Optimizing Perioperative Fluid Management in Complex Abdominal Wall Reconstruction to Prevent Postoperative Acute Kidney Injury

The American Surgeon 2022, Vol. 0(0) 1–8 © The Author(s) 2022 Article reuse guidelines: sagepub.com/journals-permissions DOI: 10.1177/00031348221082292 journals.sagepub.com/home/asu SAGE

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Abstract

Background: Acute kidney injury (AKI) is a serious postoperative complication of abdominal wall reconstruction that can significantly impact outcomes of these patients. This study examines AKI following abdominal wall hernia repair to determine incidence and risk factors and outline potential mitigation strategies.

Methods: Using a single institution IRB-approved prospective database, patients undergoing complex abdominal wall reconstruction from 2013 to 2021 were identified. Patients with AKI were compared to controls and preoperative and intraoperative characteristics were evaluated. Multivariate analysis was utilized to identify factors associated with development of AKI.

Results: 297 patients were reviewed, 21.2 % (n = 63 patients) had AKI. Patients with AKI had a greater decrease in postoperative GFR to preoperative GFR (40.5% vs 18.3%, p <0.0001). Factors associated with AKI included ASA score >2 (odds ratio (OR) = 2.10, [1.50; 5.12], p = 0.02), HTN (OR = 2.05, [1.05; 4.0], p = 0.04), higher baseline Cr (OR = 5.98, [2.56; 13.98], p <0.0001), and diabetes (OR = 0.135, [0.0275; 0.666], p = 0.01). Operative time was longer in patients who developed AKI [average 400 min (range: 278-510 min) vs 310 min (range: 260-374 min), p = 0.04] and was an independent predictor of developing AKI (OR = 319.59, [137.25; 744.65], p <0.0001).

Discussion: Preoperative identification of patients with medical comorbidities undergoing elective complex abdominal wall reconstruction continues to be imperative to improve outcomes. This study demonstrates that perioperative management for high risk patients requires flexibility, including potential adjustments to enhanced recovery after surgery protocols in order to adequately address the risks for AKI.

Keywords

hernia, plastic surgery, complex abdominal wall reconstruction, ERAS, general surgery

Introduction

Complex ventral hernias are defined as abdominal wall defects that cannot be repaired by simple suture repair technique. These defects often require complex techniques including component separation, tissue rearrangement, mesh implantation, and often a combination of multiple techniques. Surgical management of complex abdominal hernias is often managed by a multidisciplinary team,^{1,2} particularly because these patients often have undergone multiple previous repairs or have significant comorbidities that puts them at increased risk for post-operative complications.

Patients who undergo abdominal wall reconstruction (AWR) are often at risk for post-surgical complications

despite preoperative optimization.³ Complications are often related to factors that led to the development of the hernia such as obesity, diabetes, and tobacco abuse^{4–6} but can also be secondary to iatrogenic interventions during the perioperative period. We developed and executed an

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abdominal wall hernia enhanced recovery after surgery protocol (ERAS) that was aimed to optimize the perioperative care of these patients, including fluid administration. Ideal fluid balance is an important factor that can affect outcomes in abdominal hernia repair patients^{7,8} and proper management can be critical in reducing acute kidney injury (AKI), which can lead to mortality, extended length of stay, and increased hospital costs^{7,9}. Prior to and after the implementation of our AWR ERAS protocol in February 2018, there have been a subset of patients who developed postoperative AKI which required off-protocol intervention, which increased length of stay and, on occasion, impacted the ability to use other indicated adjunctive tests such as postoperative CT scans to rule out pulmonary embolism.⁶

In this article, we evaluate factors contributing to the development of AKI in complex abdominal wall hernia reconstruction patients at our tertiary referral center before and after the implementation of our ERAS protocol. Although much has been written in regards to preoperative optimization and surgical technique, there remains a paucity of literature to specifically guide perioperative fluid management in this patient population. Furthermore, this article functions to demonstrate that while ERAS protocols can serve to improve outcomes in AWR patients, it should be re-evaluated in a subset of patients at increased risk of developing AKI, and allow for individualization of the protocol to better manage this subpopulation.

Methods

Using an institutional review board-approved prospective database of all patients undergoing complex open abdominal wall reconstruction performed by the senior author at a tertiary care academic medical center, we performed a retrospective review of patients undergoing repair between August 2013 and October 2020. We evaluated patients before the institution of our ERAS protocol (2013-2018) and after its institution (2018-2020). Data extracted include age, gender, functional status, BMI (body mass index), hernia size defined by preoperative CT scan measurement, race, past medical history and comorbidities documented in their medical record (primarily hypertension, chronic kidney disease, and diabetes), preoperative and postoperative creatinine (Cr), total operative time, total number of anesthesia providers for each case, need for bowel resection during operation, intraoperative fluids administered, estimated blood loss (EBL), and total intravenous fluids given postoperatively. ASA(American Society of Anesthesiologist) classification is used to calculate preoperative fitness, and has been demonstrated to be an acceptable surrogate for functional status.^{10,11} Functional status is defined as decreased with an ASA class greater than 2 per ASA. All reconstructions included in this study were performed by a two-surgeon team at a single institution, a general surgeon who performed exploratory laparotomy, lysis of adhesions, and any indicated bowel resection and repair, and a plastic surgeon who performed the musculofascial abdominal core reconstruction, including placement of mesh and management of skin and soft tissue. Types of hernias included in this study were ventral, flank, and complex umbilical and parastomal hernias. Complex AWR was defined as any reconstruction for large defects not amenable to primary simple suture repair, as well as requirement for mesh placement, recurrent hernias, and hernias requiring component separation/musculofascial advancement flaps. The characteristics of the hernia was assessed and documented using the Kanters risk stratification system, which is a published, validated model used to predict postoperative surgical site occurrences based on patient comorbidities with statistically significant differences in outcomes between the three classifications.¹² Robotic, laparoscopic, and simple outpatient AWR cases were not included in this analysis. Development of AKI within the first 72 h postoperatively was the primary outcome in this study, and was defined using the Risk, Injury, Failure, Loss, and End-stage Kidney (RIFLE) criteria.¹³ Secondary outcomes included urine output in cc/kg/hr, need for additional postoperative intravenous fluid (IVF), need for postoperative dialysis, new diuretic use, 30-day readmission rates, and length of stay. Patients who developed AKI were compared to controls who did not develop AKI during the same time period using t test for comparison of proportions. Additionally, multivariate logistic regression analysis was performed to identify statistically significant covariates in our initial bivariate analysis independently associated with development of AKI. Data were checked for multicollinearity with the Belsley-Kuh-Welsch technique. All statistical analyses were performed

Results

We identified 297 patients that met inclusion criteria. Of these patients, n = 184 patients underwent hernia repair prior to the institution of the ERAS protocol and n = 113underwent repair after the ERAS protocol was initiated. All patients required mesh placement and mesh-reinforced primary musculofascial reapproximation was achievable in all patients included in the study. A total of 63 patients (21.2%) developed postoperative AKI. There was no significant difference between the percentage of patients who developed AKI before and after the institution of the ERAS protocol, 18.5% (n = 34) vs 25.6% (n = 29), p = .49(Table 1). The median hernia total surface area measured preoperatively by CT scan in patients who developed AKI was 132 cm^2 (IQR = 185.6 cm²) vs 136.5 cm^2 (IQR = 187.4 cm²) in patients who did not (p = 0.82) (Table 2). When comparing patients prior to and after ERAS initiation, there remained no difference in median hernia total

using SAS 9.4 (SAS Institute, Cary, NC, USA). Significance

was set at a p value lower than 0.05.

	Before ERAS	After ERAS	P-Value
Total number of patients	184	113	
Patients who developed AKI, % (n)	18.5% (n = 34)	25.6% (n = 29)	0.49
Median hernia total surface area (IQR)	131.3 cm^2 ($IQR = 183.3 \text{ cm}^2$)	132.5 cm ² (IQR =185.2 cm ²)	0.87
Patients with ASA score>2, % (n)	67.6% (n = 23)	62.1% (n = 18)	0.65
Average number of previous hernia repairs	3.9	4.2	0.92
Average total OR time in minutes (range min to max)	411 (310-510)	300 (260-426)	0.03
Total average OR IVF	3.5 L	1.75 L	<0.0001
Average IVF given postoperatively	4.3 L	3 L	0.03
Average length of stay in day (range, median)	7.5 days (5-44 days, 7 days)	5.5 days (1-32 days, 4.5 days)	0.02
30-day readmission rates, %	3.1%	1.6%	0.001
Hernia recurrence, % (n)	4.35% (8)	6.19% (7)	0.08

Table I. Perioperative characteristics for patients undergoing complex abdominal wall reconstruction before and after use of enhanced recovery after surgery protocols (ERAS).

Table 2. Baseline characteristics of patients who develop AKI vs those who do not after complex abdominal wall reconstruction.

	Patients Who Developed Postop AKI	Patients Who Did Not Develop Postop AKI	P-Value
Mean age in years, (SD)	56.0 ± 16.8	56.9 ± 16.4	0.67
Caucasian, %	90.7 %	91.5%	0.96
Gender:			
Female	41%	37%	0.42
Male	59%	63%	
Average BMI (range)	32.34 kg/m ² ± 5.6 (20.9 – 42.59)	30.88 kg/m ² ± 3.8 (19.3-53.2)	0.56
Patients with ASA>2, %	75.5% (n = 48)	63.7% (n = 149)	0.03
PMH of HTN, CKD, and DM	10.8%	4.9%	0.001
Median hernia total area (IQR)	132 cm ² (IQR = 185.6 cm ²)	136.5 cm ² (IQR =187.4 cm ²)	0.82

area in patients prior to ERAS was 131.3 cm^2 (IQR = 183.3 cm^2) vs 132.5 cm^2 (IQR = 185.2 cm^2) after ERAS initiation (p = 0.87) (Table 1). There was no difference in average age between patients with AKI compared to patients without $(56 \pm 16.8 \text{ vs. } 56.9 \pm 16.4 \text{ years old}, p =$ 0.67). There was also no difference in race, with majority of patient being Caucasian (90.7% vs 91.5%, p = 0.96), or in gender between the two groups, with majority being male patients (63% vs 59%, p = 0.42). There was no difference in the average BMI between patients who developed AKI compared to the control group (mean 32.34 kg/m^2 \pm 5.6 vs. 30.88 kg/m² \pm 3.8, p = 0.56) (Table 2). However, patients with AKI were more likely to have ASA functional status score greater than 2 (p = 0.03), with 75.5% of patients who developed AKI having an ASA >2 compared to 63.7% (Table 2). However, when looking at patients who developed AKI before and after ERAS incorporation, there was no difference in the percentage of patients who had ASA functional status score >2, 67.6% (n = 23) vs. 62.1% (n = 18), p = .65) (Table 1). Additionally, patients with AKI were more likely to have comorbidities including hypertension, chronic kidney disease, and diabetes (10.8% vs. 4.9 %, p = 0.001) (Table 2).

tWhen looking at re-operative hernias, n = 137 (46%) of the total study population had a previous hernia

operation. There was no difference in the average number of reported previous hernia repairs between patients evaluated prior to ERAS vs after (3.9 vs 4.2, p = 0.92) (Table 1). However, between those who developed AKI and those who did not, there was a difference in the average number of reported previous hernia repairs between the two groups (4.2 vs 3.0, p = 0.04) (Table 3). Total operative time was longer in patients operated on prior to initiation of ERAS [average 411 min (range: 310-510 min) vs 300 min (range: 260-426 min), p = 0.03] (Table 1). It was also longer in patients who developed AKI [average 400 min (range: 278-510 min) vs 310 min (range: 260-74 min), p = 0.04] (Table 3). Patients who developed AKI received less intravenous fluids intraoperatively (1.25 liters (L) vs 2.75 L, *p* < 0.001) (Table 3). Patients who were operated on after ERAS initiation received less intravenous fluids intraoperatively (1.75 liters (L) vs 3.5 L, p <0.0001) (Table 1).

There was also a difference in the proportion of patients who required a bowel resection, demonstrating an association between need for bowel resection and development of AKI (n = 28, 9.52% vs n = 5, 1.71%, p =0.03) (Table 3). Last, there was no difference in the reported total average intraoperative EBL [165 mL ± 45 mL vs 150 mL ± 100 mL (p = 0.07)] or the median number of total anesthesia providers for the entirety of

	Patients Who Developed Postop AKI	Patients Who Did Not Develop Postop AKI	P-Value
Previous hernia repairs (average)	4.2	3	0.04
Average total OR time in minutes (range min to max)	400 (278-510)	310 (260-374)	0.04
Total average OR IVF	1.25 L (1-2 L)	2.75 L (2-3 L)	<0.001
Need for bowel resection			
Yes/no	6 (9.52%) 57 (90.48%)	4 (1.71%) 230 (98.29%)	0.03
Average EBL (SD)	165 mL (± 45 mL)	150 mL (± 150 mL)	0.07
Median total number of anesthesia providers per case	3	2	0.77

 Table 3. Perioperative characteristics of patients who develop postop AKI vs those who do not develop postop AKI after complex abdominal wall reconstruction.

Table 4. Postoperative characteristics of patients who develop postop AKI vs those who do not develop postop AKI after complex abdominal wall reconstruction.

	Patients Who Developed Postop AKI	Patients Who Did Not Develop Postop AKI	P-Value
Average postop GFR decrease (range)	40.5% (25-75.7%)	18.3% (0.5-23.7%)	<0.0001
Average preoperative creatinine mg/DL	1.93	0.97	<0.0001
Average IVF given postoperatively	3.6 L	2.25 L	0.04
Average length of stay in day (range, median)	6.6 days (3-44 days, 10 days)	4.9 days (1-37 days, 6 days)	0.02
30-day readmission rates (%)	2.5%	1.9%	0.65
Hernia recurrence: n, (%)	7 (11.1%)	8 (3.4%)	0.03
Median follow-up in days (range)	340 days (7-2190)	340 (7-2190)	>0.05

the case between the two groups (3 vs 2, p = 0.77) (Table 3).

In regards to postoperative factors evaluated, patients who developed AKI had a greater average decrease in postoperative GFR compared to their preoperative GFR (40.5% vs 18.3%, p < 0.0001). Patients who developed AKI were associated with higher preoperative Cr. The average Cr in patients who developed AKI was 1.93 mg/dL vs 0.97 mg/dL for patients who did not develop AKI (p < 0.0001) (Table 4). Patients who developed AKI received more IVF postoperatively, over the entire course of their stay, excluding intraoperative fluids (3.6 L vs 2.25 L, p = 0.04) (Table 4). When looking comparing the two different time periods, patients who were managed prior to the institution of the ERAS protocol also received more IVF postoperatively (4.3 L vs 3 L, p = 0.03) (Table 1).

The average length of stay (LOS) for patients who developed AKI was longer, 6.6 days (3-44 days, median: 7 days), compared to the control group, 4.9 days (1-37 days, median: 6 days, p = 0.02 (Table 4). The average length of stay (LOS) for patients prior to ERAS was longer, 7.5 days (5-44 days, median: 7 days), compared to those after ERAS, 5.5 days (1-32 days, median: 4.5 days, p = 0.02) (Table 1). In the cohort of patients who did not develop AKI, two patients had an extended LOS (33 days)

and 37 days) due to postoperative pneumonia and respiratory failure. Meanwhile, in the AKI group there were two patients with protracted postoperative courses that had prolonged LOS: 42 days LOS secondary to ARDS and a 44-day LOS secondary to bowel injury requiring multiple re-operations. In regard to 30-day readmission, it remained low over the two time periods, but there was a difference between the two groups (3.1% prior to ERAS vs 1.6% after ERAS, p = 0.001) (Table 1). However, there was no difference between those who developed AKI vs those who did not develop AKI overall (2.5% vs 1.9%, p =0.65) (Table 4). There was a difference in terms of recurrence of hernia with a total of n = 15/297 (4.7%) patients that recurred. There were n = 7 patients in the group that developed AKI and n = 8 in the control group (11.1% vs 3.4%, p = 0.03) (Table 4). A total of n = 8 patients were in the group prior to ERAS and n = 7 in the post ERAS group (4.35% vs 6.19%, p = 0.08). The median follow-up duration for both groups was 340 days (range: 7-2190 days) (Table 4).

Multivariate Logistic Regression

All significant factors in our initial bivariate analysis were then evaluated for independent association with development of AKI using multivariate analysis. ASA score

	Odds Ratio	p-Value
Intercept	0.00328 [9.94e-5; 0.108]	0.00134
ASA Score >2	2.10 [1.50 ; 5.12]	0.02
HTN	2.05 [1.05 ; 4.00]	0.04
Smoking	1.34 [0.416 ; 4.32]	0.62
Diabetes	0.135 [0.0275 ; 0.666]	0.01
Baseline Cr	5.98 [2.56 ; 13.98]	<0.0001
Risk for each 1-unit increase	• • •	
CKD	0.65 [0.30 ; 1.43]	0.28
Reported recurrences	1.07 [0.88 ; 1.31]	0.47
, Risk for each I-unit increase		
Intraoperative IVF	1.49 [0.48 ; 4.64]	0.50
Operative time	319.59 [137.25 ; 744.65]	<0.0001
, Risk for each I-unit increase		
Need for bowel resection	1.45 [0.203 ; 10.31]	0.71

Table 5. Multivariate analysis of factors associated with development of postop AKI after complex abdominal wall reconstruction.

greater than 2 was associated with development of AKI (odds ratio (OR) = 2.10, [1.50; 5.12], p = 0.02), additionally HTN (OR = 2.05, [1.05; 4.0], p = 0.04), higher baseline Cr (OR = 5.98, [2.56; 13.98], p < 0.0001), and presence of diabetes (OR = 0.135, [0.0275; 0.666], p =0.01) were associated with higher rates of AKI. CKD (OR = 0.65, [0.3; 1.43], p = 0.28), number of reported prior hernia recurrences (OR = 1.07, [0.88; 1.31], p =0.47), and Smoking (OR = 1.34, [0.416; 4.32], p = 0.62) were not associated with the rate of AKI. Need for bowel resection was also not independently associated with AKI (OR = 1.45 [0.203; 10.31], p = 0.71). Surprisingly, less intraoperative IVF alone was not significantly associated with developing AKI (OR = 1.49, [0.48; 4.64], p = 0.50). However, longer operative times were an independent predictor of developing AKI (OR = 319.59, [137.25; 744.65], p <0.0001) (Table 5).

Discussion

Following complex abdominal wall hernia reconstruction, AKI can significantly impact the postoperative recovery period. ERAS protocols for AWR were developed to minimize perioperative complications, including the development of AKI, by standardizing approaches to management; however, they have been suboptimal for a small subset of patients. Using data from a high-volume tertiary referral center for abdominal wall reconstruction, our study demonstrated that low preoperative functional status, chronic kidney disease, hypertension, diabetes, prolonged operative time, and decreased intraoperative IVF administration are important risk factors for development of postoperative AKI, despite multidisciplinary-devised AWR-specific ERAS protocols. It is well known that patient selection is important for improved outcomes in elective surgical procedures. All patients included in this analysis underwent elective procedures. Patients underwent preoperative evaluation to determine eligibility for surgical intervention using Kanter's risk stratification system. Patients who were considered high risk were referred for additional preop optimization before operation was offered including weight loss counseling, smoking cessation, and diabetes management. Any additional comorbidities were evaluated and addressed utilizing outpatient preoperative anesthesia risk evaluation services at our institution. Despite preoperative optimization, comorbidities were a significant factor in development of postoperative AKI in this study. Our finding of comorbidities contributing to postoperative AKI is intuitive as these conditions are known to affect renal function and reduce renal reserve. Hypertension and diabetes specifically can result in compromised renal function secondary to heightened renal vasoconstriction and resultant decreased renal circulation. Additionally, surgery alone can serve as an insult to renal function as it is a physiological stressor.¹⁴ This particular study demonstrates that while ERAS protocols have improved outcomes for a majority of AWR patients, there is a subset of patients, particularly those who already have declining renal function and have other factors that place them at increased risk for developing AKI, who require individualization and optimization of fluid management in the perioperative period in order to decrease perioperative complications and improve outcomes.

ERAS protocols have gained success because they have been demonstrated to reduce complications and improve length of stay in patients undergoing major surgeries.^{15–19} ERAS emphasizes judicious intraoperative fluid administration. This is particularly important in complex abdominal wall reconstruction patients given

EBL, extended operative times with insensible losses, and large areas of surgically induced inflammation from tissue plane dissection. There is not always a consensus on fluid goals.²⁰ Additionally, in elective operations, there is generally no routine use of invasive hemodynamic monitoring adjuncts to assist with assessment of volume status.^{16,21}

Optimal perioperative fluid administration attempts to balance fluid overload which can lead to tissue edema and complicate closures, and under resuscitation which can lead to hemodynamic changes, AKI, increased lengths of stay, and impact on use of adjunctive tests. Our particular ERAS protocol was instituted in 2018 and focuses on appropriate fluid administration and management, early oral intake, as well as reduction in opioid use and early ambulation with the goal of improving patient outcomes and decreasing time to discharge.

This study found that AKI after complex abdominal wall reconstruction occurred in 29% of patients. While this rate is significant, it is within the 0.8-38 % range reported in the literature for AWR^{7,8}. This is noteworthy because the rate of AKI remains consistent despite the use of an ERAS protocol. Furthermore, we demonstrate that those who had preoperative underlying renal dysfunction (either previous CKD or higher levels of preoperative Cr), had a greater association with developing postoperative AKI compared to those with normal renal function. This is important as others have previously shown that perioperative AKI leads to morbidity and mortality.^{8,22} Notably, of the patients who developed AKI, no patient developed a severe enough level of AKI leading to severe hyperkalemia requiring treatment or need for dialysis.

Other studies have evaluated risk factors associated with perioperative AKI in patients undergoing hernia repair, including lower intraoperative IVF and prolonged operative time, similar to this study.^{7,16} This is likely a direct result of fluid loss and inadequate replacement resulting in hypovolemia and a prerenal insult to kidney function. Butler et al was also able to demonstrate an association between decreased intraoperative fluid administration and development of AKI, although they were looking specifically at liver transplant patients who may have other confounding factors.¹⁶ In their study, patients that developed acute kidney injury received less intraoperative volume (6 vs 8.5 mL/kg/h; p = 0.031) and the severity of postoperative renal injury was inversely related to the amount intraoperative volume given. While these results mirror our study, these studies differ in that they were not examining the impact of an ERAS protocol and represent an overview of potential causes of AKI after AWR. A key finding to highlight in our study is the impact of prolonged operative time on the development of AKI. Operative time ranged from 260 to 510 minutes which highlights the complexity of the cases encountered in this study, further supporting the potential for renal insult perioperatively. Longer case times often involve higher numbers of anesthesia providers, which may have differing fluid administration strategies or adherence to ERAS protocols. We examined for this possible confounder and found that the number of anesthesia providers did not impact the results.

Additionally, another interesting finding of note is the rate of recurrence demonstrated in this study. The overall rate of recurrence is low and remains overall stable before and after the institution of the ERAS protocol. There was however a slightly higher level of recurrence in the patients who developed AKI compared to those who did not. This is an expected finding as those patients were also associated with a higher rate comorbidities and lower functional level, which can contribute to hernia recurrence.

While this study provides valuable information, it has several limitations. Analysis of AKI is difficult because different providers use different definitions for AKI. The RIFLE criterion is a widely accepted paradigm, but is not universally utilized. This is also true for defining normal creatinine and renal dysfunction. Additionally, postoperative ileus is known to affect intravascular fluid volume and may contribute to incidence of AKI and was not able to be analyzed in this study. Because of the retrospective nature of the study, there was great variability in documentation of the incidence of ileus, hence precluding analysis. More than half of the patients in this cohort were from the time period before the ERAS protocol was instituted. This is significant because early institution of oral intake is a part of the ERAS protocol, with all patients having at least a clear liquid diet immediately postoperatively. Prior to the initiation of the protocol there was a variation on when diets were started, making it difficult to assess development of ileus based on number of days on nothing per oral diet or days until advancement to diet. Another consideration is that since AKI was defined as developing within the first 72 h postoperative, ileus is unlikely to be a contributing factor for AKI in this time period. Furthermore, our particular ERAS protocol has a recommendation for a postoperative IVF rate and a recommendation of stop date on postoperative day 2 if the patient has adequate oral intake. This leaves room for some level of provider interpretation, and could have resulted in patients receiving less fluids postoperatively than they physiologically required, thus resulting in development of AKI. Last, this study is limited by data only from a single institution.

Conclusion

The findings of this single institution, high-volume center study provide evidence that allows surgeons to be able to identify high-risk patients preoperatively and modify perioperative treatment algorithms prior to abdominal wall reconstruction. These findings will allow optimization of perioperative fluid management for these patients. This study confirms previous findings that comorbidities, prolonged operative time, and lower intraoperative IVF administration are important risk factors for postoperative AKI. More importantly, it demonstrates that for institutions that employ ERAS protocols, it provides evidence that ERAS protocols may require some adjustments for certain patients who are at higher risk of developing operative complications. More importantly, this study demonstrates that ERAS protocols may not adequately address the important complication of AKI and opens the discussion for improvement of these protocols.

Acknowledgment

R.D.S., D.E., and J.J. contributed to drafting the article and analysis of data. R.D.S. contributed to acquisition of data. All authors contributed to final approval of submitted article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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