Anal fistulae - Three-dimensional endoanal ultrasound and outcomes of collagen plug treatment

Fält, Ursula

2023

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

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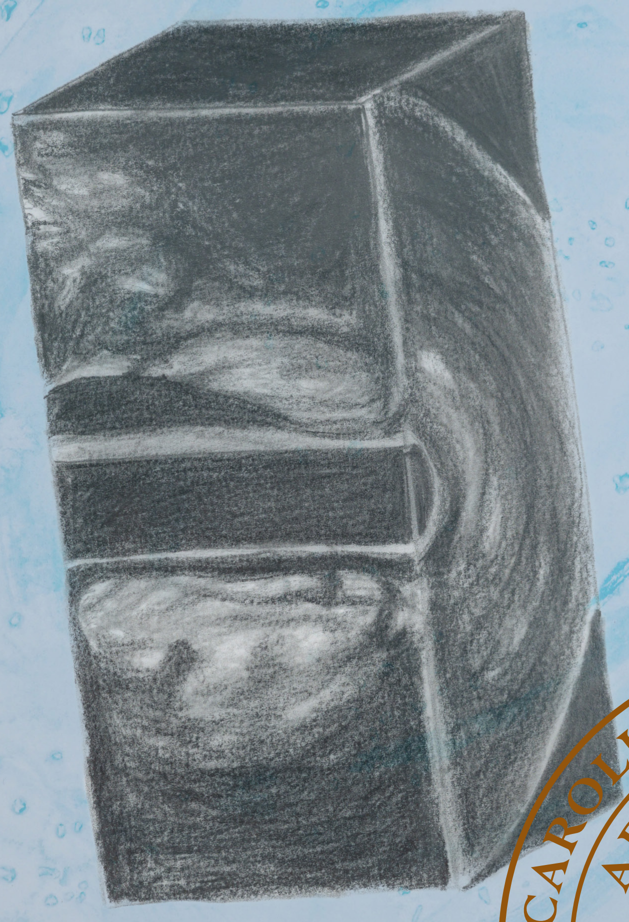
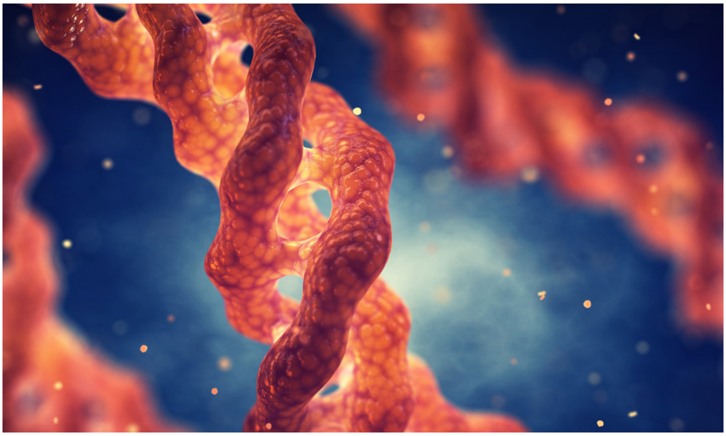
URSULA AHO FÄLT
CLINICAL SCIENCES, MALMÖ | FACULTY OF MEDICINE | LUND UNIVERSITY





Anal fistulae can cause great discomfort for patients and create major challenges for surgeons. This thesis, based on four original papers, examines the long-term results of collagen plug treatment and explores the use of three-dimensional endoanal ultrasound for the assessment and follow-up of anal fistulae, including measurements of the anal canal length with a high-resolution linear array transducer.

URSULA AHO FÄLT is a consultant surgeon at Pelvic Floor Centre, Department of Surgery, Skåne University Hospital, Malmö.

Printed by Media-Tryck, Lund 2023 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences, Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2023:138
ISBN 978-91-8021-480-3
ISSN 1652-8220

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Ursula Aho Fält

DOCTORAL DISSERTATION
Doctoral dissertation for the degree of Doctor of Philosophy (PhD)
at the Faculty of Medicine at Lund University to be publicly defended on
December 1st, 2023 at 9.00 in Lilla Aulan, Medicinskt Forskningscenter,
Jan Waldenströms gata 1, Skåne University Hospital, Malmö.

Faculty opponent
Pär Myrelid, Associate Professor
Division of Surgery, Department of Clinical and Experimental Medicine,
Faculty of Health Sciences, University Hospital Linköping

Organization: LUND UNIVERSITY

Document name: Doctoral Dissertation

Date of issue: December 1st, 2023

Author: Ursula Aho Fält

Sponsoring organization: -

Title and subtitle: Anal fistulae – Three-dimensional endoanal ultrasound and outcomes of collagen plug treatment

Abstract: Background: The treatment of complex anal fistulae remains a therapeutic challenge. Early anal fistula plug (AFP) studies showed high success rates, while later reports demonstrated success rate of 13-100% at usual follow-up of 6-24 months. Very little is known about morphological findings related to success and failure of the AFP, especially if three-dimensional (3D) endoanal ultrasound (EAUS) is used for radiological follow-up. No documentation is available for the use of a high-resolution transducer for assessment of anal fistulae and anal canal length.

Aims: I: To evaluate the long-term success rate of the AFP in the treatment of complex anal fistulae. II: To explore the utilisation of 3D EAUS for the follow-up of the AFP. III: To evaluate the accuracy of 3D EAUS alone for assessment of newly diagnosed cryptoglandular fistulae, compared to intraoperative findings in combination with intraoperative 3D EAUS (gold standard). IV: To explore the use of 3D EAUS for the measurement of anal canal length in patients with newly diagnosed cryptoglandular fistulae.

Methods: Two prospective cohort study projects, each designed to produce two papers. Papers I & II: A single-centre study of consecutive patients with complex anal fistulae treated in May 2006-October 2009. Patients with ano/rectovaginal fistulas were excluded. Papers III & IV: A single-centre study of consecutive patients with newly diagnosed cryptoglandular anal fistulae and no history of fistula surgery treated between June 2018-March 2020. Patients with inflammatory bowel disease, ano/rectovaginal fistulae, more than one fistula, or a fistula with more than one internal orifice (IO) were excluded.

Main outcomes: Paper I: AFP success rate >7 years postoperatively, Paper II: Morphological 3D EAUS findings and relation to AFP failure, Paper III: Agreement between 3D EAUS alone and the gold standard, Paper IV: the mean anal canal length in female and male with transducers 2052 and 8838.

Results and conclusions: Paper I: The overall success rate 93-138 months after the AFP was 38%. It is acceptable considering the low morbidity of the procedure in a complex disease with high recurrence rates over time.

Paper II: 3D EAUS may be utilised for AFP follow-up. Postoperative 3D EAUS at three months or later, especially in combination with clinical symptoms, can be used to predict long-term AFP failure.

Paper III: 3D EAUS alone has high precision in identifying IO of anal fistulae. The method may be utilised even by relatively inexperienced surgeons to identify fistulae suitable for fistulotomy.

Paper IV: Measurements of anal canal length in female and male patients assessed by two different transducers for 3D EAUS are reported with generally acceptable reproducibility and repeatability.

Key words: anal fistula, Crohn’s disease, cryptoglandular fistula, anal fistula plug, complex anal fistula, three-dimensional endoanal ultrasound, anal canal length, anal canal anatomy

Language: English

ISSN: 1652-8220

ISBN:978-91-8021-480-3

Recipient’s notes

Number of pages:149

Price

Security classification

Signature

Date 2023-10-19

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ISBN 978-91-8021-480-3

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University

Lund 2023

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MADE IN SWEDEN

To my parents, Aili and Matti

"A journey is a person in itself; no two are alike. And all plans, safeguards, policing, and coercion are fruitless. We find after years of struggle that we do not take a trip; a trip takes us." John Steinbeck

Table of Contents

Abstract

Background

The treatment of complex anal fistulae remains a therapeutic challenge. Early anal fistula plug (AFP) studies showed high success rates, while later reports demonstrated success rate of 13-100% at usual follow-up of 6-24 months. Very little is known about morphological findings related to success and failure of the AFP, especially if three-dimensional (3D) endoanal ultrasound (EAUS) is used for radiological follow-up. No documentation is available for the use of a high-resolution transducer for assessment of anal fistulae and anal canal length.

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List of Papers

Paper I

Aho Fält U, Zawadzki A, Starck M, Bohe M and Johnson LB. Long-term outcome of the Surgisis® (Biodesign®) anal fistula plug for complex cryptoglandular and Crohn's fistulas. *Colorectal Dis.* 2021;23:178-185. doi: 10.1111/codi.15429

Paper II

Aho Fält U, Zawadzki A, Starck M, Bohe M, Regnér S and Johnson LB. Postoperative three-dimensional endoanal ultrasound findings and relation to anal fistula plug failure. *Scand J Gastroenterology.* 2023;58:1200-1206. doi: 10.1080/00365521.2023.2212310

Paper III

Aho Fält U, Lusilla Lopez A, Zawadzki A, Starck M, Johnson LB and Regnér S. Assessment of newly diagnosed cryptoglandular anal fistulas by three-dimensional endoanal ultrasound: a prospective cohort study. *Submitted*.

Paper IV

Aho Fält U, Lusilla Lopez A, Zawadzki A, Starck M, Johnson LB and Regnér S. Anal canal length on three-dimensional endoanal ultrasound – descriptives, repeatability, and reproducibility in patients with cryptoglandular fistulae. *Manuscript*.

“For how can one know color in perpetual green,

and what good is warmth without cold to give it sweetness?”

John Steinbeck

Abbreviations

AFP	Anal fistula plug
ANOVA	Analysis of variance
BMI	Body mass index
CI	Confidence interval
EAUS	Endoanal ultrasound
EAS	External anal sphincter
ECM	Extracellular matrix
EMT	Epithelial to mesenchymal transition
EO	External fistula orifice
EUA	Examination under anaesthesia
IAS	Internal anal sphincter
ICC	Intraclass correlation coefficient
IO	Internal fistula orifice
LIFT	Ligation of intersphincteric fistula tract
MMP	Matrix metalloproteinase
MRI	Magnetic Resonance Imaging
NPV	Negative predictive value
OR	Odds ratio
PPV	Positive predictive value
PR	Puborectal muscle
SD	Standard deviation
SE	Standard error
SIS	Small intestinal submucosa
TNF	Tumour necrosis factor
VAAFT	Video-assisted anal fistula treatment
3D	Three-dimensional

"I like starting each day with a sense of possibility.

And I'm optimistic because every day I get a little more desperate.

And desperate situations yield the quickest results."

Michael Scott

Introduction

Anal fistulae

Anal fistulae remain one of the most intimidating topics in proctology. They may cause major discomfort for the patients and create daunting challenges for the treating surgeons.

Epidemiology

The prevalence of anal fistula peaks in patients aged between 30 to 50 years, and it is relatively rare to be affected before the age of 20 or after the age of 60 [1]. A Finnish study reported in 1984 the incidence to be 12.3 per 100 000 per year in male, and 5.6 per 100 000 per year in female, with overall incidence of 8.6 per 100 000 per year[2].A Spanish study published in 2007 determined the incidence of anal fistulae based on records from public hospitals in England, all private trust hospitals in Germany, public and private hospitals in Italy, and public hospitals funded by Spanish National Health Institute [3]. The incidence of anal fistulae varied among the different populations, ranging from 10.4 per 100 000 per year in Spain to 23.2 per 100 000 per year in Italy. The authors concluded that the findings, not gender-specified, indicated that the incidence of anal fistulae in the European Union is significantly higher than previously reported.

Both lifestyle factors and medical conditions may increase the risk of anal fistulae. In a case-control study, body mass index (BMI) > 25 kg/m^2, high dietary salt intake, diabetes, hyperlipidemia, alcohol intake, dermatosis, anorectal surgery, smoking, sedentary lifestyle, excessive intake of spicy or greasy food, very infrequent participation in sports and prolonged sitting for bowel opening were identified as independent risk factors for anal fistulae [4].

History

Already the Ancient Greeks acknowledged the dilemma of anal fistulae. Hippocrates (ca- 460-370 BC) documented the phenomenon and tried to interpret the mechanism of origin. He attempted to treat anal fistulae conservatively with various laxatives, and was the first to refer to surgical treatment by describing a rectal speculum [5].

John of Arderne lived in the 14th century and described himself as *'chirurgus inter medicos'* (a surgeon among physicians). He is known as The Father of British Proctology [6, 7]. Arderne devoted his attention to the treatment and cure of various fistulae and proctological conditions. He wrote his collection of notes on surgery in Latin and they were later translated into English and published under the title *"Treatises of Fistula in Ano, Haemorrhoids and Clysters"* [8]. Arderne not only provided detailed and innovative illustrations of the lesions treated, but also described the various instruments he had developed for this purpose (Figure 1). His description of surgical techniques is very similar to the methods used today. Sometimes, he treated a fistula by passing a thread through it or performed a clean incision, a lay-open, of the tract if he thought it was appropriate [6, 9].

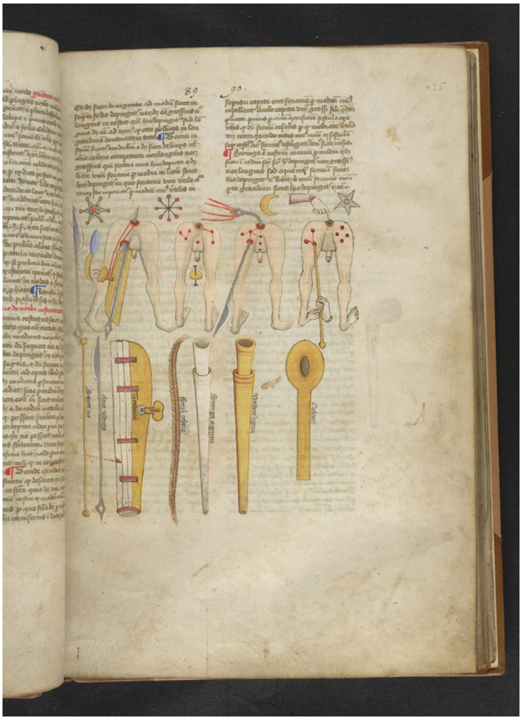

Figure 1 Illustrations of anal fistula and its surgical treatment, in John of Arderne's 'Treatises of Fistula in Ano, Haemorrhoids and Clysters'.

Louis XIV of France (1638-1715), *Le Grande Roi*, is very likely the most famous and well-documented anal fistula patient in medical history. Daily records of the King's health from his early infancy to 1711 were kept by three physicians, and published in 1862 [10]. In January 1686, the journal stated:

> "On 15 January, His Majesty complained of a small lump in his crotch, two fingerbreadths in front of the anus, which was not painful to the touch, with no redness or throbbing." [11]

Figure 2 Louis XIV of France, Hyacinthe Riagaud studio, 1701.

The condition was treated by the King's doctors with different enemas, prayers, astrological considerations, purges, bleeding, and the application of leeches. A poultice made with flour, beans, rye, barley, and flaxseed boiled in a mixture of water and vinegar was applied four times a day. [10] Attempts were made with constant treatments of red-hot iron and drying out the wound by applying compresses soaked in leaf extracts, and roses cooked in Burgundy. Louis XIV was forced to change clothes several times a day due to leakage of pus through the abscess, which after four months of treatment was suspected to develop a fistula. [11]

The situation became worse, and walking was almost impossible. In desperation, the King decided to undergo surgery. The barber-surgeon Charles-François Félix examined the patient, and since having never performed this kind of surgery before, he suggested some study and refinement time to be able to perform optimal procedure. It is said that during the months that followed, 75 operational procedures were performed, and Félix paid much attention to developing devices needed for the surgery; an anal retractor and *'le bistouri royal'*, a long, curved silver probe attached to a scalpel. On November 18, 1686, a lay-open was performed on Louis XIV and his fistula. The procedure, done without any anaesthesia, consisted of two cuts with a lancet over the probe and eight cuts with scissors. The wound was debrided twice, and another long surgery was performed on December 10. After that, the King started to feel better and made his first public appearance one month after the last operation. [10, 11]

Félix was rewarded with a noble title, a palace, and a great bonus to his yearly salary. The successful operation revolutionised the status of surgeons in the French medical society. In 1699, surgery was decreed to be a liberal art, and surgeons were regarded as more than mere day labourers. [10, 11]

Anal canal anatomy

The last part of the large intestine consists of the anal canal, which has its proximal border at the level of the levator ani muscle and its distal border at the opening of the anal verge. The average surgical anal canal (from the anorectal ring to the anal verge) length has been measured to be 4.4 cm (range: 3.2-5.3 cm) in male and 4.9 cm (range: 3.0-5.0 cm) in female [12]. In the same study, the average length of the anatomical anal canal (from the dentate line to the anal verge) was reported to be 2.2 cm (range: 1.4-3.8 cm) in male and 2.0 cm (range: 1.0-3.2 cm) in female. Figure 3 summarises the landmarks of the anal canal [13].

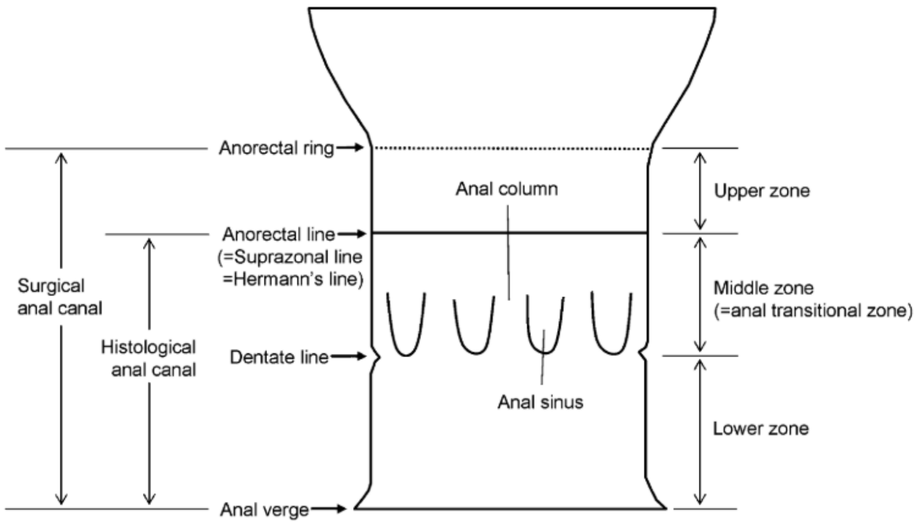

Figure 3 Schematic illustration of the landmarks of the anal canal, coronal plane.

Folds in the mucosa parallel to the anal canal located at and above the dentate line are called the columns of Morgagni. Anal crypts or sinuses draining the underlying anal glands are located between the columns. [12, 14]

The proximal anal canal closest to rectum (upper zone) is lined by simple columnar epithelium with many crypts (Figure 4). The middle zone, sometimes called the anal transitional zone, consists mainly of stratified squamous epithelium combined with stratified columnar epithelium. The anal canal stratified columnar epithelium is located in the anal sinuses, crypts, and the ducts of the anal glands whereas the actual glands consist of simple columnar epithelium. The stratified columnar cells also have a slight mucus-secreting ability. The lower zone is lined by stratified squamous epithelium. The anal verge is characterized by the presence of keratinised stratified squamous epithelium with hairs and melanin granules.[13] The portion between the dentate line and the anal verge is also called the anoderm.

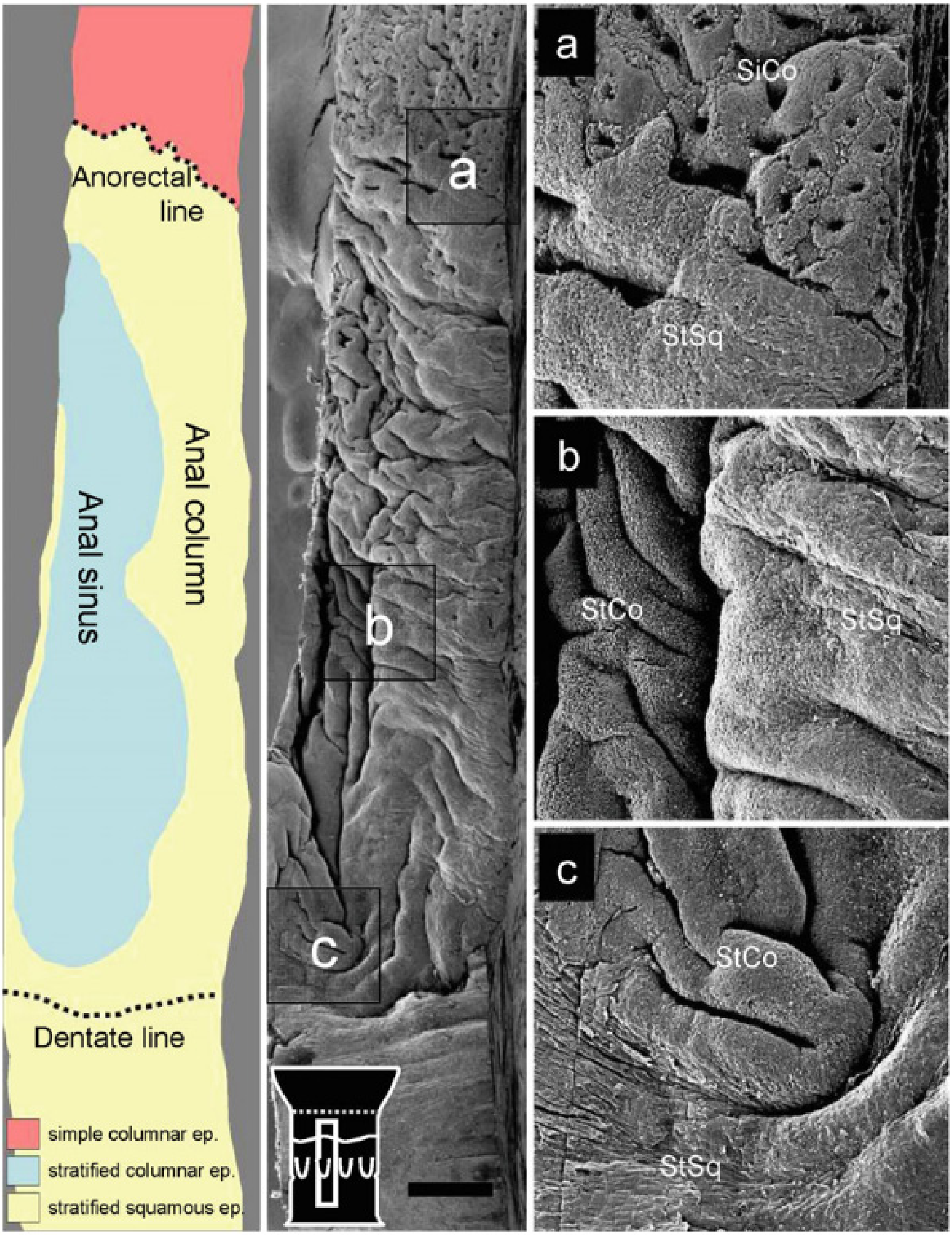

Figure 4 Illustration and scanning electron microscope findings of a tissue block containing the anal sinus and anal column.

Three types of epithelium observed. a: Anorectal line is the boundary between simple columnar epithelium (SiCo) and stratified squamous epithelium (StSq). b and c: Anal sinus consists of stratified columnar epithelium (StCo). *Bar* 1500 μm. Copyright Springer Nature. Reproduced with permission.
doi.org/10.1007/s00795-011-0541-8

The internal anal sphincter (IAS) and the external anal sphincter (EAS) surround the mucosa of the anal canal. Between the two muscle layers is the intersphincteric plane where fibrous extensions of the outer longitudinal rectal muscle layer extend into the anal canal. The EAS consists of three portions (Figure 5); the subcutaneous part, the superficial part, and the deep part, which together with the puborectal (PR) muscle form the posterior border of the anorectal ring (Figure 6). The anterior EAS complex is significantly shorter in female than male, with a mean length of 14.0 ± 3.0 mm and 27.7 ± 5.0 mm, respectively [15]. Both the EAS and the PR are composed of striated muscle fibres and are thus under voluntary control. They are innervated by the somatic pudendal nerves.

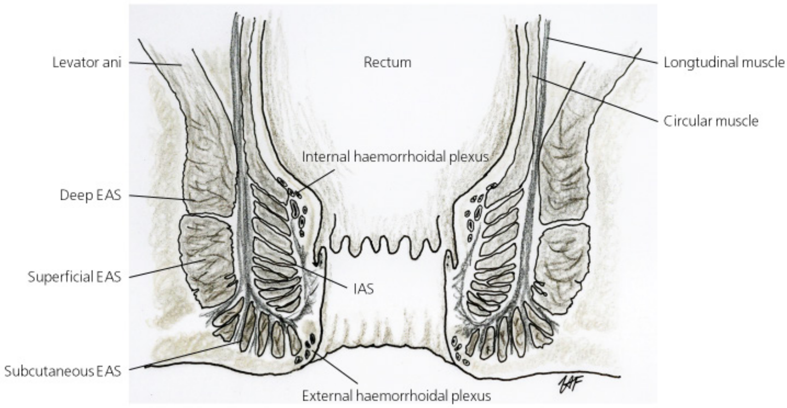

Figure 5 Schematic illustration of the muscles of the anal canal, coronal plane.
EAS: External anal sphincter, IAS: Internal anal sphincter.

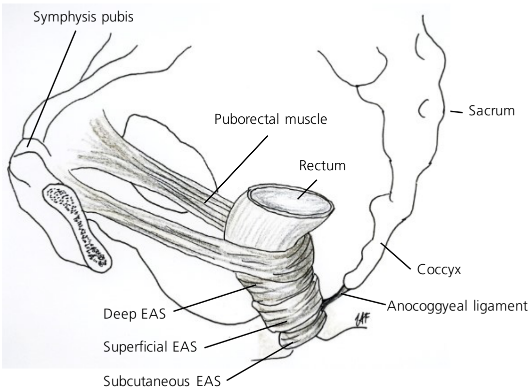

Figure 6 Schematic antero-posterior illustration of the three portions of the external sphincter and the puborectal muscle.
EAS: External anal sphincter.

The IAS is a thickened circular muscle layer extending from the inner circular muscle layer of the rectum. It surrounds the upper ¾ of the anal canal, with the distal portion being wrapped by the subcutaneous portion of the EAS. This intersphincteric junction can be identified as the anocutaneous line, also known as the white line of Hilton, where the skin transforms to the internal mucous membrane [16]. The IAS, which is composed of smooth muscle fibres, is under involuntary control and is autonomously innervated by hypogastric and sacral nerves.

A cadaver study describes that the IAS consists of circular lamellae with upper and lower flattened surfaces, similar to Venetian blinds [17]. The peripheral parts of each muscle ring are connected to the longitudinal fibromuscular band, while the central parts are free in the lumen of the anal canal (Figure 7). The muscle rings have muscle fibres and fascia at three equally spaced points around the anal canal, forming three columns in the anal canal. The authors discuss that the lamellar columnar structure plays an important role in relaxing the sphincter muscle during defecation. The anal canal is pulled proximally by the longitudinal fibres and begins to relax. The stool is peristaltically moved in the distal direction. The IAS rings are stimulated via afferent visceral stimuli through the submucosa as stool passes through the lumen, causing muscle relaxation. The stool mechanically pushes the free edges of the Venetian blinds into a vertical position and pulls the three columns toward the longitudinal fibromuscular band, opening the lumen (Figure 8). During contraction of the IAS, the annular lamellae are in a horizontal position, helping to maintain continence.

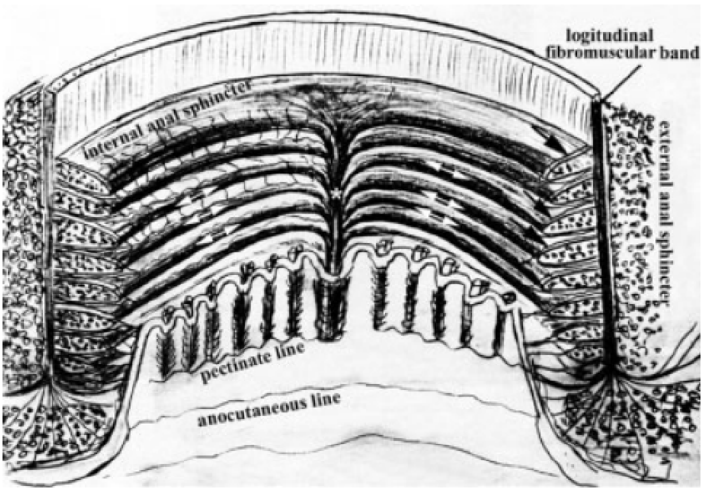

Figure 7 Schematic view of the IAS and surrounding structures.
White arrows: Rings of IAS, Black arrows: Fascia covering each leaf of IAS, Asterisk: Column. Copyright John Wiley & Sons. Reproduced with permission. doi.org/10.1002/ca.10160

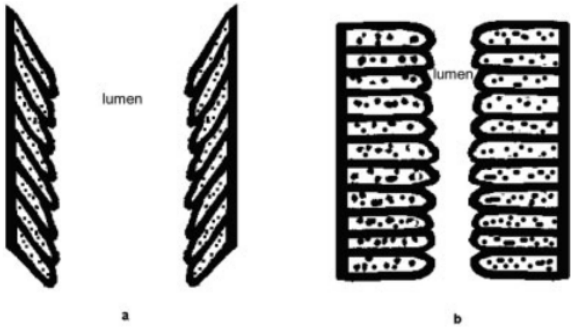

Figure 8 Longitudinal section of anal canal and the IAS.
a: Lamellae vertical during sphincter relaxation, b: Lamellae horizontal during sphincter contraction.
 doi.org/10.1002/ca.10160

Anal fistulae – pathogenesis

Arderne's lay-open procedure was based on the aetiological concept of an infection penetrating the wall of the anal canal through a fissure or other wound, and that the established infected tract is maintained by intestinal contents entering the internal fistula orifice (IO) [18]. In the late 19th and early 20th centuries, it was debated whether small glands ramifying in the IAS and submucosa could cause anal fistulae. In particular, Parks and Eisenhammer developed the basis of the cryptoglandular theory of anal sepsis and fistula formation [19]. According to this theory, anal sepsis is caused by infection of the anal gland which in turn is caused by stasis due to faecal debris. While Parks and Eisenhammer had the concept that an anal abscess originates from the intersphincteric groove and then spreads to other spaces (Figure 9), there are reports that only a minority of patients operated on for acute abscesses actually have an intersphincteric component and many of the anal glands do not reach the intersphincteric plane or extend outside the EAS [20-22]. These same patterns are thought to allow the development of an anal fistula after the acute infection has drained into the perianal skin, creating an external fistula orifice (EO).

In 2021, Gottesman published a commentary [23] questioning the classical cryptoglandular theory as the one universal mechanistic approach to perirectal infections. The background is studies reporting that some anal glands are not even near or connected to crypts, and that the intersphincteric space is partially obliterated or compartmentalised by fibrous septa originating from the longitudinal muscle fibres between IAS and EAS. He finds it difficult to understand why transsphincteric fistulae are usually shown to penetrate intact muscle rather than exit through the intersphincteric space. Because the anatomy of fibrous septa varies between individuals and the fibres penetrate the muscles at variable and unpredictable levels, the unique anatomy of the patient determines the course of the fistula. Gottesman believes it is more likely that the weakest points of the muscles are located where the septa breach the integrity of the EAS. Rather than the microbial component of perianal infection being responsible for muscle invasion or persistence of the fistula after drainage, elevated levels of cytokines produced by infection lead to disruption of tissue planes through neutrophil activation and lysozyme release. Disruption of the sphincter and bacterial seeding into the ischiorectal and perirectal spaces cause the abscess. Ongoing cytokine expression due to remaining bacterial products after drainage of the abscess promotes the development and persistence of the fistula.

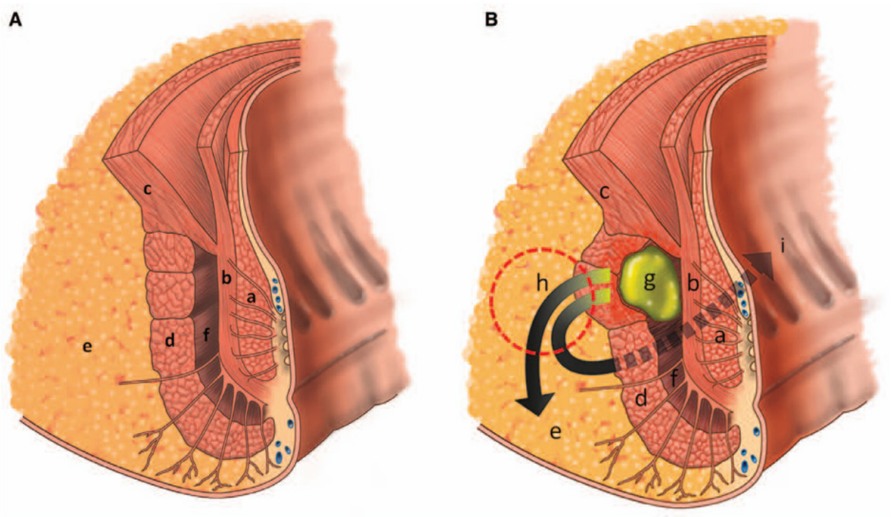

Figure 9 Illustration of the cryptoglandular theory for anorectal infection in the posterior anal canal in sagittal section.
A deep posterior intersphincteric abscess (g) forms in the intersphincteric space (f) with different expansion routes. Copyright Wolters Kluwer Health, Inc. Reproduced with permission.
doi.org/10.1097/DCR.0000000000000629

Although the exact mechanisms still disputed, it seems to be generally accepted that most fistulae originate near the anal crypts. They are therefore referred to as *'cryptoglandular fistulae'*. Approximately 90% of perirectal abscesses are thought to be of cryptoglandular origin; the remainder are caused by Crohn's disease, trauma (including iatrogenic fistulae), human immunodeficiency virus, sexually transmitted diseases, or local irradiation [24]. The cumulative probability of perianal fistula at 20 years of Crohn's disease has been estimated to be 26-28% [25, 26]. There are reports that 44-45% of patients develop a fistula at or before the time of diagnosis of Crohn's disease [26, 27].

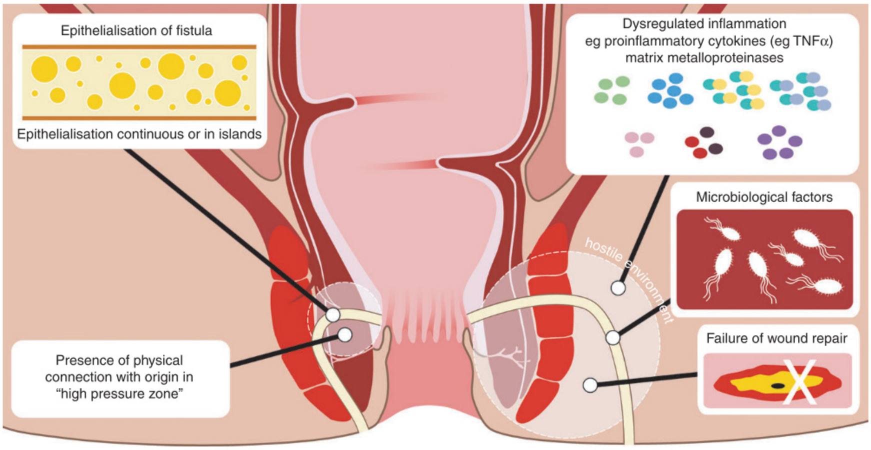

Figure 10 Key factors promoting or facilitating perianal fistulation in Crohn's disease.

Aetiological studies on fistulising perianal Crohn's disease have their limitations due to heterogeneous patients, mix of medical therapies, and differences in inclusion criteria and experimental methods [28]. Current evidence supports a multifactorial hypothesis that the process of persistent perianal Crohn's fistula formation is influenced by multiple pathophysiologic elements (Figure 10). Several genetic predispositions have been identified, some of them which lead to early onset of the inflammatory disease with severe perianal engagement [28]. An effective host response to bacterial inflammation, in which cellular bacterial remnants continue to elicit an inflammatory response in the fistula lumen even after bacterial destruction, has been demonstrated in Crohn's fistulae [28-30]. Draining loose setons, a commonly performed intervention in patients with Crohn's fistulae prior to treatment with anti-tumour necrosis factor (TNF) agents to reduce the risk of abscess formation, likely stimulates epithelisation of the fistula tract, which in turn may impair fistula healing [28]. A dysregulated and persistent inflammatory response with proinflammatory cytokines, epithelial to mesenchymal transition (EMT), elevated levels of matrix metalloproteinases (MMPs), and abnormal distribution of luminal myofibroblasts likely prevent fistula healing. These inflammatory phenomena, as well as elevated TNFα levels, have been shown to be observed not only in Crohn's fistulae, but the broader immunologic dysfunction creates an environment in which the odds of healing are diminishing. [28, 31-35] In Crohn's disease, the abnormal distribution of myofibroblasts together with EMT leads to an imbalanced tissue repair process and poor wound repair [28]. The microbiota and its remnants not only trigger and maintain inflammation, but are also thought to be involved in EMT. Activated MMPs lead to further tissue damage, inflammation, and fistula formation. [36] All these factors combined with the physical fact that IO of a fistula is usually located in the high-pressure zone of the anal canal mean that multiple pathophysiologic mechanisms must be addressed to successfully treat anal fistulae associated with perianal Crohn's disease.

Anal fistulae – classification and complex fistulae

Parks' classification

Parks published his classification system for anal fistulae in 1976 as a result of analysis of 400 patients treated over a 15-year period, based on the cryptoglandular pathogenesis of fistula disease in combination with the muscular anatomy of the anal canal and pelvic floor [37]. The classification presents not only the four commonly known main types of fistulae, but also 14 different subtypes. The classification describes different fistulae in relation to EAS and is still the most commonly used classification system for anal fistulae. The four main types are:

1) Intersphincteric
2) Transsphincteric
3) Suprasphincteric
4) Extrasphincteric,

as shown in Figure 11.

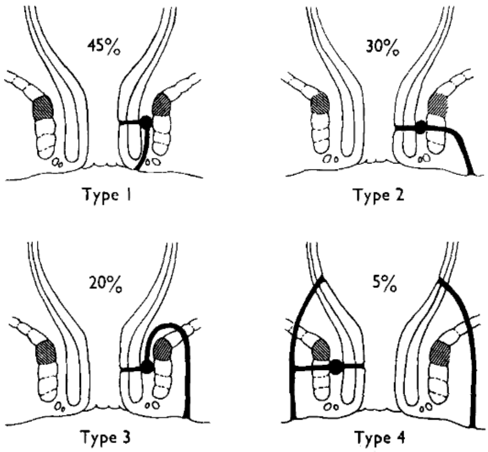

Figure 11 The four main anatomical types of fistulae according to Parks.
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Over the years, several fistula classifications have been published, for example by Eisenhammer, who divided intermuscular fistulae into high and low, but also into posterior and anterior, and even including the three-dimensional component for possible horseshoe extension, as well as high ischiorectal components [38]. There is a modification of Parks' classification based on magnetic resonance imaging (MRI) findings, also called St James Hospital University classification [39], as well as a proposed classification system based on natural fistula patterns (low intersphincteric, low transsphincteric, anterior high transsphincteric, posterior high transsphincteric or high intersphincteric) [21].

Complex fistulae

The New classification was published by Garg in 2017 [40] and assessed for validation three years later [41] (Figure 12). He stated that the purpose of any classification is to give guidance regarding not only the severity of the disease but also the management. Grade I and II fistulae were considered simple and thus safe to treat by fistulotomy without risk of incontinence. Grade III-V fistulae, on the other hand, were complex and should be treated only with sphincter-preserving surgical procedure or fistulectomy with primary sphincter reconstruction (see *Treatment of anal fistulae*). In the assessment study, Garg compared amenability of fistulae for fistulotomy according to classification and found that 42.7% of fistulae classified as *'complex'* according to Parks' classification and equally many complex fistulae according to St James classification were actually amenable to fistulotomy, while 1% of fistulae classified as *'complex'* according to the New classification were suitable for fistulotomy [41].

In his work Garg uses the same terms as the 2005 American Society of Colon and Rectal Surgeons' fistula guidelines, also called Standard Practice Task Force classification, which classified anal fistulae into two categories; *'simple'* or *'complex'* [42]. Simple fistulae, involving less than one third of the sphincter length, were suitable for fistulotomy, whereas treatment of complex fistulae posed a high risk of incontinence.

According to The New classification, all anterior fistulae in female are automatically of grade III, as are fistulae in patients with Crohn's disease, sphincter injury, or a history of radiation therapy. Standard Practice Task Force also defines these fistulae as *'complex'*. The difference between the classifications considering the complex fistulae is that the New classification considers, for example, fistulae with multiple tracts or high fistulae with horseshoe extensions as more complex (Grade IV). It even classifies low fistulae with horseshoe tracts as Grade II, which are still suitable for fistulotomy.

The concept of complex fistulae has been adopted by several clinical studies assessing fistula treatment by anal fistula plug (AFP) [43-45].

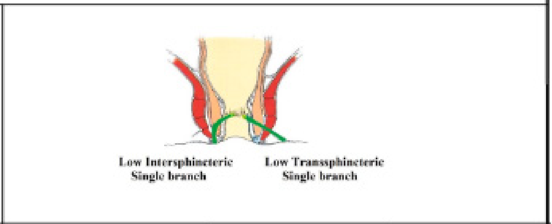
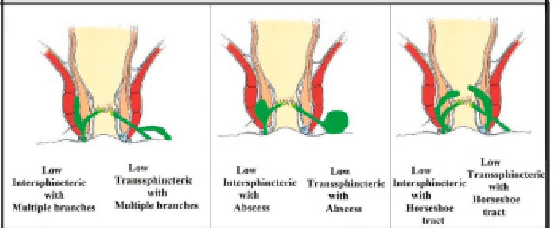
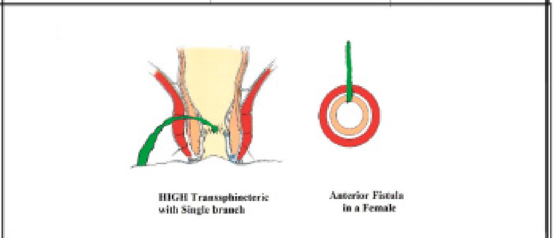
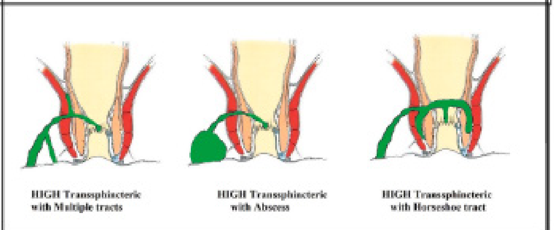
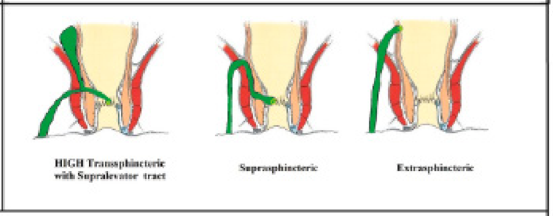
New Classification (2017)		
Grade I	LOW Fistula with Single Branch Intersphincteric or Transsphincteric	
Grade II	LOW Fistula with Multiple tracts, Abscess or Horseshoe Intersphincteric or Transsphincteric	
Grade III	HIGH Transsphincteric Fistula with Single Branch Anterior Fistula in Female or Impaired Continence or Crohn's disease or Previous radiation	
Grade IV	HIGH Transsphincteric with Multiple tracts or Abscess or Horseshoe tract	
Grade V	HIGH Transsphincteric with Supralevator tract or Suprasphincteric or Extrasphincteric	
LOW - Involving < 1/3 of External Sphincter, HIGH - Involving > 1/3 of External Sphincter		

Figure 12 The New classification according to Garg.

doi.org/10.1016/j.amsu.2020.09.022

Assessment of anal fistulae

In the last two decades, radiological diagnostic methods have revolutionised the assessment of anal fistulae. The new millennium brought with it the long-awaited additions to the diagnostic arsenal. Until then, and even today in clinical settings with limited resources, the methods used to assess fistulae were basically the same as those used by Hippocrates, Arderne, Parks and Eisenhammer. Examination under anaesthesia (EUA) allows palpation of the fistula and surrounding tissues, use of probes and injection of hydrogen peroxide, sodium chloride or methylene blue solution into the fistula tract to assess fistula anatomy and plan surgical treatment. This highly accessible method is associated with not only the risk of over- or underestimating the portion of the sphincter apparatus engaged by the fistula, but also with the risk of missing complicating factors, such as secondary extensions, multiple tracts, or cavities, or creating iatrogenic fistulae through careless use of probes.

The Clinical Practice Guidelines Committee of the American Society of Colon and Rectal Surgeons published in 2016 state that simple fistulae, in general, do not require diagnostic imaging to guide treatment [46]. The same guidelines conclude that there is moderate-quality evidence for the strong recommendation for the use of radiological methods (ultrasound, MRI, or fistulography) in patients with complex anal fistula or perianal Crohn's disease.

Magnetic resonance imaging (MRI)

MRI is a method commonly used for preoperative assessment of anal fistulae. The fistulae are generally identified as linear hyperintense structures on fat-saturated T2-weighted images (Figure 13). Pelvic fat surrounding the anal canal appears hyperintense as well, meaning that fat-suppression is required for optimal fistula diagnostics. Fibrosis associated with the fistula is generally hypointense in both T1 and T2-weighted images and have no or minimal enhancement on postcontrast images. Intense postcontrast enhancement of the fistula wall and surrounding soft tissues is often seen. [47, 48]

Studies on the use of MRI in the diagnosis of anal fistulae emerged in the 1990s and showed promising results [49-51]. A study of 56 patients published in 2001 compared preoperative high-spatial-resolution MRI with intraoperative findings during fistula surgery [52]. Primary fistula tract was identified with 100% sensitivity and 85% specificity, whereas sensitivity and specificity for identifying IO were 96% and 90%, respectively. Cohen's kappa (κ) values for interobserver agreement between three raters were 0.75-0.85 (95% CI: 0.67-0.91) for fistula detection, and 0.74-0.76 (95% CI: 0.66-0.84) for IO identification. Analysis of intraobserver agreement for one rater had κ = 0.87 (95% CI: 0.69-1.00) for fistula detection and κ = 0.82 (95% CI: 0.64-1.00) for IO identification. Interobserver agreement for fistula classification between three raters varied between κ = 0.60-0.71 (95% CI: 0.38-0.85), and intraobserver agreement for the same parameter was κ = 0.75 (0.59-0.91).

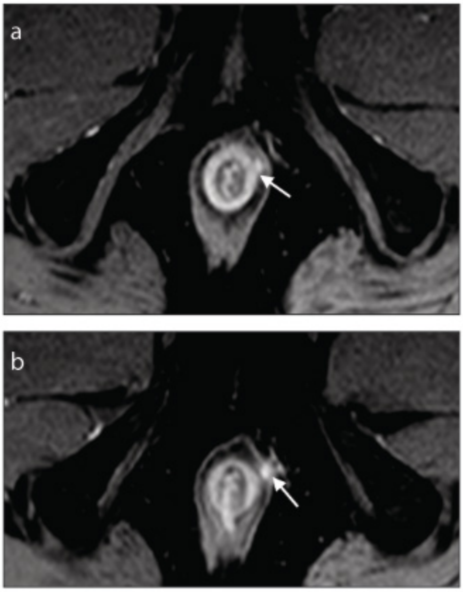


Figure 13 Axial contrast-enhanced, fat-suppressed T1-weighted images of an anal fistula at the 2 o'clock position.
White arrow: Fistula. Copyright Balci et al, published by Turkish Society of Radiology. Reproduced under Creative Commons CC-BY license. doi.org/10.5152/dir.2018.17499

In 2002, it was reported that in 71 patients with recurrent fistula, surgery guided by MRI was estimated to reduce further recurrence by 75% [53]. The authors concluded that MRI is beneficial in fistula patients with recurring disease or complex fistulae, but also noted that MRI missed some superficial extensions and had difficulty locating the precise level of the IO.

Dynamic contrast-enhanced MRI has been shown to be useful in determining disease activity in perianal Crohn's disease [54]. Response to medical therapy can be monitored using an MRI-based score for severity of perianal Crohn's fistulas. The score takes into account both the local extent of fistulae (number of tracts, location in relation to IAS, EAS, and levator ani muscles) and fistula characteristics (hyperintensity on T2-weighted images, presence of concomitant abscesses/cavities and presence of rectal wall involvement) [48, 55]. A recent review concluded that MRI is the gold standard for evaluation and quantification of perianal Crohn's disease [56].

Three-dimensional (3D) endoanal ultrasound (EAUS)

On ultrasound, an anal fistula is usually seen as a hypoechoic tract that can be followed from the mucosa through and along the sphincter apparatus. Debris or gas may be seen as hyperechoic spots or streaks in the fistula (Figure 14).

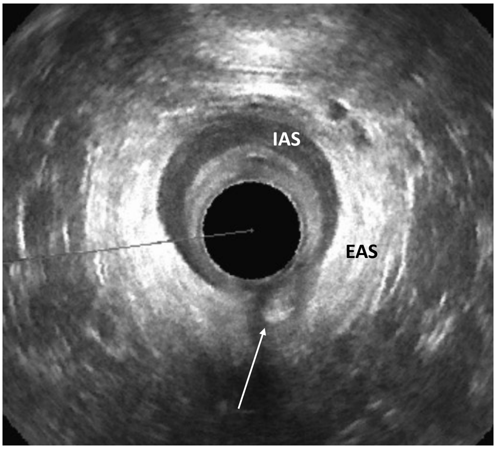


Figure 14 Axial 3D EAUS image of a posterior fistula in female.
Arrow: Fistula, IAS: Internal anal sphincter, EAS: External anal sphincter

In 1989, Law et al published a study of 22 patients with anal fistulae and perianal sepsis in which they compared endoanal ultrasound (EAUS) with intraoperative findings [57]. The authors concluded that the examination was well-tolerated, rapid, and generally accurate. The first study comparing EAUS and digital examination for the evaluation of anal fistulae was published in 1991 [58]. Subsequent reports confirmed that the use of EAUS increased the accuracy of defining fistula anatomy in relation to the anal sphincter [58-61]. It was concluded that EAUS may be utilised for preoperative fistula assessment [62].

Because of the limited tissue differentiation on EAUS, the use of hydrogen peroxide (H_2O_2) injected through the EO for fistula enhancement in two-dimensional (2D) EAUS has been studied with a reported accuracy of 71-95% for primary tracts and 63-96% for secondary tracts (standard EAUS: 50-92% for primary tracts, and 60-68% for secondary tracts). Even the identification of IO is improved by the use of H_2O_2. [63]. Disadvantages of H_2O_2 injection are the strong reflection at the gas/tissue interface which may hide peripheral details and that tissue reaction due to peroxide irrigation may lead to oedema that obliterates the fistula tract. This, in turn, may make it impossible to establish a safe connection between the IO and the EO often needed to treat the fistula. A study published in 2002 compared EAUS with intraoperative findings in 86 patients, 66 of whom underwent H_2O_2 enhancement, and concluded that 87% of fistula types and 81% of IO locations were correctly diagnosed [64]. In another study of 401 patients with anal fistulae, published in 2008, H_2O_2.enhancement was used “if needed and feasible” in 20 patients (4.9%). EAUS was significantly more accurate than physical examination without anaesthesia in identifying the primary fistula tract, horseshoe extension and IO [65].

The introduction of three-dimensional (3D) imaging systems has generally improved the accuracy of EAUS. A study of 61 patients with “not simple low” fistulae showed that 3D EAUS had significantly higher accuracy in detecting the primary fistula tract, identifying secondary extensions, and localising IO compared with physical examination [66]. The same study showed that injection of H_2O_2 in the fistula tract for enhancement did not cause a significant difference in 3D EAUS accuracy. Studies comparing 3D reconstructions with 2D EAUS showed improved diagnostic accuracy of IO localisation from 66.7-87.9% to 89.5-96.4% using 3D EAUS [63].

A study published in 2010 evaluated the role of 3D EAUS in the choice of surgical technique in 33 patients with anterior transsphincteric fistulae and found that ultrasound assessment was useful in quantifying the length of muscle to be transected, and thus helped to reduce postoperative anal incontinence [67].

A Spanish study published in 2012 examined 36 patients with simple perianal fistulae before and 8 weeks after fistulotomy [68]. There was a strong correlation between the preoperative 3D EAUS measurement of fistula height and the intraoperative and postoperative 3D EAUS measurements of the longitudinal extent of the IAS and EAS division.

Preoperative use of 3D EAUS has been shown to have a favourable impact on surgical outcome, especially in patients with complex anal fistulae. A study published in 2015 analysed the long-term results of fistula surgery in 196 patients assigned to either the 3D EAUS group or the EUA at the discretion of patient’s preference [69]. One year after surgery, overall recurrence rate was 8.8% in the 3D EAUS group and 13.8% in the EUA group. The number of patients who developed postoperative anal incontinence was lower in the 3D EAUS group (6.7%) than in the EUA group (33.3%).

Studies conclude that 3D EAUS is a safe and reliable technique for assessment of perianal sepsis [70] and accurate and reproducible in assessing the type and height of anal fistulae [71]. Brillantino et al demonstrated very good agreement ($\kappa = 0.93$) for classification of primary tracts and identification of IO ($\kappa = 0.97$) between preoperative 3D EAUS and EUA [70]. Kolodziejczak et al showed an overall accuracy of 91% for fistula type and 92% for fistula height for 3D EAUS compared with intraoperative findings [71]. Fistula types were identified with good or very good agreement, and low and high fistulae were identified with very good agreement compared with intraoperative findings. The results even show very good interobserver agreement for fistula type and height between two raters.

A meta-analysis published in 2021 analysing 12 studies and 1057 fistulae evaluated the use of 3D EAUS to assess anal fistulae and concluded that ultrasound had high accuracy in most types of anal fistulae and can be used in the first line of diagnosis [72].

In a study published in 2012, Zawadzki et al reported a unique 3D EAUS feature of perianal Crohn's fistula; the Crohn's ultrasound fistula sign (CUFS) [73]. This is defined as a hypoechogenic fistula track surrounded by a well-defined hyperechogenic area with an extension in the perianal tissue with a thin regular hypoechogenic border. This phenomenon has a sensitivity of 69% and a specificity of 98% for Crohn's disease, with a positive predictive value (PPV) of 87% and a negative predictive value (NPV) of 93%, with good interobserver agreement. Another study reported lower sensitivity of 44% but high specificity of 98% for CUFS in perianal Crohn's disease [74]. A study of 2D EAUS reported that ultrasound influenced patient management in 86% of patients with perianal Crohn's disease [75]. 75% of the patients in the study were diagnosed with complex fistulae.

MRI vs 3D EAUS

The results of studies comparing MRI and EAUS for assessment of anal fistulae have varied widely [63]. A meta-analysis published in 2012 by Siddiqui et al analysed four studies published between 1999-2004 comparing 2D EAUS with MRI, including 241 fistulae in the EAUS group and 240 fistulae in the MRI group [76]. The results suggest that MRI and EAUS had comparable sensitivities (both 87%) for fistula detection, whereas MRI had higher specificity, 69% vs. 43%. Three of the studies included in the analysis reported on identification of fistula IO. The sensitivity of MRI ranged from 19-97% and specificity from 71-100%. The sensitivity of EAUS to detect IO ranged from 88-91% and specificity from 43-100%. The authors concluded that EAUS showed a trend toward being better than MRI in detecting IOs, but also that the large heterogeneity of the data and lack of applicable studies prevents any firm clinically relevant conclusions.

As early as 2004, West et al published a study of 40 patients who were examined preoperatively with both MRI using an endoanal coil and H_2O_2 -enhanced 3D EAUS [77]. The methods agreed in 88% in determining the primary fistula tract ($\kappa = 0.45$), in 90% in determining the IO location ($\kappa = 0.83$), and in 78% in secondary tracts ($\kappa = 0.62$). There was no patient preference for one procedure over the other, and both were associated with similar discomfort.

A retrospective study published in 2014 compared MRI and H_2O_2 -enhanced 3D EAUS for preoperative assessment of high anal fistulae in 14 patients [78]. The authors concluded that MRI was more accurate in identifying high fistulae and secondary tracts, while 3D EAUS was more accurate in assessing IO localisation.

In a study published in 2016, Alabiso et al compared 3D EAUS and MRI in 51 patients with Crohn's fistulae [79]. Results showed no significant difference between modalities in identifying transsphincteric fistulae, abscesses, or secondary extensions. 3D EAUS was more accurate in detecting intersphincteric fistulae, whereas MRI was more accurate in detecting supra- and extrasphincteric fistulae. The authors concluded that both 3D EAUS and MRI play a critical role in the evaluation and detection of perianal Crohn's disease.

Treatment of anal fistulae

A persisting or recurring EO with discharge or signs of infection indicate the presence of anal fistula, for which surgery remains the definitive therapeutic approach for cure. The main principles for the treatment of anal fistulae are to drain the infection and eradicate the fistula tract with minimal impact on faecal continence.

The first stage of fistula treatment is usually to perform a draining loose seton by placing a silastic thin vascular loop into the fistula tract. Once the acute infection has resolved, the fistula can be treated with a curative intent.

Surgery

Fistulotomy, a lay-open of the fistula tract, means that the entire fistula tract is opened up and allowed to heal by secondary intention. Marsupialisation of the edges may be performed to reduce the size of the wound. Depending on the degree of infection and inflammation, it may be possible to perform a lay open without preceding drainage with a loose seton. Fistulotomy is generally considered to be the gold standard surgical treatment for low fistulae. Reported overall success rates after fistulotomy vary between 80-100%, with faecal incontinence rates between 0-62% [80]. A study from Imperial College in London published data on 50 fistulotomies at a tertiary referral centre with focus on recurring fistula disease with overall success rate of 93% [81]. Deterioration of continence was experienced by 20%. In the group of patients referred from a surgeon in secondary care, the success rate was 91% and continence impairment rose from 32% at referral to 40% after surgery. The authors conclude that very high rate of healing can be achieved in patients after recurrence, when fistulotomy is offered to appropriate patients. This comes with 20% additional risk of continence impairment, but majority of patients experience only minor incontinence.

Fistulotomy may be combined with fistulectomy, a complete excision of the fistula tract. The American Clinical Practice Guidelines state that the procedure is associated with longer healing times, larger defects, and a higher risk of incontinence without lowering the recurrence rate compared to fistulotomy [46]. An Italian study has reported that fistulectomy combined with primary sphincteroplasty for complex anal fistulae had overall success rate of 96% in 72 patients at mean follow-up of 29 months [82]. 12% of patients with no baseline incontinence reported de novo soiling after defecation.

Sphincter-preserving procedures

The most common treatment options for high and complex fistulae consist of sphincter-preserving surgical procedures in order to minimise the risk of faecal incontinence. Techniques are based on careful assessment of fistula anatomy, adequate drainage and simplification of the tract (when necessary), secure closure of the IO, and avoidance of tension, ischaemia, dead space, and abscess [83].

In endoanal advancement flap procedure a flap of distal rectum containing healthy mucosa, submucosa and smooth muscle is interposed to cover the IO after curettage of the fistula tract and suture closure of the IO. A systematic review of 40 studies and 2333 patients shows success rates of 20-100%, with the success rate tending to decrease over time [83]. The American Clinical Practice Guidelines give a strong recommendation based on moderate-quality evidence to treat complex fistula with advancement flap [46]. They also note that mild to moderate incontinence is reported in up to 35% of patients, which has been verified by manometry.

Both simple and complex anal fistulae may be suitable for treatment with ligation of intersphincteric fistula tract (LIFT) procedure, in which the fistula tract is divided and closed with a suture in the intersphincteric plane. Systematic reviews and meta-analyses report healing in 61-94% of patients [84-87]. The procedure generally has little effect on faecal continence. Risk factors for failure have been documented as obesity, smoking, multiple previous surgical procedures, and length of the fistula tract [87]. When 3 cm is chosen as an arbitrary cutoff point, fistulae <3 cm have been shown to have a significantly higher primary healing rate (85%) than longer fistulae (48%) [87]. The American Clinical Practice Guidelines give a strong recommendation based on moderate-quality evidence to treat anal fistulae with LIFT [46].

In the 1990s, there were initial reports of the use of fibrin glue to repair anal fistulae after curettage of the fistula tract. A systematic review of 31 studies involving 871 patients reported success rates ranging from 0-86%, with success rates decreasing over time [83]. The American Clinical Practice Guidelines state that fibrin glue is a relatively ineffective treatment for anal fistulae but is still a minimally invasive option that may be considered [46].

The anal fistula plug (AFP)

The Surgisis® (Biodesign®) anal fistula plug (AFP) (Cook Medical, Bloomington, Indiana, USA) was registered in 2005 to treat cryptoglandular and Crohn's fistulae. Early studies published by Georgia Colon & Rectal Surgical Clinic in Atlanta, Georgia, reported success in 85% of fistula tracts after a median follow up of 12 months (range: 6-24 months) for cryptoglandular fistulae [88], and in 83% of fistula tracts in anorectal Crohn's disease [44]. Subsequent studies and systematic reviews have shown that success rates vary widely (reported from 13-100%), with AFP failure rates increasing with longer follow-up time [83, 89-100]. Many studies include follow-up during the first postoperative year, but few include longer follow-up periods. A systematic review of sphincter-preserving procedures by Kontovounisios et al, published in 2016, highlighted the need for studies with defined fistula types, homogeneous study groups, and long-term follow-up [83].

In 2010, Ellis et al published their long-term results for 63 patients with anal fistulae, 12 (19%) of whom had Crohn's disease, and reported an 81% clinical healing after a follow-up period of at least 12 moths (range: 12-24 months) [43]. Multivariate analysis showed that smoking, posterior fistula, and prior AFP treatment failure predicted procedure failure. The authors concluded that AFP was effective for long-term closure of complex anal fistulae, but randomised trials comparing this method with other sphincter-preserving procedures were needed.

In the same year, McGee et al published a study of 41 patients with a mean follow-up of 25 months in which they reported an overall closure rate of 43% and found that AFP failure occurred in 79% of fistula tracts <4 cm [101].

Lenisa et al reported a global success rate of 72% without continence impairment in 60 consecutive patients with cryptoglandular fistulae with a mean follow-up of 19 months (range: 6-34 months) [102]. The authors concluded that AFP was a viable surgical option that should be offered to patients with complex fistulae.

A meta-analysis published in 2012 compared AFP with advancement flap and found similar success rates and incidences of fistula recurrence [103]. Both the risk of incontinence and the incidence of other complications were lower in the AFP group. The authors concluded that AFP was an effective treatment for complex anal fistulae. A systematic review from the same year summarised the AFP literature for cryptoglandular and Crohn's fistulae and included 20 studies and 530 patients (42 with Crohn's fistulae) [97]. The pooled proportion of patients achieving fistula closure was 0.54 (95% CI: 0.50-0.59) in cryptoglandular fistula group and 0.55 (95% CI: 0.39-0.70) in the Crohn's fistula group. The authors concluded that AFP was not adequately evaluated for the treatment of Crohn's disease.

A Swedish retrospective multicentre study from four Stockholm hospitals included 126 patients, 84% of whom had fistulae of cryptoglandular origin [89]. They reported an overall success rate of 24%, with results varying between 13-33% among the four participating units. For anterior fistulae, the success rate was 12%, whereas for posterior fistulae, the success rate was 32%, with a hazard ratio (HR) 2.98 for successful healing.

A randomised clinical trial comparing AFP and endoanal advancement flap published in 2017 included 94 patients: 48 in the AFP group and 46 in the advancement flap group [90]. After a median follow-up of 12 months (range: 9-24 months), success rates were 44% in the AFP group and 62% in the advancement flap group. This difference was statistically significant. Patients reported reduced anal pain, unaffected continence, and improved quality of life after surgery, with no differences between groups.

The American Clinical Practice Guidelines state that AFP is a relatively ineffective treatment for anal fistulae but is still a sphincter-preserving option that may be considered [46].

Collagen plug

Small intestinal submucosa (SIS) is usually obtained from porcine jejunum and it is one of the oldest and most widely studied and applied biomaterials. Several decellularization procedures remove the cellular components, but collagen type I, glycosaminoglycans, elastin, fibronectin, fibroblastic growth factor, vascular endothelial growth factor, and transform growth factor remain in the extracellular matrix (ECM) (Figure 15). [104] The ECM acts as a natural scaffold for tissue development and repair with inherent resistance to infection [105]. Experience from hernia repair surgery and abdominal defect repair in contaminated fields supports the scaffold theory, in which fibroblasts may penetrate through the porous structure of the ECM implant, leading to formation of native, site-specific tissue [102]. The bioprosthetic collagen material has the advantage of minimising the risk of foreign body reaction [106].

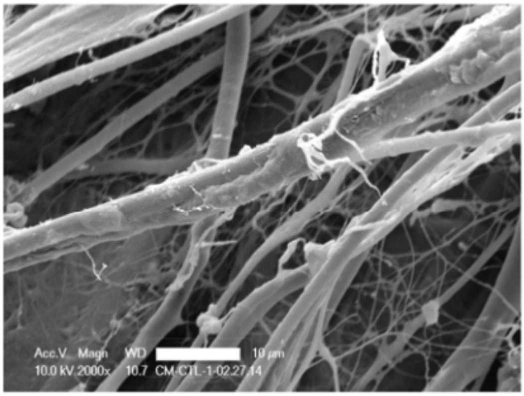


Figure 15 Scanning electron microscope image of porcine SIS-extracellular matrix (ECM) containing well-preserved collagen fibres.

doi.org/10.1371/journal.pone.0153412

Collagens are the most abundant proteins in mammals (about 30% of the total protein mass) [108], and collagen type I is the most common type of collagen of the human body [109]. The common structural feature of collagen I is the presence of a triple helix (Figure 16), and it provides structure in skin, bones, tendons, and ligaments. Collagen I is considered to be highly resistant toward proteolytic degradation, but collagenases such as MMP-1, MMP-8, and MMP-13 are capable of degrading certain types of collagen, including collagen I. [108, 109] Collagen I is widely used in tissue engineering as a regenerative agent for nerve deficits, bone regeneration, chondrogenic differentiation, vascular grafts, and skin substitutes [109].

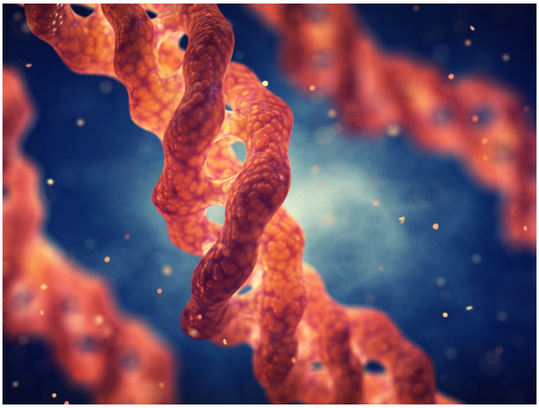


Figure 16 3D illustration of collagen triple helix.

The major collagen type in healthy intestine is type I (68%), followed by type III (20%), and type V (12%). In intestinal strictures due to Crohn's disease, both the collagen content and the relative amount of Collagen V have been shown to be significantly increased compared to healthy intestine [110]. This is thought to be related to smooth muscle cell proliferation. Another study demonstrated an increase in the biosynthesis of Collagen III in the intestine of patients with Crohn's disease [111].

The AFP is a 0.6 × 9.5 cm long cone-formed structure of SIS (Figure 17).

Figure 17 The Anal fistula plug (AFP).

AFP Procedure

The AFP procedure is usually performed under general anaesthesia in the prone jackknife or lithotomy position. The draining loose seton is cut and the fistula tract is debrided with a curette or a brush. In the first procedures, the fistula was irrigated with H_2O_2.followed by 0.9% sodium chloride; later, only sodium chloride is used to flush the fistula. A surgical probe is inserted into the fistula tract, and the AFP is completely immersed in sodium chloride for a maximum of 2 minutes and rehydrated. A suture is placed at the end of the plug which is then pulled through the IO into the fistula tract until resistance is encountered and the AFP blocks the IO (Figure 18). After proper positioning, the excess plug is trimmed from IO and EO, if necessary. The IO is sutured with a figure-of-eight suture through the adjacent tissue and the AFP to prevent leakage of intestinal contents into the fistula tract and to ensure adequate fixation of the AFP. [88, 112, 113]

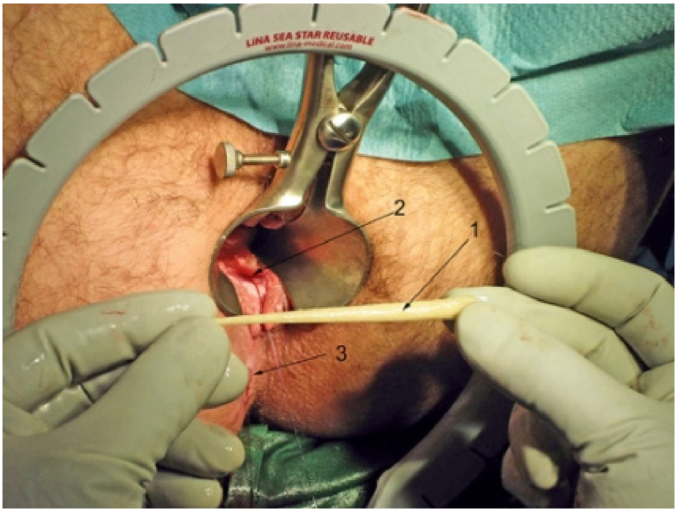


Figure 18 Intraoperative picture of the AFP procedure.
After the AFP has been immersed in sodium chloride for a maximum of 2 minutes, it is pulled into the anal fistula using a suture that runs through the tract and is attached to the narrow end of the plug. 1: AFP, 2: IO, 3: EO. Copyright Läkartidningen. Reproduced with permission. *Läkartidningen* 2008;105:1489-91.

Radiological follow-up

In a small number of studies, some of the participants have been followed up by MRI after the AFP procedure. In their study published in 2010, Ellis et al followed up eight patients with clinical fistula healing by MRI at least one year after treatment [43]. In six of them (75%), no residual fistula tract or fluid was found in the area of the procedure. Senejoux et al studied 106 patients with Crohn's disease who were drained with a loose seton and then, after more than one month of drainage, randomised to either seton removal or the AFP [99]. In the 54 patients treated with AFP, the fistula closure rate at 12 weeks was 32%, compared with 23% in the group of seton removal alone, with no statistically significant difference between the groups. 25 patients in the AFP group underwent MRI 12 weeks postoperatively, and healing of the fistula tract was noted in six patients (24%). The examinations were assessed using the previously mentioned MRI-based score for severity of perianal Crohn's fistulae [55].

Garg reported a study of 702 patients who were evaluated with preoperative MRI and then operated on with either fistulotomy or transanal laying-open of the intersphincteric fistula tract [114]. Postoperative MRI performed in 108 of these patients showed that granulation tissue and postoperative inflammation were difficult to distinguish from an active fistula, as both were hyperintense on T2-weighted images and short tau inversion recovery (STIR) sequences, especially during the first eight postoperative weeks. After healing, the fistula became hypointense but was still visible.

In 2018, Murad-Regadas et al published a study on assessment of anal fistulae with 3D EAUS in preoperative and postoperative setting [115]. 73 patients were treated with either LIFT or seton placement with subsequent fistulotomy and followed up with 3D EAUS within 4 months after the wound was completely healed. 3D EAUS was helpful in quantifying the extent of sphincter division, identifying healing tissue, and the type of fistula recurrence.

In summary, there are no systematic studies describing the morphological findings related to healing and failure after the AFP. The documented knowledge on radiological follow-up is very limited, especially for 3D EAUS.

3D Endoanal ultrasound (EAUS)

In 1989, Law and Bartram published an article reporting on endoanal ultrasound in 30 study subjects and 2 resected specimens [116]. The authors described the examinations as rapid, simple, and well tolerated, providing high-resolution images of the anal canal, including the mucosa, submucosa, IAS, intersphincteric plane, and EAS. They also found that the configuration of the anterior EAS differed between male and female study subjects. Sonomorphologic studies on cadavers, surgical specimens, and patients without anal pathology followed to clarify the anatomy [117-119].

Modern ultrasound technology offers a variety of transducers and post-processing options that make ultrasound an attractive option for assessment of pelvic floor and proctological disorders. 3D technology allows automatic collection of 3D data without the need to move the transducer in relation to the tissue under examination. The 3D cube created may be freely rotated, tilted, sliced, and saved for later analysis. The patient can be positioned in lithotomy, lateral or prone position during the examination. For the examination of the anal canal, the examination should extend from the oral aspect of the puborectal muscle to the anal verge. [120]

The use of 3D EAUS is influenced by local factors, such as clinical traditions, economic aspects, and availability. The advantages of 3D EAUS are that the examination is quick, easily accessible, and usually well-tolerated by patients. It can be easily performed as part of a clinical examination in the outpatient clinic as well as in the operation theatre before, after, or during surgery. In addition, the fact that the surgeon can perform the examination allows continuity and eliminates waiting time between radiology and surgery.

Transducer 2052

Transducer 2052 is the "traditional" transducer used for 3D EAUS examinations. It is a mechanical multifrequency (6-16 MHz) transducer with a build-in 3D mover. A double crystal assembly with crystals back-to-back is placed inside the transducer head. The crystals rotate at 4-6 cycles per second to give a 360° scan of the surrounding tissues (Figure 19). The built-in 3D automatic motorised system moves the crystal system allowing acquisition of 300 transaxial images over a distance of 60 mm in 60 seconds. The examination is presented as a 3D cube (Figure 20) that can be examined in detail from any angle and archived for offline analysis.

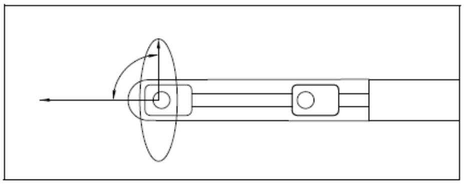


Figure 19 BK Medical 2052 Anorectal 3D transducer.
Imaging plane for the transducer with a built-in 3D mover. The assembly rotates inside the transducer to give a 360° field of view. Used with permission of GE HealthCare.

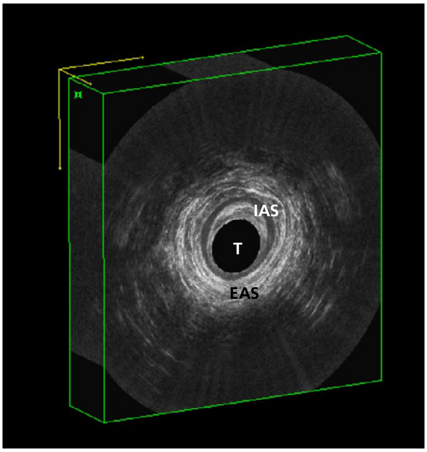


Figure 20 3D cube acquired by transducer 2052.
IAS: Internal anal sphincter, EAS: External anal sphincter, T: Transducer.

Transducer 8838

Transducer 8838 was initially introduced to be used in transrectal prostate and transvaginal imaging, but it is possible to use the transducer for 3D EAUS as well. This 6-12 MHz high-resolution transducer contains a linear array of 192 ultrasound elements that first gives a dynamic radial 2D image used for anatomical orientation and interventions. The built-in 3D system rotates the element array inside the transducer (Figure 21) immediately reconstructing the acquired 2D images into a 3D cube (Figure 22). The created 3D cube is similar to the one acquired by transducer 2052 but a cube from transducer 8838 has a stich where the rotational plane has started. The position of the plane is chosen by the operator before the 3D function is activated.

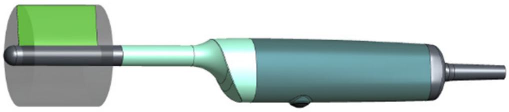


Figure 21 BK Medical 8838 3D *ART* transducer
Imaging plane for the transducer containing a linear array of ultrasound crystals that rotates covering an adjustable angle up to 360° to produce a 3D image. Used with permission of GE HealthCare.

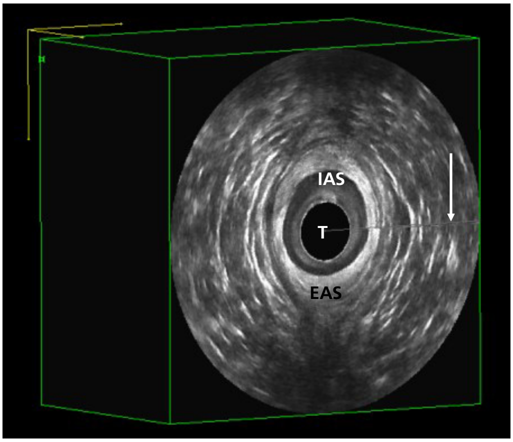


Figure 22 3D cube acquired by transducer 8838 with the stich at the 3 o'clock position.
IAS: Internal anal sphincter, EAS: External anal sphincter, T: Transducer, Arrow: Stich of the rotational plane.

3D EAUS for anatomical measurements

Assessment of the anatomy and length of the anal canal is important in the management of anal fistulae and obstetric injuries. The focus of the gynaecologic ultrasound of the anal canal is to diagnose the degree of sphincter disruption. The documented knowledge of anal canal morphology in modern 3D EAUS is very limited, especially in male. In addition, to the best of our knowledge, there are no studies in which the length of the anal canal was measured with the 8838 transducer.

In 2005, Starck et published a study on the length of the anal canal in women of different ages and parity [121]. A 2D EAUS transducer was mounted on a step unit which moved the transducer in 5 mm steps. The mean posterior sphincter length (EAS+PR) varied from 29.6 ± 2.63 mm to 31.5 ± 6.19 mm, depending on the age group. Mean anterior sphincter length (EAS) varied between 22.9 ± 3.83 mm and 25.0 ± 2.94 mm.

In 2007, Regadas et al examined 12 healthy male and 14 healthy female volunteers with a similar transducer to 2052 [122]. The authors reported that the mean anterior EAS length was significantly shorter in female compared with male, 22.0 ± 3.1 mm vs. 34.2 ± 1.8 mm. They found that the mean posterior EAS length was also significantly shorter in female compared to male, 32.0 ± 4.7 mm vs. 36.6 ± 1.8 mm. Another study by the same group investigated 165 patients, both male and female, using transducer 2052 [123]. The mean anterior EAS length was 35 ± 0.6 mm in male and 20 ± 0.5 mm in female (*P* <0.001). The mean posterior length (EAS+PR) was 40 ± 0.6 mm in male and 36 ± 1.0 mm in female (*P* = 0.0023). The study did not exclude cryptoglandular fistula patients with history of proctological surgery.

In a recent study, Norderval et al examined 43 healthy nulliparous women with a similar transducer to 2052 [124]. They reported that the posterior anal canal length (EAS+PR) was 34.8 mm (95% CI: 33.6-36.0 mm). The mean anterior length (EAS) was reported to be 15.8 mm (95% CI: 14.9-16.7 mm). Interclass correlation coefficients between two raters were 0.56 (95% CI: 0.31-0.74) for posterior anal canal length and 0.64 (95% CI: 0.41-0.79) for anterior length.

Rostaminia et al compared the 2052 and 8838 transducers in diagnostics of obstetric injuries in 85 patients for a validation study of the latter [125]. They did not take length measurements but focused on the evaluation of levator ani muscles and anal sphincter complex defects. The authors detected more EAS and IAS defects with transducer 8838 but the difference with transducer 2052 was not statistically significant. They concluded that both transducers can be used interchangeably for endoanal imaging of the sphincter complex.

Aim and Objectives

The overall aim of this thesis was to investigate the long-term outcome of the AFP procedure and to explore the utilisation of 3D EAUS for the assessment and follow-up of anal fistulae.

Specific objectives:

Paper I Primary: To evaluate the long-term success rate of the AFP in the treatment of complex anal fistulae.

Secondary: To assess AFP failure over time and compare success rates of the cryptoglandular fistula group and the Crohn's fistula group.

Paper II Primary: To explore the utilisation of 3D EAUS for the follow-up of the AFP.

Secondary: To describe the morphological findings in postoperative 3D EAUS at different time points after the AFP and evaluate whether postoperative 3D EAUS in combination with clinical symptoms may be used to predict AFP failure.

Paper III Primary: To evaluate the accuracy of 3D EAUS alone for the assessment of newly diagnosed cryptoglandular fistulae, compared to intraoperative findings in combination with intraoperative 3D EAUS (gold standard).

Secondary: To analyse interobserver agreement between three surgeons with different levels of experience in 3D EAUS, determine interobserver agreement between two different types of 3D EAUS transducers and analyse intraobserver agreement for one rater.

Paper IV Primary: To explore the use of 3D EAUS for the measurement of anal canal length in patients with newly diagnosed cryptoglandular fistulae.

Secondary: To analyse reproducibility between two surgeons, assess repeatability, and determine agreement between two different types of 3D EAUS transducers.

"-- for it is better that the term be lengthened than the cure,

for prolongation of the cure giveth

the cause of despairing to the patients."

John of Arderne

Materials and Methods

This doctoral thesis consists of two prospective cohort study projects, each designed to produce two papers.

Study design

Paper I

This was a single-centre study of consecutive patients with complex anal fistulae treated at the Pelvic Floor Centre, Department of Surgery, Skåne University Hospital, Malmö, between May 2006 and October 2009. Patients with ano/rectovaginal fistulae were excluded.

Surgical procedure

Patients were assessed by clinical examination combined with 3D EAUS and treated with a draining loose seton for at least 6 weeks before the AFP surgery. All procedures were performed by four surgeons with extensive experience in fistula surgery; two of them were present during each procedure. A mini enema was administered for preoperative bowel preparation. On the day of surgery, a single dose of oral or intravenous combination of metronidazole and trimethoprim/sulfamethoxazole was administered. General anaesthesia and the lithotomy position were used for surgical procedures. Another 3D EAUS was performed immediately preoperatively to ensure that no undrained abscesses or further tracks were present.

The Surgisis® (Biodesign®) AFP was placed in the fistula tract according to the manufacturer's instructions. However, the original procedural protocol was adjusted in one aspect: two double-armed absorbable monofilament 3-0 sutures on UR-6 needles were placed as a cross through the plug and over the IO and IAS. Instead of a figure-of-eight suture, the AFP was fixed on the IO by tying the crossed sutures (Figure 23). Special care was taken to ensure that the AFP was adequately fixed and covered by the mucosa when closing the IO (Figure 24).

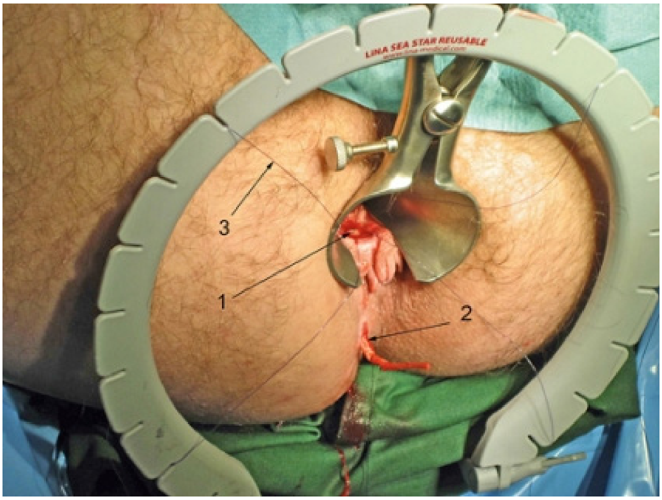


Figure 23 The AFP pulled into the fistula tract.
The AFP has been attached with cross sutures created by two double-armed sutures. 1: The AFP at the IO, 2: The excessive portion of the AFP is trimmed to skin level, 3: The sutures fix the AFP in the IAS and the mucosa. Copyright Läkartidningen. Reproduced with permission. *Läkartidningen* 2008;105:1489-91.

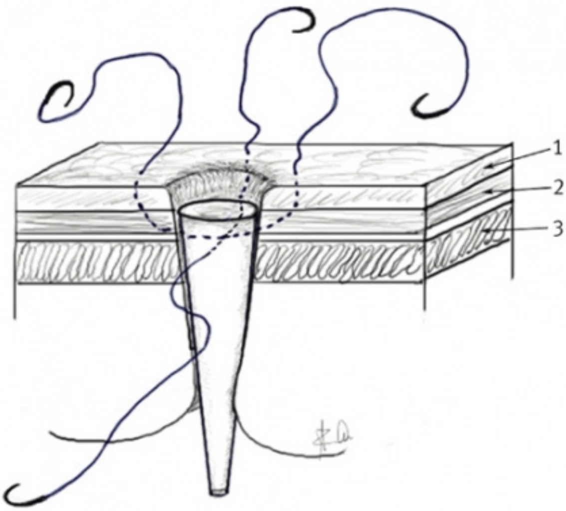


Figure 24 Sketch of the AFP in the fistula tract.
The AFP has been attached with cross sutures created by two double-armed sutures. Special attention was paid to ensure adequate fixation and that the AFP was covered by mucosa. 1: Mucosa and submucosa, 2: IAS, 3: EAS. Copyright Läkartidningen. Reproduced with permission. *Läkartidningen* 2008;105:1489-91.

Follow-up

Patients were assessed by clinical examination combined with 3D EAUS in the outpatient clinic 2 weeks, 3 months and between 6-12 months postoperatively.

After the last outpatient visit, the patients were instructed to contact the clinic if any symptoms of AFP failure occurred. All patients who contacted the clinic because of new symptoms underwent reassessment by clinical examination and 3D EAUS. In case of a persisting or recurring fistula, a new loose seton was inserted for drainage within 1-3 weeks. After 6 weeks of drainage, either a new plug was inserted, or another surgical procedure was performed according to the best clinical practice. If a new AFP was inserted, the same surgical procedure and follow-up were performed as described above.

Long-term follow-up

Long-term follow-up was performed between August and December 2017. All patients who were not known to have undergone further fistula surgery were sent a questionnaire about any symptoms of fistula recurrence. Patients who did not respond to the initial questionnaire were sent a reminder, and attempts were made to contact patients by telephone if no response was received. Patients who reported any symptoms were assessed by clinical examination combined with 3D EAUS.

Paper II

Documented 3D EAUS examinations of the study cohort in **Paper I** formed the basis of investigation of post-surgery and healing states assessed in this paper.

3D EAUS

Clinical follow-up after the AFP procedure included a standardised 3D EAUS at 2 weeks, 3 months, and 6-12 months ('late control') after the procedure. A 3D Pro Focus device (BK Medical, Herlev, Denmark) and a 360° rotating 13 MHz transducer were used for the ultrasound examinations. The transducer was covered with a condom containing water-soluble ultrasound gel (Lectro Derm 1, Handelshuset Viroderm AB, Solna, Sweden) and water-soluble lubricant was applied to the condom before insertion of the probe into the anal canal.

The 3D EAUS examinations were coded and retrospectively analysed by a team of two colorectal surgeons with extensive experience in pelvic floor ultrasound and fistula surgery. First, an exploratory analysis of 10 randomly selected patients was performed to determine the most common findings for each time point. Based on the exploratory analysis, a protocol for the analysis of 3D EAUS examinations was established and used for all examinations. The reviewers, who were blinded to the outcome, analysed the examinations together, and in case of disagreement, consent was resolved by discussion.

Paper III

This was a single-centre study of consecutive patients with newly diagnosed anal fistulae treated at the Pelvic Floor Centre, Department of Surgery, Skåne University Hospital, Malmö, between June 2018 and March 2020. Patients with cryptoglandular fistulae and no history of fistula surgery were eligible for inclusion. Patients with inflammatory bowel disease, ano/rectovaginal fistulae, more than one fistula, or a fistula with more than one IO were excluded. All patients were assessed with 3D EAUS at the outpatient clinic as part of the routine clinical practise, and a preliminary plan for surgery was made.

3D EAUS

The 3D EAUS examinations were performed by using a 3D Flex Focus 500 device (BK Medical, Herlev, Denmark). Two different types of ultrasound transducers, BK Medical 2052 and 8838, were used for all examinations in the study. Figure 25 shows the 3D EAUS equipment used for the study procedures. The 2052 Anorectal 3D transducer is a mechanical 360° rotating probe with a double crystal assembly (Figure 19). A frequency of 16 MHz was selected for the study. The 8838 3D *ART* transducer is a high-resolution probe containing a linear array of crystals that rotates 360° to produce a 3D image (Figure 21). A frequency of 12 MHz was chosen for the study. The transducers were prepared and operated in a similar manner as in **Paper II**.

Figure 25 The 3D EAUS equipment used for the study.
The 3D Flex Focus 500 machine with the transducer 8838 on the left and transducer 2052 on the right. Both transducers are covered with a condom containing ultrasound gel.

Complete assessment: 3D EAUS and intraoperative findings

A mini enema was administered for preoperative bowel preparation. The 3D EAUS and surgical procedure were performed with the patient in the lithotomy position and under general anaesthesia. All procedures were performed by a total of five experienced fistula surgeons. Surgery was always performed by two surgeons.

The surgeons performed 3D EAUS examinations with both study transducers at the beginning of each surgical procedure. The examinations were evaluated intraoperatively and depending on the clinical situation, the surgeons then performed a fistula procedure according to best clinical practise.

Postoperatively, surgeons completed the study protocol based on the results of both 3D EAUS examinations and all intraoperative findings. In case of disagreement, consent was resolved by discussion. The fistula was assessed for orientation of IO (anterior, posterior, or lateral right/left), orientation of passage through the EAS (anterior, posterior, lateral right/left, or no penetration), fistula height defined by the highest level of the main fistula tract (subcutaneous, low, high, or ultra-high), and fistula tract length (<3 cm or ≥3 cm). The results were documented in the study protocol which was saved as the gold standard for each patient.

3D EAUS assessment

The coded 3D EAUS examinations were archived in the study database. The examinations were retrospectively analysed by three surgeons ('raters') with varying degrees of experience in 3D EAUS and proctology. One surgical specialist, who had been working at the clinic for one year, had no experience with the 2052 transducer and only limited experience with the 8838 transducer. One consultant had five years of experience using both transducers, and a senior consultant had more than 20 years of extensive experience using multiple ultrasound transducers.

The raters used offline analysis software BK 3DViewer, version 7.0.0.601 (BK Medical, Herlev, Denmark) for 3D EAUS assessment. They worked individually, had access only to the coded examinations, and were completely blinded to patient history and symptoms. It was also not possible to compare the two examinations performed with different transducers on the same patient. The 3D EAUS protocol for each transducer, which was identical to the gold standard protocol, was completed by each rater for each examination.

To facilitate the analysis of intraobserver agreement, the consultant repeated the ratings more than three months after the first round of assessments.

Paper IV

3D EASUS examinations from the cohort of **Paper III** formed the basis for investigation and analysis of the anal canal length in this paper.

3D EAUS measurement

The 3D EAUS examinations were retrospectively analysed by two surgeons ('raters') using the same offline analysis software as in **Paper III**. The raters worked individually and had access only to the coded examinations. It was not possible to access any patient data other than patient gender or to compare the two examinations performed with different transducers on the same patient.

The raters were instructed to work systematically, identifying specific anatomic landmarks for measurements, and documenting them in the study protocol for each transducer and patient. Because the anal verge sometimes being difficult to identify, it was decided that the measurements should be made by identifying both the anal border of the IAS and the anal border of the EAS as distal measurement points. The proximal measurement point was defined by the oral border of the EAS in the anterior segment and the oral midline border of the PR muscle in the posterior segment. The measurements were performed in the sagittal plane, while the identification of the anatomical landmarks was performed using the biplanar technique.

Rater 1 had five years of experience in the use of both transducers whereas Rater 2 had no experience with the 2052 transducer and only limited experience with the 8838 transducer. Rater 1 performed all measurements twice, with more than three months between assessment rounds. Rater 2 acted as an external control.

Definitions

Papers I and II

Complex fistula

'Complex fistula' was defined as a fistula where a simple lay-open was not the first treatment option. In practice, a tract of a complex fistula involved >30-50% of the anal sphincter length (depending on fistula orientation) or the patient had a history of Crohn's disease, incontinence, or local irradiation. All anterior fistulae in female patients were also considered to be complex.

Fistula healing / AFP success

Fistula healing or AFP success 6-12 months postoperatively was clinically defined by the absence of discharge and a dry scar at the EO.

In long-term follow-up, healing of the fistula tract or AFP success was clinically defined by no need for further fistula surgery, a closure of the fistula EO and no discharge. Patients who had been evaluated in the outpatient clinic or the Department of Gastroenterology in the previous year without clinical evidence of an active fistula were considered to have AFP success.

Fistula recurrence / AFP failure

AFP failure was classified clinically by the entire plug falling out up to 2 weeks after surgery, signs of inflammation and/or infection of the surgical site, and discharge of faeces or gas through the EO at any time. Persistent, but decreasing fluid discharge without other clinical findings 3 months postoperatively was not automatically classified as AFP failure.

Patients with clinical symptoms of late fistula recurrence or a fistula that required surgery on the same side as the previously treated fistula were considered to have AFP failure.

Paper III

Fistula assessment protocol

The same protocol was used for both the complete assessment and the 3D EAUS assessment of the anal fistula. The fistula assessment model is shown in Figure 26. The assessment model for female had a shorter anterior anal canal than that for male.

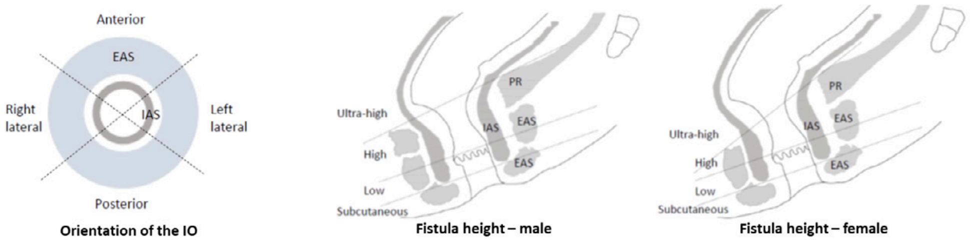


Figure 26 The models for fistula assessment for the orientation of the IO and the highest level of the main fistula tract.
IAS: Internal anal sphincter, EAS: External anal sphincter, PR: Puborectal muscle.

Paper IV

Reproducibility

Reproducibility is defined as variation in measurements under changing conditions [126, 127]. In this study, the changing conditions were two different raters and transducers.

Repeatability

Repeatability is defined as the variation in successive measurements made on the same subject under identical conditions [126, 127]. In this study, this was applicable to repeated measurements made under the same conditions by Rater 1.

Reliability

Reliability relates to the magnitude of the measurement error in observed measurements to the inherent variability in the underlying level of the quantity between subjects [126]. In this study, this was applicable to repeated measurements made by Rater 1

Anterior anal canal length – IAS

The anterior length of the anal canal where the proximal measurement point was defined by the border of the EAS where the circular muscle fibres were seen in the midline. The distal measurement point was defined by the border of the IAS where the circular hypoechogenic ring of the IAS was no longer seen (Figure 27).

Anterior anal canal length – EAS

The anterior length of the anal canal where the proximal measurement point was defined by the border of the EAS where the circular muscle fibres were seen in the midline. The distal measurement point was defined by the border of the EAS at the muscle-gel/air interface (Figure 27).

Posterior anal canal length – IAS

The posterior length of the anal canal where the proximal measurement point was defined by the border of the PR muscle at midline where the U-formation of the muscle fibres was identified. The distal measurement point was defined by the border of the IAS where the circular hypoechogenic ring of the IAS was no longer seen (Figure 27).

Posterior anal canal length – EAS

The posterior length of the anal canal where the proximal measurement point was defined by the border of the PR muscle at midline where the U-formation of the muscle fibres was identified. The distal measurement point was defined by the border of the EAS at the muscle-gel/air interface (Figure 27).

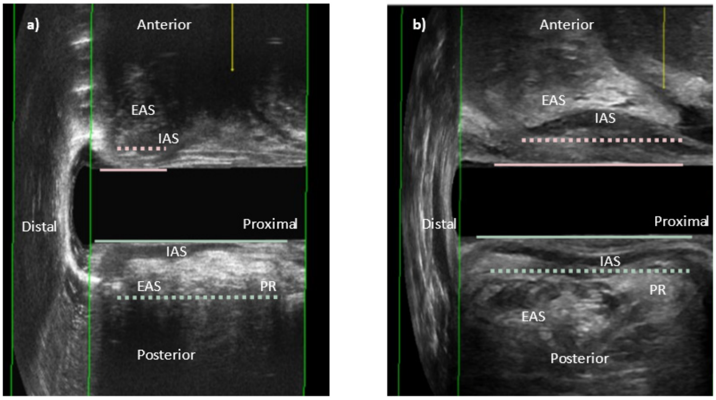


Figure 27 Multiplanar 3D EAUS images of the measurement methods used in the sagittal plane to investigate anterior and posterior anal canal length. a) Female; transducer 2052 b) Male; transducer 8838
IAS: Internal anal sphincter, EAS: External anal sphincter, PR: Puborectalis muscle, Solid pink line: Anterior anal canal length – EAS, Dashed pink line: Anterior anal canal length – IAS, Solid green line: Posterior anal canal length – EAS, Dashed green line: Posterior anal canal length – IAS.

Ethical considerations

All study subjects provided informed consent. The studies of this thesis were approved by the Regional Ethics Committee in Lund, Sweden; **Paper I and II** (Dnr 2010/77) and **Paper III and IV** (Dnr 2018/201). Both projects are registered in ClinicalTrials.gov; **Paper I and II** (*NCT03961984*) and **Paper III and IV** (*NCT03937752*).

Statistical methods

Patient characteristics in all studies are expressed as frequency (percent) or median (range), as appropriate. A value of $P < 0.05$ was considered statistically significant.

In **Paper IV**, the length of the anal canal was calculated using all three measurements for each parameter; rounds 1 and 2 by Rater 1, and the control round by Rater 2. First, the mean of the two measurements by Rater 1 was calculated for each study subject. Then, an overall mean was formed by calculating a new mean after the measurement from Rater 2 was added.

The statistical analysis, methods, and results of **Paper III and IV** have been discussed with a medical statistician to ensure that appropriate methods were used, and correct results reported. The results of **Paper II** were also discussed with a statistician.

Statistical analyses were performed using IBM® SPSS® Statistics for Windows®, version 25/27/28 (IBM Corp., Armonk, New York, USA), except for Hotelling's *T*-square statistics which were performed by using the delta method of Multiagree package in RStudio, version 4.2.3 (The R Foundation for Statistical Computing, Vienna, Austria) and bootstrapping analysis of intraclass correlation coefficients which were performed by using the irr and boot packages.

Chi-squared test

Pearson's chi-squared (χ^2) test is most commonly used test to examine whether the distribution of individuals among the categories of one variable is independent of distribution of the same individuals among categories of another variable [128]. The test compares the differences of expected (E) and observed (O) cell values in a contingency table with the null hypothesis of no difference [128, 129] :

$$\chi^2 = \sum \frac{(O-E)^2}{E} \text{, df = 1 for a 2 x 2 table}$$

The value of χ^2 is compared to the probability for that value of the chi-squared distribution. For $P < 0.05$ this means that the probability is less than 5% that the observed difference could have arisen by chance. Thus, there is evidence against the null hypothesis.

Pearson's χ^2 assumes simple random samples that are independent of each other. A sufficient overall sample size is >40, regardless of the expected values, or all the expected values ≥5 when the overall total is 20-40 samples. [128, 129]

In this thesis, the χ^2 test was used in **Paper I** to analyse success rate per plug for patients who received the AFP as the first definitive treatment compared to patients who received the AFP after previous fistula surgery on curative intent. It was even utilised to compare success rate per plug in the group of patients reporting fluid discharge through EO 3 months postoperatively to patients experiencing no discharge.

Fisher's exact test

Fischer's exact test is needed to compare two proportions when the values in a 2 x 2 table are too small to be analysed with a chi squared test [128]. The test analyses, given the marginal total, the probability of obtaining data as or more extreme than the one observed [129]. For a 2 x 2 table consisting of cells A, B, C, and D, with a total number of N samples, this is calculated by:

$$Exact\ probability = \frac{(A+B)!\,(A+C)!\,(B+D)!\,(C+D)!}{A!\,B!\,C!\,D!\,N!}$$

where the exclamation mark denotes the factorial [128, 129].

Given a null hypothesis of no difference, not only the probability of the observed table, but also the probability of tables more extreme than that, should be calculated. The *P*-value can be calculated by either defining more extreme as less probable (Approach I) or by restricting the calculation to extreme tables showing differences in the same direction as observed, and then double the probability (Approach II) [128]:

P (Approach I) = $\text{probability}_{\text{observed}} + \text{probability}_{\text{less\_probable\_tables}}$

P (Approach II) = $2 \times (\text{probability}_{\text{observed}} + \text{probability}_{\text{more\_extreme\_tables\_in\_the\_same\_direction}})$

In this thesis, the comparisons between categorical variables in **Paper II** were made using Fischer's exact test.

Kaplan-Meier estimate of the survival curve

Kaplan-Meier estimate is a survival analysis method for longitudinal studies that does not assume a constant rate of events over the study period. The survivor function *S(t)* is derived by considering the risk sets of individuals still being studied at each time (*t*) when an event (such as AFP failure) occurs. The estimated survival probability at *t* is calculated by:

$$s_t = 1 - r_t = \frac{n_t - d_t}{n_t}$$

where n_t is the number of individuals at risk at time t, and d_t is the number of events at that precis time, whereas r_t accounts for estimated risk at t.

The general product-limit formula for the survival probability up to and including event j is:

$$S(t_j) = S(t_{(j-1)}) \times s_{t_j} = s_{t_1} \times s_{t_2} \times \ldots s_{t_j} \text{ [128].}$$

The estimate is based on assumptions that censoring is unrelated to prognosis, the survival probabilities are the same for subjects no matter when they are recruited to the study, and the timepoint of the event can be specified [128, 130]. The results are usually presented as a graph of the cumulative survival probability against time from diagnosis or treatment start.

In this thesis, the Kaplan-Meier estimate was used to present success rate over time for the cryptoglandular group and the Crohn's fistula group in **Paper I**.

Log-rank test

The log-rank test is based on the same assumptions as the Kaplan-Meier estimate. It can be used to test the null hypothesis that there is no difference between groups in the probability of an event at any time point, thus providing a comparison of the total survival [128, 130]. The analysis is based on a χ^2 test of observed and expected events. First, the Mantel-Cox estimate of a hazard ratio (HR) is calculated based on a combination of separate 2 x 2 tables for each time an event occurs. The null hypothesis of the Mantel-Cox HR (HR_{MC}) being equal to 1 is tested by calculating the Mantel-Cox χ^2 statistic:

$$\chi^2_{MC} = \frac{U^2}{V}$$

where V is the sum across the strata of the variances for the number of exposed individuals experiencing the outcome event, and

$$U = \sum(d_{1i} - E_{1i}), \text{ and } E_{1i} = \frac{d_i \times n_{1i}}{n_i}$$

where d_{1i} is the number of exposed individuals observed to have experienced the event and E_{1i} is the expected number in this category if there was no difference in HR_{MC}. [128]

The log-rank test is a pure test of significance and does not provide any estimates of the size of the difference between the groups [130].

In this thesis, the Kaplan-Meier estimate of success rate over time from **Paper I** was analysed with the log-rank test.

Logistic regression

Logistic regression is used for the analysis of binary outcomes [128]. It describes the relation between an outcome of interest and one or more explanatory variables [131]. The principle of logistic regression is similar to linear regression analysis that attempts to fit a line to the data analysed by the equation $y = A + Bx$, where A is the point where the regression line crosses the vertical axis, and B is the slope of the line. In logistic regression, each patient has a probability (p) of achieving a particular outcome, modelled as:

$$\log\left(\frac{p}{1-p}\right) = A + Bx \text{ [131]}$$

Since odds is the measure of the likelihood of an outcome, it means that:

$$\log(\text{Odds}) = A + Bx,$$

which can be transformed into:

$$\text{Odds} = e^{A + Bx}$$

The model can be used to calculate odds ratio (OR) that is defined as:

$$OR = \frac{Odds\ in\ exposed\ group}{Odds\ in\ unexposed\ group} \text{ [128]}$$

Meaning that the OR for $x = 1$ vs $x = 0$ is:

$$OR = \frac{e^{A+B}}{e^{A}} = e^{B}$$

Thus, the logistic regression model can be interpretated to give the OR for the outcome of interest based on the explanatory variable.

In this thesis, the variables identified as statistically significant in **Paper II** were analysed with a logistic regression model.

Cohen's kappa coefficient

The extent of reproducibility of categorical variables is usually assessed using a kappa (κ) coefficient [128]. This method of giving a quantitative measure of agreement between observers is often referred to as Cohen's κ, since it was first described by Jacob

Cohen [132]. The coefficient represents the proportion of agreement after agreement by chance is removed from consideration:

$$\kappa = \frac{p_O - p_C}{1 - p_C}$$

where p_o is the proportion observed, and p_c is the proportion expected by chance [132].

At complete agreement κ = 1, and at agreement no more than what could be expected by chance κ = 0 [128, 133]. The results can be interpreted using different criteria. This thesis uses the criteria slightly modified from criteria presented by Altman [134]:

κ	Strength of Agreement
0.81-0.99	Very good
0.61-0.80	Good
0.41-0.60	Moderate
0.21-0.40	Fair
≤0.20	Poor

The κ coefficient is based on assumptions that the raters operate independently, the units are independent, and the categories of the nominal scales are independent, mutually exclusive, and exhaustive [132].

For ordinal variables, weighted Cohen's κ may be more useful, since it assigns less weight to agreement as categories are further apart [133, 135]. The observed and expected proportions of agreement are modified to include partial agreements [128].

In this thesis, the Cohen's κ was analysed for interobserver agreement between raters and the gold standard regarding parameters for fistula orientation and length in **Paper III**. Linear weighted Cohen's κ was used to analyse data for fistula height.

The agreement was even presented as proportion of agreement, meaning the observed percentage of agreement where the rater was in unison with the gold standard. The same methods were used to analyse intraobserver agreement.

Fleiss' kappa coefficient

Since Cohen's κ coefficient is restricted to analyse between two observations, Fleiss reported a method for several raters, now referred to as Fleiss' κ [136]. Simplified, the calculations are based on the means of rater-rater pairs in agreement relative to the number of possible rater-rater pairs [136, 137]:

$$\kappa = \frac{\bar{P} - \bar{P}_e}{1 - \bar{P}_e}$$

where $\bar{P}$ equals the mean of proportion of agreeing pairs, and $\bar{P} - \bar{P_e}$ is the degree of agreement actually observed in excess of chance, whereas $1 - \bar{P_e}$ measures the agreement attainable over and above what would be predicted by chance [136].

In this thesis, the Fleiss' κ coefficient was utilised in **Paper III** to assess the reliability of agreement between the three raters.

Hotelling's *T*-square distribution

Kappa coefficients are coefficients of relative agreement, and dependent on the prevalence of the trait under study [133, 138]. Need of analysis methods applicable on multilevel data has extended the use of κ coefficients for new study designs. Hotelling's *T*-square (T^2) can be used to compare several dependent multilevel components [138, 139]. The test was introduced in 1930 as the multivariate generalisation of Student's *t*-test [140]. Instead of performing separate *t*-tests for each variable in a comparison, it is possible to use the T^2 distribution to test the null hypothesis that the mean vector (the vector containing all means) is equal for the two groups being tested [141, 142].

In this thesis, Hotelling's T^2 statistics was applied in **Paper III** for pairwise dependent κ-comparisons by using the delta method for analysis of asymptotic variance-covariance of the κ coefficients between the different raters and transducers.

Bland-Altman plot

In 1983, Altman and Bland introduced an analysis appropriate for method comparison studies [128, 143]. Instead of producing a scatter plot with a line of equality between two measurements, it is often easier to visually assess the disagreements between the methods by plotting the difference in the measurements from one subject against the mean of their measurements, now commonly referred as the Bland-Altman plot [126]. The plots are usually presented with limits of agreement; the range within which 95% of future differences in measurements between the two methods are expected to lie [126, 144]. This is calculated by

$$\textit{mean difference} \pm 1.96SD \text{ [144].}$$

Since limits of agreement are just estimates, it is essential to calculate the 95% confidence intervals (CI) [126, 144].

If the SD of paired differences in not uniform across the range of measurements, the calculations are possible to perform after a log-transformation. Since difference of two logarithms is equal to the logarithm of the ratio, the results instead provide geometric mean ratios and limits of agreement for the ratio [126].

In this thesis, the Bland-Altman plots were used in **Paper IV** to analyse reproducibility of measurements between the two raters and transducers.

Shapiro-Wilk test

The Shapiro-Wilk test, a test based on comparing ordered sample values to the ones expected on normal distribution, is one of the methods used as a general test of the assumption of normality [128, 145].

In this thesis, the Saphiro-Wilk test was used as a test of normality for calculated variables in **Paper IV**.

Student's *t*-test

The null hypothesis of equal means of two groups with small sample sizes is often tested by Student's *t*-test, most commonly known from a paper published under a pseudonym in 1908 [146]. The method can be utilised to calculate the *P*-value, the probability for the null hypothesis to be true [128]. The two estimates of the common SD from two samples, s_1 and s_0, are combined to give a common estimate of the population standard deviation, s:

$$s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_0 - 1)s_0^2}{n_1 + n_0 - 2}}$$

which, in turn, is used to calculate the t statistic of an unpaired t test by:

$$t = \frac{\bar{x}_1 - \bar{x}_0}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_0}}}$$

where $\bar{x}_1 - \bar{x}_0$ is the difference in means of the groups consisting of n_1 and n_0 individuals or measurements, and df = $n_1 + n_0 - 2$ [128]. The t value is then compared to the probability for that value of the t distribution.

In hypothesis testing for paired means:

$$t = \frac{\bar{x}}{s/\sqrt{n}}, \text{ df} = n - 1$$

where $\bar{x}$ is the mean of paired differences, and n is the number of pairs [128].

Being a parametric test method, the *t*-test is based on assumptions of independence, normally distributed data and uniform variance [147].

In this thesis, mean comparisons of normally distributed parameters between genders and between transducers within gender were performed by unpaired and paired Student's *t*-test, respectively, as a part of **Paper IV**. Paired *t*-test was even used to assess bias between Rater 1 and Rater 2, and between transducers.

Wilcoxon signed rank test

Wilcoxon rank sum-test [148] is the non-parametric counterpart of the Student's *t*-test examining whether the median of difference between pairs of observations is equal to zero [128].

In this thesis, Wilcoxon signed rank test was utilised in **Paper IV** for median comparisons of parameters that were not normally distributed between transducers within gender.

Mann-Whitney U -test

Mann-Whitney *U*-test [149] is the non-parametric counterpart of the unpaired Student's *t*-test examining the null-hypothesis that the probability of two randomly selected values from two populations being greater than the other is equal [128].

In this thesis, Mann-Whitney *U*-test was used in **Paper IV** for comparisons of parameters that were not normally distributed between genders and in length ratio comparisons between raters.

One-way analysis of variance

One-way analysis of variance (ANOVA) is a method applicable to compare mean numerical outcomes between subgroups defined by one variable. The method analyses the effect of the overall variation in the outcome on differences between group means [128].

In this thesis, one-way ANOVA was used for repeatability analysis of measurements by Rater 1 in **Paper IV**. The groups were defined by study subjects, and the ANOVA model was fitted to estimate within-subject SD. This made it possible to estimate how much of the variation in the measurements was caused by true differences between subjects (between-subject SD) with the remainder being caused by measurement error. This was then used to calculate the estimated repeatability coefficient by:

$$\textit{Repeatability coefficient} = 1.96 \times \sqrt{2} \times \textit{within\_subject SD} \text{ [126, 150].}$$

The repeatability coefficient estimates the maximum absolute difference between any two future measurements made by Rater 1 on 95% of occasions. 95% CI for the repeatability coefficient was calculated by multiplying the 95% CI limits of *within_subject SD* by 1.96 × √2 [126, 150].

The use of ANOVA model assumes constant SD throughout the true values, and that the measurement errors are independent of the true values [126, 151].

Intraclass correlation coefficient

Since repeated measurements made by one rater violates the assumption of independence, data generated by multiple measurements on a same subject is considered to be clustered. The amount of clustering can be analysed by using the intraclass correlation coefficient (ICC), defined as the ratio of the between-cluster variance to the total variance:

$$ICC = \frac{variance_{between}}{variance_{between} + variance_{within}} \text{ [128].}$$

This can also be expressed as:

$$ICC = \frac{SD^2_{true}}{SD^2_{true} + SD^2_{error}}$$

where SD_{true} is SD of the true values, and SD_{error} is SD of the measurement error [126]. The ICC values may range between 0 and 1, and with no variation within cluster ICC = 1. This means that high values of ICC represent strong reliability. For example, ICC of 0.80 can be interpreted as that 80% of the variability in the measurements is estimated to depend on true differences whereas 20% of the result variability is due to errors in the measurement process and the rater. The numerical values is somewhat subjectively interpreted, but a common suggested model is [152]:

ICC	Reliability
≥0.91	Excellent
0.76-0.90	Good
0.51-0.75	Moderate
≤0.50	Poor

It should be noted that reliability depends on the measurement errors and the true heterogenicity in the population studied, whereas repeatability (agreement between repeated measurements) in a characteristic of the method [126].

In this thesis, ICC estimates and their 95% CI were calculated based on a single-rating, absolute-agreement, two-way, mixed effects model to assess reliability of measurements by Rater 1 in **Paper IV.** Because some of the parameters were not normally distributed, the ICC and their 95% CI estimates for these parameters were calculated employing bootstrapping technique. This means that the observed data is analysed combined with a simulation of resampled data where repeated draws of new observations with replacement from the original data are generated to increase sample size [142].

“There are no bad ideas, Lemon,

only great ideas that go horribly wrong.”

Jack Donaghy

Results

Paper I

Patient characteristics

In total, 95 patients were included in the study, 61 male and 34 female. Thirty (32%) patients had quiescent Crohn's disease, and biological medication was used on 19 (63%) of them during the study period. Sixty-five (68%) patients with no history of Crohn's disease were included in the cryptoglandular fistula group. The median patient age at inclusion was 44 years (range: 10-80 years) in the total study population. Sixty-six (70%) patients had not been exposed to any fistula surgeries on curative attempt at study inclusion, meaning that 30% of the patients had a persisting or recurring fistula already when they went through their first AFP procedure. Detailed characteristics are summarised in Table 1.

Table 1. Characteristics of participants in the study presented in Paper I and Paper II.
Characteristics in total, the cryptoglandular and the Crohn's fistula groups.

Characteristic	Total (*N* = 95)	Crohn's group (*n* = 30)	Cryptoglandular group (*n* = 65)
Age	44 (10-80)	34 (10-66)	46 (21-80)
Gender			
Female	34 (36)	12 (40)	22 (34)
Male	61 (64)	18 (60)	43 (66)
Earlier procedure			
None	66 (70)	19 (63)	47 (72)
Fistulotomy	12 (13)	5 (17)	7 (11)
Advancement flap	3 (3)	-	3 (5)
Other	13 (14)	5 (17)	8 (13)
Unknown	1 (1)	1 (3)	-
Internal orifice			
Anterior	30(32)	7 (23)	23 (35)
Lateral	15 (16)	8 (27)	7 (11)
Posterior	49 (50)	15 (50)	34 (52)
Unknown	1(1)	-	1 (2)

Values are given as median (range) or n (%).

Follow-up

Four patients in the cryptoglandular fistula group and one patient in the Crohn's fistula group were lost to follow-up, leading to complete follow-up in 90 (95%) patients. The five patients were lost after 4-30 months.

The median duration of follow-up for all patients with AFP success was 110 months (range: 93-138 months).

AFP success

AFP success was reported in 28 (31%) patients after the first procedure, while AFP failure occurred in 62 (69%) patients. A second AFP procedure was performed in 37 patients after failure of the first AFP, success noted in five (14%) of these. AFP success was reported in one patient after a third operation. AFP procedure was performed more than twice in a total of 11 patients; no success was seen in patients receiving four or five AFPs. The treatment process and total results are summarised as an algorithm below (Figure 28).

Thus, after a median follow-up of 110 months (range 93-138 months), the overall healing rate was 38% (34/90).

There was no statistical difference between the outcomes of the AFP procedures performed during the first half of the study compared with the second half (χ^2 test, $P = 0.24$). The early procedures had success rate of 27% (20/73) and the late procedures had success rate of 19% (14/73).

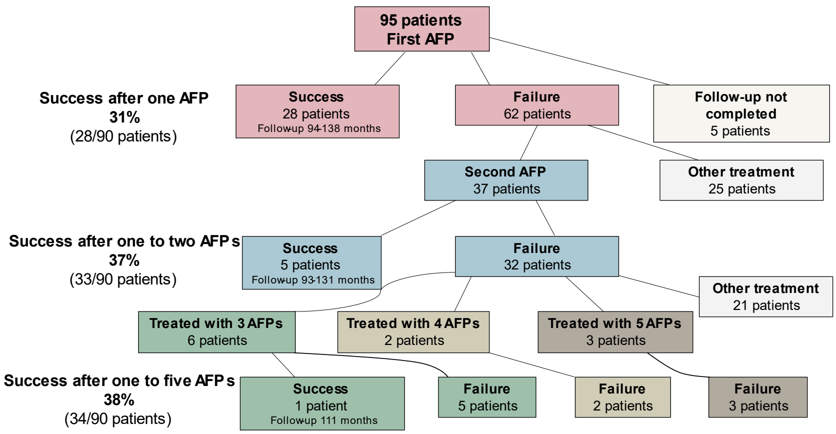


Figure 28 Treatment and result algorithm for Paper I.
Modified from doi.org/10.1111/codi.15429.

Treatment and result algorithms for the cryptoglandular fistula group (Figure 29) and the Crohn's fistula group (Figure 30) are presented below.

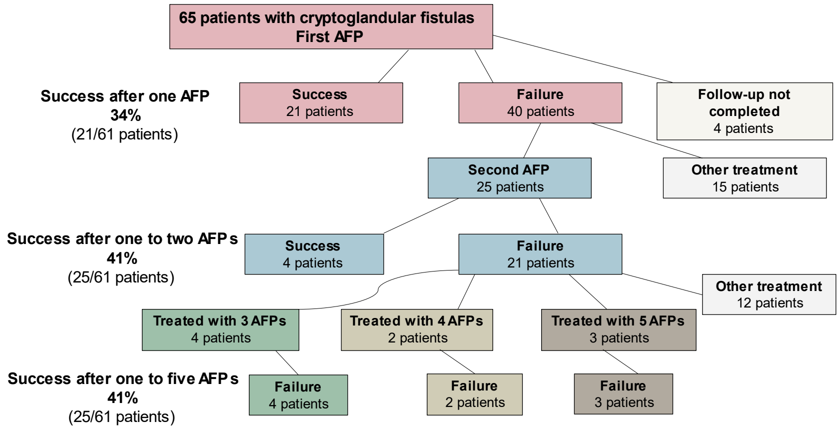


Figure 29 Treatment and result algorithm for patients in the cryptoglandular fistula group. Modified from doi.org/10.1111/codi.15429.

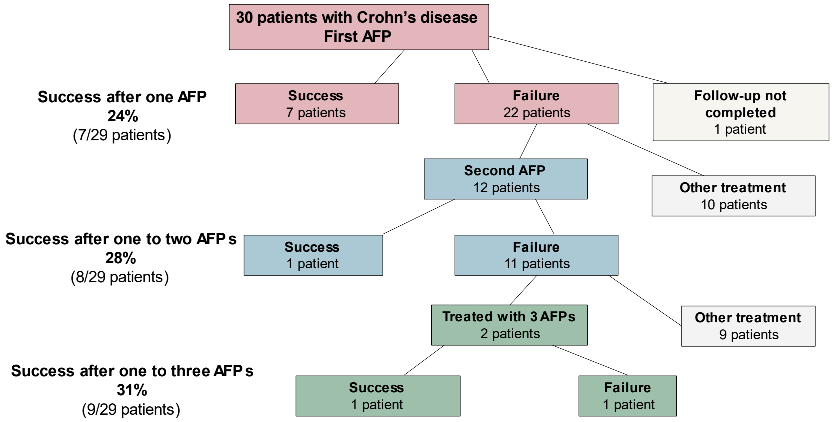


Figure 30 Treatment and result algorithm for patients in the Crohn's fistula group. Modified from doi.org/10.1111/codi.15429.

Cryptoglandular vs Crohn's fistulae

Success rate per plug in the cryptoglandular fistula group (103 AFP procedures) was 24 % (25 AFPs with success, 78 AFPs with failure). Success rate per plug in the Crohn's fistula group (43 AFP procedures) was 21% (9 success, 34 failure).

Kaplan-Meier estimates for success rate over time for the 146 AFP procedures included in the study are presented in Figure 31. A log-rank test showed no statistically significant difference between the groups ($P = 0.37$).

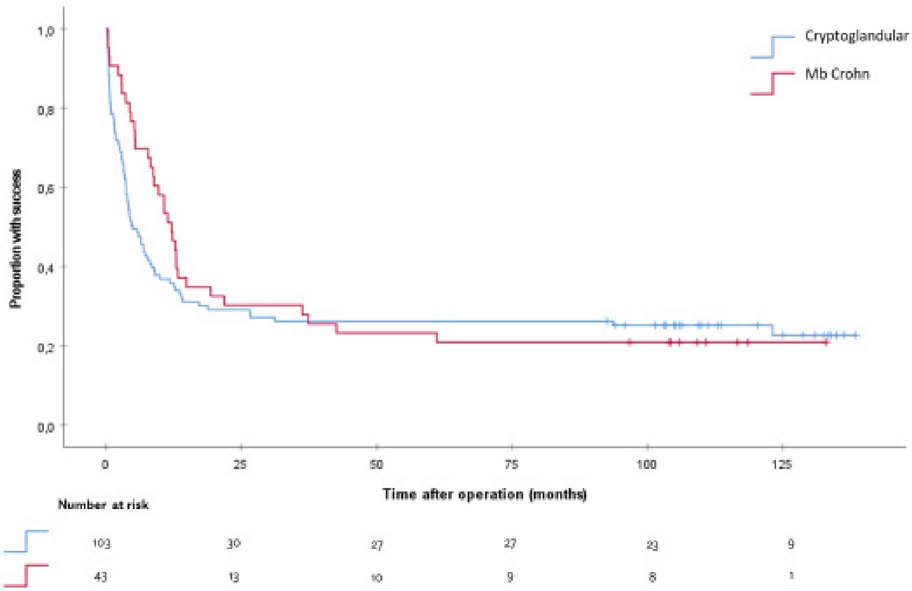


Figure 31 Kaplan-Meier estimates for success rate over time for the 146 AFP procedures included in the study: 103 in the cryptoglandular fistula group and 43 in the Crohn's fistula group.
A log-rank test showed no statistically significant difference between success rate distributions ($P = 0.37$). Mb Crohn: Crohn's fistula group. Modified from doi.org/10.1111/codi.15429.

AFP as the first definitive treatment

Of the total of 146 AFP procedures performed on patients included in the study, 62 operations were performed as the first attempt at definitive fistula treatment. Success rate per plug in this group was 37% (23 success, 39 failure). Succes rate per plug in the group of 84 procedures performed for recurring or persisting fistula after any type of fistula surgery was 13% (11 success, 73 failure). A χ^2 test showed that the difference was statistically significant ($P < 0.001$).

Discharge as a prognostic factor

The clinical symptom of presence or absence of fluid discharge through the EO three months postoperatively was documented for 113 AFP procedures (missing for one patient with AFP success). Discharge at three months was reported in 68 cases with success rate per plug at 10% (7 success, 61 failure). No discharge was documented in 45 cases with success rate per plug at 58% (26 success, 19 failure). The difference between these groups was statistically significant (*P < 0.001)*.

The presence of discharge three months postoperatively had 76% sensitivity and 77% specificity for AFP failure. The positive predictive value (PPV) for AFP failure was 90%, and the negative predictive value (NPV) was 58%.

Paper II

Patient characteristics

Since **Paper I** and **Paper II** are based on the same patient cohort, the main characteristics of the patients studied in **Paper II** can be seen in Table 1.

3D EAUS parameters

The 3D EAUS findings identified as relevant for the different time points in the exploratory analysis of the examinations of ten randomly selected patients are listed in Table 2.

Table 2. 3D EAUS parameters identified as relevant for different timepoints.
Based on the exploratory analysis of 10 random patients.

2 weeks	3 months	Late control
Gas	Gas	Gas
Residual fluid	Residual fluid	Residual fluid
Inflammation	Inflammation	-
Visible sutures	Visible sutures	-
-	Visible fistula	Visible fistula

Examples of relevant 3D EAUS findings are shown in Figures 32 and 33.

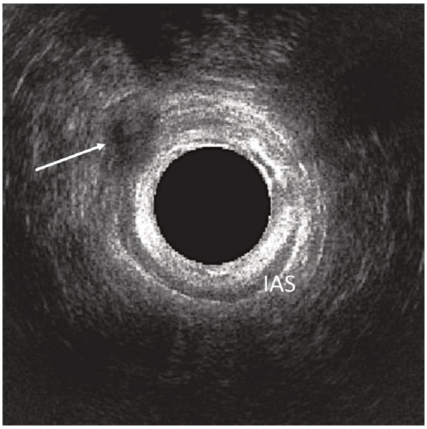


Figure 32 3D EAUS in axial plane after the AFP procedure. The visible fistula is seen as a hypoechogenic area (white arrow).
IAS: Internal anal sphincter. Reproduced from doi.org/10.1080/00365521.2023.2212310.

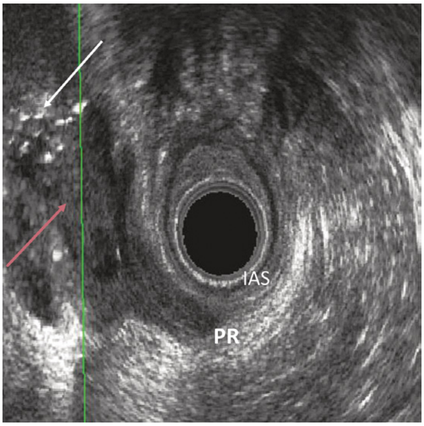


Figure 33 3D EAUS in multiplanar reconstruction after the AFP procedure. Strongly hyperechogenic dots (white arrow) represent gas bubbles in the fistula. Inflammation is seen as a slightly hyperechogenic area (red arrow).
IAS: Internal anal sphincter, PR: Puborectalis muscle. Reproduced from 10.1080/00365521.2023.2212310.

Postoperative 3D EAUS

The relevant 3D EAUS parameters and their relationship to the long-term results of the AFP procedure are summarised in Table 3.

Table 3. The relevant 3D EAUS parameters related to the results of the AFP procedure as identified in the long-term follow-up.

3D EAUS parameter	Time	Total	Failure	Success	Not possible to analyse	EAUS missing	*P*
Gas	2 weeks	65/121 (54)	49/94 (52)	16/27 (59)	20/141 (14)	5/146 (3)	0.66
	3 months	55/98 (56)	47/71 (66)	8/27 (30)	14/112 (13)	7/119 (6)	*0.0014*
	Late control	35/64 (55)	32/43 (74)	3/21 (14)	13/70 (19)	7/77 (9)	*<0.001*
Inflammation	2 weeks	71/125 (57)	57/97 (59)	14/28 (50)	16/141 (11)	5/146 (3)	0.52
	3 months	51/98 (52)	43/71 (61)	8/27 (30)	14/112 (13)	7/119 (6)	*0.0072*
Visible fistula	3 months	89/98 (91)	69/73 (95)	20/25 (80)	14/112 (13)	7/119 (6)	*0.045*
	Late control	52/64 (81)	43/45 (96)	9/19 (47)	6/70 (9)	7/77 (9)	*<0.001*

Values are given as *n*(positive parameter)/*n*(plugs in group) and (%). *P* values based on comparisons made by Fisher's exact test.

None of the parameters were statistically significant for AFP failure at 2 weeks. Gas in the fistula, surgical site inflammation, and a visible fistula three months postoperatively were significantly associated with failure. Gas in the fistula and visible fistula at the late control were statistically significant findings for AFP failure.

Neither visible sutures nor residual fluid in the fistula tract were statistically significant parameters for AFP failure.

The diagnostic probabilities for AFP failure based on different 3D EAUS parameters are shown in Table 4.

Table 4. The diagnostic probabilities for AFP failure based on 3D EAUS findings.

3D EAUS parameter	Time	Sensitivity	Specificity	PPV	NPV
Gas	3 months Late control	66% 74%	70% 86%	85% 91%	44% 62%
Inflammation	3 months	61%	70%	84%	40%
Visible fistula	3 months Late control	95% 96%	20% 53%	76% 83%	56% 83%

PPV: positive predictive value, NPV: negative predictive value.

The parameters found to be statistically significant by using the Fischer's exact test were analysed by using a logistic regression model (Table 5).

Table 5. Odds ratio (OR) for AFP failure based on logistic regression model analysis of the relevant 3D EAUS findings.

3D EAUS parameter	Time	*P*	OR	95% CI
Gas	3 months Late control	*0.0017* *<0.001*	4.65 17.46	1.78-12.16 4.30-70.86
Inflammation	3 months	*0.0078*	3.65	1.41-9.46
Visible fistula	3 months Late control	*0.0042* *<0.001*	4.31 23.89	1.06-17.59 4.45-128.13

95% CI: 95% confidence interval.

AFP failure: clinical symptoms and 3D EAUS

In this part of the study, the clinical symptom of fluid discharge through EO three months postoperatively (discussed in **Paper I**) was analysed together with the 3D EAUS parameter of the presence of gas in the fistula tract at the same time point. In 47 AFP procedures (43 failure, 4 success), the patient reported fluid discharge and gas was detected in the fistula tract. Meanwhile, 19 AFP procedures (15 success, 4 failure) were negative for both. This relationship between 'the double positive plugs' and 'the double negative plugs' was statistically significant. The results are presented in Figure 34.

The presence of both gas and discharge three months postoperatively had 91% sensitivity and 79% specificity for AFP failure. The PPV for AFP failure was 91% and the NPV was 79%.

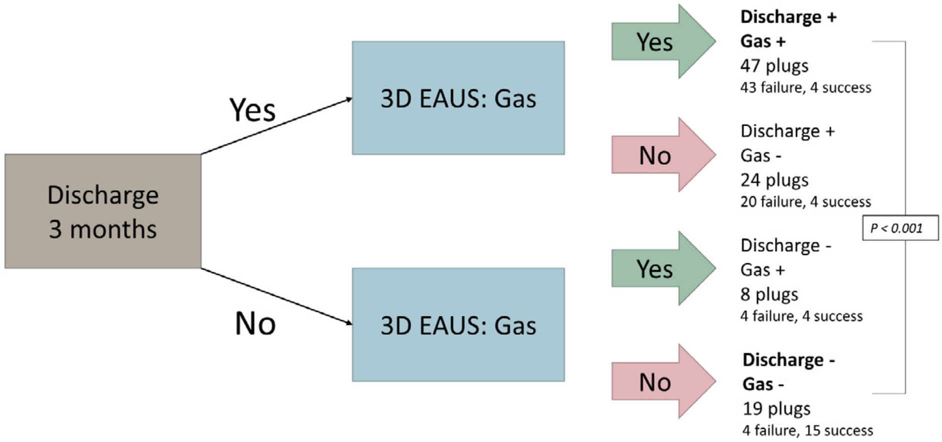


Figure 34 Algorithm for the relationship between fluid discharge through the EO combined with 3D EAUS parameter of gas in fistula three months postoperatively analysed in relation to AFP failure for 'the double negative plugs' and 'the double negative plugs'.

P-value based on comparison made by Fischer's exact test.

Reproduced from doi.org/10.1080/00365521.2023.2212310

Paper III

Patient characteristics

In total, 111 consecutive patients were initially included. Figure 35 shows the flow diagram of the exclusion of patients from the study, which resulted in the analysis of data from 93 patients. Sixty-four (69%) patients were male and 29 (31%) were female. The median age of patients was 43 years (range: 17-79 years).

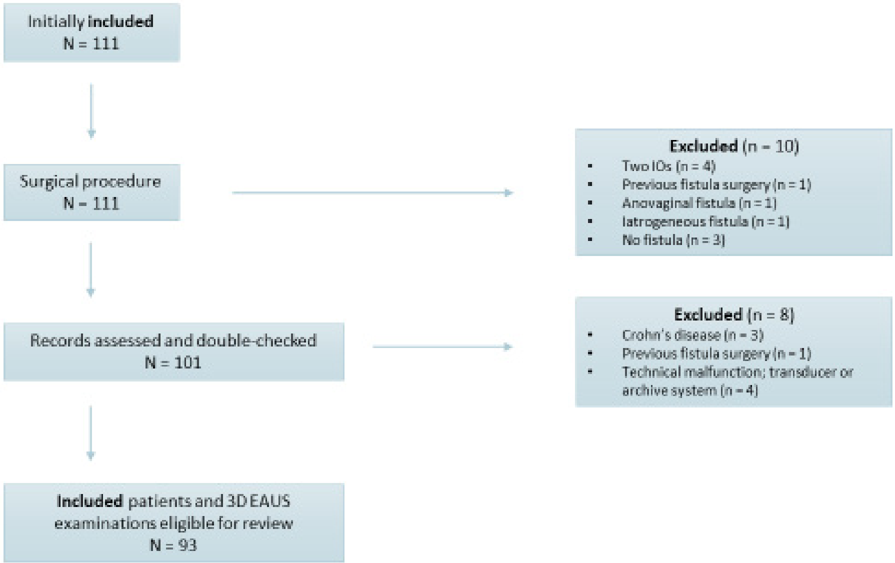


Figure 35 Exclusion flowchart for patients in Paper III and Paper IV.

Complete assessment: 3D EAUS and intraoperative findings

Complete assessment of patients was based on both 3D EAUS examinations (transducers 2052 and 8838) in combination with surgical findings during the procedure ('gold standard'). The distribution of these parameters is shown in Table 6 (page 80). Eighty (86%) fistulae were classified as either subcutaneous or low.

3D EAUS assessment

The overall results of the 3D EAUS assessment by three raters with different levels of experience are included in Table 6 and presented separately for the two transducers, 2052 and 8838.

The specialist defined 75 (81%) and 68 (74%) fistulae as subcutaneous or low using transducers 2052 and 8838, respectively. The consultant assessed this type of fistulae in 70 (75%) and 62 (66%) patients using transducers 2052 and 8838, respectively. The senior consultant identified 73 (79%) subcutaneous or low fistulae using transducer 2052, and 78 (84%) using transducer 8838.

Table 6. Fistula parameters according to complete assessment (Gold standard) and 3D EAUS assessment by three raters.

Parameter		Gold standard	Specialist 2052	Specialist 8838	Consultant 2052	Consultant 8838	Senior consultant 2052	Senior consultant 8838
IO Orientation	Anterior	43 (46)	41 (44)	44 (47)	46 (50)	49 (53)	39 (42)	46 (50)
	Posterior	38 (41)	37 (40)	37 (40)	39 (42)	38 (41)	36 (39)	37 (40)
	Lateral left	4 (4)	6 (7)	3 (3)	3 (3)	1 (1)	4 (4)	3 (3)
	Lateral right	8 (9)	5 (5)	7 (8)	3 (3)	5 (5)	7 (8)	7 (8)
	Not rated	-	4 (4)	2 (2)	2 (2)	-	7 (8)	-
Orientation through EAS	Anterior	35 (38)	39 (42)	38 (41)	36 (39)	46 (50)	18 (19)	30 (32)
	Posterior	32 (34)	37 (40)	33 (36)	31 (33)	36 (39)	23 (25)	24 (26)
	Lateral left	9 (10)	6 (7)	12 (13)	3 (3)	4 (4)	3 (3)	5 (5)
	Lateral right	8 (9)	4 (4)	5 (5)	3 (3)	5 (5)	3 (3)	5 (5)
	Does not pass	9 (10)	3 (3)	4 (4)	18 (19)	2 (2)	39 (42)	29 (31)
	Not rated	-	4 (4)	1 (1)	2 (2)	-	7 (8)	-
Fistula height	Subcutaneous	23 (25)	6 (7)	6 (7)	19 (20)	3 (3)	46 (50)	45 (48)
	Low	57 (61)	69 (74)	62 (67)	51 (55)	59 (63)	27 (29)	33 (36)
	High	12 (13)	12 (13)	21 (23)	17 (18)	27 (29)	11 (12)	14 (15)
	Ultra-high	1 (1)	2 (2)	3 (3)	4 (4)	4 (4)	2 (2)	1 (1)
	Not rated	-	4 (4)	1 (1)	2 (2)	-	7 (8)	-
Fistula length	<3 cm	50 (54)	28 (30)	41 (44)	70 (75)	37 (40)	70 (75)	56 (60)
	≥3 cm	43 (46)	11 (12)	22 (24)	20 (22)	54 (58)	19 (20)	37(40)
	Not rated	-	55 (58)	32 (30)	3 (3)	2 (2)	4 (4)	-

Values are given as *n* (%).

IO: Internal fistula orifice, EAS: External anal sphincter.

Complete assessment vs 3D EAUS assessment

The results of the agreement analysis between the raters and the gold standard are summarised in Table 7 (next page).

IO was completely misidentified (opposite sector compared with the gold standard) six (7%) times by the specialist and once (1%) by the consultant using transducer 2052. This occurred three times (3%) for the specialist using transducer 8838. All other IOs were identified either in the correct sector or in the adjacent one with 100% accuracy.

Table 8 shows the diagnostic probabilities for identification of subcutaneous or low fistulae for each rater.

Table 8. 3D EAUS diagnostic probabilities for identifying the lowest fistulas (subcutaneous and low) for both transducers and each rater compared to the gold standard.

Transducer	Rater	Sensitivity	Specificity	PPV	NPV
2052	Specialist	90%	50%	92%	43%
	Consultant	86%	77%	96%	48%
	Senior consultant	93%	67%	95%	62%
8838	Specialist	82%	77%	96%	42%
	Consultant	78%	100%	100%	42%
	Senior consultant	93%	69%	95%	60%

PPV: positive predictive value, NPV: negative predictive value.

Table 7. 3D EAUS assessment compared to the gold standard, analysed by Cohen's kappa. Agreement implies the proportion of ratings coherent with the gold standard. 'Low fistula' summarises the two lowest fistula categories (subcutaneous and low).

Transducer	Parameter	Specialist		Consultant		Senior consultant	
		Kappa (95% CI)	Agreement (%)	Kappa (95% CI)	Agreement (%)	Kappa (95% CI)	Agreement (%)
2052	IO Orientation	0.63 (0.49-0.76)	69/89 (78)	0.81 (0.71-0.92)	81/91 (89)	0.85 (0.75-0.95)	78/86 (91)
	Orientation through EAS	0.52 (0.38-0.65)	60/89 (67)	0.50 (0.38-0.63)	59/91 (65)	0.44 (0.32-0.55)	48/86 (56)
	Fistula height^	0.23 (0.05-0.41)	47/83 (57)	0.27 (0.11-0.43)	48/90 (53)	0.31 (0.16-0.45)	40/86 (47)
	Fistula length	0.28 (0.0-0.58)	26/39 (67)	0.18 (0.0-0.36)	55/90 (61)	0.25 (0.07-0.42)	57/89 (64)
	Low fistula^	0.42 (0.15-0.69)	71/83 (86)	0.52 (0.30-0.75)	77/90 (86)	0.58 (0.33-0.83)	77/86 (90)
8838	IO Orientation	0.78 (0.67-0.89)	79/91 (87)	0.82 (0.71-0.92)	83/93 (90)	0.86 (0.76-0.95)	85/93 (91)
	Orientation through EAS	0.54 (0.42-0.67)	63/93 (68)	0.60 (0.48-0.72)	68/93 (73)	0.44 (0.32-0.56)	54/93 (58)
	Fistula height^	0.29 (0.14-0.45)	50/89 (56)	0.34 (0.21-0.47)	55/93 (59)	0.44 (0.30-0.58)	56/93 (60)
	Fistula length	0.49 (0.27-0.71)	48/63 (76)	0.35 (0.17-0.53)	61/91 (67)	0.35 (0.16-0.54)	63/93 (68)
	Low fistula^	0.46 (0.25-0.67)	73/89 (82)	0.49 (0.31-0.67)	75/93 (81)	0.58 (0.35-0.81)	83/93 (89)

^: Analysed by linear weighted Cohen's kappa, data for fistula height excluded for examinations with completely wrong IO orientation.

IO: Internal fistula orifice, EAS: External anal sphincter.

Interobserver agreement between three raters

Analysis of Fleiss' kappa showed overall good agreement between the three raters for the orientation of IO using transducer 2052, κ = 0.73 (95% CI: 0.63-0.83), and very good agreement using transducer 8838, κ = 0.84 (95% CI: 0.74-0.93). Overall agreement for fistula length was moderate for transducers 2252 and 8838, κ = 0.48 (95% CI: 0.30-0.67) and κ = 0.47 (95% CI: 0.33-0.61), respectively. Moderate overall agreement was noted for identification of the lowest fistulae (subcutaneous and low), with κ = 0.47 (95% CI: 0.34-0.59) for transducer 2052, and κ = 0.52 (95% CI: 0.40-0.64) for transducer 8838.

Hotelling's T^2-test comparing κ-values between raters was statistically significant for transducer 2052 (P = *0.006*) and not significant for transducer 8838 (P = 0.90). No statistically significant difference in κ was found between raters for the ability to detect the lowest fistulae (P = 0.32 and P = 0.93 for 2052 and 8838, respectively).

Agreement between different transducers

Cohen's kappa analysis showed good agreement, κ = 0.64 (95% CI: 0.50-0.78), for IO orientation between transducers 2052 and 8838 when the examinations were assessed by the specialist. The scores of the consultant and senior consultant showed very good agreement for IO orientation, with κ = 0.88 (95% CI: 0.79-0.97) and κ = 0.90 (95% CI: 0.82-0.98), respectively.

The results of Hotelling's T^2 comparisons between transducers 2052 and 8838 are shown in Table 9.

Table 9. Comparisons of κ-values between transducers 2052 and 8838 for the raters.

Parameter	*P*-value Specialist	Consultant	Senior consultant
IO Orientation	*0.004*	0.53	0.56
Low fistula	0.53	0.13	0.55

Analysed by pairwise dependent Hotelling's T^2 with the delta method.

Figures 36 and 37 show examples of 3D EAUS examinations performed on the same patients using the two different transducers.

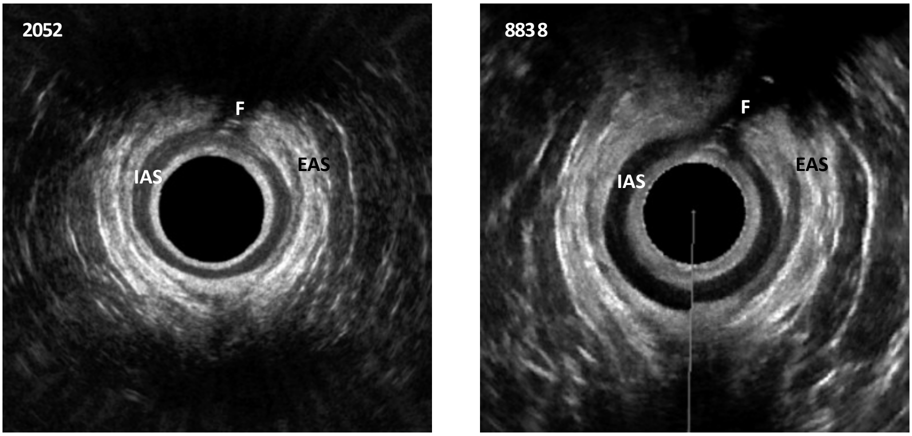


Figure 36 Axial 3D EAUS images of an anterior fistula in female acquired with transducers 2052 and 8838.
IAS: Internal anal sphincter, EAS: External anal sphincter, F: Fistula.

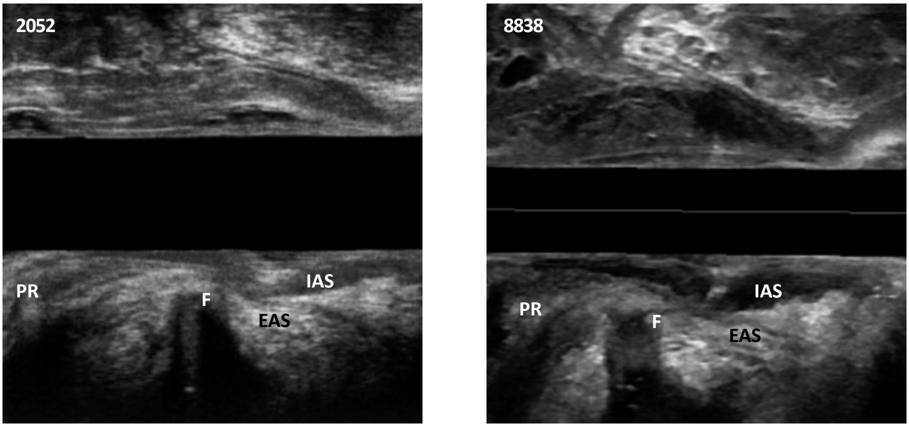


Figure 37 Sagittal 3D EAUS images of a posterior fistula in male acquired with transducers 2052 and 8838.
IAS: Internal anal sphincter, EAS: External anal sphincter, PR: puborectal muscle, F: Fistula.

Intraobserver agreement

The results of repeated ratings by the consultant are summarised in Table 10. All IOs were identified in either the correct or the adjacent sector with 100% accuracy.

Table 10. Results of repeated 3D EAUS assessment by the consultant, analysed by Cohen's kappa. Agreement implies the proportion of ratings coherent with each other. 'Low fistula' summarises the two lowest fistula categories (subcutaneous and low).

Transducer	Parameter	Kappa (95% CI)	Agreement (%)
2052	IO Orientation	0.84 (0.73-0.94)	83/89 (93)
	Fistula length	0.70 (0.53-0.87)	78/88 (89)
	Low fistula^	0.56 (0.37-0.75)	73/89 (82)
8838	IO Orientation	0.79 (0.68-0.90)	82/93 (88)
	Fistula length	0.43 (0.25-0.61)	65/91 (71)
	Low fistula^	0.56 (0.38-0.73)	74/93 (80)

^: Analysed by linear weighted Cohen's kappa, IO: Internal fistula orifice, EAS: External anal sphincter.

Paper IV

Patient characteristics

In total, the same 93 patients included in **Paper III** were analysed in this study. The detailed characteristics are summarised in Table 11.

Table 11. Characteristics of the participants in the study presented in Paper III and Paper IV.

Characteristic	Total (*N* = 93)	Female (*n* = 29)	Male (*n* = 64)
Age	43 (17-79)	45 (21-79)	41 (17-71)
Length (m)	1.74 (1.51-1.91)	1.67 (1.51-1.80)	1.77 (1.56-1.91)
BMI	27.4 (18.3-44.5)	26.5 (19.6-44.5)	27.5 (18.3-41.6)
Weight (kg)	84 (53-141)	75 (53-141)	86 (54-129)
Parity status			-
Nulliparous	6 (6)	6 (21)	
≥1 Vaginal delivery	19 (20)	19 (66)	
Caesarean section	2 (2)	2 (7)	
Unknown	2 (2)	2 (7)	

Values are given as median (range) or n (%).

Anal canal length

Boxplots of the three measurements for each length parameter for both genders and transducers are presented in Figures 38 and 39.

The overall descriptive statistics for the overall mean length of the anal canal are summarised in Table 12. Measurements of the anterior length of the anal canal were significantly shorter in female with transducers 2052 and 8838 than in male ($P<0.001$). The length of the posterior anal canal with transducer 8838 measured by IAS was shorter in male than in female, and the difference was statistically significant ($P = 0.027$).

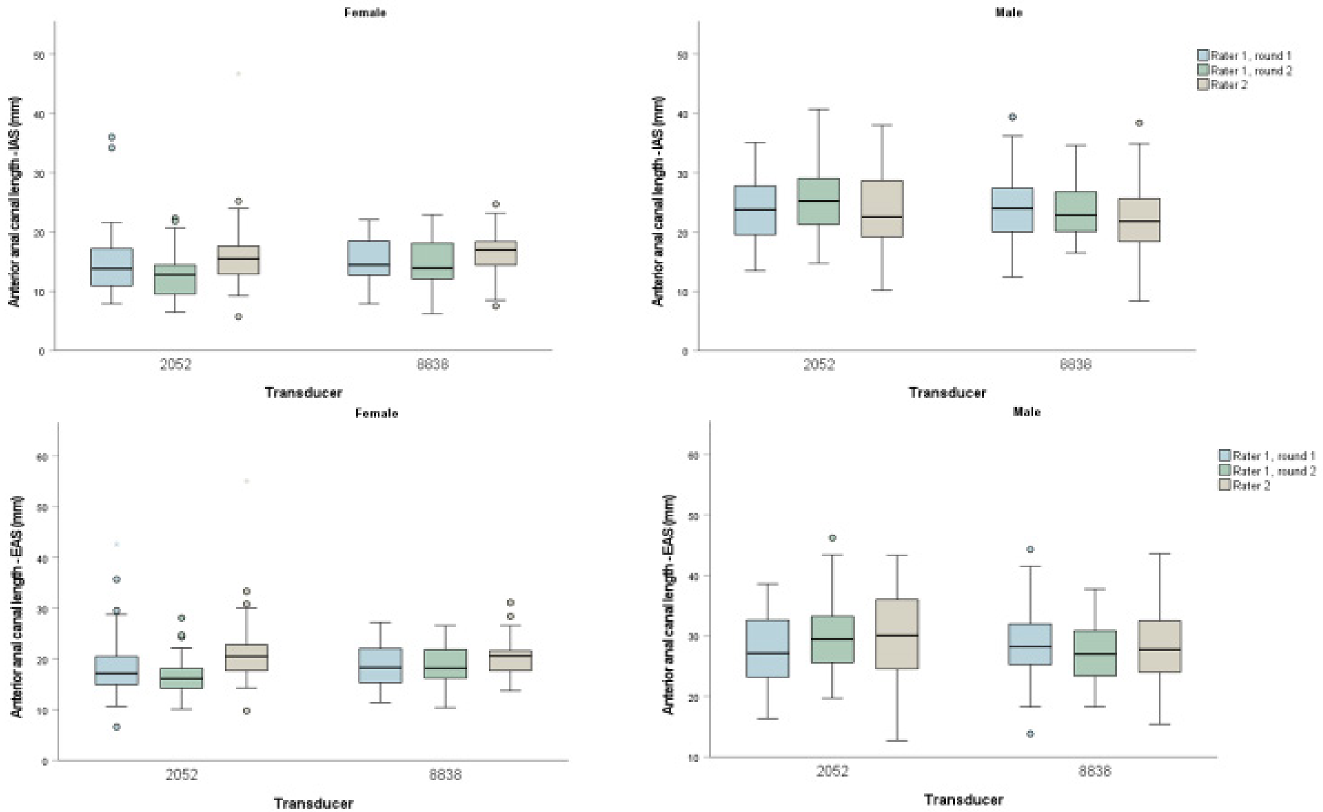


Figure 38 Clustered boxplots of the anterior length of the anal canal (measured by IAS and EAS) by Rater 1 and Rater 2 with transducers 2052 and 8838.

IAS: Internal anal sphincter, EAS: External anal sphincter.

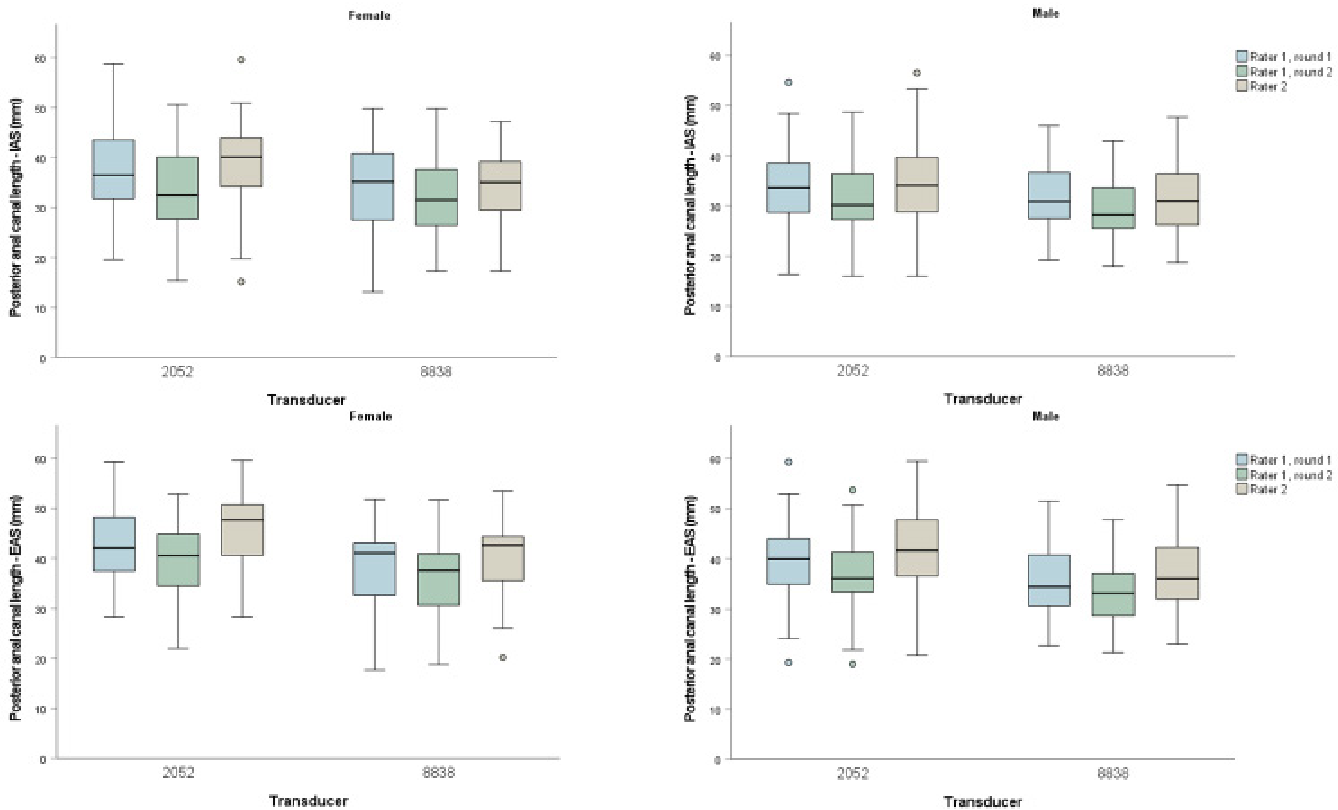


Figure 39 Clustered boxplots of the posterior length of the anal canal (measured by IAS and EAS) by Rater 1 and Rater 2 with transducers 2052 and 8838.

IAS: Internal anal sphincter, EAS: External anal sphincter.

Table 12. Anal canal length parameters presented as the overall mean values.

	Female Transducer			Male Transducer			Female vs male *P*	
Parameter	2052	8838	*P*	2052	8838	*P*	2052	8838
Mean (mm)								
Anterior – IAS	15.4	15.5	0.48[a]	24.1	23.2	0.20[a]	*<0.001*[b]	*<0.001*[b]
Anterior – EAS	19.9	19.2	0.69[a]	29.2	28.2	0.11[c]	*<0.001*[b]	*<0.001*[c]
Posterior – IAS	36.3	33.9	*0.039*[c]	33.6	30.6	*<0.001*[c]	0.13[c]	0.027[c]
Posterior – EAS	43.1	39.0	*<0.001*[c]	40.2	36.0	*<0.001*[c]	0.067[c]	0.056[c]
Median (mm)								
Anterior – IAS	14.1	15.3		24.1	22.6			
Anterior – EAS	18.6	19.3		29.9	27.7			
Posterior – IAS	38.1	35.5		32.8	30.4			
Posterior – EAS	44.3	41.7		39.7	35.4			
SD (mm)								
Anterior – IAS	5.07	3.97		5.26	4.61			
Anterior – EAS	5.82	3.43		5.08	4.88			
Posterior – IAS	7.94	7.33		7.52	6.02			
Posterior – EAS	6.71	7.67		6.98	6.61			
5th percentile (mm)								
Anterior – IAS	8.8	7.9		16.2	16.2			
Anterior – EAS	12.6	12.9		21.2	20.3			
Posterior – IAS	19.2	19.0		21.8	21.0			
Posterior – EAS	29.0	22.6		29.7	25.9			
95th percentile (mm)								
Anterior – IAS	29.0	23.1		32.3	33.6			
Anterior – EAS	36.5	27.3		37.1	38.1			
Posterior – IAS	47.4	43.2		48.4	41.2			
Posterior – EAS	52.4	49.5		52.5	49.4			

[a]: Wilcoxon signed rank test, [b]: Mann-Whitney *U*-test, [c]: Student's *t*-test.
IAS: Internal anal sphincter, EAS: External anal sphincter.

Reproducibility: agreement between two raters

The Bland-Altman plots generated and utilised to assess agreement between raters are provided in Appendix (Figures A1-A8). The resulting mean differences in absolute lengths for parameters with uniform difference distributions together with limits of agreement are listed in Table 13. Mean of length ratios for parameters with variability in difference distributions together with limits of agreement are listed in Table 14.

Differences were statistically significant for posterior EAS measurement with transducer 2052 and for anterior IAS and EAS measurements with transducer 8838 in female. In male, the differences were statistically significant for anterior and posterior EAS measurements with transducer 2052 and for anterior IAS and posterior EAS measurements with transducer 8838.

For transducer 2052, Rater 1 tended to give shorter measurements than Rater 2. In four of the eight comparisons, this bias was statistically significant. The tendences were more mixed with transducer 8838. We have interpreted this as a demonstration of how difficult it is to identify the correct measurement points.

Table 13. Mean of paired differences (mm) between measurements in anal canal length by the two raters ($length_{Rater1round1} - length_{Rater2}$) and limits of agreement (mm) with 95% confidence interval (CI).

Parameter	2052 Mean Difference	Low limit of agreement (95% CI)	High limit of agreement (95% CI)	8838 Mean Difference	Low limit of agreement (95% CI)	High limit of agreement (95% CI)
Female						
Anterior – IAS	*Table 14*			-1.2	-6.0 (-6.6 - -5.3)	3.7 (3.0 - 4.3)
Anterior – EAS	*Table 14*			-1.8	-6.7 (-7.3 - -6.0)	3.1 (2.4 – 3.7)
Posterior – IAS	*Table 14*			*Table 14*		
Posterior – EAS	*Table 14*			1.5	-10.6 (-12.2 - -9.0)	13.6 (12.0 – 15.2)
Male						
Anterior – IAS	-0.0	-12.7 (-13.8 - -11.6)	12.6 (11.5 – 13.7)	2.2	-11.8 (-13.0 - -10.6)	16.1 (14.9 – 17.3)
Anterior – EAS	-2.1	-17.5 (-18.9 - -16.1)	13.4 (12.0 – 14.7)	0.2	-14.8 (-16.2 - -13.5)	15.3 (14.0 – 16.6)
Posterior – IAS	*Table 14*			0.5	-9.0 (-9.9 - -8.2)	9.9 (9.1 – 10.8)
Posterior – EAS	*Table 14*			-1.4	-11.1 (-12.0 - -10.2)	8.4 (7.5 – 9.3)

IAS: Internal anal sphincter, EAS: External anal sphincter.

Table 14. Ratio of paired differences between measurements in anal canal length by the two raters ($length_{Rater1round1}$ / $length_{Rater2}$) and limits of agreement with 95% confidence interval (CI).

Parameter	2052 Difference Ratio	Low limit of agreement (95% CI)	High limit of agreement (95% CI)	8838 Difference Ratio	Low limit of agreement (95% CI)	High limit of agreement (95% CI)
Female						
Anterior – IAS	0.93	0.40 (0.36 – 0.45)	2.15 (1.93 – 2.41)	*Table 13*		
Anterior – EAS	0.86	0.38 (0.34 – 0.42)	1.95 (1.75 – 2.18)	*Table 13*		
Posterior – IAS	0.98	0.73 (0.70 – 0.76)	1.30 (1.25 – 1.35)	0.98	0.64 (0.61 - 0.68)	1.50 (1.42 - 1.60)
Posterior – EAS	0.94	0.74 (0.72 – 0.76)	1.20 (1.16 – 1.23)	*Table 13*		
Male						
Anterior – IAS	*Table 13*			*Table 13*		
Anterior – EAS	*Table 13*			*Table 13*		
Posterior – IAS	0.97	0.70 (0.68 – 0.72)	1.34 (1.30 – 1.38)	*Table 13*		
Posterior – EAS	0.94	0.70 (0.68 – 0.72)	1.26 (1.22 – 1.29)	*Table 13*		

IAS: Internal anal sphincter, EAS: External anal sphincter.

Repeatability of measurements by one rater

The results of the ANOVA models for repeated measurements by Rater 1 are presented in Table 15.

Reproducibility: agreement between transducers 2052 and 8838

The Bland-Altman plots generated and utilised to assess agreement between transducers 2052 and 8838 can be found in Appendix (Figures A9-A16). The resulting mean differences in absolute lengths are listed in Table 16 along with limits of agreement.

The differences in length measurements between transducer 2052 and transducer 8838 were statistically significant for female for the posterior EAS measurement by Rater 1 and for both posterior measurements by Rater 2. For male, both posterior measurements by both raters were significantly longer with transducer 2052.

Table 15. Agreement for repeated measurements by Rater 1 including within-subject SD (mm), estimated repeatability coefficient (mm) with 95% CI and ICC with 95% CI.

		2052			8838	
Parameter	**SD**	**Repeatability**	**ICC (95% CI)**	**SD**	**Repeatability**	**ICC (95% CI)**
Female						
Anterior – IAS	4.79	13.3 (10.5-18.0)	0.31 (0.03-0.61)[b]	1.87	5.2 (4.2-7.1)	0.82 (0.66-0.92)
Anterior – EAS	4.96	13.8 (11.1-19.0)	0.38 (0.02-0.64)[b]	1.73	4.8 (3.9-6.6)	0.81 (0.66-0.92)
Posterior – IAS	2.86	7.9 (6.3-10.7)	0.88 (0.48-0.96)	3.68	10.2 (8.1-13.8)	0.79 (0.53-0.89)[b]
Posterior – EAS	3.40	9.4 (7.6-13.1)	0.80 (0.44-0.96)	3.85	10.7 (8.5-14.4)	0.78 (0.46-0.90)
Male						
Anterior – IAS	3.33	9.2 (7.82-11.06)	0.65 (0.48-0-78)	3.69	10.2 (8.7-12.2)	0.52 (0.30-0.67)[b]
Anterior – EAS	3.65	10.1 (8.6-12.1)	0.61 (0.38-0.78)[b]	3.54	9.8 (8.2-11.6)	0.59 (0.39-0.75)[b]
Posterior – IAS	3.06	8.5 (7.1-10.1)	0.85 (0.75-0.91)[b]	2.44	6.8 (5.7-8.1)	0.85 (0.40-0.94)
Posterior – EAS	3.85	9.0 (7.6-10.7)	0.82 (0.46-0.92)	2.84	7.9 (6.7-9.4)	0.83 (0.40-0.93)

IAS: Internal anal sphincter, EAS: External anal sphincter. [b]: analysed by bootstrapping method.

Table 16. Mean of paired differences (mm) between measurements in anal canal length by transducers 2052 and 8838 ($length_{2052} - length_{8838}$) and limits of agreement with 95% confidence interval (CI) for Rater 1 and Rater 2.

Parameter	Rater 1 Mean difference	Low limit of agreement (95% CI)	High limit of agreement (95% CI)	Rater 2 Mean difference	Low limit of agreement (95% CI)	High limit of agreement (95% CI)
Female						
Anterior – IAS	0.2	-12.3 (-13.9 - -10.7)	12.7 (11.0 – 14.3)	0.1	-13.0 (-14.7 - -11.3)	13.2 (11.4 – 14.9)
Anterior – EAS	0.6	-13.4 (-15.3 - -11.6)	14.5 (12.7 – 16.4)	1.4	-14.7 (-16.8 - -12.6)	17.5 (15.4 – 19.6)
Posterior – IAS	2.8	-15.1 (-17.4 - -12.7)	20.7 (18.3 – 23.0)	4.1	-9.7 (-11.6 - -7.9)	18.0 (16.1 – 19.8)
Posterior – EAS	4.4	-12.1 (-14.3 - -9.9)	20.9 (18.7 – 23.1)	5.6	-5.9 (-7.4 - -4.4)	17.1 (15.5 – 18.6)
Male						
Anterior – IAS	-0.6	-11.4 (-12.3 - -10.4)	10.2 (9.2 – 11.1)	1.6	-12.9 (-14.2 - -11.7)	16.1 (14.8 – 17.4)
Anterior – EAS	-0.6	-11.8 (-12.8 - -10.8)	10.5 (9.6 – 11.5)	1.7	-14.0 (-15.4 - -12.6)	17.3 (16.0 – 18.7)
Posterior – IAS	2.4	-8.9 (-9.9 - -7.9)	13.7 (12.7 – 14.7)	3.8	-10.0 (-11.2 - -8.7)	17.5 (16.3 – 18.7)
Posterior – EAS	3.7	-8.2 (-9.3 - -7.1)	15.7 (14.6 – 16.7)	5.0	-9.0 (-10.2 - -7.8)	19.0 (17.8 – 20.2)

IAS: Internal anal sphincter, EAS: External anal sphincter.

Discussion

The aim of this thesis was to investigate the long-term outcome of the AFP procedure and to explore the utilisation of 3D EAUS for the assessment and follow-up of anal fistulae. We report that the long-term success rate of the AFP was 38%. Our study was the first to report the most common morphological findings on postoperative 3D EAUS that can be utilised for AFP follow-up.

Our results show that 3D EAUS can be mastered with more than satisfactory results, even at early stages of the surgical career, to identify fistulae suitable for fistulotomy. The thesis also pinpoints challenges of measuring anal canal length in 3D EAUS.

Sphincter-preserving procedures

When the study plan for **Paper I** and **Paper II** was established, AFP was an emerging and exciting bioprosthetic alternative for the treatment of complex fistulae. Over the course of the study, many lessons were learned by both our research group and proctologists and fistula surgeons in general. Long-term observation of patients has given us the opportunity to learn even more, but also realising diminishing success rate over time.

In hindsight, some of the patients treated in the study should not have been treated with an AFP. During the inclusion and surgical phases of the study, fistula length <4 cm was not known to significantly increase the risk of failure. For this reason, there are no data on fistula length to allow subgroup analysis. With modern 3D EAUS devices, it is possible to examine the sphincter apparatus in more detail than with the equipment used in the study. This means that fistulotomies have replaced many of the borderline cases that used to receive the AFP as a precaution.

In **Paper I**, the surgeons ensured that no abscesses or further tracts were present by preoperatively examining the patients with 3D EAUS immediately before the AFP procedure. This may explain the unexpectedly high success rate in the Crohn's fistula group, which resulted in no difference between the two study populations. As mentioned in the introduction, it is not uncommon for Crohn's disease to present as perianal fistula disease several months before diagnosis. This was also the case in some of the Crohn's patients in our study, which partly explains the low proportion of patients receiving biologic treatment. In general, the theoretical aspects of the AFP treatment in Crohn's fistulae are extremely complicated, considering that both the disease and its treatment may affect collagen synthesis and composition in the intestinal wall, while at the same time elevated MMP levels have the ability to degrade the collagen I scaffold of the AFP.

During the long-term follow-up and publication of **Paper I**, the use of AFP was greatly reduced. One factor that may have contributed was the randomised multicentre FIAT Trial, published in early 2021 that reported that AFP was associated with similar faecal incontinence and success rates one year after surgery as surgeon's preference [153]. The study group concluded that higher cost and uncertain gain in quality-adjusted life years did not motivate the AFP as a cost-effective treatment. Clinical healing was seen in 54% (66/122) in the AFP group and 55% (66/119) in the surgeon's preference group (advancement flap, cutting seton, fistulotomy, or LIFT) at 12 months. Somewhat surprisingly, the study reported complication and reinvention rate of 35% in the AFP group, significantly higher than in the control group (18%) with a reintervention rate of 21% in the AFP group at 6 weeks. This was mainly because more patients in the AFP group had unexpected pain (65% vs. 36%). This could be explained in part by the fact that 11% of fistulae were superficial or intersphincteric and 27% fistulae had extensions, and patients were assessed by MRI six months before the procedure. Further, the FIAT trial included fistula lengths >2 cm, which were considered much too short for successful AFP after the study began. 45% of the patients included in the trial had history of previous fistula surgery, putting them at significantly higher risk for AFP failure, according to **Paper I**. In addition, the report states that the AFPs used were either 4 or 7 mm in diameter and that the button of the plug was secured by a suture, meaning that the AFP used in the study was not the traditional plug which is both longer and softer in the part that covers the IO. The FIAT trial confirmed the results of **Paper I** by showing that what could initially be termed as failure after AFP should not be decided on too early after the procedure: clinical healing increased from 39% to 54% from six to 12 months after the AFP.

In an interesting retrospective study published in 2013, Sayers et al reported on 113 AFP patients, 23 of whom were treated with a four-sided pyramidal AFP manufactured from acellular dermal collagen matrix derived from porcine dermis [154]. The rest of the patients were treated with the traditional cone-shaped SIS-AFP. The dermal AFP had significantly higher healing rate (57%) compared to the SIS-AFP (12%), but also shorter median follow-up time; 43 weeks (range: 4-196 weeks) vs. 152 weeks (range:5-619 weeks). AFP was not successful in any of the 14 Crohn's patients included in the study. 28 patients (24.5%) experienced complications in the first 30 postoperative days, 26 of them in the SIS-AFP group. Of these, 21 experienced AFP extrusion which occurred within 72 hours after surgery. The authors concluded that the study demonstrated differing outcomes according to the AFP-type used, as shown by inferior healing rate and higher complication rate in SIS-AFP compared to dermal AFP.

During the progress of this thesis, several novel or established treatment options for complex fistula have gained in popularity. A systematic review and meta-analysis of 14 studies and 1201 patients treated with video-assisted anal fistula treatment (VAAFT) reported a success rate of 83% with a median follow-up of 16.5 months (range: 8-48 months) [155]. The authors concluded that VAAFT, with its long-term benefits of reducing anal incontinence and surgical morbidity combined with the possibility of earlier healing and accelerated rehabilitation, may be a valuable alternative to fistulotomy or seton in the treatment of complex anal fistulae. Laser ablation of the fistula tract with a radial-emitting laser probe (FiLaC) has been reported to have primary healing rates of 33-89% with a median follow-up of 11-30 months [156].

Further developments of fibrin glue and AFP refilling the fistula tract after closure of the IO include methods in which autologous plasma is injected. Platelet-rich plasma (PRP) consisting of bioactive elements, such as growth factors, peptides, and cytokines, stimulates tissue regeneration and has been discussed as a low-cost implement in healing complex fistulae. Healing rates of 42-81% have been reported, but further research work and methodological development is needed to ensure standardised techniques for PRP preparation, activation, and follow-up. [157] Vivostat® platelet-rich factor, also known as Obsidian® regenerative fistula treatment, is an autologous, bioactive matrix with thrombocytes with reported 62% success rate in a study of 23 patients [158]. Further studies are needed to evaluate the long-term results of the treatment.

Injection of adipose-derived mesenchymal stem cells has shown promising results, particularly in the treatment of fistulising Crohn's disease, with reported success rates of 56-83%, through their ability to produce and release anti-inflammatory and immunoregulatory mediators, such as chemokines, complement factors, immunosuppressive molecules, and growth factors. However, the treatment has been criticised for being expensive and time-consuming. [157] Series of injections around the IO and the fistula tract after freshly collected autologous fat grafting have been described as a readily available and cost-effective alternative to harvesting and culturing mesenchymal stem cells. Two studies on 52 and 77 patients reported clinical closure rates of 51-64%, with additional patients experiencing symptom improvement despite persistent or recurrent fistula [159, 160].

A randomised controlled PISA Trial aimed to compare medical and surgical treatment of perianal Crohn's fistulae [161]. Patients were randomly assigned to treatment groups of chronic loose seton, anti-TNF treatment or surgery with either LIFT or advancement flap after anti-TNF treatment start. The trial was terminated early due to significantly higher reintervention rate in the seton group. The authors concluded that seton treatment should not be recommended as the sole treatment for perianal Crohn's fistulae. In a follow-up trial of 94 patients, PISA II, patients were randomly assigned to either surgical closure group with short-term anti-TNF or the anti-TNF treatment alone group or could self-select [162]. The results were followed up by MRI. The authors concluded that short-term anti-TNF treatment in combination with surgical closure was more likely to result in long-term healing on MRI than anti-TNF alone.

The experience from **Paper I** and **Paper II**, and from the FIAT Trial indicates that regenerative sphincter-preserving fistula treatments should not be followed up or deemed to failure too early in the healing process. If the fistula has been properly assessed, drained, and appropriately simplified (if necessary), the risk of severe infection should be minimised. Therefore, it may be worthwhile to wait until the fluid discharge subsides and eventually disappears, as long as the patient is instructed to contact the clinic if signs of severe infection should occur.

Radiological follow-up

MRI is often considered the method that should be used for radiological follow-up after anal fistula surgery. Apart from the large fistulotomy/partial lay-open study by Garg [40], it has been difficult to find studies describing fistula healing after surgery. The FIAT study followed 72-74% of patients with MRI [153] but did not specify how healing was defined.

Considering the cost and inconvenience of MRI it is understandable that it may be difficult to motivate patients without symptoms to undergo a postoperative MRI scan. Ultrasound is often criticised for being operator-dependent, but optimal anal fistula examination and assessment on MRI is also highly dependent on the protocols, image quality, and experience of the assessing radiologist. 3D EAUS is a practical method that can be performed on an outpatient basis as part of a clinical checkup. Results from **Paper II** show that it would be interesting to follow sphincter-preserving treatments even in patients without clinical symptoms to learn more about the natural history of fistula healing. We also believe that the knowledge gained from AFP treatment would be applicable to other sphincter-preserving fistula treatment methods.

Fistula assessment

The Clinical Practice Guidelines Committee of the American Society of Colon and Rectal Surgeons published in 2016 state that simple fistulae, in general, do not require diagnostic imaging to guide treatment [46]. This is reassuring for patients with superficial fistulae, but the reported faecal incontinence rates of up to 60% after fistulotomy speak for themselves; the clinical reality may not be as black and white as we would like it to be.

A problem identified during the course of this thesis is the persistence of classifying fistulae according to a 50-year-old classification system that has been shown to be clinically irrelevant in >40% of fistula cases. On the other hand, classifying fistulae as 'simple' or 'complex' is not sufficient, as many patients end up in a grey zone that does not exist in the Standard Practice Task Force classification. Many clinical studies have adopted this, classifying complex fistulae as more than 30-50% sphincter engagement, anterior fistula in women, and a history of inflammatory bowel disease, faecal incontinence, or local radiation. There are definitely anterior fistulae in female where the only option for cure is fistulotomy, as well as patients with quiescent Crohn's disease who heal well after a lay open procedure with no effect on faecal continence. Based on 3D EAUS length measurements from **Paper IV**, the subcutaneous portion of the EAS in the posterior anal canal accounts for 13-16% of the length of the surgical anal canal (EAS+PR). Fistulae with IO involving 30% of the length of the anal canal pass through both the IAS and EAS when they are transsphincteric. The results of **Paper III**, as well as many of the 3D EAUS studies mentioned, show that only 25-29% of the fistulae included in the studies were intersphincteric, but proportions as high as 62-67% have been reported [62, 65, 69-71]. Many low fistulae may be mistaken as intersphincteric on EUA or MRI when in fact they involve not only the IAS but also the subcutaneous portion of the EAS. Thus, following the recommendation not to perform diagnostic imaging for simple fistula, a fistula surgeon should perform a fistulotomy of all posterior fistulae where the IO is up to 12-14 mm from the anal verge, for example, based on the measurements in **Paper IV,** without the need for preoperative radiology. In many cases, the patient would likely come away with mild incontinence, but the surgeon may not be as comfortable with this decision. It should be noted that many fistulae classified as transsphincteric by Parks (type 2) actually do not penetrate the entire extent of the EAS at the level of the IO, especially in posterior fistulae. The natural course of many fistulae appears to follow more closely the pattern described by Gottesman. The fistula runs both intersphincterically and transsphincterically, and it runs into and along the EAS rather than transversally through it.

The challenge for all classification systems is the three-dimensional complexity of fistulae with primary tracts that do not follow a simple pattern, secondary extensions, and cavities. In particular, the role of cavities deserves more attention in pathophysiologic, diagnostic and management studies.

A study published in 2019 compared 3D EAUS with intraoperative findings in 86 fistula patients and reported that 43% of them had fistula associated anal sphincter defects [163]. 57% of the defects were located in the IAS, with the remaining defects distributed approximately equally between the EAS or both sphincters. Unfortunately, the paper does not specify how many patients had previous fistula surgery. The authors concluded that 3D EAUS is particularly useful in identifying sphincter defects before surgery. This finding is interesting because both raters in **Paper IV** reported that identification of the distal IAS measurement point was sometimes difficult due to a defect in the IAS. We suspect that some fistulae originate from anal fissures that damage the IAS and lead to a fistula, usually located at upper end of the muscle defect near the dentate line. This aspect is also of interest, as LIFT is obviously not an option for patients with an IAS defect. A meta-analysis published in 2019 analysed 30 studies and 1295 patients to evaluate the outcomes of endorectal advancement flap and LIFT in patients with cryptoglandular and Crohn's fistulae [164]. No significant difference was found between advancement flap and LIFT in cryptoglandular fistulae, with weighted overall success rates of 75% and 69%, respectively. No differences between methods were noted in patients with Crohn's fistulae. Incontinence rates were significantly higher after the advancement flap (7.8%) than after LIFT (1.6%).

A recently published multicentre study of 253 patients showed good agreement in the identification of the main fistula characteristics between 3D EAUS performed with the 2052 transducer and intraoperative findings [165]. The ultrasound examinations were performed by a surgeon or a radiologist with at least 10 years of experience in 3D EAUS. The reported κ-values were 0.69 for IO-identification, 0.90 for primary tract classification, and 0.87 for secondary extensions. The authors concluded that 3D EAUS may have a relevant impact on the outcome of a defined group of patients undergoing anal fistula surgery, as careful evaluation of ultrasound results could simplify intraoperative detection of IO and improve the success rate. The results of Paper III provide an indication that surgeon's experience indeed has very little influence on outcome, and that 3D EAUS is a viable alternative even for surgeons without 10 years of experience.

Ultimately, it all boils down to the question of how to minimise the number of patients in the grey zone. The reason that 86% of patients included in **Paper III** at a tertiary centre have subcutaneous or low fistulae (the low ones engaging the IAS) is simple; general surgeons and proctologists who only have access to EUA and MRI are not comfortable performing fistulotomies unless they have detailed knowledge of both the level of sphincter involvement and the presence of complicating factors, such as sphincter defects, secondary extensions and cavities. Not only the level of the fistula, but also the depth of the EAS engagement, is critical to fistulotomy. It is sometimes very difficult to assess this on MRI and palpation because infection and inflammation can cause significant oedema of the tissue. On the other hand, if both clinical assessment during EUA and radiology lead the surgeon to perform a fistulotomy that turns out to be deeper and higher than expected, it is reassuring for both the patient and the surgeon to know that the surgeon's expertise allows him or her to perform a fistulectomy with direct sphincteroplasty if needed. The weakness of MRI is accurate identification of IO and depending on the waiting time from radiology to surgery, the status may have changed when it is time to perform the procedure. The advantage of the 3D EAUS is not only that it is easily accessible for immediate preoperative evaluation with more efficient identification of IO in the operating room, but also the ability to perform ultrasound-guided drainage of cavities and secondary extensions with the 8838 transducer. This is a good option for the sometimes tricky and time-consuming VAAFT procedure, and it is even possible to combine the modalities for advanced intervention. A multidisciplinary expert consensus document published in 2022 described the development of a minimum dataset for MRI reporting of anorectal fistula [166]. The final minimum dataset consisted of general characteristics, features of IOs and EOs, fistula course through the sphincters, and any associated extensions and/or collections. Additional surgical and perianal Crohn's disease subsets were also developed. The panel discussed that the lower limit of IAS is the only fixed landmark on MRI for assessing fistula height and that measurements can be difficult to specify because of differences in patient positioning between MRI and surgical procedures. The upper limit should accordingly be defined as the top border of the puborectal muscle. In addition, the panel discussed if the terms *'simple'* and *'complex'* should be defined such that a simple fistula is synonymous with a fistula where cure can be achieved, whereas a complex fistula indicates that symptom control would be the appropriate goal. Advances in new generation of 3D EAUS devices, transducers, software, and post-processing of examinations would allow the same parameters to be assessed by ultrasound. However, studies are needed to evaluate the efficacy of the new products.

Length of the anal canal

Both raters in **Paper IV** found measuring the length of the anal canal to be more difficult than expected. The raters met for consensus discussions before beginning measurements, but the anatomical landmarks were challenging to identify when working alone. Compared to the latest models of ultrasound machines with noise reduction protocols and transducers in use today, the 3D Flex Focus device and the 2052 and 8838 transducers have some interference at the air-tissue interface at the anal verge. In addition, in is sometimes very difficult to distinguish the anterior EAS from the surrounding tissues.

The proximity of the transducer to the tissues under examination is an advantage of 3D EAUS that cannot be ignored. In most cases, the differences between the measurements of Rater 1 and Rater 2, or the repeated measurements of Rater 1, were relatively small. The main reason to the wide limits of agreement was the extreme differences due to the cases with difficult anatomy or suboptimal image quality.

It is important to recognise the importance of assessing the length of the anal canal as part of fistula treatment. In particular, the anterior portion of the female anal canal is sometimes extremely short, so that even relatively low but deep fistulae may involve an unexpectedly large portion of the sphincter apparatus. These often-short fistulae can be difficult to assess with MRI.

Methodology and limitations

The study of **Paper I** and **Paper II** began when AFP was a new procedure. The limitations of the method were not yet fully known. To opt for a randomised controlled clinical trial instead of a descriptive cohort study was discussed, but clinical experience with the advancement flap showed low success rates. Therefore, there was actually no suitable alternative treatment arm into which patients could be randomised. The long follow-up period and few patients lost to follow-up are the strengths of the AFP study. The Swedish health care system with computerised patient records and the fact that our hospital is the only clinic in the region performing this type of advanced fistula surgery made it possible that the information collected during long-term follow-up was secure. Selection bias was minimised by including consecutive patients in need of a surgical procedure other than fistulotomy. There is some risk of selection bias in the patients lost to follow-up, but because three of them still live near the clinic, it is likely that they would have contacted the clinic in the event of AFP failure. This may also introduce some information bias, as may the 3D EAUS examinations in **Paper II** which were either missing or could not be evaluated.

It can be discussed whether the use of two raters is a limitation of **Paper II**. This method was chosen after the exploratory evaluation of ten randomly selected patients when the raters realised how difficult it was to evaluate the 3D EAUS examinations without knowing in advance how to assess the parameters. The decision to use two raters was based on the conclusion that the assessment of postoperative morphology was more accurate when performed by two raters in a team.

Some studies exploring 3D EAUS and fistula assessment use agreement between 3D EAUS and intraoperative findings alone (blinded to 3D EAUS) instead of 3D EAUS agreement to 3D EAUS in combination with intraoperative findings as done in **Paper III**. Since 3D EAUS has been a part of the clinical routine at Pelvic Floor Centre in Malmö for several years, it was not ethical to expose the study participants to a treatment that could be expected to be of lower quality than regular fistula treatment.

The challenge of all research work in ultrasound is the rapid technical development in the field of medical diagnostics and radiology. By the end of the inclusion period of the study that produced **Paper III** and **Paper IV,** the hospital had already purchased new equipment and transducers with subjectively much better image quality. The strength of our ultrasound studies is the use of the high-resolution 8838 transducer, which has not been reported in previous proctology studies. Also, the use of raters with different levels of experience has shown that 3D EAUS is a modality that can be mastered early in the surgical career. One could debate whether it would have been informative to even record the healing rate of the fistulae included in **Paper III**. Since some of the fistulae were complex and still undergoing treatment, this was not considered to add value to the main purpose of the study. It should also be noted that the documented mean anal canal lengths were obtained from unoperated fistula patients and not from healthy volunteers. As mentioned previously, some of the anal measurements may have been influenced by a fistula in the measurement area or a fissure that affected the IAS. Because the 3D EAUS examinations with the 8838 transducer were performed for fistula diagnosis, the stitch was sometimes placed in the 6 o'clock position, which made it more difficult to assess the exact level of the muscle borders.

The challenge in the study of anal fistulae is the discrepancy between different classification systems, guidelines, and definitions. This thesis has sought to balance this situation and manage it in a way that makes standardisation of future studies less difficult.

"Perplexity is the beginning of knowledge."

Kahlil Gibran

Conclusions

This thesis investigated the long-term outcome of the AFP procedure and explored the utilisation of 3D EAUS for the assessment and follow-up of anal fistulae. The main conclusions are:

Paper I The long-term success rate of 38% after the AFP is acceptable considering the low morbidity of the procedure in a complex disease with high recurrence rates over time. There was no statistically significant difference between the success rates in the cryptoglandular group and the Crohn's fistula group.

Paper II 3D EAUS may be utilised for AFP follow-up. Postoperative 3D EAUS at three months or later, especially in combination with clinical symptoms, can be used to predict long-term AFP failure. Future studies are needed to evaluate the role of 3D EAUS in radiological follow-up of sphincter-preserving fistula treatments.

Paper III 3D EAUS alone has high precision in identifying IO of anal fistulae. The method may be utilised even by relatively inexperienced surgeons to identify fistulae suitable for fistulotomy. Both transducers included in the study are suitable for fistula diagnostics.

Paper IV The study reports the measurements of anal canal length in female and male patients with newly diagnosed anal fistulae assessed by two different transducers for 3D EAUS. Repeatability and reliability are generally acceptable but the intervals for limits of agreement between two raters and different transducers are wide. Accurate identification of the anatomical landmarks used for the measurements, especially in the anterior sector, was a major challenge for transducers available during the study.

“Että minä menisin ja eläisin täysin.
Enkä ketään kumartaisi turhan syvään.
Enkä ketään en mitään pelkäisi
paitsi omaa jäätymistäni”
Arja Tiainen

Future perspectives

This thesis has discussed the pathogenesis, assessment, classification, and treatment of anal fistulae. Several sphincter-preserving treatment options are in the pipeline, and only time will tell if they can achieve better long-term success rates than the AFP.

Despite years of research, there is still much to discover about the pathogenesis of anal fistulae. Should we focus more on the mechanisms of fistula development and maintenance? By better understanding the pathogenesis, it would be possible to identify fistula subtypes that are amenable to different types of treatments. Much more attention should be paid to the role of inflammation and the microbiota, as well as mechanisms for the formation of persistent cavities. For example, bacterial biofilm inhibitors would be an interesting option to pursue in the future.

The latest generation of 3D EAUS devices and transducers have much higher resolution than the equipment used in this thesis. Similarly, modern MRI scanners are far more effective than the devices used for the aforementioned studies. Both methods have their advantages and challenges, and large studies are needed on the effective use of radiological examinations.

In parallel with improved radiologic capabilities, new opportunities for novel fistula classification systems are emerging. But first, fistula surgeons would have to agree on whether classification between 'simple' and 'complex' fistulae is necessary and how it should be defined. Since it is unlikely that a single surgical procedure will be the optimal treatment for all the different subtypes of fistulae that are now classified as 'complex', more effort should be made to define fistulae in a way that is clinically relevant. Modern technology offers opportunities for more advanced assessment methods that can actually lead to simplified classification and treatment algorithms.

AFP is one of the treatments that has emerged from advanced regenerative medicine, and advances in tissue engineering and stem cell therapy are likely to continue to provide surgeons with novel treatment techniques for anal fistulae. Tissue-engineered setons [167] are one possibility, and antimicrobial microsphere scaffolds [168] are currently being investigated in feasibility studies. Similar suggestions have also been made for anti-TNFα-encapsulated scaffolds [169].

In Crohn's disease, the key to improving the management and outcomes of anal fistulae may be the combination of multiple medical and/or surgical therapies [170]. Perhaps some of the patients with complex fistulae should be treated more with a multimodal approach, similar to patients with Crohn's fistulae, because some mechanisms of chronic inflammation are very similar. In addition, it is possible that patients may have Crohn's disease that manifests only as a fistula. Also, more consideration may need to be given to differences in the natural collagen composition of patient populations, as individual tissue types may respond differently to regenerative treatments.

On the other hand, the development and assessment of new treatments takes time. One of the quickest ways to improve the treatment of anal fistulae is to figure out how to optimise current radiologic modalities and surgical procedures to provide the most effective treatment possible using established methods. Simplifying complex fistulae with different types of "bridge to fistulotomy" approaches may be the easiest way.

Populärvetenskaplig sammanfattning

En analfistel är en falsk gång mellan analkanalen och huden vid analöppningen. Fisteln tros oftast uppstå genom en infektion i körtlar som är belägna i området nära den inre slutmuskeln och kallas då för *kryptoglandulära fistlar*. Efter ofta ett akut insjuknande med böld nära analöppningen har patienterna kvar en öppning på huden där det tömmer sig blod, vätska, var eller avföring. Dessutom finns det risk att utveckla nya bölder. De flesta patienter är unga, 30–50 år, vid insjuknandet. Enligt olika rapporter insjuknar 9-23 per 100 000 européer i anal fistelsjukdom varje år. En fistel läker i princip aldrig av sig självt utan för bot krävs någon form av kirurgi. Många patienter upplever tillståndet som plågsamt och det kan påverka såväl yrkesliv som social tillvaro. Analfistlar kan även uppstå i samband med till exempel inflammatorisk tarmsjukdom, framför allt vid Crohns sjukdom.

På Bäckenbottencentrum Kirurgi, Skånes universitetssjukhus, Malmö, bedrivs bland annat högspecialiserad behandling och vård av patienter med anala fistlar. Komplicerade analfistlar kännetecknas av att de antingen omfattar mer än 30-50 % av slutmuskelns längd alternativt föreligger samtidigt som känd avföringsinkontinens, Crohns sjukdom eller efter strålbehandling nära analkanalen. Även fistlar belägna i främre delen av analkanalen (mot slidan) hos kvinnliga patienter betraktas vara komplicerade. Eftersom fistlarna ofta löper genom eller mycket nära till såväl den inre som den yttre slutmuskeln krävs det oftast stor erfarenhet hos fistelkirurgen för att möjliggöra lyckat operationsresultat. En effektiv metod att operera fistlar är fistelklyvning där man öppnar upp fisteln och låter den läka från botten. Komplicerade fistlar fortsätter dock att vara en stor utmaning. Då fistelklyvning oftast inte är lämpligt måste dessa fistlar handläggas med muskelbevarande operationsmetod med stor risk för återfall eller med muskelengagerande kirurgi med risk för avföringsinkontinens och risk för återfall.

År 2005 introducerades en ny muskelbevarande behandlingsmetod för komplicerade analfistlar; kollagenplugg (AFP, *anal fistula plug*). Tidiga studier visade mycket goda resultat men senare har variation i behandlingsframgång och läkningsfrekvens från 13–100 % rapporterats. Återfallsfrekvensen visar tendenser att öka med uppföljningstid, samtidigt som de flesta studier endast följt patienterna under det första året efter operationen. En systematisk genomgång har dragit slutsatsen att kollagenplugg inte är tillräckligt väl utvärderat som behandling för fistlar som orsakas av Crohns sjukdom.

För att minska risk för anal inkontinens behöver sfinktermuskulaturens relation till fisteln kartläggas före operationen. Magnetisk resonanstomografi (MR) kan användas, men är dyr, tidskrävande och svårtillgänglig. Dessutom är vissa fistlar svårbedömda med MR. Tredimensionellt (3D) endoanalt ultraljud (EAUS) har därför undersökts som ett lättillgängligt alternativ till MR. Till skillnad från MR kan operatören själv göra ultraljudsundersökningen före och/eller under operationen för omedelbar bedömning av situationen. Undersökningen används för att skräddarsy den bästa behandlingsmetoden för varje enskild patient. Genom att kartlägga fisteln noga vill man undvika återfall, nya operationer och anal inkontinens. Det finns dock väldigt lite forskning kring uppföljning efter kollagenplugg med MR och framför allt 3D EAUS i relation till både fistelsjukdom och kollagenplugg. Det finns väldigt lite kunskap om faktorer som påverkar tillförlitligheten och för vilka patienter metoden brister. Det finns heller ingen forskning om hur enkelt eller svårt det är att lära sig undersökningsmetoden och väldigt lite forskning kring hur samstämmiga olika undersökare är. För att veta hur stor del av analkanalen påverkas av fisteln måste man också kunna bedöma hela analkanalens längd med hjälp av 3D EAUS. Det finns inga studier över analkanalens längd hos icke-opererarede män och kvinnor, framför allt inte med den nyare ultraljudsutrustningen med högre upplösning.

I denna avhandling undersöks långtidsresultat av kollagenplugg och utforskas användning av 3D EAUS för diagnostik och bedömning av analfistlar samt för uppföljning av fistelbehandling.

I **Delarbete I** undersöktes långtidsresultat av kollagenplugg i 95 patienter mer än sju år efter operationen. 90 patienter genomförde hela uppföljningen, de andra fem följdes upp i 4-30 månader. Efter 93-138 månaders uppföljningstid var 38% av patienterna fortfarande fria från återfall. Ingen skillnad sågs mellan patienter med kryptoglandulära fistlar och patienter med fistlar orsakade av Crohns sjukdom.

Delarbete II utforskade 3D EAUS-uppföljning efter operation med kollagenplugg. Syftet var att beskriva ultraljudsfynd efter operationen och analysera om fynd på 3D EAUS tillsammans med patientens symptom kan förutse långtidsresultat. Vår slutsats är att 3D EAUS kan användas för uppföljning efter kollagenplugg, och eventuellt även för andra liknande operationsmetoder. Ultraljudsundersökning vid tre månader efter operationen, eller senare, tillsammans med patientens symptom kunde i över 90% av fallen identifiera de patienter som hade hög risk för återfall.

I **Delarbete III** utvärderades tillförlitlighet av 3D EAUS för diagnostik av 93 nyinsjuknade patienter med kryptoglandulära fistlar. Resultat av enbart 3D EAUS jämfördes med sammansatta resultat från patientens operationsfynd tillsammans med 3D EAUS. Dessutom gjordes ultraljudsundersökningarna med två olika undersökningsmetoder och undersökningarna eftergranskades av kirurger med olika grader av erfarenhet. Studien visade att 3D EAUS i hög grad stämmer överens med fistelns inre mynnings placering vid operationen. Metoden kan även användas redan tidigt under den kirurgiska karriären för att identifiera fistlar som kan behandlas med fistelklyvning. Båda av de undersökta metoderna för 3D EAUS var lämpliga för att diagnostisera fistlar.

Delarbete IV hade sitt huvudsyfte i att mäta analkanalens längd vid undersökningsmetoderna använda i **Delarbete III**. Då patienterna inte hade blivit opererade tidigare, var det möjligt att bilda sig en uppfattning om hur lång analkanalen brukar vara hos både kvinnor och män när man använder sig av dessa mätmetoder. Mätningarna utfördes av två kirurger, den ena upprepade mätningarna. Metoderna kan acceptabelt upprepas med viss pålitlighet men intervallerna för överensstämmelse blir breda vid mätningar av två eftergranskare eller mellan de två undersökta metoderna. Det finns utmaningar i att identifiera de anatomiska landmärkena för mätpunkterna. Man kan tänka sig att detta blir lättare med den nya generationens ultraljudsmaskiner som nu lanserats, men fler studier behövs inom detta område.

"-- for that he was strong-hearted

and suffered well sharp things,

and that he was of good complexion

and had able flesh to heal."

John of Arderne

Acknowledgements

Sara Regnér, my main supervisor, for your courageous attitude that broadened horizons and gave me the opportunity to write this thesis. Thank you for your optimism, encouragement, and structured guidance through this process. You are, and will forever remain, my idol in scientific research work and administration.

Marianne Starck, my co-supervisor, for your tireless work with me over the past few years. I am truly impressed with your efforts in the field of ultrasound research, and I am honoured that you have allowed me to carry on your legacy. Your attention to detail has made me a better scientist.

Louis Johnson, my co-supervisor, for being one of the most intriguing people I have ever worked with. Your unique skills in academic thinking and writing have helped me greatly during this process. Thank you for occasionally making me feel super-organised, and for always working your magic when we operate together!

Antoni Zawadzki, my co-author, for all your efforts in all four papers included in this thesis. I am grateful for your encouragement that led me to make this career choice. Your commitment to your patients and to the field of fistula surgery should serve as an example.

Måns Bohe, my co-author, for enriching the quality of my scientific and managemental work with your experience. In addition, I learned valuable and unforgettable lessons about fistula surgery during the weeks I spent with you in the operating theatre early in my proctology career.

Alejandro Lusilla Lopez, my co-author, for all your hard work with the never-ending stock of 3D EAUS examinations. You are an essential part of our team and I really enjoy working, operating, and driving in the car with you.

Martin Lindsten, Maria Torper, Konstantinos Charonis and **Winfried Seiler** for being a part of the wonderful team at the Pelvic Floor Centre. Our daily interactions during the workdays are what makes it so much fun. Thank you for your patience with my physical and mental absence due to this thesis!

Patricia Maldonado for being the one that got away. I still dream that one day we will work together!

Mari Dahlberg and **Sara Strandberg** for all your support throughout the years. Our thriving collaboration has made me a better doctor, and the challenges we face together are so incredibly rewarding. I am looking forward to our pantomime performance!

Krystyna Korona and **Silvia Baquedano** for all your help in my daily work, for taking care of me and always making my day. It is a pleasure to work with colleagues who know me so well that everything runs smoothly without the need for words!

Britt Günther and **Kerstin Fisher** for your daily help and reminding me when I forget something. **Johanna Sjögren** for helping my most challenging patients. Our newest addition, **Emma Andersson** for being a good ally.

Kerstin Olsson for keeping me sane throughout the administration and for always being so understanding. **Ingrid Palmquist** for your help with practical issues and bookings.

Lil Valentin for the invaluable help in planning the 3D EAUS study. **Helene Jacobsson** for all the patient advice on statistics and for encouraging me to conquer the mountain of R Statistics.

Victor Verwaal, Head of Colorectal and Pelvic Floor Unit, for supporting and challenging me, and for encouraging me to continue with the research work.

Stefan Santén, Head of Department of Surgery, for supporting my research work by allowing me to apply for grants from the Regional Research Funds.

Jenny Brändstedt, my former boss lady, for believing in me, challenging me, and supporting me, for being there for me in difficult times.

Tobias Axmarker for all your support in so many ways during the years we have worked together. I am glad to have a friend like you.

All my **colleagues at the Department of Surgery, Skåne University Hospital, Malmö**, for being the greatest colleagues a girl could ask for! Special thanks to **Mikael Ekelund, Åsa Olsson** and **Per Olofsson** for always making feel welcome during my brief visits to emergency surgery.

My roommates **Eva Ekström, Mathilde Delorme** and **Selma Medic** for putting up with the organised chaos also known as my desk.

Riffat Cheema and **Claudia Bruss** for the good collaboration over all the years.

All the **colleagues at the Surgical Unit and the Surgical ward, Landskrona Hospital**, for your patience with this research work and our daily surgeries. Special thanks to **Kristina Nilsson Wall** for your help during the patient inclusion process.

All **patients at Pelvic Floor Centre**, Department of Surgery, Malmö, for your unique contributions to this research work. It exists only because of you.

Roland Andersson and **Bobby Tingstedt** for making me to want to become a surgeon in the first place. I am forever grateful for everything I have learned from you.

Ulrika Olsson-Rosén and **Jenny Rydstedt** for your mentorship, clinical guidance, and support during my early surgical years.

Colleagues at the Department of Surgery, Skåne University Hospital, Lund, and Ystad for hours of surgery and all the lessons learned in the years we have worked together.

Ellen Andersson with family for all the inspiration in surgery and science, and for being a true friend. I will never forget our Fridays at Flormansgatan.

Lisa Prahl Wittberg with family for a friendship that has lasted decades. Our discussions about academic life and juggling motherhood and work are truly valuable.

Anders Jedholt and **Karin Andersson Jedholt** with family for amazing “häng i gäng”, sweet memories and the best breaks from this sometimes-nerve-wracking work.

Friends with families, if you remember me after three kids in four years, a long pandemic, and an insanely stressful year… I promise to get back to cooking dinner, throwing parties, and drinking coffee for purposes other than caffeine trips!

Österlen for being our happy place and sanctuary and giving me clarity when I needed it most.

My brother-in-law **Johan** and **Anna** for all the incredible family gatherings, and for always welcoming my side of our extended family as well, creating entirely new Swedish-Finnish hybrid traditions for each holiday. Your wonderful children, **Elsa** and **Elof**, for all your practical help over the years. You are wonderful role models for your cousins!

My mother-in-law **Lena**, for your invaluable help during this process. Our children are truly lucky to have such an active grandmother! Your contribution to this thesis over all these years cannot be measured in any way. I would also like to acknowledge my late father-in-law, **Lasse**. The last ten years have gone by so quickly. I often see glimpses of you in our children.

Our extended family **Hörman-Levmo**, for the best birthday and other celebrations.

My siblings **Elina, Kaisa, Pekka, Mari** and **Laura with their families** for all the love and care. I feel so blessed to have all of you in my life! And I am so glad that your kids motivate mine to speak Finnish.

My parents, **Aili** and **Matti**, who have always supported and trusted me to find my way through life. This thesis, which is the greatest accomplishment of my academic career, is rightly dedicated to you. You know that my greatest achievements lie elsewhere, and thanks to you, I am aware of that. I grew up knowing that success in life is not measured by academic merit, but by love and happiness.

My husband **Tobias**, for the life-altering love and infinite patience. There are no words that can express my gratitude for all you have done for me and the kids over the years; early mornings as I leave for surgery, cargo bike school runs and bedtime stories as I write… You are my rock, my partner, my everything. I. Love. You.

Alvar, Frans and **Edit**, the greatest miracles of our lives. I am so incredibly happy and proud to be your mother. I admire you for being so supportive through all of this and being in the thick of it from the day you were born. Now that I have my own book with a barcode on the back cover, which obviously is super cool, I am going to focus on hugging you so tight. Rakastan teitä!

Financial support

This work has been supported by The Department of Surgery, Skåne University Hospital.

The thesis was supported by grants from Helge B Wulffs Stiftelse, Landshövding Per Westlings minnesfond (Grant number RMv2021-0007) and Region Skåne Regional Research Fund (Grand number 2022-1305).

The design, conduct of the research, collection and analysis of the data, writing or editing of the manuscripts, or publication process were not influenced in any way by the funders.

"Huvudsaken är att du vet vad du vill."

Snusmumriken

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Appendix

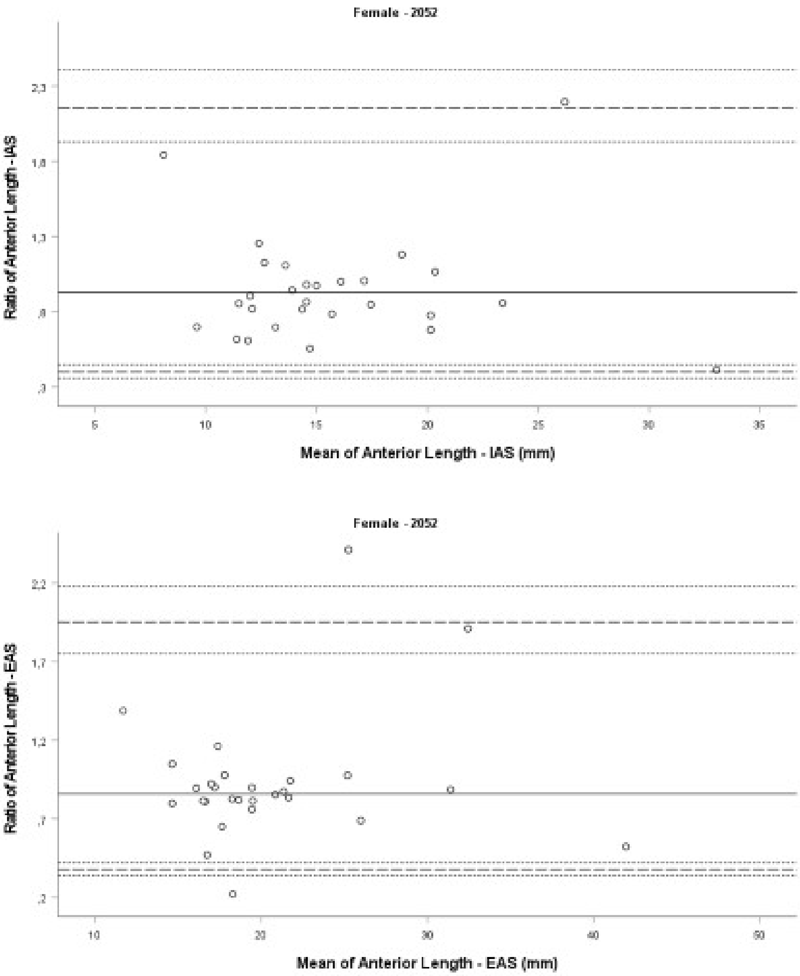
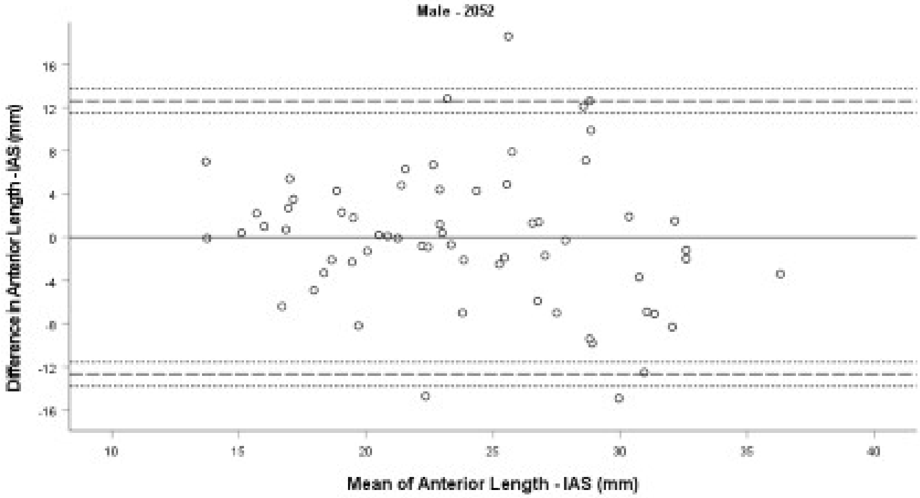


Figure A1 Bland-Altman plots of ratio of anterior anal canal length for transducer 2052 by Rater 1 and by Rater 2 (log scale) against mean of anterior anal canal length for female, defined by both IAS and EAS.
Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). An extreme ratio value of 3.7 is excluded from the IAS graph but included in the calculations. IAS: Internal anal sphincter, EAS: external anal sphincter.



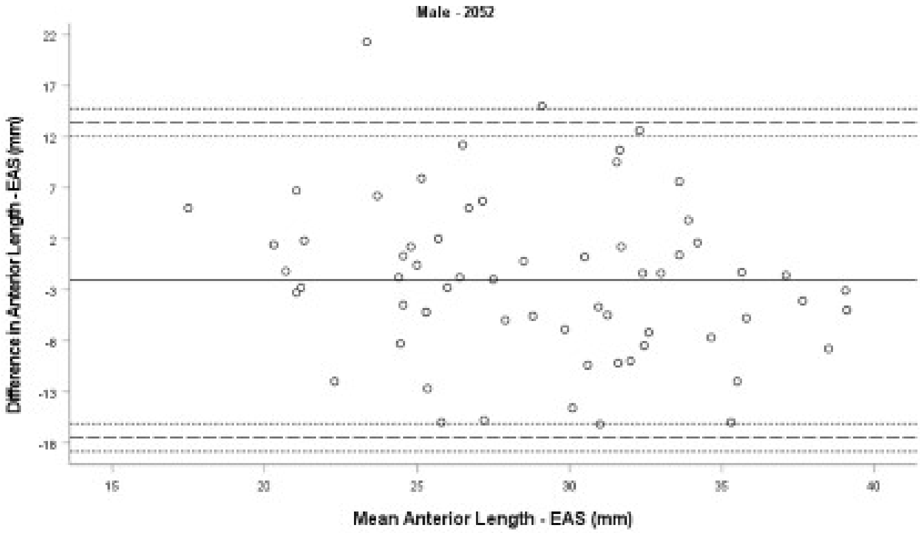
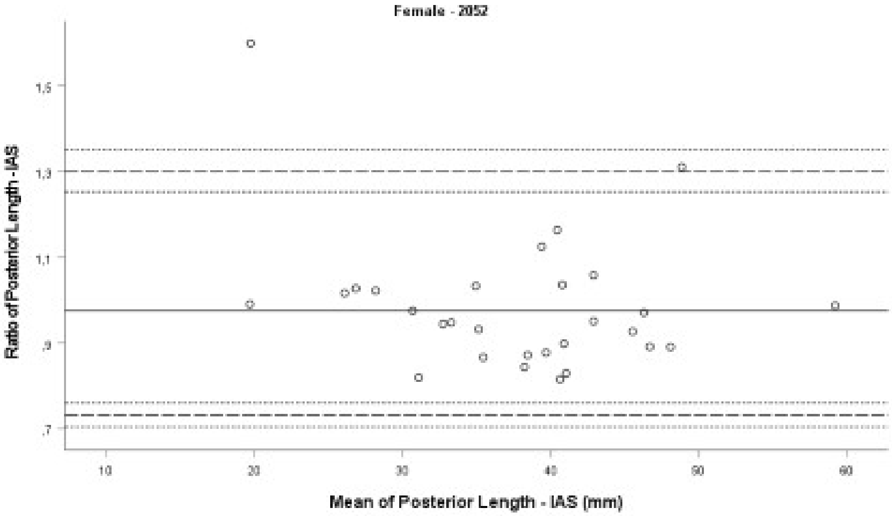


Figure A2 Bland-Altman plots of difference in anterior anal canal length for transducer 2052 by Rater 1 and by Rater 2 against mean of anterior anal canal length for male, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.



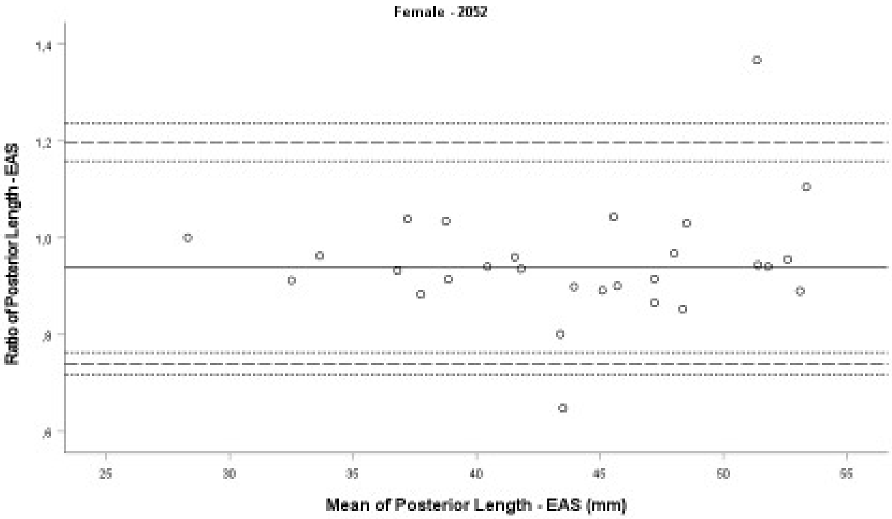


Figure A3 Bland-Altman plots of ratio of posterior anal canal length for transducer 2052 by Rater 1 and by Rater 2 (log scale) against mean of anterior anal canal length for female, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

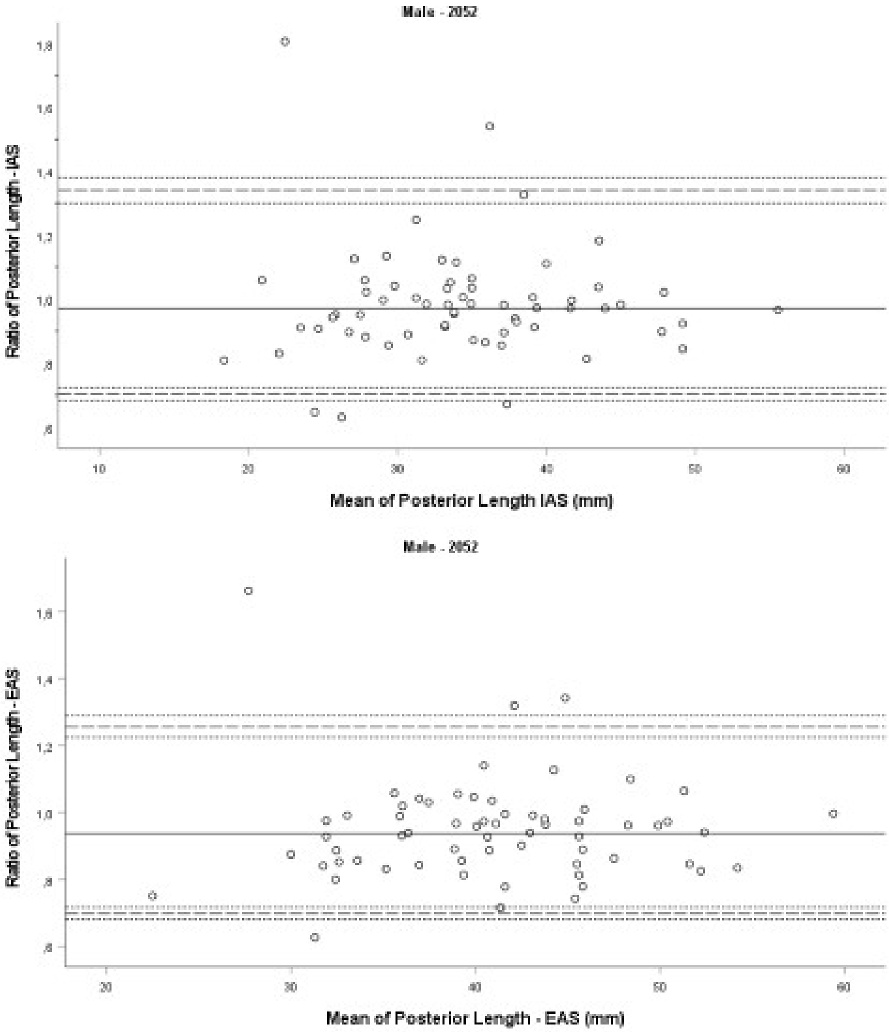


Figure A4 Bland-Altman plots of ratio of posterior anal canal length for transducer 2052 by Rater 1 and by Rater 2 (log scale) against mean of anterior anal canal length for male, defined by both IAS and EAS.
Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

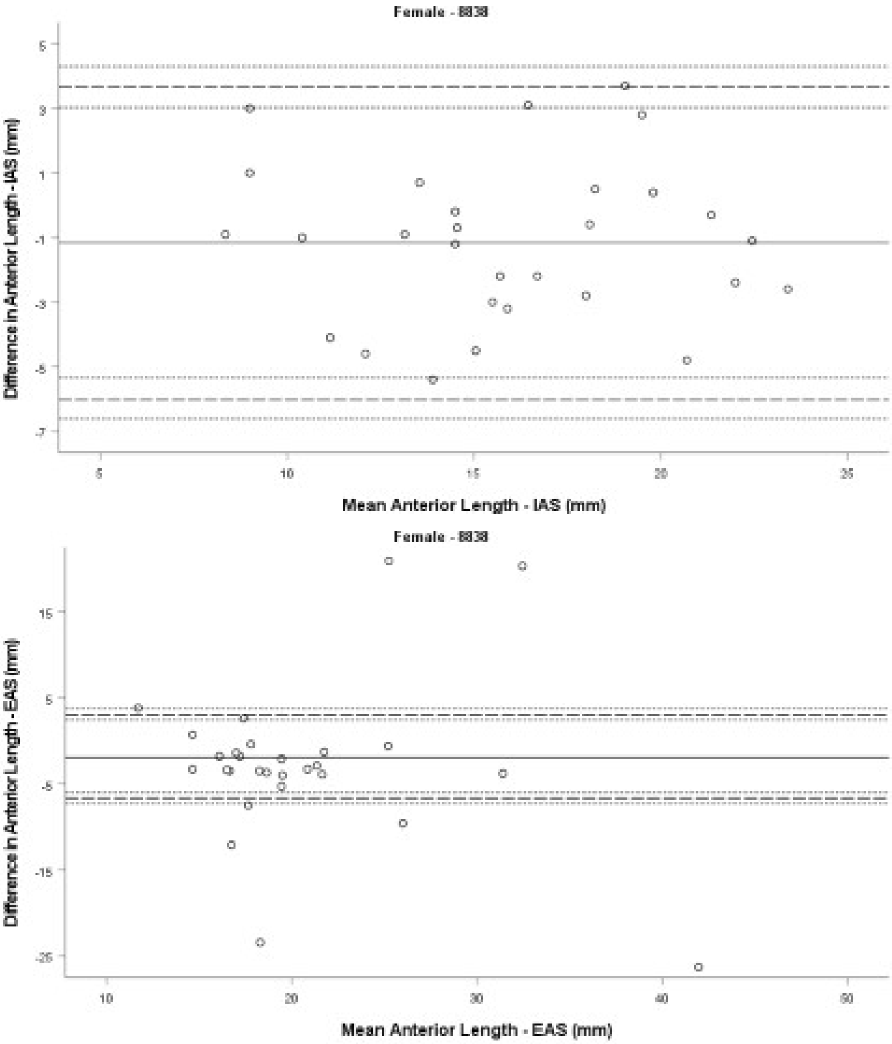


Figure A5 Bland-Altman plots of difference in anterior anal canal length for transducer 8838 by Rater 1 and by Rater 2 against mean of anterior anal canal length for female, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

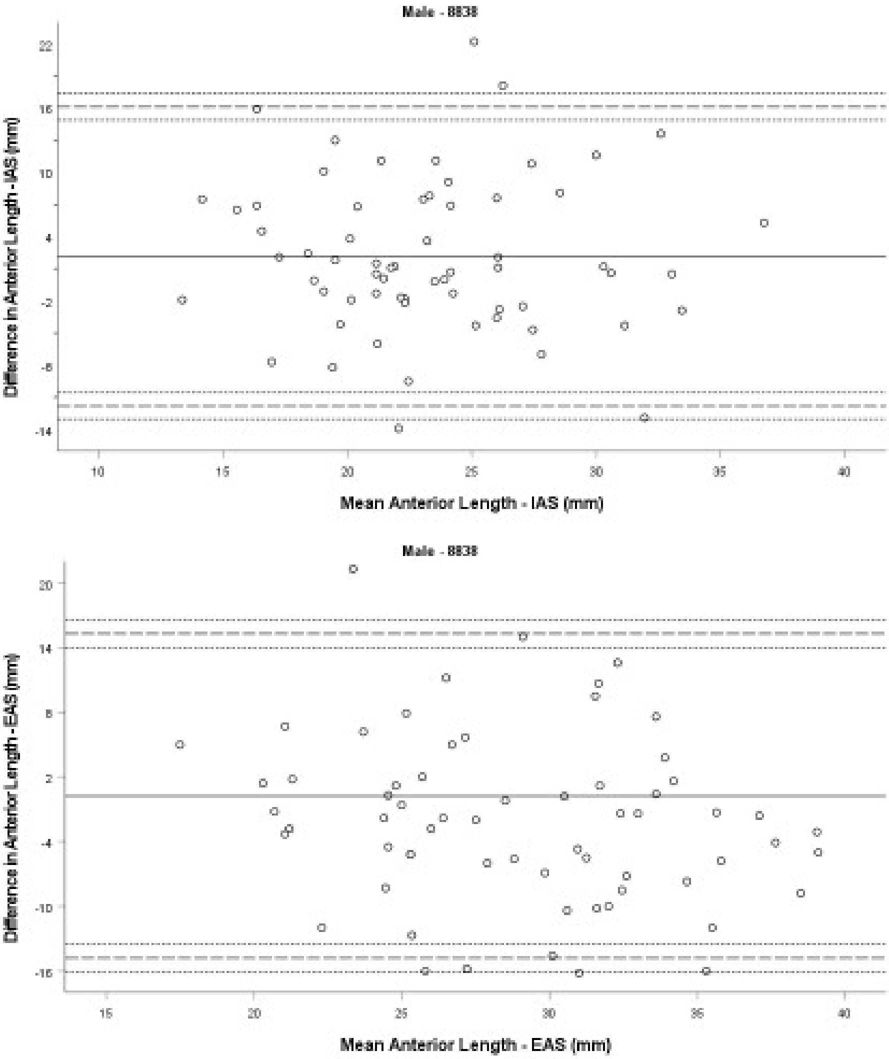


Figure A6 Bland-Altman plots of difference in anterior anal canal length for transducer 8838 by Rater 1 and by Rater 2 against mean of anterior anal canal length for male, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

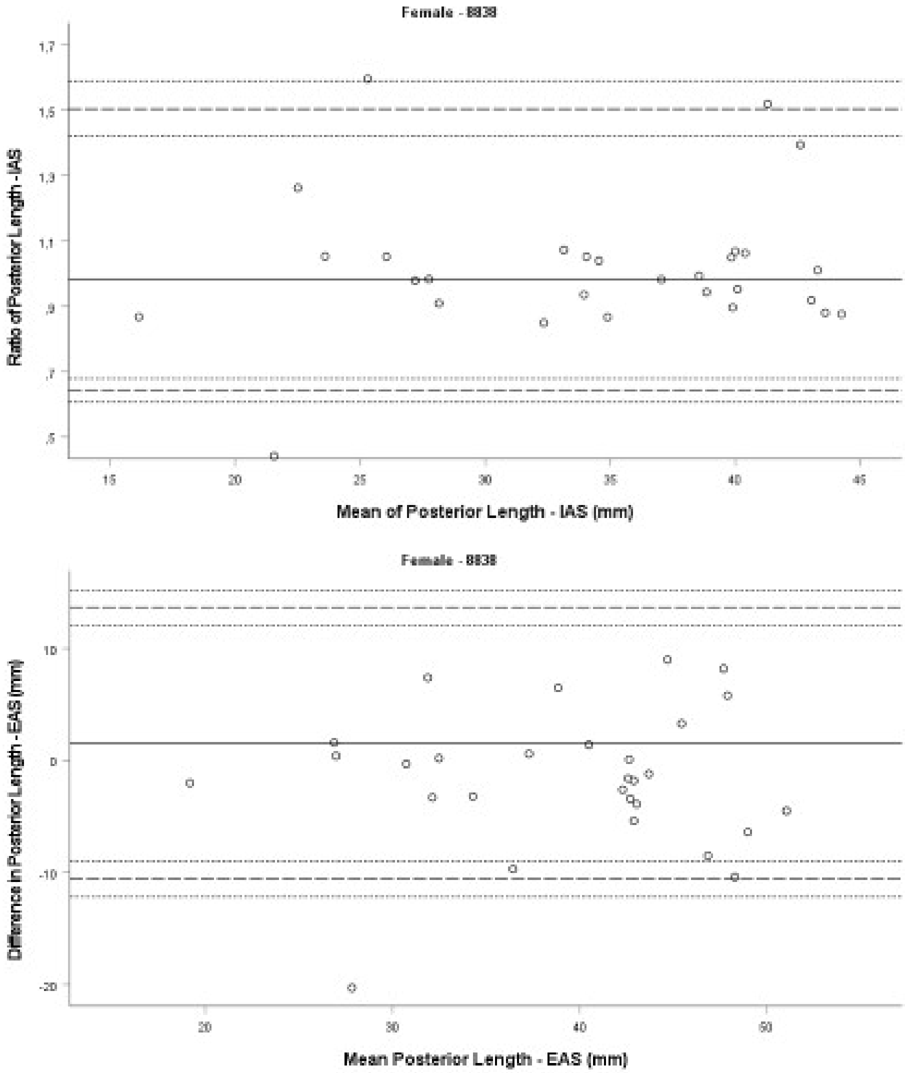


Figure A7 Bland-Altman plots for female and transducer 8838 with ratio of posterior anal canal length by Rater 1 and Rater 2 against mean of posterior anal canal length defined by IAS and of difference in posterior anal canal length by Rater 1 and by Rater 2 against mean of posterior anal canal length defined by EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

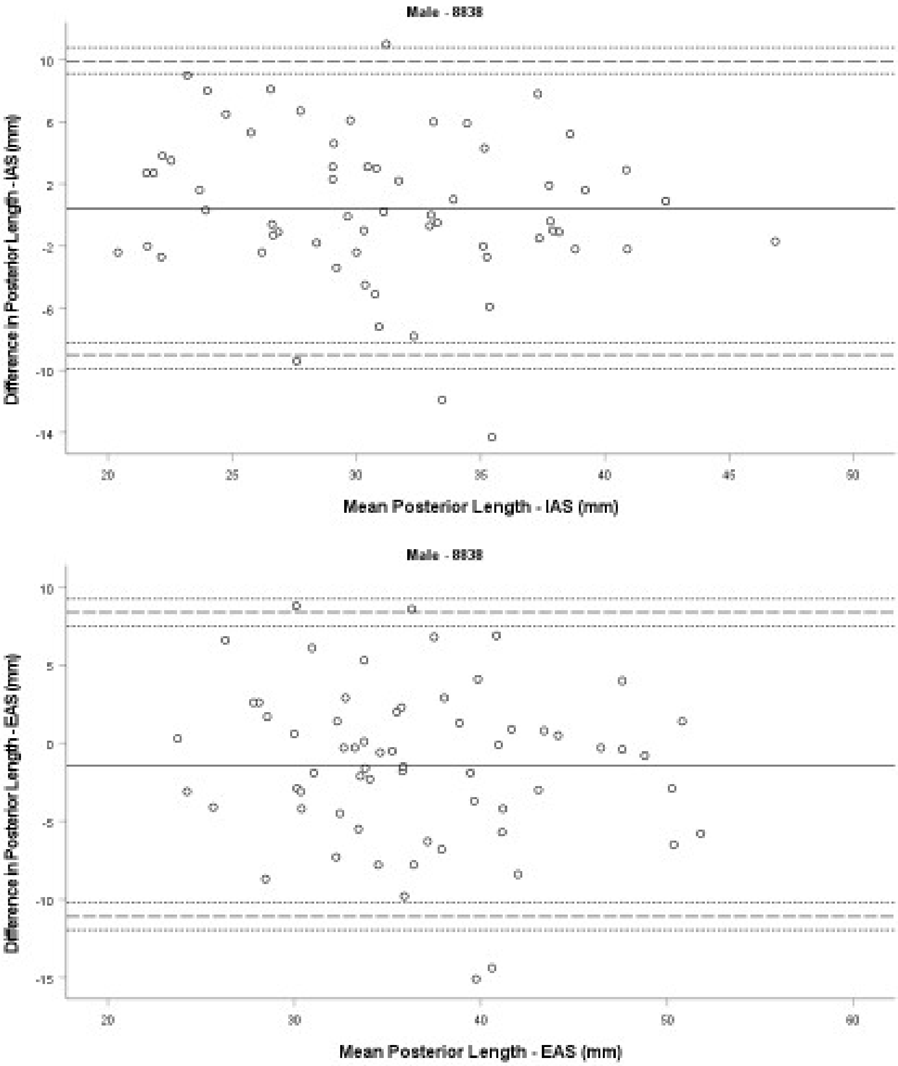


Figure A8 Bland-Altman plots of difference in posterior anal canal length for transducer 8838 by Rater 1 and by Rater 2 against mean of anterior anal canal length for male, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

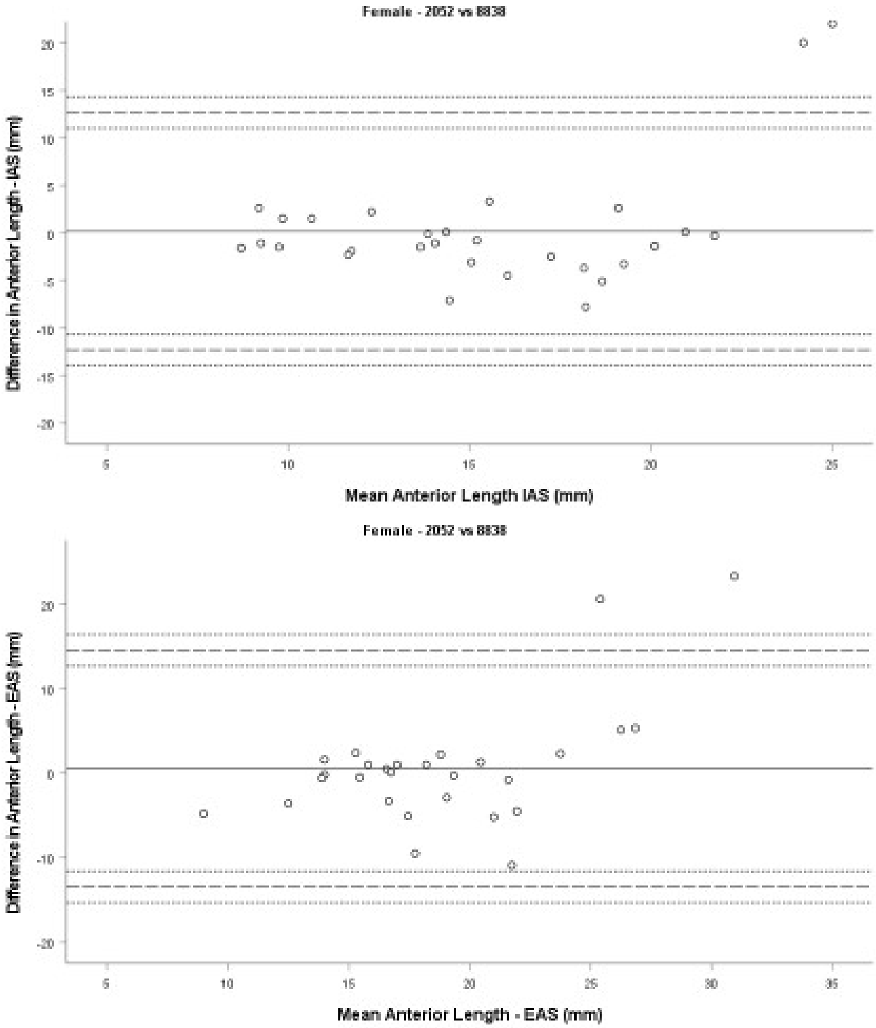
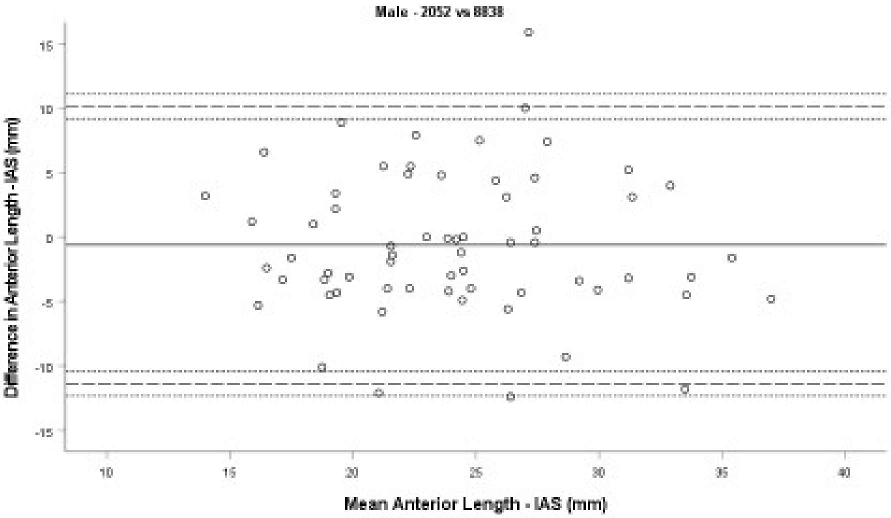


Figure A9 Bland-Altman plots of difference in anterior anal canal length for transducers 2052 and 8838 by Rater 1 against mean of anterior anal canal length for female, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.



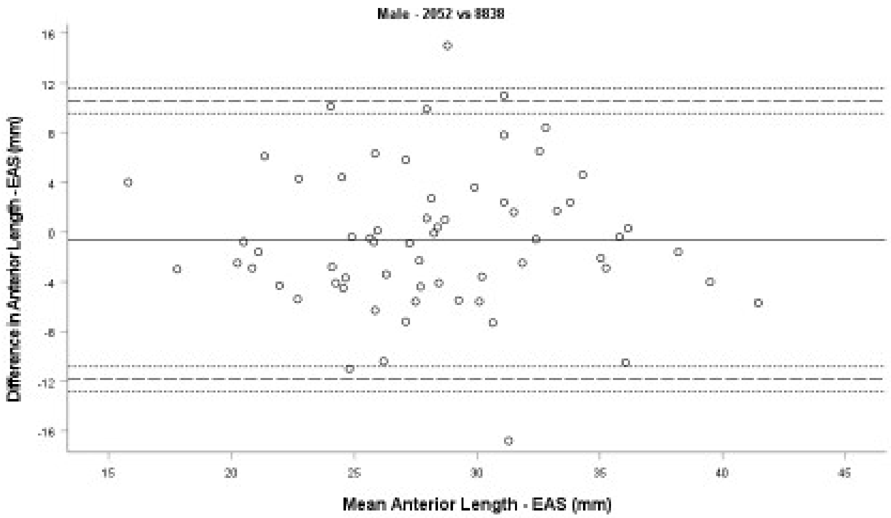
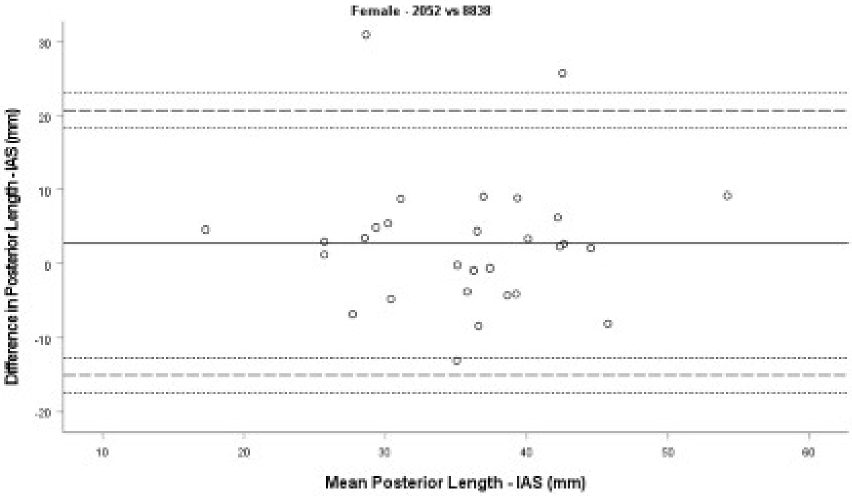


Figure A10 Bland-Altman plots of difference in anterior anal canal length for transducers 2052 and 8838 by Rater 1 against mean of anterior anal canal length for male, defined by both IAS and EAS. Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.



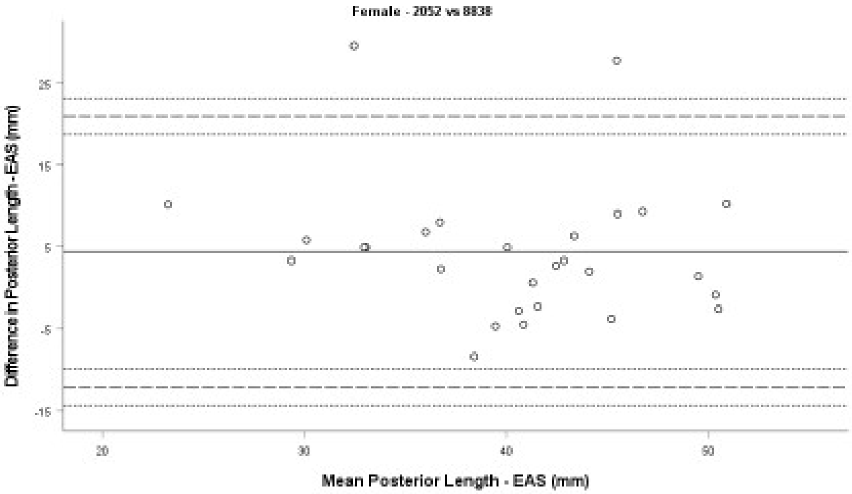


Figure A11 Bland-Altman plots of difference in posterior anal canal length for transducers 2052 and 8838 by Rater 1 against mean of posterior anal canal length for female, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

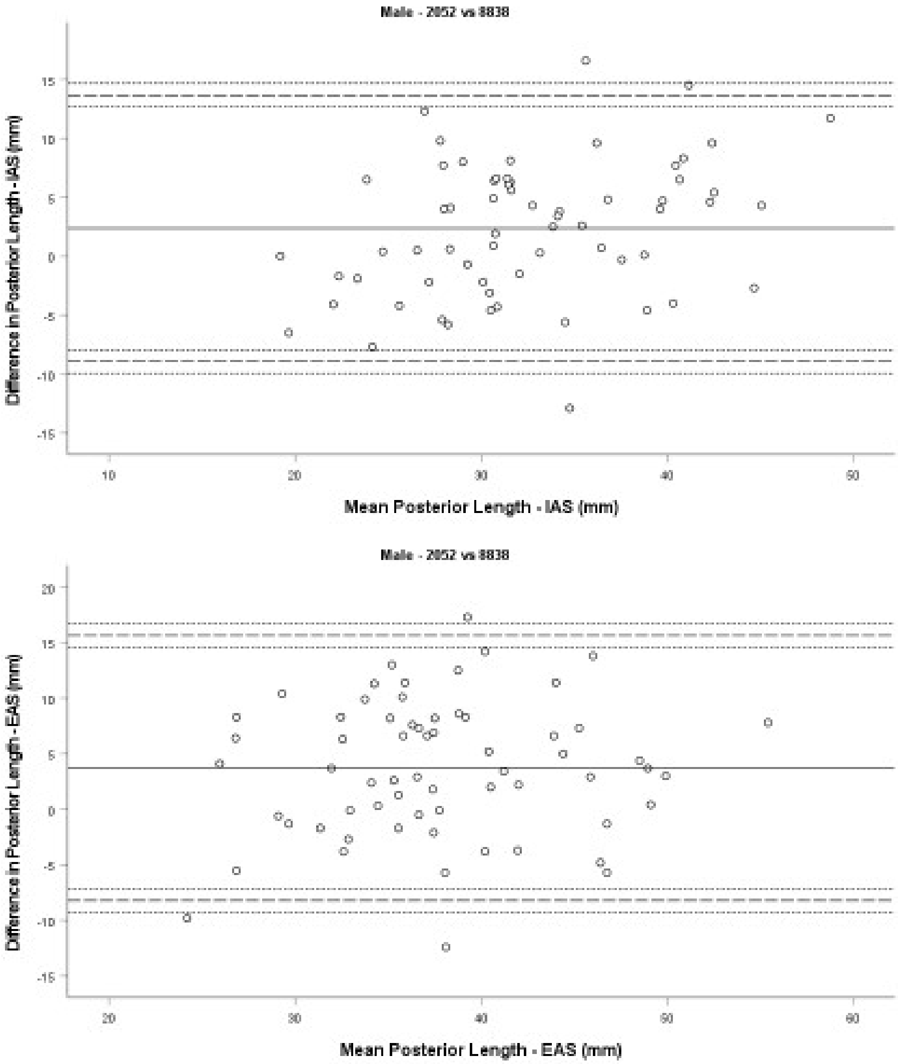
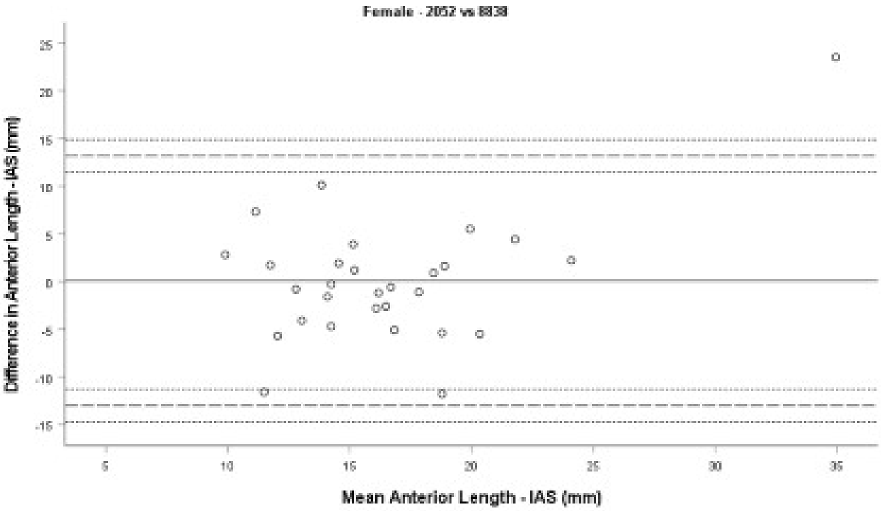


Figure A12 Bland-Altman plots of difference in posterior anal canal length for transducers 2052 and 8838 by Rater 1 against mean of posterior anal canal length for male, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.



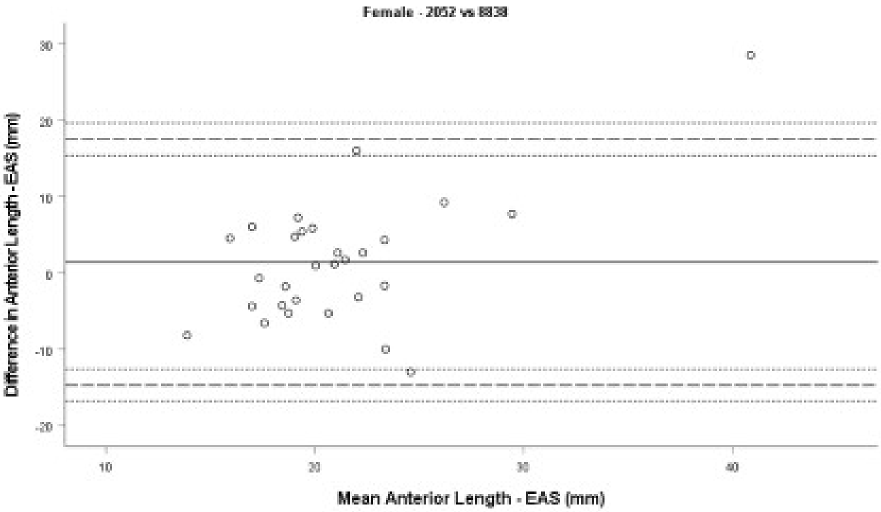
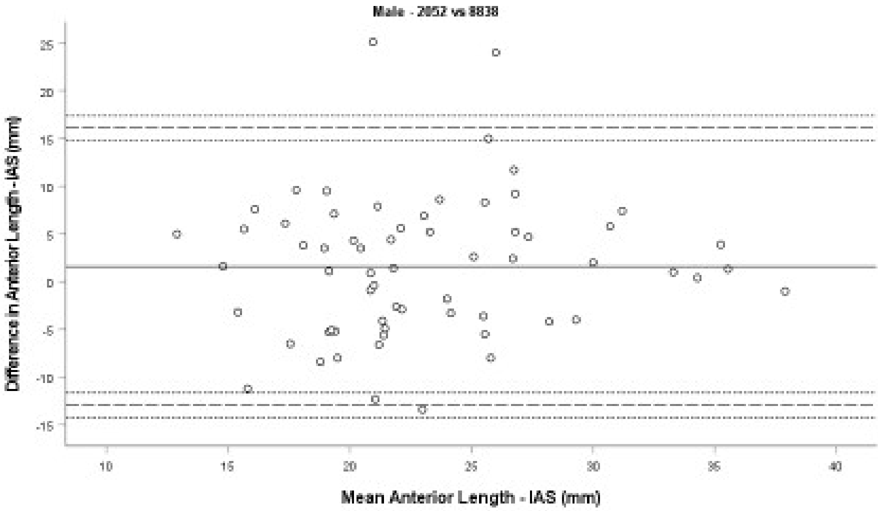


Figure A13 Bland-Altman plots of difference in anterior anal canal length for transducers 2052 and 8838 by Rater 2 against mean of anterior anal canal length for female, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.



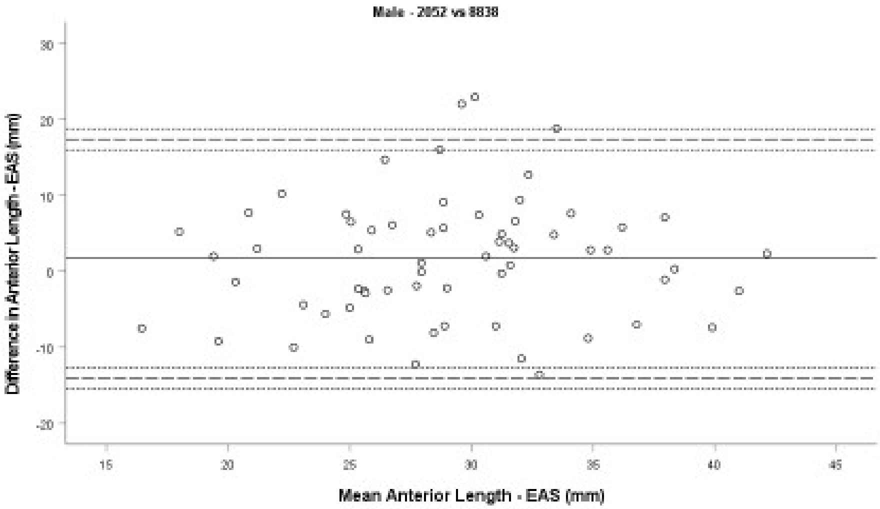


Figure A14 Bland-Altman plots of difference in anterior anal canal length for transducers 2052 and 8838 by Rater 2 against mean of anterior anal canal length for male, defined by both IAS and EAS. Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

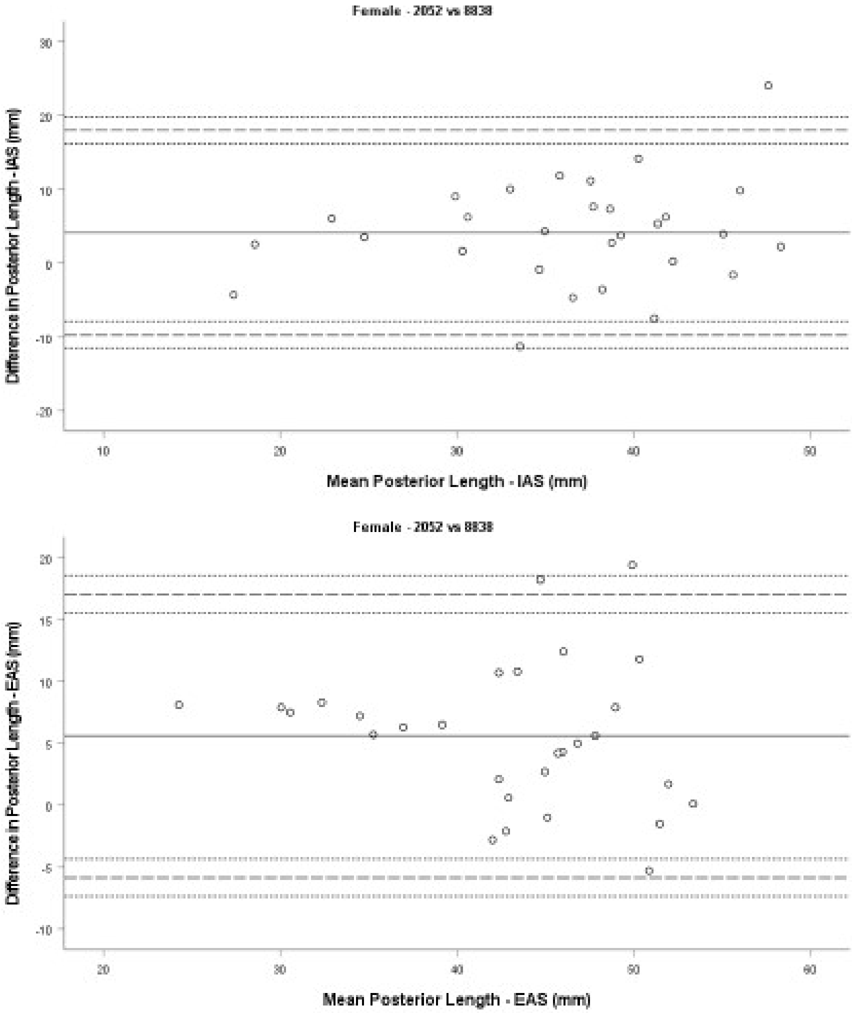


Figure A15 Bland-Altman plots of difference in posterior anal canal length for transducers 2052 and 8838 by Rater 2 against mean of posterior anal canal length for female, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

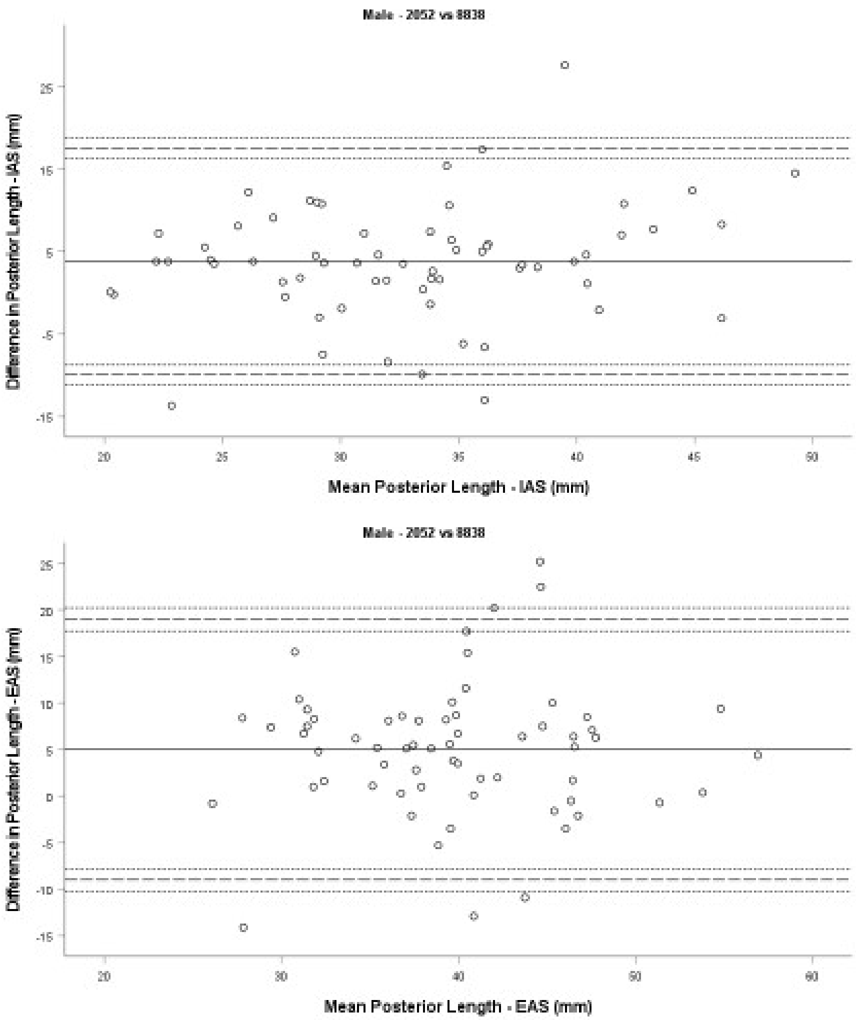


Figure A16 Bland-Altman plots of difference in posterior anal canal length for transducers 2052 and 8838 by Rater 2 against mean of posterior anal canal length for male, defined by both IAS and EAS.

Solid line represents mean; dashed lines the mean ± 1.96SD with 95% CI (dotted lines). IAS: Internal anal sphincter, EAS: external anal sphincter.

Errata

Paper I

Materials and methods – Surgical procedure

Now reads: “monofilament absorbable 2-0 sutures”

Should be: “monofilament absorbable 3-0 sutures”

Results – Patient characteristics – Table 1

Now reads: “Fistulectomy”

Should be: “Fistulotomy”

Paper II

Results – Table 4

Now reads: “Odds”

Should be: “Odds Ra tio (OR)”