Minimal invasive approach in the management of perianal suppuration disease

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DOI:
10.21996/wn2d-6t49

Publication date:
2021

Document version:
Final published version

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This Ph.D. thesis is based on the following three manuscripts:

1. Needle aspiration treatment vs. incision of acute simple perianal abscess: randomized controlled study. International Journal of Colorectal Disease (IF 2.108) Pub Date: 2021-01-15, DOI: 10.1007/s00384-021-03845-6 Karam Matlub Sørensen, Sören Möller, Niels Qvist

2. The outcome of minimal invasive treatment of high crypto-glandular anal fistula. A randomized clinical study. Karam Matlub Sørensen, Sören Möller, Niels Qvist
Submitted to BJS 13.05.2021, revised and resubmitted to BJS *OPEN* 17.06.2021

3. Treatment of fistulizing perianal Crohn's disease by autologous microfat enriched with Adipose-Derived Regenerative Cells, ADRC.
Karam Matlub Sørensen, Charlotte Harken Jensen, Søren Paludan Sheikh, Niels Qvist, Jens Ahm Sørensen
Submitted to *Inflammatory Bowel Disease*® journal on 18.08.2021

Preface and acknowledgements

The present Ph. D. thesis is based on three studies conducted at the Department of Surgery, Odense University Hospital, during period between November 2015 and June 2021. The studies were part of the Ph.D. program at the Department of Clinical Research of Health Science, University of Southern Denmark.

First, I would like to thank the patients who participated in these studies. Having a perianal abscess or fistula is not a life-threatening condition but can trouble the patient with protracted complicated course. These patients dared to take the chance to improve the treatment of themselves and future patients with complex anal fistula.

Secondly, I would like to thank:

My main supervisor Professor Niels Qvist who has given me personal support and been inspiring mentor and colleague, with unlimited guidance in both clinical and academic research questions.

My co-authors Sören Möller, Charlotte Harken Jensen, Søren Paludan Sheikh and Jens Ahm Sørensen for helping me with the advanced statistical analysis and manuscript writing.

Chief Surgeon, Claus Christian Vinther, Department of Surgery, Odense University Hospital, for the continuous support during the conduction of the three studies.

The doctors, nursing staff and secretaries at the outpatient clinic, emergency department and operating theatre of Department of Surgery, Odense University Hospital.

Our collaborators at the Department of Plastic Surgery and Laboratory of Molecular and Cellular Cardiology, Department of Clinical Biochemistry and Pharmacology, Odense University Hospital.

Technicians Tina Kjærgaard Andersen and Tonja Lyngse Jørgensen (Laboratory of Molecular and Cellular Cardiology, Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark), who contributed to the third study by ADRC isolation and characterization.

Musa Büyükuslu (Department of Surgery, Southwest Jutland Hospital in Esbjerg) and Kristina Safir-Hansen (Department of Surgery, Slagelse Hospital), who contributed to the first study, by patient recruitment and follow-up data collection.

The University of Southern Denmark, Odense University Hospital, Danish Crohn Colitis Association CCF, Fionia Fund and the Region of Southern Denmark for Their financial support.

Finally, a special thanks to my wife, Tina and our three beloved children Maja, Adam, and Anna for giving me time and space to prepare and report the studies during the last six years.

Karam Matlub Sørensen, August 2021

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English summary

Background

About one-fifth of the anal fistula are of the complex type, involving a substantial part of the sphincter or associated with inflammation as in Crohn's disease. The treatment of this type is often associated with relative high recurrence and protracted course with potential risk of serious complication.
This Ph. D project was conducted to evaluate three minimal invasive treatment approaches.

- Needle aspiration of an acute simple perianal abscess may be an alternative to conventional incision drainage with potential advantages in wound healing, functional outcome, and quality of life.

- Video-Assisted Anal Fistula Treatment VAAFT may have a recurrence rate comparable to fistulectomy and sphincter repair FSR in the treatment of high anal fistula and with potential advantages in wound healing, functional outcome, and quality of life.

- Stem-cell enriched fat grafting is thought to be more efficient than standard surgical therapy in the treatment of Crohn's anal fistula.

Aim and Objectives

The aim and objectives of the first study are to compare the outcome of needle aspiration and postoperative antibiotics with conventional surgical incision drainage of acute perianal abscess. Primary outcome was abscess recurrence. Secondary outcomes were fistula formation, wound healing, quality of life and fecal continence.

The aim and objectives of the second study are to compare the outcome of VAAFT with FSR of high cryptoglandular anal fistula. Primary outcome was fistula recurrence. Secondary outcomes were anal manometry, quality of life and fecal continence.

The aim and objectives of the third study are to evaluate short-term efficacy and safety of using fat graft enriched with Adipose-Derived Regenerative Cells (ADRC) as a treatment of Crohn's high anal fistula, in terms of healing rate and adverse events.

Methods and Results

First study
A three-center randomized controlled trial, including adults with acute perianal abscess. The needle aspiration group received Clindamycin for one week postoperatively. All included patients were scheduled for follow-up at 2, 12 and 52 weeks postoperatively including physical examination, quality of life assessment (SF 36 questionnaire) and fecal continence (Wexner score)

A total of 98 were included. Recurrence rate was 41% in needle aspiration and 15% in incision drainage, with HR of 3.033 (p=0.014). Fistula formation was 15% without significant difference between the groups. There was no significant difference in wound healing, quality of life or fecal incontinence scores.

Second study

A single center randomized controlled trial, including adults with high anal fistula. The surgical procedures were performed as one-day surgery and with standard postoperative regimen. All included patients were scheduled for follow-up at six months postoperatively including physical examination, MR scanning, anal manometry, quality of life assessment (RAND SF 36 questionnaire) and fecal continence (Wexner score).

A total of 45 patients were included. Recurrence rate was 65% in VAAFT and 27% in FSR, with HR 4.18 (p= 0.016), and the length of the fistula as a risk factor had a significant association with recurrence with HR 1.8 (p=0.02). There was a significant difference in quality of life in favor of FSR and in anal manometry in favor of VAAFT and a significant improvement in Wexner score in both groups.

Third study

Adult patients with transsphincteric anal fistula and Crohn's disease in remission were included. Two simultaneous procedures were performed as a same-day surgery, starting with liposuction from the abdominal wall followed by debridement of the fistula tract and closure of the internal fistula opening. About 30-50 ml lipoaspirate was then re-injected around the fistula tract. Using an automated processing Celution® 800/IV system, ADRC were prepared and injected around the fistula tract (average of 30 million stem cells). Postoperative clinical and MRI follow-up were performed at six months.

12 adult patients were included and nine (75%) had complete clinical healing and eight (67%) radiological healing of the fistula by a single treatment. Complete wound healing was achieved at 12-weeks follow-up in 67% of the patients treated. There was clear and significant improvement in the fecal incontinence score and no major adverse events were observed.

Conclusions

Needle aspiration with postoperative antibiotics cannot be recommended as alternative for surgical incision in the treatment of acute perianal abscess. Fistula formation following acute anal abscess is not affected by the treatment type.

Fistulectomy and sphincter repair is more effective than VAAFT in the treatment of high anal fistulas.

ADRC-enriched autologous lipoaspirate can be safely used in the treatment of high anal fistula in patients with Crohn's disease with high rate of success.

Dansk resume'

Baggrund

Omkring en femtedel af den anale fistel er af kompleks type, der involverer en væsentlig del af lukkemusklen eller er forbundet med betændelse som i Crohn's sygdom. Behandling af denne type er ofte forbundet med relativt høj recidivrate og langvarig forløb med potentiel risiko for alvorlige komplikationer.
Dette ph.d.-projekt blev gennemført for at evaluere tre minimale-invasive behandlingsmetoder.

• Nåleaspiration af en akut anal absces kan være et alternativ til konventionel kirurgisk drænage med potentielle fordele i sårheling, funktionelt resultat og livskvalitet.

• Video-assisted Anal Fistula Treatment VAAFT kan have sammenlignelig recidivrate med fistulektomi og sfinkter reconstruktion FSR som behandling af høj anal fistel og med potentielle fordele i sårheling, funktionelt resultat og livskvalitet.

• Stamcelleberiget fedttransplantation kan være mere effektiv end standard kirurgisk behandling af Crohn's anal fistel.

Formål

Formålet med den første studie er at sammenligne resultatet af nålespiration og postoperative antibiotika med konventionel kirurgisk drænage af akut perianal absces. Primært endpoint var recidiv af absces. Sekundære endpoints var fisteldannelse, sårheling, livskvalitet og fækal kontinens.

Formålet med den anden studie er at sammenligne resultatet af VAAFT med FSR for høj kryptoglandular anal fistel. Primært endpoint var recidivaf fistel. Sekundære endpoints var anal manometri, livskvalitet og fækal kontinens.

Formålet med den tredje studie er at evaluere kortvarig effektivitet og sikkerhed ved anvendelse af fedttransplantat beriget med fedtvævs regenerative celler (ADRC) som behandling af Crohn's høje anal fistel med hensyn til heling og komplikationer.

Metode og Resultater

<u>Første studie</u>
Et randomiseret, kontrolleret forsøg ved tre centre, og inkluderede voksne med akut perianal absces. Nålaspirations gruppen modtog Clindamycin i en uge postoperativt. Alle inkluderede patienter var planlagt til opfølgning 2, 12 og 52 uger postoperativt inklusive klinisk undersøgelse, livskvalitetsvurdering (SF 36 spørgeskema) og fækal kontinens (Wexner score)

I alt 98 blev inkluderet. Recidivrate var 41% i nålespiration og 15% i kirurgisk incision med HR på 3,033 (p = 0,014). Fisteldannelse var 15% uden signifikant forskel mellem grupperne. Der var ingen signifikant forskel i sårheling, livskvalitet eller fækal inkontinens.

<u>Anden studie</u>
Et randomiseret kontrolleret forsøg ved et enkelt center, inkluderede voksne med høj anal fistel. De kirurgiske procedurer blev udført som en dags operation og med standard postoperativt regime. Alle

inkluderede patienter var planlagt til opfølgning seks måneder postoperativt inklusive klinisk undersøgelse, MR-scanning, anal manometri, livskvalitetsvurdering (RAND SF 36 spørgeskema) og fækal inkontinens (Wexner score).
I alt 45 patienter blev inkluderet. Recidivrate var 65% i VAAFT og 27% i FSR med HR 4,18 (p = 0,016), og fistelens længde som en risikofaktor var signifikant associeret med fistel recidiv med HR 1,8 (p = 0,02). Der var en signifikant forskel i livskvalitet til fordel for FSR og i anal manometri til fordel for VAAFT og en signifikant forbedring i Wexner-score i begge grupper.

Tredje studie
Voksne patienter med transsfincterisk anal fistel og Crohn's sygdom i remission blev inkluderet. To samtidige procedurer blev udført som en operation samme dag, startende med fedtsugning fra bugvæggen efterfulgt af debridering af fistelkanalen og lukning af den indre fistelåbning. Omkring 30-50 ml lipoaspirat blev derefter injiceret igen omkring fistelkanalen. Ved hjælp af Celution® 800 / IV-system blev ADRC forberedt og injiceret omkring fistelkanalen (gennemsnit på 30 millioner stamceller). Postoperativ klinisk opfølgning og MR-opfølgning blev udført efter seks måneder.
12 voksne patienter blev inkluderet, og ni (75%) havde fuldstændig klinisk heling og otte (67%) radiologisk heling af fistlen ved en enkelt behandling. Komplet sårheling blev opnået ved 12 ugers opfølgning hos 67% af de behandlede patienter. Der var en klar og signifikant forbedring i fækal inkontinens score, og der blev ikke observeret nogen større bivirkninger.

Konklusioner

Nåleaspiration med postoperative antibiotika kan ikke anbefales som alternativ til kirurgisk incision i behandlingen af akut perianal absces. Fisteldannelse efter akut anal absces er uafhængig af behandlingstypen.
Fistulektomi og reparation af lukkemuskel er mere effektiv end VAAFT til behandling af høje anale fistler.
ADRC-beriget autologt lipoaspirat kan anvendes sikkert til behandling af høj anal fistel hos patienter med Crohn's sygdom med høj succesrate.

Introduction

The anal fistula is an abnormal tract between the anal canal and the perianal skin. Most anal fistulas arise following an abscess and represents the chronic form of perianal suppuration. Anal fistulas can also be caused by inflammatory conditions as anal Crohn`s disease, trauma, malignancy, and radiation. In about one-fifth of the cases, the fistula is of the complicated type and is still presenting a challenging surgical problem [1].

Surgical anatomy (Fig. 1)

The understanding of the anatomy of the anal canal is crucial for the proper management of anal fistula. The anal canal is about 2-4 cm long and is surrounded by the sphincter complex; where the internal muscle is a continuation of the inner circular muscle of the lower rectum, and the external muscle is a thickened continuation of the pelvic floor muscle. The epithelial lining of the anal canal is divided at the dentate line (mucocutaneous junction) into mucosa (cuboidal and then columnar) above and stratified non keratinized squamous epithelium below. The anal crypts or sinus are situated at the dentate line, where the anal glands empty into these crypts [2]. The anal glands are shown to be situated in the submucosa, inside and between the two sphincter muscles [3,4], producing a small amount of mucin.

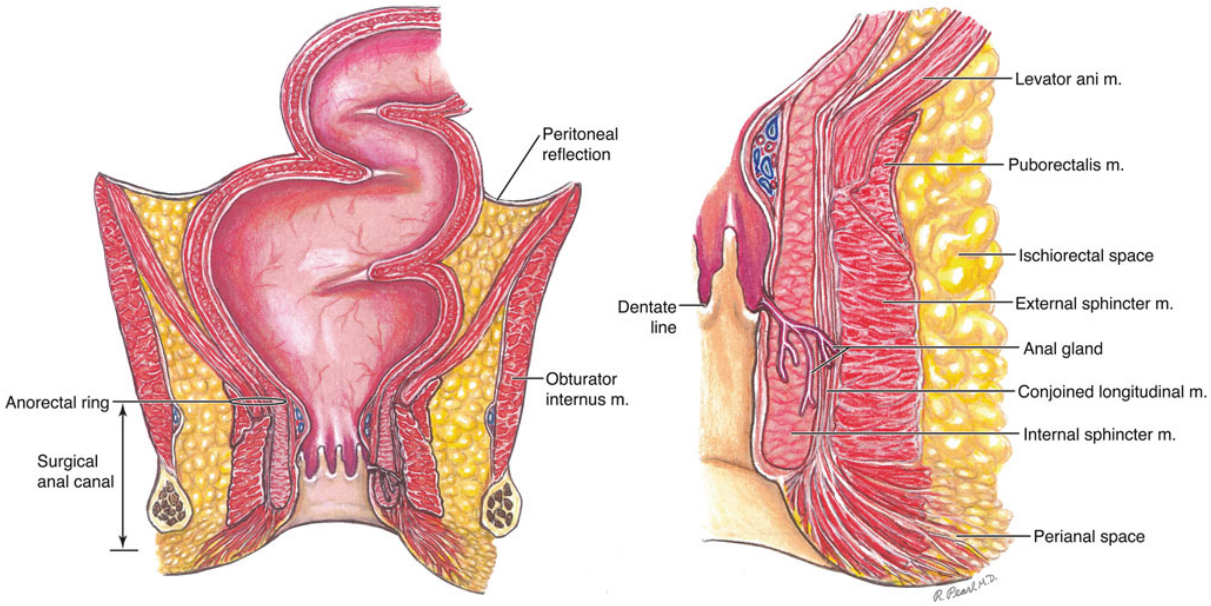


Fig. 1: Coronal view of the anal canal, lower rectum, and surrounding spaces. The enlarged view on the right highlights the architecture of the sphincter muscles and anal glands (From Anal Fistula, Principles and management, H. Abcarian, Springer, 2014)

Pathogenesis

The anal glands are thought to be the origin of infection which leads to abscess-fistula formation [5,3]. This theory of gland infection, first introduced by Parks and Eisenhammer, is widely accepted as explanation of the origin of idiopathic fistula, although it has been previously challenged [6].
The pathophysiology of perianal fistulizing Crohn's disease is more complicated and still not fully clear. The anal fistula in perianal Crohn's disease might be the result of infection/inflammation in the glands or a deep penetrating ulceration in the rectum or anal canal, with several underlying

mechanisms including differentiated epithelial cells migration (epithelial-to-mesenchymal transition EMT) and matrix remodeling enzymes [7,8].

Classification of anal fistula

The anatomical classification of anal fistulas was introduced by Parks, Gordon and Hardcastle in 1976 [5], in which the anal fistulas were divided into four main types according to their relation to the anal sphincter complex: intersphincteric, transsphincteric, suprasphincteric and extrasphincteric fistulas and each was further subdivided into more complicated subtypes depending on the presence of side branches or blind extensions (Fig. 2). Anal fistulas can be also divided into low and high fistulas according to the amount of sphincter muscle involved. By taking the possibility of surgical treatment into consideration, the anal fistulas can be divided into simple and complex [9,10]; where simple fistulas can be treated by incision without major risk of fecal incontinence as they involve less than 30% of the anal sphincter (intersphincteric or low transsphincteric), whereas the complicated type requires other surgical procedure to preserve the continence and it includes fistulas involving more than one-third of the anal sphincter, anterior fistulas in female patients, recurrent fistulas, the presence of impaired anal sphincter function, inflammatory bowel disease or radiation [1,9,10].

Clinical manifestation

In adults, the anal fistula presents usually with an elevated opening near the anus with discharge and might be associated with discomfort, pain, itching and bleeding. There might be an adjacent swelling if there is an associated abscess or fluid retention (Fig.3). There is often a history of previous perianal abscess. Mean age of presentation is 40 years and male to female ratio is 2:1 [11]. Patients with fistulizing perianal Crohn’s disease are younger with median age of 30 and males are slightly affected more than females [12].

Diagnosis

The diagnosis of anal fistula is made by examination under anesthesia EUA and anoscopy looking for the internal opening [9]. The careful probing combined with fluid injection (isotonic saline or hydrogen peroxide) can be useful in localization of the internal opening [11]. EUA can be combined with transanal endoluminal ultrasound and/or magnetic resonant imaging MRI of the anal canal to ensure the correct identification and classification of the fistula tract.

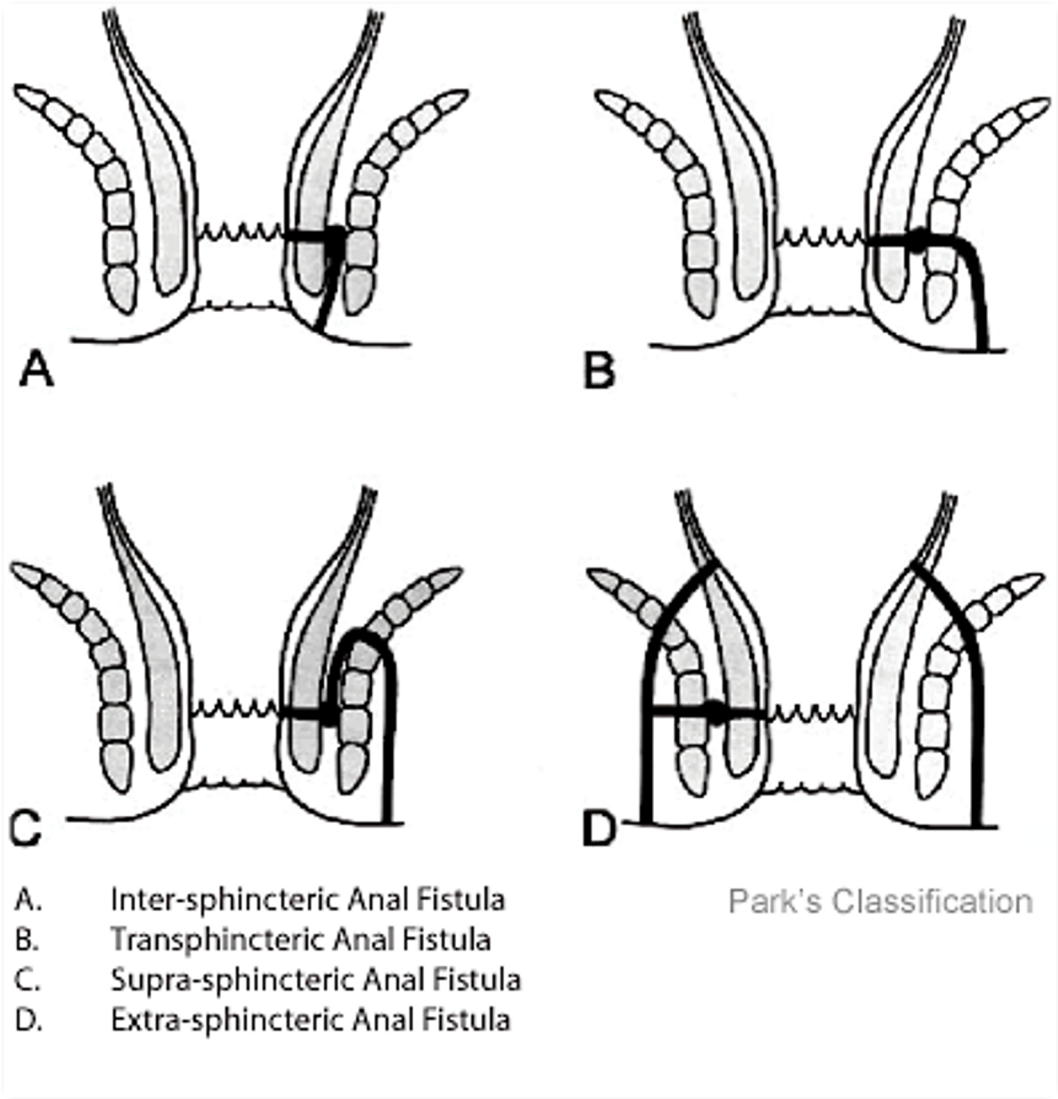


Fig. 2: Parks' classification of anal fistula.

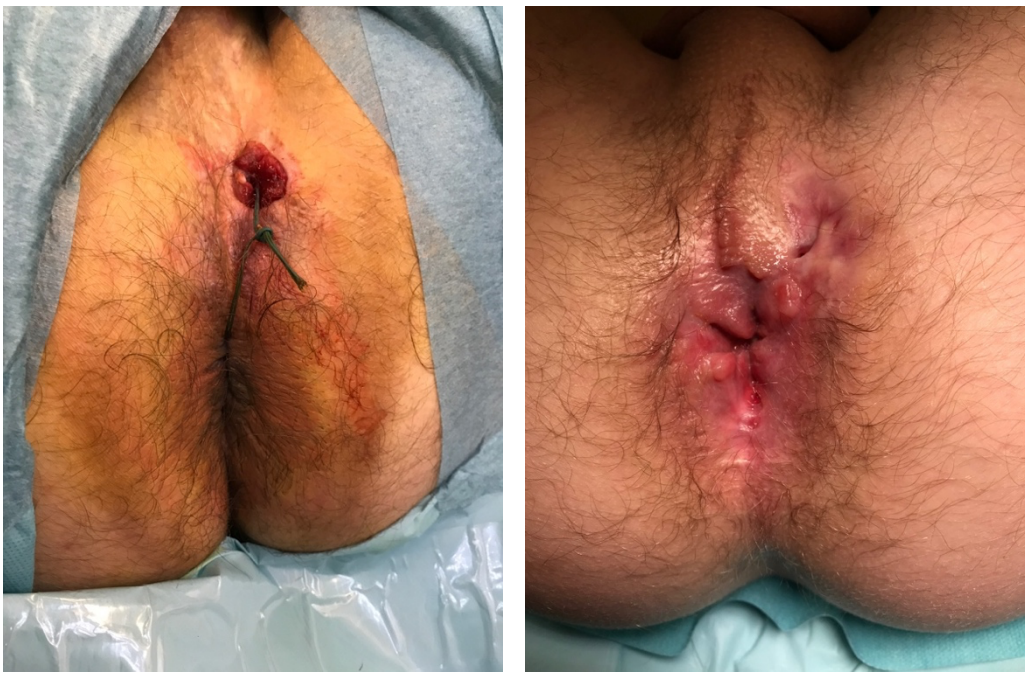


Fig.3: Anal fistula drained by a Seton (left). Perianal fistulizing Crohn’s disease (right).

Background

The three studies comprising this Ph.D. project are conducted to investigate the minimal invasive approaches in three different cases of the perianal suppuration disease.

The anal abscess-fistula sequence (the first study)

Recurrence of perianal abscess and subsequent fistula formation occurs in relatively high percentage of the patients after standard surgical incision drainage. The incision drainage of perianal abscesses can be followed by pain and discomfort, prolonged wound healing and excessive healthcare and societal cost [13]. Several risk factors for recurrence and fistula formation, are previously reported with contradicting results [14-19]. In uncontrolled studies, similar results in terms of recurrence and fistula formation with lesser discomfort have been previously reported using catheter drainage [20-24]. Needle aspiration under ultrasound guidance has not been reported as alternative treatment for perianal abscess. The first study is conducted to verify whether the type of treatment of anal abscess affects abscess recurrence and fistula formation.

The minimal invasive treatment option for high anal fistula (the second study)

The surgical treatment of complex fistulas is difficult and ideally aims to completely heal the fistula and prevent recurrence without affecting the anal sphincter function. There are few randomized trials in the literature on the treatment of complex anal fistulas treatment and there is no conclusive evidence of which method is the best [25]. Furthermore, the knowledge of changes in quality of life and functional results in terms of standardized continence evaluation and manometric studies are either contradictive or simply lacking after the surgery for anal fistulas [24-29]. Success rates of more than 90% [26-28] can be achieved by fistulectomy and sphincter repair (FSR), but with a risk of postoperative delayed wound healing and impaired fecal continence in more than 20% [29,27,30,31] . The minimal invasive sphincter-preserving procedure Video Assisted Anal Fistula Treatment (VAAFT) [32] might be an alternative treatment method, with previously shown promising results [33-37,32] and success rates reported between 70-85%. The second study was conducted to compare a sphincter-saving (VAAFT) with a sphincter-cutting (FSR) methods as a treatment of cryptoglandular high anal fistula.

A new treatment option for fistula in perianal Crohn’s disease (the third study)

Several surgical treatment methods for high fistula associated with perianal Crohn's disease (pCD) in patients with failed medical therapy have been described but all with relative high recurrence and failure rates [38,39]. The use of biologicals did not alter the risk of anal fistula formation in perianal Crohn’s disease [40]. The need for repeated surgery [41], significant impairment of quality of life [42-44] and a relatively high risk of proctectomy [41,45,46] emphasize the need of an alternative treatment approach. The combination of surgical closure of the fistula internal opening and the use of adult stem cell injection in or around the fistula [47-51], or the use of autologous adipose tissue graft [52,53] have been shown to be safe and feasible but with various healing rates. The stromal vascular fraction (SVF), also referred to as Adipose Derived Regenerative Cells (ADRC), is isolated from freshly harvested autologous adipose tissue (by liposuction) and includes several cell types with regenerative potentials. The third study was conducted to test the possibility of enhancing the surgical treatment of fistulizing pCD with autologous fat graft enriched with ADRC.

Aim and objectives

The aim of the first study was to compare the outcome of needle aspiration and postoperative antibiotics with standard surgical incision and drainage. The primary objective was recurrence of abscess. The secondary objectives were fistula formation, time to wound healing, fecal incontinence as measured by Wexner fecal incontinence score and quality of life scoring, using Short Form (Rand SF36).

The aim of the second study was to compare the outcome of VAAFT (intervention) with FSR (control) in the treatment of high anal fistula. The primary objective was to compare recurrence after initial treatment. The secondary objectives were comparing fecal continence evaluated with Wexner fecal incontinence score, anal manometry and quality of life measured with Rand Short Form SF36.

The aim of third study was to evaluate the outcome and safety of treating fistulizing pCD with autologous fat graft enriched with ADRC. The primary objective was the healing rate defined as absence of discharge and closure of external fistula opening by clinical examination. The secondary objectives were time to healing (weeks), fecal incontinence measured by Wexner Fecal Incontinence Score and radiological recurrence of fistula on MRI scanning.

Ethical approvals and study registrations

The three studies were conducted in accordance with the rules of the Helsinki Declaration. Data collection and processing were performed according to the Act of Processing of Personal Data and Health Act and were approved by Region of Southern Denmark´s joint review of the Data Protection Agency (20/18031). All studies were approved by the local Research Ethics Committee and were registered on Clinicaltrial.org

Methods and Results

The first study

This multicenter study was a randomized controlled open label trial to compare the outcome of treatment of acute perianal abscess by needle aspiration drainage (minimal invasive) and single broad-spectrum antibiotic with standard incision drainage. Table 1 shows the inclusion- and exclusion criteria.

Included patients were randomly allocated using REDCap online database tools into intervention (needle aspiration drainage) or control (incision drainage). Both procedures were done under general anesthesia and ultrasound was done to ensure diagnosis, measuring the largest diameter of the abscess and to exclude horseshoeing. Pus obtained from patients, when possible, was examined for bacterial growth. The abscess was drained either by incision de-roofing and curettage and irrigation of the cavity (control group) or by aspiration of the content and saline irrigation by large caliber needle with one week of postoperative oral antibiotic monotherapy (intervention group).

Inclusion criteria	Exclusion criteria
Adults (≥ 18 years), admitted to hospital with an acute perianal abscess and where surgical drainage was indicated	1. recurrent perianal abscess or a history of perianal fistula within the last six months 2. immune suppressive treatment 3. malignancy within the last five years 4. previous pelvis radiotherapy 5. pregnancy or lactation 6. known allergy for Clindamycin. 7. an abscess with a diameter less than 2 cm 8. the presence of a horseshoe abscess by per-operative ultrasonography

Table 1: The inclusion and exclusion criteria of the first study.

Included patients were assigned to a scheduled clinical follow up at 2, 12 and 52 weeks after treatment, which included clinical wound assessment for healing, palpation for suppuration and the patients were asked to fill out Rand SF-36 questionnaire and Wexner fecal incontinence score. Whenever there was a suspicion of recurrence or fistula formation, examination under general anesthesia was performed. A recurrence was treated by incision drainage irrespective of the primary treatment.

Data collection

Patient's demography, surgical and ultrasonic findings, microbiology results and follow-up findings as well as Wexner Fecal Incontinence score and Rand SF-36 score data were collected.

The following factors were examined in the analysis as risk factors for recurrence and fistula formation; patient risk factors (age, gender, Body Mass Index BMI, tobacco, alcohol, use of antibiotics before admission, duration of symptoms, health status) and abscess risk factors (allocated treatment, size of abscess, location of abscess, bacterial growth).

Statistical analyses

Desired sample size was determined for comparison of two proportions, assuming a clinically accepted rate of recurrence of 5% in the incision drainage group, and 25% recurrence rate in the needle aspiration group, resulting in a desired sample size of 49 patients in each group of the study for obtaining a significance level of 5% at a power of 80%.

Survival models were used in the analysis of abscess recurrence and fistula formation as well as the risk factors. T-test, two sample Wilcoxon rank-sum (Mann-Whitney) test and Pearson χ^2 -test were used when appropriate in the analysis of the covariates. P-values below 0.05 were considered statistically significant.

Results

98 patients were included in the study with 46 patients allocated in the needle aspiration group and 52 patients in the incision group (Fig. 4). Complete case analysis without missing data was possible for 78 out of 98 patients (80%). Missing data were mainly from follow-ups.

The demographic characteristics of the two groups are shown in Table 2. No major difference between the groups were observed. Pus samples were possible to obtain in 83 (85%) patients, and

bacterial growth was shown in 64 samples (77%), and without significant association between bacterial growth and recurrence of the abscess or fistula formation. No treatment-demanding surgical or medical complications were observed.

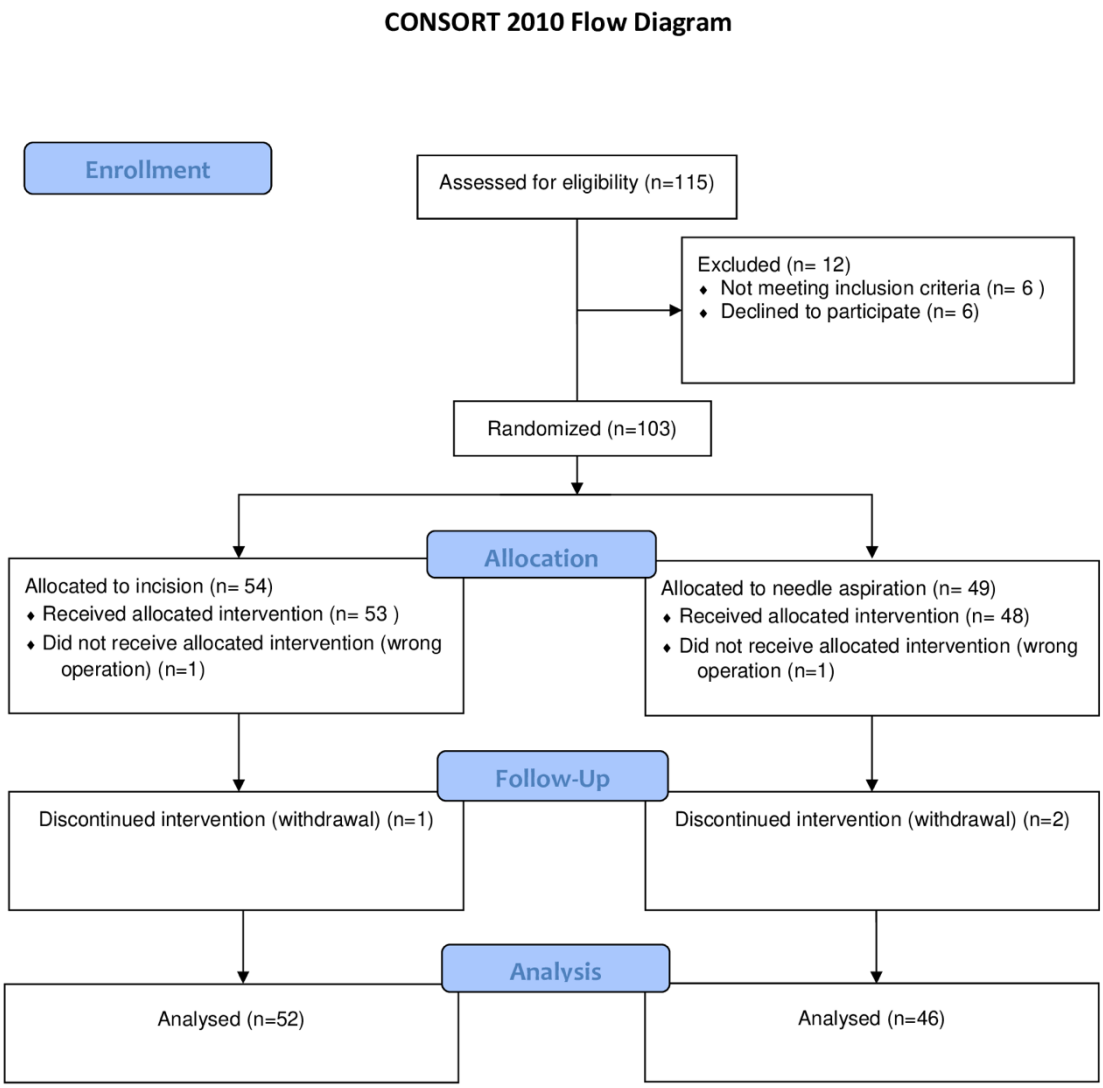


Fig. 4: The first study CONSORT flowchart.

	Incision (n=52)			Needle aspiration (n=46)			
Variables	Frequency	Mean	95%CI	Frequency	Mean	95%CI	Total
Gender							
Male	34 (65.4%)			29 (63%)			63 (64.3%)
Female	18 (34.6%)			17 (37%)			35 (35.7%)
Age		45.59	41.52 - 49.66		46.41	41.68 - 51.14	45.98
BMI		27.57	26.34 - 28.81		26.59	24.66 - 28.53	27.11
Health status							
Healthy	39 (75.00%)			33 (71.74%)			72 (73.47%)
Co-morbidity	13 (25.00%)			13 (28.26%)			26 (26.53%)
Tobacco							
Non-smoker	12 (23.08%)			17 (36.96%)			29 (29.59%)
Smoker	33 (63.46%)			22 (47.83%)			55 (56.12%)
Quit	7 (13.46%)			7 (15.22%)			14 (14.29%)
Alcohol							
Null	17 (32.69%)			8 (17.39%)			25 (25.51%)
=<7/14 pints/week	30 (57.69%)			32 (69.57%)			62 (63.27%)
> 7/14 pints/week	5 (9.62%)			6 (13.04%)			11 (11.22%)
Duration of symptoms (days)		6.48	4.47 - 8.49		6.97	4.85 - 9.06	6.70
7 days	42 (80.77%)			38 (82.61%)			80 (81.63%)
> 7 days	10 (19.23%)			8 (17.39%)			18 (18.37%)
Prior use of antibiotics							
Prior use of antibiotics	22 (42.31%)			13 (28.26%)			35 (35.71%)
No prior use of antibiotics	30 (57.69%)			33 (71.74%)			63 (64.29%)
Size of abscess		3.43	3.04 -3.82		3.70	3.20 - 4.19	3.55
2-5 cm	48 (92.31%)			40 (86.96%)			
> 5 cm	4 (7.69%)			6 (13.04%)			
Microbiology study							
Growth of bacteria	29 (72.5%)			35 (81.4%)			64 (77.1%)
No growth of bacteria	11 (27.5%)			8 (18.6%)			19 (22.9%)

Table 2: Demographic characteristics of the two groups. (The first study)

Recurrence of perianal abscess

27 (28%) patients were treated for recurrences: 19 (41%) in the needle aspiration group and 8 (15%) in the incision group (p=0.006). Most of the recurrences occurred during the first four months of observation time in both groups (Fig. 5). The estimated cumulative hazard of recurrence was 0.02 in both groups at two weeks follow-up, and it increased to 0.20 in the incision group and 0.60 in intervention group at 52 weeks follow-up. Multivariate analysis showed significant higher risk of recurrence following needle aspiration with HR 3.033 (95% Confidence Interval CI 1.251-7.357, and p= 0.014), and none of the other risk factors analyzed had a significant association with recurrence.

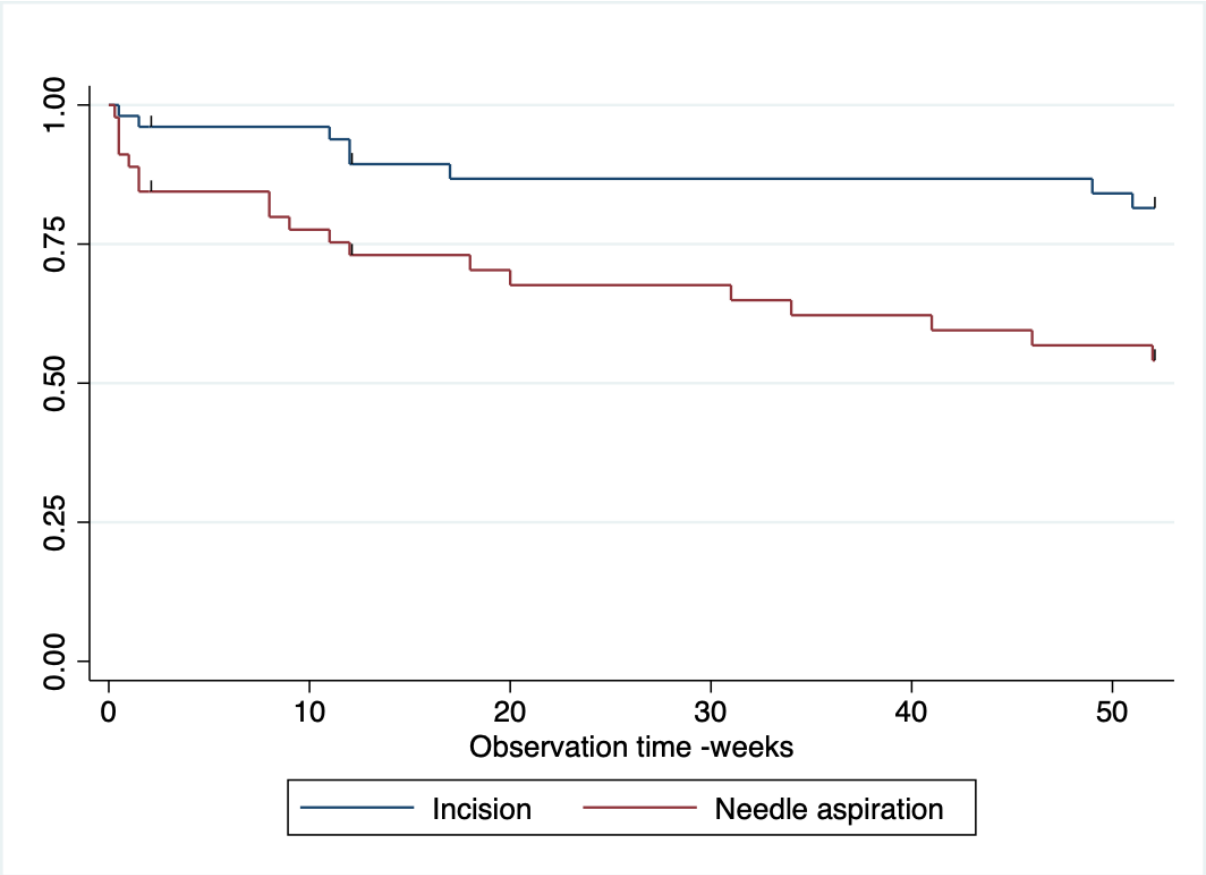


Fig. 5: Kaplan-Meier survival estimates of perianal abscess recurrence

Fistula formation

Four patients were found to have fistula at the time of operation. Of the remaining 94 patients, 14 patients (15%) developed subsequent fistula; 8 (16%) in the incision drainage group and 6 (14%) in the intervention group (p=0.780). The estimated cumulative hazard was similar in both groups, being 0.29 at 52 weeks after treatment. Univariate analysis showed that older age was associated with lower risk of fistula formation, with HR 0.342 (95% CI .131-.891, p= 0.028), while multivariate analysis showed anteriorly located abscess was associated with lower risk of fistula formation, with HR 0.264 (95% CI .072-.970, p= 0.045).

Wound healing

Wound healing was obtained in 98 % of the patients. Wound healing time was longer in incision drainage group, as 68% of needle aspiration group patients healed at 2 weeks follow-up and 67% of the patients in incision drainage group healed at 12 weeks follow-up.

Fecal incontinence score

The mean Wexner fecal incontinence score at baseline was comparable between the two groups (p= 0.86) with ¾ of the patients having no symptoms of incontinence. There were no differences in the mean Wexner fecal incontinence score between groups throughout the three clinical follow-ups at 2, 12 and 52 weeks (Fig. 6).

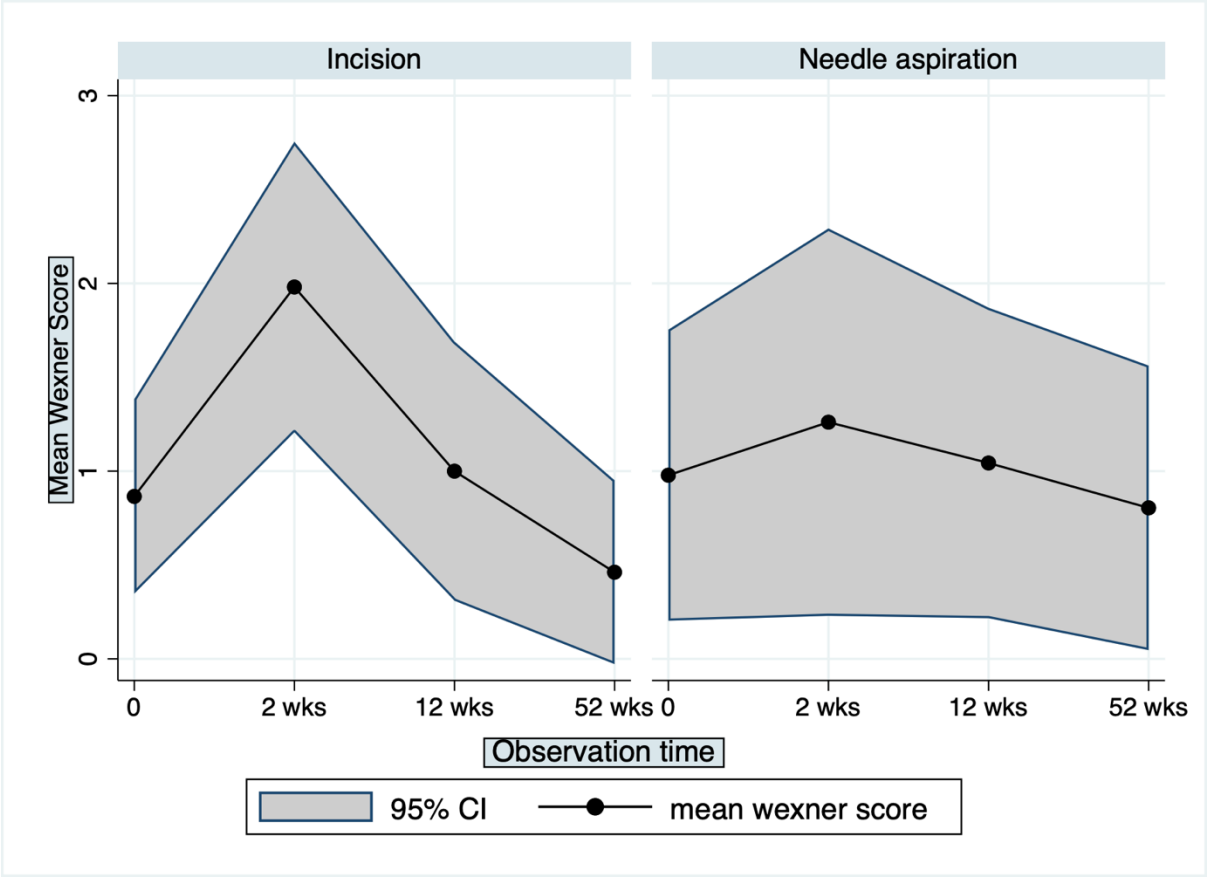


Fig. 6: Mean profile of differences in Wexner Fecal incontinence Score over the observation period. (X axis: 0 baseline, 2-12-52 weeks follow-up. Y axis: mean Wexner Fecal incontinence score). CI: Confidence Interval

Quality of Life score

Emotional well-being score was significantly lower (i.e., more disability) in the control group with incisional drainage at 2 weeks follow-up (p= 0.033), otherwise there were no significant differences. The pain score was higher (i.e., lower disability) at 52 weeks follow-up compared to baseline (p<0.001), without differences between the two groups.

Complete case analysis

Complete case analysis showed similar results of recurrence and fistula formation and analysis of Wexner score and Rand SF36 data showed no further differences.

The second study

This study was conducted as a randomized controlled open label trial to compare the outcome of treatment of complex cryptoglandular anal fistula by Video-Assisted Anal Fistula Treatment VAAFT (minimal invasive) with Fistulectomy and primary Sphincter Repair FSR.

Adults (≥ 18 years) referred with complex cryptoglandular anal fistula, with intention of surgical treatment were eligible patients. Patients with Crohn disease, signs of suppuration and cavitation, immune suppressive treatment, malignancy within the last five years, previous pelvis radiotherapy or a rectovaginal fistula were excluded. Included patients were randomly allocated into intervention (VAAFT) or control (FSR), using REDCap online database tools.

All included patients had undergone pre-operative work-up including anal examination under general anesthesia, endoluminal ultrasound, baseline MRI scanning of the anal canal as well as baseline high resolution anal manometry. All allocated procedures were carried out as one-day surgery and all excised fistula tissue was sent for histopathology.

The fistula tract was excised in its entire length after dividing the involved part of the anal sphincter in the FSR group. The sphincter complex including the anal canal was reconstructed using interrupted absorbable sutures. The internal and external anal sphincters were repaired separately. The lateral part of the incision was left open for drainage. In the VAAFT group, the procedure was performed according to the original technique described by Meinero and Mori [32], using Meinero fistuloscope (Karl-Storz, Tuttlingen, Germany). The internal orifice was secured with two-layer closure using interrupted absorbable sutures for both the muscle and anal mucosa layers. The external orifice was excised leaving the wound for secondary healing.

Included patients were assigned to a scheduled clinical follow up at 6 months after treatment, which included clinical wound assessment for healing and sign of fistula by physical examination. Patients were asked to fill out Rand SF-36 questionnaire and Wexner fecal incontinence score. MRI scanning of the anal canal, endoanal ultrasound and high-resolution anorectal manometry were performed.

Data collection

Patient's demography, surgical and ultrasonic findings, anal manometry, and follow-up findings as well as Wexner Fecal Incontinence score and Rand SF-36 score data were collected.

The following factors were examined in the analysis as risk factors for recurrence of the fistula; patient risk factors (age, gender, Body Mass Index (BMI), tobacco, alcohol, duration of symptoms, health status) and fistula risk factors (allocated treatment, length of fistula in centimeter (measured by standard per operative probing), location of fistula).

Statistical analyses

Desired sample size was determined for comparison of two proportions, assuming a rate of clinically accepted recurrence of 5% in the FSR group, and 30% recurrence rate in the VAAFT group, resulting in a necessary sample size of 33 patients in each group of the study for obtaining a significance level of 5% at a power of 80%. The high recurrence rate accepted for VAFT was mainly due to the mini-invasive character of VAFT compared to standard treatment.

Differences in recurrence of the fistula were analyzed using survival models and univariate and multivariate analysis of the risk factors were performed using Cox proportional hazards regression. Continuous and categorical covariates were compared between groups using t-test, two sample Wilcoxon rank-sum (Mann-Whitney) test and Pearson χ^2 -test when appropriate. P-values below 0.05 were considered statistically significant.

Results

It was possible to include and analyze 45 patients with high transsphincteric anal fistula with 23 patients allocated in the VAAFT group and 22 patients in the FSR group (Fig. 7). Table 3 shows the distribution of the demographic characteristics in the two groups. No major difference between the groups were observed, and none of the patients had a stoma. Mean length of the fistula tract was 4.3 cm, 19 fistulas were located posteriorly to the anus and mean duration of symptoms was 14.6 months. Histopathological study of the fistula tissue was possible in 43 patients (96%), and none of them showed inflammatory bowel disease. Only two patients, one in each group, had a previous history of anal fistula surgical treatment. Besides recurrences, there were no other medical or surgical complications and none of the patients needed a diverting stoma.

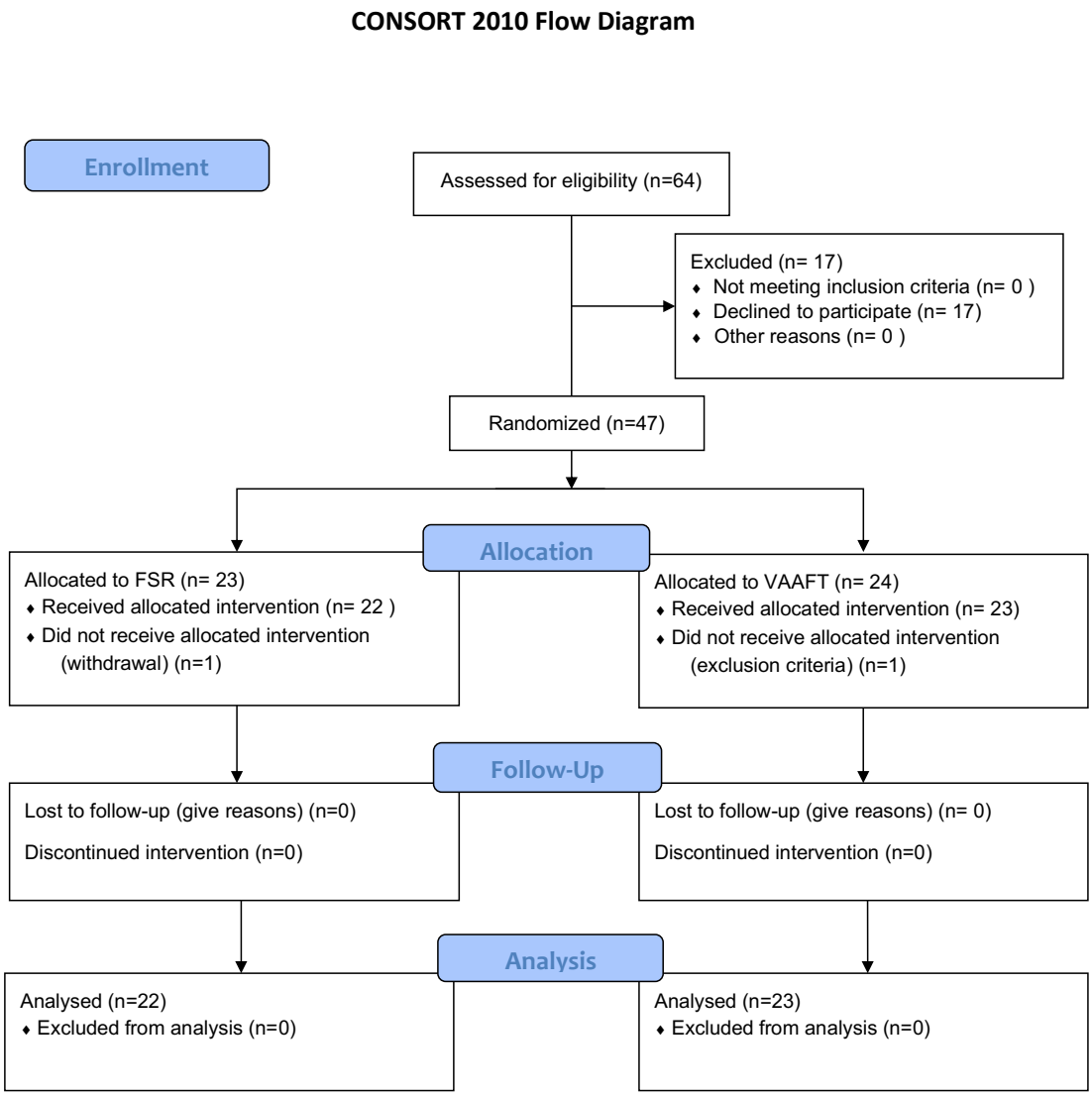


Fig. 7: The second study CONSORT flowchart.

Variables	FSR n=22		VAAFT n=23		Total n=45	
	Mean (freq.)	**95% CI**	**Mean (freq.)**	**95% CI**	**Mean (freq.)**	**95% CI**
Age	45.05	38.93-51.16	42.65	37.17-48.14	43.82	39.88-47.77
Young	(8) – 36%		(11) – 48%		(19) – 42%	
Old	(14) – 64%		(12) – 52%		(26) – 58%	
Gender						
male	(15) – 68%		(17) – 74%		(32) – 71%	
female	(7) – 32%		(6) – 26%		(13) – 29%	
BMI	29.25	27.56-30.95	28.07	26.21-29.92	28.65	27.43-29.87
Normal weight	(1) – 4.6%		(7) – 30.4%		(8) – 17.8%	
Overweight	(13) – 59%		(8) – 34.8%		(21) –46.6%	
Obese	(8) – 36.4%		(8) – 34.8%		(16) –35.6%	
Tobacco						
None	(9) – 40.9%		(16) –69.6%		(25) –55.6%	
Smoker	(7) – 31.8%		(2) – 8.7%		(9) – 20%	
Quit	(6) – 27.3%		(5) – 21.7%		(11) –24.4%	
Alcohol						
0	(2) – 9.1%		(2) – 8.7%		(4) – 8.9%	
≤7/14 unit/wk.	(18) –81.8%		(20) – 87%		(38) –84.4%	
> 7/14 unit/wk.	(2) – 9.1%		(1) – 4.3%		(3) – 6.7%	
Health status						
Healthy	(19) –86.4%		(19) –82.6%		(38) –84.4%	
Co-morbidity	(3) – 13.6%		(4) – 17.4%		(7) – 15.6%	
Duration (m)	11.6	8.11-15.16	17.4	12.38-22.40	14.6	11.47-17.67
Fistula location						
Anterior	(14) – 63.6%		(12) – 52.2%		(26) – 57.8%	
Posterior	(8) – 36.4%		(11) – 47.8%		(19) – 42.2	
Length of fistula (cm)	4.41	3.73-5.09	4.24	3.62-4.85	4.32	3.88-4.76

Table 3: Patients demographic characteristics of the second study. unit alcohol=12 g alcohol. Maximal 7 units for females and 14 units for males per week as Danish health administrations recommendation for alcohol consumption. (m) = month. (cm) = centimeter. (freq.) = frequency.

Recurrence of the fistula

VAAFT group had 15 (65%) fistula recurrences compared to six (27%) in the FSR group (p=0.01). The recurrences occurred throughout the observation time in both groups (Fig. 8) and all recurrences

occurred at the operation site. The estimated cumulative hazard of recurrence was 0.30 in the FSR group and 0.98 in VAAFT group at 6 months follow-up.
Multivariate analysis showed significant higher risk of recurrence following VAAFT with HR 4.18 (95% confidence interval (CI) 1.30-13.42, and p= 0.016), and analysis of risk factors showed a significant association between length of the fistula and recurrence with HR 1.8 (95% CI 1.097-2.984, p=0.02), while higher BMI was associated with lower risk of recurrence, HR 0.76 (95% CI 0.633-0.91, p=0.003) and the obese category with HR 0.11 (95% CI 0.019-0.618, p=0.012).
Clinically obscured recurrence was revealed by MR scanning at follow-up in six patients in FSR group and four in VAAFT group. At follow-up, three patients in FSR group and ten patients in VAAFT group had not achieved wound healing (epithelialization) (p=0.027).

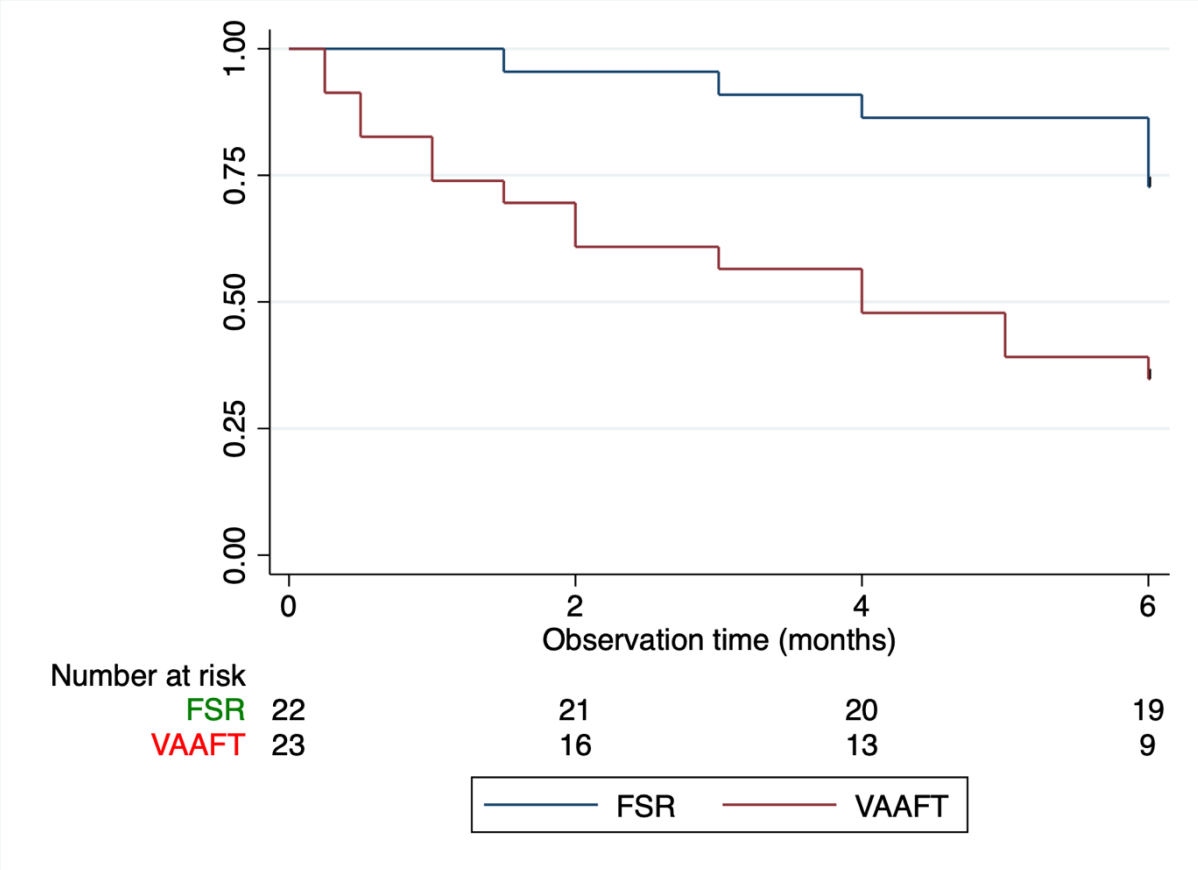


Fig. 8: Kaplan-Meier estimate of fistula recurrence. FSR= Fistulectomy and sphincter repair. VAAFT= Video-Assisted Anal Fistula Treatment.

Fecal incontinence

The mean Wexner fecal incontinence score at baseline was comparable between the two groups (p= 0.13) with 36% of the patients having mild or no symptoms of incontinence. There was a significant improvement in the mean Wexner fecal incontinence score when comparing baseline and follow-up measurements for both groups (FSR p=0.022 and VAAFT p=0.011) (Fig. 9).

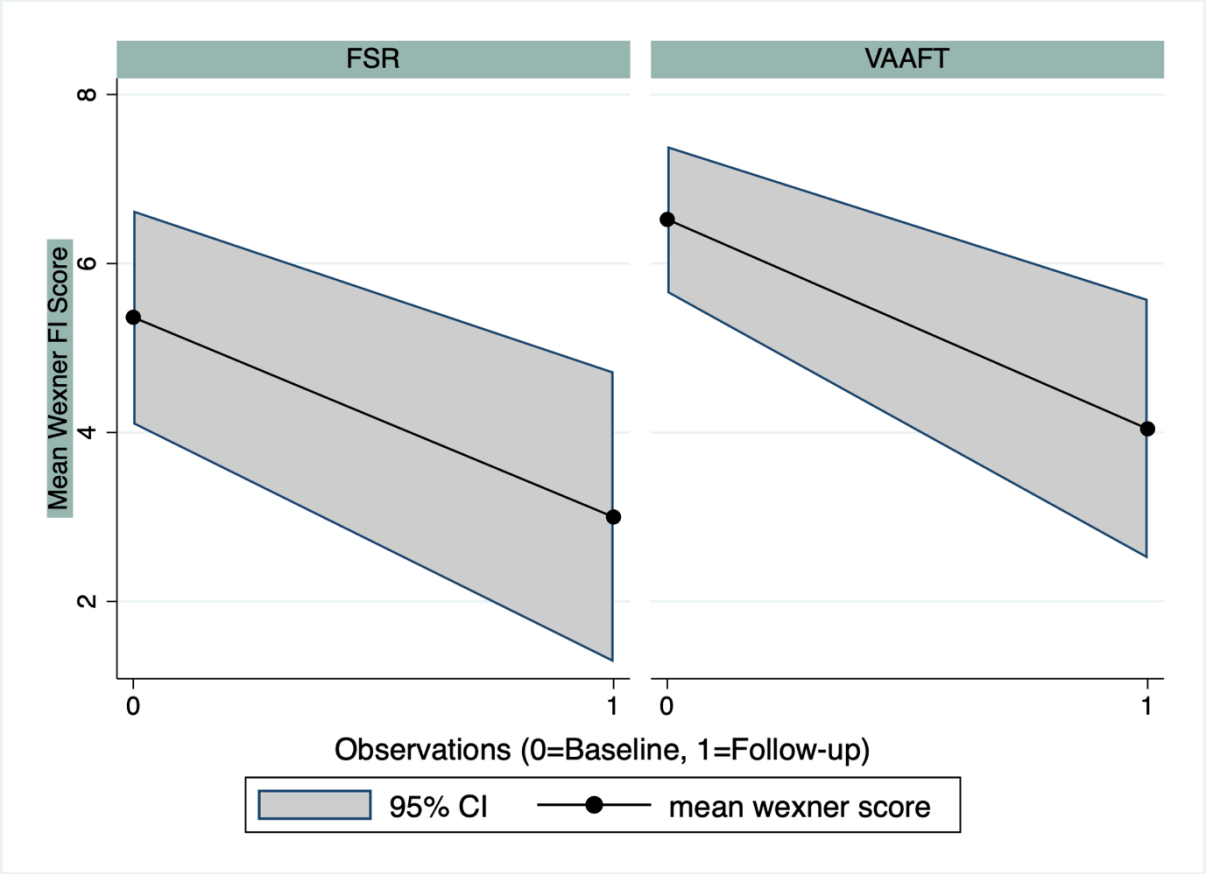


Fig.9: Mean profile of differences in Wexner Fecal incontinence Score over the observation period. (X axis: 0 baseline, 1 follow-up. Y axis: mean Wexner Fecal incontinence score). CI: Confidence Interval

Anal manometry measurement showed a decrease in the mean resting and squeezing pressures in both groups at the follow-up but only statistically significant for the mean squeezing pressure in the FSR group (p=0.018). At follow-up, endoluminal ultrasound revealed defect of the internal anal sphincter in nine (41%) patients in FSR group and one patient (4%) in VAAFT group (p=0.003). The presence of sphincter defect did not have significant impact on the results of anal manometry or fecal incontinence score.

Quality of Life

Analysis of mean of the eight parameters of RAND SF-36 score (Table 4) revealed significant increase (i.e., lower disability) of all the parameters in the FSR group and in two parameters (Physical Function Score and Pain Score) in the VAAFT group. By comparing the two groups at the follow-up measurement, significant differences were found in favor for FSR group in three of the parameters (Energy/fatigue Score, Social functioning Score, Pain Score).

Rand SF-36	Fistulectomy and sphincter repair FSR		Video-assisted Anal Fistula Treatment VAAFT		FSR & VAAFT		FSR vs VAAFT
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Follow-up
Physical Function Score	81.36	94.32 **P=0.0019**	74.78	85.65 **P=0.0024**	78	89.89 **P<0.0001**	P=0.0625
Role limitations due to physical Health Score	62.12	86.36 **P=0.0171**	65.21	68.12 P=0.7753	63.70	77.04 P=0.0626	P=0.1159
Role limitations due to emotional problems Score	62.12	86.36 **P=0.0171**	65.22	68.12 P=0.7753	63.70	77.04 P=0.0626	P=0.1159
Energy/fatigue Score	53.18	75.90 **P=0.0005**	49.34	59.13 P=0.0501	51.22	67.33 **P=0.0001**	**P=0.0121**
Emotional well-being Score	70	82.54 **P=0.0043**	67.36	74.26 P=0.0520	68.62	78.31 **P=0.0005**	P=0.1318
Social functioning Score	81.25	93.18 **P=0.0496**	65.22	75 P=0.0647	73.06	83.89 **P=0.0061**	**P=0.0117**
Pain Score	68.07	88.64 **P=0.0002**	57.83	73.91 **P=0.0019**	62.83	81.11 **P<0.0001**	**P=0.0171**
General health Score	69.77	79.55 **P=0.0051**	67.17	69.57 P=0.4095	68.44	74.44 **P=0.008**	P=0.0767

Table 4: Quality of life measurements of study population. The figures are the mean of each score of the eight parameters of Rand SF 36. Significant p values are highlighted.

Early cessation of the study

It was necessary to perform a premature analysis of the results due to the observed higher recurrence rate in the intervention group throughout the study, which showed a significant statistical difference in the primary objective, which could not be altered by continuing the study (futility analysis), leading to a serious ethical consideration of a premature closing of the study which was decided due to safety and benefit concerns [54].

The third study

The study was conducted as a prospective single center pilot study. The inclusion criterium was adult patient (≥18 years) with fistulizing perianal Crohn's disease not responding to medical therapy for at least six months and a loose seton suture for at least three months. Exclusion criteria were multiple (two or more tracts) fistulas, anal stenosis, suppuration around the fistula tract, a subcutaneous perianal fistula, active intestinal Crohn's disease not in remission, Body Mass Index BMI < 18.5, coagulopathy, previous radiotherapy to the abdomen and pelvis, any malignancy within five years and verified syphilis, Human Immune deficiency virus **(**HIV) infection or hepatitis on screening test.

After informed consent all included patients were scheduled for preoperative work-up with MRI scanning of pelvis/rectum, serological screening test for syphilis, hepatitis and HIV and physical examination under general anesthesia including trans-anal ultrasound with anatomical mapping of the fistula tract. Patients' current Crohn's medications were not altered or postponed during the period of treatment and follow-up.

Surgical procedures and ADRC preparation

All procedures were performed as a same day surgery. The fatty tissue was harvested by liposuction form anterior abdominal wall and followed by surgical debridement of the fistula tract and double layered closure of the internal opening using absorbable suture material and leaving the excised external opening open for drainage. Then 30-50 milliliters of the freshly harvested lipoaspirate were injected around the entire length of fistula tract, taking care not to perforate the fistula tract (Fig. 10). The patient was observed at the recovery unit for 120-150 minutes. Meanwhile, ADRC were isolated from the remaining lipoaspirate using an automated processing Celution® 800/IV system (Lorem Cytori, San Diego, California, USA) according to the manufacturer's instructions. Four ml of isolated ADRC suspension were injected and evenly distributed around the fistula tract corresponding to the locations of previous injections of lipoaspirate under mild sedation with propofol.

The patients were discharged the same day with prescription of postoperative oral antibiotics (metronidazole 1500 milligram/day and cefuroxime 1000 milligram/day) for seven days.

Follow-up

All included patients were scheduled for three postoperative follow-ups at two weeks, three and six months, for clinical evaluation of healing as well as Wexner fecal incontinence score. MRI scanning was performed at 6-months follow-up, for patients with clinical healing and no sign of recurrence at physical examination. Whenever there was a suspicion of recurrence or abscess formation, examination under general anesthesia was performed. Further clinical follow-up for healing and recurrence was done at 12 months, for patients who achieved clinical and radiological healing at 6-months follow-up.

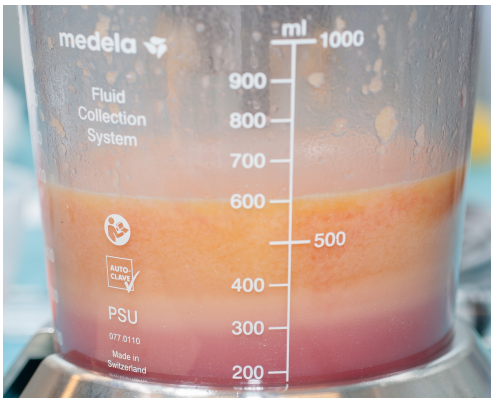
Data Collection

The collected data included patients' demography, Crohn's disease, surgical findings, ADRC analysis, follow-up clinical findings, Wexner Fecal Incontinence Score and MRI scanning.

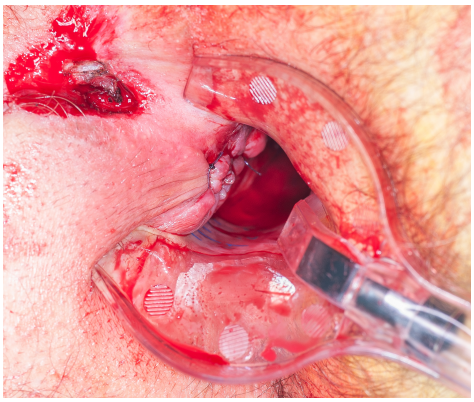
Statistical analysis

Continuous variables were analyzed using simple descriptive statistical tests (*t*-test and Wilcoxon rank-sum (Mann-Whitney) test) and were expressed in frequencies and mean when appropriate. Following Normality tests, cell data were analyzed using unpaired *t*-test and Pearson correlation (r).

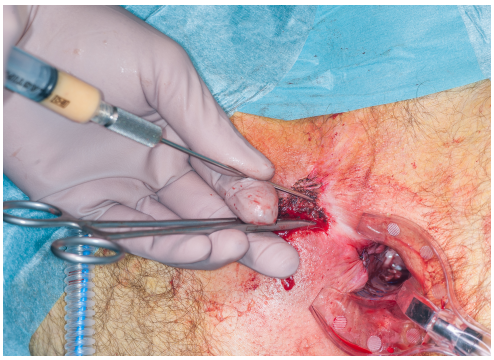
P values below 0.05 were considered significant. Stata statistical software package version 16.0 and GraphPad Prism 9 were used.



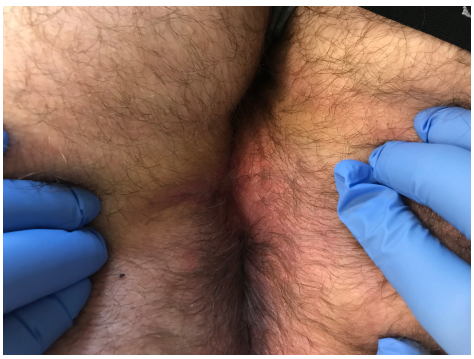
a: Lipoaspirate (upper layer) harvested with water-jet liposuction from the anterior abdomen



b: while the lipoaspirate left to sediment at the operating theatre, the fistula is curated, and internal opening is closed with two-layer absorbable sutures



c: the lipoaspirate is directly injected around the entire length of fistula tract. When the ADRC suspension is ready, it is injected into the same site of lipoaspirate injection



d: Complete healing at 6 months follow-up

Fig. 10: Combined injection of fat-graft enriched with ADRC and surgical debridement and closure of the anal fistula.

Results

During the study period, 13 patients were found eligible for the study. One patient was excluded due to exacerbation of Crohn disease and weight loss (BMI below 18.5) despite intensive medical treatment. 12 patients received the intended treatment and completed the observation period.

Demography and surgical data

Table 5 shows the demographic characteristics of the study population. All but three patients received adjuvant medical therapy (biological treatment alone in six and biological treatment plus immune modulation in three). The mean length of fistula was 4.5 cm (95% CI 3.2-5.8), all fistulas were high transsphincteric and in two patients there were two external openings. One patient had a diverting colostomy.
The mean total volume of lipoaspirate was 263 ml (95% CI 220-307) and the mean volume of lipoaspirate injected was 40 ml (95% CI 31-49.6). A mean of 233 ml (95% CI 174-292) of the lipoaspirate was used for ADRC isolation. The average number of injected ADRC cells was 37.6 million (95% CI 29.3-45.8).

Gender Male Female	 3 9
Age Mean in years (Min-Max)	 33 (22-51)
BMI Mean (Min-Max)	 27.9 (20.07-37.03)
Smoking habits	
Smokers	3
Quit	3
Non-smokers	6
Alcohol consumption	
Within recommended	11
Above recommended	1
Co-morbidity	1
Duration of fistula Mean in months (Min-Max)	 42.2 (8-132)
Duration of Crohn Mean in years (Min-Max)	 8.3 (1-23)

Table 5: Demographic characteristics of the participants

Recurrence and healing

Three patients had fistula recurrence within the 2-weeks follow-up. Nine patients (75%) had a complete closure of the fistula tract and were free of symptoms at 6-months and at one-year follow-up. Apart from the recurrences, there were no serious surgical complications (Calvien-Dindo grade III or above) observed in any cases. Wound healing was achieved in eight of the twelve patients (67%) at

12-weeks follow-up. The complete healing of the fistula was confirmed by MRI scanning at 6-months follow-up in eight of the nine patients, and the last patient had regression of the fistula without fluid collection.

ADRC characterization revealed no significant differences in the cell yields between male and female patients in the present study (p=0.911) and importantly, also no relation to medical therapy (p=0.552). Medical therapy did not appear to have an impact on any of the analyzed cell parameters that all were within normal range. Hemorrhage due to the liposuction procedure showed to have a negative correlation to the percentages of CFU-Fs (p=0.0088) and nucleated, non-hematopoietic CD31-CD34+ stromal cells (p=0.0209). As the CD34 defines the stromal progenitors capable of clonal expansion in the CFU-F assay[55], this finding suggests that the total yield of stromal cells may be affected by hemorrhage. However, as for all other cell parameters, the number of injected nucleated, non-hematopoietic CD31-CD34+ ADRC was not significantly different between responders and non-responders.

Fecal Incontinence

The mean Wexner Fecal Incontinence score was reduced by more than 50% at 6-months follow-up compared to baseline measurement (Fig. 11).

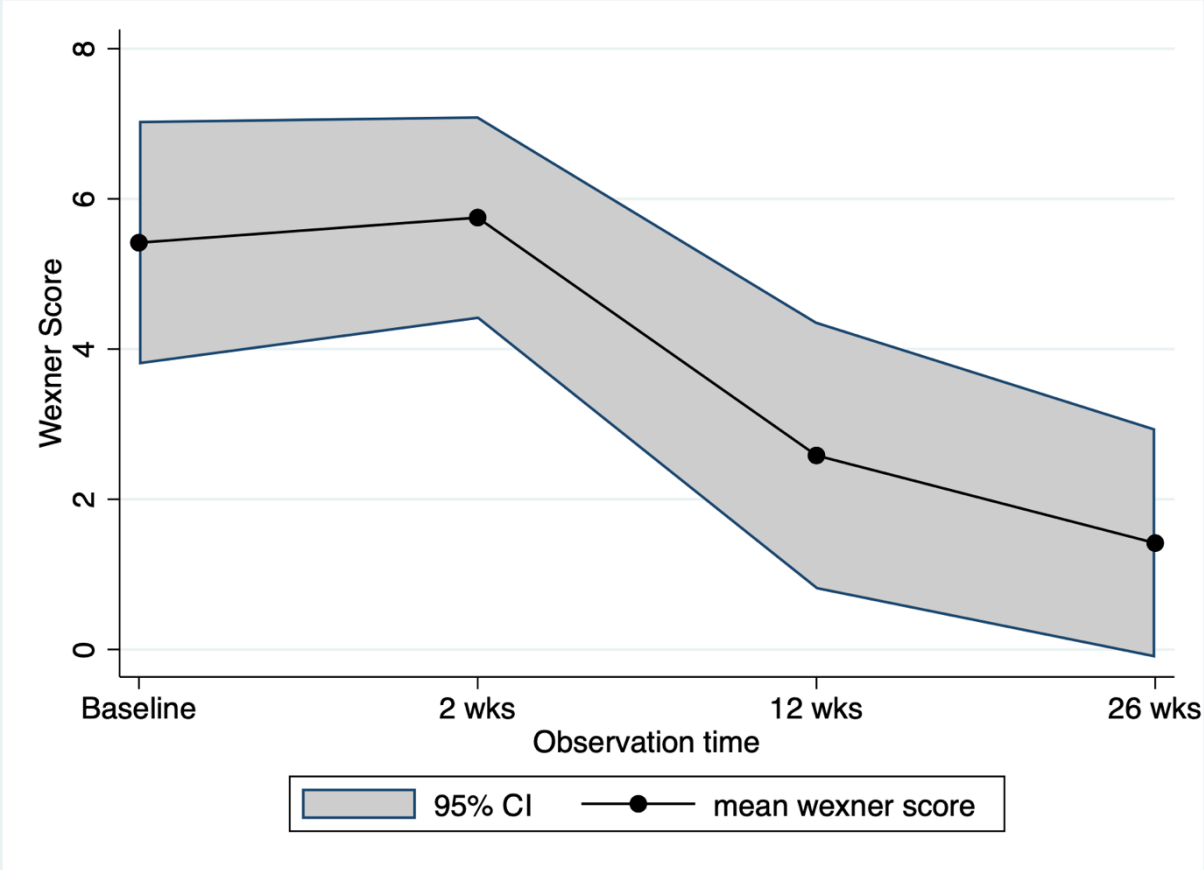


Fig.11: Changes in Wexner Fecal Incontinence Score.

Discussion

The anal abscess-fistula sequence (the first study)

The minimal invasive approach in the treatment of acute perianal abscess using needle aspiration was associated with significantly higher recurrence rate of (41%) compared to conventional incision drainage (15%), and with comparable rates of subsequent fistula formation of (14%) and (16%) respectively. The results of the current study lie within previously reported variable rates of recurrence and fistula formation following conventional incision drainage [1,5,6,7,26][18] and might be explained by the study design and patients' selection as patients with a history of anal abscess/fistula within 6 months were excluded. Apart from treatment type, none of the analyzed risk factors was significantly associated with recurrence of the abscess.

Apart from earlier wound healing in the needle aspiration group, no major advantages regarding the secondary outcomes were observed, using needle aspiration as a minimal invasive treatment. However, when taking into consideration the previously reported high cost of treatment and follow up of anal fistula [56], early wound healing can be considered an important advantage when using needle aspiration, especially when 59% of the patients healed.

Methodological considerations

The absence of analysis of resistance to Clindamycin, the multicenter approach [57], the treatment by junior surgical residents and missing data might be considered study limitations. However, complete case analysis was possible in 80% of the patients and with similar results. The present study mirrored however the daily clinical situation.

The minimal invasive treatment option for high anal fistula (the second study)

This randomized study showed significantly higher recurrence rate of the fistula after VAAFT (65%) compared with FSR (27%). Only one of the risk factors (length of the fistula) investigated was significantly associated with fistula recurrence. The demographic characteristics of the study population were similar to those reported in previous studies [1,26,29] with males being affected 2.5 times more than females and mean age in the fifth decade of life.

The recurrence rate after FSR in the present study was higher than previously reported [27,28,26] and might be explained by inclusion of only patients with high fistula and that the previously reported recurrence included the results from re-operations. The recurrence of fistula after VAAFT was considerably higher (65%) in this study compared to previously reported studies, which also included patients with non-complex fistulas. High recurrence rate was previously reported after VAAFT for high transsphincteric anal fistula [58]. Recurrences occurred at the operation site. MRI scanning was not performed in recurrent fistula patients. Therefore, it was not certain whether the recurrences are missed secondary tracts or original fistulas. It is easier to believe that recurrence of the original tract occurs in the VAAFT group.

Considering the secondary functional outcome, this study failed to show any advantage in applying the minimal invasive treatment of VAAFT compared to FSR. No impairment of the fecal continence following both treatments was shown in this study. Despite the decrease in pressure measurements by anal manometry, this was not reflected in fecal continence as evaluated by the Wexner score. The

presence of an anal sphincter defect and the size of the defect along with mean squeeze pressure were previously found to correlate to fecal incontinence score [59]. However, the presence of a defect in the internal anal sphincter at follow-up did not significantly affect the results of anal manometry or fecal incontinence score in the present study and might be explained by the two different study populations. Improvement in quality-of-life following treatment with VAAFT was previously reported [33] but this study showed improvement in quality-of-life measurements was in favor for FSR. This might be due to the significantly higher recurrence rate and delayed wound healing in the VAAFT group.

Methodological considerations

The ethical consideration of safety and benefit [54] led to early cessation of the study based on the fact of significantly higher rate of recurrence in the VAAFT group. This with the actual number of included patients, might be considered as study weakness. Although the surgical procedures were all performed by dedicated fistula surgeon with the necessary training in both procedures, learning curve might be a confounder. Other limitations might include the study being a single surgeon single centre study with lacking external validity and the study was also underpowered as the desired sample calculation was focused on having 25% difference between the groups with a low recurrence rate for FSR. The fistula recurrence rate was higher in both groups than anticipated.

A new treatment option for fistula in perianal Crohn's disease (the third study)

By combining surgical debridement and closure of the anal fistula with ADRC enriched fat graft, it was possible to achieve 75% clinical healing and 67% radiological healing of the fistula at 6-months follow-up and no clinical sign of recurrence at one-year follow-up. Similar results were shown by Serrero et. al [60] using a comparable method, with clinical response of 80% and fistula healing confirmed by MRI scanning in 60% at 48-weeks follow-up. Both studies were carried out independently in different countries but were similar and apart from minor differences in ADRC isolation and characterization, both applied the same treatment principle with comparable outcomes suggesting that the method is robust. A negative correlation between hemorrhage due to liposuction and the yield of CD34+ cells was shown in the present study, suggesting that a regenerative effect cannot be ascribed to this population alone, although it should be interpreted with caution due to the restricted number of participants.

The ADRC/g fat tissue yield in the present study was similar to a previous study of female lymphedema patients following mastectomy [61] but higher than in older males with erectile dysfunction following prostatectomy [62]. This probably reflected gender related effects on the cellular composition of subcutaneous adipose tissue as Haahr et. al [63] and others [64] had previously shown a lacking correlation between age and ADRC yield.

The results of the study might be positively influenced by long effect of unaltered Crohn's medication and the meticulous surgical debridement of the fistula tract and closure of the internal opening, with special attention to avoid disruption of the fistula tract during fat and ADRC injection. The improvement in the incontinence score can be explained by the successful closure of the internal opening and healing of the fistula tract by epithelialization, leading to cessation of secretion and the need of diapers.

The commercially available product, Cx601(darvadstrocel)® has been shown to have clinical and radiological remission in 56% after one year compared to 35% with placebo in patients with Crohn's

disease and a single fistula tract [65,66]. The present study strongly supported an alternative approach with the advantage of using autologous ADRC-enriched freshly collected lipoaspirate injection. It will be important to develop an autologous procedure for fistula treatment to reduce the risk of adverse effects since many of the patients are treated with immunosuppressive medication.

Methodological considerations

The major limitation of the study is the small population of selected patients, without control group and a short observation time.

Conclusions

Minimal invasive approach in the treatment of three different cases of perianal suppuration disease showed that:

1. Needle aspiration with postoperative antibiotics resulted in a high recurrence rate of abscess formation compared to surgical incision and cannot be recommended as routine. Subsequent fistula formation was not affected by the type of the treatment.

2. Fistulectomy and sphincter repair is more effective than VAAFT in the treatment of high cryptoglandular anal fistula.

3. ADRC-enriched autologous lipoaspirate can be safely used in the treatment of high anal fistula in patients with Crohn's disease with high rate of success.

Results perspectives

There was a significant high recurrence of the abscess after needle aspiration treatment, which also failed to show a better outcome regarding quality of life and fecal incontinence. However, needle aspiration with postoperative antibiotics can be offered for patients with acute perianal abscess, as it is not associated with complications or higher risk of fistula formation but with earlier wound healing, keeping in mind the significant higher risk of recurrence.

Although VAAFT was followed by a significant higher recurrence rate of anal fistula compared to FSR, both procedures were safe regarding the functional outcomes. Repeated VAAFT in case of fistula recurrence might be considered as a treatment option, as it was shown to be safe and not associated with major complication.

Relatively high rate of successful closure of anal fistula in patients with Crohn’s disease can be achieved applying minor surgical debridement combined with ADRC enriched fat graft. Further evaluation of these results by larger setting in a randomized trial is highly recommended. Future clinical trials should consider the following issues: 1) The optimal approach in case of recurrence suggesting re-injection of ADRC against surgical excision alone in a randomized trial.
2) The choice of transplantation suggesting comparison of the treatment outcome using fat graft alone against the use of autologous stem cells alone against the use of allogenic stem cells alone in a randomized trial.

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Appendix

The original manuscript of the three studies.

International Journal of Colorectal Disease
https://doi.org/10.1007/s00384-021-03845-6

ORIGINAL ARTICLE

Needle aspiration treatment vs. incision of acute simple perianal abscess: randomized controlled study

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Accepted: 8 January 2021

Abstract

Purpose Needle aspiration of an acute simple perianal abscess may be an alternative to conventional incision drainage with potential advantages in wound healing, functional outcome, and quality of life. The aim and objectives of the study are to compare the outcome of needle aspiration and postoperative antibiotics with that of conventional surgical incision drainage of acute perianal abscess. The primary outcome was abscess recurrence. Secondary outcomes were fistula formation, wound healing, quality of life, and fecal continence.

Methods This is a three-center randomized controlled trial, including adults with acute perianal abscess. The needle aspiration group received clindamycin for one week postoperatively. All included patients were scheduled for a follow-up at 2, 12, and 52 weeks postoperatively including physical examination, quality of life assessment (SF 36 questionnaire), and fecal continence (Wexner score).

Results A total of 98 patients were included. The recurrence rate was 41% in needle aspiration and 15% in incision drainage, with HR of 3.033 ($p = 0.014$). Fistula formation was 15% without significant difference between the groups. There was no significant difference in wound healing, quality of life, or fecal incontinence scores.

Conclusion Needle aspiration with postoperative antibiotics cannot be recommended as an alternative for surgical incision in the treatment of acute perianal abscess.

Trial registration number ClinicalTrials.org with identification number NCT02585141, initial release on 15 October 2015.

Keywords Perianal abscess recurrence · Anal fistula · Needle aspiration · Fecal incontinence · Quality of life

Background

Surgical incision drainage is widely accepted as a standard treatment of perianal abscesses, but it is often associated with postoperative pain and discomfort, prolonged wound healing, and excessive healthcare and societal cost [1]. Short-term and long-term fecal incontinence problems in various degrees have been reported in retrospective studies with lack of standardized and comparable data [2, 3]. Recurrence of perianal abscess after surgical incision drainage has been reported with great discrepancy in the literature, varying from 4% to 68% [2–7]. The incidence of subsequent fistula formation is reported to occur in a quarter to one third of patients treated by incision and drainage [1, 5, 6, 8, 9]. Most of these published papers are retrospective studies with various observation periods and incomparable data. The risk factors associated with recurrence and fistula formation, including age, gender, comorbidity, and smoking and alcohol habits, are previously reported with contradicting results [2, 5, 8, 10–12].

A minimally invasive approach with catheter drainage for the treatment of acute perianal abscess has also been reported [13]. This may be conducted as an outpatient regimen, with less discomfort and with similar results to those of the conventional incisional treatment in terms of recurrence and subsequent fistula formation [13–17]. The method has not gained wide acceptance, as there may be some problems with securing the catheter in place. Needle aspiration under ultrasound

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Published online: 15 January 2021

guidance has been shown to be safe and effective in the treatment of breast abscess [18–20], but it has not been reported as an alternative treatment for perianal abscess.

The aim of the present study was to compare the outcome of needle aspiration and postoperative antibiotics with that of standard surgical incision and drainage. The primary outcome was recurrence of abscess. The secondary outcomes were fistula formation, time to wound healing, fecal incontinence as measured by the Wexner fecal incontinence score [21], and quality of life scoring, using Short Form (Rand SF-36) [22].

Methods

This study was a randomized controlled open-label trial to compare the outcome of treatment of acute perianal abscess by needle aspiration drainage (minimally invasive) and single broad-spectrum antibiotic with standard incision drainage. The study was reported in accordance with the CONSORT Statement [23]. The study was conducted at three surgical departments, one university hospital (Odense University Hospital) and two regional hospitals (South West Jutland Hospital in Esbjerg and Slagelse Hospital), during the period between November 2015 and June 2020.

The primary end point was the recurrence of the abscess at 52-week follow-up. The secondary end points (at 52-week follow-up) were fistula formation demonstrated by probing, time to wound healing (defined as an epithelialized wound), fecal incontinence (Wexner fecal incontinence score), and quality of life (Rand SF-36).

Eligible patients were adults (≥ 18 years), admitted to the hospital with an acute perianal abscess where surgical drainage was indicated. Exclusion criteria were recurrent perianal abscess or a history of perianal fistula within the last six months, immunosuppressive treatment, malignancy within the last five years, previous pelvis radiotherapy, pregnancy or lactation, and known allergy for clindamycin. Inflammatory bowel disease (IBD) diagnosis was not an exclusion criterion, but disease activity with recurrent abscess/fistula in the last six months and immunosuppressive treatment were exclusion criteria. Other exclusion criteria were an abscess with a diameter less than 2 cm or the presence of a horseshoe abscess by peroperative ultrasonography.

After informed consent was obtained, patients were randomly allocated into intervention (needle aspiration drainage) or control (incision drainage) groups, using REDCap[1] electronic data capture tools hosted at OPEN (Open Patient data Explorative Network) [24, 25]. Randomization was performed online as 1:1 randomization without stratification. Resident doctors at the surgical emergency ward were responsible for patients' assessment for eligibility and inclusion, and randomization was performed prior to treatment under general anesthesia.

All procedures were done under general anesthesia in the lithotomy position. Percutaneous and transanal ultrasound was done by the surgical resident to ensure diagnosis, measuring the largest diameter of the abscess, and to exclude horseshoeing. All surgical residents, who performed the ultrasound, had received basic training in using ultrasound as a part of their standard surgical training. An anoscopy was performed to exclude any other pathology and to determine the presence or absence of an internal fistula opening. Pus obtained from patients, when possible, was examined for bacterial growth.

In the incision group, the abscess was drained by deroofing the abscess with radial incision, followed by curettage and irrigation of the cavity, and the wound was left open for secondary healing, without dressing. The patients were instructed to have a sitz bath or common hygiene until wound healing.

In the intervention group, the abscess content was aspirated using a large-caliber needle (MEDIPLAST®, Sweden, 13G, 2.5 × 110 mm). The cavity was irrigated with saline until clear fluid was obtained. Postoperative oral antibiotics were prescribed for seven days with Dalacin C® (clindamycin) (Pfizer, Denmark) 300 mg tablets, three times a day.

The included patients were assigned to a scheduled clinical follow-up at 2, 12, and 52 weeks after treatment, which included clinical wound assessment for healing and palpation for suppuration, and the patients were asked to fill out the Rand SF-36 questionnaire and Wexner fecal incontinence score.

Whenever there was a suspicion of recurrence or fistula formation, examination under general anesthesia was performed. Recurrence was treated by incision drainage irrespective of the primary treatment.

Data collection

At inclusion, baseline data were registered, including patients' demography (age, gender, height, and weight), comorbidities (diabetes, cardiovascular disease, lung disease, renal disease, and immune/connective tissue disease), smoking habit (smoker—and how many, quit—and for how long, and never) and alcohol consumption (0, ≤ 7/14 units[2]/week, and > 7/14 units/week), duration of symptoms, and use of antibiotics before admission. Baseline data for quality of life and fecal

[1] RedCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture, (2) audit trails for tracking data manipulation and export procedures, (3) automated export procedures for seamless data downloads to common statistical packages, and (4) procedures for data integration and interoperability with external sources.

[2] Unit alcohol = 12 g alcohol. Seven units for females and 14 units for males per week according to Danish health administration recommendation for alcohol consumption

incontinence were obtained by filling out the Rand SF-36 questionnaire and Wexner fecal incontinence score scheme, respectively. No occupational data was collected in this study. Peroperative data collection included the diameter (cm) of the abscess as assessed by ultrasonography (transanal and percutaneous) and location of the abscess (1–12 o'clock) and the presence of fistula.

Clinical follow-up data included visual evaluation of the wound healing (healed, with scar or hypergranulation formation or visible discharge), recurrence, and fistula formation. Data for quality of life and fecal incontinence were obtained by filling out the Rand SF-36 questionnaire and Wexner fecal incontinence score scheme, respectively.

The following factors were examined in the analysis as risk factors for recurrence and fistula formation: patient risk factors (*age*[3], gender, body mass index (BMI), tobacco, alcohol, use of antibiotics before admission, *duration of symptoms*, and health status) and abscess risk factors (allocated treatment, *size of abscess*, location of abscess, and bacterial growth).

Statistical analyses

Desired sample size was determined for comparison of two proportions, assuming a clinically accepted rate of recurrence of 5% in the incision drainage group and a 25% recurrence rate in the needle aspiration group, resulting in a desired sample size of 49 patients in each group of the study for obtaining a significance level of 5% at a power of 80%.

Differences in recurrence and fistula formation were analyzed using survival models applying the Kaplan–Meier method. Cox proportional hazards regression was performed to obtain hazard ratios (HR) with 95% confidence intervals for recurrence and fistula formation, as well as for risk factors. The Nelson–Aalen estimator was applied to obtain cumulative hazard rates for recurrence and fistula formation. The eight parameters of the Rand SF-36 questionnaire and Wexner fecal incontinence score were compared between groups using the Mann–Whitney *U* test. Demographic covariates were compared using the two-sample Wilcoxon rank-sum (Mann–Whitney) test and Pearson χ^2 test when appropriate. *p* values below 0.05 were considered statistically significant. Stata software v.16 was used.

Ethical considerations

Participants did not have any financial gain by participating in the study. Participation was voluntary, and the patients could withdraw their consent without consequences at any time. The advantage for the patients was that those who were randomized to needle aspiration might experience less discomfort postoperatively compared to the conventional treatment. The disadvantage might be a higher risk of recurrence. Data collection and processing were performed according to the Act of Processing of Personal Data and Health Act. The project was approved by the local Research Ethics Committee (S-20140191) and by the Region of Southern Denmark's joint review of the Data Protection Agency (20/18031). The trial was registered on Clinicaltrial.org with identification number NCT02585141.

[3] Variables with a cursive style were analyzed as both continuous and categorical variables.

Results

A total of 320 patients were admitted for perianal abscess, 115 were assessed for eligibility, and 103 were included. Five patients were excluded (three withdrawals and two did not receive allocated treatment), leaving 98 patients with 46 patients allocated in the needle aspiration group and 52 patients in the incision group (Fig. 1).

Complete case analysis without missing data was possible for 78 out of 98 patients (80%). Missing data were mainly from follow-ups (5/98 patients at 2-week follow-up, 16/98 patients at 12-week follow-up, and 20/98 patients at 52-week follow-up). Medical records for these patients were examined at the end of the observation time for any data of recurrence noticed in the patient record during the observation period. Medical records were accessible for all included patients in the region.

Table 1 shows the distribution of the demographic characteristics in the two groups. The male-to-female ratio was 1.8:1 and the mean age 45.9 years. When converting the age and BMI (body mass index) variables to categorical variables (age < 40 years and ≥ 40 years, BMI: underweight, normal weight, overweight and obese), no major difference between the groups was observed. All abscesses were of ischioanal type. There were two patients with inflammatory bowel disease (IBD), one with stable ulcerative colitis at the inclusion and one with Crohn disease after recurrence of the abscess. Both patients were allocated to needle aspiration treatment.

Twenty-six patients (27%) had one or more comorbidities equally distributed between the two groups. Pus samples were possible to obtain in 83 (85%) patients, and bacterial growth was shown in 64 samples (77%), with no statistically significant difference between the two groups. There was no significant association between bacterial growth and recurrence of the abscess or fistula formation. The most frequent isolated bacteria were *Escherichia coli* and *Enterobacter fragilis* (Table 2). Except abscess recurrences, no treatment-demanding surgical or medical complications were observed.

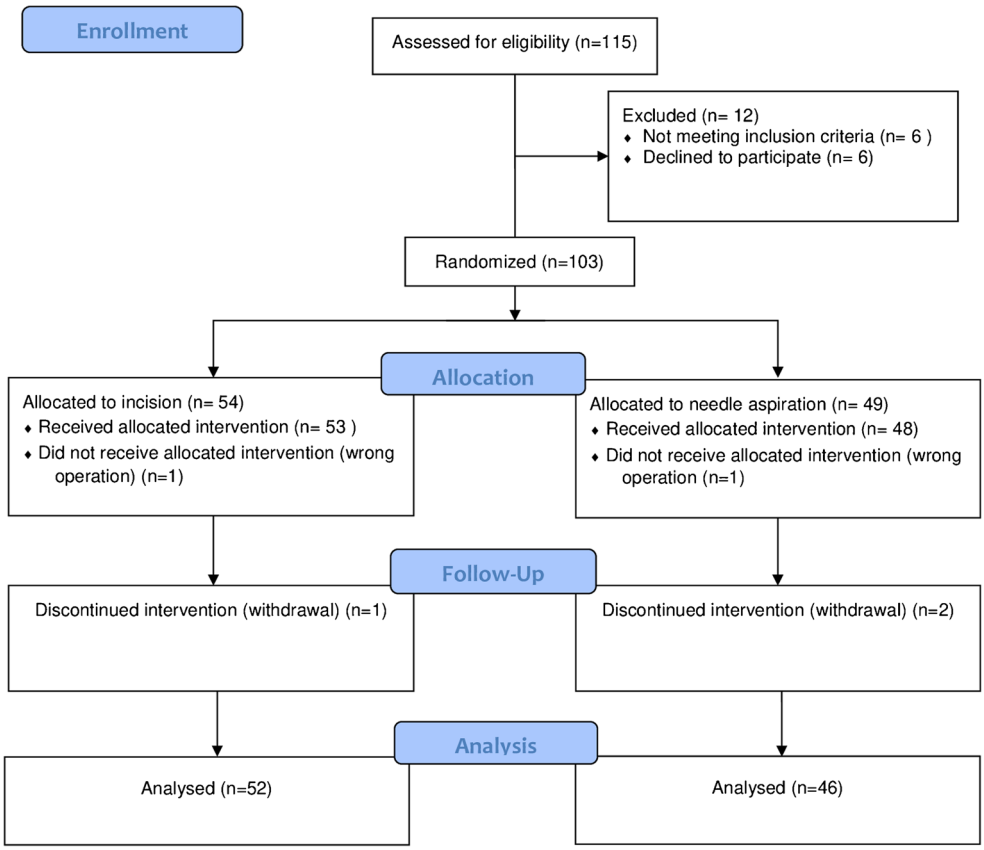

Fig. 1 CONSORT study flowchart

Recurrence of perianal abscess

Of the 98 patients analyzed, there were 27 (28%) treated for recurrences: 19 (41%) in the needle aspiration group and eight (15%) in the incision group ($p = 0.006$). Most of the recurrences occurred during the first four months of the observation time in both groups (Fig. 2). The estimated cumulative hazard of recurrence was 0.02 in both groups at two-week follow-up, and it increased to 0.20 in the incision group and 0.60 in intervention group at 52-week follow-up.

Univariate analysis showed none of the risk factors of interest being significantly associated with recurrence in both groups. Multivariate analysis showed significant higher risk of recurrence following needle aspiration with HR of 3.033 (95% confidence interval (CI) 1.251–7.357, $p = 0.014$), and none of the other risk factors analyzed had a significant association with recurrence.

Fistula formation

Four patients were found to have fistula at the time of operation. Of the remaining 94 patients, 14 patients (15%) developed subsequent fistula: eight (16%) in the incision drainage group and six (14%) in the intervention group ($p = 0.780$). Most of the subsequent fistula formation was diagnosed within four months after surgery in both groups. The estimated cumulative hazard was similar in both groups, being 0.29 at 52 weeks after treatment.

Univariate analysis showed that older age was associated with lower risk of fistula formation, with HR of 0.342 (95% CI 0.131–0.891, $p = 0.028$), while multivariate analysis showed that anteriorly located abscess was associated with lower risk of fistula formation, with HR of 0.264 (95% CI 0.072–0.970, $p = 0.045$).

Wound healing

In two patients (2%), wound healing was not obtained within the observation period of 52 weeks, one patient in each group. Wound healing time was longer in the incision drainage group, as it was obtained in 30 patients (68%) in the needle aspiration group at two-week follow-up, in comparison with 34 patients (67%) in the incision drainage group at 12-week follow-up.

Fecal incontinence score

The mean Wexner fecal incontinence score at baseline was comparable between the two groups ($p = 0.86$) with 3/4 of the patients having no symptoms of incontinence (Fig. 3). There were no differences in the mean Wexner fecal incontinence score between groups throughout the three clinical

Table 1 Demographic characteristics of the two groups

Variables	Incision (n=52)			Needle aspiration (n=46)			Total
	Frequency	Mean	95% CI	Frequency	Mean	95% CI	
Gender							
Male	34 (65.4%)			29 (63%)			63 (64.3%)
Female	18 (34.6%)			17 (37%)			35 (35.7%)
Age		45.59	41.52–49.66		46.41	41.68–51.14	45.98
BMI		27.57	26.34–28.81		26.59	24.66–28.53	27.11
Health status							
Healthy	39 (75.00%)			33 (71.74%)			72 (73.47%)
Comorbidity	13 (25.00%)			13 (28.26%)			26 (26.53%)
Tobacco							
Nonsmoker	12 (23.08%)			17 (36.96%)			29 (29.59%)
Smoker	33 (63.46%)			22 (47.83%)			55 (56.12%)
Quit	7 (13.46%)			7 (15.22%)			14 (14.29%)
Alcohol							
Null	17 (32.69%)			8 (17.39%)			25 (25.51%)
≤7/14 pints/week	30 (57.69%)			32 (69.57%)			62 (63.27%)
>7/14 pints/week	5 (9.62%)			6 (13.04%)			11 (11.22%)
Duration of symptoms (days)		6.48	4.47–8.49		6.97	4.85–9.06	6.70
7 days	42 (80.77%)			38 (82.61%)			80 (81.63%)
>7 days	10 (19.23%)			8 (17.39%)			18 (18.37%)
Prior use of antibiotics							
Prior use of antibiotics	22 (42.31%)			13 (28.26%)			35 (35.71%)
No prior use of antibiotics	30 (57.69%)			33 (71.74%)			63 (64.29%)
Size of abscess		3.43	3.04–3.82		3.70	3.20–4.19	3.55
2–5 cm	48 (92.31%)			40 (86.96%)			
>5 cm	4 (7.69%)			6 (13.04%)			
Microbiology study							
Growth of bacteria	29 (72.5%)			35 (81.4%)			64 (77.1%)
No growth of bacteria	11 (27.5%)			8 (18.6%)			19 (22.9%)

follow-ups at 2, 12, and 52 weeks. Furthermore, no differences were observed by stratifying the score into a categorical variable (none, mild, moderate, and severe incontinence).

Quality of life score

The emotional well-being score was significantly lower (i.e., more disability) in the control group with incisional drainage at two-week follow-up ($p = 0.033$); otherwise, there were no significant differences. The pain score was higher (i.e., lower disability) at 52-week follow-up compared to baseline ($p < 0.001$), without differences between the two groups.

Complete case analysis

Complete case analysis showed similar results, along with lower risk of recurrence and fistula formation in the older age in both univariate and multivariate analyses, and higher risk of recurrence in obese patients in multivariate analysis and analysis of the Wexner score and Rand SF-36 data showed no further differences.

Discussion

This is the first randomized clinical trial on the outcome of surgical treatment of perianal abscesses with needle aspiration plus postoperative antibiotics compared to conventional incision drainage. The recurrence rate was significantly higher after needle aspiration (41%) compared to incision drainage (15%). The rates of subsequent fistula formation were 14% and 16%, respectively. All the recurrences of abscess and fistula formation were observed during the first four months of the observation period. None of the patient risk factors of interest were significantly associated with abscess recurrence and fistula formation. The demographic characteristics of the

Table 2 The results of the bacteriological study of the obtained pus samples ($n = 64$)

Bacteria	Incision (%)	Needle aspiration (%)	Total (%)
Escherichia coli	14 (48)	13 (37)	27 (42)
Bacteroides fragilis	4 (14)	6 (17)	10 (16)
Prevotella	4 (14)	4 (11)	8 (12.5)
Streptococcus anginosus	2 (7)	5 (14)	7 (11)
Bacteroides thetaiotaomicron	2 (7)	4 (11)	6 (9.4)
Haemolytic streptococci	1 (3.5)	4 (11)	5 (8)
Normal flora	3 (10)	2 (5.7)	5 (8)
Actinomyces	2 (7)	2 (5.7)	4 (6.3)
Peptoniphilus	2 (7)	2 (5.7)	4 (6.3)
Staphylococcus aureus	2 (7)	2 (5.7)	4 (6.3)
Fusobacterium	2 (7)	1 (3)	3 (5)
Gram-positive cocci	1 (3.5)	1 (3)	2 (3)
Anaerobic bacteria	1 (3.5)	1 (3)	2 (3)
Bacteroides ovatus	0 (0)	2 (5.7)	2 (3)
Staphylococcus epidermidis	2 (7)	0 (0)	2 (3)
Enterobacter	0 (0)	2 (5.7)	2 (3)
Others	6 (21)	12 (34.3)	18 (28)

study population were similar to those reported in previous studies [12, 26] with males being affected as twice as females and mean age in the fifth decade of life.

The recurrence rate of abscess after conventional incision drainage treatment was previously reported to vary between 3.7% and 11% [1, 5–7, 26]. The rate of subsequent fistula formation was previously reported to be 15.5% to 37% [12]. The results of the current study lie within this range and might be explained by the study design and patients' selection. The recurrence rate of the abscess with needle aspiration was higher than this range and would be difficult to accept.

There were no serious surgical complications (Clavien–Dindo grade III or above) observed in any cases. The incontinence score was better during the early stage of observation, in favor of needle aspiration. This may be explained by longer wound healing time in the incision drainage group, and wound discharge observed by the patients might have been interpreted as fecal incontinence. At one-year follow-up, 98% of all wounds were healed. Quality of life assessment did not show any major differences between the groups, and the reported lower pain at one-year follow-up compared to baseline just reflected that both groups ended up with a satisfactory treatment result.

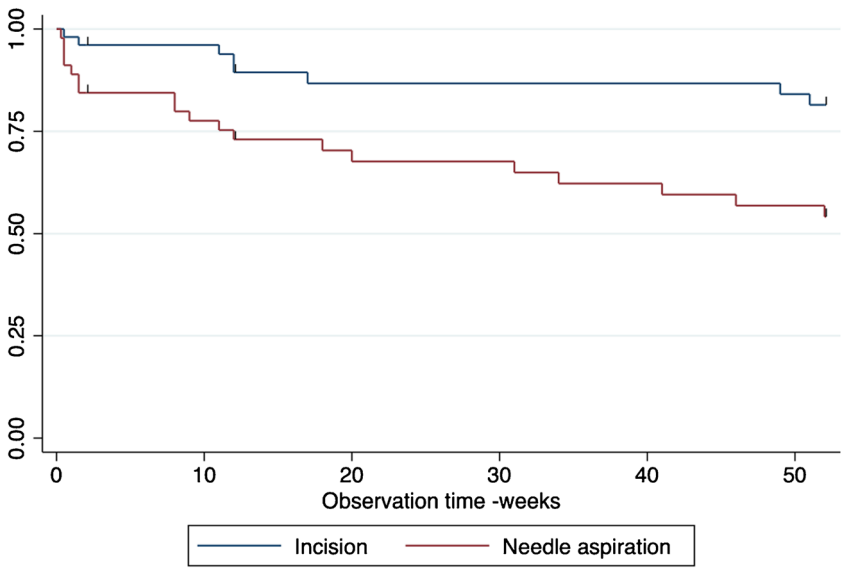

Fig. 2 Kaplan–Meier survival estimates of perianal abscess recurrence

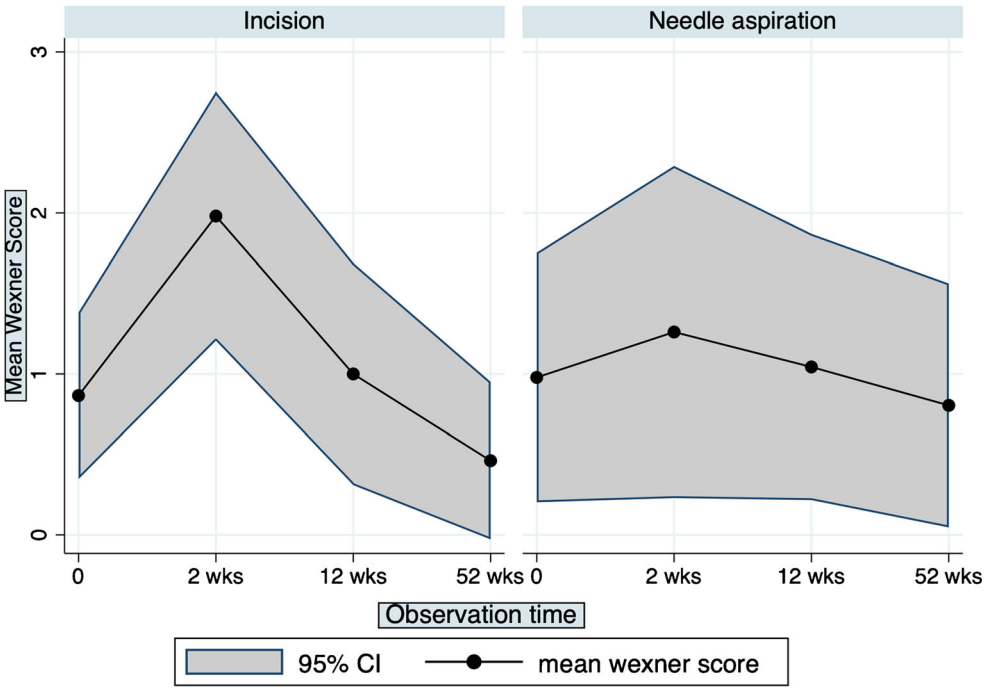

Fig. 3 Mean profile of differences in the Wexner fecal incontinence score over the observation period (*X* axis: 0 baseline, 2, 12, and 52-week follow-up; *Y* axis: mean Wexner fecal incontinence score). CI confidence interval

As a supplement for needle aspiration, Dalacin C® was chosen as a monotherapy broad-spectrum antibiotic covering both aerobic and anaerobic bacteria, with documented results in the treatment of soft tissue infections [27]. Postoperative antibiotic treatment was not prescribed in the incision group, as this was not a part of standard practice. A bacteriological growth study of the pus sample retrieved showed bacterial growth in 77% of the samples, with *Escherichia coli* and *Enterobacter fragilis* as the most frequent isolated bacteria. It is a weakness that there was no analysis of resistance to clindamycin, which to some degree could explain the high recurrence rate of the abscess in the intervention group.

The multicenter approach is a weakness of the present study together with the relative low number of patients that were evaluated for inclusion, which might be a confounder [28]. Another weakness is that the treatment was carried out mainly by junior surgical residents, operating under everyday emergency conditions. In spite of the huge effort done to ensure strict adherence to the study protocol, heterogenicity in daily clinical and surgical practice may have led to slower and heterogeneous patient recruitment together with a risk of case selection. Another limitation of the study is missing follow-up data. However, complete case analysis was possible in 80% of the patients and with similar results. The strength of the present study was that it mirrored the daily clinical situation.

The present study showed significant high recurrence of the abscess after needle aspiration treatment, which also failed to show a better outcome regarding quality of life and fecal incontinence. However, needle aspiration with postoperative antibiotics can be offered for patients with acute perianal abscess, as it is not associated with complications or higher risk of fistula formation but keeping in mind the significant higher risk of recurrence.

Conclusion

Needle aspiration with postoperative antibiotics resulted in a high recurrence rate of abscess formation compared to surgical incision and cannot be recommended as a routine.

Availability of data and material and code availability The corresponding author is responsible for the availability of data material and custom code.

Authors' contributions Karam Matlub Sørensen and Niels Qvist contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Karam Matlub Sørensen. The first draft of the manuscript was written by Karam Matlub Sørensen, and all authors commented on the previous versions of the manuscript. All authors read and approved the final revised manuscript. Musa Büyükuslu (Department of Surgery, South West Jutland Hospital in Esbjerg) and Kristina Safir-Hansen (Department of Surgery, Slagelse Hospital) contributed to the study, by patient recruitment and follow-up data collection.

Funding The study was part of Karam Matlub's Ph.D. project, which was funded by the University of Southern Denmark; the Region of Southern Denmark; Surgical Department A, Odense University Hospital; Odense University Hospital Fund; Danish Crohn Colitis Association CCF; and Fionia Fund.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the local Research Ethics Committee (S-20140191).

Consent to participate Informed consent was obtained from all individual participants included in the study.

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The outcome of minimal invasive treatment of high crypto-glandular anal fistula: a randomized clinical study.

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Abstract

Background

Video-assisted anal fistula treatment (VAAFT) may have a recurrence rate comparable to fistulectomy and sphincter repair (FSR) in the treatment of high anal fistula and with potential advantages in wound healing, functional outcome, and quality of life. The aim and objectives of the study are to compare the outcome of VAAFT with FSR for high cryptoglandular anal fistula.

Methods

Single centre randomized controlled trial, including adults with high anal fistula, compared FSR with VAAFT. Primary outcome was fistula recurrence. Secondary outcomes were results of anal manometry, quality of life and faecal continence. A power calculation of 33 patients in each arm (1:1) was based on recurrence in the FSR and VAAFT groups of 5% and 30% respectively. Follow-up at six months postoperatively including physical examination,

MRI, anal manometry, quality of life assessment (RAND SF 36 questionnaire) and faecal continence (Wexner score).

Results

The study terminated early due to high recurrence rates in both groups. A total of 45 patients were included. Recurrence rate was 65% in VAAFT and 27% in FSR, with HR 4.18 (p= 0.016), and the length of the fistula as a risk factor had a significant association with recurrence with HR 1.8 (p=0.020). There were significant differences in quality of life in favour of FSR and in anal manometry in favour of VAAFT with a significant improvement in Wexner score in both groups.

Conclusion

FSR was associated with a lower recurrence rate than VAAFT in the management of complex anal fistulae in this single centre study.

Trial registration number

ClinicalTrials.org with identification number NCT02585167, initial release on 20th October 2015.

Introduction

About one-fifth of anal fistulas are classified as complex including either transsphincteric or *high* fistulas [1]. Fistulectomy and primary sphincter repair (FSR) has been described as an effective treatment for the complex anal fistula, with success rates of more than 90% in non-randomised studies[2-4]. It is the standard approach for fistula management in this department for the last 20 years. This method carries the risk of delayed wound healing and impaired faecal continence in more than 20% [3, 5-7]. Anal fistula surgery involving the sphincter reduced anal canal pressure resulting in impaired anal continence [8]. The results of quality-of-life measurements and functional studies after anal sphincter surgery are contradictory [7-12].

An alternative to FSR may be the minimal invasive sphincter-preserving procedure 'video assisted anal fistula treatment' (VAAFT) [13]. Prospective studies have demonstrated promising

results [13-18] with low recurrence rates of 12.5-17% and success rates after long term (2-3 years) follow-up between 70-85% [19-22], although these figures includes re-operation. The VAAFT method has the advantage of direct vision of the fistula tract allowing identification of secondary tracts and cavities, sphincter preservation and potentially reduced postoperative discomfort. The recurrence rate of the fistula is higher when VAAFT is performed to treat complex transsphincteric fistulas [23].

The aim of the present study was to compare the outcome of VAAFT (intervention) with FSR (control) in the treatment of high anal fistula.

Methods

This study was performed as a randomized controlled open label trial to compare the outcome of treatment of complex cryptoglandular anal fistula by video-assisted anal fistula treatment VAAFT (minimal invasive) with fistulectomy and primary sphincter repair FSR. The study was reported in accordance with the CONSORT statement [26] and conducted at the surgical department, Odense University Hospital, between February 2016 and May 2021. The first author received the basic and advanced training for VAAFT procedure and performed several procedures prior to the study initiation. All allocated procedures were performed by the first author.

The primary objective was to compare recurrence after initial treatment at 6-months follow-up. The secondary objectives (at 6-months follow-up) were time to wound healing (defined as epithelialized wound), faecal continence evaluated with Wexner fecal incontinence score [24], along with changes in anal manometry (changes in maximal resting and squeeze pressures) and quality of life measured with Rand Short Form SF36 [25].

Eligible patients were adults (≥ 18 years), referred to Odense University Hospital surgical department with complex cryptoglandular anal fistula, with intention of surgical treatment. High anal fistula, involving more than one-third of the external anal sphincter, was considered of the complex type according to the current Danish guidelines [27] (based on the original Parks' classification of anal fistula). Exclusion criteria were Crohn disease, signs of suppuration and cavitation, immune suppressive treatment, malignancy within the last five years, previous pelvic radiotherapy and a rectovaginal fistula.

After informed consent, patients were randomly allocated into intervention (VAAFT) or control (FSR), using REDCap [i] electronic data capture tools hosted at OPEN (Open Patient data Explorative Network) [28, 29]. Randomization was performed online as 1:1 randomization without stratification. The corresponding author was responsible for patients' assessment for eligibility, inclusion, and preoperative randomization.

All included patients had undergone anal examination under general anaesthesia to ensure the anatomical classification of the fistula and adequate drainage by a loose seton suture. All patients underwent endoanal ultrasound to exclude undetected cavitation and suppuration as well as defects in the anal sphincter complex. All had the fistula adequately drained by a loose seton suture for at least three months prior the allocated treatment. Preoperative baseline MRI scanning of the anal canal was performed and colonoscopy when indicated.

Preoperative baseline measurement of faecal incontinence and quality-of-life using the Wexner fecal incontinence score and Rand SF-36 score, respectively, were performed in all included patients, as well as baseline anal manometry using the MANOSCAN™ AR high-resolution anorectal manometry system (Medtronic, Minneapolis, USA). All allocated procedures were carried out as one-day surgery, with moderate preoperative bowel preparation (Bisacodyl) and per operative broad-spectrum antibiotics (single intravenous doses of metronidazole and cefuroxime). Endoluminal ultrasound was repeated per operatively prior to allocated treatment to ensure fistula classification. All excised fistula tissue was sent for histopathology.

In the FSR group, the fistula tract was excised in its entire length after dividing the involved part of the anal sphincter. The sphincter complex including the anal canal was reconstructed using interrupted absorbable sutures. The internal and external anal sphincters were repaired separately. The lateral part of the incision was left open for drainage.

In the VAAFT group, the procedure was performed according to the original technique described by Meinero and Mori [13], using Meinero fistuloscope (Karl-Storz, Tuttlingen, Germany). The internal orifice was secured with two-layer closure using interrupted absorbable sutures for both the muscle and anal mucosa layers. The external orifice was excised leaving the wound for secondary healing.

Standard postoperative regimen was analgesia, oral broad-spectrum antibiotics (ciprofloxacin and metronidazole) for five days, oral laxative (magnesium oxide) for 14 days and patients were instructed to avoid heavy physical straining for at least four weeks postoperatively. The wounds were kept clean by repeated washing and no dressing was used.

Included patients were assigned to a scheduled clinical follow up at six months after treatment, which included clinical wound assessment for healing and sign of persistent fistula by physical examination and endoanal ultrasound. Patients were asked to fill out Rand SF-36 questionnaire and Wexner fecal incontinence score. MRI scanning of the anal canal, endoanal ultrasound and high-resolution anorectal manometry were performed.

Whenever there was a suspicion of recurrence or fistula formation, examination under general anesthesia was performed. A recurrence was treated by FSR irrespective of the primary treatment.

Data collection

At inclusion, baseline data were registered, including patients' demography (age, gender, height, and weight), co-morbidities (diabetes, cardiovascular, lung, renal and immune/connective tissue disease), smoking habit (smoker, quit, never) and alcohol consumption (0, ≤7/14 units[ii]/week, >7/14 units/week), duration of symptoms and location of the fistula. No occupational data were collected. Operative data included length (cm) and location of the fistula (anterior/posterior), and anorectal manometric measurement including maximal resting pressure and maximal squeezing pressure.

Clinical follow-up data included visual evaluation of the wound healing (healed, with scar or hyper granulation formation or visible discharge) and recurrence of fistula. Radiological follow-up data of fistula recurrence and presence of a sphincter defect were obtained by endoanal ultrasound and MRI scanning. Quality of life, faecal incontinence and manometric data were recorded at 6 months after the allocated surgery.

The following factors were examined in the analysis as risk factors for fistula recurrence; age, gender, Body Mass Index (BMI), tobacco, alcohol, duration of symptoms, health status, allocated treatment, length of fistula in centimetres, and fistula location.

Statistical analyses

Desired sample size was determined for comparison of two proportions, assuming a rate of recurrence of 5% in the FSR group, and 30% recurrence rate in the VAAFT group, resulting in a necessary sample size of 33 patients in each group of the study for obtaining a significance level of 5% at a power of 80%. The assumed fistula recurrence rates for FSR and VAAFT were assigned to ensure a 25% difference in the fistula recurrence between the two groups, and the high recurrence rate accepted for VAAFT was mainly due to the minimally invasive nature of VAAFT compared to FSR treatment with previously reported low recurrence rates.

Differences in recurrence of the fistula were analysed using survival models applying the Kaplan-Meier method. Cox proportional hazards regression was performed to obtain hazard ratios (HR) with 95% confidence intervals for recurrence with respect to the intervention as well as for risk factors. The Cox proportional hazards regression model was applied both as univariate and multivariate analysis (adjusted for age, gender, BMI, tobacco, alcohol, duration of symptoms, health status, allocated treatment, length of fistula, and fistula

location) for risk factors for fistula recurrence. The Nelson-Aalen estimator was applied to obtain cumulative hazard rates for recurrence. The eight parameters of Rand SF-36 questionnaire, anorectal manometric measurements and Wexner fecal incontinence score were compared between groups using Mann-Whitney U-test and *t*-test when appropriate. Demographic covariates were compared using two sample Wilcoxon rank-sum (Mann-Whitney) test and Pearson χ^2 -test when appropriate. *P*-values below 0.050 were considered statistically significant. Stata software v.16.1was used.

The study protocol did not initially include an interim analysis, but as it was unblinded study, high recurrence rate was observed in the intervention group VAAFT, that the study group had the obligation to undertake an early analysis before concluding to end the study.

Ethical considerations

Participation was voluntary. Patients could withdraw their consent at any time and they received no remuneration. Data collection and processing were performed according to the Act of Processing of Personal Data and Health Act. The project was approved by the local Research Ethics Committee (S-20150053) and by Region of Southern Denmark's joint review of the Data Protection Agency (20/18031). The trial was registered on Clinicaltrial.org (identification number NCT02585167).

Results

During the study period, a total of 536 patients were referred for the assessment of a complex anal fistula of whom 64 had a high transsphincteric anal fistula and were assessed for eligibility. Of these 47 were included (17 patients declined to take part). Two patients were excluded (one withdrawal, one with excessive suppuration at time of operation), leaving 45 patients for analysis with 23 patients allocated in the VAAFT group and 22 patients in the FSR group. (Fig. 1)

Table 1 shows the distribution of the demographic characteristics in the two groups. Male to female ratio was 2.5:1 and mean age 43.8 years (22-75). Groups were well matched for age and BMI and 37 patients were either in the overweight (BMI 25-29.9) or obese (BMI $\geq$ 30) categories. None of the patients had a stoma. Mean length of the fistula tract was 4.3 cm, 19 fistulas were located posteriorly to the anus and mean duration of symptoms was 14.6 months, without major differences between the groups. Missing data included three follow-up MRI scans and one follow-up anal manometry measurement. Histopathological study of the fistula tissue was possible in 43 patients (96%), and none showed inflammatory bowel disease. Only two patients, one in each group, had a previous history of anal fistula surgical treatment. Besides recurrences, there were no other medical or surgical complications and no patient needed a diverting stoma.

Recurrence of the fistula

Of the 45 patients analysed, 21 (47%) had fistula recurrence: 15 (65%) in the VAAFT group and six (27%) in the FSR group (p=0.016). Recurrences occurred throughout the observation time in both groups (Fig. 2), and all were at the operation site. The estimated cumulative hazard of recurrence was 0.30 in the FSR group and 0.98 in VAAFT group at 6-months follow-up.

Multivariable analysis demonstrated a significant higher risk of recurrence following VAAFT with HR 4.18 (95% confidence interval (CI) 1.30-13.42, and p= 0.016), and analysis of risk factors showed a significant association between length of the fistula and recurrence with HR 1.8 (95% CI 1.097-2.984, p=0.020), while higher BMI was associated with lower risk of recurrence, HR 0.76 (95% CI 0.633-0.91, p=0.003) and the obese category with HR 0.11 (95% CI 0.019-0.618, p=0.012).

Clinically obscured recurrence was revealed by MR scanning at follow-up in six patients in the FSR group and four in the VAAFT group. At follow-up, three patients in the FSR group

BJS CONSORT diagram

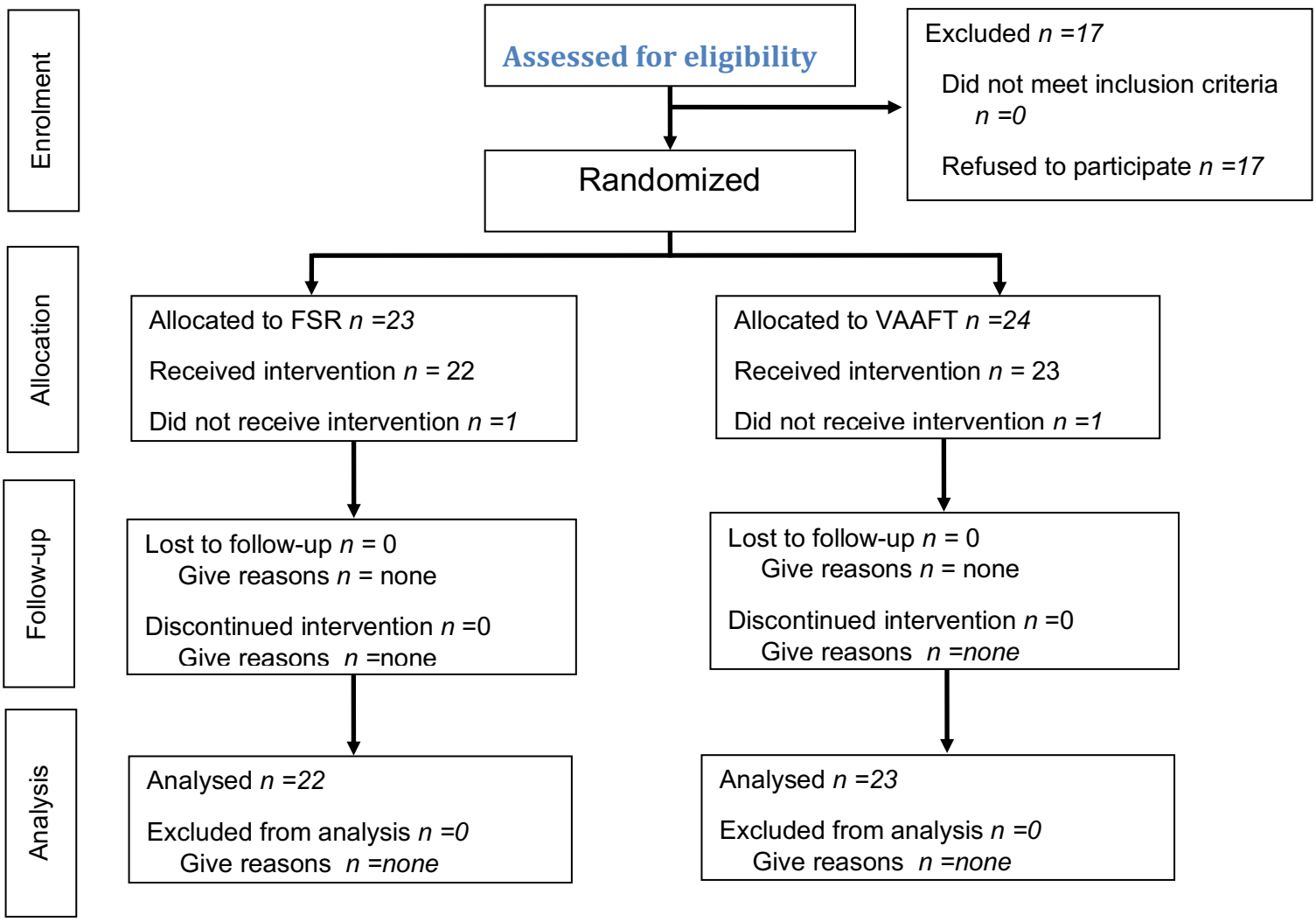

Fig.1: CONSORT study flowchart.

Variables	FSR n=22		VAAFT n=23		Total n=45	
	Mean (freq.)	**95% CI**	**Mean (freq.)**	**95% CI**	**Mean (freq.)**	**95% CI**
Age	45.05	38.93-51.16	42.65	37.17-48.14	43.82	39.88-47.77
Young	(8) – 36%		(11) – 48%		(19) – 42%	
Old	(14) – 64%		(12) – 52%		(26) – 58%	
Gender						
male	(15) – 68%		(17) – 74%		(32) – 71%	
female	(7) – 32%		(6) – 26%		(13) – 29%	
BMI	29.25	27.56-30.95	28.07	26.21-29.92	28.65	27.43-29.87
Normal weight	(1) – 4.6%		(7) – 30.4%		(8) – 17.8%	
Overweight	(13) – 59%		(8) – 34.8%		(21) – 46.6%	
Obese	(8) – 36.4%		(8) – 34.8%		(16) – 35.6%	
Tobacco						
None	(9) – 40.9%		(16) – 69.6%		(25) – 55.6%	
Smoker	(7) – 31.8%		(2) – 8.7%		(9) – 20%	
Quit	(6) – 27.3%		(5) – 21.7%		(11) – 24.4%	
Alcohol						
0	(2) – 9.1%		(2) – 8.7%		(4) – 8.9%	
<= 7/14 unit/wk	(18) – 81.8%		(20) – 87%		(38) – 84.4%	
> 7/14 unit/wk	(2) – 9.1%		(1) – 4.3%		(3) – 6.7%	
Health status						
Healthy	(19) – 86.4%		(19) – 82.6%		(38) – 84.4%	
Co-morbidity	(3) – 13.6%		(4) – 17.4%		(7) – 15.6%	
Duration (m)	11.6	8.11-15.16	17.4	12.38-22.40	14.6	11.47-17.67
Fistula location						
Anterior	(14) – 63.6%		(12) – 52.2%		(26) – 57.8%	
Posterior	(8) – 36.4%		(11) – 47.8%		(19) – 42.2	
Length of fistula (cm)	4.41	3.73-5.09	4.24	3.62-4.85	4.32	3.88-4.76

Table 1: Patients demographic characteristics. unit alcohol=12 g alcohol. Maximal 7 units for females and 14 units for males per week as Danish health administrations recommendation for alcohol consumption. (m) = month. (cm) = centimeter. (freq.) = frequency.

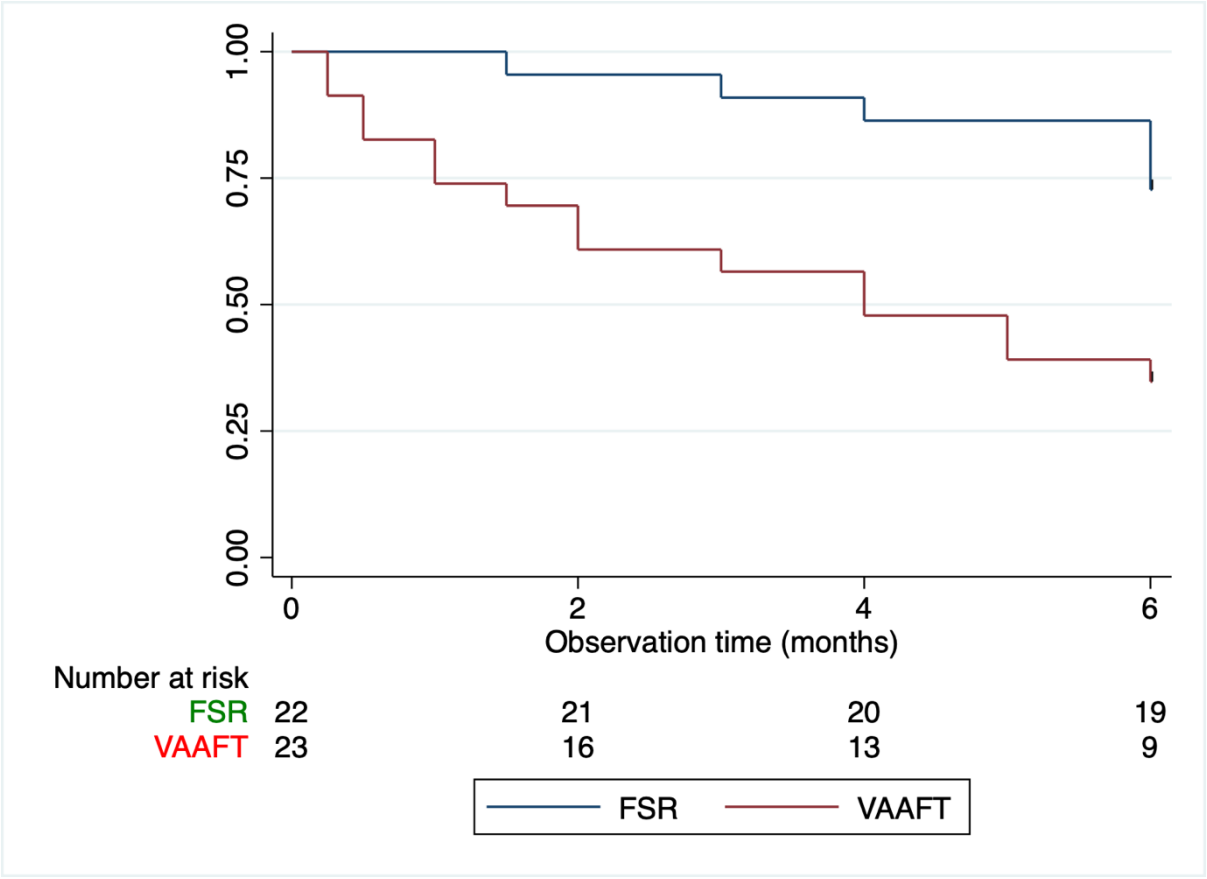

Fig.2: Kaplan-Meier estimate of fistula recurrence. FSR= Fistulectomy and sphincter repair. VAAFT= Video-Assisted Anal Fistula Treatment.

and ten patients in the VAAFT group had not achieved wound healing (epithelialization) (p=0.027).

Faecal incontinence score

The mean Wexner fecal incontinence score at baseline was comparable between the two groups (p= 0.135) with 36% of the patients having mild or no symptoms of incontinence (Fig. 3). However, 18 patients in VAAFT group had moderate incontinence at baseline compared to eight in FSR group (p=0.028). There was a significant improvement in the mean Wexner fecal incontinence score when comparing baseline and follow-up measurements for both groups (FSR p=0.022 and VAAFT p=0.011). There was improvement of the continence in both groups without difference when stratifying the score into categorical variable (none, mild, moderate, and severe incontinence).

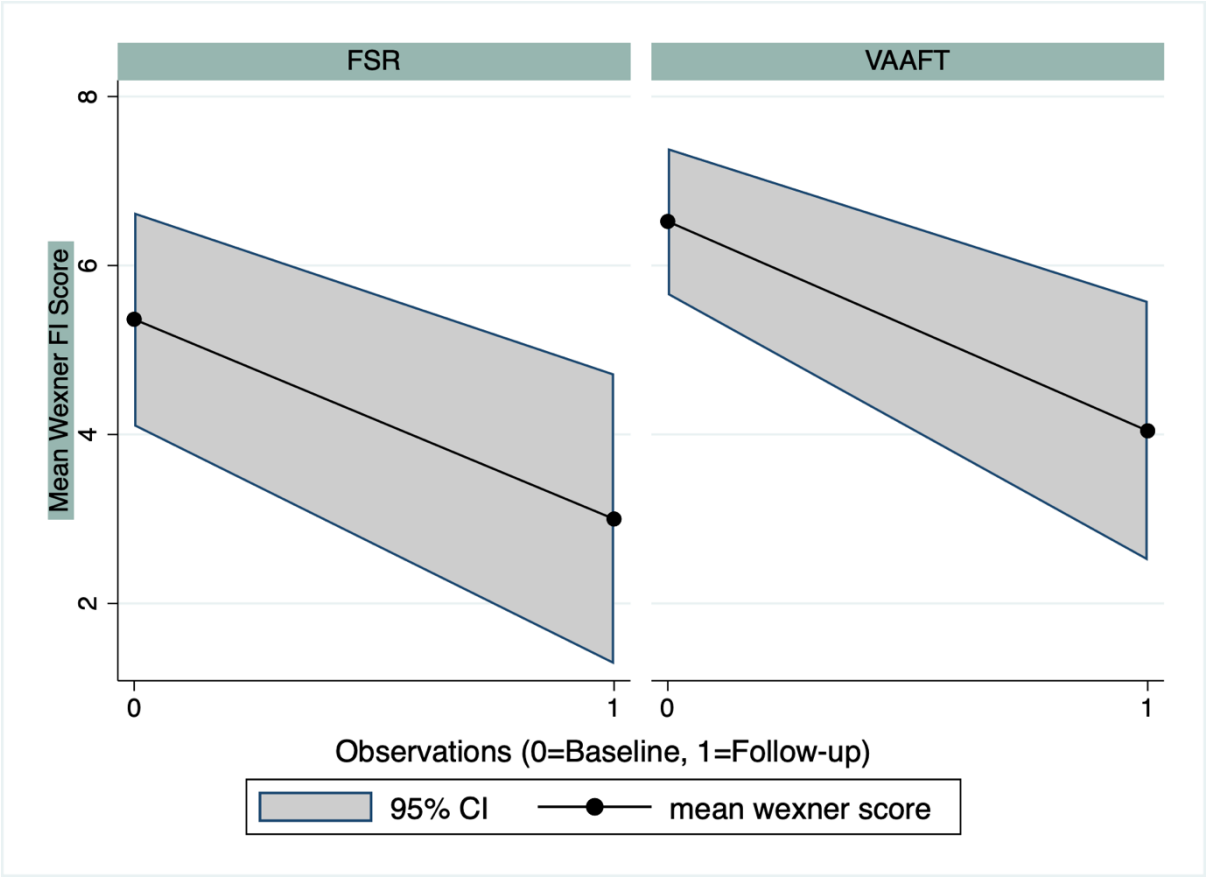

Fig.3: Mean profile of differences in Wexner Fecal incontinence Score over the observation period. (X axis: 0 baseline, 1 follow-up. Y axis: mean Wexner Fecal incontinence score). CI: Confidence Interval.

Anal manometry measurements

There were no differences between the groups in baseline measurements of maximum resting pressure and maximum squeezing pressure. A decrease in the mean resting and squeezing pressures was observed in both groups at the follow-up but only statistically significant for the mean squeezing pressure in the FSR group (p=0.018). At follow-up, endoluminal ultrasound revealed a defect of the internal anal sphincter in nine (41%) patients in the FSR group and one (4%) in VAAFT group (p=0.003). The presence of a sphincter defect was unrelated to the results of anal manometry or faecal incontinence score.

Quality of Life score

Analysis of the means of the eight parameters of RAND SF-36 score (Table 2) revealed a significant increase (less disability) in all the parameters in the FSR group and in two

Rand SF-36	Fistulectomy and sphincter repair FSR		Video-assisted Anal Fistula Treatment VAAFT		FSR & VAAFT		FSR vs VAAFT
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Follow-up
Physical Function Score	81.36	94.32 **P=0.0019**	74.78	85.65 **P=0.0024**	78	89.89 **P<0.0001**	P=0.0625
Role limitations due to physical Health Score	62.12	86.36 **P=0.0171**	65.21	68.12 P=0.7753	63.70	77.04 P=0.0626	P=0.1159
Role limitations due to emotional problems Score	62.12	86.36 **P=0.0171**	65.22	68.12 P=0.7753	63.70	77.04 P=0.0626	P=0.1159
Energy/fatigue Score	53.18	75.90 **P=0.0005**	49.34	59.13 P=0.0501	51.22	67.33 **P=0.0001**	**P=0.0121**
Emotional well-being Score	70	82.54 **P=0.0043**	67.36	74.26 P=0.0520	68.62	78.31 **P=0.0005**	P=0.1318
Social functioning Score	81.25	93.18 **P=0.0496**	65.22	75 P=0.0647	73.06	83.89 **P=0.0061**	**P=0.0117**
Pain Score	68.07	88.64 **P=0.0002**	57.83	73.91 **P=0.0019**	62.83	81.11 **P<0.0001**	**P=0.0171**
General health Score	69.77	79.55 **P=0.0051**	67.17	69.57 P=0.4095	68.44	74.44 **P=0.008**	P=0.0767

Table 2: Quality of life measurements of study population. The figures are the mean of each score of the eight parameters of Rand SF 36. Significant p values are highlighted.

parameters (Physical Function Score and Pain Score) in the VAAFT group. Comparing the two groups at follow-up, significant differences were found in favour of the FSR group in three of the parameters (Energy/fatigue Score, Social functioning Score, Pain Score).

Early cessation of the study

It was necessary to perform a premature analysis of the results due to the observed higher recurrence rate in the intervention group throughout the study, which showed a significant statistical difference in the primary objective, which could not be altered by continuing the study (futility analysis), leading to a serious ethical consideration of a premature closing of the study which was decided due to safety and benefit concerns [31].

Discussion

This is the first reported randomized clinical trial on the outcome of surgical treatment of high cryptoglandular anal fistula with VAAFT compared with FSR. The recurrence rate of the fistula was significantly higher after VAAFT (65%) compared with FSR (27%). Only one of the risk factors (length of the fistula) investigated was significantly associated with fistula recurrence. The demographic characteristics of the study population were similar to those reported in previous studies [1, 2, 5] with males being affected 2.5 times more than females and mean age in the fifth decade of life. In comparison to previous reports, about 58% of the fistula were anteriorly located to the anus. The patients were included according to clearly defined inclusion criteria and selection bias cannot be rejected or confirmed.

The recurrence rate after FSR was previously reported to be between 1-13% in non-randomized trials [2-4]. The recurrence rate after FSR in the present study was higher and might be explained by inclusion of only patients with high fistula and that the previously reported recurrence rates included results from re-operations. The recurrence of fistula after VAAFT was considerably higher (65%) in this study compared to previously reported studies, which also included patients with non-complex fistulas. High recurrence rate was previously reported after VAAFT for high transsphincteric anal fistula [23]. Recurrences occurred at the operation site. MRI scanning was not performed in recurrent fistula patients. Therefore, it was not certain whether the recurrences were missed secondary tracts or original fistulas. It is easier to believe that recurrence of the original tract occurs in the VAAFT group. There were no serious surgical complications (Calvien-Dindo grade III or above) observed in any patients and there was no need for a diverting stoma.

Impairment of faecal continence following both treatments was not demonstrated in this study. Despite the decrease in pressure measurements by anal manometry, this was not reflected in continence as evaluated by the Wexner score. There was a significant improvement in Wexner score in both groups, without the predicted advantage for the VAAFT group. The presence of an anal sphincter defect and the size of the defect along with mean squeeze pressure were previously found to correlate to faecal incontinence score [32], but the presence of a defect in the internal anal sphincter at follow-up in the present study did not significantly affect the results of anal manometry or faecal incontinence score and might be explained by different study populations.

VAAFT was previously reported to be associated with improvement in quality-of-life [14]. Despite the minimal invasive nature of VAAFT, this study demonstrated that improvement in quality-of-life measurements was in favour for FSR. This might be due to the significantly higher recurrence rate and delayed wound healing in the VAAFT group.

The early cessation of the study was due to significantly higher rate of recurrence in the VAAFT group [31]. Although the surgical procedures were all performed by a dedicated fistula surgeon (first author) with the necessary training in both procedures, the learning curve might be a confounder. Another limitation of the study is being a single centre study with lacking external validity. The study was also underpowered as the desired sample calculation was focused on having 25% difference between the groups with a low recurrence rate for FSR. The fistula recurrence rate was higher in both groups than anticipated.

This randomized study for high cryptoglandular anal fistula required early cessation due to a significantly higher recurrence rate after VAAFT compared to FSR.

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[i] RedCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

[ii] unit alcohol=12 g alcohol. Maximal 7 units for females and 14 units for males per week as Danish health administrations recommendation for maximal alcohol consumption

Treatment of fistulizing perianal Crohn's disease by autologous microfat enriched with Adipose-Derived Regenerative Cells, ADRC.

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Disclosures
Søren Paludan Sheikh is owner and CEO of Blue Cell Therapeutics, https://blue-cell.com. The other authors declare that they have no conflict of interest.

This research was funded by The University of Southern Denmark, The Region of Southern Denmark, Surgical Department A, Odense University Hospital, Odense University Hospital Foundation, the Danish Crohn Colitis Association CCF and the Fionia Foundation

Summary

In this pilot study, short-term efficacy and safety of fat graft enriched with Adipose-Derived Regenerative Cells (ADRC) in the treatment of Crohn's high anal fistula was evaluated. (75%) complete clinical healing of the fistula was achieved by a single treatment.

Key words: Rectal Fistula, Stem Cells, Crohn Disease, Cell Therapy.

Introduction
Surgical treatment of fistulizing perianal Crohn's disease (pCD) in patients with failed medical therapy is challenging with relative high recurrence and failure rates [1]. The disease has a significant impairment on quality of life [1] and carries a relatively high risk of proctectomy [2]. The risk of developing a Crohn's perianal fistula has not altered during the last two decades despite the increased prescription of biologicals [3].

The use of stem cell injection in or around the fistula has been shown to be safe and feasible but with variable healing rates [4, 5]. Encouraging results have been reported with autologous adipose tissue graft combined with surgical closure of the internal fistula opening [6]. The stromal vascular fraction (SVF), also referred to as Adipose Derived Regenerative Cells (ADRC), is isolated from freshly harvested autologous adipose tissue (by liposuction). SVF includes several cell types with regenerative potential as demonstrated in experiments of erectile dysfunction following radical prostatectomy [7] and breast cancer related lymphedema [8].

The aim of the present study was to evaluate the outcome and safety of treating fistulizing pCD with autologous fat graft enriched with ADRC. The primary outcome was the healing rate defined as absence of discharge and closure of external fistula opening by clinical examination. The secondary outcomes were time to healing (weeks), fecal incontinence measured by Wexner Fecal Incontinence Score and radiological recurrence of fistula on MRI scanning.

Materials and Methods

The study was designed as a prospective single center pilot study and was conducted between June 2018 and December 2020. The inclusion criterium was adult patient (≥18 years) with fistulizing perianal Crohn's disease not responding to medical therapy for at least six months and a loose seton suture for at least three months. Exclusion criteria were multiple (two or more tracts) fistulas, anal stenosis, suppuration around the fistula tract, a subcutaneous perianal fistula, active intestinal Crohn's disease not in remission, Body Mass Index BMI < 18.5, coagulopathy, previous radiotherapy to the abdomen and pelvis, any malignancy within five years and verified infection on screening test.

After informed consent all included patients were scheduled for preoperative work-up with MRI scanning of pelvis/rectum, serological screening test for syphilis, hepatitis and HIV and physical examination under general anesthesia including trans-anal ultrasound with anatomical mapping of the fistula tract. Patients' current Crohn's medications were not altered or postponed during the period of treatment and follow-up.

Surgical procedures and ADRC preparation

All procedures were performed as a same day surgery. The liposuction and ADRC isolation had been previously described in detail [7, 8]. Liposuction was followed by surgical debridement of the fistula tract and double layered closure of the internal opening using absorbable suture material. The external opening was excised and left open for drainage. Then 30-50 milliliters of the freshly harvested lipoaspirate were injected around the entire length of fistula tract, taking care not to perforate the fistula tract (Figure 1a-e). The patient was observed at the recovery unit for 120-150 minutes. Meanwhile, ADRC were isolated from the remaining lipoaspirate using an automated processing Celution® 800/IV system (Lorem Cytori, San Diego, California, USA) according to the manufacturer's instructions. Four ml of isolated ADRC were injected and evenly distributed around the fistula tract corresponding to the locations of previous injections of lipoaspirate under mild sedation with propofol.

The patients were discharged the same day with prescription of postoperative oral antibiotics (metronidazole 1500 milligram/day and cefuroxime 1000 milligram/day) for seven days.

Follow-up

All included patients were scheduled for three follow-ups at two weeks, three and six months, for clinical evaluation of healing (inspection, digital palpation and anoscopy) as well as Wexner fecal incontinence score. MRI scanning was performed at 6-months follow-up, for patients with clinical healing and no sign of recurrence at physical examination. Whenever there was a suspicion of recurrence or abscess formation, examination under general anesthesia was performed. Further clinical follow-up for healing and recurrence was done at 12 months, for patients who achieved clinical and radiological healing at 6-months follow-up.

Statistical analysis

Continuous and categorical variables were analyzed using descriptive statistical tests (*t*-test, Wilcoxon rank-sum (Mann-Whitney) test and Pearson correlation (r)) when appropriate. *P* values below 0.05 were considered significant. Stata statistical software package version 16.0 and GraphPad Prism 9 were used.

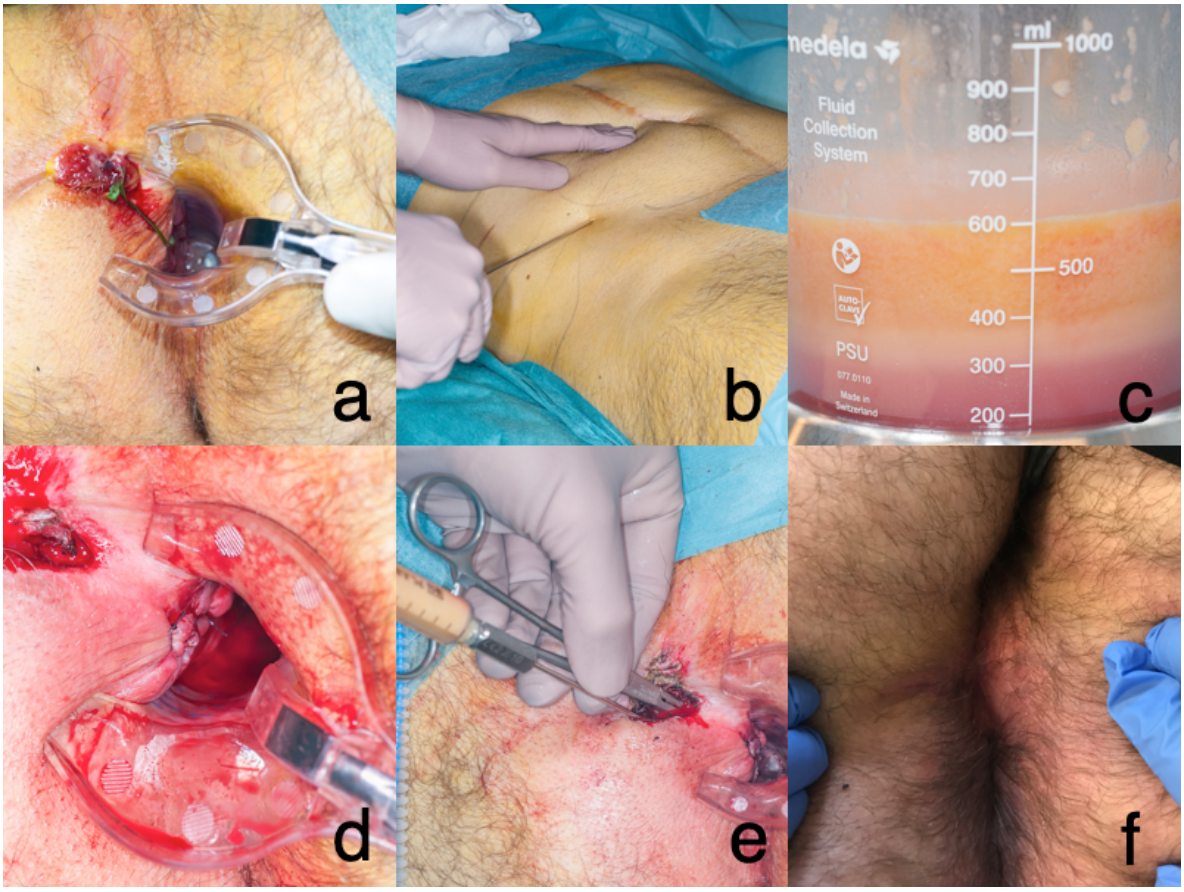

Figure 1:

a: A high transsphincteric Crohn's anal fistula on the right side

b: Liposuction from anterior abdomen using waterjet assisted liposuction (body-jet®, Human med AG, Schwerin, Germany)

c: Lipoaspirate (upper layer) harvested with waterjet liposuction from the anterior abdomen

d: while the lipoaspirate left to sediment at the operating theatre, the fistula is curated, and internal opening is closed with two-layer absorbable sutures

e: the lipoaspirate is directly injected around the entire length of fistula tract. When the ADRC suspension is ready, it is injected into the same site of lipoaspirate injection

f: Complete healing at 6-months follow-up

Ethical considerations

The study was conducted in accordance with the rules of the Helsinki declaration. The study was approved by the local Research Ethics Committee (S-20170140). ADRC isolation was performed at an authorized tissue establishment (Authorization no. 29035, Department of Clinical Biochemistry and Pharmacology, Odense University Hospital). The study was registered at ClinicalTrials.org with identification number NCT03466515.

Results

Of the 76 patients with Crohn disease and anal fistula evaluated for surgical treatment during the study period, 13 patients were found eligible for the study. One patient was excluded due to exacerbation of Crohn disease and weight loss (BMI below 18.5) despite intensive medical treatment. 12 patients received the intended treatment and completed the observation period.

Demography, ADRC characterization and surgical data

Table 1 shows the demographic characteristics of the study population. All but three patients received adjuvant medical therapy (biological treatment alone in six and biological treatment plus immune modulation in three). The mean length of fistula was 4.5 cm (95% CI 3.2-5.8), all fistulas were high transsphincteric and in two patients there were two external openings. One patient had a diverting colostomy. The mean volume of lipoaspirate injected was 40 ml (95% CI 31-49.6). The average number of injected ADRC cells was 37.6 million (95% CI 29.3-45.8).

The cell yields were not affected by patients' gender in the present study (p=0.911) and importantly, also no relation to medical therapy (p=0.552). Medical therapy did not appear to have an impact on any of the analyzed cell parameters that all were within normal range. A negative correlation was observed between percentage of erythrocytes and percentage of CFU-Fs (p=0.0088) and CD31-CD34+ stromal cells (p=0.0209), suggesting that the total yield of stromal cells may be affected by hemorrhage. However, as for all other cell parameters, the number of injected nucleated, non-hematopoietic CD31-CD34+ ADRC was not significantly different between responders and non-responders.

Recurrence and healing

Fistula recurrence occurred in three patients within the 2-weeks follow-up, mainly due to failure of the closure of the internal opening. Nine patients (75%) had a complete closure of the fistula tract and were free of symptoms at 6-months (Figure 1f) and at one-year follow-up. Apart from the recurrences, there were no serious surgical complications (Calvien-Dindo grade III or above) observed in any cases. Wound healing was achieved in eight of the twelve patients (67%) at 12-weeks follow-up. The complete healing of the fistula was confirmed by MRI scanning at 6-months follow-up in eight of the nine patients, and the last patient had regression of the fistula without fluid collection.

Fecal Incontinence

The mean Wexner Fecal Incontinence score was reduced by more than 50% at 6-months follow-up compared to baseline measurement.

Patients' demographic characteristics N=12	
Gender	Male=3, Female=9
Mean age in years (range)	33 (22-51)
Mean BMI (range)	27.9 (20.07-37.03)
Smoking habits	
Smokers	3
Quit	3
Non-smokers	6
Alcohol consumption[1]	
Within recommended	11
Above recommended	1
Co-morbidity[2]	1
Mean duration of fistula in months (range)	42.2 (8-132)
Mean duration of Crohn in years (range)	8.3 (1-23)
ADRC characteristics	
Mean lipoaspirate total volume (95% CI)	263 ml (220-307)
Nucleated ADRC/g fat tissue (95% CI)	2.59×10^5 (1.93-3.24)
Mean lipoaspirate for ADRC isolation (95% CI)	233 ml (174-292)
Mean volume of lipoaspirate injected was (95% CI)	40 ml (31-49.6)
Average number of injected ADRC cells (95% CI)	37.6×10^6 (29.3-45.8)
ADRC subpopulation	
CD34 mean (95% CI)	58.5% (55.8-61.3)
CD90 mean (95% CI)	53.9% (39.1-68.6)
CD31 mean (95% CI)	30.5% (0.5-60.6)
CD73 mean (95% CI)	39.5% (24.4-54.9)
Erythrocytes %	5.8-57.8%

Table 1 showing demographic characteristics of the participants and the results of cell analysis. (BMI: Body Mass Index, ADRC: Adipose-Derived Regenerative Cells, CI: confidence interval).

Discussion

The present study showed 75% clinical healing and 67% radiological healing of the fistula at 6-months follow-up and no clinical sign of recurrence at one-year follow-up. Similar results were shown by Serrero et. al [9] using a comparable method, with clinical response of 80% and fistula healing confirmed by MRI scanning in 60% at 48-weeks follow-up. Apart from minor differences in ADRC isolation and characterization, both studies applied the same treatment principle with comparable outcomes suggesting that the method is robust. A negative correlation between hemorrhage due to liposuction and the yield of CD34+ cells was shown in the present study, suggesting that a regenerative effect cannot be ascribed to this population alone, although it should be interpreted with caution due to the restricted number of participants.

[1] The recommended weekly alcohol consumption by the Danish health council.
[2] only one patient had significant co-morbidity (impaired renal function).

Unaltered Crohn's medication during the study might have a positive influence on the results of the study due to continuous long effect of medical therapy. A similar positive influence could also be attributed to the meticulous surgical closure of the internal opening, with special attention to avoid disruption of the fistula tract during fat and ADRC injection. The improvement in Wexner score can be explained by healing of the fistula, leading to cessation of secretion and the need of diapers.

The commercially available product, Cx601(darvadstrocel)® has been shown to have clinical and radiological remission in 56% after one year compared to 35% with placebo in patients with Crohn's disease and a single fistula tract [10]. The present study strongly supported an alternative approach with the advantage of using autologous ADRC-enriched freshly collected lipoaspirate injection.

The major limitation of the study is the small population of selected patients, without control group and a short observation time. Further evaluation of the results of the present study by larger setting in a randomized trial is highly recommended,

Conclusion

ADRC-enriched autologous lipoaspirate can be safely used in the treatment of high anal fistula in patients with Crohn's disease with high rate of success.

Acknowledgments

We thank Tina Kjærgaard Andersen and Tonja Lyngse Jørgensen (LMCC, Odense University Hospital) for excellent technical assistance in relation to ADRC isolation and characterization.

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