#### **ORIGINAL ARTICLE**



# Short-term postoperative outcomes following robotic versus laparoscopic ileal pouch-anal anastomosis are equivalent

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#### Abstract

**Background** Minimally invasive approaches have become the standard of care for ileal pouch-anal anastomoses (IPAA). There are few reports comparing outcomes following a laparoscopic versus robotic approach. Our aim was to determine if there were any differences in the 30-day postoperative outcomes following IPAA performed laparoscopically versus robotically.

**Methods** A retrospective chart review of all laparoscopic and robotic IPAA performed between January 1, 2015 and June 30, 2018 was carried out. Patients included were adult patients who underwent a proctectomy and IPAA utilizing either a laparoscopic or robotic approach. Data collected included patient demographics, operative variables, and 30-day postoperative outcomes.

**Results** A total of 132 patients had a minimally invasive IPAA; 58 were performed laparoscopically and 74 robotically. Less than half the patients were female (n=55; 41.7%) with a median age of 37 years (range 18–68 years). The majority of patients had a diagnosis of ulcerative colitis (n=103; 78.0%) with medically refractory disease (n=87; 65.9%). A greater proportion of patients in the laparoscopic cohort had a prolonged length of stay (n=27; 46.6% versus n=18; 24.3%; p<0.001) and a two-stage approach (n=56; 96.6% versus n=37; 50%; p<0.001), but there were no differences in the rates between the laparoscopic versus robotic cohorts of superficial surgical site infection (6.9% versus 6.8%; p=0.99), peripouch abscess (15.5% versus 6.8%; p=0.11), anastomotic leak (6.9% versus 2.7%; p=0.21), pelvic abscess (15.5% versus 6.8%; p=0.11), and pelvic sepsis (15.5% versus 6.8%; p=0.11), readmission (24.1% versus 17.6%; p=0.35) or reoperation (6.9% versus 5.4%; p=0.72). On multivariable analysis, only male sex remained predictive of prolonged length of stay, and a robotic approach trended toward a decreased rate of prolonged length of stay.

**Conclusions** Laparoscopic and robotic IPAA have equivalent postoperative morbidity underscoring the safety of the continued expansion of the robotic platform for pouch surgery.

**Keywords** Ileal pouch-anal anastomosis · Proctocolectomy · Restorative · Minimally invasive surgery · Robotic surgical procedures

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## Introduction

A restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the surgical treatment of choice for ulcerative colitis (UC) and familial adenomatous polyposis (FAP) [1, 2]. Since the advent of IPAA in 1978, the operative approach has evolved from only an open technique to a variety of minimally invasive approaches including either hand assisted or straight laparoscopy, a transanal pelvic dissection with a single stapled anastomotic technique, or a robotic platform [3–5]. The evolution toward a less invasive approach has largely been driven by the clear patient benefits including a shorter length of postoperative stay [6, 7], improved body image [8], decreased infertility rates [9, 10], and decreased intravenous narcotic use [6].

Despite these advantages of conventional laparoscopy [11], there are also several limitations including reduced degrees of freedom of movement within the bony confines of the pelvis, poor visibility when performing the distal rectal dissection and subsequent ileal pouch anastomosis, and the need for an open incision to construct the IPAA. To overcome these limitations, some surgeons have begun using a transanal proctectomy with a single stapled anastomosis [3–5, 12], and others having begun using the robotic platform [13–15].

The Da Vinci<sup>®</sup> Surgical System (Intuitive Surgical, Mountain View, Sunnyvale, CA, USA) is being increasingly used for operations in colorectal surgery [16, 17]. The improved dexterity, visualization, and ergonomics of the robotic platform, and extensive body of literature reporting equivalent safety and efficacy outcomes as compared to conventional laparoscopy, has contributed to the surge in its use for rectal cancer [18, 19]. The same advantages are seen using the robotic platform for IPAA, where it allows improved visualization during the distal rectal dissection to preserve the neurovascular structures in the pelvis, as well as during the intracorporeal double stapled anastomotic technique for the IPAA. These proposed advantages may outweigh the potential increased cost [20] and limitations related to lack of haptic feedback [21, 22]. In fact, early literature suggests this technique is feasible and has comparable outcomes to both open and laparoscopic approaches [13, 14]. Due to its expanding use of the robotic platform for IPAA, we sought to compare the 30-day outcomes among patients undergoing a laparoscopic IPAA versus robotic IPAA to understand if a robotic approach is (1) associated with a reduced rate of 30-day postoperative complications, readmission and reoperation or (2) a reduced length of hospital stay.

#### Materials and methods

#### Patients

Following Institutional Review Board approval, all adult patients (age  $\geq$  18 years) with UC, indeterminate colitis (IC), or FAP who had a laparoscopic or robotic IPAA between January 1, 2015 and June 30, 2018 were identified using a prospectively maintained pouch database. Hand assisted laparoscopy was defined as performing the proctectomy through a hand port and pouch in an open fashion; straight laparoscopy was defined as performing the majority of the proctectomy laparoscopically followed by a Pfannenstiel or lower midline incision to construct the pouch anastomosis; robotic was defined as performing the proctectomy robotically and the anastomosis intracorporeally under direct visualization. The following current procedural terminology (CPT) codes were used to identify cases from the database: 44157, 44158, and 44211, 45113. As there are currently no CPT codes specific to robotic surgery versus laparoscopic surgery, laparoscopic operative reports were individually read for the type of case performed to identify the use of the robotic platform.

Data collected included patient demographics [age, sex, body mass index (BMI)], smoking status, duration of disease, indication for operation, American Society of Anesthesiologists (ASA) score, diabetes mellitus (DM), preoperative clinical variables [serum laboratory values within 2 weeks prior to surgery and exposure to immunosuppressive medications preoperatively (corticosteroids and/or immunomodulators within 4 weeks of surgery, and anti-TNF, vedolizumab, or ustekinumab within 12 weeks of surgery)], operative characteristics (number of stages, anastomotic technique, operation length, estimated blood loss), and 30-day postoperative outcomes. The primary endpoint was the rate of 30-day postoperative complications including superficial surgical site infection (sSSI) defined as need to open the incision or prescribe antibiotics, peripouch abscess seen on abdominopelvic imaging, anastomotic leak seen on abdominopelvic imaging or upon return to the operating room, pelvic sepsis (composite of both abscess and/or anastomotic leak), urinary tract infection (UTI) defined by a positive urinary culture, venous thromboembolism (VTE), ileus (defined as need for insertion of nasogastric tube), and small bowel obstruction (SBO) as defined by need for reoperation. Secondary endpoints were length of stay after the initial operation, and the 30-day rate of reoperation, unplanned hospital readmission, and pelvic sepsis. Prolonged length was defined as a stay greater than the 75th percentile of the entire cohort, which was 6 days.

#### **Statistical analysis**

Summary statistics are presented as frequencies and percentages for categorical variables and median and interquartile ranges for continuous variables. Univariate analysis was performed using unequal variance *t* tests for continuous variables and Pearson's Chi-squared tests for categorical variables. Univariate logistic regression analysis of potential risk factors for complications including laparoscopic versus robotic platform, gender, ASA, BMI, smoking, and diabetes was also performed. Additionally, the sample was separated by preoperative diagnosis of FAP or UC and the univariate logistic regression analysis was again performed based on either a laparoscopic or a robotic approach. Multivariate logistic regression analysis of prolonged length of stay included the following risk factors laparoscopic versus robotic surgery, gender, and n-stage. Significance was set at  $p \le 0.05$ . Data analysis was performed using SAS 9.4 software (Institute, Cary, NC,USA).

## Results

A total of 132 patients had a minimally invasive IPAA; 58 were performed laparoscopically and 74 robotically. Less than half the patients were female (n = 55; 41.7%) with a median age of 37 years (range 18–68 years). The majority of patients had a diagnosis of UC (n = 103; 78%); a minority were taking corticosteroids (n = 18; 13.6%), immunomodulators (n = 7; 5.3%) or biologics (n = 15; 11.4%) for their medically refractory disease (n = 87; 65.9%). The median hemoglobin level was 13.0 mg/dL (range 11.0–14.0 mg/dL), median leukocytes was 7.4 mg/ dL (range 5.9–9.1 mg/dL), median platelet count was  $273 \times 10^9$ /L (range 238–320 10<sup>9</sup>/L), median albumin level 4.4 g/dL (range 4.0–4.6 g/dL), and median C-reactive protein level 4.4 mg/L (range4.0–4.6 g/dL). There was a significantly greater proportion of patients with FAP in the laparoscopic cohort as well as more patients having IPAA for dysplasia and polyposis. There were no significant differences in the laparoscopic versus robotic group with regard to age, sex, ASA, BMI, smoking status, DM, preoperative immunosuppressive regimens, or serum laboratory values (Table 1).

The majority of the cases were performed with a twostage approach (n = 93;70.5%). In the majority of laparoscopic cases a lower midline incision was used for the anastomosis (n = 40; 69%) compared to a Pfannenstiel incision (n = 18; 31%). The robotic cohort had a significantly longer operating time than laparoscopic (315 min versus 281 min; p < 0.001), but a lower estimated blood loss (75 mL versus 100 mL; p < 0.001) (Table 2).

Table 1 Demographics, preoperative treatments, and laboratory values

	All patients $(n = 132)$	Robotic $(n = 74)$	Laparoscopic $(n=58)$	р
Age (years), median (IQR)	37 (28–50)	40 (30-50)	36 (26–48)	0.20
Gender (female) (%)	55 (41.7)	29 (39.2)	26 (44.8)	0.51
BMI $(kg/m^2)$ (mean $\pm$ SD)	$24.6 \pm 4.7$	$24.5 \pm 3.9$	$24.8 \pm 5.7$	0.73
ASA group				0.63
1 (%)	7 (5.3)	4 (5.4)	3 (5.2)	
2 (%)	90 (68.2)	48 (46.9)	42 (72.4)	
3 (%)	35 (26.5)	22 (29.7)	13 (22.4)	
Diagnosis				< 0.001
Ulcerative colitis/Indeterminate colitis (%)	104 (78.8)	67 (91.9)	36 (62.1)	
FAP (%)	28 (21.2)	6 (8.1)	22 (37.9)	
Indication				< 0.001
Medically refractory (%)	87 (65.9)	59 (79.7)	28 (48.3)	
Dysplasia/adenocarcinoma (%)	24 (18.2)	10 (13.5)	14 (24.1)	
Polyposis (%)	21 (15.9)	5 (6.8)	16 (27.6)	
Smoking (%)	9 (6.8)	3 (4.1)	6 (10.3)	0.15
Diabetes (%)	5 (4.8)	3 (4.1)	2 (3.4)	0.86
Preoperative steroids (%)	18 (13.6)	9 (12.2)	9 (15.5)	0.58
Preoperative IMM (%)	7 (5.3)	2 (2.7)	5 (8.6)	0.13
Preoperative biologics (%)	15 (11.4)	8 (10.8)	7 (12.1)	0.82
Preoperative hemoglobin (g/dL)	13 (11–14)	12 (11–14)	13 (12–15)	0.08
Preoperative leukocyte count ( $\times 10^{9}/L$ )	7.4 (5.9–9.1)	7.4 (5.6–9.3)	7.8 (6.3–9.1)	0.80
Preoperative platelet count ( $\times 10^{9}/L$ )	273 (238–320)	228 (236-315)	278 (239–362)	0.49
Preoperative albumin (g/L)	4.4 (4-4.6)	4.4 (4.1–4.5)	4.4 (4-4.6)	0.37
Preoperative CRP (mg/L)	4.4 (4-4.6)	4.4 (4.1–4.5)	4.4 (4-4.6)	0.37

Laboratory values expressed as median (IQR)

Bold characters indicate significant values (p < 0.05)

Baseline demographics, preoperative treatments and preoperative laboratory values of patients undergoing laparoscopic IPAA (n=58) and patients undergoing robotic IPAA (n=74)

*IQR* interquartile range, *BMI* body mass index, *ASA* American Society of Anesthesiology, *FAP* familial adenomatous polyposis, *IMM* immunomodulator, *CRP* C-reactive protein

Table 2 Operative characteristics

	All patients $(n = 132)$	Robotic $(n=74)$	Laparoscopic $(n=58)$	р
n stages				
2 (%)	93 (70.5)	37 (50)	56 (96.6)	< 0.001
3 (%)	39 (29.5)	37 (50)	2 (3.4)	
Operating time (minutes), median (IQR)	314 (252–349)	315 (276–365)	281 (235–335)	0.002
Estimated blood loss(ml), median (IQR)	75 (50–100)	75 (50–100)	100 (50–175)	0.002

Operative characteristics of patients undergoing laparoscopic IPAA (n=58) and patients undergoing robotic IPAA (n = 74)

Bold characters indicate significant values (p < 0.05)

A greater proportion of patients in the laparoscopic cohort had a prolonged length of stay (n = 27; 46.6% versus n = 18; 24.3%; p = 0.01). There were no differences in the rates of ileus or small bowel obstruction, for which the overall rates were 18% and 3%, respectively. There were no differences between the laparoscopic and robotic cohorts in the rates of superficial SSI (6.9% versus 6.8%; p = 0.98), peripouch abscess (15.5% versus 6.8%; p = 0.11), anastomotic leak (6.9% versus 2.7%; p = 0.21), pelvic abscess (15.5% ver-)sus 6.8%; p = 0.11), and pelvic sepsis (15.5% versus 6.8%; p = 0.11), Thirty-day intensive care unit admission, (2.7%) versus 1.7%; p = 0.72) readmission (24.1% versus 17.6%; p = 0.35) or reoperation (6.9% versus 5.4%; p = 0.72) rates were also not significantly different in the two cohorts (Table 3).

Univariate logistic regression for the 30-day postoperative outcome variables including ileus, superficial SSI, peripouch abscess, anastomotic leak, pelvic sepsis, reoperation, and readmission did not identify any significant associations for each outcome considered. Laparoscopic approach, male sex and a 2 stage IPAA were all associated with a prolonged length of stay (p=0.01) (Table 4). On multivariable analysis for prolonged length of stay, only male sex remained significant (*p* < 0.01) (Table 5).

Table 3	30-Day postoperative
outcome	es

	All patients $(n = 132)$	Robotic $(n=74)$	Laparo- scopic $(n=58)$	Р
Ileus (%)	24 (18.2)	11 (14.9)	13 (22.4)	0.2644
SBO (%)	4 (3)	4 (5.4)	0	0.0722
Superficial SSI (%)	9 (6.8)	5 (6.8)	4 (6.9)	0.9748
Peripouch abscess (%)	14 (10.6)	5 (6.8)	9 (15.5)	0.1047
Anastomotic leak (%)	6 (4.5)	2 (2.7)	4 (6.9)	0.2509
Pelvic sepsis (%)	14 (10.6)	5 (6.8)	4 (6.9)	0.1047
UTI (%)	2 (1.5)	0	2 (3.4)	0.1075
VTE (%)	2 (1.5)	2 (2.7)	0	0.2071
30-day ICU admission (%)	3 (2)	2 (3)	1 (2)	0.7081
Readmission (%)	27 (20.5)	13 (17.6)	14 (24.1)	0.3530
Reoperation (%)	8 (6.1)	4 (5.4)	4 (6.9)	0.7216
Length of stay (days), median (IQR)	5 (3–6)	4 (3–5)	5 (4–7)	0.0503
Prolonged stay (%)	45 (34.1)	18 (24.3)	27 (46.6)	0.0075

30-day complications of patients undergoing laparoscopic IPAA (n=58) and patients undergoing robotic IPAA (n=74)

Bold characters indicate significant values (p < 0.05)

SBO small bowel obstruction, SSI surgical site infection, UTI urinary tract infection, VTE venous thromboembolism, ICU intensive care unit, IQR interquartile range

Table 4 Logistic regression for discrete outcome variables

Dependent variable	Dependent variable Variable O		95% CI		р
			Lower	Upper	
Ileus	Laparoscopic	1.66	0.68	4.03	0.27
	Male gender	1.00	0.41	2.45	1.00
	ASA	1.42	0.61	3.31	0.41
	BMI	1.09	1.00	1.20	0.06
	Smoking	0.54	0.07	4.56	0.57
	Diabetes	3.18	0.50	20.18	0.22
	n stages	0.42	0.13	1.31	0.14
	Diagnosis-UC	2.55	0.96	6.78	0.06
	Diagnosis-FAP	0.50	0.04	6.68	0.60
Superficial SSI	Laparoscopic	1.02	0.26	3.99	0.97
	Male gender	1.46	0.35	6.13	0.60
	ASA	1.60	0.45	5.74	0.47
	BMI	1.06	0.93	1.21	0.40
	Smoking	1.80	0.20	16.20	0.60
	Diabetes	0.88	0.04	22.53	0.98
	n stages	0.66	0.13	3.35	0.62
	Diagnosis-UC	1.58	0.40	6.27	0.52
	Diagnosis-FAP	_	_	_	_
Pelvic abscess	Laparoscopic	2.54	0.80	8.03	0.11
	Male gender	1.32		4.19	0.63
	ASA	1.01	0.35	2.91	0.99
	BMI	1.06		1.19	0.29
	Smoking	1.06	0.12	9.14	0.96
	Diabetes	2.19		21.12	0.50
	n stages	1.37	0.43	4.39	0.54
	Diagnosis-UC	3.04	0.89	10.40	0.08
	Diagnosis-FAP	1.59	0.05	46.78	0.96
Reoperation	Laparoscopic	1.30	0.31	5.42	0.72
F	Male gender	0.70	0.17	2.92	0.62
	ASA	1.86	0.48	7.18	0.37
	BMI	0.94	0.80	1.11	0.48
	Smoking	2.07	0.23	18.97	0.52
	Diabetes	0.78	0.03	20.15	0.98
	n stages	0.78	0.15	4.07	0.52
	Diagnosis-UC	2.00	0.47	8.52	0.35
	Diagnosis-FAP	_	_	_	_
Prolonged length of	Laparoscopic	2.71	1.29	5.68	0.01
stay	Male gender	2.69	1.23	5.88	0.01
-	ASA	1.74	0.87	3.50	0.12
	BMI	1.05	0.97	1.13	0.12
	Smoking	0.96	0.23	4.05	0.20
	Diabetes	1.30	0.23	8.09	0.78
	n stages	0.32	0.21	0.79	0.78
	Diagnosis-UC	2.14	0.13	5.07	0.01
	-		0.91	60.14	0.08
	Diagnosis-FAP	6.00	0.00	00.14	0.15

Table 4	(continued)
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Dependent variable	Variable	OR	95% CI		р
			Lower	Upper	
Readmission	Laparoscopic	1.49	0.64	3.49	0.35
	Male gender	0.60	0.25	1.39	0.23
	ASA	1.05	0.47	2.35	0.91
	BMI	1.00	0.91	1.09	0.91
	Smoking	2.06	0.48	8.85	0.33
	Diabetes	0.97	0.10	9.06	0.98
	n stages	1.25	0.51	3.09	0.52
	Diagnosis-UC	1.56	0.59	4.14	0.38
	Diagnosis-FAP	1.47	0.14	15.68	0.75
Pelvic sepsis	Laparoscopic	2.54	0.80	8.03	0.11
	Male gender	1.32	0.42	4.19	0.63
	ASA	1.01	0.35	2.91	0.99
	BMI	1.06	0.95	1.19	0.29
	Smoking	1.06	0.12	9.14	0.96
	Diabetes	2.19	0.23	21.12	0.50
	n stages	1.37	0.43	4.39	0.54
	Diagnosis-UC	3.04	0.89	10.40	0.08
	Diagnosis-FAP	1.59	0.05	46.78	0.96

Bold characters indicate significant values (p < 0.05)

ASA American Society of Anesthesiology, BMI body mass index, SSI surgical site infection

Table 5	Multivariable anal	vsis for	prolonged	length of stay

Dependent variable	Variable	OR	95% CI		р
			Lower	Upper	
Prolonged Length of	Robotic	0.44	0.18	1.07	0.07
stay	Male gender	3.00	1.32	6.83	< 0.01
	N stages $= 3$	0.49	0.17	1.42	0.19

## Discussion

Compared with conventional laparoscopy, we found that a robotic approach was associated with a clinically significant decrease in the rate of pelvic sepsis, with no change in other 30-day morbidity.

When laparoscopy was first applied to colon and rectal pathology, there was initial skepticism about its utility due to prolonged operative times and high conversion rates. However, widespread adoption has shortened operative times, and randomized controlled trials comparing laparoscopic versus open approaches for colon cancer have demonstrated faster recovery, decreased morbidity, and reduced hospital length of stay, and comparable oncological survival outcomes with a laparoscopic approach [27–29]. Laparoscopy does have limitations around the

view available and freedom of movement; largely overcome using a robotic platform.

These advantages of the robotic approach in forming the IPAA may help reduce the rate of pelvic sepsis, which is the most dreaded complication of pouch surgery due to known increased rates of pouch failure [9, 12]. Pelvic sepsis following original IPAA occurs in 5–25% [1, 30–32] of patients, and most commonly is a result of a peripouch abscess or anastomotic leak from the pouch-anal staple line. This inflammation eventually leads to scarring and pelvic fibrosis, causing decreased distensibility of the pouch, which ultimately manifests as poor long-term pouch function or eventual need for pouch reconstruction [33]. With a robotic approach, one can visualize the dissection down to the pelvic floor, ensure the height of the rectal transection with regard to the levator ani muscles, and then perform the anastomosis intracorporeally under direct visualization, which improves the alignment of the stapler ends, pouch orientation and mesenteric orientation. This in turn means that technical contributors to leak are minimized and this may lead to a reduction the rate of pelvic sepsis. While underpowered, there was a trend towards decreased pelvic sepsis within this study. As more outcomes after robotic IPAA are reported, it will enable the determination of whether robotic IPAA should be the preferred approach when a difficult pelvic dissection and anastomosis is anticipated.

Using a transanal approach to the distal proctectomy and pouch anastomotic construction [12, 34] may allow an exact identification of where to transect the rectum for the anastomosis, and allow a single stapled rather than a double stapled technique thus avoiding crossing staple lines. While the transanal approach may be useful, the learning curve is steep, and it remains unknown if the anastomotic leak rate following IPAA is lowered.

There are few reports of robotic IPAA. Miller et al. [14] compared 17 robotic cases to 17 laparoscopic cases and found no differences in complications or short-term functional results. Interestingly the return to bowel function and the length of stay was longer in the robotic than the laparoscopic group. Rencuzagullari et al compared 21 robotic completion proctectomy cases to 21 laparoscopic proctectomy cases, with IPAA performed in 18 patients in each cohort, and found that the operative time and blood loss was higher in the robotic group, but that the length of stay and postoperative outcomes were similar in the two cohorts [35]. Mark-Christensen et al compared 81 robotic IPAA cases to 170 open IPAA cases and found no difference in the rate of postoperative complications but the robotic group did have an increased rate of readmission [13]. We found equivalent rates of postoperative morbidity in the laparoscopic and robotic cohorts.

Future research will be needed to gather larger numbers of patients treated by each minimally invasive approach to better answer whether one approach is better than another with regard to perioperative outcomes, functional outcomes, fertility outcomes, and quality of life outcomes. We anticipate that if lower leak rates are achieved with one particular approach functional outcomes will also be improved. Similarly, we anticipate less adhesion formation when using a technique that does not require an incision for pouch formation (a robotic platform or a transanal approach) versus even laparoscopy which uses an incision for the anastomotic construction. With less adhesions, we would anticipate that fertility would be improved. Similarly, without an incision, there is less risk of hernia formation, postoperative SBO, and need for future surgical intervention to address these complications. Longitudinal longer-term studies will be useful in determining whether these advantages are seen with these minimally invasive approaches.

There are several limitations to this study. First this is a retrospective review of a prospectively maintained database, limiting the analysis of postoperative outcomes. Second, we are an inflammatory bowel disease referral center and our patient population may be different from that of outside institutions, limiting the applicability of our findings. Third, many of our patients travel long distances. Therefore, we do not have routine postoperative visits, but often rely on patient phone calls and nursing communication to gather information on postoperative outcomes and complications. Thus, our 30-day complication rate may be under-reported. Fourth, there were not enough events for a multivariable analysis of pelvic sepsis to determine whether a robotic approach results in decreased rates of pelvic sepsis, which may then have implications on long term functional outcomes. Fifth, we do not yet have data on long-term functional outcomes following a robotic versus laparoscopic approach, which are arguably more significant than 30-day postoperative outcomes.

### Conclusions

Laparoscopic and robotic IPAA have equivalent postoperative morbidity. The choice of operative instrument should be based on the individual surgeon's skill and preference, availability of technology, and any patient preferences. Since the application remains novel, we will have to wait for long-term prospective functional and quality of life data.

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#### **Compliance with ethical standards**

**Conflict of interest** Amy Lightner receives consultant fees from Takeda; all other authors: declare that they have no conflict of interest.

**Ethical approval** All human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the 1965 Declaration of Helsinki.

**Informed consent** For this type of study, informed consent was not necessary.

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