**Acute kidney injury risk in orthopaedic trauma patients pre and post surgery using a biomarker algorithm and clinical risk score**

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Acute kidney injury (AKI) after major trauma is associated with increased mortality. The aim of this study was to assess if measurement of blood biomarkers in combination with clinical characteristics could be used to develop a tool to assist clinicians in identifying which orthopaedic trauma patients are at risk of AKI. This is a prospective study of 237 orthopaedic trauma patients who were consecutively scheduled for open reduction and internal fixation of their fracture between May 2012 and August 2013. Clinical characteristics were recorded, and 28 biomarkers were analysed in patient blood samples. Post operatively a combination of H-FABP, sTNFR1 and MK had the highest predictive ability to identify patients at risk of developing AKI (AUROC 0.885). Three clinical characteristics; age, dementia and hypertension were identified in the orthopaedic trauma patients as potential risks for the development of AKI. Combining biomarker data with clinical characteristics allowed us to develop a proactive AKI clinical tool, which grouped patients into four risk categories that were associated with a clinical management regime that impacted patient care, management, length of hospital stay, and efficient use of hospital resources.

Acute kidney injury (AKI) after major trauma is associated with increased mortality. In a recent meta-analysis, from 17 articles describing AKI outcomes in 24,246 trauma patients, the pooled incidence of AKI was 20.4%1. The Kidney Disease: Improving Global Outcomes (KDIGO) criteria have reported that the incidence of AKI is 7–11% after orthopaedic surgery2,3. Orthopaedic trauma patients in general already have longer hospital stay, higher post-operative morbidity and mortality, and reduced quality of life4.

The most recent criteria developed for the diagnosis of AKI is the 2012 KDIGO5, which combines Risk/Failure/Injury/Loss/End-stage (RIFLE) and Acute Kidney Injury Network (AKIN) and relies on changes in serum creatinine (sCr) levels and urine output. Urine output may be compromised as a result of surgery and if used as an indicator of AKI could misclassify patients6. Creatinine measured in serum or plasma is the most commonly used biomarker to determine estimated glomerular filtration rate (eGFR). However, an increase in creatinine levels after renal insult is often delayed and a change in plasma creatinine levels may not be evident until two to three days after the initial insult7. Work in animals and humans has shown that although AKI due to ischemia can be prevented and treated, preventative therapy must be started very early after the renal injury8.

Single biomarkers are unlikely to predict or diagnose AKI due to the heterogeneity involved in the pathogenesis of AKI. Identification of biomarkers for AKI, have focussed on cardiac surgery populations9–11. Indeed, a recent publication identified a biomarker combination (heart-type fatty acid binding protein (H-FABP), midkine (MK) and soluble tumour necrosis factor (sTNFR) 1 or 2) that predicted AKI both pre and post cardiac surgery11. Clinical risk factors for AKI have been reported to include age, male gender, pre-existing chronic kidney disease (CKD), diabetes, heart failure and surgery15–17. Interestingly, clinical risk factors, pre and post cardiac surgery, identified in a recent publication, when combined with biomarkers could predict the risk of AKI11. However,

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biomarker combinations together with clinical risk factors have not been identified for orthopaedic trauma patients.

Therefore, we considered (1) could measurement of blood biomarkers pre and post surgery be used to stratify risk of AKI in orthopaedic trauma patients? and (2) could biomarker data combined with clinical characteristics be used to develop a tool to assist clinicians in identifying orthopaedic trauma patients at risk of AKI and guide patient management?

Methods

Study population. This prospective study of 237 patients was performed within the Fracture Unit of the Royal Victoria Hospital, Belfast, UK between May 2012 and August 2013. 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. Orthopaedic trauma patients who were consecutively scheduled for open reduction and internal fixation (ORIF) of their fracture, were recruited into the study. Patients were excluded if they were <18 years of age, had preoperative or pre-trauma dialysis-dependent renal failure or had a history of significant renal disease prior to recruitment. Of the n = 237 patients recruited to the study, pre and post operative samples were available for 201/237 (84.8%) patients. Patient samples were not available for 36/237 (15.2%) 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, comorbidities and current medications.

Sampling and laboratory methods. Patient blood samples (10 ml) were collected preoperatively and on day 1 post operatively. Patient blood samples were centrifuged, and serum and plasma were aliquoted within 30 min of collection and stored at −80 °C.

Patient blood samples were analysed in duplicate by Randox Clinical Laboratory Services (RCLS) (Antrim, UK) using cytokine arrays (Randox Laboratories Ltd, Crumlin, UK), ELISAs or IT assays, as described previously. Biomarker values below the limit of detection (LOD) were recorded as 90% of LOD. A total of 27 biomarkers (IL-2 (LOD 4.9 pg/ml), IL-4 (LOD 3.5 pg/ml), IL-6 (LOD 0.4 pg/ml), IL-8 (LOD 2.3 pg/ml), VEGF (LOD 10.8 pg/ml), IFNy (LOD 2.1 pg/ml), TNFα (LOD 3.7 pg/ml), IL-1α (LOD 0.9 pg/ml), MCP-1 (LOD 25.5 pg/ml), EGF (LOD 2.5 pg/ml), IL-10 (LOD 1.1 pg/ml), IL-1β (LOD 1.3 pg/ml), IL-1Ra (LOD 16.83 pg/ml), PDGF-BB (LOD 16.16 pg/ml), IP-10 (LOD 7.81 pg/ml), IL12-p40 (LOD 7.81 pg/ml), sIL-2A (LOD 0.12 ng/ml), sIL-6R (LOD 0.62 ng/ml), sTNFR1 (LOD 0.09 ng/ml), sTNFR2 (LOD 0.2 ng/ml), MMP-9 (LOD 3.03 ng/ml), CRP (LOD 0.67 mg/l), D-Dimer (LOD 2.1 mg/l), NSE (LOD 0.26 ng/ml), NGAL (LOD 17.8 ng/ml), MK (LOD 8.0 pg/ml) and H-FABP (LOD 2.94 ng/ml) were measured by RCLS. Serum creatinine (LOD 5 μm/L) was measured in the Kelvin Laboratory, Belfast Royal Victoria Hospital, Belfast.

Outcome definition. Patients did not have a baseline eGFR measurement prior to trauma but were assumed to have a normal renal function with a baseline eGFR of at least 60 ml/min/1.73m². A value of <45 ml/min/1.73m² was used to define a patient as AKI positive on any of the recorded pre and post operative sampling days, in accordance with the RIFLE classification; any patient with an eGFR result at any time (day 0, 1, 2, and 5) > 25% of 60 ml/min/1.73m² (45 ml/min/1.73m²) were determined to have AKI.

Statistical analysis. Statistical analyses were performed using R. Wilcoxon rank sum test was used to identify differentially expressed biomarkers. Biomarkers with a p<0.05 were considered significant. The ability of the biomarkers to predict AKI was further investigated using logistic regression (Lasso regression). For each biomarker and biomarker combination, areas under the receiver operator characteristic (AUROC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were generated pre and post operatively to identify models that differentiated between the two diagnostic groups (non AKI vs. AKI).
Results
Clinical characteristics for patients involved in the study are presented in Table 1. Of the 28 biomarkers that were investigated, sTNFR1 and H-FABP had the highest AUROC pre surgery to stratify risk of AKI in orthopaedic
trauma patients (Table 2) (sTNFR1 sensitivity 76.4%; specificity 59.7%; AUROC 0.729 (CI 0.654–0.804); H-FABP sensitivity 62.1%; specificity 71.2%; AUROC 0.712 (CI 0.637–0.786) (Fig. 2A,B)). LASSO regression identified a combination of 3 biomarkers post operatively to stratify risk of AKI, namely H-FABP, sTNFR1 and MK (Table 2) (H-FABP, sTNFR1 and MK combined sensitivity 80.5%; specificity 86.0%; AUROC 0.885 (CI 0.825—0.944) (Fig. 3A,B)).

To develop a tool to assist clinicians in identifying orthopaedic trauma patients at risk of AKI and guide patient management, the clinical characteristics between non AKI and AKI patients were investigated. We used biomarker combinations to determine a biomarker risk score (BRS) pre and post surgery (based on AUROC determined by LASSO regression) that could be used to identify patients at risk of AKI. Establishing a biomarker set point (cut-off) pre and post surgery categorised patients either positive or negative for AKI i.e. if a post surgery patient had a BRS above the set point they would be predicted to be positive for AKI (Table 3).

Three clinical characteristics were identified for patients at potential risk of AKI pre and post operatively (Table 4). Each clinical characteristic was given a score 0 or 1 (0 = no risk, 1 = risk). Each clinical characteristic was then added to give a cumulative risk score (CRS). For example, pre surgery patients who score ≥ 1 e.g. an ≥ 80-year-old patient with dementia and hypertension would have a cumulative CRS of 3 and would therefore be categorized high risk for AKI. The cut-off for age was based on significance, where patients > 80 years were at greater risk of AKI, based on our patient cohort.

To translate the results of the BRS and CRS into a proactive clinical AKI tool, the BRS and CRS were combined. Combining BRS with CRS either pre or post surgery identified 4 risk categories for patient management (Table 5). Categories 1 and 2 = low risk; Categories 3 and 4 = high risk. Two worked examples for a non AKI and AKI patient, are shown in the Supplementary Notes S1–S3 and Supplementary Tables S1–S6. The distribution of non AKI and AKI within the patient cohort, for each risk category, is shown in Supplementary Note S4.

**Discussion**

Hip fracture is the most common serious injury reported in the elderly resulting in long hospital stays, high post-operative morbidity and mortality, and reduced quality of life. Furthermore, AKI after trauma, such as hip fracture is associated with a poor prognosis.

Diagnosis of AKI using sCr and urine output can often result in misdiagnosis. The aim of this study was to further investigate if blood biomarkers and clinical risk factors could be used to identify AKI risk in orthopaedic trauma patients pre and post ORIF surgery in a similar fashion to those identified in patients undergoing cardiac surgery.

Interestingly, the same blood biomarkers, H-FABP, Midkine, sTNFR1 or sTNFR2, that predicted AKI
**Figure 3.** (A) Post operative serum biomarker model predicted probabilities for non AKI and AKI patients. Predicted probabilities for post surgery serum levels for H-FABP, MK and sTNFR1 individually and combined. Wilcoxon rank sum statistical significance is indicated by: **p < 0.01, ****p < 0.0001. AKI acute kidney injury, H-FABP heart-type fatty acid-binding protein, MK midkine, sTNFR soluble tumour necrosis factor receptor. (B) Receiver operator characteristics for post surgery serum biomarkers and model. H-FABP (AUROC 0.829), MK (AUROC 0.678), sTNFR1 (AUROC 0.795) and model H-FABP + MK + sTNFR1 (AUROC 0.885). AUROC area under the receiver operator characteristic, H-FABP heart-type fatty acid-binding protein, MK midkine, sTNFR soluble tumour necrosis factor receptor.

| BRS          | Patient score* |
|--------------|----------------|
| Negative     | < -1.05        |
| Positive     | ≥ -1.05        |

| Clinical factor | Level | Clinical factor score |
|-----------------|-------|-----------------------|
| Age             | < 80  | 0                     |
|                 | ≥ 80  | 1                     |
| Dementia        | No    | 0                     |
|                 | Yes   | 1                     |
| Hypertension    | No    | 0                     |
|                 | Yes   | 1                     |

**Table 3.** Post surgery patient score calculation and BRS determination. The patient score equation was derived from logistic regression. The cut-off (closest top left) of − 1.05 was determined using the following equation: closest top left = min((1 − sensitivities)^2 + (1 − specificities)^2). If patient score < − 1.05 then BRS is negative, if patient score ≥ − 1.05 then BRS is positive. BRS biomarker risk score, H-FABP heart-type fatty acid-binding protein, sTNFR soluble tumour necrosis factor receptor, MK midkine, min minimum. *Patient Score = − 8.185 + 2.037*ln(H-FABP) + 2.373*ln(sTNFR1) + 0.056*ln(MK).

**Table 4.** Clinical risk factors. If total clinical factor score = 0 then CRS is low, if total clinical factor score ≥ 1 then CRS is high. CRS clinical risk score.
in pre and post cardiac surgery patients also identified AKI risk in orthopaedic trauma patients undergoing ORIF surgery. The biomarkers combined with clinical characteristics (age, dementia and hypertension) identified from the study, delivered a proactive clinical AKI tool that could assist clinicians with patient management.

A total of 28 blood biomarkers were investigated. However only two biomarkers, sTNFR1 or H-FABP, were identified as predictive of AKI pre surgery and a combination of three biomarkers, sTNFR1, H-FABP and MK, were predictive for AKI post surgery. Remarkably these biomarkers represent three main pathological processes of AKI. Mechanisms contributing to AKI include (1) perioperative episodes of under perfusion, followed by (2) ischemia reperfusion injury during restoration of normal blood pressure. Inflammatory mediators (3) contribute to and augment the renal injurious effects of this twofold process. Accordingly, an additional separate inflammatory insult arising from other perioperative factors such as coagulation disturbance (which is an important proinflammatory mechanism), can augment the renal injurious effect of hypotension and ischemia reperfusion. Biomarkers have been associated with identification of underlying processes of hypotension (VEGF and H-FABP), IRI (MK) and inflammation (sTNFR1 and 2), which as anti-inflammatory biomarkers are taken as surrogates for the underlying proinflammatory response which drives them.

Elevated sTNFR1 levels have been identified in many clinical conditions e.g. kidney disease, neuropathy, cardiovascular disease and diabetes, and circulating levels of sTNFR1 have been shown to be an independent predictor of CKD progression in elderly patients. Tumour necrosis factor alpha (TNFa) and TNFR2 are almost undetectable in the kidneys of healthy subjects unlike TNFR1 which is expressed within the trans-golgi network of the glomerular endothelium. An increase in the level of sTNFRs in CKD patients has been implicated in declining eGFR. Moreover, TNFa acting through TNFR1 has a damaging effect on renal endothelial cells, possibly through iNOS, which would generate intratubular toxic levels of NO, as demonstrated by increased urinary nitrate levels in a porcine model of ischaemia reperfusion-mediated AKI. The elevated anti-inflammatory sTNFR1 response in blood may be driven by an underlying proinflammatory response which includes TNFa. Since monomeric TNFa is much smaller than sTNFR1 and 2, it is more readily filtered by the glomerulus. Accordingly, TNFa is able to cause glomerular injury once it escapes from the moderating biological effect of the TNFRs.

H-FABP was also predictive of AKI pre operatively, and in combination with sTNFR1 and MK, post operatively. H-FABP, associated with cardiac injury, is released into the bloodstream 30 min after an ischaemic event and peaks at 6 h before returning to normal levels after 24 h. H-FABP has been reported to predict AKI pre and post cardiac surgery, however, this is the first time that H-FABP has been demonstrated to predict AKI in patients pre and post ORIF surgery.

H-FABP is predominantly expressed in the heart but also at lower levels in skeletal muscle, kidney, stomach, brain and testis. The levels of H-FABP in skeletal muscle have been shown to be almost half that found in the heart. Moreover, kidney H-FABP levels are almost two-thirds that found in skeletal muscle. While it is known that H-FABP levels increase in the blood, this may arise from skeletal muscle or renal sources, but it is more likely to be from the heart, which is the largest reservoir of H-FABP in the body. In elderly patients, acute coronary insufficiency is common and would be reflected in elevated H-FABP. Any transient hypoperfusion, which such an event would provoke, could result in a significantly heightened risk of AKI. This is the most likely reason why H-FABP was predictive in this orthopaedic trauma patient cohort.

In addition to sTNFR1 and H-FABP, MK was also identified in the biomarker combination to predict AKI post operatively. The pathophysiological roles of MK are diverse, ranging from AKI to progression of CKD, accompanied by hypertension, renal ischaemia and diabetic nephropathy. After ischaemic reperfusion MK is immediately induced in the proximal tubules, leading to the upregulation of macrophage inflammatory protein-2 for neutrophils and monocyte chemotactic protein-1 for macrophages. Eventually, infiltrated inflammatory cells cause severe tubulointerstitial injury. Silencing renal MK expression with anti-sense oligos prevents kidney damage and increases osteogenic activity. Midkine is also involved in chondrogenesis and fracture healing. Interestingly, MK-deficient mice have been shown to display increased bone formation rate and volume. This is the first study, to our knowledge which has identified MK as a biomarker for stratifying patients at risk of AKI following orthopaedic trauma and ORIF surgery.

Three important pathways involved in the pathogenesis of AKI were identified, namely hypoperfusion (H-FABP), ischaemia reperfusion injury (MK) and proinflammatory insult (sTNFR1) (Fig. 4).

Risk factors that have previously been reported for AKI include age, pre-existing CKD, male gender, diabetes, heart failure and surgery. In this study, three clinical risk factors were identified for patients who were at potential risk for the development of AKI, pre and post operatively; age, dementia and hypertension (Table 4). Using biomarker data and clinical factors we developed a BRS and a CRS, respectively. Combining BRS (Table 3)
with CRS (Table 4) grouped patients into four risk categories, each of which is associated with a clinical management regime (Table 5). Deployment of this proactive clinical AKI tool would allow clinicians to stratify patients at risk of AKI enabling early intervention and improving patient outcomes. Use of a cardiac proactive clinical AKI tool has been described previously. In the study cohort 63/201 (31.3%) orthopaedic trauma patients developed AKI post ORIF surgery. The incidence of AKI in this patient cohort is higher than previously reported. Patients who developed AKI were significantly older and more likely to have hypertension and/or dementia. Advanced age is frequently reported as a risk factor for AKI however, to our knowledge, this is the first report that identified dementia as a potential risk factor in the development of AKI. Recently, an association of heightened proinflammatory activity in patients with dementia has been reported. Our results are consistent with these findings. Interestingly, patients surviving AKI have a higher probability of developing dementia in the long-term compared to patients who did not develop AKI.

The time between presentation and surgery was 2 days and was not significant between non AKI and AKI patients. An optimal operation time of between 24–48 h after orthopaedic trauma has been identified for lower extremity fracture fixation to reduce complications. Operations performed outside of this timeframe are associated with increased morbidity and mortality. AKI patients stayed an additional two days in hospital compared to non AKI patients (12.0 (3.7–20.3) days for AKI vs. 9.8 (1.9–17.7) days for non AKI patients), consistent with previous findings. Patients that develop AKI following elective total joint arthroplasty also have increased hospital stay. The management of patients with AKI is a significant burden to the healthcare service. Earlier diagnosis and management of patients at risk of AKI will potentially reduce the financial burden on healthcare systems in addition to improving patient outcomes and welfare.

Surprisingly previous work has failed to identify hypotension as a serious risk factor in AKI. Since intraoperative blood pressure modulation is a readily available strategy for anaesthetologists, inability to show a link between hypotension and AKI at orthopaedic surgery could arguably generate a false sense of complacency. However, the reason for lack of the relationship between perioperative blood pressure and subsequent AKI could be because most patients have non-invasive blood pressure measurements where blood pressure readings are obtained by an arm cuff measurement every 5 min whereas more critically ill patients have continuous arterial blood pressure measurements which detect and record all hypotensive episodes. In Bräunier’s study they recorded lowest blood pressure measurement intraoperatively. Their work suggested that this was not a useful marker in terms of AKI prediction. However, we argue that clinically significant hypotensive episodes may have been missed in this study if they happened in between measurements. This means that transient, albeit clinically significant, hypotension could be missed in between these times. In summary, the use of clinical data alone (including perioperative hypotensive events) to predict perioperative AKI is of limited usefulness in hip fracture surgery. It has already been shown in cardiac surgery that biomarkers of ischaemia reperfusion (MK) or hypotension (VEGF or HFABP) and inflammation augmented clinical parameters. This present work suggests that this principle is also applicable to hip fracture surgery.

![Figure 4. Pathogenesis of AKI. Three important pathways in the pathogenesis of AKI are represented by biomarkers in the model: (1) hypoperfusion (H-FABP), (2) proinflammation (sTNFR1 as a surrogate for the transient TNFα response) and (3) ischaemia reperfusion injury (MK). Adapted from McBride et al. AKI acute kidney injury, BP blood pressure, H-FABP heart-type fatty acid-binding protein, MK midkine, sTNFR soluble tumour necrosis factor receptor, TNFα tumour necrosis factor alpha.](https://www.nature.com/scientificreports/)
In a meta-analysis it was demonstrated that perioperative hemodynamic optimization in surgery patients, reduces post-operative acute renal injury. Preoperative prediction would allow for enhanced perioperative hemodynamic optimization i.e. provision of Level 2 care provided post operatively, and invasive hemodynamic monitoring intraoperatively rather than blood pressure measurements every 5 min, as is routine for such cases in many centres. It could also be taken as a contraindication to noradrenaline anti-inflammatory use post operation.

Biomarkers are not a substitute to the classical approach to using low-cost information—but add to the information available to the clinician. However, it must be noted that a clear clinical history in these elderly patients can sometimes be unreliable. Hence the need for the objective information that biomarkers provide.

Limitations of the study. Clinical characteristics were not reliably available for everyone in this patient group including a guaranteed history of normal renal function pre trauma; patients were assumed, based on available clinical history, to have a normal renal function prior to their trauma and a baseline eGFR of at least 60 ml/min/1.73m². Therefore, patients who had undiagnosed pre-trauma renal dysfunction could have been included in the study. Nevertheless, subsequent fluctuations in renal function were still detectable using our proactive clinical AKI tool, demonstrating the clinical utility of our proposed method in this patient cohort, where obtaining clinical history is sometimes challenging and unreliable.

Conclusion
In conclusion, serum H-FABP and sTNFR1 measured pre operatively and serum H-FABP, MK and sTNFR1 measured post operatively, identified orthopaedic trauma patients at risk of developing AKI during ORIF surgery. Utilisation of the proactive clinical AKI tool, which combines BRS with CRS, would allow clinicians to stratify patients into one of four AKI risk categories with related treatment regimens that could impact patient care and management, length of hospital stay, and the efficient use of hospital resources.

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.M., M.J.K., G.M., M.R. and J.I.L. made substantial contributions to conception and design, analysis and interpretation of data, revising the manuscript for IP and given final approval of the version to be published. Furthermore, G.M. 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.P. provided conceptual support and contributed to manuscript revision. All authors have reviewed the manuscript.

Competing interests
YES, M.J.K., A.D.M., 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. G.M. was funded by Randox Laboratories Ltd, 55 Diamond Road, Crumlin BT29 4QY for 2 years to complete his MD. W.M. has no competing interests. A patent has been submitted by Randox to protect the biomarkers identified from this work.

Additional information
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