Association of plasma and urine NGAL with acute kidney injury after elective colorectal surgery: A cohort study

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ABSTRACT

Background: Acute kidney injury (AKI) is common in surgical patients. We aimed to investigate the validity of plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) in the detection of AKI and prediction of outcomes in patients undergoing major colorectal surgery.

Materials and methods: This was a pre-specified post-hoc analysis of a randomized controlled trial comparing oesophageal doppler and lithium dilution cardiac output monitoring in high risk patients undergoing major colorectal surgery as part of an Enhanced Recovery After Surgery protocol in a tertiary care hospital. Plasma and urine samples for NGAL measurement were taken before surgery (T1), immediately after surgery (T2), and on postoperative day 1 (T3). AKI was defined according to the KDIGO criteria.

Results: A total of 89 patients were included of whom 12 (13.5%) developed AKI. Plasma NGAL significantly increased from T1 to T3 in both AKI (p < 0.001) and non-AKI (p = 0.048) patients, while urine NGAL did not change over time. There were no significant differences in plasma and urine NGAL in patients with and without AKI at all time points. Postoperative day 1 urine NGAL concentrations were significantly higher in non-survivors than survivors (41.2 versus 25 ng/mL, p = 0.026). One-year mortality was significantly higher in AKI patients with a raised urine NGAL compared to AKI patients without elevated urine NGAL levels.

Conclusions: Plasma and urine NGAL poorly predicted AKI post-colorectal surgery. Non-survivors had higher urine NGAL results. More research is required to explore the association between NGAL and long-term outcomes.

1. Introduction

Acute kidney injury (AKI) is common in surgical patients and is associated with an increased risk of postoperative complications, longer stay in hospital, and higher mortality [1–6]. There are multiple potential causes [7]. Survivors of AKI are more likely to develop chronic kidney disease (CKD) and have a higher risk of cardiovascular complications and premature mortality [8–11]. Consequently, it is important to diagnose AKI early to initiate appropriate management and potentially prevent AKI-related complications.

The incidence of postoperative AKI after colorectal surgery varies from 3 to 13% [12–16]. Enhanced Recovery After Surgery (ERAS) protocols are multi-modal, multidisciplinary programs aiming to reduce surgical stress and restore physiological functions early. The key components of ERAS protocols are fluid administration guided by goal-directed haemodynamic monitoring, more liberal use of vasopressors, multimodal pain management, early resumption of oral intake, and early mobilisation. These programs are well established in colorectal surgery and have shown to hasten the return of bowel function, reduce postoperative complications and decrease length of hospital stay [16, 17]. Whether the implementation of an ERAS program affects the risk of AKI is still controversial [15,16,18–20].

A recent expert consensus meeting recommended using validated AKI biomarkers to identify patient populations who may benefit from...
early interventions to improve outcomes [21]. Neutrophil gelatinase-associated lipocalin (NGAL) is one of the most extensively studied biomarkers for AKI. It is considered a marker of tubular damage and has shown promising results for early AKI prediction in various settings such as post-cardiac surgery, coronary angiography, kidney and liver transplantation, and critically ill patients [22-24]. Nevertheless, certain conditions such as inflammation, sepsis, and cancer can increase both systemic and urine NGAL concentrations, thus mitigating its robustness as an indicator of renal damage [24].

The utility of plasma NGAL (pNGAL) and urine NGAL (uNGAL) as markers of early postoperative AKI and predictors of outcomes in patients with colorectal malignancy or inflammatory bowel disease is unknown. This study aims to evaluate the association of pNGAL and uNGAL with postoperative AKI in patients undergoing major elective colorectal surgery applying ERAS protocols.

2. Methods

2.1. Study design

This was a pre-specified post-hoc analysis of a prospective single-centre randomized controlled trial at a tertiary academic hospital in patients undergoing elective colorectal surgery using ERAS protocols from December 2011 until September 2012 (Trial registration: ISRCTN 50251697 and 24020298; Unique identification number: researchregistry6434. Available at https://www.researchregistry.com/browse-the-registry#home/registrationdetails/5fb1ee4acaca01b8c35b3/). The first series compared intra-operative goal-directed fluid optimization using pulse pressure waveform analysis (LiDCOrapid) with Oesophageal Doppler-guided oxygen delivery index (DO2i)-targeted fluid optimization in patients undergoing elective colorectal surgery using ERAS protocols (DO2i)-targeted fluid optimization was extended to 16 h after surgery versus standard post-operative care [25,26]. The study was approved by South East London Research Ethics Committee (REC) 2 (Reference No: 11/H0802/9). All patients gave written informed consent and the study was conducted according to the principles of the Declaration of Helsinki. Reporting is consistent with the STROCSS guidelines [27].

2.2. Population

We enrolled adults undergoing major high-risk colorectal surgery using ERAS protocols at a tertiary care center in the UK. Criteria for high-risk patients were 1) pre-operative American Society of Anesthesiologists (ASA) physical classification grade III and above, 2) anaerobic threshold <11 ml/min/kg, or 3) undergoing major complex surgery (as per Office of Population, Censuses and Surveys (OPCS) 4.8 Classification of Surgical Operations and Procedures) and planned for overnight intensive recovery (OIR) admission [28]. Exclusion criteria were 1) emergency operations, 2) lack of capacity to consent, 3) pregnancy, 4) contraindication to oesophageal Doppler probe, 5) contraindication to arterial access for LiDCORapid monitoring, 6) previous renal replacement therapy (RRT), or 7) kidney transplant.

2.3. Data collection

Baseline characteristics, comorbidities, and perioperative data were collected prospectively from the hospital electronic medical records (EMR) and paper anaesthetic and operation records. Baseline serum creatinine (lowest value within 3 months preceding surgery) and daily serum creatinine up to 3 days post-surgery were recorded. Glomerular filtration rate (GFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKDEpi) creatinine formula. Chronic kidney disease (CKD) was defined as estimated GFR (eGFR) < 60 ml/min/1.73 m² persisting for 3 months [29]. CardioQ ODM or LiDCORapid were used for haemodynamic cardiac output monitoring to guide fluid management, and additional vasopressor support if required.

2.4. Sample collection

Blood and spot urine samples for measurement of pNGAL and uNGAL were prospectively collected pre-operatively after induction of anaesthesia and before the start of surgery (T1), immediately after surgery (T2), and on post-operative day 1 (T3). Samples were centrifuged at 3000 g for 4 min and the supernatants were stored at −80 °C until measurement.

2.5. NGAL measurement

Plasma NGAL and uNGAL were measured immediately upon study completion using enzyme-linked immunosorbent assay (ELISA) (Bio-Porto diagnostic A/S, Copenhagen, Denmark). The upper limits were 250 ng/mL for pNGAL and 100 ng/mL for uNGAL as advised by the manufacturer [30].

2.6. Outcomes

The primary outcome was new AKI within 72 h after surgery as defined by the serum creatinine (SCr) criteria of the Kidney Disease Improving Global Outcome (KDIGO) classification [31]. Secondary outcomes included post-operative complications according to the Clavien-Dindo classification, re-admission and mortality at 30 days, 6 months, and 1, 5, and 8 years. The data were collated and checked against the patients’ notes following patient discharge. Long-term mortality was collected from the medical electronic patient records.

2.7. Statistical analysis

Categorical data were expressed in frequencies and compared using Chi-square or Fisher’s exact test, where appropriate. Continuous data were expressed in mean ± standard deviation if normally distributed and compared using independent t-test. Non-parametric data were expressed in median (interquartile range, IQR) and compared using Wilcoxon rank-sum test or Kruskall-Wallis test. Univariable logistic regression analysis was performed to assess an association between risk factors and AKI. Multicollinearity was tested, and parameters with p < 0.05 were used for multivariate logistic regression analysis. Differences between pNGAL and uNGAL values with respect to time were analysed using a linear generalized estimating equation (GEE) comparing between AKI and non-AKI groups and changes over time. Spearman’s rank correlation was determined between baseline pNGAL and uNGAL and other parameters. To examine the diagnostic performance of pNGAL and uNGAL for the development of AKI, the area under the receiver operating characteristic (AUROC) curve was determined. Unadjusted survival data were plotted as a Kaplan-Meier estimate and compared using the log rank test. A univariate cox-regression analysis was undertaken to assess factors related to time to death. Variables with p < 0.05 were included in the multivariate analyses. A 2-tailed p value < 0.05 was considered statistically significant. All statistical analyses were performed using Stata 14.0 (Statacorp, College Station, Texas, USA).

3. Results

3.1. Patient characteristics

Ninety-two patients participated in the study. After exclusion of two patients who had had RRT prior to surgery and one patient due to missing data, 89 patients were included in the final analysis (51.7% male, mean age 56.5 ± 18.0 years) (Fig. 1). Their main diagnoses were malignancy (49.4%), inflammatory bowel disease (22.5%), and other benign conditions (28.1%) (Table S1).
in malignancy, inflammatory bowel diseases, and other benign diagnoses. Plasma NGAL was higher in CKD patients and in more severe AKI patients (p = 0.019). NGAL was also significantly higher in AKI versus non-AKI patients. Spearman’s rank correlation analysis (Table 3) showed that plasma NGAL was significantly associated with AKI severity score (r = 0.45, p = 0.009), but there was no association with other parameters including blood transfusion and pre-morbid anemia. The area under the ROC curve (AUROC) of plasma NGAL for predicting post-operative AKI was 0.54 (0.31–0.8). Neither plasma NGAL nor uNGAL was associated with AKI development. The AUROC curve of pNGAL for predicting post-operative AKI was 0.54 (0.31–0.8). There was no significant increase in pNGAL concentrations from T1 to T2 (p = 0.048). However, there was a significant increase in pNGAL concentrations from T1 to T3 (p = 0.019), indicating that pNGAL concentrations were significantly higher in AKI versus non-AKI patients.

### 3.3. Trajectories of NGAL

Trajectories of NGAL were analyzed using repeated measure ANOVA. Serial uNGAL values did not significantly change and there was no significant increase in uNGAL concentrations from T1 to T2 (p = 0.048) and from T2 to T3 (p = 0.034). There was a trend towards higher 8-hour uNGAL in AKI versus non-AKI patients. The maximal AUROC curve of uNGAL was 0.54 (0.31–0.77) at T2 (Fig. S1). There was no significant difference between groups (p = 0.884) (Table 2, Fig. 2).

### 3.4. Risk factors for AKI

Multivariate logistic regression identified log10CRP and intra-operative blood transfusion as independent predictors for AKI. The AUROC curve of pNGAL for predicting post-operative AKI ranged from 0.43 (95% CI 0.17–0.69) (T3) to 0.46 (0.19–0.74) (T2). The maximal AUROC curve of uNGAL was 0.54 (0.31–0.77) at T2 (Fig. S1 and Fig. S2).

### 3.5. Association of NGAL and underlying disease

The median concentrations of plasma and urine NGAL were similar in malignancy, inflammatory bowel diseases, and other benign diagnoses. Plasma NGAL was higher in CKD patients and in more advanced colorectal cancer, whereas urinary NGAL was significantly higher in metastatic cancer (M1) (Table 4; Fig. S3). Spearman’s rank correlation demonstrated baseline pNGAL to be positively associated with tumour staging (p = 0.017) and uNGAL (p = 0.006). (Table S2; Fig. S4).

### 3.6. NGAL and long-term outcome

Twenty-four patients died within 8 years after surgery (27.0%), of whom 20.8% had postoperative AKI compared with 10.8% among survivors (Table S9). Causes of death were cancer progression (n = 10), sepsis (n = 3), and stroke (n = 2); the cause of death of the remaining 9 patients could not be determined. There was a trend towards higher 8-year mortality in patients with more severe AKI. In non-survivors, pNGAL (T1 and T2) and immediate postoperative uNGAL (T2) were marginally higher, and postoperative day 1 uNGAL was significantly higher, and postoperative day 1 uNGAL was significantly higher, and postoperative day 1 uNGAL was significantly higher, and postoperative day 1 uNGAL was significantly higher.
categorized into 4 categories; NGAL-/Cr-, NGAL+/Cr-, NGAL-/Cr+, NGAL+/Cr+. (Table S4). Of all patients, 78.7% and 65.2% patients were classified as pNGAL+/Cr+ and uNGAL+/Cr+. One-year mortality was highest in AKI patients with a raised uNGAL concentration (uNGAL+/Cr+) but there was no statistically significant difference in 8-year mortality between AKI patients with and without raised NGAL concentrations. The crude HR for pNGAL+/Cr+ and uNGAL+/Cr+ for 8-year mortality are 4.30 (95% confidence interval (CI) 0.48–38.47; p = 0.192) and 5.53 (95% CI 1.38–22.16; p = 0.016) compared to other groups, respectively (Fig. S5 and Fig. S6).

4. Discussion

Our study demonstrated a 13.5% incidence of AKI post-elective major colorectal surgery. Most patients had AKI stage 1, and none required RRT. There was an increase in pNGAL and uNGAL between baseline and the immediate postoperative period, but no significant difference between AKI and non-AKI patients. Both plasma and urine NGAL concentrations were higher in non-survivors, and AKI patients with uNGAL positivity demonstrated the worst long-term prognosis.

NGAL has been studied extensively post cardiac surgery [32]. Less is known about its role in major non-cardiac surgery; in addition, the results are conflicting [33]. A previous study in 22 postoperative oncological patients, including 15 patients with gastrointestinal tract malignancy, showed that uNGAL was an independent predictor of AKI. None of the patients had CKD, and the association with tumour staging was not explored [43].

NGAL is not organ specific. The employed NGAL assay used in our study measures pNGAL and uNGAL simultaneously. NGAL is not organ specific. The employed NGAL assay used in our study measures pNGAL and uNGAL simultaneously.

Abbreviations: AKI, acute kidney injury; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; Cr, creatinine; GFR, glomerular filtration rate; CKD, chronic kidney disease; CMO, complex major operation; MAJ, major surgery; POSSUM, Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity; IBD, inflammatory bowel disease; ASA, American Society of Anaesthesiology; PCA, patient-controlled analgesia; FTC, corrected flow time; DO2I, oxygen delivery index; CRP, c-reactive protein; OIR, overnight intensive recovery.

Table 1 (continued)

| Parameters                        | All patients | No AKI (n – 77) | AKI (n = 12) | P value |
|-----------------------------------|--------------|-----------------|-------------|---------|
| Intra-operative fluid (mL)        |              |                 |             |         |
| - Crystalloids                    | 2000         | 2000            | 2000        | 0.651   |
|                                  | (1100, 3000) | (1000, 3000)    | (1400, 3000)|         |
| - Colloids                        | 1000         | 1000            | 500         | 0.491   |
|                                  | (500, 2000)  | (500, 2000)     | (500, 2000)|         |
| - Blood products                  | 0 (0, 440)   | 0 (0, 0)        | 0 (0, 560)  | 0.508   |
| - Total                           | 3562.5       | 3625 (2500, 5000)| 3500 (1900, 4500)| 0.771   |

Outcomes

Table 1 (continued)

| CRP***                         | 138 (92, 237) | 131 (59, 234) | 205 (151, 289) | 0.024   |
|                                | 80 (61, 100)  | 74 (59, 89)   | 130 (103, 222.5)| <0.001  |
| Highest post-operative SCr (μmol/L)* |              |                |                |         |
| Clavien-Dindo classification   |               |                |                |         |
| 0                               | 34 (38.2)     | 34 (44.2)      | 0              | 0.003*  |
| 1 - II                          | 52 (58.4)     | 40 (52)        | 12 (100)       | 0.001*  |
| III - IV                        | 15 (16.9)     | 8 (10.4)       | 7 (58.3)       | <0.001  |
| ORL length of stay (hours)      | 18.5 (16.8)   | 18.5 (16.4)    | 18.5 (17.5)    | 0.694   |
| Hospital length of stay (days)  | 21.5          | 21.3           | 22.5           |         |
| 30-day readmission (%)          | 13 (14.6)     | 9 (11.7)       | 4 (33.3)       | 0.070*  |
| 30-day mortality (%)            | 1 (1.1)       | 0              | 1 (8.3)        | 0.135*  |
| 6-mo mortality (%)              | 3 (3.4)       | 1 (1.3)        | 2 (16.7)       | 0.047*  |
| 1-year mortality (%)            | 6 (6.7)       | 2 (2.6)        | 4 (33.3)       | 0.003   |
| 5-year mortality (%)            | 20 (22.5)     | 15 (19.5)      | 5 (41.7)       | 0.087   |
| 8-year mortality (%)            | 24 (27.0)     | 19 (24.7)      | 5 (41.7)       | 0.217   |

Abbreviations: AKI, acute kidney injury; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; Cr, creatinine; GFR, glomerular filtration rate; CKD, chronic kidney disease; CMO, complex major operation; MAJ, major surgery; POSSUM, Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity; IBD, inflammatory bowel disease; ASA, American Society of Anaesthesiology; PCA, patient-controlled analgesia; FTC, corrected flow time; DO2I, oxygen delivery index; CRP, c-reactive protein; OIR, overnight intensive recovery.

* Fisher’s exact test.

# Highest creatinine within 72 h after surgery.

Table 2

| Outcome | No AKI | T1 (n = 75) | T2 (n = 75) | T3 (n = 26) | p within groups* |
|---------|--------|-------------|-------------|-------------|------------------|
| pNGAL [ng/ ml] | 164.6 (128, 220.9) | 188.1 (149.9, 246.5) | 336.6 (236.4, 465.6) | 0.048 |
| uNGAL [ng/ ml] | 25 (25, 52.6) | 25.7 (25, 67.8) | 27.3 (25, 45.7) | 0.447 |

Abbreviations: AKI, acute kidney injury; pNGAL, plasma neutrophil gelatinase-associated lipocalin; uNGAL, urine neutrophil gelatinase-associated lipocalin.

T1 = Before surgery; T2 = Immediately after surgery; T3 = Post-operative day 1.

* All analyses were performed by generalized estimated equations.

3.7. Sub-group analyses

Using a cut-off value of 250 ng/mL for pNGAL and 100 ng/mL for uNGAL (NGAL+) and presence of AKI (Cr+), patients were further analyzed.
study measures a monomeric form of NGAL which is predominantly synthesized and released from kidney tubules, but small amounts are also released from inflammatory cells [44]. As a result, NGAL concentrations might have been influenced in patients with pre-existing malignancy or systemic inflammatory conditions. In our patient cohort, pNGAL increased significantly over time, which might suggest tissue injury or direct renal insult intraoperatively and post-surgery but there was no association with development of AKI. However, confounding may have occurred, for example, by malignant disease or the presence of CKD.

Our results suggest an association of NGAL and malignant disease. Pathologically, NGAL expression in colorectal tissue has been shown to

Table 3
Univariate and multivariate logistic regression for AKI prediction.

| Parameters | Crude odds ratio (95% CI) | p value | Adjusted odds ratio (95% CI) | p value |
|------------|--------------------------|---------|-----------------------------|---------|
| Diabetes   | 5 (1.20, 20.89)          | 0.027   | 3.87 (0.52, 28.68)          | 0.185   |
| Pre-operative haemoglobin | 0.65 (0.47, 0.91) | 0.012 | 0.64 (0.36, 1.14) | 0.130 |
| POSSUM physiological score | 1.17 (1.05, 1.32) | 0.007 | 0.98 (0.83, 1.22) | 0.829 |
| POSSUM total score | 1.11 (1.03, 1.20) | 0.010 | |
| Analgesia  |                          |         |                             |         |
| PCA only   | 1                        | Reference | 1                         | Reference |
| Epidural/spinal anaesthesia | 3.03 (0.32, 28.81) | 0.335 | 9.26 (0.55, 155.62) | 0.122 |
| Combined   | 9.21 (1.04, 81.36)       | 0.046   | 10.04 (0.69, 145.27)       | 0.091   |
| Log10 CRP  | 3.73 (1.10, 12.62)       | 0.034   | 6.05 (1.27, 28.89)         | 0.024   |
| Intra-op blood transfusion units | 1.45 (1.04, 2.02) | 0.028 | 1.59 (1.04, 2.41) | 0.031 |
| Log10 pNGAL at baseline | 0.77 (0.22, 2.69) | 0.681 | 1.59 (1.04, 2.41) | 0.031 |
| Log10 uNGAL at baseline | 2.80 (0.94, 8.38) | 0.067 | |

Abbreviations: POSSUM, Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity; PCA, patient-controlled analgesia; CRP, c-reactive protein; pNGAL, plasma neutrophil gelatinase-associated lipocalin; uNGAL, urine neutrophil gelatinase-associated lipocalin.

Table 4
Relationship of baseline pNGAL and uNGAL and clinical characteristics.

| Characteristics | Number of patients | pNGAL [ng/ml] | p value | uNGAL [ng/ml] | p value |
|-----------------|--------------------|---------------|---------|---------------|---------|
| Diagnosis       |                    |               |         |               |         |
| Malignancy      | 44                 | 161.6         | 0.744   | 25 (25, 42.9) | 0.868   |
| IBD             | 20                 | 163.1         | 0.342   | 25 (25, 31.8) |         |
| Others          | 25                 | 176.3         | 0.778   | 25 (25, 28)   |         |
| Diabetes        |                    |               |         |               |         |
| No              | 78                 | 161.3         | 0.549   | 25 (25, 31.8) | 0.176   |
| Yes             | 11                 | 196.2         |         | 30.1 (25, 59.1) |         |
| CKD             |                    |               |         |               |         |
| No              | 79                 | 161.3         | 0.035   | 25 (25, 31.9) | 0.547   |
| Yes             | 10                 | 277.4         |         | 25 (25, 42.9) |         |
| Type of operation |                |               |         |               |         |
| Open            | 74                 | 164.6         | 0.484   | 25 (25, 32.6) | 0.627   |
| Laparoscopy     | 15                 | 139.3         |         | 25 (25, 48.9) |         |
| Malignancy staging |              |               |         |               |         |
| T stage a       |                    |               |         |               |         |
| T1/T2           | 12                 | 116.6         | 0.019   | 25 (25, 28.1) | 0.489   |
| T3              | 25                 | 176.9         |         | 25 (25, 42.9) |         |
| T4              | 7                  | 177.6         |         | 25.8 (25, 184.8) |         |
| N stage b       |                    |               |         |               |         |
| N0              | 26                 | 138.4         | 0.056   | 25 (25, 45.2) | 0.932   |
| N1/2            | 18                 | 178.5         |         | 25 (25, 33.3) |         |
| M stage b       |                    |               |         |               |         |
| M0              | 40                 | 146.5         | 0.587   | 25 (25, 33)  | 0.007   |
| M1              | 4                  | 195.8         |         | 56.2 (39.916.6) |         |
| IBD             |                    |               |         |               |         |
| UC              | 10                 | 153.1         | 0.722   | 25 (25, 25)  | 0.214   |
| Crohn’s disease | 10                 | 178.9         |         | 27.6 (25, 33.6) |         |

Abbreviations: IBD, inflammatory bowel diseases; CKD, chronic kidney disease; T, tumour; N, nodes; M, metastases; UC, ulcerative colitis; pNGAL, plasma neutrophil gelatinase-associated lipocalin; uNGAL, urine neutrophil gelatinase-associated lipocalin.

a- Kruskal wallis test; b-Wilcoxon rank-sum test.
Table 5
Univariate and multivariate cox-regression analysis and 8-year mortality.

| Parameters                      | Crude HR (95% CI) | p value  | Adjusted HR (95% CI) | p value |
|---------------------------------|-------------------|----------|----------------------|---------|
| Age*                            | 1.06 (1.03, 1.09) | <0.001   | –                    | –       |
| Diabetes                        | 3.01 (1.19, 7.59) | 0.019    | 1.04 (0.22, 4.90)    | 0.956   |
| Malignancy                      | 2.80 (1.16, 6.77) | 0.022    | 1.37 (0.43, 4.39)    | 0.597   |
| T stage                         |                   |          |                      |         |
|   • T1/T2                       | 1.81 (0.50, 6.58) | 0.368    | –                    | –       |
|   • T3                          | 4.25 (0.95, 19.09)| 0.059    | –                    | –       |
| N stage                         |                   |          |                      |         |
|   • N0                          | 1.43 (0.51, 4.01) | 0.501    | –                    | –       |
|   • N1                          | 1.91 (0.41, 8.88) | 0.409    | –                    | –       |
| Metastases                      | 9.35 (2.58, 33.89)| 0.001    | –                    | –       |
| IBD                             | 0.13 (0.02, 0.96) | 0.045    | 0.30 (0.03, 2.92)    | 0.299   |
| Pre-operative haemoglobin (per each 1 g/dL) | 0.71 (0.58, 0.88) | 0.001 | –                    | –       |
| Pre-operative anaemiaa          | 3.10 (1.36, 7.07) | 0.007    | –                    | –       |
| Hypertension                    | 4.38 (1.96, 9.82) | <0.001   | 1.44 (0.44, 4.73)    | 0.546   |
| CKD                             | 3.45 (1.43, 8.34) | 0.006    | 0.92 (0.20, 4.17)    | 0.916   |
| Previous CVA                    | 4.31 (1.27, 14.68)| 0.019    | 2.14 (0.30, 15.10)   | 0.445   |
| POSSUM Physiological score     | 1.21 (1.12, 1.30) | <0.001   | 1.19 (1.04, 1.36)    | 0.010   |
| POSSUM total scoreb            | 1.09 (1.04, 1.14) | <0.001   | –                    | –       |
| Clavien-Dindo classification III-IV | 4.34 (1.89, 9.97) | 0.001    | 2.87 (0.63, 13.07)   | 0.173   |
| Highest Cr (per each 1 μmol/L) | 1.01 (1.01, 1.02) | <0.001   | 1.00 (0.99, 1.01)    | 0.931   |
| AKI                             |                   |          |                      |         |
|   • No AKI                      | 1.53 (0.45, 5.16) | 0.495    | –                    | –       |
|   • AKI stage 1                 | 3.49 (0.47, 26.07)| 0.224    | –                    | –       |
|   • AKI stage 2                 | 18.55 (2.15, 160.17)| 0.008    | –                    | –       |
| Log10 pNGAL (T1)                | 0.042             | 0.62 (0.12, 3.13) | 0.566 |
| Log10 pNGAL (T2)                | 0.020             | 1.54 (0.30, 7.93) | 0.604 |
| Log10 pNGAL (T3)                | 0.442             | –                    | –       |
| Log10 uNGAL (T1)                | 0.140             | 1.50 (0.86, 2.61) | 0.151 |
| Log10 uNGAL (T2)                | 0.040             | 1.50 (0.86, 2.61) | 0.151 |
| Log10 uNGAL (T3)                | 0.047             | –                    | –       |

Abbreviations: T, tumour; N, nodes; IBD, inflammatory bowel diseases; CKD, chronic kidney disease; CVA, cerebrovascular accident; POSSUM, Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity; CRP, c-reactive protein; Cr, serum creatinine; AKI, acute kidney injury; pNGAL, plasma neutrophil gelatinase-associated lipocalin; uNGAL, urine neutrophil gelatinase-associated lipocalin.

* Not included in multivariable cox regression analysis because these factors comprise POSSUM physiological score.

† Not included in multivariable cox regression analysis because POSSUM total score showed multicollinearity with POSSUM physiological score.

‡ Not included in multivariable cox regression analysis because there were 32 observations.

correlate with advanced TNM stage, depth of tumor invasion, presence of lymph node metastases, and decreased disease-free survival [45,46]. Several studies have shown that serum NGAL concentrations were higher in cancer patients compared with benign diagnoses or healthy controls, and that serum NGAL correlated with T staging, maximal tumor size, and lymph node involvement [47-50]. Previous studies also suggested that serum NGAL was able to distinguish active inflammatory bowel disease from inactive disease [51,52]. In our study, about one-fourth of our patients with inflammatory bowel disease had similar NGAL concentrations to those with malignancy.

Interestingly, non-survivors at 8 years were characterized by higher baseline and postoperative pNGAL concentrations and significantly higher postoperative uNGAL (T3) values compared to survivors. pNGAL was shown an independent predictor for long-term mortality in cancer settings [46]. Our study showed that AKI patients with uNGAL positivity (NGAL+/Cr-) had worse 1-year prognosis than those with AKI and normal uNGAL concentrations (NGAL-/Cr-). This could possibly be explained by association between uNGAL and metastases. Whether NGAL can predict long-term prognosis independent of cancer progression and AKI needs to be determined in future studies.

This is the largest study to date which explored the role of NGAL after major colorectal surgery. However, it is important to acknowledge some limitations. This was a single-center study with limited sample size and small event rates, and a type II error cannot be excluded. Urine output was not used for AKI diagnosis and staging, which might have underestimated the true incidence of AKI. We did not collect any long-term creatinine results and cannot comment on the association between NGAL and development of CKD. Finally, causes of death could not be determined for all patients; therefore, we cannot associate NGAL with long-term morbidity independent of cancer progression. Nevertheless, our study is the first to examine the utility of both plasma and urine NGAL for AKI prediction after colorectal surgery in the era of ERAS protocols. Both, pNGAL and uNGAL were measured at three time points to compare changes within and between groups. All patients underwent rigorous haemodynamic monitoring before and after surgery.

In summary, in patients undergoing colorectal surgery under ERAS protocol, the impact of underlying malignant disease and chronic comorbidities precludes NGAL as a reliable biomarker for AKI prediction.

Ethical approval

The study has ethical approval from the Research & Ethics Committee South East London 2 (Reference 11/H0802/9). Substantial amendment (version 1.2) of addition of urinary NGAL, assays, with paired serum NGAL & CRP assays, to increase sensitivity of acute kidney injury has also been approved.

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Author contribution

MO, JvD and AW designed the study. JvD and NL collected the data and performed the data analysis. All authors contributed to the interpretation of the results. NL wrote the first draft of the manuscript. MO, AW and JvD edited the manuscript. All authors met criteria for authorship and approved the final manuscript.

Trial registration

ISRCTN 50251697 and ISRCTN 24020298, UIN
Marlies Ostermann accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

Consent

Informed consent was obtained from all individual participants included in the study.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.01.060.

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