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Using Minimally Invasive Sphincter-Sparing Techniques of Surgical Treatment of Transsphincteric Anal Fistulas: A Focus on Preserving Anal Sphincter Function

Igor A. Kryvoruchko^{1*}, Valeriy V. Boyko^{2,3}, Massimo Sartelli⁴, Tetayna M. Firsyk¹, Marina S. Antonova¹

ABSTRACT

Background: The existing recommendations for the diagnosis and treatment of anal cryptoglandular transsphincteric fistulas (AF) are contradictory, which makes practical decisions in its treatment extremely difficult. **Methods:** 82 patients were examined after surgery for AF. All the patients were divided into three groups: the first included patients who underwent fistulotomy (n = 17) and fistulectomy (n = 9); the second, who underwent ligation of the intersphincteric fistula (LIFT, n = 29); and the third, treated with bio-welding of the AF (n = 27). **Results:** Positive short-term results were obtained in all groups, in 53.8%, 82.8%, and 85.2% of cases, respectively. The effect of bio-welding of AF was achieved due to electrothermal denaturation and the appearance of common space between protein molecules with a small area of heat propagation, less than 1–2 mm. The immunohistochemical studies showed that cells had produced neutrophilic granulocytes and pro-inflammatory interleukins in both the intra- and extrasphincteric parts of AF. The average follow-up period for patients after bio-welding was 8.8±4.6 after LIFT was 9.6±4.6, and in the group, who underwent fistulotomy and fistulectomy, was 9.8±4.8 months; satisfactory long-term results of treatment were noted in 92.6%, 89.7% and 62.2% of cases, respectively. **Conclusion:** Suggested surgery techniques in AF have made it possible to personalize surgical tactics and improve the treatment. The cryptoglandular theory of AF seems controversial considering the data of our study.

Keywords: transsphincteric AF, intersphincteric fistula ligation, seton, fistulectomy, bio-welding, cryptoglandular theory of AF.

1. INTRODUCTION

Descriptions of anal fistula (AF) are found in the oldest known medical literature, since at one time, Hippocrates described in detail the method of

treating the rectum using a ligature, for which he used horsehair. John of Ardene gave a detailed description of the disease back in the XIV century (Parks, 1961), and modern researchers define AF as a pathological tract thought of as the rectum communicating with the skin, which is clinically accompanied by pain, mucopurulent discharge in this area with a significant decrease in the patient's quality of life, which worsens with a relapse of the disease (Owen et al., 2016). It is known that men get sick more often than women, and the first manifestations of the AF are 40 years old (Abcarian, 2011). Although in 90–95% of cases, AF is classified as cryptoglandular (Alasari & Kim, 2014), the cause is also Crohn's disease, while their frequency ranges from 17% to 50%. Approximately 35% of patients had one AF during their disease and approximately 30% of these have had complex healing (Lee et al., 2017). In addition, AF is also associated with sexually transmitted diseases in 7% of the general population of patients, tuberculosis (80-91% of patients), surgery and trauma (0.3%-1.2% of patients), radiation therapy, which can lead to deep damage to the mucous membrane of the rectum or anal canal with development of the fistula (Felt-Bersma & Bartelsman, 2009).

The incidence in the four countries of the European Union ranges from 10.4 per 100,000 in Spain to 23.2 per 100,000 in Italy (Zanotti et al., 2007), and a recent study estimates the prevalence of AF in Europe to be 1.69 per 10,000 patients (Hokkanen et al., 2019). There is still a need to analyze well-designed and conducted studies comparing different treatments for AF nowadays (Jacob et al., 2010). As well-known, AF is divided into simple, including a small part of the anal complex or the complex that may not be affected, and complex (Bleier & Moloo, 2011). Simple fistulas are small, including intrasphincteric or low transsphincteric fistula. In this case, the message is carried out through one tract, it is associated with cryptoglandular inflammatory processes, but not with other causes that lead to their occurrence. The next type is complex AF, which affects a significant sphincter part and can have multiple paths of development, affect other organs (for example, the vagina), and be associated with many causes leading to its formation, including radiation injury to the intestine or inflammatory disorders. However, recurrent fistulas are usually classified as complex.

The three main goals of the current treatment of transsphincter AF are (1) elimination of the fistula by closing it, (2) preservation of sphincter function, and (3) minimization of healing time, and over the past decades, there have been quite a lot of methods of sphincter-preserving operations for this pathology. For example, the movement of an endorectal flap (ERAF) (Oh, 1983); plugging AF with fibrin glue (Hjortrup et al., 1991) and various sealing “tampons” (Fistula plug) (Heydari et al., 2013); introduction of stem cells derived from adipose tissue into the fistulous tract (Garcia-Olmo et al., 2008); interphincteric fistula tract ligation (LIFT) (Manoj Kumar et al., 2021); video-assisted treatment using a fistuloscope (VAAFT) (Meinero & Mori, 2011); laser closure of fistulas (FiLaC™) (Wilhelm, 2011); fistulous tract bio-welding technique (Boyko et al., 2019); application of the OTSC® proctological system; staged combined use of gentle treatment methods (Boyko et al., 2019). Although many surgeons consider fistulotomy/fistulectomy to be the treatment of choice for AF, there are still many concerns about adapting surgical options to more complex clinical situations (Limura & Giordano, 2015). There are several recommendations worldwide for surgical approaches for AF (Ommer et al., 2017), but lots of them are controversial (de Groof et al., 2016), which in practical terms makes the decision extremely difficult to treat.

Objectives

Our study aimed to compare and analyze the standard surgical methods for the treatment of transsphincteric AF with the method of LIFT and bio-welding of the fistula.

2. MATERIALS AND METHODS

All patients were treated in 2014-2021, they were divided into three groups: patients who were treated with fistulotomy (n = 17) and fistulectomy with primary sphincter reconstruction (n = 9) were included in the first group; the second group included patients, treated with LIFT (n = 29); and the third group included patients treated with bio-welding (n = 27).

2.1. Identification of patients, operative technique, and postoperative period

Criteria of inclusion

Age ≥ 18 years; low (involvement of less than 1/3 of the sphincter complex) and high linear (involvement of more than 1/3 of the sphincter) transsphincteric AF with the penetration of the fistular tract through the superficial or deep part of the external anus; the informed consent of the patient.

Exclusion criteria

From the study were the presence of superficial AF; AF with infiltrates; the appearance of AF and Crohn's disease, ulcerative colitis, or tuberculosis, or actinomycosis, or oncological process, etc., and the presence of gross cicatricial changes in the layers of the rectum.

When the diagnosis was made, the patients underwent laboratory and instrumental examinations. Laboratory methods were presented with a standard set of analyses and bacteriological examination of the purulent contents of the fistula. Instrumental examination methods include those such as anoscopy, rectoromanoscopy, fistulography and MRI. The functional state of the muscles of the sphincter of the rectum was assessed by electromyography. The internal anal electrode was used to record the contractile capacity of the external anal sphincter and pelvic floor muscles (Garcia-Olmo et al., 2008). Integral indicators of bioelectrical activity were evaluated at rest, arbitrary rapid contraction and relaxation of muscles of the sphincter, and also in stress tests. To identify patients with symptoms of deficiency anal sphincter when taking anamnesis before surgery, and also to assess the effect of surgery on the mechanism of holding, a clinical survey and questioning of patients were carried out using the Cleveland score for assessing anal incontinence (Jorge & Wexner, 1991), where 0 points meant adequate maintenance of all components of intestinal contents without impairing the lifestyle, and 20 points - incontinence of all components of intestinal contents for regular use of pads due to aggravated anal incontinence.

To characterize patients, a database was used, including records from medical records with such indicators as the age and gender of the patient, body mass index, comorbidities, colon and rectal disease, duration of rectal fistula, distance from the outer opening of the rectal fistula to the anus. Low transsphincteric fistulas used fistulotomy, and high linear transsphincteric fistulae used LIFT, bio-welding procedures and fistulectomy with primary sphincter reconstruction. Surgical treatment of AF with bio-welding consisted of the elimination of the intrasphincteric part, removal of the extrasphincteric part of the fistula (or without it) after preliminary contrast through the external opening. The EK-300M1 multifunctional machine (Ukraine) was used for bio welding, and special probes were used to supply current directly to the fistula tract, the end part of which has a bipolar configuration electrode. The probe was selected according to the fistula for adequate welding (Figure 1). Bio-welding was completed in the mode of "manual welding" with a capacity of 50%, the removal rate of the zone reached 0.5 cm/sec (Boyko et al., 2019). In fact, with the application of bio-welding, we have managed to achieve a reliable separation of the epithelium of the proximal AP, as well as obturation of the tract of the fistula. Evidence of removal of the intrasphincteric part of AF was the impossibility of re-introducing the probe into the intestinal lumen. All the surgeries we performed were under spinal anaesthesia. For 24 hours, antibacterial prophylaxis was administered. After surgical procedures, all patients received analgesics and syrups to soften their faeces. Postoperative care included the use of antiseptic baths, dressings with ointment, and rectal suppositories. We have also identified the main parameters to compare the surgeries: there were such parameters as a relapse, functionality of the sphincter, terms of wound healing, and at the hospital stay. The results were evaluated by several criteria: the duration of the operation, the presence of postoperative complications, the duration of complete wound healing, success rate, and amount of pain in patients. Full wound healing, absence of sphincter complex failure, or disease recurrence was considered a successful procedure.

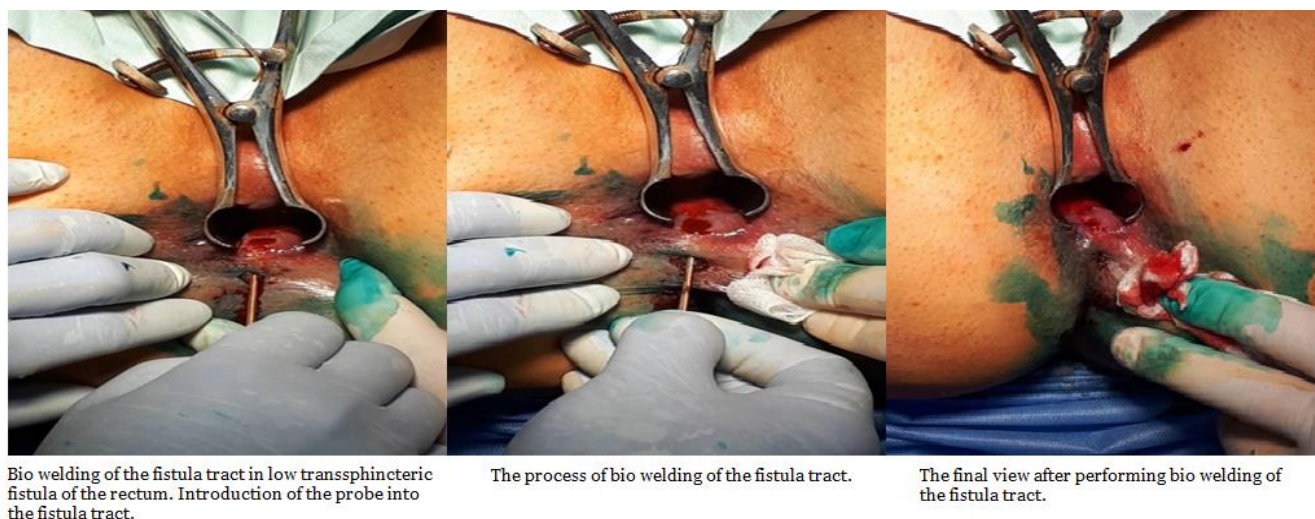


Figure 1. Treatment of AF with bio-welding procedure

2.2. Data analysis and processing

The materials for morphological examination were represented by the AF that were obtained during the operations. With these interventions, different amounts of material were obtained, which were conveniently divided into intrasphincteric and extrasphincteric parts of AF and evaluated qualitative and quantitative results of the histological and immunohistological examination in the presented parts. The resulting material was fixed in a 10% formalin solution, followed by passing through alcohols of different concentrations and pouring into paraffin. Prepared serial sections with a thickness of 4-5x10⁶m. Histological examinations were conducted on all patients according to standard methods. All micropreparations were subjected to staining with hematoxylin and eosin, picrofuchsin according to van Gieson, Mallory's trichrome stain, and studied on an Olympus VX-41 microscope. The immunohistochemical study was carried out on paraffin sections by the indirect immunofluorescence reaction with monoclonal antibodies (MCAs) to collagen types I and III (IMTEK Ltd.), as well as MCAs to CD18, CD16, TNF- α , IL-6 (Novocastra Laboratories Ltd.). Immune cells were counted in the field of view at a magnification of 400 times, for which all micro preparations were examined under an Olympus BX-41 microscope (Japan).

2.3. Assessment of the sort of treatment

To evaluate the type of treatment, all patients underwent follow-up examinations. Similar examinations were carried out every two weeks after discharge from the hospital in the first of two months after surgery. After that monitoring after surgery was carried out for 3 to 12 months with conducted a survey and clinical examinations of patients at each visit to clarify complaints and complications.

2.4. Statistical data

Statistical studies had done using Statistical studies were carried out with StatSoft Statistica 6.0 statistical software package. To determine the differences distributed to the methods of surgery and such criteria as of Student's, Mann-Whitney's, and χ^2 tests were used. In all cases, the verification of statistical hypotheses was conducted with a confidence probability of more than 95%.

3. RESULTS

Patient characteristics are reviewed in Table 1. As follows from the presented data, most of the analyzed patients were of working age, so it was important for us to reduce the terms of treatment and their rehabilitation, which would be made it possible to reduce the bed-day and economic costs in general.

Table 1. Evaluation criteria of AF

EVALUATION CRITERIA	INDICATORS
Type of AF (n = 82)	Transsphincteric (100%)
Low	38 (46.3%)
High	44 (53.7%)
Male/ Female	68/14
Average age	41.5 \pm 8.79
Median BMI (kg/m ²)	27.8 \pm 2.42
Previous operations (1 st /2 nd /3 ^d)	26 (18/6/2)
Localization of an internal opening:	
Anterior	22 (26.8%)
Posterior	58 (70.8%)
Lateral	2 (2.4%)
Localization of an internal hole:	
Anterior	21 (25.6%)
Posterior	50 (61%)
Lateral	11 (13.4%)
Distance between the external opening of the external hole and the anus (sm)	4.7 \pm 0.95
Duration of the disease:	
before 3 months	12 (14.6%)
from 3 to 12 months	42 (51.2%)

more than 12 months	28 (34.2%)
Types of surgery:	
fistulotomy / fistulectomy with the	17 (20.7%)/
primary reconstruction of the sphincter	9 (11%)
LIFT procedure	29 (35.4%)
Bio-welding of the AF	27 (32.9%)

Notes: * - reliably with data before surgery ($p < 0.05$)

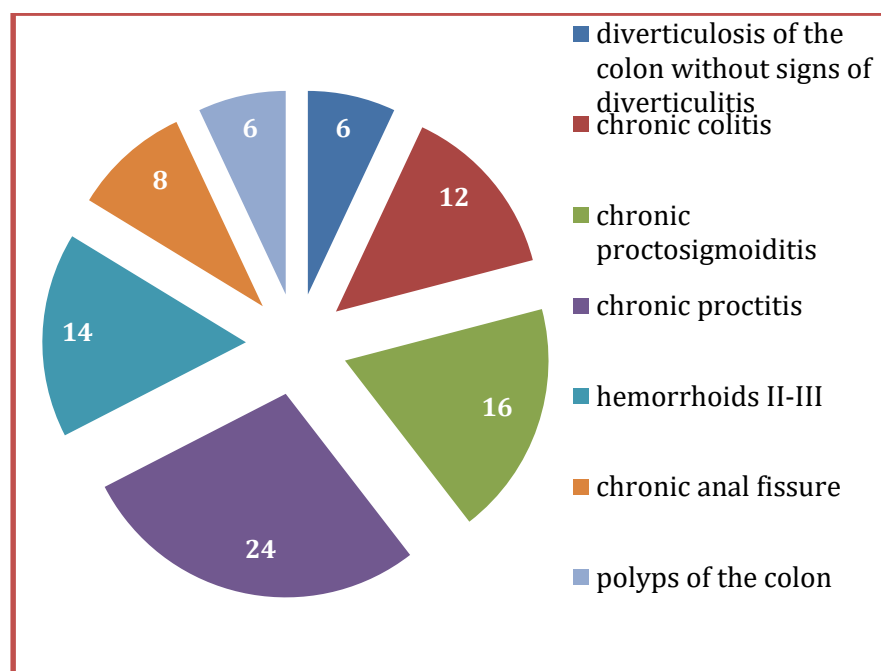


Figure 2. The structure of concomitant diseases of the colon

Among the studied patients, 34 (41.5%) had concomitant systemic diseases, including coronary heart disease (11%), hypertensive heart disease (46.3%), chronic kidney disease (17.71%), diabetes mellitus (13.4%), and chronic nonspecific lung disease (6.1%). However, in our opinion, availability of comorbidity had little effect on wound healing and the postoperative period as a whole. In 56 patients, 68.3% were found to have colon diseases (Figure 2). According to this data, it was the following diseases of the colon: chronic colitis (14.6%), chronic proctosigmoiditis (19.5%) and chronic proctitis (29.3%). It is connected to the long duration of the disease, which not only contributes to the exacerbation of chronic pathology but also contributes to the emergence of additional comorbidities. Since the patient had a fistula in the rectum, this area was a source of permanent infection. Perhaps this contributed to the emergence of patients with related pathology of the genitourinary tract.

Using the data obtained after the analysis of the questionnaires, we made the following conclusions: In 9 (36.6%) patients of the 1st group, 11 (52.2%) of the 2nd and 8 patients of the 3rd group: in the 1st group, 7 patients had recurrent incontinence gas less than one time a week, but more than one time a month, and in two patients sometimes gas and fluid incontinence were noted without wearing gaskets; in group 2, eight patients also had gas and fluid incontinence without wearing pads, two patients usually wore pads in connection with this, and one had not only gas and fluid incontinence but also intermittent incontinence of hard faeces. In group 3, seven patients had gas incontinence, and one patient sometimes had gas and fluid incontinence.

Previously, 18 (65.5%) patients in the 1st group, 6 (69.5%) in the 2nd group, and 2 (7.4%) patients in the 3rd group underwent surgical interventions along the drainage channel of an acute abscess to fistula formation. Spontaneous opening of an acute abscess was noted in 3 (6.9%) and 2 (13.0%) cases in the 1st and 2nd groups, respectively.

An analysis of some of the treatment outcomes can be seen in Table 2. As can be seen from the above data, in the of patients undergoing LIFT and bio-welding procedures, the number of days spent in the hospital was significantly less than in the patients who used non-sphincter-sparing methods of surgery ($p < 0.05$). It was due to the less traumatic nature of the treatment method for this patient group and using of bio-welding to 'remove' the intrasphincteric part of the fistula by its complete occlusion together

with the AF inner hole allowed to avoid damage to the sphincter. As is well-known, the effective method of bio-welding the fistulous tract had achieved due to electrothermal denaturation and the appearance of common space between protein molecules. In this case, the zone of propagation of the thermal effect did not exceed 1–2 mm. Thus, the epithelial lining of the AF was destroyed without excessive injury to the rectal sphincter during surgery.

It was also important to reduce pain in patients after surgery. A questionnaire to assess the postoperative period in these patients using the level of pain on a scale from 1 to 10 points on 1, 3, 7 days after surgery was used (Table 2). The pain in the first of the two groups was moderate from 2 to 8 points, and in the third group, there were been minor pains after surgery from 1 to 4 points which were significantly different from the indicators in the first and second groups of patients ($p < 0.05$). By the 7th day, the intensity of pain in all groups decreased slightly and was 0-5 points on a visual-analog scale. In the 3rd group, there was fallen down in the intensity of pain, which did not exceed the level of 3 points by the 7th day after the operation ($p < 0.05$). To sum up, the dynamics of the pain in the patients were similar but statistically significantly different in terms of normalization after surgery.

According to the obtained generalized data, a satisfactory short-term outcome of treatment in the 1st group was 53.8% of cases (12 (46.2%) of patients with postoperative complications), in the 2nd in 82.8% (5 (17.2%) of patients with complications after surgery), and in the 3rd in 85.2% of cases (4 (14.8%) of patients with complications after surgery). The patients with complications after surgery were treated using conservative methods. Although the differences for a given sample size were statistically insignificant ($\chi^2 = 4.678$, $p = 0.096$), there is a clear trend in favour of better healing rates after using bio-welding of the AF.

After surgery, the follow-up period for all patients was almost the same. It was noted that the recurrence of the AF after surgery within twelve months after fistulotomy/fistulectomy was in 8 (30.8%), in the second in 3 (10.3%), and in the third in 2 (7.4%) patients, respectively. Satisfactory long-term treatment outcomes were found in 15.4%, 44.8%, and 81.5% of patients (Table 2). Moreover, the satisfactory long-term treatment outcomes obtained in the patients were statistically different ($\chi^2 = 9.465$, $p = 0.05$).

Table 2. Analysis of the results of surgical treatment

Indicators	1 st group	2 nd group	3 rd group
Number of patients	26	29	27
Operation time (min)	61.7±11.2	53.6±8.4 *	22.8±5.8 **
Number of bed-days	14.1±2.2	11.8±1.7 *	7.9±0.9 **
Evaluation of pain on (points):			
1 st day	5.7±0.84	5.5±0.81	3.1±0.82 **
3 rd day	4.3±0.83	3.6±0.72 *	2.5±0.82 **
7 th day	3.5±0.91	2.7±0.85 *	1.5±0.6 **
Postoperative complications	12 (46.2%)	5 (17.2%)	4 (14.8%)
Postoperative mortality	0	0	0
**Satisfactory short-term treatment outcomes (%)	53.8%	82.8%	85.2%
Terms of observation after surgery (months)	9.8±4.8	9.6±4.6	8.8±4.2
Recurrence of the AF after surgery (%)	8 (30.8%)	3 (10.3%)	2 (7.4%)
***Satisfactory long-term treatment outcomes (%)	62.2%	89.7%	92.6%

Note: *-reliability between groups ($P < 0.05$); ** satisfactory short-term treatment outcomes included treatment without purulent-septic complications after surgery; *** satisfactory long-term treatment outcomes included no severe insufficiency of the anal sphincter and recurrence of AF.

The morphological study of the removed areas of the AF and surrounding tissues showed the priority of the direction we chose, which was aimed at applying techniques that do not damage the sphincter structures of the rectum. The material obtained in the patients after fistulotomy/fistulectomy was represented by intrasphincteric and extrasphincteric parts of the rectal fistula. In the intrasphincteric part of the micro preparation, the connective tissue component, muscular and vascular, is defined mainly. Connective tissue was represented by both mature and granulation tissue, regardless of localization, oedema, with areas of disorganization, as of fibrinoid oedema and necrosis, determined by Mallory's trichrome staining. Inflammatory infiltration represented by lymphocytes, macrophages, and neutrophils was seen in the stroma. Granulation tissue was represented by connective tissue and immune cells, microvessels, which were visible along the inner wall of the AF. The position of the cells varied and was remarkable in that they were located predominantly in the zone of granulation tissue. The presence of squamous epithelium with keratinization and acanthosis was seen in the extrasphincteric part of the AF. The basement membrane of the epidermis in places due to multiple sclerosis. Fuchsinophilic bundles of collagen fibres with signs of disorganization of fibrinoid changes were determined in the dermis during Mallory's trichrome staining (Figure 3).

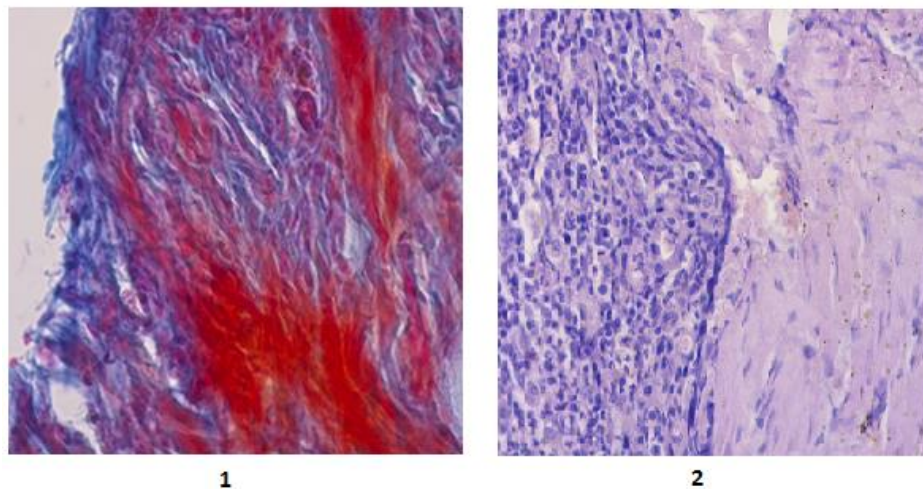


Figure 3. The morphological study of the transsphincteric AF: **1:** Oedema, fibrinoid swelling, and fibrinoid necrosis of connective tissue in the wall of the fistula tract: Mallory's trichrome stain, x 400. **2:** granulation tissue in the wall of the fistula tract in the first group: staining with hematoxylin and eosin, x 400.

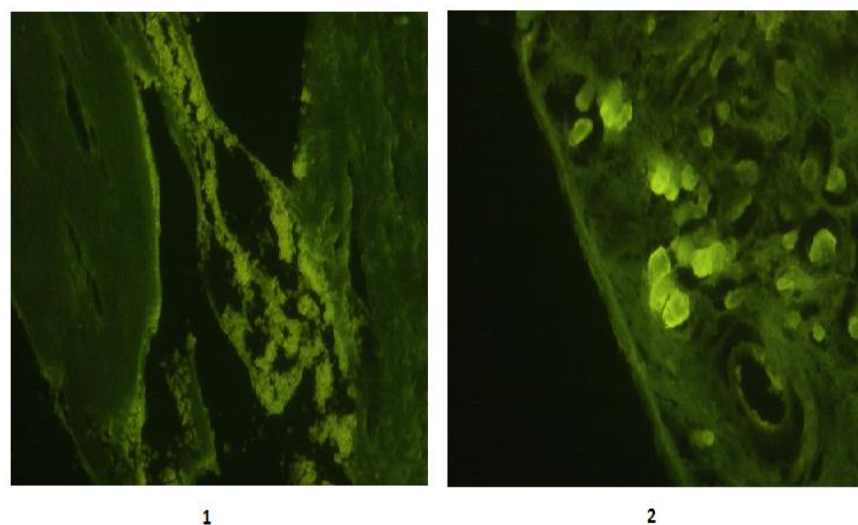


Figure 4. The immunohistochemical study of the AF: **1:** The abundance of neutrophilic leukocytes (CD18) in the inflammatory infiltrate of the fistula tract wall: indirect immunofluorescence reaction with MCA to CD18, x100; **2:** Cells-producers of TNF α in the granulation tissue of the wall of the fistula tract: indirect Immunofluorescence reaction with MCA to TNF- α , x 600.

The connective tissue elements of the stroma were represented by fibroblasts, fibrocytes, and macrophages. Massive growths of granulation tissue, as well as young connective tissue, were detected. An immunohistochemical study of the connective tissue component of the AF walls located near the internal opening with MCA to interstitial collagen revealed collagens I and III types. We would like to point out that, the mature type I collagen had predominated in it which was revealed as a linear and focal bright glow. Less mature type III collagen was predominantly defined as focal weak or moderate luminescence intensity in the zones of localization of granulation and maturing connective tissue. Infiltration of focal inflammatory cells was located directly near and among connective tissue fibers, as well as perivascular areas. The cell infiltrate was dominated by CD18 neutrophilic granulocytes (Figure 4), NK-cells (CD16), as well as IL-6 and TNF α -producing cells, were quite often detected. Just as in the tissues of the layers of the fistula located near the inner opening, the number of NK-cells (CD16) was large around the areas of disorganization of the connective tissue. The cells producing IL-6 and TNF α (Figure 4) were localized more around and in the granulation tissue. The immunohistochemistry study was revealed to present both interstitial collagens with a predominance of collagen of type I, which is characteristic of mature connective tissue, and also the collagen of type III, as weak or moderate focal immunofluorescence was localized in granulation tissue and young maturing connective tissue.

Table 3. The number of individual clones of immune cells in the fistula tracts.

	IL-6		TNF- α		CD-16	
	1	2	1	2	1	2
The 1st group	30.83 \pm	25.63	23.7	18.73	13.9	13.73
	1.62	\pm	\pm	\pm	\pm	\pm
		1.99	1.83	1.82	1.63	1.41
	95% CI 4.3262-6.138, P = 0.000		95% CI 0.027- 5.913, P = 0.000		95% CI 0.6177-0.9577, P = 0.667	
The 2nd group	30.3	27.53	21.93 \pm	18.4	14.17 \pm	12.27
	\pm	\pm	2.03	\pm	1.39	\pm
	2.84	1.89		1.35		1.48
	95% CI 1.523 -6.138, P = 0.000		95% CI 2.639- 4.421, P = 0.000		95% CI 1.158- 2.642, P = 0.000	

Note: 1 – intrasphincteric part of the AF; 2 – extrasphincteric part of the AF; CI – 95% confidence interval.

An immunohistochemical study revealed the predominance of neutrophilic granulocytes (CD18) in the connective tissue and muscle components (Figure 4). In addition, cells expressing receptors for IL-6 and TNF- α , and natural killer cells, CD16, were noted. The number of CD16-NK cells was high around areas of connective tissue disorganization and myocytolysis (Figure 4). Thus, the average number of these cells was 13.9 ± 1.63 and 13.73 ± 1.41 , cells producing IL-6 was 30.83 ± 1.62 and 25.63 ± 1.99 , TNF was 23.7 ± 1.83 and 18.73 ± 1.82 , and they were more often determined in the zone of localization of granulation tissue (Table 3). Between the connective tissue fibres, the patients had moderately focal and, in places significantly pronounced inflammatory infiltration, represented by lymphocytes, macrophages, and neutrophils. The growth of granulation tissue with a lot of number of microvessels and cellular elements of connective tissue origin with an admixture of immune cells was found out on the internal superficies of the AF. The perivascular proliferation of the connective tissue led to a sharp narrowing of the vessels and in some places to their obliteration, which aggravated the alternative changes caused by chronic hypoxia. The muscle component was presented as transversely and longitudinally oriented muscle fibres. The focal normal orientation of muscle fibres was disturbed. The phenomena of oedema, fibrillation, and atrophy of muscle fibres were revealed. Between myocytes, individual muscle fibres and their bundles, the growth of fibrous connective tissue was observed, and it was stained blue according to Mallory and red according to van Gieson (Figure 5). This immunohistochemical study of the AF walls, which are located near the inner opening, revealed the luminescence of I and III interstitial collagens with a noticeable predominance of mature type I collagen. In addition, less mature type III collagen was found in granulation and maturing connective tissue, and near the external opening of the fistula, the keratinization of squamous multi-row epithelium with signs of acanthosis was noted. According to van Gieson, the basement membrane of the epidermis was uneven in thickness due to sclerotic changes, as evidenced by the focal bright fuchsinophilia. The

vessels of the subepidermal zone were expanded unevenly and are mostly full-blooded. The dermis was fibrous connective tissue with signs of disorganization as of fibrinoid swelling and fibrinoid necrosis, which was detected as an orange colour when stained according to Mallory's trichrome.

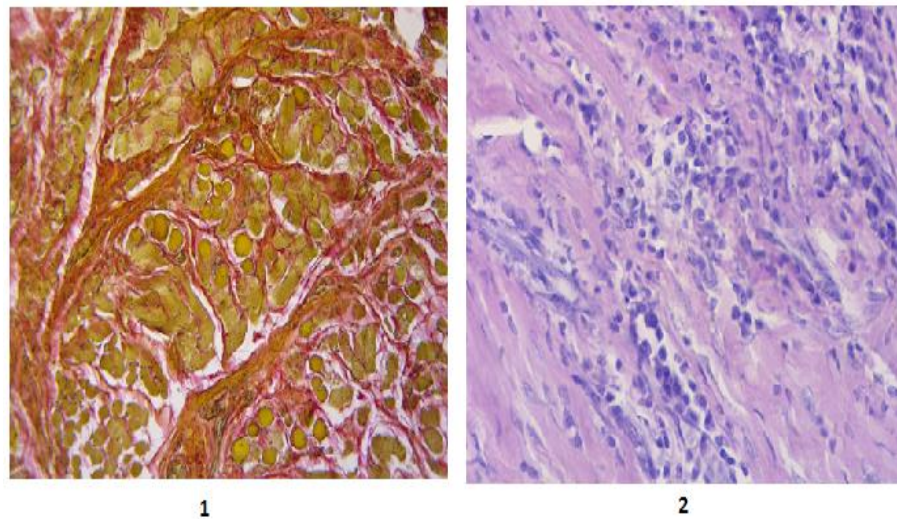


Figure 5. A morphological study of the AF in the first group: **1:** Oedema, atrophy, and disorientation of muscle fibres against the background of the proliferation of fibrous connective tissue: paint according to van Gieson, x 100; **2:** Diffuse inflammatory cellular infiltration between muscle and connective tissue fibres in the tissues of the fistula tract wall: staining with hematoxylin and eosin, x 400.

The immunohistochemical study revealed the predominance of neutrophilic granulocytes (CD18) and cells expressing receptors for IL-6 and TNF α (Figure 6). The connective tissue fibres were intensely stained red according to the van Gieson method, and diffuse inflammatory infiltration was observed among fibers (Figure 6, 1). In the described tissues of the AF layers, both types of interstitial collagen were detected, and collagen of type I predominated (Figure 6, 2). Focal collagen of type III luminescence was observed mainly in granulation tissue and maturing connective tissue, and also along with IL-6-producing cells in the fistula tract (Figure 6, 3). As in the observations of the first group of patients, the numbers of NK cells (CD16) were accompanied by large areas of disorganization of connective tissue, myocytolysis and on average were 14.17 ± 1.39 and 12.27 ± 1.48 , respectively. Nevertheless, IL-6-producing cells (30.3 ± 2.84 and 27.53 ± 1.89 , respectively) and TNF- α (21.93 ± 2.03 and 18.4 ± 1.35 , respectively) were more determined in the zone of localization of granulation tissue (Table 3).

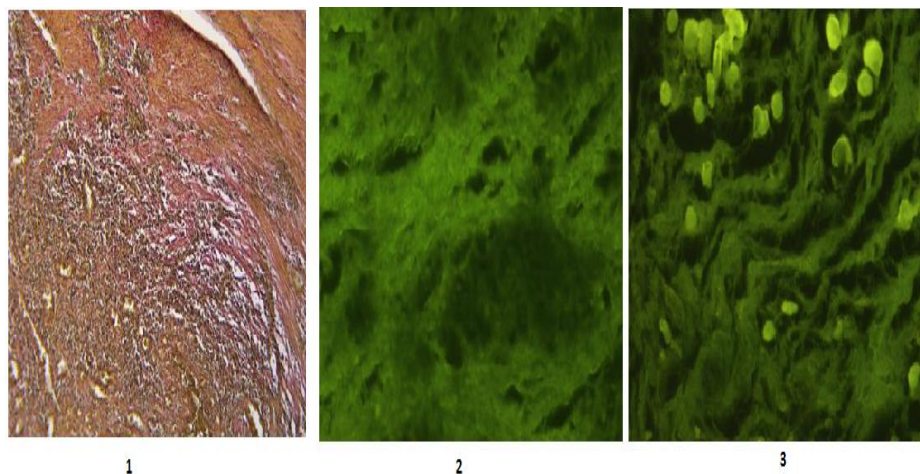


Figure 6. The morphological and immunohistochemical studies of the AF in the 2nd group: **1:** Severe inflammatory infiltration in the tissues of the wall of the fistulous tract and pronounced fuchsinophilia of the connective tissue fibres, according to van Gieson, x 100; **2:** Bright immunofluorescence of collagen of type I in the wall of the fistula tract: indirect immunofluorescence reaction with

MCA to collagen of type I, x 600; 3: IL-6-producing cells in the inflammatory infiltration of the walls of the fistula tract: indirect immunofluorescence reaction with MCA to IL-6, x 600.

4. DISCUSSION

AF is commonly defined as an unusual connection between the anorectal canal and the perianal area, for which plenty of surgical techniques have been proposed, often including a seton procedure, fibrin glue, collagen sutures, rectal valves, fistulotomy with sphincter regeneration, redirecting the AF tract, etc. (Rizzo et al., 2010). However, the results were variable, and no procedure's effectiveness exceeded the others. The purpose of any treatment in these variants of AF is to destroy the path and reduce the frequency of relapses while maintaining the full functionality of the sphincter of the rectum. Fistulotomy is now recognized by many coloproctologists as the standard of care for the surgical treatment of AF, as this operation leaves fewer surfaces without epithelialization, which speeds up wound healing, but the rate of the treatment had always been limited by the incidence of faecal incontinence, which could be up to 40% (Han et al., 2013) when the transsphincteric AF is opened and the sphincters are cut using this method of surgery. It has long been known that the significant condition in satisfactory results after any surgical intervention in AF is the choice of an adequate method of operation, taking into account the data on the ratio of the fibres of the sphincter apparatus of the rectum to the fistula before surgery. If the fistula spreads to most of the sphincter complex, the operation might contribute to the insolvency of the sphincter after surgery. If the fistula extends over most of the sphincter complex, surgery may exacerbate postoperative anal sphincter insufficiency. In our study, there were 14 (53.8%) cases of complications from the fistulotomy procedure in the short term and 8 cases of fistula recurrence (30.8%) at different times after surgery.

In 2007, Rojanasakul et al. described a new surgical variant of surgical intervention aimed at ligating the AF in the intermuscular space with very good initial results (Rojanasakul et al., 2007). Since then, this type of surgery has been used with satisfactory early results in lots of hospitals, and to date, there are more than six variants of the LIFT operation, and success rates varied from 47 to 95%. (Wallin et al., 2012; Limura & Giordano, 2015). But it is necessary to note that no studies have been conducted to liken the efficiency of these methods, and now difficult to determine the true value of both the classical technique LIFT and its modifications (Mushaya et al., 2012; Campbell et al., 2013; Liu et al., 2013) for the presentation of full reports after the use of this method of surgery. When using an improved LIFT technique, we observed 5 (17.2%) cases of complications in the immediate outcome and 3 (10.3%) cases of AF recurrence. When analyzing the results obtained, some causes of relapses and insufficiency of the sphincter were identified: a common significant factor was errors in the selection of the method of treating AF associated with preoperative diagnosis, namely, incomplete contrasting of AF, which contributed to an incorrect definition of the binding between the fistula and the muscle frame of the sphincter. Therefore, some authors recommended the application of MRI (Pomerri, 2010), which we applied only to 3 patients, since it assists to identify purulent perirectal foci, and also allows you to obtain information about the state of the anal sphincter, which contributes to the right choice of surgery. Among the technical mistakes, we noted two main ones: first, excessive excision of the sphincter tissue; and secondly, wound suppuration, which is the general basis of suture divergence. These factors led to the creation of a coarse postoperative scar that reduced the functional ability of the sphincter. Some authors have used drainage before LIFT as an effective way to prevent such postoperative complications, but a few of the research have shown that prior use of drainage before LIFT had no significant effect on outcomes in these patients (Araújo, et al., 2017).

In the study we conducted, it was shown that 82.8% of successful wound healing was achieved with the modified LIFT technique, and 85.2% of satisfactory immediate results were achieved with the bio-welding technique without prior drainage. This study has shown, at least two links are revealed in the morphogenesis of sclerosis of the tissues of the AF wall: The study showed that at least two links are distinguished in the morphogenesis of sclerosis of the AF walls: 1) the presence of a high and different from the normal cytokine-stimulating function of immune cells expressing receptors for IL-6 and TNF- α 2) the presence of chronic hypoxia, which leads to induction of the synthesis of interstitial fibroblasts, fibrocytes and collagen. Pronounced alternative changes in the overdeveloped connective tissue component manifested by fibrinoid changes, namely fibrinoid swelling and fibrinoid necrosis, in addition, the presence of foci of myocytolysis could be an effect of both tissue chronic hypoxia, also the activating of lysosomal enzymes which contained in neutrophilic granulocytes and also the cytotoxic effects of NK-cells (CD16). Note that morphological studies showed that in the AF there is always an inflammatory process with exacerbation on the background of preliminary remodelling of the connective tissue and muscle components against the backdrop of the development of severe sclerosis. Moreover, all these processes were accompanied by the production of IL-6 and TNF- α -producing cells not only in inflammatory infiltration but also in granulation tissue and immature connective tissue. This indicates an active role of these cytokines in stimulating collagenogenesis in AF, and their high expression may facilitate to the recurrence of fistulas after surgery. In 1961, Parks put forward the hypothesis that anal fistulas develop from the anal glands that originate in the intersphincteric

region and then penetrate the internal sphincter along the path of least resistance for AF occurrence (Parks, 1961). The theory was called “cryptoglandular”, and the implication of the proctodeal glands in this process can facilitate the creation of fistulas in these pathological processes. The theory of Parks was also confirmed clinically, and in the opinion of numerous proponents of it, since most of the internal openings of the AF were located within the crypt-dentate line, this theory fitted in well with the clinical findings. First of all, in the initial stages, an abscess in this area is formed, which is usually caused by blockage of the outlet duct of the slimy capsule of the anal gland with faecal material or trauma, which naturally can lead to local inflammation. In lack of blocking of the glands and while maintaining their good drainage function, the secret of the submucous glands will be freely released into the anal passage including when its inflammation is present. In cases where blockage of the anal crypt and inflammation occur, the submucous glands are expanding with an excessive accumulation of secretions in them. Because these glands end much deeper than the crypts of the rectum, their secrets cannot be freely released into the anal passage due to the tonic contraction of the sphincter. Therefore, the infected glandular secret, in this case, can go through places with the least resistance, along with muscle tissue. With these pathological changes, there are two ways to evacuate the secret of the glands: the promotion of the secret into the sciatic-anal fossa, or through the external sphincter due to the least resistance for the deflation of secretion of glands among the fibres of these muscles (Seow-Choen & Ho, 1994). However, back in 1967, the usefulness of Parkes's hypothesis about the cryptoglandularity of the AF was questioned, since the authors found a connection between the abscess in this area and the crypt only in five out of twenty-nine observations (Goligher et al., 1967), which was also confirmed by morphological studies of fifty-three tissues removed during surgery, and none of the preparations of the AF revealed anal tissue with cells-mucin producers (Mitalas et al., 2012). The generally accepted hypothesis that the cause of AF recurrence is its excessive epithelialization has also been questioned due to the foundation of granulation tissue and the virtual lack of epithelialization in this area (van Koperen et al., 2010). According to plenty of researchers, this is due to the conditions of fistula healing, namely, the fact that the healing of the AP is an unsuccessfully healed purulent wound with all the ensuing consequences since there is practically no good healing under these conditions (Gosselink et al., 2015) since suggested that under these conditions, local mucosal immunity provides effective protection, but does not stimulate tissue remodelling and repair (van Onkelen et al., 2013). In this way, according to some conclusions and our morphological studies, only the theory of Parks in the AF creation seems controversial.

To sum up, cure of AF is challenging, and no definitive opinions to date on the surgical approaches among colorectal surgeons to the cure. The disadvantage long-term results using fistulotomy as the “gold standard” for the management of transsphincter AF is that (1) many patients before the operation already have clinical signs of dysfunction of the perianal sphincter complex, which was often not taken into account before, and (2) in a few patients after fistulotomy, it is most often possible to develop dysfunction of the sphincter after surgery. We have used and demonstrated the effectiveness of surgical procedures for the cure of transsphincteric AF, which, in our opinion, these sphincter-preserving surgical techniques are optimal for the use of this category of patients compared to fistulotomy, since they allow good therapeutic effects with minimal impact on the functional state of the sphincter of the rectum. The aetiology of the AF is currently unknown, but our study has shown that the inflammatory process plays an important, if not decisive, role in the causes of AF and the relapse of its symptoms after surgery. The morphological studies showed that only the cryptoglandular theory of the formation of AF seems unlikely and controversial, what should be used when choosing a method of cure intervention for a particular patient in a particular clinical situation.

Limitations

Our research has several limitations. Primarily, the number of cases in our series was low, reflecting only clinical cases of transsphincteric AF. The limitations of our analysis also include its design and the incomplete records in hindsight, which we used. No medication records were made completely, these MRI descriptions for this pathology are not standardized, and the parameters of its choice for the analysis were not based on the famous literature. Secondly, many of the above-mentioned of surgery may become irrelevant shortly or have become solutions for a certain type of AF in the cause of conducting surgery. But issues of study for causes of AF will still be relevant in the coming years, and the causes of their recurrence after any interventions at this stage of studies will be really important and relevant too.

5. CONCLUSION

The pathogenesis of cryptoglandular AF has not been completely understood. Research had spent in the latest decades has identified the crucial roles of many factors, such as microbiological, morphological, and molecular, in the evolution of cryptoglandular AF. The data obtained to date are the foundation for the development and use of new approaches and treatment methods, which are fundamental for achieving better treatment results since all available research data can help found out the

causes of various types of cryptoglandular AF and define reliable markers of its treatment. First, given the results of our study, with low transsphincteric AF, we could advise applying fistulotomy or one of the options for sphincter-preserving procedures, for example, closure of the AF using high-energy approaches (laser and bio-welding closure procedures, etc.), since our results showed the higher efficiency of bio-welding as a thermal obliteration in this situation. Undoubtedly, more convincing conclusions will require further study of the effectiveness of this method on a larger amount of clinical material and a longer evaluation of the supervision of patients after surgery, including the study of their good character of life. Secondly, fistulotomy is not indicated for highly linear transsphincteric AF, since after this operation, anal incontinence occurs in most patients. In this category of patients, sphincter-preserving operations are the optimal method of surgical intervention to seek favourable results both in the early and the long term. Also, in some cases, with this AF, fistulectomy with a primary reconstruction of the rectal sphincter can be applied, but the usage of this operation in such patients should be minimal.

Compliance with ethical standards

The work has been cleared by the Ethics Committee of Kharkiv National Medical University, Ukraine (the protocol №3, September 20, 2021). The number of state registration is 0116u00499.

Competing interests

The authors declare that they have no competing interests. All authors have contributed equally to this work. All authors have read and approved the final manuscript.

Informed consent

Informed consent was obtained from all participants included in the study.

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Conflict of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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