



# MRI defecography of the ileal pouch-anal anastomosis—contributes little to the understanding of functional outcome

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## Abstract

**Purpose** Variability in functional outcome after ileal pouch-anal anastomosis (IPAA) is to a large extent unexplained. The aim of this study was to use MRI to evaluate the morphology, emptying pattern and other pathology that may explain differences in functional outcome between well-functioning and poorly functioning pouch patients. A secondary aim was to establish a reference of normal MRI findings in pelvic pouch patients.

**Methods** From a previous study, the best and worst functioning patients undergoing IPAA surgery between 2000 and 2013 had been identified and examined with manovolumetric tests ( $N=47$ ). The patients were invited to do a pelvic MRI investigating pouch morphology and emptying patterns, followed by a pouch endoscopy.

**Results** Forty-three patients underwent MRI examination. We found no significant morphological or dynamic differences between the well-functioning and poorly functioning pouch patients. There was no correlation between urge volume and the volume of the bony pelvis, and no correlation between emptying difficulties or leakage and dynamic MRI findings. Morphological MRI signs of inflammation were present in the majority of patients and were not correlated to histological signs of inflammation. Of the radiological signs of inflammation, only pouch wall thickness correlated to endoscopic pouchitis disease activity index scores.

**Conclusion** It seems MRI does not increase the understanding of factors contributing to functional outcome after ileal pouch-anal anastomosis. Unless there is a clinical suspicion of perianal/peripouch disease or pelvic sepsis, MRI does not add value as a diagnostic tool for pelvic pouch patients. Endoscopy remains the golden standard for diagnosing pouch inflammation.

**Keywords** Ulcerative colitis · Ileal pouch-anal anastomosis · Functional outcome · MRI dephography

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What does this paper add to the literature?

MRI has to a small extent been evaluated as a tool for better understanding of functional variability among patients with restorative proctocolectomy. This paper explores different aspects of morphological and dynamic MR imaging without finding any major investigational benefits to explain variations in functional outcome. However, normal values were established.

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## Introduction

Ileal pouch-anal anastomosis (IPAA) was first introduced by Nicholls and Parks in 1978 [1] and is today considered the treatment of choice in patients with ulcerative colitis and familial adenomatous polyposis in need of surgery. The procedure is done to restore a normal route of defecation, hence improving quality of life in a young patient group by avoiding a permanent stoma. IPAA is considered safe with a very low mortality rate; however, there is a considerable post-operative short- and long-term complication rate [2–4]. The 10-year failure rate varies between 9 and 16%, commonly caused by septic pelvic complications and poor functional outcome, with Crohn's disease and chronic pouchitis being less common reasons for failure [5–8]. Between good function and failure, there is a wide range in pouch function, and the reasons for this variability are poorly understood. However, some factors are known predictors of functional outcome. Septic pelvic

complications and pouchitis not contributing to failure is known to impair pouch function [5–7]. Age, the type [9–11] and level [12, 13] of the pouch-anal anastomosis are other factors known to impact pouch function, with high age, hand-sewn anastomosis and a long rectal cuff being negatively correlated to function. The most dominant predictors of good function seem to be a large pouch volume and good compliance [14–21]. However, there is a large variability in pouch volume in patients with pouches constructed of the same length of ileum [19]. The factors contributing to these differences in pouch volume remain to a large extent unknown, and still the major source of functional variability remains undefined.

A possible explanation to the variation in pouch volume might be anatomical limitations of the pelvis. Other differences in pelvic morphology and emptying patterns might also explain functional outcome to some extent. Fluoroscopic enema, computer tomography, magnetic resonance (MR) enterography, and conventional or MRI defecography, are all imaging techniques used to investigate IPAA patients. However, the literature is scarce, and there is no consensus on the optimal investigation algorithms. Only a limited number of studies have investigated the correlation between radiological findings versus endoscopic findings and histology [22–25]. To our knowledge, no study has compared pelvic volume with manovolumetric findings of urge volume, nor compared radiologic findings in a poorly functioning pouch group with a well-functioning pouch group as controls.

The primary aim of this study was hence to evaluate the morphology and emptying patterns of the ileal pouch with a pelvic MRI in well-functioning and poorly functioning pouches, comparing the results with findings from endoscopy and manovolumetric examinations of volume. In addition, as this is the first study published on MRI findings of pouch patients with a well-functioning control group, our secondary aim was to establish a reference of normal MRI findings in pelvic pouch patients.

## Methods

In previous studies [26, 27], all patients undergoing IPAA at our unit from 2000 to 2013 ( $n = 114$ ) had been interviewed regarding their pouch function, using a pouch functioning score (PFS) according to Oresland et al. reaching from 0 to 16 (low scores indicate good function) [28]. The variables included in the score and how they are defined can be seen in Table 1. The best and worst functioning patients ( $N = 47$ ) were further examined with manovolumetric tests measuring pouch volume at urge (April–June 2016), followed by a pouch endoscopy [29]. Good function was defined as  $PFS \leq 3$ , poor function as  $PFS \geq 7$  and  $PFS \geq 6$  in patients also claiming that their pouch gave them a social handicap. The manovolumetric

tests were conducted using a barostat (G&J Electronics Inc., Toronto, Canada) measuring pouch volumes at preset filling pressure. The volume where the patients noted an urge to defecate is referred to as the urge volume. The pouchitis disease activity index (PDAI) [30] was used to investigate endoscopic signs of pouchitis, and biopsies were secured for histological signs of acute inflammation.

In the present study, the 47 patients were invited to do a pelvic MRI. Endoscopic and histological findings of acute inflammation from the previously mentioned study [29] were compared with morphological MRI signs of inflammation, and pouch volume was compared with pelvic measures. The MRI and pouch endoscopy were performed on the same day, with the MRI being performed prior to the endoscopy to avoid irritation of the pouch. All patients were examined within a timeframe of 3 months (October–December 2016).

From medical record files and previously conducted studies [26, 27], pre-/per and post-operative information was gathered. The following variables were analysed: age, gender, indication for surgery, pouch formation and post-operative complications. Pelvic sepsis was defined as anastomotic leaks, parapouch abscesses (pelvic abscesses) and pouch-anal fistulas [2], perianal fistulas not communicating with the pouch were defined as perianal disease. At the outpatient clinic, patients were asked about pouchitis, including those treated by their general practitioner or at other hospitals. Previous episodes of pouchitis were defined as an episode with increased frequency of defecation and/or bloody stool, responding on antibiotics [31] in patients with an incidence endoscopy verified pouchitis.

## MRI

There was no bowel preparation before the MRI scans, but the patients were asked to empty the pouch before imaging. The bladder was emptied 2 h before the imaging. The patients were examined in the supine position with a pillow underneath the knees. The first part of the exam consisted of morphological MRI sequences (sagittal turbo spin echo (TSE) T2-weighted sequence, an axial 3 dimensional (3D) TSE T2-weighted sequence, an axial 3D TSE-weighted sequence with fat suppression (FS)). Then contrast (methylcellulose) was installed in the pouch from the anal canal through a continent ileostomy catheter. The amount of methylcellulose was individualised, installing the volume at which the patients reported an urge to defecate during previous manovolumetric tests. After the installation, the axial 3D TSE FS sequence was repeated and then finally dynamic MRI sequences were undertaken with an initial thick slab sagittal balanced fast field echo (BFFE) sequence performed with the patient squeezing the sphincter musculature, after which a sagittal BFFE was performed while the patient was straining. Finally, both a sagittal and coronal

**Table 1** Demographics, complications and functional outcome in patients with well-functioning and poor functioning pouches

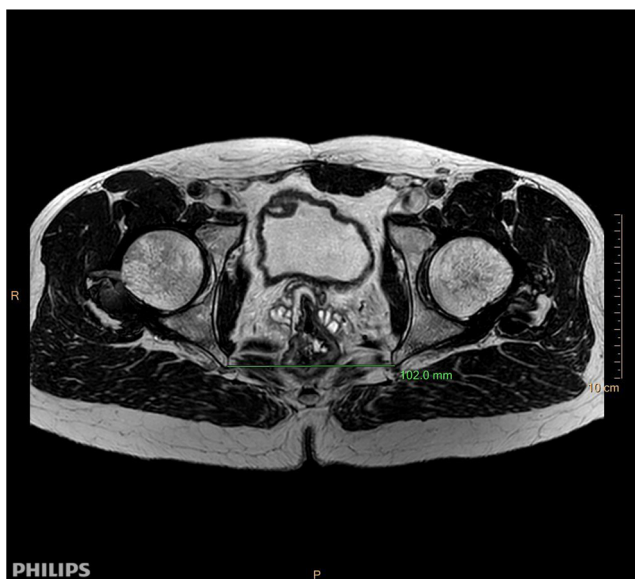
	Good function ( <i>n</i> = 21)	Poor function ( <i>n</i> = 22)	<i>p</i> value
Demographics			
J-pouch	10	12	0.496
Female	6	9	0.239
Age (median, range)	51 (28, 70)	54 (14, 72)	0.814
Follow-up in years (median, range)	9 (4, 16)	11 (3, 16)	0.723
Complications			
Septic pelvic complication	0	2	0.180
Perianal fistula/abscess	0	3	0.097
Pouch inflammation			
≥ 1 pouchitis episode	4	11	0.061
Histological signs of acute inflammation	10	17	0.069
Endoscopic PDAI (median, range)	0 (0, 4)	0.5 (0, 6)	0.516
Functional outcome according to the variables included in the pouch functioning score			
No. of daily bowel movements (median, range)	5 (3, 7)	7 (5, 13)	< 0.001
No. of nightly bowel movements (median, range)	0 (0, 1)	1.5 (0, 4)	< 0.001
Urgency (inability to defer evacuation > 30 min)	0	4	0.043
Evacuation difficulties (> 15 min spent in toilet on any occasion during the week)	2	3	0.678
Soiling or seepage > 1/week			
Day	1	8	0.012
Night	1	11	0.001
Use of protective pad > 1/week			
Day	0	10	< 0.001
Night	2	14	< 0.001
Perianal soreness			
Occasional	10	11	0.877
Always	0	9	0.001
Reporting pouch as social handicap	0	13	< 0.001
Inability to release flatulence safely	13	15	0.670
Dietary restrictions	3	10	0.028
Use of medication	5	15	0.004

dynamic imaging was performed while the patient emptied the pouch.

Two radiologist consultants assessed the MRI scans separately to validate the findings with a final consensus reading if disagreement; both were blinded for the patient histories. The following parameters were noted: morphological pathology and signs of inflammation in the pouch and in the pelvis, anatomical characteristics of the pelvis including size, and dynamic evaluation of the pouch and pelvic floor. The different parameters evaluated are defined in Table 3. The most prominent morphological MRI signs of inflammation included pouch wall oedema and increased pouch wall thickness ( $\geq 3$  mm) [22], and if both were present, the radiologists concluded on pouch inflammation based on morphological MRI findings. Additional MRI signs of inflammation included enlarged lymph nodes, peripouch free fluid—oedema—fatty proliferation and sinuses, abscesses and fistulas. The results from the MRI exams were compared with volume from the previously

conducted manovolumetric examination, endoscopic PDAI scores and histology.

The pelvic volume was calculated from standardised pelvimetry of the bony pelvis [32–34] (Figs. 1, 2, 3 and 4). In addition, the angle between a line drawn from the promontory to the upper border of the pubic symphysis and a line drawn along the upper border of the pubic symphysis was calculated, as a narrower angle and hence less accessible pelvis has been found to correlate to the quality of total mesorectal incision performed through a transabdominal approach [34]. According to a study by Jones et al., the mentioned measurements were used to calculate the pelvic inlet ( $\pi \times$  anatomical transverse distance  $\times$  the distance from the promontory to the upper border of the pubic symphysis), pelvic outlet ( $\pi \times$  the intertuberous distance) and pelvic height (distance from superioposterior pubis to the posterior anorectal junction, at the level of tuber). The volume was then calculated using a formula for frustum.



**Fig. 1** The anatomical limitations in the axial plane used to measure the interischial distance (IS)

### Surgical technique

All patients operated before 2008 were given a stapled J-pouch [35], and all patients operated in 2008 and onwards had a hand-sewn, double-folded K-pouch according to the technique of the continent ileostomy (Kock pouch) [36]. For the construction of both pouch designs, the distal 30 cm of the ileum was folded into two loops. In the J-pouches, the loops were stapled longitudinally. To form the K-pouch, the apexes of both loops were folded transversely to form a sphere. The pouch was completed by a transverse suture, and then pushed through the mesentery obtaining its final pear shape. The

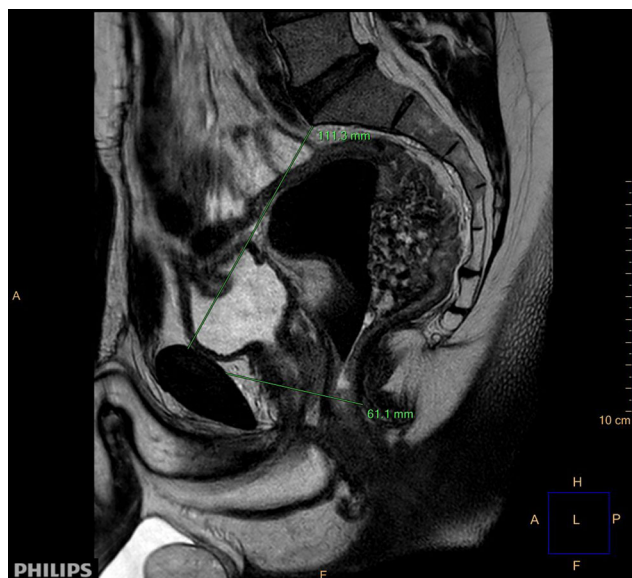


**Fig. 2** The anatomical limitations in the axial plane used to measure the intertuberos distance (IT)



**Fig. 3** The anatomical limitations in the coronal plane used to measure the transverse distance of the pelvis (IP; the cranial line) and the interacetabular distance (IA; the caudal line)

pelvic dissection (done in the mesorectal plane) was the same in both groups, and all patients were diverted with a loop ileostomy. The median time to stoma closure was 98 (23,209) days. The majority of the included patients ( $N=25$ ) was operated on in a three-stage procedure, with most of them having undergone laparoscopic subtotal colectomy prior to the IPAA. The pouch surgery was performed with open technique through a low midline incision in all patients, and the ileal pouch-anal anastomosis was created within a maximum of



**Fig. 4** The anatomical limitations in the sagittal plane used to measure the distance between the promontory (Pr) and the upper border of the pubic symphysis (SyU), the line distance is referred to as PrSyU, and the angle between PrSyU and a line drawn along the upper border of the pubic symphysis

2 cm above the dentate line. The indication for surgery was in most patients ulcerative colitis, with the exception of one patient with Hirschsprung's disease, one with FAP and one patient with CAP polyposis [37].

## Statistics

All analyses were performed using SPSS statistics (IBM Corp. Released 2013. IBM SPSS statistics version 24.0. Armonk, NY, USA). A value of  $p < 0.05$  was considered to be statistically significant. Mann-Whitney  $U$  test was used to calculate differences between the well-functioning and poorly functioning pouch groups, and linear regression and binary logistic regression were used to investigate the correlation when the dependent factor was linear or binary respectively.

The study was approved by the regional and local ethics committee (REK no 2014/2206).

## Results

Of the 47 patients eligible who had already undergone manovolumetry analysis, 44 agreed to further examination with a functional MRI. One image series was lost in the system and this patient had to be excluded ( $N = 43$ ). The patient's demographics, complications, and pouch function can be seen in Table 1. Median PFS was three in the well-functioning and 9 in the poor-functioning group ( $p < 0.001$ ). The groups were comparable in terms of demographics.

The correlation between volume at urge and the measurements of the bony pelvis can be seen in Table 2. As can be seen from the table and Fig. 5, the calculated total pelvic volume was not correlated to urge volume. However, some of the transverse measures of the bony pelvis were significantly correlated to volume at urge. The PrSyU angle was not correlated to function ( $p = 0.098$ ) or the presence of septic pelvic complications ( $p = 0.093$ ).

The MRI findings from the morphological and dynamic MRI scans are illustrated in Table 3. There were no differences between the well-functioning and poorly functioning pouch groups. In total, 36 patients had one or more radiological finding of pouch inflammation (14 in the well-functioning and 22 in the poorly functioning group). Only two in the well-functioning group and four in the poorly functioning group had a normal pouch wall thickness of  $< 3$  mm [22]. There was no difference between the groups in number of patients with both increased pouch wall thickness and pouch wall oedema indicating pouch inflammation based on morphological MRI findings ( $p = 0.293$ ). In Table 4, radiological signs of pouch inflammation are correlated against histological signs of acute inflammation, PDAI scores and a history of one or more episodes of pouchitis. None of the patients presented with symptoms of pouchitis at the time of examination.

There was no difference in pelvic descent during emptying in patients with and without emptying difficulties (four of five patients with and 23 of 38 without emptying difficulties displayed descent  $\geq 30$  mm during emptying,  $p = 0.489$ ). One patient with emptying difficulties had a stricture of the pouch inlet. None of the patients with emptying difficulties displayed outlet stricture, obstructive intussusception or sphincter dysfunction. Three patients displayed small intestine dilated  $> 30$  mm, all were in the poorly functioning group. None of them suffered from emptying difficulties or abdominal pain.

None of the dynamic parameters were correlated to leakage. Four of the 13 patients suffering from soiling or seepage during the day and/or night and four of the 30 patients not experiencing soiling displayed a dysfunctional external sphincter ( $p = 0.055$ ). None of the patients reporting soiling displayed sphincter atrophy.

Four patients suffered from urge symptoms, of whom all displayed descent  $\geq 30$  mm during emptying compared to 23 of 39 patients without urgency displaying descent during emptying. Only one of the patients with urge displayed a dysfunctional sphincter. None of the four displayed levator or sphincter atrophy, or obstructive intussusception.  $p$  values have not been calculated as the numbers are small.

## Discussion

In this study, we have examined 43 well-functioning and poorly functioning pelvic pouch patients with MRI scans to investigate potential dynamic, morphological or anatomical differences between well-functioning and poorly functioning pouches, looking for explanations for the known variation in functional outcome after IPAA. Interestingly, there were no significant differences in radiological findings between the groups.

MRI defecography has not been used routinely as a diagnostic tool to investigate pouch function, and its value in diagnosing mechanisms for malfunction is unclear. In the present study, MRI examinations could not explain malfunction, as none of the radiological findings were correlated to emptying difficulties, urge or soiling. However, a limitation is the small number of patients suffering from the mentioned symptoms; there might be correlations the present study was underpowered to find. A recent study investigating non-relaxing pelvic floor dysfunction (N-RPFD) found this to be a common explanation for evacuation difficulties. However, MRI defecography only detected 25% of patients with known N-RFD [38]. Conventional defecating pouchography has been reported to be useful in identifying anismus and pelvic floor descent in patients complaining of straining, anal pain or incontinence, but not in patients complaining of high frequency of bowel movements or abdominal pain [39]. Several previous

**Table 2** Measurements of the bony pelvis

	<i>N</i> = 43 mean (95% CI)	<i>p</i> value (linear regression with pouch volume at urge as dependant variable)
Pelvic volume (cm <sup>3</sup> )	1313.7 (1248.3–1374.7)	0.839
IP (cm)	13.3 (13.0–13.6)	0.114
IA (cm)	13.3 (13.1–13.6)	0.023
IS (cm)	9.8 (9.4–10.1)	0.009
IT (cm)	12.2 (11.8, 12.7)	0.029
PrSuY (cm)	11.7 (11.5–12.0)	0.886

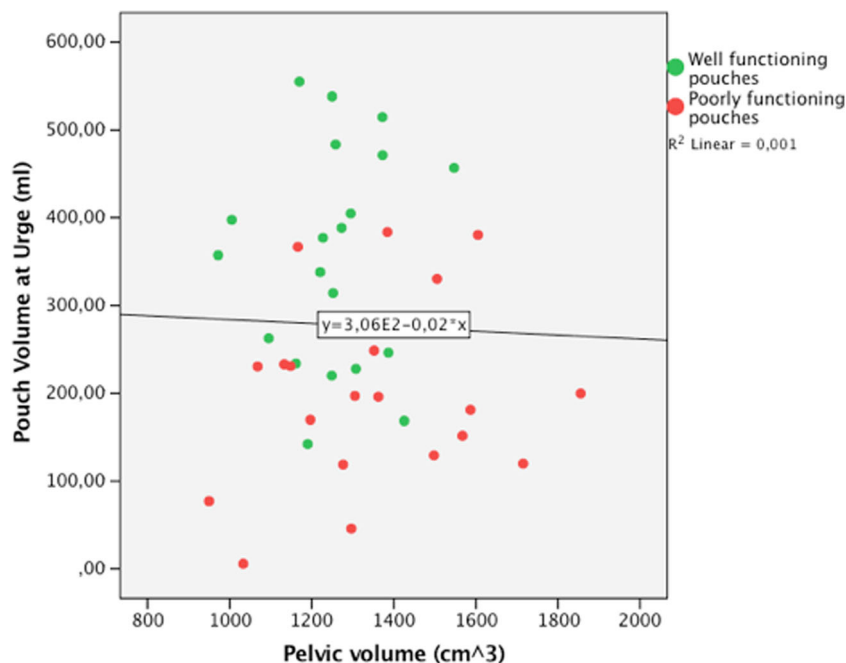
*IP* anatomical transverse distance, *IA* interacetabular distance, *IS* interischial distance, *IT* intertuberous distance, *PrSyU* line from the upper border of the promontory to the pubic symphysis, *APrSyU* angle between a line drawn from the promontory to the upper border of the pubic symphysis and a line drawn along the upper border of the pubic symphysis

studies have investigated evacuation fractions using pouch scintigraphy, reporting a wide range in evacuation fraction (28–77%) [39–43], with a recent study finding patients with more than 33% barium retention complaining of incomplete emptying [39]. In the present study, we were unable to quantify the evacuation fraction as we did not have a reliable measure of the amount of emptied gel; hence, we could not correlate the subjective feeling of emptying difficulties with the evacuation fraction. It is unclear whether findings from conventional pouchographies also apply for MRI pouchographies. A limitation with the dynamic MRI series compared to conventional fluoroscopic defecating pouchogram is that the patients are laying down in the MRI machine while emptying the pouch, as this is both an uncomfortable and non-physiological position for emptying.

There were no significant differences in the presence of inflammation signs on MRI between the good and poorly

functioning pouches, and none of the morphological MRI signs of inflammation were correlated to histological findings of acute inflammation. Pouch wall thickness was correlated to the endoscopic PDAI score. Other retrospective studies without a well-functioning control group have found MRI findings of inflammation to be correlated with endoscopic findings but not with histological findings [22, 23], and in cases of suspected severe and complicated pouchitis, the MRI findings correlated to both [24]. In the mentioned studies, intravenous contrast was used and probably improved the MRI evaluation of inflammation. In our study, the patients were not given intravenous contrast. However, it is important to note that it seems the presence of morphological MRI signs of inflammation, such as enlarged lymph nodes, pouch wall oedema and increased pouch wall thickness  $\geq 3$  mm, is to be considered a normal finding also in well-functioning pouches. The MRI evaluation of morphological findings indicating the presence

**Fig. 5** This figure illustrates the correlation between pouch volume at urge and the pelvic pouch volume calculated from bony limitations on MRI (created in SPSS)



**Table 3** MRI findings from the morphological and dynamic MRI series in well-functioning and poorly functioning pouch patients

		Pouch function		<i>p</i> value
		Good ( <i>n</i> = 21)	Poor ( <i>n</i> = 22)	
Largest diameter of pre pouch small intestine loops before emptying (mm)	Median (range)	18.5 (10.5, 28)	21.5 (9.5, 40)	0.053
Presacral width (> 15 mm was considered pathological)	Median (range)	4.5 (2, 15)	6 (2.5, 18)	0.209
Presacral width > 15 mm	<i>N</i>	0	1	0.329
Pouch wall thickness (mm) (measured in a well-distended nondependent portion of the pouch to avoid falsely elevated measurements; $\geq 3$ mm was considered pathological)	Median (range)	4 (2, 6)	3.5 (1.5, 5)	0.236
Enlarged lymph nodes	<i>N</i>	4	6	0.807
	1–5 mm	3	3	
	$\geq 5$ mm	14	13	
Pouch wall oedema	<i>N</i>	11	8	0.142
Peripouch free fluid	<i>N</i>	4	5	0.770
Peripouch oedema	<i>N</i>	1	0	0.306
Peripouch fatty proliferation	<i>N</i>	0	2	0.162
Presacral sinus/abscess	<i>N</i>	0	1	0.329
Peripouch/perianal fistulas	<i>N</i>	2	0	0.069
Inlet stricture of the distal small bowel	<i>N</i>	0	1	0.162
Outlet stricture of the ileoanal anastomosis	<i>N</i>	0	0	0.143
Pelvic floor descent* before dynamic imaging (mm)	Median (range)	2.5 (0, 21)	4.25 (0, 21)	0.607
	$N \geq 30$ mm	0	0	1.000
Pelvic floor descent* during valsalva (mm)	Median (range)	18 (1.5, 48)	20.5 (0, 44)	0.504
	$N \geq 30$ mm	6	5	0.664
Pelvic floor descent* during emptying (mm)	Median (range)	38 (11.5, 60)	37.5 (0, 64)	0.679
	$N \geq 30$ mm	12	15	0.459
Atrophic sphincter	<i>N</i>	1	0	0.306
Atrophic levator	<i>N</i>	2	1	0.962
Dysfunction of external sphincter (failure to open during emptying)	<i>N</i>	3	5	0.668
Obstructive intussusception (pouch infolding in anal canal during emptying)	<i>N</i>	1	1	1.000

\*Descent was defined as a downward movement of the anorectal junction on straining of more than 2 cm below the pubococcygeal line.  $\geq 30$  mm was considered pathological

of pouch inflammation was correlated to neither PDAI scores nor histological findings of acute inflammation; hence, in this study, morphological MRI findings alone were not suited to detect pouch inflammation. The use of intravenous contrast is

recommended for this purpose. Endoscopy remains the golden standard for diagnosing inflammation of the pouch.

We did not find the total pelvic volume to be correlated to pouch volume at urge, although some transverse bony

**Table 4** Comparison between MRI findings and different aspects of pouch inflammation [29]

	Histological signs of acute inflammation (binary regression)	PDAI score (linear regression)	History of $\geq 1$ episodes of pouchitis (binary regression)
Pouch wall thickness	0.270	0.017	0.253
Pouch wall oedema	0.292	0.549	0.196
Enlarged lymph nodes	0.341	0.116	0.552
Peripouch free fluid	0.276	0.278	0.913
Radiologist conclude on pouch inflammation*	0.418	0.458	0.287

\*If increased pouch wall thickness ( $\geq 3$  mm) and pouch wall oedema was present, the radiologists concluded on pouch inflammation based on morphological findings on MRI (statistical analyses were not calculated on the following inflammation parameters, as they were displayed in few patients; peripouch oedema (*n* = 1), peripouch fatty proliferation (*n* = 2), presacral sinus/abscesses (*n* = 1) or peripouch or perianal sinuses (*n* = 3))

limitations were correlated to urge volume. The significance of this finding is unclear. A limitation with the comparison is the difficulty to calculate the total pelvic volume due to the geometric form of the bony pelvis. Another limitation with the volume comparison is that soft-tissue structures and organs in the pelvis taking up space in addition to the pouch have not been considered in this correlation. The PrSuY angle was not correlated to function or septic pelvic complications; hence, it does not seem a smaller pelvis and more difficult access has had a negative impact on the quality of surgery and functional outcome in this study.

Previous studies of the value of conventional and dynamic MRI in pouch patients have been retrospective with the lack of a control group. Thus, it is not possible to know whether or not apparently abnormal patterns of the morphological findings, or on the defecating pouchograms, are variations within the normal range. The mentioned studies are also susceptible for selection bias as the diagnostic procedures were not standardised, different methods of data recording by different practitioners were used and complex symptomatology potentially complicated the evaluation. Our study is the first to our knowledge comparing findings between well-functioning and poorly functioning pouches, and hence establishing a reference for normal findings in pouch patients. Although two different pouch designs were used, they were equally represented in the well-functioning and poorly functioning groups. For interpretation and usefulness of MRI to evaluate functional outcome, the different designs should not be of significance.

In conclusion, it seems MRI does not increase the understanding of factors contributing to functional outcome after IPAA surgery. Unless there is a clinical suspicion of perianal or peripouch disease, pelvic sepsis or strictures, morphological MRI with defecography does not add value as a diagnostic tool for pelvic pouch patients. Endoscopy remains the golden standard for diagnosing pouch inflammation.

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**Author contributions** All authors participated in conceiving the study and its design. MLS coordinated the study, performed the data analyses and statistical analyses and drafted the manuscript. AN and NB analysed and described the MRI images. All have read and approved the final manuscript.

### Compliance with ethical standards

The study was approved by the regional and local ethics committee (REK no 2014/2206).

**Conflict of interest** The authors declare that they have no conflicts of interest.

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