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Success rate and utility of ultrasound guided synovial biopsies in clinical practice.

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Abstract (249 WORDS)

The utility of synovial biopsy in increasing our understanding of the pathogenesis of inflammatory arthropathies, as well as in evaluating treatments is well established. Ultrasound allows synovial assessment and therefore assists in biopsying synovial tissue in a safe and well-tolerated manner.

Objectives: (a) To determine the rate of success in retrieving synovial tissue using ultrasound guidance; (b) to describe the indications for US guided synovial biopsies in the clinical setting; (c) to determine how frequently the synovial biopsy can lead to a clear diagnosis and (d) to assess the quality of the synovial tissue obtained using this technique.

Methods: Synovial biopsies of small and large joints were performed under ultrasound guidance between January 2007 and December 2014 using a semi-automatic core biopsy needle. The biopsy procedure was considered successful if synovial tissue was found at histological examination.

Results: Seventy-four patients with undifferentiated arthritis underwent 76 synovial biopsies. The success rate in retrieving synovial tissue was 81.6% (62/76). One patient taking salicylic acid at 75mg at the time the biopsy presented with hemarthrosis 48 hours after the procedure, which resolved following simple arthrocentesis. A definite diagnosis was achieved in 16.1% of the patients where synovial tissue was sampled successfully.

Conclusion: Ultrasound guided synovial biopsies in clinical practice can be performed safely on patients with undifferentiated arthritis and with heterogeneous presentations. The rate of success in acquiring synovial tissue is high. The procedure usually retrieves quality tissue and leads to a definite diagnosis in a significant minority of patients.

Key indexing items: biopsy, synovial membrane, ultrasonography, diagnosis, early arthritis.

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Short running footline: Ultrasound guided synovial biopsy.

Introduction

Synovial tissue is the principal target and end organ involved in the pathogenesis of multiple articular disease processes (1,2). Synovial tissue analysis has been widely used for basic science, translational and clinical research. Moreover, synovial assessment allows for studying many aspects of disease processes including pathogenesis (3), the identification of relevant targets clinical features (4), diagnosis, prognosis (5) as well as in assisting in assessments of response to treatment (6–8).

Histological and immunohistological synovial assessment is also used as a diagnostic tool (9). Indeed, it is especially useful for identifying arthritis of an infectious aetiology, when synovial fluid or blood analysis (Gram, Ziehl) and cultures are negative or in cases where empiric antimicrobial therapy has been commenced before it has been possible to examine the synovial fluid (10). The bacterial broad range 16S ribosomal RNA can also be tracked down by polymerase chain reaction (PCR) on synovial tissue (11). The same methods allow identification of fungal, mycobacterial, spirochetes and Tropheryma Whipplei in the joint. False negative for monosodium urate crystals (MSU) and calcium pyrophosphate (PPC) occur frequently at microscopic examination of the synovial fluid (12), and synovial tissue assessment can be helpful with typical histological features. Finally, synovial benign tumours such as primary or secondary osteochondromatosis or villonodular synovitis can be diagnosed as well, showing specific macroscopic and histological pattern.

There are several techniques to obtain synovial tissue from the joints. Synovial biopsy was performed by Forestier in 1932 using a needle blindly introduced in the knee joint (13). Polley (14) and Parker (15) described new smaller diameter needles that have been widely used over the past years for knee synovial biopsies. Beaulé (16), Parlier and Cuau (17) then described a technique of synovial biopsy under direct visualisation under flurorscopy with a semi-automatic Tru-cut needle. This technique allows performing multi-sites biopsies such as hips, shoulders, elbows, ankles and wrists. Synovial biopsies were later performed under direct vision using 2 portals via an arthroscope (18). Although this technique is usually well tolerated (9), it remains invasive, expensive and not yet widely available. Moreover, it has been shown that microscopic measurements of synovial inflammation does not differ between biopsies taken blindly or under guided vision (19).

More recently, ultrasound guided synovial biopsies have been developed. Musculoskeletal ultrasound (US) is very commonly used nowadays, especially for guiding interventional procedures (20,21). This technique has the benefit of being low cost, rapidly and easily performed without the need for exposing the patient to ionising radiation, and is widely available (22). It is more practical than arthroscopy for biopsying small joints and allows guidance to the thickest synovial zones. Moreover, Kelly et al (23), reported that increasing synovial thickness on ultrasound correlated with increasing grades of synovitis on histological examination. However, few studies have reported on synovial biopsies performed in routine clinical practice (24,25). It is unknown if the success and the quality of the biopsy are the same as the one performed in a research setting. Finally, their clinical utility is still a matter of debate.

The aims of our study are (a) to describe the indications for US guided synovial biopsies in the clinical setting, (b) to determine the rate of success in acquiring synovial tissue using this approach and to report the complications, (c) to determine how frequently the synovial biopsy can lead to a clear diagnosis and (d) to assess the quality of the synovial tissue obtained using this technique.

Material and methods

Patients and histological diagnosis

We included all patients who underwent a US guided synovial biopsy between February 2007 and December 2014 in Nantes University hospital for arthritis without definite diagnosis based on the history, clinical examination or imaging. During this service evaluation study, we collected epidemiological (age, sex) and clinical data (clinical presentation, indication, biopsied joint, complications) using a standardized form. Final histological diagnosis was reported by 3 pathologists who had an expertise in assessing synovial tissue in a formal report based on a Hematoxylin and Eosin staining. Patients were followed to determine the clinical course of their symptoms.

US guided synovial biopsies

Synovial biopsies were performed under US guidance using a Philips HD11 XE ultrasound machine and a 7-13MHz transducer from Philips Healthcare. They were performed in an outpatient and inpatient setting depending on the patient's presentation. All patients underwent a thorough assessment of the joint to be biopsied. Vascular and nervous structures nearby were identified and synovial thickness was assessed.

All the biopsy procedures were performed by one operator (BLG) who had an expertise in US examination, under sterile technique (wearing gown, sterile gloves, mask and a surgical cap). Skin disinfection was processed with a 5 steps protocol using Iodine polyvidone or Hibiscrub if the patient had Iodine past history of allergy. The joint was draped and a sterile field thus generated. The transducer was covered with sterile gel and sterile sheath. Anaesthesia was performed injecting 5 to 10 ml of lidocaine 2% in the subcutaneous tissue and up to the joint capsule. If an effusion was present, synovial fluid was withdrawn and sent to the laboratory for cell count, crystal microscopy, bacteriological, mycobacteriological and/or fungal analysis depending on the patient clinical history and features. A semi-automatic guillotine biopsy "Tru-cut®" needle from TEMNOS has been used for all the biopsies. The calibre used was 16 Gauge (G) for small and intermediate joints or 14G for large joints such as hips, shoulders and knees. Coaxial needle was inserted under US guidance through the skin until reaching the articular cavity. The coaxial needle was positioned in intimate contact with the synovium. The semi-automatic guillotine biopsy "Tru-cut®" needle was then inserted through the cannula of the co-axial needle, still under US guidance. Once positioned within the zone of interest of the

synovial tissue, the Tru-cut® needle was triggered collecting a piece of synovial tissue according to the size of the joint. This Tru-cut® needle was repeatedly inserted through the co-axial needle and triggered to obtain the appropriate number of samples. Then, these two needles were removed and a classical bandage was applied. Patients were recommended to have 48 hours rest after the procedure.

Depending on the indication of the biopsy and the size of the joint, 3 to 8 biopsies were performed per procedure and sent for bacteriological, mycobacteriological and/or fungal examination in appropriate laboratories. At least 1 sample was fixed in formalin 4%, embedded in paraffin and sent to the pathology laboratory. When the clinical history was relevant extra samples were sent for universal bacterial polymerase chain reaction (PCR) (ARN 16S), universal fungal PCR (ARN 18S) and Trophyrema Whipplei or Lyme PCR.

Analysis of the quality and quantity of the synovial tissue retrieved during synovial biopsies

All the synovial biopsies were blindly read by one rheumatologist (AN). The number of samples per patient, the presence or absence of synovial tissue, the presence or absence of a synovial lining layer, the length and the width, the total area of the biopsy (mm2), the area of proper synovial tissue (mm2), was assessed in standardized manner with the NDP viewer® software. These findings were compared to the histological findings described on the pathologist reports which were the gold standard. In case of disagreement between rheumatologist and pathologist, an expert reader (DV) was responsible for final decision. We considered the biopsy successful when synovial tissue was seen at the histological examination. Good quality was defined as: sufficient size (>0,5 mm^2) (26), preserved tissue allowing assessment by pathologists and presence of lining layer.

Statistical analysis

Mean and median were used to describe quantitative data according to their Gaussian distribution. Number and percentage were used to report qualitative data. Fisher test has been used to compare percentage. Kappa coefficient calculation was used to assess the interobserver reliability for histological analysis. $p<0.05$ was considered as statistically significant. All statistics were made through GraphPad Prism 6.0® software.

Results

Patient characteristics

Seventy-four patients underwent 76 US guided synovial biopsy procedures. Demographic and clinical features of patients included in the study are shown in Table 1. Mean age was 57 years (Range 13-86 years) and there were 39 (52.7%) men. Most of the patients presented with an undifferentiated chronic monoarthritis (54.6%, n=41). The biopsied joints were reparsed as followed: 46 knees (60.5%), 6 ankles (8%), 6 wrists (8%), 5 shoulders (7%), 4 hips (5%), 2 elbows, 2 sternoclavicular joints, 2 metatarso-phalangeal joints and one pubic symphysis, one acromio-clavicular joint and one peroneal tenosynovitis. Patients were mainly referred to rule out the diagnosis of septic arthritis (82.4%, n=61).

US guided biopsy procedure was safe and successful.

Overall, 62 of the 76 biopsies (81.6%) yielded synovial tissue according to the pathologists' analysis. Within these 62 biopsies, the main histological finding was a non-specific inflammatory mononuclear cell infiltrate (lymphocyte, monocytes and plasma cells) (81%, n=50). A mild neutrophil infiltrate was seen in 24 (50%) of these biopsies. 8 (13%) biopsies showed specific histological lesions (Figure 1). A major neutrophil cell infiltrate consistent with a septic arthritis was found in 2 cases. 2 biopsies showed a synovial infiltration of positive Perls' siderophages (villo-nodular synovitis). 1 biopsy showed vascular and interstitial deposits of Sirius red staining protein consistent with amyloidosis AL. 1 biopsy contained tophi surrounded by lymphocytes and giant cells. 1 biopsy found dystrophic cartilage inside the synovial tissue; consistent with synovial osteochondromatosis. Finally, 1 biopsy showed an articular localisation of lymphoma. Four biopsies retrieved normal synovial tissue without any inflammatory cell infiltrate (Table 2).

The 14 failed biopsies occurred in both small and large joints. Percentages of failed biopsies per joint were as follows: Glenohumeral joints n=3/5 (60%), ankle n=3/6 (50%), hip n=2/4 (50%), wrist n=2/6 (38.3%), elbow n=1/2 (50%), sternoclavicular joint n=1/2 (50%), knees n=2/46 (4.3%). In case of failure, histological analysis showed mainly connective and adipose tissue in 10 cases, fibrin and leucocytes in 3 cases, tendon in 1 case. Tolerance per procedure was excellent. One patient taking acetyl salicylic acid at the time of the biopsy presented with

a haemarthrosis 48 hours after the procedure, which resolved following arthrocentesis within one week.

Overall, 10 (16.2%) definitive diagnoses were made based only on synovial tissue histological or PCR analysis.

Long term follow-up (mean 34.9 months (Range; <1 month-96 months) and final diagnosis were available for 66 of the 74 patients (Table 3). No patient has since been diagnosed with an infectious arthritis or villo-nodular synovitis or developed any complication of the biopsy procedure. In three of the cases where the diagnosis remained unclear despite the US guided biopsy and in two case of failed biopsy, patients underwent secondary procedures. One of them had an arthroscopic examination after the US guided biopsy and four of them had an open synovectomy. One of those synovectomy allowed a diagnosis of chondrocalcinosis on pathological examination.

Quality and quantity of the synovial tissue retrieved after US guided synovial biopsies.

Finally, the synovial tissue retrieved was assessed for quality and quantity. For this purpose, we analysed the histological characteristics per sample retrieved during the procedure (Figure 2). The median number of sample taken per patient was 1 (IQR 1-3) leading to a total of 125 samples available for analysis. Mean length and width of the biopsy samples were 6.34 millimetres (mm) (+/- 3.60) and 1.70 mm (+/- 0.77) respectively. The mean total area of the samples was 8.77mm^2.

Biopsies showed synovial tissue at the histological examination in 102 samples (80.1%). The average area of synovial tissue is these samples was 6.36 mm^2 corresponding to 72.5% of the total area of biopsied tissue. The other type of tissue present on these biopsies were connective tissue in 101 cases (80.8%), adipose tissue in 42 cases (33.6%), tendon in 14 cases (11.2%) and fibrin in 24 cases (19.2%). The 23 samples retrieving no synovial tissue were composed of fibrin in 15 cases (12%), conjunctive and adipose tissue in 17 cases (13.6%), tendinous tissue in 3 cases (3.15%), cartilage in 3 cases (3.15%) and muscle in one case (0.8%).

Synovial lining layer was found in 92.6% of the successful biopsies.

We finally compared our histological final finding regarding presence or absence of synovial tissue with the ones given by the pathologist and found 97.1% of agreement. Interobserver

reliability for presence/absence of synovial tissue was high with a kappa coefficient of 0.90 (95% CI = 0.763 to 1).

Discussion

Given the fact that synovial tissue analysis has been mostly used for research purposes, our study highlights the potential diagnostic role of synovial biopsy in routine clinical practice. In order to develop this technique in clinical practice, the patient needs to be offered a well-tolerated technique with an acceptable rate of success.

To date, two different techniques of US guided synovial biopsies have been described. Both have been shown to be safe, and well tolerated by the patients (22). The first method requires a single portal with a flexible or rigid biopsy forceps. The portal is directly introduced inside of the joint to perform biopsies (27). The second technique as outlined above, requires an empty co-axial needle that is inserted inside of the joint and a semi-automatic guillotine-type needle that is inserted through the co-axial. The procedure is not painful after the local anaesthesia and once the co-axial needle is settled and this technique allows retrieving several biopsies during the same procedure without moving the co-axial needle. To our knowledge, five other studies, reporting their experience of US guided synovial biopsies, have been published to date. Two reported their experience using the first technique (27,28), one of them a technique using semi-automatic guillotine-type needle without co-axial needle (23) and two of them using the second technique outlined above (24,25).

The success rates in retrieving synovial tissue described by other authors vary from 89% to 100% (23,25,27–29). Although, the rate of success in our cohort was slightly lower, for which there are several potential reasons. Our patients comprised a heterogeneous group regarding clinical features and the joints that have been biopsied among those studies and there were also minor differences in techniques in 2 of the studies referenced above. Moreover, no biopsies have been done prior 2007 in our centre and 43% of the failures occurred within the first 18 months (6 on 14), especially in more challenging joints such as ankles, wrists, hips or shoulders. This might correspond to the operator learning curve. However, our success rate remains equivalent to the highest rates described for synovial biopsies with blind needle (48 to 85%) (30).

In our study, patients were referred mostly by their GPs or their rheumatologist with no clear diagnosis despite multiple punctures with synovial fluid analysis and imaging consisting in computed tomography scanner (CT-scan) or magnetic resonance imaging (MRI). Given the fact that low-grade infection often evolve in chronic arthritis with joint destruction, it is very important to pursue atypical germs such as tuberculosis, fungi, Tropheryma Whipplei, Borrelia Burgdorferi. Moreover, some of the more common bacteria can be responsible of low-grade infection in some rheumatic patients because of immunosuppression. In all these situations, the biopsy allows a quick bacteriological examination with Gram staining, then later culture and PCR analysis for atypical organism. Indeed, 2 patients were diagnosed with Lyme and articular Whipple disease by PCR analysis. Interestingly, the Whipple PCR that was performed on the synovial fluid collected during procedure was negative. There is one previously reported similar cases where synovial fluid PCR failed to demonstrate the presence of Tropheryma Whipplei but the synovial tissue PCR was positive (31).

Bacterial culture in both synovial fluid and synovial membrane is a key examination for septic arthritis diagnosis. However, using those methods, infectious agents was isolated in only 41,2% of the patients (38.7 % of synovial fluid and 23.5 % of synovial membrane positive cultures) (32).Therefore, histological synovial cell infiltrate analysis is also relevant for septic arthritis assessment. A neutrophilic cellular infiltrate, has been showed to be highly associated with septic arthritis (33). Their presence inside of the synovial tissue is considered as a sufficient evidence for the diagnosis of septic arthritis. Regarding the data we present, the diagnosis of septic arthritis was established following the histological examination of 2 patients. Interestingly, after empiric antimicrobial therapy was commenced in these 2 patients, no relapse occurred within at least 6 years follow-up for both. This analysis can also be useful in fibrocartilagenous joints (acromio-clavicular, pubic symphysis) where fluid is rarely found even in case of inflammation. Furthermore, we can conclude from our data, that no patient of our cohort has been further diagnosed with infectious arthritis. This technique can therefore be considered as reliable to rule out septic arthritis assessment, permitting thus for local treatments such as steroids injections.

More rarely, synovial biopsy can be performed for synovial tumour assessment, especially villo nodular synovitis or osteochondromatosis. The 2 patients in our cohort diagnosed with

villo-nodular synovitis underwent surgical synovectomy. The histological examination of the tissue confirmed those findings.

For the biopsy to be useful in clinical practice, the quality of the biopsies retrieved has to be good. Quality of a synovial biopsy has been defined for research recently (23). But no definition has been given for the clinical setting yet. In our study, we defined good quality as: sufficient size defined by synovial tissue area > 0,5mm^2, preserved tissue allowing assessment by pathologists and presence of lining layer. In our cohort, the quality was good enough to allow a histological examination in all biopsies retrieving synovial tissue. Lining layer was found in 92.2% of the cases. In some instances, the lining layer could be identified but was not connected to the main biopsy, which may have occurred during tissue processing or may represent separation due to fibrin deposition in case of ulcerative synovitis.

No study has thus far demonstrated a predictive clinical value for histological findings in identifying those with early arthritis or those that will go on to have an aggressive disease course (6,9,10) . Indeed, multiple studies tried to determine histological cell infiltrates patterns matching with different rheumatologic conditions. There is undeniable differences between RA and Psoriatic arthritis (34,35), RA and Ankylosing Spondylitis (AS) (36) and RA and osteoarthritis (OA) (37,38). OA synovial membrane is known to show less inflammatory infiltrate and less vascularity than their inflammatory counterparts (RA, PsA, AS). RA synovium has been described to show a higher number of B cells and more rarely ectopic follicles, helping in the diagnosis. The high grade synovitis features are more consistent with RA (39). However, despite those differences, no algorithm is able to predict the evolution in early arthritis (33).

Given this, the histopathologist was rarely able to determine the type of inflammatory arthritis. However, by ruling out or confirming infectious arthritis or synovial tumour, it is clear enough that US guided synovial biopsy is helpful on patients with remaining unknown diagnosis despite synovial fluid analysis, X-ray, CT scan and/or MRI examinations. In our setting, synovial biopsies allowed to treat some patients by achieving a definite diagnosis, or to give systemic immunosuppressive or local therapies such as intraarticular steroid injections. We acknowledge that our work has limitations. One limitation is the monocentric design of our study. The biopsies were performed by a trained investigator and the pathologists in our centre have an expertise in biopsy assessment. This could be a limit for the

generalization of those results. Although all patients had 3 to 8 biopsies taken, 55% of them had a single fragment sent to pathology department. This might be another limitation.

Finally, one of the main concerns about any procedure is its tolerance. In our cohort, one patient treated with salicylic acid presented with knee haemarthrosis 48 hours after the procedure. Overall, in our cohort, the adverse effects rate was 1.35% (IC 95 -1.3-4) (1/74) and no severe adverse event (life-threatening, leading to patient admission in hospital or with a risk of sequelae) occurred. The arthroscopic biopsies have the advantage to be retrieved under direct vision and therefore allow a histological analysis of the inflamed areas within the joint. However, this procedure is more invasive and has multiple adverse effects (joint infection; wound infection; haemarthrosis; deep venous thrombosis; neurological damage, thrombophlebitis) (40).

Conclusion

Our study highlights the potential diagnostic role of synovial biopsy. To our knowledge, it is the first study describing indications, tolerability, rate of success, diagnosis role and quality of ultrasound guided synovial biopsy in the clinical setting. Ultrasound guided synovial biopsy is performed in clinical practice in a heterogeneous population with variant clinical features. The success rate of the procedure remains high with only rare and minor complications. 13.3% achieved a definitive diagnosis leading to a specific treatment. In other patients, we could rule out the diagnosis of septic arthritis. Therefore, this procedure should not only be used for research purposes, but may also be used routinely in undifferentiated arthritis.

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Legends for illustrations:

Figure 1 A, B, C, D, E. Synovial biopsies of 5 specific histological lesions. **A.**Fibrin deposits with neutrophils infiltrate (asterix). Septic arthritis. **B.** Villo nodular synovitis. Hematoxylin and Eosin staining.**C.**Villo nodular synovitis with Perl's staining showing siderophages (arrow head). **D.** Cell infiltrate within synovial tissue in an articular lymphoma. **E.** Amyloids (cross) revealed by Sirius red staining. AL amyloidosis. **F.** Micro tophi surrounded by giant cells and lymphocytes (black arrow) leading to gout diagnosis.

Figure 2. Example of the sample histological analysis. Black line is the global area measurement; red line is the width measurement and white line in the length measurement.

Table 1. Demographic and clinical features of the patients.

		No.	(%)
Gender			
	Female	35	47,3
	Male	39	52,7
Mean age, years (Range)		57	(13-86)
Indications			
	Undifferentiated chronic monoarthritis	41	54,7
	Acute monoarthritis	18	24,0
	Chronic undifferentiated oligoarthritis	7	9,3
	Chronic polyarthritis	6	8,0
	Chronic bursitis	1	1,3
	Chronic tenosynovitis	1	1,3
	Acute polyarthritis	1	1,3

No: number. %: percentage

Table 2. Histopathological analysis.

Histopathological findings	Number of biopsy
Normal synovium	4
Inflamed synovium	50
Cell infiltrate	
Lymphocytes	50
Plasma cells	22
Neutrophils	24
Specific lesions	8
Villonodular synovitis (shoulder and knee)	2
Infectious arthritis [1]	2
Amyloid arthritis (knee)	1
Articular localization of mantle B cell lymphoma (ankle)	1
Gout (first MTP)	1
Osteochondromatosis (knee)	1
Failure	14

[1] 2 infectious arthritis (hip, ankle) treated on typical histological aspect with no relapse after 6 weeks of empiric antibiotics; MTP: metatarsophalangeal.

Table 3. Overall final diagnosis after follow up

Final diagnosis	No.	(%)
Rheumatoid arthritis	7	9,5
Ankylosing spondylitis	2	2,7
Psoriatic arthritis	5	6,8
Degenerative arthropathy	12	16,2
Crystal arthropathy	4	5,4
Chondrocalcinosis	2	2,7
Gout	3	4,1
Villo-nodular synovitis	2	2,7
Osteochondromatosis	1	1,4
Giant cell arthritis	1	1,4
Behcet's disease	1	1,4
Latent infectious arthritis	4	5,4
Others	2	2,7
Undifferentiated arthritis	21	28,4
Lost to follow-up	7	9,5
Total	74	100

No: number; %: percentage

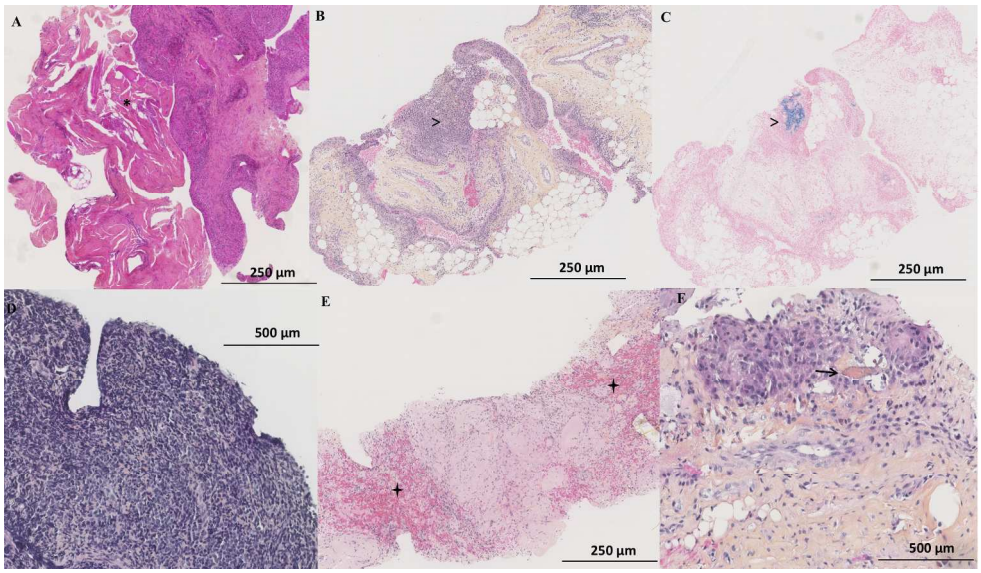

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Figure 1

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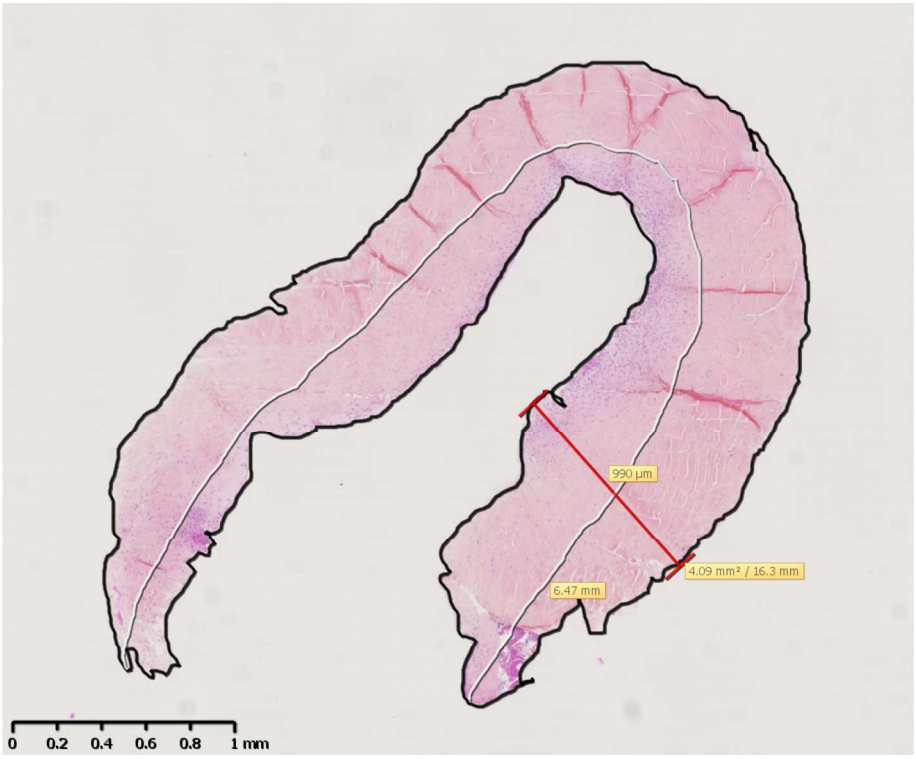

Figure 2. Example of the sample histological analysis. Black line is the global area measurement; red line is the width measurement and white line in the length measurement.
Figure 2
243x182mm (300 x 300 DPI)