRS are assigned to category 3 (high risk for the development of AKI) (Table 7) (See Supplementary Notes 1–3 and Supplementary Tables 1–8 for worked examples).

Clinical utility; combining BRS with CRS: pre and postoperative management of patients at potential risk for the development of AKI. Combining BRS with CRS could assist with pre and postoperative management of patients at potential risk for the development of AKI. Four categories of risk were identified (Table 7): Categories 1 and 2 = low risk; Categories 3 and 4 = high risk. Combining the biomarkers with the clinical risk factors, preoperative and postoperative, improved the AUROC (See Supplementary Note 4 and Supplementary Table 9 for distribution of non AKI and AKI patients within the risk categories and Supplementary Note 5 and Supplementary Table 10 for further statistical analysis of biomarkers and clinical factors).

Discussion

The aim of this study was to investigate whether a combination of biomarkers and clinical characteristics/risk score could predict AKI earlier than SCr and oliguria in patients undergoing cardiac surgery. Although a large range of biomarkers were studied, the mediators identified in our model interestingly represented three important pathways for the pathogenesis of renal dysfunction, namely hypoperfusion (H-FABP), ischaemia reperfusion injury (MK) and proinflammatory insult (sTNFR1 or sTNFR2).

Of the n = 30 biomarkers investigated in the patient samples undergoing cardiac surgery, only serum sTNFR1 or sTNFR2 on their own proved to be the best predictive biomarkers pre surgery, whereas serum TNFα was not significant. Soluble TNFR1 and sTNFR2 are the soluble forms of their membrane-bound counterparts (mTNFR1 and mTNFR2) through which TNFα acts. When sTNFR1 and sTNFR2 are released from the membrane,
they bind free TNFα thus limiting its biological proinflammatory effects. Soluble TNFR1 and sTNFR2 are thus anti-inflammatory agents. Similarly, postoperative serum sTNFR1 and sTNFR2 had biopredictive utility in combination with MK and H-FABP for AKI whereas TNFα did not.

There are several reasons why this may occur. Firstly, perioperative serum TNFα exhibits different kinetics to serum sTNFR1 and sTNFR2 responses. Serum TNFα has a transient and small increase prior to CPB followed by a second transient and small increase at the end of CPB. These small transient increases may be caused in part, by surgically-induced coagulation disturbances, interaction of blood with the foreign surface of the CPB machine, and retransfusion of unwashed shed mediastinal blood perioperatively. The un-sustained transient nature of the TNFα response reflects efficient mechanisms to clear blood TNFα from the circulation.

Kinetically, unlike TNFα, the serum sTNFR1 and sTNFR2 anti-inflammatory response is larger and more sustained lasting over 24 hours. Moreover, soluble sTNFR2 in blood increases progressively following cardiac surgery over at least a 2-day follow-up period. In this regard, serum sTNFR1 and sTNFR2 responses differ from the blood response of other important anti-inflammatory cytokines such as IL-10 and IL-1Ra which rise and fall to baseline 24 hours perioperative. Furthermore, it may be argued that because the blood IL-10 and IL-1Ra responses at cardiac surgery have been shown to be transient, this may explain why these anti-inflammatory mediators lack biopredictive utility in our model.

The second reason may lie in the underlying pathogenesis of perioperative inflammatory-mediated renal failure. It has been suggested that perioperative increases in filtered TNFα, if unsuccessfully handled by the kidney, could directly injure renal tubules.

Due to the transient nature of TNFα, it is not clinically practicable to measure its exact peak in serum or TNFα recovery from urine. Moreover, serum sTNFR1 and sTNFR2 are > 20 kDa and thus not as readily filtered by the tubules as monomeric TNFα. Therefore, increases in sTNFR1 and sTNFR2 are not likely to have a direct protective effect against tubular damage mediated by filtered TNFα. This may explain in part why blood increases in sTNFR1 and sTNFR2 were not linked with reduced AKI risk in our model. However, increased blood sTNFR1 and sTNFR2 were linked with AKI risk. This could be because the sustained increases in blood sTNFR1 and sTNFR2 are a proportionate and compensatory response to transient increases in blood TNFα.

As already discussed, serum TNFα is barely detectable in preoperative blood in healthy individuals, whereas baseline preoperative serum sTNFR1 and sTNFR2 concentrations are constitutively expressed. This should be understood in the context of other conditions known to modulate serum sTNFR1 and sTNFR2 levels. For example, both sTNFR1 and sTNFR2 were demonstrated as potential biomarkers for the identification of patients presenting with chronic kidney disease (CKD) by predicting outcome in either those with diabetic nephropathy, or early or moderate CKD, or underlying malignancy.

However, in this study we show for the first time that higher baseline sTNFR1 and sTNFR2 in patients who have normal preoperative renal function may predict postoperative AKI risk. Elevated baseline serum sTNFR1 and sTNFR2 preoperatively is driven by a heightened proinflammatory response due to underlying cardiovascular disease processes e.g. atheroma which would constitute a perioperative AKI risk. Alternatively, a reduced preoperative renal ability to clear preoperative episodic TNFα pulses could lead to a requirement for higher compensatory sustained increased levels in baseline sTNFR1 and sTNFR2.

When the biomarkers (n = 30) were measured in patient serum samples post cardiac surgery at any time the combination of H-FABP, MK and sTNFR1 or sTNFR2 had the highest predictive ability for detecting patients at risk of developing AKI (AUROC 0.817 for H-FABP, MK and sTNFR1 and AUROC 0.836 for H-FABP, MK and sTNFR2 (Table 3)). While serum sTNFR1 and sTNFR2 in our model may be an indirect reflection of the relative contribution of proinflammatory factors in pathogenesis of AKI, H-FABP in our model may reflect under perfusion of the kidney. Firstly, this increase in serum H-FABP could be secondary to the peri and postoperative myocardial dysfunction which commonly accompanies cardiac surgery. Schaub et al. reported a 6-fold increase in H-FABP measured in blood from patients who experienced AKI at any time point (day 1–day 5 post cardiac operation). Moreover, H-FABP is released into the blood 30 minutes after an ischaemic event from myocytes. The resulting suboptimal cardiac output could lead to renal hypoperfusion and AKI. Secondly, H-FABP is also produced by kidney distal tubular cells. However, H-FABP expression in the myocardium is 20 times higher than renal tissue so H-FABP measured in the serum is more likely of myocardial origin. Thirdly, because serum H-FABP is renally cleared, patients with diminished renal function, whether acute or chronic, have compromised H-FABP renal clearance which may further contribute to the elevated H-FABP levels.

Our model also identified MK as a significant factor in the postoperative biomarker combination to detect AKI. Midkine is a pleiotropic, heparin-binding growth factor involved in the pathogenesis of ischemia reperfusion injury. Necrosis and autophagy occur after ischemic reperfusion injury resulting in vascular endothelial dysfunction and vascular congestion and oedema, reduced blood flow and migration of inflammatory cells to the kidney. Infiltrating inflammatory cells release cytokines, reactive oxygen species (ROS) and other chemokines adding further insult to the already compromised kidney. Midkine promotes this process and is normally expressed at low levels in proximal tubules. However, it is up-regulated in proximal tubules after ischaemic reperfusion. Of note, the absence of MK in MK-deficient mice protects against experimentally induced renal ischaemic reperfusion injury.

The existing method of measuring Scr (eGFR) evaluates the result of AKI. In contrast, our biomarker combination of serum H-FABP, MK and sTNFR1 or sTNFR2, is based largely on the processes initiating and underlying the pathogenesis of AKI. Thus, the information provided by the biomarker combination has the potential to assist with earlier diagnosis and prediction of AKI.

In summary, three main factors in perioperative AKI at cardiac surgery, namely proinflammatory-mediated tubular injury, renal under perfusion and ischemia reperfusion injury are utilised in our model. Soluble TNFR1 and sTNFR2 indicate perioperative proinflammatory load, H-FABP indicates the risk of renal under perfusion.
### Table 2. Summary of baseline and clinical characteristics of the study patients. Data are presented as mean ± standard deviation or number/total (percentages). Note that patients presented with multiple comorbidities. BMI, body mass index; ACE, angiotensin-converting-enzyme; CPB, cardio pulmonary bypass; CABG, coronary artery bypass graft; ICU, intensive care unit; HDU, high dependency unit.

| Patient characteristics | non AKI (n = 273) | 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/m²)             | 28.9 ± 10.2       | 31.0 ± 6.0  | 0.001   |

| Comorbidities           |                  |             |         |
|-------------------------|------------------|-------------|---------|
| Myocardial Infarction   | 73/268 (27.2%)   | 13/68 (19.1%) | 0.171   |
| Ischemic Heart Disease  | 218/268 (81.3%)  | 65/68 (95.6%) | 0.760   |
| Hypertension            | 35/268 (13.1%)   | 10/68 (14.7%) | 0.722   |
| Diabetes                | 29/268 (10.8%)   | 16/68 (23.5%) | 0.006   |
| Chronic Obstructive Pulmonary Disease | 9/268 (3.4%) | 3/68 (4.0%) | 0.676   |
| Diverticulitis          | 8/268 (3.0%)     | 3/68 (4.4%)  | 0.555   |
| Asthma                  | 6/268 (2.2%)     | 2/68 (2.9%)  | 0.735   |
| Transient Ischemic Attack | 6/268 (2.2%) | 1/68 (1.5%) | 0.692   |
| Peripheral Vascular Disease | 4/268 (1.5%) | 2/68 (2.9%) | 0.421   |
| Cerebrovascular Accident | 4/268 (1.5%) | 1/68 (1.5%) | 0.989   |

| Pre surgery medications |                  |             |         |
|-------------------------|------------------|-------------|---------|
| Beta blockers           | 186/266 (70.0%)  | 47/68 (69.1%) | 0.897   |
| Calcium antagonists     | 49/265 (18.5%)   | 18/68 (26.5%) | 0.144   |
| Nitrates                | 61/265 (23.0%)   | 13/68 (19.1%) | 0.491   |
| Potassium channel blockers | 39/265 (14.7%) | 8/68 (11.8%) | 0.533   |
| ACE inhibitors          | 131/265 (49.4%)  | 36/68 (52.9%) | 0.606   |
| Angiotensin II blocker  | 9/265 (3.4%)     | 2/68 (2.9%)  | 0.852   |

| Intraoperative conditions |                  |             |         |
|---------------------------|------------------|-------------|---------|
| Dopamine                  | 136/267 (50.9%)  | 40/67 (59.7%) | 0.200   |
| Noradrenaline             | 158/267 (59.2%)  | 42/67 (62.7%) | 0.601   |
| Adrenaline                | 10/266 (3.8%)    | 4/67 (6.0%)  | 0.421   |
| Milrinone                 | 33/267 (12.4%)   | 15/67 (22.4%) | 0.037   |
| CPB time (min)            | 132.8 ± 50.7     | 152.1 ± 61.3 | 0.018   |
| Cross clamp time (min)    | 91.7 ± 40.2      | 105.3 ± 47.6 | 0.018   |
| Operation time (min)      | 296.3 ± 125.4    | 319.7 ± 108.5 | 0.029   |
| Intra-aortic balloon pump | 9/266 (3.4%)     | 7/67 (10.4%) | 0.016   |
| Packed red blood cells    | 126/266 (47.4%)  | 41/67 (61.2%) | 0.043   |
| Fresh frozen plasma       | 20/266 (7.5%)    | 7/67 (10.4%) | 0.433   |
| Platelet bags             | 25/266 (9.4%)    | 8/67 (11.9%) | 0.534   |

| Operative method          |                  |             |         |
|---------------------------|------------------|-------------|---------|
| Valve Surgery             | 118/267 (44.2%)  | 47/68 (69.1%) | <0.001  |
| CABG                      | 178/267 (66.7%)  | 44/68 (64.7%) | 1.000   |
| Valve Surgery + CABG      | 40/267 (15%)     | 21/68 (30.9%) | 0.002   |

| Postoperative conditions  |                  |             |         |
|---------------------------|------------------|-------------|---------|
| Dopamine                  | 156/268 (58.2%)  | 51/67 (76.1%) | 0.008   |
| Noradrenaline             | 163/268 (60.8%)  | 51/67 (76.1%) | 0.023   |
| Adrenaline                | 12/267 (4.5%)    | 12/67 (17.9%) | <0.001  |
| Milrinone                 | 39/267 (14.6%)   | 21/67 (31.3%) | 0.001   |
| Packed red blood cells    | 110/267 (41.2%)  | 36/67 (53.7%) | 0.065   |
| Fresh frozen plasma       | 39/266 (14.7%)   | 13/67 (19.4%) | 0.340   |
| Platelet bags             | 39/266 (14.7%)   | 18/67 (26.9%) | 0.018   |
| Resternotomy              | 11/267 (4.1%)    | 10/67 (14.9%) | 0.001   |
| Readmitted to intensive care | 1/267 (0.4%) | 0/68 (0.0%) | 0.614   |
| Length of admission (days)| 11.0 ± 8.0       | 13.1 ± 7.2  | <0.001  |
| Length of ICU admission (days) | 2.4 ± 3.0 | 4.1 ± 3.5 | <0.001  |
| Length of stay HDU (days) | 1.3 ± 1.0        | 1.6 ± 1.0   | 0.001   |
### Table 3. Serum biomarkers for predicting AKI pre and post cardiac surgery. AUROC, Sensitivity and specificity for serum biomarkers for predicting AKI pre and post cardiac surgery. AKI, acute kidney injury; AUROC, area under receiver operating characteristic; CI, confidence interval; sTNFR2, soluble tumour necrosis factor receptor 2; sTNFR1, soluble tumour necrosis factor receptor 1; MK, midkine; H-FABP, heart-type fatty acid-binding protein.

|                  | Biomarkers       | AUROC | CI               | Sensitivity | Specificity |
|------------------|------------------|-------|------------------|-------------|-------------|
| **Anytime**      |                  |       |                  |             |             |
| Preoperative     | sTNFR2           | 0.713 | 0.647–0.778      | 65.6% (42/64) | 65.9% (170/258) |
|                  | sTNFR1           | 0.748 | 0.684–0.812      | 70.3% (45/64) | 68.5% (178/260) |
|                  |                  |       |                  |             |             |
| Postoperative    | MK               | 0.704 | 0.633–0.775      | 70.7% (41/58) | 61.3% (130/212) |
|                  | H-FABP           | 0.729 | 0.663–0.794      | 63.1% (41/65) | 68.1% (175/257) |
|                  | sTNFR2           | 0.762 | 0.699–0.825      | 69.2% (45/65) | 69.2% (175/253) |
|                  | sTNFR1           | 0.774 | 0.708–0.840      | 72.3% (47/65) | 74.0% (188/254) |
|                  |                  |       |                  |             |             |
|                  | H-FABP + sTNFR1  | 0.817 | 0.761–0.872      | 81.0% (47/58) | 67.8% (141/208) |
|                  | H-FABP + MK + sTNFR2 | 0.836 | 0.785–0.888      | 75.9% (44/58) | 69.1% (143/207) |

**Figure 2.** Preoperative biomarker for detecting AKI risk. (A) Serum sTNFR1 pre cardiac surgery was significantly higher in patients who developed AKI. (B) Soluble TNFR1 had the highest predictive ability to identify patients at risk of developing AKI (AUROC 0.748). AKI, acute kidney injury; sTNFR1, soluble tumour necrosis factor receptor 1; AUROC, area under receiver operating characteristic.

**Figure 3.** Postoperative biomarker-based algorithm for detecting AKI risk. (A) Serum H-FABP, MK and sTNFR2 any time post surgery were significantly higher in patients who developed AKI. (B) H-FABP, MK and sTNFR2 had the highest predictive ability to identify patients at risk of developing AKI (AUROC 0.836). AKI, acute kidney injury; H-FABP, heart-type fatty acid-binding protein; MK, midkine; sTNFR2, soluble tumour necrosis factor receptor 2; AUROC, area under receiver operating characteristic.
**Table 4.** Clinical factors identified for patients at risk of developing AKI pre cardiac surgery and CRS (result). AKI, acute kidney injury; CRS, clinical risk score; BMI, body mass index.

| Clinical Factors | Parameter | Result |
|------------------|-----------|--------|
| Age              | <65 < 65 | 0 1    |
| BMI              | <25 < 25 | 0 0.5  |
|                  | ≥25 ≥25 | 0 1    |
|                  | <30 < 30 | 0 1    |
|                  | ≥30 ≥30 | 0 1    |
| Diabetes         | No No | 0 1    |
|                  | Yes Yes | 1 1    |
| CPB time (min)   | <130 <130| 0 1    |
|                  | ≥130 ≥130| 0 1    |
| Cross clamp time (min) | <90 <90 | 0 1 |
|                  | ≥90 ≥90 | 0 1    |
| Operation time (min) | <296 <296| 0 1 |
|                  | ≥296 ≥296| 0 1    |
| Intra-aortic balloon pump | No No | 0 1 |
|                  | Yes Yes | 1 1    |
| Packed red blood cells | No No | 0 1 |
|                  | Yes Yes | 1 1    |
| Platelet bags    | No No | 0 1    |
|                  | Yes Yes | 1 1    |
| Resternotomy     | No No | 0 1    |
|                  | Yes Yes | 1 1    |

**Table 5.** Clinical factors identified for patients at risk of developing AKI 24 hours post cardiac surgery and CRS (result). AKI, acute kidney injury; CRS, clinical risk score; BMI, body mass index; CPB, cardio pulmonary bypass.

| Clinical Factors | Parameter | Result |
|------------------|-----------|--------|
| Age              | <65 < 65 | 0 1    |
| BMI              | <25 < 25 | 0 0.5  |
|                  | ≥25 ≥25 | 0 1    |
|                  | <30 < 30 | 0 1    |
|                  | ≥30 ≥30 | 0 1    |
| Diabetes         | No No | 0 1    |
|                  | Yes Yes | 1 1    |
| CPB time (min)   | <130 <130| 0 1    |
|                  | ≥130 ≥130| 0 1    |
| Cross clamp time (min) | <90 <90 | 0 1 |
|                  | ≥90 ≥90 | 0 1    |
| Operation time (min) | <296 <296| 0 1 |
|                  | ≥296 ≥296| 0 1    |
| Intra-aortic balloon pump | No No | 0 1 |
|                  | Yes Yes | 1 1    |
| Packed red blood cells | No No | 0 1 |
|                  | Yes Yes | 1 1    |
| Platelet bags    | No No | 0 1    |
|                  | Yes Yes | 1 1    |
| Resternotomy     | No No | 0 1    |
|                  | Yes Yes | 1 1    |

**Table 6.** Post surgery patient score calculation and BRS. *Patient Score = 7.322 + 1.773 *log(H-FABP) + 1.120 *log(MK) + 3.510 *log(sTNFR2).* The patient score equation was derived from logistic regression. The cut-off (0.200) was manually determined to optimise sensitivity while maintaining specificity. If the patient score was <0.200 then BRS is negative for AKI, if the patient score ≥0.200 then BRS is positive for AKI. BRS, biomarker risk score; H-FABP, heart-type fatty acid-binding protein; MK, midkine; sTNFR2, soluble tumour necrosis factor receptor 2; AKI, acute kidney injury.

| BRS     | Patient score |
|---------|---------------|
| Negative | <0.200        |
| Positive | ≥0.200        |

**Table 7.** Proactive AKI clinical tool for management of patients pre- and post-cardiac surgery. BRS biomarker risk score: negative = non AKI, positive = AKI. CRS clinical risk score pre cardiac surgery: low 0–1, high 1.5–3. CRS clinical risk score post cardiac surgery: low 0–4, high 4.5–10. AKI, acute kidney injury; BRS, biomarker risk score; CRS, clinical risk score.

| Category | BRS     | CRS     | Clinical Management |
|----------|---------|---------|---------------------|
| 1        | Negative| Low     | Routine pre or postoperative management |
| 2        | Negative| High    | Assign to low risk management |
| 3        | Positive| Low     | Assign to higher risk management |
| 4        | Positive| High    | Assign to highest risk management |
secondary to myocardial dysfunction, and MK suggests renal ischemia reperfusion injury. A potential mechanism of action for the biomarker combination is described in Fig. 4.

The onset of AKI is multifactorial so in addition to biomarkers, clinical characteristics including age, BMI and diabetes were identified as risk factors for patients at potential risk for the development of AKI preoperatively (Table 4). These three clinical factors were also identified together with surgery-related factors, which included CPB time, cross-clamp time, operation time, whether the patient needed intra-aortic balloon pump, transfusion of blood or platelets and resternotomy, for identifying AKI in patients postoperatively (Table 5). To translate both the biomarker data and clinical characteristics into a proactive AKI clinical tool, the information was converted into a BRS and CRS, respectively (Tables 4, 5, 6 and 7). The results from the BRS and CRS combination will allow the clinician to identify patients at risk of AKI, administer appropriate treatments and monitor treatment efficacy. Thus, if a patient was identified as risk category 2, the current author (WMcB) would monitor the patient’s expected increases in creatinine and urea concentrations over several days retaining an expectation that dialysis requirement would be unlikely. A diuretic, if necessary, would be considered. If the patient was identified as category 3, the dialysis machine would be made available but not primed for potential use post surgery. The present author would be more hesitant to give a diuretic to this patient. However, if the patient was identified as category 4, the author would request that the dialysis machine was ready for use once the operation was completed.

The patients who developed AKI in the current study stayed an extra 2 days in hospital and 2 days longer in ICU ($p=0.000$ and $p=0.000$, respectively). Similarly, AKI patients had significant increased length of stay in the HDU ($p=0.001$). Additional hospital stay is associated with increased costs. However, the low risk patients could potentially have been moved out of the HDU to a ward which would improve patient flow and free up beds and staff to accept new patients, with associated savings (2018/2019 costs per day in HDU is £1400 (excluding medication))40. Earlier diagnosis of AKI benefits the patient, clinician and improves use of hospital resources.

Limitations of the study. Patients undergoing cardiac surgery were included in the study and, therefore, AKI resulting from other serious diseases such as sepsis or drug-induced AKI, were not represented. This was an observational study, where biomarker analysis was completed post event. This limits conclusions since patient interventions were not influenced by our results. Strengths of the study include; the patients were considered not renally impaired preoperatively which enabled measurement of baseline biomarkers. This assisted with determination of biomarker levels post surgery and an understanding of the role of biomarkers in AKI development.

Conclusion
Measurement of sTNFR1 or sTNFR2 preoperatively predicted risk of a patient developing AKI following cardiac surgery. Measurement of a combination of biomarkers, namely H-FABP, MK, sTNFR1 or sTNFR2, at any time postoperatively identified patients with increased risk of developing AKI. Furthermore, deployment of a BRS in combination with CRS in routine practice could assist the clinician with appropriate patient management. This would allow identification of patients at higher risk of developing AKI pre and immediately postoperatively.
Adoption of this novel proactive AKI clinical tool will (1) facilitate early identification of patients at risk of AKI, (2) allow timelier clinical decision-making, (3) alter current patient pathways, resulting in more efficient hospital resources utilisation and reduced hospital/ICU/HDU stay.

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

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**Author contributions**

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

**Competing interests**

M.J.K., A.D., D.M., J.W. 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.

**Additional information**

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