TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Urinary biomarkers after donor nephrectomy

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Keywords
acute kidney injury, donor nephrectomy, urinary biomarkers.

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Conflicts of interest
The authors have declared no conflicts of interest.

Received: 10 December 2013
Revision requested: 4 April 2014
Accepted: 4 January 2015
Published online: 2 February 2015

doi:10.1111/tri.12523

Summary

As the beginning of living-donor kidney transplantation, physicians have expressed concern about the possibility that unilateral nephrectomy can be harmful to a healthy individual. To investigate whether the elevated intra-abdominal pressure (IAP) during laparoscopic donor nephrectomy causes early damage to the remaining kidney, we evaluated urine biomarkers after laparoscopic donor nephrectomy. We measured albumin and alpha-1-microglobulin (α-1-MGB) in urine samples collected during and after open and laparoscopic donor nephrectomy and laparoscopic cholecystectomy and colectomy. Additionally, kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) were measured in urine samples collected during and after laparoscopic donor nephrectomy and colectomy. The same biomarkers were studied in patients randomly assigned to standard or low IAP during laparoscopic donor nephrectomy. We observed a peak in urinary albumin excretion during all procedures. Urine α-1-MGB rose in the postoperative period with a peak on the third postoperative day after donor nephrectomy. Urine α-1-MGB did not increase after laparoscopic cholecystectomy and colectomy. After laparoscopic nephrectomy, we observed slight increases in urine KIM-1 during surgery and in urine NGAL at day 2–3 after the procedure. After laparoscopic colectomy, both KIM-1 and NGAL were increased in the postoperative period. There were no differences between the high- and low-pressure procedure. Elevated urinary α-1-MGB suggests kidney damage after donor nephrectomy, occurring irrespective of IAP during the laparoscopic procedure.

Introduction

Kidney transplantation remains the treatment of choice for patients with end-stage renal disease (ESRD). However, the gap between the demand and supply of deceased donor kidneys continues to grow. Living-donor programs have gradually become an attractive strategy to expand the donor pool for kidney transplantation. Grafts from living-related donors display superior function and longer survival than those obtained from deceased donors [1,2]. Living-donor surgery has changed radically in the past decade as laparoscopic techniques have supplanted open nephrectomy. In a meta-analysis, laparoscopic donor nephrectomy was found to be associated with reduced analgetic consumption, shorter hospital stay, and faster return to normal physical functioning as compared to the open technique [3]. As the beginning of living-donor kidney transplantation, physicians have expressed concern about the possibility that unilateral nephrectomy can be harmful to a healthy individual although survival and the risk of

ESRD in carefully screened kidney donors appear to be similar to those in the general population [4]. To monitor renal problems after donor nephrectomy, we closely monitored the urine of the donor after donor nephrectomy.

We observed increased levels of urinary alpha-1-microglobulin (α-1-MGB), a marker of renal tubular dysfunction, in several patients after laparoscopic donor nephrectomy. This suggested that the procedure may cause some harm to the remaining kidney. It is well known that the kidney is especially vulnerable to the effects of intra-abdominal pressure (IAP) due to its anatomical position and blood supply. It is now also accepted that the adverse effects of elevated IAP on renal function can occur at lower levels of IAP, long before development of overt abdominal compartment syndrome [5]. To investigate whether the elevated IAP during laparoscopic donor nephrectomy causes early damage to the remaining kidney, we evaluated urine biomarkers after laparoscopic donor nephrectomy in two studies.

Patients and methods

Study 1

Patients

Between January 2010 and July 2011, we included 10 donors who underwent a laparoscopic donor nephrectomy at the Radboud University Medical Center. For comparison, we studied nine patients who underwent a laparoscopic cholecystectomy in the Isala Zwolle, and 12 donors who underwent an open donor nephrectomy in the University Medical Center Utrecht. Inclusion criteria were as follows: age >18 years and estimated GFR >60 ml/min/1.73 m^2 (abbreviated MDRD formula). Exclusion criteria were as follows: blood pressure >160/90 mmHg and/or use of antihypertensive medication and/or the presence of (micro) albuminuria before kidney donation.

Between July and September 2014, we additionally included eight patients who underwent a laparoscopic colectomy as a second control group, because of the short duration of laparoscopic cholecystectomy in comparison with laparoscopic nephrectomy.

Procedure

In all patients, a urinary catheter was inserted directly preoperatively. In laparoscopic procedures, IAP during the period of pneumoperitoneum was maintained at 12–14 mmHg. A combination of remifentanil, fentanyl, sufentanil, propofol, and rocuronium was used for anesthesia.

Urine samples were obtained preoperative, at start operation (after insertion of the urinary catheter), directly postoperative (all urine produced during the procedure), on the first, second, and third postoperative day and finally at about 4–6 weeks postoperatively.

Outcome measures

Serum creatinine was measured at baseline (day 0) and postoperative at day 1, 2, 3, and at about 4–6 weeks postoperatively. Urine samples were centrifuged and urine supernatants were stored in 1 ml aliquots at −80 °C until further use. Creatinine, albumin and α-1-MGB were measured in all collected urine samples. After analysis of all data, frozen-stored urine samples of the laparoscopic procedures were used for measurement of kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL).

Patients provided informed consent. The local ethical committee waived the requirement for formal approval of this study.

Study 2

Patients

The second study was performed using stored urine samples obtained from 20 donors who participated in the recent Leopard study [6]. In summary, this randomized, double-blinded study included 20 donors undergoing a laparoscopic donor nephrectomy between September 2011 and January 2012 at the Radboud University Medical Center. This study investigated the effect of low-pressure pneumoperitoneum (7 mmHg) versus normal pressure pneumoperitoneum (14 mmHg) on donors' comfort [6]. All Dutch speaking individuals who were medically suitable for donation were eligible. Exclusion criteria were a history of kidney or adrenal gland surgery at the side of donation. Potential participants were informed about the study at the outpatient clinic. Informed consent was mandatory for inclusion. Approval for this study was given by the Central Committee on Research involving Human Subjects of the Radboud University Medical Center Nijmegen and has therefore been performed in accordance with the ethical standards laid down in an appropriate version of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008.

Procedure

Randomization using a single block was used to create 20 sealed envelopes numbered from 1 to 20, containing instructions for the setting of the carbon dioxide inflator, at, respectively, 7 mmHg for the experimental and 14 mmHg for the control arm. Prior to the operation, a nurse who was not involved in the operation installed the pneumoperitoneum pressure. All other personnel were blinded for the allocation of treatment.

General anesthesia was induced using a standardized protocol, which included remifentanil, propofol, and rocuronium. Further details concerning the randomization,

operation, and anesthesia procedures were described earlier [6].

Urine samples were taken preoperatively (day −1), directly postoperatively (urine produced during the procedure from insertion of a urinary catheter till closure), the first, second, and third postoperative day, and finally 1 week postoperative. All samples were centrifuged and urine supernatants were stored in 1 ml aliquots at −80 °C until further use. No urine samples were obtained directly preoperatively (after insertion of a urinary catheter) in this study.

Outcome measures

Donor serum creatinine was measured at baseline (day −1) and postoperative at day 1, 2, and 7. Urinary output and saline infusion were also registered carefully in the periods from first incision to insufflation, from insufflation to desufflation (pneumoperitoneum phase), and from desufflation to closure.

Creatinine, albumin, α-1-MGB, KIM-1, and NGAL were measured in all collected urine samples. All outcome assessments were performed on coded samples by investigators who were blinded for the treatment group to which each sample belonged.

Statistical analysis

Parameters between groups were compared using independent *t*-test or ANOVA for parametric continuous data. Mann–Whitney *U*-test or Kruskal–Wallis test was used for nonparametric continuous data. Chi-square test was used for categorical data. Parameters within groups were compared using related-samples Wilcoxon's signed-rank test. Categorical variables are presented as numbers and continuous variables as mean values (± standard deviation) or median values [range]. *P*-values <0.05 were considered significant. Data on urinary biomarkers are presented in figures as median values. To test significance of changes between groups, a quadratic mixed model analysis was used. All statistics were performed using SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Study 1

Patients' characteristics

Patients' characteristics and outcome measures are presented in Table 1. The average time of elevated IAP was 34 ± 19 min in laparoscopic cholecystectomy compared with 99 ± 19 min in laparoscopic donor nephrectomy ($P < 0.001$). Most baseline characteristics of patients in the laparoscopic colectomy group, including the average time of elevated IAP of 86 ± 35 min, were comparable with the characteristics of the patients in the laparoscopic donor nephrectomy group, with the exception of older age ($P = 0.026$) and higher ASA classification ($P = 0.023$) in the colectomy group.

Urinary biomarkers

Urinary biomarkers are presented in Table 1 and Figs 1a, 2a, 3a and 4a. Increased levels of urine albumin were observed in the urine samples collected during the procedure in the open donor nephrectomy, laparoscopic donor nephrectomy as well as in laparoscopic cholecystectomy and colectomy group (Fig. 1a). Albuminuria disappeared rapidly after the procedure.

Urine α-1-MGB followed a different time course. We observed a significant increase of urine α-1-MGB after laparoscopic donor nephrectomy, with a peak on the third postoperative day (Fig. 2a). A similar although more variable pattern was observed in donors after open nephrectomy. In contrast, in patients who underwent a laparoscopic cholecystectomy or colectomy urine, α-1-MGB was not significantly elevated in the postoperative period.

In view of these discordances, we analyzed the recently identified urinary biomarkers KIM-1 and NGAL in the stored urine samples of patients in the laparoscopic nephrectomy and laparoscopic colectomy group. Remarkably, after laparoscopic nephrectomy, urinary excretion of KIM-1 increased slightly in the perioperative period, in parallel with the albuminuria. In contrast, urine KIM-1 increased more after laparoscopic colectomy and exceeded normal values in the postoperative period. After laparoscopic nephrectomy, urine NGAL followed the pattern of α-1-MGB with a peak in excretion on the 2nd or 3rd postoperative day. Similarly, variable increases in urine NGAL were noted after colectomy (Figs 3a and 4a).

There was no relationship between the duration of IAP during laparoscopic donor nephrectomy and the amount of urinary α-1-MGB excretion in the postoperative period. Urinary α-1-MGB excretion did not correlate with postoperative rise in serum creatinine after laparoscopic donor nephrectomy. There was no correlation between urine α-1-MGB and urine NGAL.

Study 2

Main outcome data have been reported [6].

Patients' characteristics

Patients' characteristics and outcome measures are presented in Table 2. Operation time and time of elevated IAP were significantly longer in patients operated with low-pressure pneumoperitoneum compared with normal pressure pneumoperitoneum. Patients operated with low-pressure pneumoperitoneum showed a higher urine output

Table 1. Baseline characteristics and outcome measures study 1.

	Laparoscopic cholecystectomy (n = 9)	Laparoscopic colectomy (n = 8)	Laparoscopic nephrectomy (n = 10)	Open Nephrectomy (n = 12)	P-value (between groups)
Age (years)	46.6 (16.4)	65.7 (8.9)	55.6 (8.1)	47.5 (11.3)	0.005
Male gender	3	4	6	3	ns
BMI (kg/m^2)	26.4 (3.7)	28.3 (5.5)	25.3 (3.3)	26.7 (2.5)	ns
Serum creatinine (μmol/l)	66 (12)	78 (14)	69 (10)	78 (9)*	ns
ASA classification†					
I (healthy)	9	4	10	12	<0.001
II or III	0	4	0	0	
Operation length (min)					
Skin-to-skin time	61 (18)	132 (57)	136 (22)	179 (26)	<0.001
Pneumoperitoneum	34 (19)	86 (35)	99 (19)	–	<0.001
Mean arterial pressure					
During operation	75 (11)	77 (4)	79 (11)	71 (6)	ns
Urine output (ml/h)					
During operation	121 (81)	101 (94)	55 (29)	56 (52)	ns
Infusion volume (ml/h)					
During operation	1098 (387)	459 (318)	485 (224)	776 (185)	<0.001
Serum creatinine (μmol/l)					
4–6 weeks postoperatively	75 (17)	n.d.	106 (18)	113 (17)	<0.001
Albuminuria (mg/mmol creatinine)‡					
Preoperative	n.d.	0.5 [0.2–8.8]	0.9 [0.4–2.1]	0.3 [0.2–5.0]	ns
Start operation	0.7 [0.0–2.9]	1.3 [0.4–14.0]	1.4 [0.3–4.2]	0.5 [0.2–21.6]	ns
During operation	3.0 [0.2–31.2]#	9.2 [2.3–18.6]	10.7 [5.5–39.9]#	3.4 [0.9–25.0]#	ns
First postoperative day	0.6 [0.3–6.3]	2.0 [1.0–14.1]	2.6 [0.5–7.9]#	1.6 [0.4–6.8]	0.041
Second postoperative day	n.d.	3.1 [1.2–5.2]	4.5 [0.7–13.5]#	1.9 [0.3–8.4]	ns
Third postoperative day	0.7 [0.3–1.3]	1.4 [0.6–5.8]	2.1 [0.6–22.4]	2.2 [0.9–13.0]	0.013
4–6 weeks postoperative	0.6 [0.3–2.0]	0.9 [0.4–2.0]	0.9 [0.5–2.8]	0.7 [0.2–3.5]	ns
Urinary α-1-MGB (mg/mmol)§					
Preoperative	n.d.	0.3 [0.2–3.1]	0.7 [0.3–1.6]	0.6 [0.1–2.5]	ns
Start operation	0.6 [0.4–2.6]	1.1 [0.1–2.3]	0.3 [0.1–1.0]	0.3 [0.1–6.3]	ns
During operation	0.6 [0.2–1.4]	0.9 [0.1–2.6]	0.4 [0.1–0.9]	0.8 [0.2–8.6]	ns
First postoperative day	0.3 [0.2–1.0]	0.7 [0.2–3.3]	7.6 [3.5–12.1]#	5.6 [1.9–16.4]#	<0.001
Second postoperative day	n.d.	1.9 [0.4–10.6]	10.5 [5.3–19.1]#	7.2 [1.7–25.5]#	0.003
Third postoperative day	0.4 [0.2–1.2]	2.1 [0.1–9.4]	11.2 [3.1–13.9]#	7.2 [3.0–21.6]#	<0.001
4–6 weeks postoperative	0.4 [0.2–0.9]	0.4 [0.3–2.3]	2.3 [1.2–5.9]#	2.9 [0.9–17.5]	0.001
KIM-1 (μg/mmol creatinine)¶					
Preoperative	n.d.	0.16 [0.08–0.33]	0.02 [0.00–0.09]	n.d.	<0.001
Start operation	n.d.	0.03 [0.00–0.38]	0.08 [0.00–0.15]	n.d.	ns
During operation	n.d.	0.23 [0.04–0.73]#	0.08 [0.00–0.28]	n.d.	ns
First postoperative day	n.d.	0.24 [0.07–1.51]#	0.03 [0.00–0.20]	n.d.	0.003
Second postoperative day	n.d.	0.86 [0.02–4.93]#	0.05 [0.00–0.17]	n.d.	0.001
Third postoperative day	n.d.	0.23 [0.00–0.44]#	0.04 [0.00–0.17]	n.d.	0.012
4–6 weeks postoperative	n.d.	0.10 [0.03–0.31]	0.00 [0.00-0.05]	n.d.	0.005
NGAL (μg/mmol creatinine)¶					
Preoperative	n.d.	0.7 [0.0–3.5]	0.9 [0.0–3.2]	n.d.	ns
Start operation	n.d.	0.5 [0.0–2.1]	0.3 [0.0–3.3]	n.d.	ns
During operation	n.d.	0.2 [0.0–12.0]	0.0 [0.0–3.3]	n.d.	ns
First postoperative day	n.d.	0.0 [0.0–2.6]	1.5 [0.0–4.9]#	n.d.	ns
Second postoperative day	n.d.	5.2 [0.0–19.4]#	2.6 [0.0–18.2]#	n.d.	ns
Third postoperative day	n.d.	1.6 [0.0–27.1]#	1.8 [0.8–7.3]#	n.d.	ns
4–6 weeks postoperative	n.d.	1.7 [0.0–2.3]	0.7 [0.0–20.5]	n.d.	ns

Categorical variables are presented as numbers and continuous variables as mean values (standard deviation) or median values [range].
n.d., not determined; KIM-1, kidney injury molecule 1; α-1-MGB, alpha-1-microglobulin; NGAL, neutrophil gelatinase-associated lipocalin.
*Serum creatinine was imbalanced between groups due to differences in determination method (Jaffe method in open nephrectomy group).
†American Society of Anesthesiologists classification.
In some cases, the urine sample for a particular time point could not be collected due to insufficient urine production during surgery, or because it was impossible to collect a urine sample on a specific postoperative day: ‡24 of 225 samples missing; §29 of 225 samples missing; ¶4 of 108 samples missing.
#$P < 0.05$ compared with start operation.

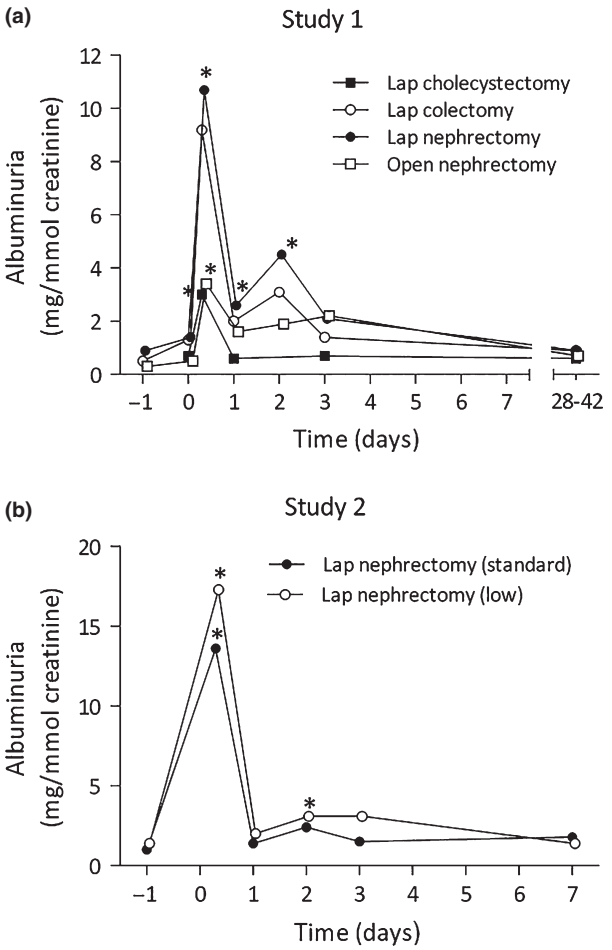

Figure 1 Albuminuria. Albuminuria during the perioperative period (mg/mmol creatinine; median). * $P < 0.05$ versus baseline. (a) Study 1: laparoscopic cholecystectomy, laparoscopic colectomy, open donor nephrectomy, and laparoscopic donor nephrectomy. (b) Study 2: laparoscopic donor nephrectomy with standard pressure pneumoperitoneum (14 mmHg) and laparoscopic donor nephrectomy with low-pressure pneumoperitoneum (7 mmHg).

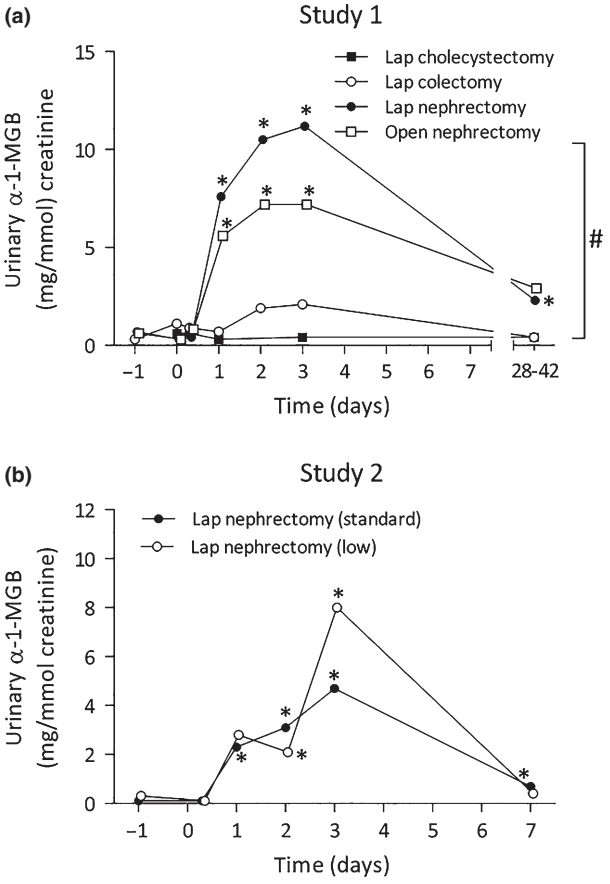

Figure 2 Urinary excretion of alpha-1-microglobulin (α-1-MGB). Urinary excretion of α-1-MGB (mg/mmol creatinine; median) *$P < 0.05$ versus baseline. #$P < 0.05$ laparoscopic nephrectomy versus laparoscopic cholecystectomy and laparoscopic colectomy in quadratic mixed model analysis. (a) Study 1: laparoscopic cholecystectomy, laparoscopic colectomy, open donor nephrectomy, and laparoscopic donor nephrectomy. (b) Study 2: laparoscopic donor nephrectomy with standard pressure pneumoperitoneum (14 mmHg) and laparoscopic donor nephrectomy with low-pressure pneumoperitoneum (7 mmHg).

during the pneumoperitoneum phase compared with normal pressure pneumoperitoneum ($P = 0.041$).

Urinary biomarkers

Urinary biomarkers are presented in Table 2 and Figs 1b, 2b, 3b and 4b. The data closely resembled the results of study 1. We observed a significant increase in urinary albumin excretion during the procedure, whereas urine α-1-MGB was not increased during surgery, but rose significantly in the postoperative period with a peak on the third postoperative day (Figs 1b and 2b). Urine KIM-1 paralleled albumin, whereas NGAL followed the same pattern as α-1-MGB.

There were no significant differences between the high- and low-pressure procedure (Figs 1b, 2b, 3b and 4b).

Discussion

Our study illustrates the effect of laparoscopic donor nephrectomy on urinary biomarker excretion. A short-lasting increase of albumin and KIM-1 excretion was noted during the procedure. In contrast, there was a slow rise in urine α-1-MGB and NGAL, which peaked on day 2 or 3, and in some patients remained elevated at 4–6 weeks after the procedure. The latter finding is suggestive for the occurrence of subtle tubular damage after donor nephrectomy, a finding that warrants further study. Of note, there was no evidence of acute kidney injury in any patient.

Serum creatinine and e-GFR are imprecise and insensitive markers of kidney injury. Therefore, urine biomarkers

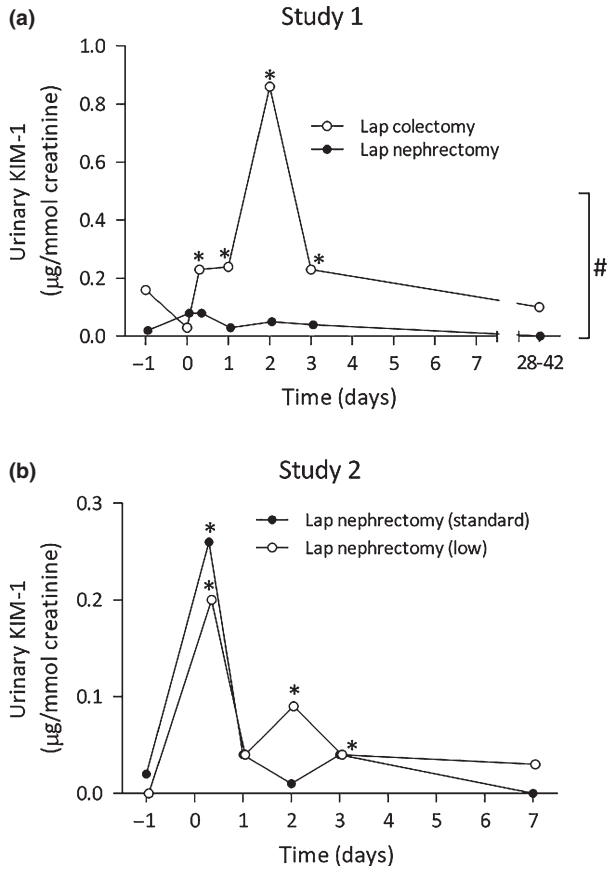

Figure 3 Urinary excretion of kidney injury molecule 1 (KIM-1). Urinary excretion of KIM-1 (µg/mmol creatinine; median) $^{*}P < 0.05$ versus baseline. $^{\#}P < 0.05$ laparoscopic nephrectomy versus laparoscopic colectomy in quadratic mixed model analysis. (a) Study 1: laparoscopic donor nephrectomy and laparoscopic colectomy. (b) Study 2: laparoscopic donor nephrectomy with standard pressure pneumoperitoneum (14 mmHg) and laparoscopic donor nephrectomy with low-pressure pneumoperitoneum (7 mmHg).

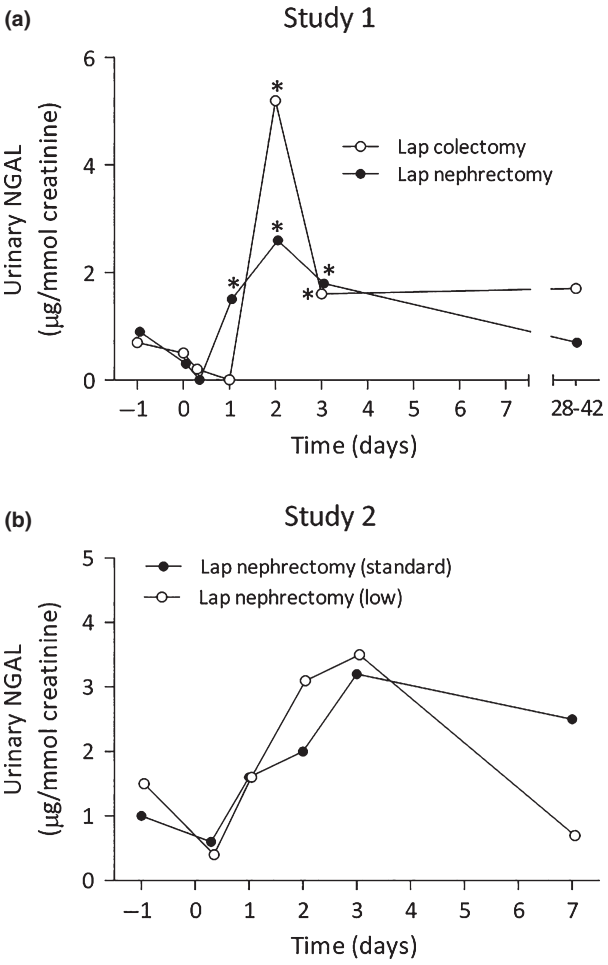

Figure 4 Urinary excretion of neutrophil gelatinase-associated lipocalin (NGAL). Urinary excretion of NGAL (µg/mmol creatinine; median) $^{*}P < 0.05$ versus baseline. (a) Study 1: laparoscopic donor nephrectomy and laparoscopic colectomy. (b) Study 2: laparoscopic donor nephrectomy with standard pressure pneumoperitoneum (14 mmHg) and laparoscopic donor nephrectomy with low-pressure pneumoperitoneum (7 mmHg).

are advocated to allow an early diagnosis of acute kidney injury. For many years, albumin and low molecular weight proteins such as α-1-MGB have been used as markers of kidney injury. Urine albumin reflects glomerular injury, although increased urine albumin excretion can be observed in patients with severe tubular injury. The excretion of urine low molecular weight proteins reflects tubular injury.

The observation that urinary albumin excretion but not α-1-MGB excretion was elevated during surgery indicates that during surgery glomerular permeability is altered. The rapid normalization suggests that this is a reversible process, either the result of the anesthesia or hemodynamic changes that occur during surgery.

In addition to glomerular changes, there was also the suggestion of tubular injury after open and laparoscopic donor nephrectomy, as indicated by a peak in α-1-MGB excretion on the third postoperative day. Remarkably α-1-MGB excretion was still elevated 4–6 weeks postoperative. To support these findings, we performed additional studies. We analyzed novel biomarkers of kidney injury. In addition, we studied a control group of patients who underwent a laparoscopic procedure either cholecystectomy or colectomy.

The novel biomarkers included KIM-1 and NGAL. KIM-1 is a type 1 transmembrane protein, not detectable in normal kidney tissue, but is expressed at high levels in kidneys with dedifferentiated proximal tubule epithelial cells after ischemic or toxic injury. NGAL is synthesized mostly in the distal nephron of the kidney in response to nephrotoxic and/or ischemic injury. However, in response to renal injury, NGAL is also systemi-

Table 2. Baseline characteristics and outcome measures study 2.

	Standard pressure (n = 10)	Low-pressure (n = 10)	P-value
Age (years)	50.7 (8.9)	51.6 (10.2)	ns
Male gender	3	7	ns
BMI (kg/m^2)	24.9 (2.5)	25.7 (3.9)	ns
Serum creatinine (μmol/l)	64 (13)	70 (14)	ns
ASA classification*			
I (healthy)	10	10	ns
II or III	0	0	
Operation length (min)			
Skin-to-skin time	111 (19)	149 (86)	0.003
Pneumoperitoneum	86 (16)	126 (27)	0.001
Mean arterial pressure			
Pneumoperitoneum phase	73 (8)	76 (9)	ns
Urine output (ml/h)			
Pneumoperitoneum phase	11 (20)	23 (35)	0.041
Infusion volume (ml/h)			
Pneumoperitoneum phase	746 (253)	693 (134)	ns
Donor eGFR (ml/min/1.73 m^2)			
Postoperative week 1	56.3 (10.6)	56.0 (10.4)	ns
Albuminuria (mg/mmol creatinine)†			
Preoperative	1.0 [0.3–2.4]	1.4 [0.2–4.7]	ns
During operation	13.6 [8.2–95]#	17.3 [10.0–43.0]#	ns
First postoperative day	1.4 [0.4–5.8]	2.0 [0.2–35.4]	ns
Second postoperative day	2.4 [0.7–8.5]	3.1 [0.8–11.2]#	ns
Third postoperative day	1.5 [0.5–4.0]	3.1 [1.0–11.9]	ns
One week postoperative	1.8 [0.3–21.3]	1.4 [0.5–4.9]	ns
Urinary α-1-MGB (mg/mmol)†			
Preoperative	0.1 [0.0–0.5]	0.3 [0.1–0.6]	ns
During operation	0.1 [0.1–0.4]	0.1 [0.0–0.2]	ns
First postoperative day	2.3 [0.1–12.3]#	2.8 [0.1–9.3]	ns
Second postoperative day	3.1 [0.3–23.7]#	2.1 [0.5–10.1]#	ns
Third postoperative day	4.7 [0.7–8.2]#	8.0 [0.5–31.5]#	ns
One week postoperative	0.7 [0.1–5.4]#	0.4 [0.1–6.7]	ns
KIM-1 (μg/mmol creatinine)‡			
Preoperative	0.02 [0.00–0.07]	0.00 [0.00–0.12]	ns
During operation	0.26 [0.02–0.52]#	0.20 [0.08–0.41]#	ns
First postoperative day	0.04 [0.00–0.28]	0.04 [0.00–0.21]	ns
Second postoperative day	0.01 [0.00–0.10]	0.09 [0.00–0.12]#	ns
Third postoperative day	0.04 [0.00–0.08]	0.04 [0.00–0.13]#	ns
One week postoperative	0.00 [0.00–0.31]	0.03 [0.00–0.06]	ns
NGAL (μg/mmol creatinine)‡			
Preoperative	1.0 [0.0–12.0]	1.5 [0.0–5.7]	ns
During operation	0.6 [0.0–1.7]	0.4 [0.1–2.2]	ns
First postoperative day	1.6 [0.5–10.1]	1.6 [0.0–8.2]	ns
Second postoperative day	2.0 [0.5–10.7]	3.1 [0.7–11.4]	ns
Third postoperative day	3.2 [0.6–7.0]	3.5 [0.7–27.1]	ns
One week postoperative	2.5 [0.0– 5.7]	0.7 [0.0–5.0]	ns

Categorical variables are presented as numbers and continuous variables as mean values (standard deviation) or median values [range]. KIM-1, kidney injury molecule 1; α-1-MGB, alpha-1-microglobulin; NGAL, neutrophil gelatinase-associated lipocalin.

*American Society of Anesthesiologists classification.

In some cases, the urine sample for a particular time point could not be collected due to insufficient urine production during surgery, or because it was impossible to collect a urine sample on a specific postoperative day: †11 of 60 samples missing; ‡8 of 60 samples missing.

#$P < 0.05$ compared with preoperative.

cally produced. After glomerular filtration, most of this NGAL is endocytosed by the proximal tubule epithelia, and little is secreted in the urine. NGAL is an early predictor of acute kidney injury after cardiac surgery, contrast-induced nephropathy, and in critically ill patients [7]. After laparoscopic nephrectomy, we

observed a slight increase of urinary excretion of KIM-1 in the immediate perioperative period, in parallel with albuminuria. In contrast, urine NGAL followed the pattern of α-1-MGB after laparoscopic nephrectomy with a peak in excretion on the 2nd or 3rd postoperative day. It has been shown that anesthesia can be associated with increased urinary excretion of biomarkers. Patients undergoing breast surgery, anesthetized with ketorolac and sevoflurane, developed increased urinary excretion of α-1-MGB in the first postoperative day [8]. Another study after elective surgery showed elevated excretion of total protein, β-2-microglobulin, and N-acetyl-β-d-glucosaminidase after anesthesia with sevoflurane [9].

It is unclear whether the anesthetics used in our studies could be responsible for the increased excretion of the biomarkers that we have observed. To further evaluate this, we have performed additional studies in patients who underwent laparoscopic cholecystectomy and colectomy. The laparoscopic cholecystectomy patients showed a slight increase in albuminuria during the operative procedure. There was no increase in urinary α-1-MGB. However, the laparoscopic cholecystectomy group might not be a good control group because the laparoscopic procedure was of significantly shorter duration. Therefore, we performed additional studies in patients who underwent laparoscopic colectomy. These patients also showed the perioperative increase in albuminuria. However, the patterns of urinary excretion of the tubular biomarkers were clearly different from that observed after laparoscopic nephrectomy. Specifically, after laparoscopic colectomy, there was no increase in urinary α-1-MGB excretion, and there were clear increases in urinary KIM-1 and NGAL occurring in the 2nd and 3rd day after the procedure. Taken all together, our data indicate that the increase in urinary α-1-MGB excretion is specific for nephrectomy. The typical and unique pattern of increased urinary α-1-MGB after nephrectomy is supported by the absence of a correlation between the excretion of α-1-MGB and the excretion of urine NGAL. Clearly, the increased urinary α-1-MGB excretion thus cannot be attributed to the anesthesia nor to the elevated intra-abdominal pressure.

This points to a specific injury after donor nephrectomy, situated in the proximal tubules. We can only hypothesize that an alteration in blood supply, possibly mediated by renal reflexes, to the remaining kidney might be an explanation for the damage observed. Another possible explanation might be that nephrectomy causes hypertrophy and dedifferentiation of tubular cells in the remaining kidney. These dedifferentiated tubular cells may have more limited reabsorption capacity resulting in an increased urinary excretion of otherwise reabsorbed low molecular weight proteins. Our study also illustrates that the new biomarkers of kidney injury respond differently to the various operative procedures. More detailed studies are needed to find explanations for these specific expression patterns.

In adult patients undergoing elective cardiac surgery, Koyner *et al.* measured urinary biomarkers, such as KIM-1 and NGAL [10]. They demonstrated that the 6-h postoperative urinary NGAL best detected early stage 3 acute kidney injury. They also demonstrated that preoperative KIM-1 was predictive for the development of stage 1 and stage 3 acute kidney injury. Potential sources of this preoperative elevation include exposure to radiocontrast, hypotensive events, or pre-existing chronic kidney disease. The population of healthy kidney donors included in the present study differs from patients undergoing cardiac surgery or critically ill patients in which most biomarker studies were performed. Therefore, profiles which accurately detect cardiac surgery-related acute kidney injury or acute kidney injury in septic patients are not necessarily useful in other patient groups such as kidney donors.

Limitations of our study are mainly related to its design as a pilot. Without a power calculation, the conclusions are preliminary and should be confirmed in a larger trial.

Urinary biomarkers are very sensitive for true histological tubular damage, and in research conditions, these high-sensitivity markers accurately predict potential nephrotoxicity. However, in clinical practice, we need to define what degree of subclinical damage as detected by biomarkers is clinically relevant, in terms of complications and long-term renal function. Therefore, studies linking acute tubular damage as assessed by biomarkers to long-term outcomes are needed.

Conclusion

Elevated α-1-MGB suggests kidney damage after open and laparoscopic donor nephrectomy. This occurs irrespective of intra-abdominal pressure during the laparoscopic procedure. Further studies are required to assess the mechanism underlying biomarker elevation after donor nephrectomy.

Authorship

JMHA: designed study, collected data, analyzed data, and wrote paper. MCW: designed study, collected data, analyzed data, and wrote paper. ADZ: designed study, and collected data. HJK: designed study, and collected data. KEW: designed study, collected data, analyzed data, and wrote paper. FCHd'A: designed study, collected data, analyzed data, and wrote paper. DMDÖ: collected data, and wrote paper. JFMW: designed study, analyzed data, and wrote paper. AJH: designed study, analyzed data, and wrote paper.

Funding

The authors have declared no funding.

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