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Predictive accuracy of progesterone receptor B in young women with atypical endometrial hyperplasia and early endometrial cancer treated with hysteroscopic resection plus LNG-IUD insertion

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ABSTRACT

Study Objective: Immunohistochemical expression of the isoform B of progesterone receptor (PRB) has shown promising results in predicting the response of atypical endometrial hyperplasia (AEH) and early endometrial cancer (EEC) to conservative treatment. We aimed to calculate the accuracy of PRB as a predictive marker of conservative treatment outcome in AEH or EEC.

Design: Retrospective cohort study.

Setting: University of Naples Federico II, Naples, Italy.

Patients: Thirty-six consecutive premenopausal women <45 years of age with AEH (n=29) or EEC (n=7) conservatively treated from January 2007 to June 2018 were retrospectively assessed.

Interventions: All patients had been treated with hysteroscopic resection + LNG-IUD insertion and followed for at least 1 year. Immunohistochemical expression of PRB was separately assessed in glands and stroma of the lesion and dichotomized as “weak” or “normal”.

Measurement and Main Results: Treatment outcomes considered were: 1) treatment failure (i.e. a combined outcome including no regression or recurrence); 2) no regression; 3) recurrence. Predictive accuracy of PRB immunohistochemistry was assessed by calculating sensitivity (SE), specificity (SP) and area under the curve (AUC) on receiver operating characteristic curve.

A weak glandular PRB expression showed:

- SE=70%, SP=77%, AUC=0.74 for treatment failure;
- SE=66.7%, SP=70%, AUC=0.68 for no regression;
- SE=75%, SP=68.8%, AUC=0.72 for recurrence.

A weak stromal PRB expression showed:

- SE=100%, SP=53.8%, AUC=0.77 for treatment failure;

- SE=100%, SP=46.7%, AUC=0.73 for no regression;
- SE=100%, SP=43.8%, AUC=0.72 for recurrence.

Conclusions: A weak stromal PRB expression is a highly sensitive predictive marker of both no response and recurrence of AEH and EEC conservatively treated.

KEYWORDS

hysteroscopy; fertility-sparing; endometrioid adenocarcinoma; progestogen; progesterone; progestin; LNG-IUS; levonorgestrel.

INTRODUCTION

Background

Endometrial hyperplasia is a lesion characterized by irregularly proliferating endometrioid glands. In the absence of cytologic atypia, endometrial hyperplasia is regarded as a benign proliferation due an unbalanced action of estrogens. On the other hand, atypical endometrial hyperplasia (AEH) is considered a precancerous lesion and the precursor of endometrial endometrioid carcinoma [1,2]. On this account, the treatment of choice for AEH is total hysterectomy with bilateral salpingo-oophorectomy. However, a conservative approach can be adopted in young women who wish to preserve their fertility [3]. Conservative treatment can still be used in the case of well-differentiated early endometrioid carcinoma limited to the endometrium (EEC) [4]. Several conservative treatments have been used for AEH and EEC [5]. Among progestins, levonorgestrel-releasing intrauterine device (LNG-IUD) has appeared as the most effective one [6,7]. Moreover, the combination of hysteroscopic resection with progestins has been shown to be more effective than progestins alone [8,9].

Knowledge gap

In spite of the effectiveness of progestins, a variable percentage of patients show either no response to conservative treatment or recurrence after an initial response, implying a risk of progression to myoinvasive disease. To date, there are no reliable clinical or pathological markers for predicting the response of AEH and EEC to conservative treatment. Immunohistochemical expression of progesterone receptor has been proposed as the most obvious candidate predictive marker, as it mediates the action of progestins. Nonetheless, it has been shown that progesterone receptor expression is not accurate enough to be clinically useful. More promising results derived from the study of the isoform B of progesterone receptor (PRB). However, the accuracy of PRB as predictive marker of response to conservative treatment has never been calculated. Furthermore, most studies in this field are affected by many possible sources of bias in the study population,

such as non-atypical hyperplasia lumped together with AEH and EEC, different types of conservative treatment, premenopausal lumped together with postmenopausal patients, insufficient follow-up time [10].

Objective

The aim of this study was to assess the predictive value of PRB on a selected cohort of patients with conservatively treated AEH or EEC who are homogeneous regarding age (<45 years), premenopausal status, pathological diagnosis (AEH and EEC), type of treatment (hysteroscopic resection plus LNG-IUD insertion) and follow-up time (at least 1 year). In particular, we aimed to calculate the accuracy of PRB as a predictive marker of response to conservative treatment of AEH or EEC.

MATERIALS AND METHODS

Study protocol

The protocol defining study methods was *a priori* defined. The study was designed as a single-center observational study assessing a retrospective cohort, and was reported following The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines and checklist [11].

We reviewed medical records and pathology reports of all consecutive premenopausal patients up to 45 years of age diagnosed with AEH or ECC who underwent conservative treatment with hysteroscopic resection followed by LNG-IUD insertion at the Department of Neuroscience, Reproductive Sciences and Dentistry and at the Department of Public Health of University Federico II, Naples, Italy, from January 2007 to June 2019. Regarding EEC patients, we only included women with endometrial carcinoma with endometrioid histotype, International Federation of Gynecology and Obstetrics (FIGO) tumor grade 1, absence of extrauterine metastases, and absence of lymphovascular space, myometrial or cervical invasion [4].

Histological slides of all patients satisfying the selection criteria were reviewed in order to confirm the initial diagnosis of AEH and EEC. Paraffin blocks of pre-treatment endometrial biopsy were retrieved in order to obtain *ad hoc* sections for immunohistochemical assessment of PRB expression. Lastly, we assessed the accuracy of PRB immunohistochemistry in the prediction of the response to conservative treatment.

Based on *a priori* defined selection criteria, we excluded: patients treated with hysterectomy, patients with a follow-up period < 1 year; patients not providing a written informed consent for the use of own biospecimens for research purposes; patients with no available tissue for *ad hoc* immunohistochemistry.

Pre-operative management, treatment and follow-up were performed as previously described [12] (Figures 1 and 2).

Study outcomes

The primary outcome was the accuracy of PRB expression in predicting the failure of conservative treatment in AEH or EEC.

Secondary outcomes were:

- the accuracy of a weak immunohistochemical expression of PRB in the prediction of no regression of AEH or EEC after the conservative treatment;
- the accuracy of a weak immunohistochemical expression of PRB in the prediction of recurrence of AEH or EEC after an initial regression to conservative treatment.

Regression was defined as the absence of AEH or EEC at histological examination of two consecutive follow-up hysteroscopic biopsies; no regression of the disease was defined as the persistence of AEH or EEC 12 months after the beginning of the treatment. Recurrence of the disease was defined as the presence of AEH or EEC after a previous regression. The failure of the conservative treatment was defined as a composite adverse outcome including 1) no regression, or 2) recurrence of the disease.

The immunohistochemical expression of PRB was assessed separately in endometrial glands and stroma of the lesion according a total score obtained from the product of staining intensity by staining distribution. In particular, the staining intensity was categorized according a score from 0 to 6, where 0 indicated absence of nuclear staining, 1 minimal nuclear staining, 2 slight nuclear staining, 4 moderate nuclear staining, 6 strong nuclear staining, while 3 and 5 indicated intermediate staining intensity between 2 and 4, and 4 and 6, respectively. On the other hand, the staining distribution was assessed according to a score between 0 and 100, where 0 indicated no cell were stained, and 100 all cells were stained in the lesion. Thus, the total score was between 0 and 600. In endometrial glands of the lesion, PBR expression was considered as weak if the total score was <400, while in the stroma of the lesion, it was considered weak if the total score was <200. Total scores higher than these thresholds indicated normal PRB expression.

Histological and immunohistochemical methods

Histological and immunohistochemical procedures were performed as previously described [13]. An anti-PRB Rabbit Monoclonal Antibody (dilution 1/600; Cell Signalling, Clone C1A2) was used. Histological specimen of proliferative endometrium was used as a positive control. Histological examinations were performed by two blinded authors (LI and AT). Scoring of PRB expression was performed by eye at a light microscope, by counting the cells in all the area of the lesion (AEH or EEC); disagreements were resolved by discussion at a two-headed microscope. The thresholds of PRB expression were defined based on areas of normal proliferative endometrium and non-atypical endometrial hyperplasia, which showed a total score between 400 and 600 for glandular expression, and between 200 and 500 for stromal expression in all cases. Thus, we considered total scores below these thresholds as abnormal.

Statistical analysis

Agreement among the two blinded pathologists was assessed by using Cohen's k. Agreement was a priori categorized as null for $k \leq 0$, low for $0 < k \leq 0.4$, moderate for $0.4 < k \leq 0.6$, high for $0.6 < k \leq 0.8$, and excellent for $0.8 < k \leq 1$.

The association of clinico-pathological factors with treatment failure was assessed by using logistic regression, with a significant p-value<0.05.

PRB predictive accuracy was assessed by calculating sensitivity, specificity and area under the curve (AUC) on receiver operating characteristic (ROC) curves. PRB predictive accuracy was *a priori* categorized as null for $AUC \leq 0.5$, low for $0.5 < AUC \leq 0.75$, moderate for $0.75 < AUC \leq 0.9$, high for $0.9 < AUC < 0.97$, very high for $AUC \geq 0.97$, as previously reported [14].

Statistical analyses were performed using SPSS 19.0 package (SPSS Inc., Chicago, IL, USA).

Ethical statement

Before the beginning of the study, it received approval by the Institutional Review Board of the University of Naples Federico II (no. 138/19). All included patients signed an informed written consent for the use of their biospecimens for research purposes, and all data were anonymized in order to prevent the identification of the subjects.

RESULTS

Patients' characteristics

A total of 36 patients meeting the selection criteria were included in the study: 29 (80.6%) were diagnosed with AEH and 7 (19.4%) with EEC (Table 1). Characteristics of patients with EEC are shown in Table 2.

The mean ± standard deviation (SD) of age was 35.5 ± 5.5 years. The mean ± SD of Body Mass Index was 28.6 ± 7.9 Kg/m^2. 41.6% of patients had previous pregnancies, 5.4% a familiar history of endometrial carcinoma, and 16.6% a familiar history of other cancers. Among other diseases, hypertension was reported in 13.9% of patients, diabetes mellitus in 2.8%, thyroid diseases in 22.3%, endometriosis in 5.6%, and infertility in 5.6%. Among symptoms, 33.3% of patients showed heavy menstrual bleeding with or without prolonged menstrual bleeding, 5.6% frequent irregular non-menstrual vaginal bleeding, 5.6% frequent menstrual bleeding (Table 1).

About treatment outcomes, 27.8% of patients showed the failure of the conservative treatment of AEH or EEC. In particular, 16.7% of women showed no regression, and 13.3% of responding patients showed recurrence of the disease. The mean ± SD of follow-up length was 69.9 ± 36.3 months (Table 1).

PRB as predictive marker

On immunohistochemistry, 13 patients (36.1%) showed a weak PBR expression in the glands of the lesion, while 22 patients (61.1%) showed a weak PBR expression in the stroma (Figure 3). The agreement among the two pathologists was high for glandular PRB (agreement=86.1%; k=0.72) and excellent for stromal PRB (agreement=91.7%; k=0.82) (Table 3). Treatment failure was significantly associated with glandular PRB expression (p=0.009), stromal PRB expression (p=0.003) and EEC diagnosis (p=0.004).

In predicting the failure of the conservative treatment, a weak PRB immunohistochemical expression showed sensitivity=70%, specificity=77%, and AUC=0.74 (95%CI: 0.54-0.93) when assessed in the glands of the lesion (Figure 4), and sensitivity=100%, specificity=53.8%, and AUC=0.77 (95%CI: 0.62-0.92) when assessed in the stroma of the lesion (Figure 5).

In predicting no regression of the disease to conservative treatment, a weak PRB immunohistochemical expression showed sensitivity=66.7%, specificity=70%, and AUC=0.68 (95%CI: 0.44-0.92) when assessed in the glands of the lesion (Figure 6), and sensitivity=100%, specificity=46.7%, and AUC=0.73 (95%CI: 0.56-0.91) when assessed in the stroma of the lesion (Figure 7).

In predicting recurrence of the disease after a regression to conservative treatment, a weak PRB immunohistochemical expression showed sensitivity=75%, specificity=68.8%, and AUC=0.72 (95%CI: 0.45-0.99) when assessed in the glands of the lesion (Figure 8), and sensitivity=100%, specificity=43.8%, and AUC=0.72 (95%CI: 0.52-0.92) when assessed in the stroma of the lesion (Figure 9).

DISCUSSION

Main findings and interpretation

This study showed that the accuracy of PRB as stand-alone predictive marker of response to conservative treatment of AEH and EEC was low-to-moderate; however, a weak stromal expression of PRB showed 100% sensitivity in predicting both no response and recurrence.

Progesterone receptor, also known as nuclear receptor subfamily 3, group C, member 3 (NR3C3), is a protein encoded by a single PGR gene [15,16]. Progesterone receptor has two isoforms, A and B, that differ in their molecular weight [17]. Given its crucial role in mediating the effects of progestogens, immunohistochemical expression of progesterone receptor has been assessed as a possible predictive marker of response in conservatively treated AEH and EEC. However, the results in this field appear conflicting [10,14]. In our previous meta-analysis, we found that progesterone receptor expression was associated with the response of AEH and EEC to LNG-IUD insertion [14]. However, we found that the predictive accuracy was insufficient to be clinically useful as stand-alone marker [14,18]. We identified several limitations in the published studies that might have affected the results, and we provided suggestions for further studies in order to overcome them. In fact, the previously published studies included both non-atypical hyperplasia (which is a benign proliferation) and AEH and EEC (which are monoclonal lesions driven by specific mutations), adopted different treatments (oral progestins, subcutaneous progestins, LNG-IUD, hysteroscopic resection) which may have different effectiveness, reported a follow-up time not always adequate, and enrolled both premenopausal and postmenopausal women (although this did not necessarily affect the results) [10,14,18]. Furthermore, results from previous studies suggested that PRB may be more reliable than progesterone receptor as a predictive marker in this field [10].

On this account, we assessed the predictive value of PRB on only premenopausal patients with AEH and EEC, treated with hysteroscopic resection plus LNG-IUD and followed for at least 1 year.

We found that PRB accuracy in predicting the response to conservative treatment of AEH and EEC was low-to-moderate accuracy in all analyses. In particular, moderate accuracy was only found for a weak PRB expression as a predictor of treatment failure (AUC=0.77). These results support that PRB assessment cannot be used as a stand-alone predictive marker in conservatively treated AEH and EEC. However, our analysis showed that a weak stromal PRB expression had a 100% sensitivity in predicting both no response and recurrence. This might imply that, in the case of normal PRB expression in the stroma, patients with AEH or EEC will respond to conservative treatment without risk of relapse. Therefore, immunohistochemical assessment of PRB might identify patient at low-risk of treatment failure, who might be encouraged in attempting a conservative approach. Moreover, in the future, the management of these patients might be tailored requiring shorter length of treatment and follow-up.

In contrast to our findings, a previous study assessing patients with conservatively treated endometrial hyperplasia found that the risk of recurrence was higher in the case of strong stromal PRB expression; however, in that study most patients had non-atypical hyperplasia and were treated with oral progestins [19]. In particular, PRB may have a completely different role in non-atypical hyperplasia, which is a benign lesion. Such condition is a functional proliferation which is promoted by hormonal unbalance rather than underlying genetic mutations [1,2]. Therefore, its recurrence might be determined by hormonal factors unrelated to the lesion.

It should be remarked that other immunohistochemical markers showed an association with the response of AEH and EEC to progestins [10]. Combining PRB with other relevant predicting markers might allow defining a predictive method to estimate the probability of response and the risk of recurrence in patients with conservatively treated AEH and EEC. In the era of the precision medicine, this method might be useful to tailor the patient management, providing the right treatment (e.g. oral progestins, LNG-IUD insertion, hysteroscopic resection, metformin, bariatric surgery, or their combination), the right length of therapy, and the right type and length of follow-up in the right patient [20,21]. We plan to test further promising markers on larger cohorts, in order to achieve a more tailored management of patients with AEH and EEC.

Strengths and limitations

The main strength of our study lies in the homogeneity of the study population. First, we only included premenopausal patients, who are the main candidates to conservative treatment. Second, we did not include non-atypical hyperplasia, since it is considered a benign proliferation which lacks the typical mutations of AEH and EEC [1,2,22,23]. Third, all patients were treated with hysteroscopic resection plus LNG-IUD insertion, which may currently be the most effective conservative treatment for AEH and EEC [12]. Finally, all patients were followed at least for 1 year, which is twice the minimum time required to reliably assess the response [3]. Another strength may be the method to quantify PRB expression, which takes into account both the intensity of staining and the percentage of stained cells. Moreover, the evaluation of immunohistochemistry was performed by two blinded pathologists, with high agreement for glandular expression and excellent agreement for stromal expression.

Limitations of our study lie in the retrospective design and in the overall small sample size. In fact, the low number of patients included lead to a broad 95% CI for our results, requiring a confirmation on larger cohorts. However, given the intention to assess a homogenous study population and the rarity of the condition (only 20-25% of endometrial cancer and AEH occur in premenopausal women, and only 3-5% of women with endometrial cancer are in reproductive age [24]), it appears difficult to perform a prospective study on a greater study population.

CONCLUSION

In conservatively treated AEH and EEC, PRB showed an accuracy insufficient to be used as a stand-alone predictive marker of response in the clinical practice. However, a weak stromal expression of PRB showed 100% sensitivity in predicting both no response and recurrence. This may help to tailor patient management, by identifying patients at low-risk of treatment failure in the case of a normal stromal PRB expression.

In the future, combining PRB with other markers may allow developing a more accurate predictive methods for directing the management of patients conservatively treated for AEH and EEC. Further studies are encouraged in this regard.

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LEGEND FOR TABLES

Table 1. Characteristics of the included patients and outcomes of conservative treatment of atypical endometrial hyperplasia and early endometrial carcinoma.

Table 2. Characteristics of the 7 patients with early endometrial cancer.

EEC: early endometrial cancer; AEH: atypical endometrial hyperplasia; MMR: mismatch repair.

Table 3. Results of the interpretation of glandular and stromal PRB expression performed by two blinded pathologists.

Figure 1. Conservative treatment of atypical endometrial hyperplasia: before treatment (**a**), superficial endometrial resection preserving the basal layer of the endometrium (recognized by distinct signs of punctuation indicating glandular tissue) (**b**, **c, d, e**); final appearance of uterine cavity after treatment **(f)**; 3-months follow-up with LNG-IUD in situ (**g**) and endometrial biopsy (**h**).

Figure 2. Conservative treatment of early endometrial carcinoma according the three steps technique firstly described by Mazzon et al.: before treatment (**a**); removal of the exophytic tumor lesion (**b, c**); removal of the endometrium adjacent (4-5 mm outside) to the lesion (**d, e**); removal of the muscle layer beneath (3-4mm) the lesion (**f**); one of multiple random biopsies of endometrium (**g**); final appearance of uterine cavity after treatment (**h**).

Figure 3. Immunohistochemical expression of PRB in atypical endometrial hyperplasia specimens (red arrows indicate glands; green arrows indicate stroma; magnification 200X). a) Strong PRB expression in both glands and stroma. b) Weak PRB expression in both glands and stroma. c) PRB expression strong in glands and weak in stroma. d) PRB expression weak in glands and strong in stroma.

Figure 4. Area under the curve (AUC) on receiver operating characteristic (ROC) curve of PRB immunohistochemical expression in the glands of atypical endometrial hyperplasia or early endometrial carcinoma in predicting the failure of the conservative treatment.

Figure 5. Area under the curve (AUC) on receiver operating characteristic (ROC) curve of PRB immunohistochemical expression in the stroma of atypical endometrial hyperplasia or early endometrial carcinoma in predicting the failure of the conservative treatment.

Figure 6. Area under the curve (AUC) on receiver operating characteristic (ROC) curve of PRB immunohistochemical expression in the glands of atypical endometrial hyperplasia or early endometrial carcinoma in predicting no regression of the lesion to conservative treatment.

Figure 7. Area under the curve (AUC) on receiver operating characteristic (ROC) curve of PRB immunohistochemical expression in the stroma of atypical endometrial hyperplasia or early endometrial carcinoma in predicting no regression of the lesion to conservative treatment.

Figure 8. Area under the curve (AUC) on receiver operating characteristic (ROC) curve of PRB immunohistochemical expression in the glands of atypical endometrial hyperplasia or early endometrial carcinoma in predicting recurrence of the disease after an initial response to conservative treatment.

Figure 9. Area under the curve (AUC) on receiver operating characteristic (ROC) curve of PRB immunohistochemical expression in the stroma of atypical endometrial hyperplasia or early endometrial carcinoma in predicting recurrence of the disease after an initial response to conservative treatment.

PATIENTS CHARACTERISTICS	N (%)	MEAN ± SD
Age, years	-	35.5 ± 5.5
BMI, kg/m^2	-	26.6 ± 7.9
Diagnosis of early endometrial carcinoma	7 (19.4)	-
Diagnosis of atypical endometrial hyperplasia	29 (80.6)	-
Previous pregnancies	15 (41.6)	-
Spontaneous delivery	2 (5.6)	
Cesarean section	10 (27.8)	
Miscarriages	12 (33.4)	
Familiar history of endometrial carcinoma	2 (5.4)	-
Familiar history of other cancers	6 (16.6)	-
Other diseases		-
Blood hypertension	5 (13.9)	
Diabetes mellitus	1 (2.8)	
Thyroid diseases	8 (22.3)	
Endometriosis	2 (5.6)	
Infertility	2 (5.6)	
Symptoms		-
Heavy menstrual bleeding with or without prolonged menstrual bleeding	12 (33.3)	
Frequent irregular non-menstrual vaginal bleeding	2 (5.6)	
Frequent menstrual bleeding	2 (5.6)	
Treatment outcomes		-
Treatment failure	10 (27.8)	
No regression	6 (16.7)	
Recurrence	4 (13.3)	
Follow-up length, months	-	69.9 ± 36.3

CASE NO.	AGE	BIOPSY RESULT	PRB IN GLANDS	PRB IN STROMA	MMR PROTEINS IMMUNOHISTOCHEMISTRY	REGRESSION	RECURRENCE
1	34	G1 EEC	low	low	proficient	no	-
2	34	AEH+G1 EEC	high	low	proficient	no	-
3	43	G1 EEC	low	low	deficient	no	-
4	31	G1 EEC	low	low	proficient	no	-
5	29	AEH+G1 EEC	low	low	proficient	yes	no
6	34	AEH+G1 EEC	high	low	proficient	no	-
7	24	AEH+G1 EEC	high	high	proficient	yes	no

MARKER	FIRST REVIEWER'S JUDGEMENT	SECOND OBSERVER AGREED	SECOND OBSERVER DISAGREED	RESULTS
PRB in glands	low	13	3	Agreement=86.1%
	high	18	2	Cohen's k=0.72
PRB in stroma	low	22	2	Agreement=91.7%
	high	11	1	Cohen's k=0.82

Figure 1a

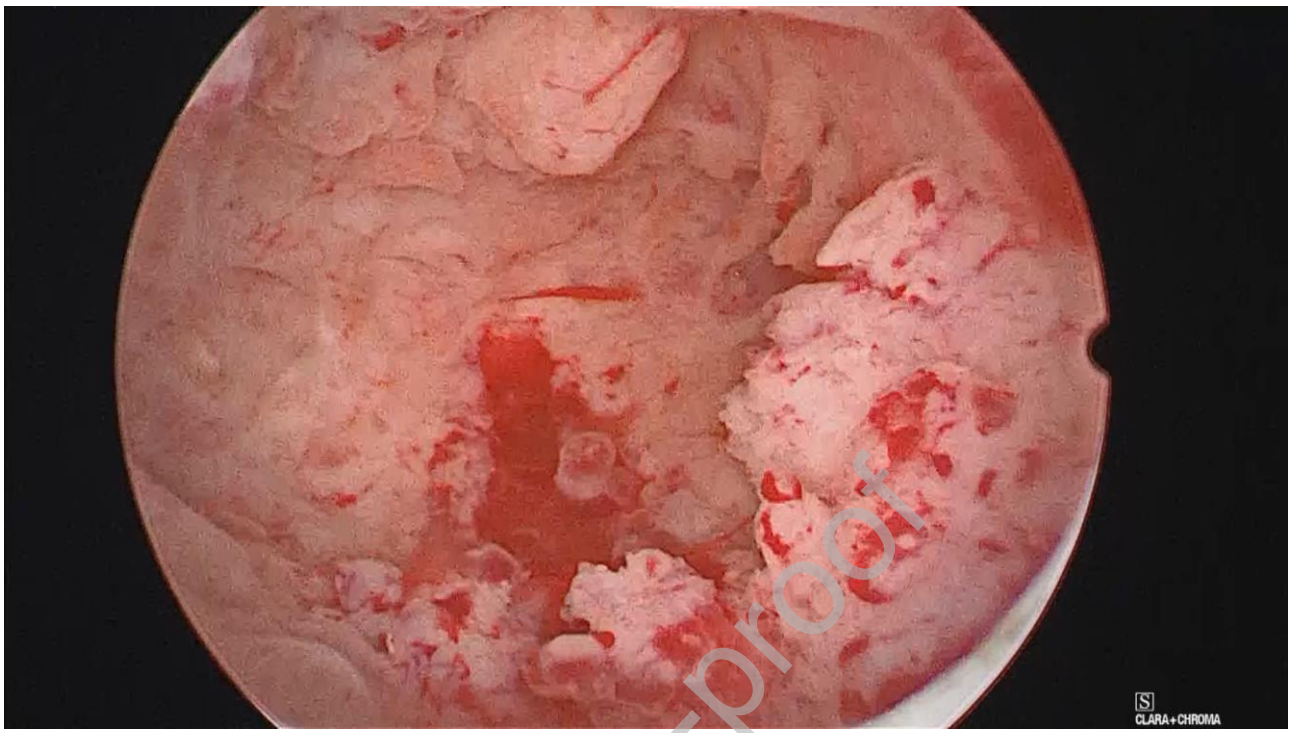

Figure 1b

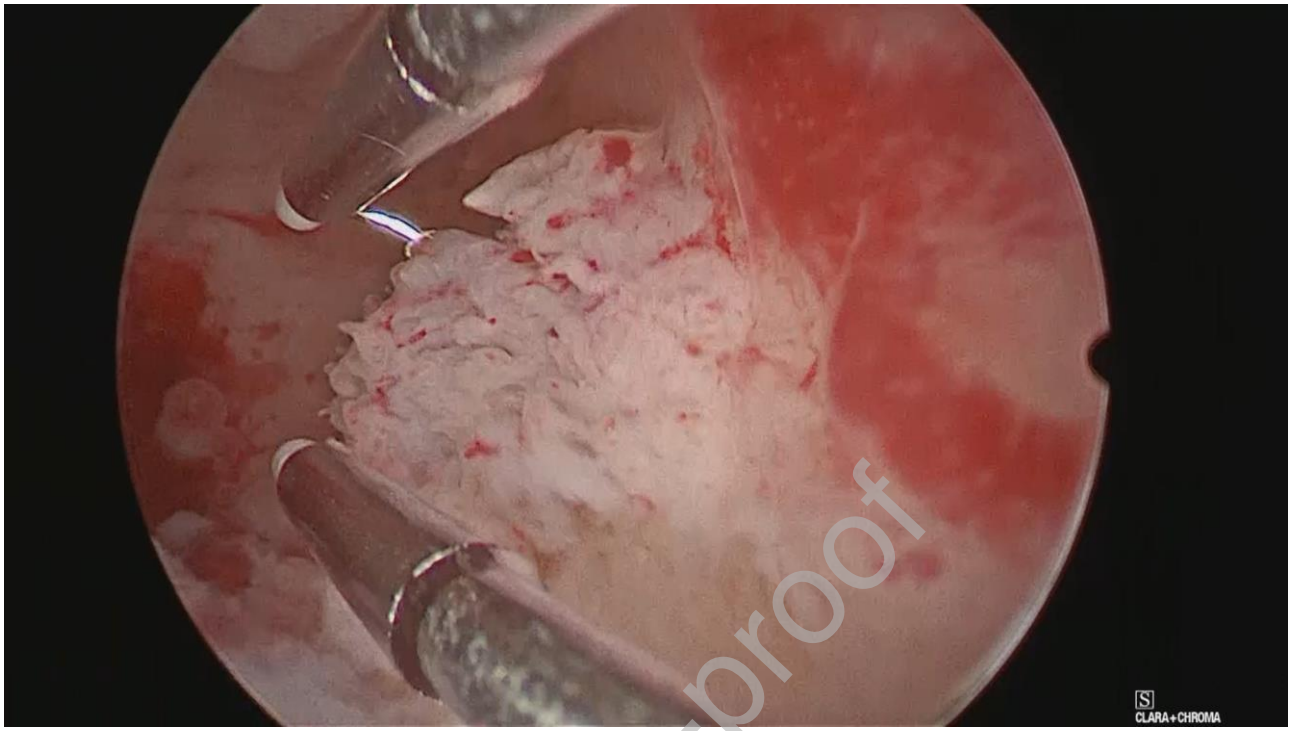

Figure 1c

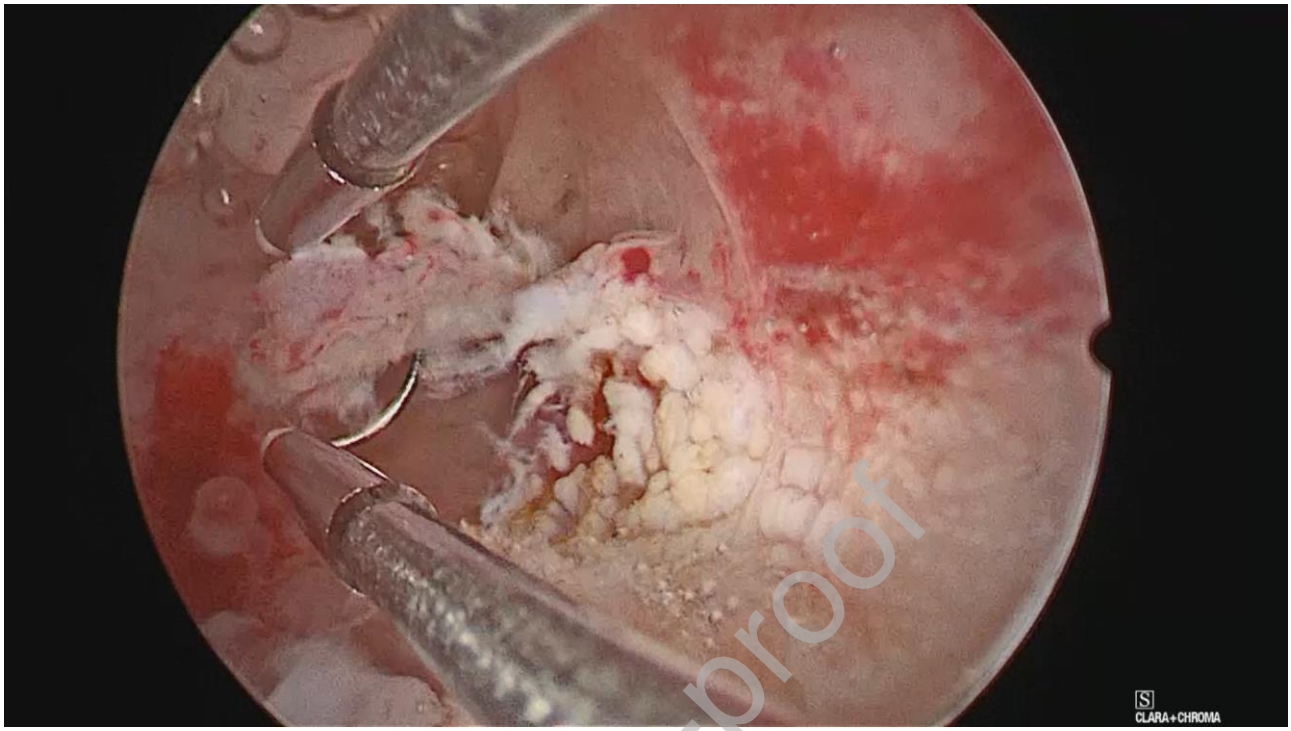

Figure 1d

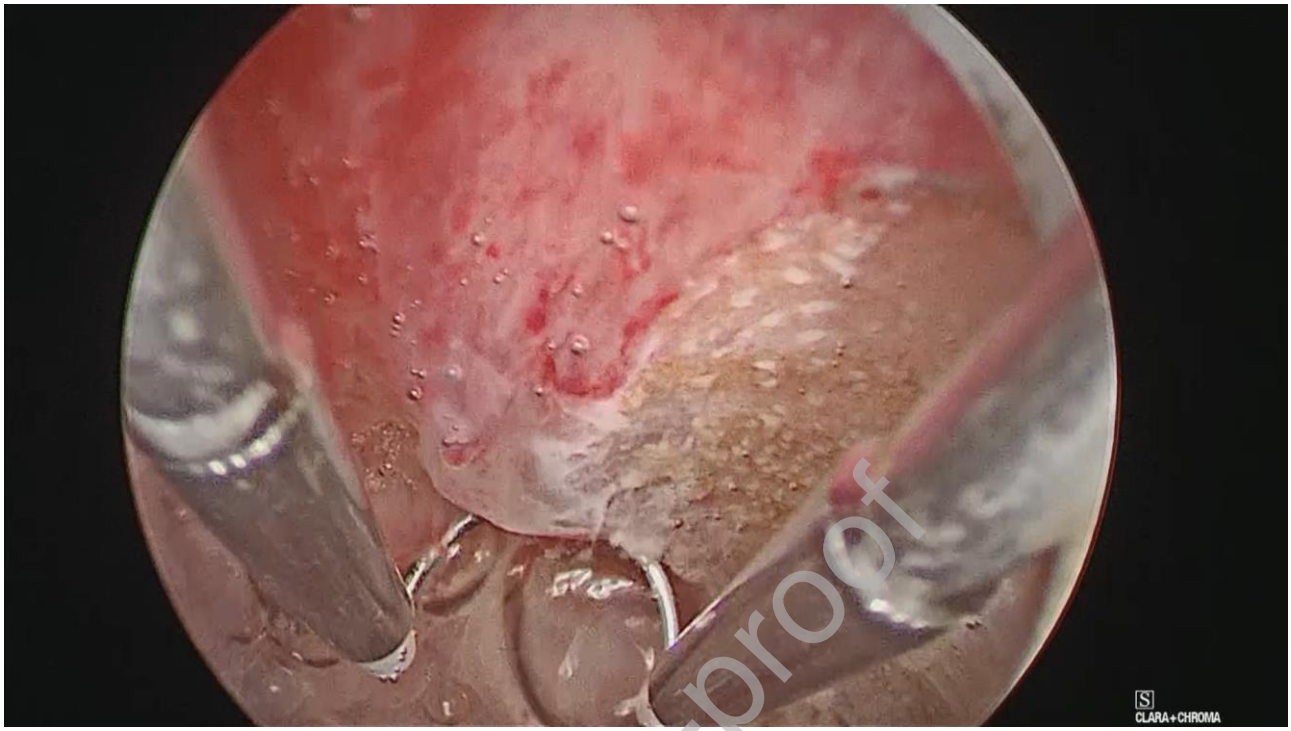

Figure 1e

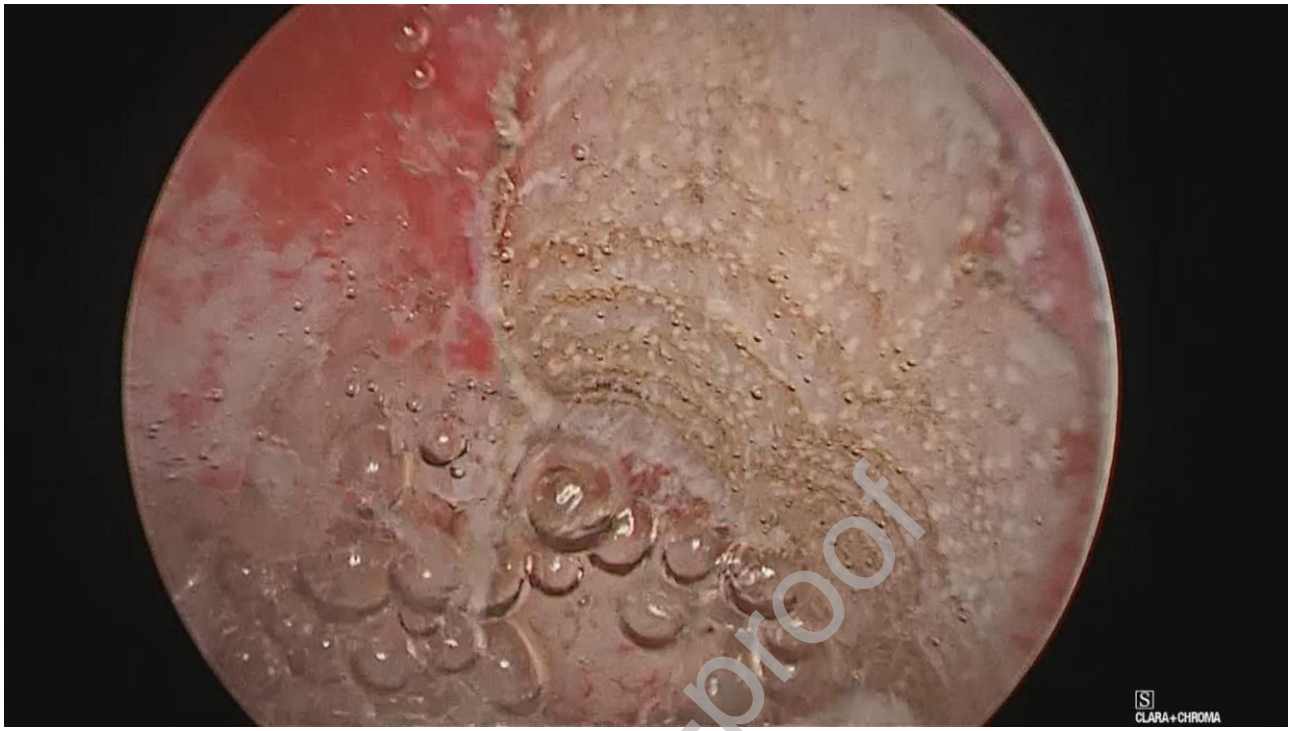

Figure 1f

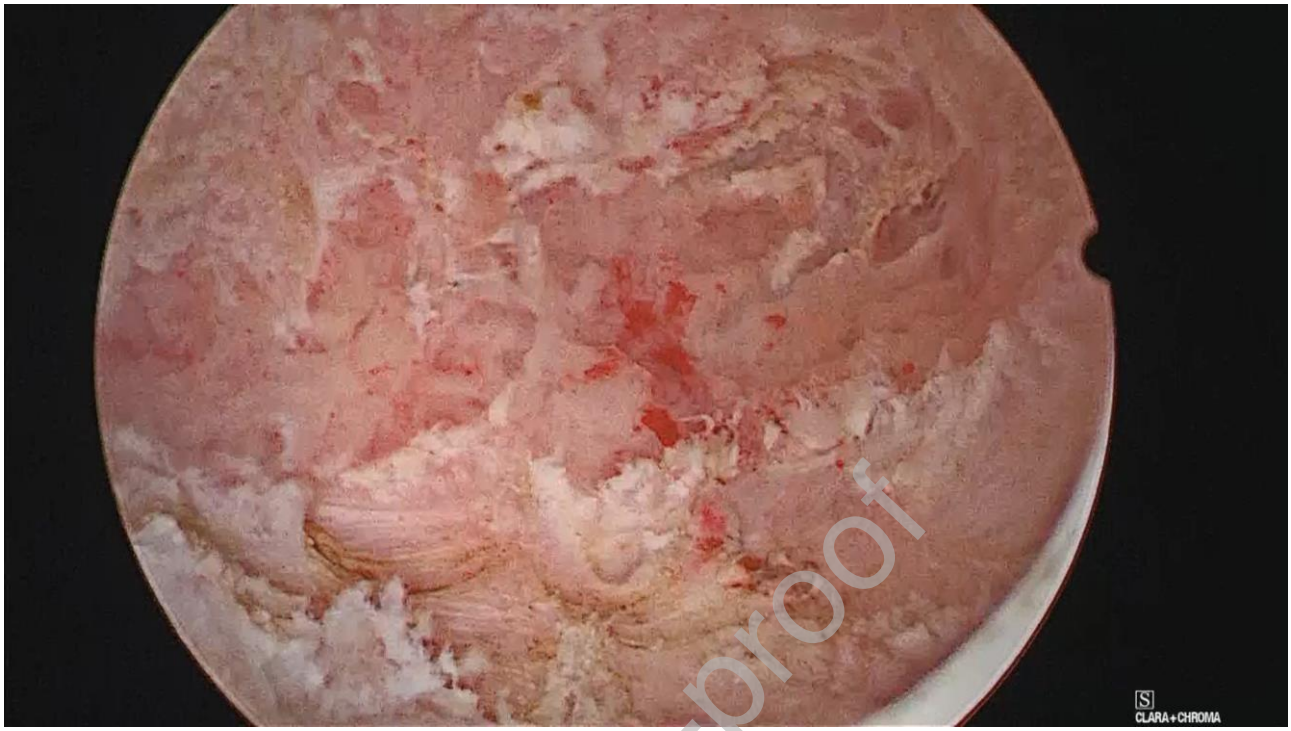

Figure 1g

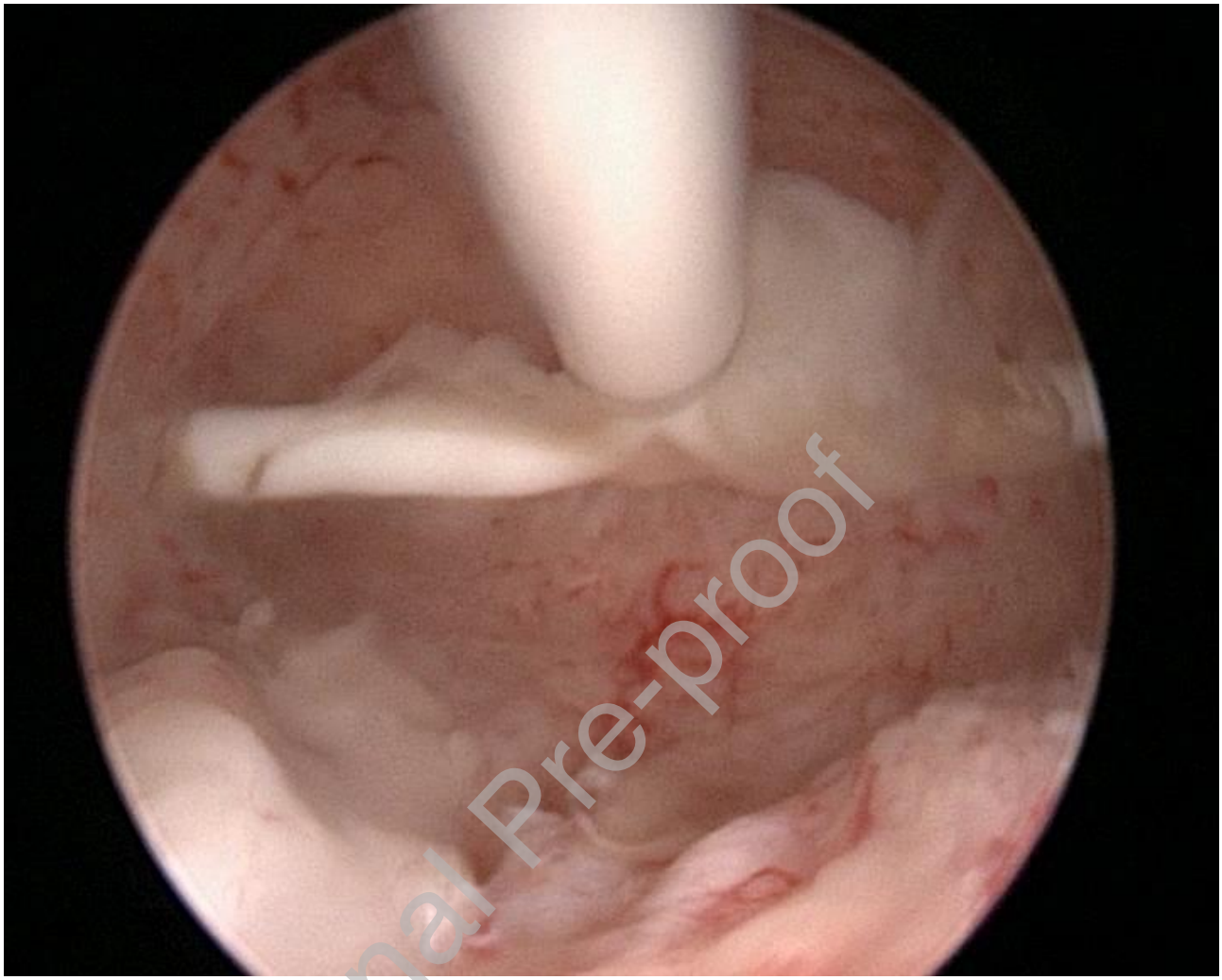

Figure 1h

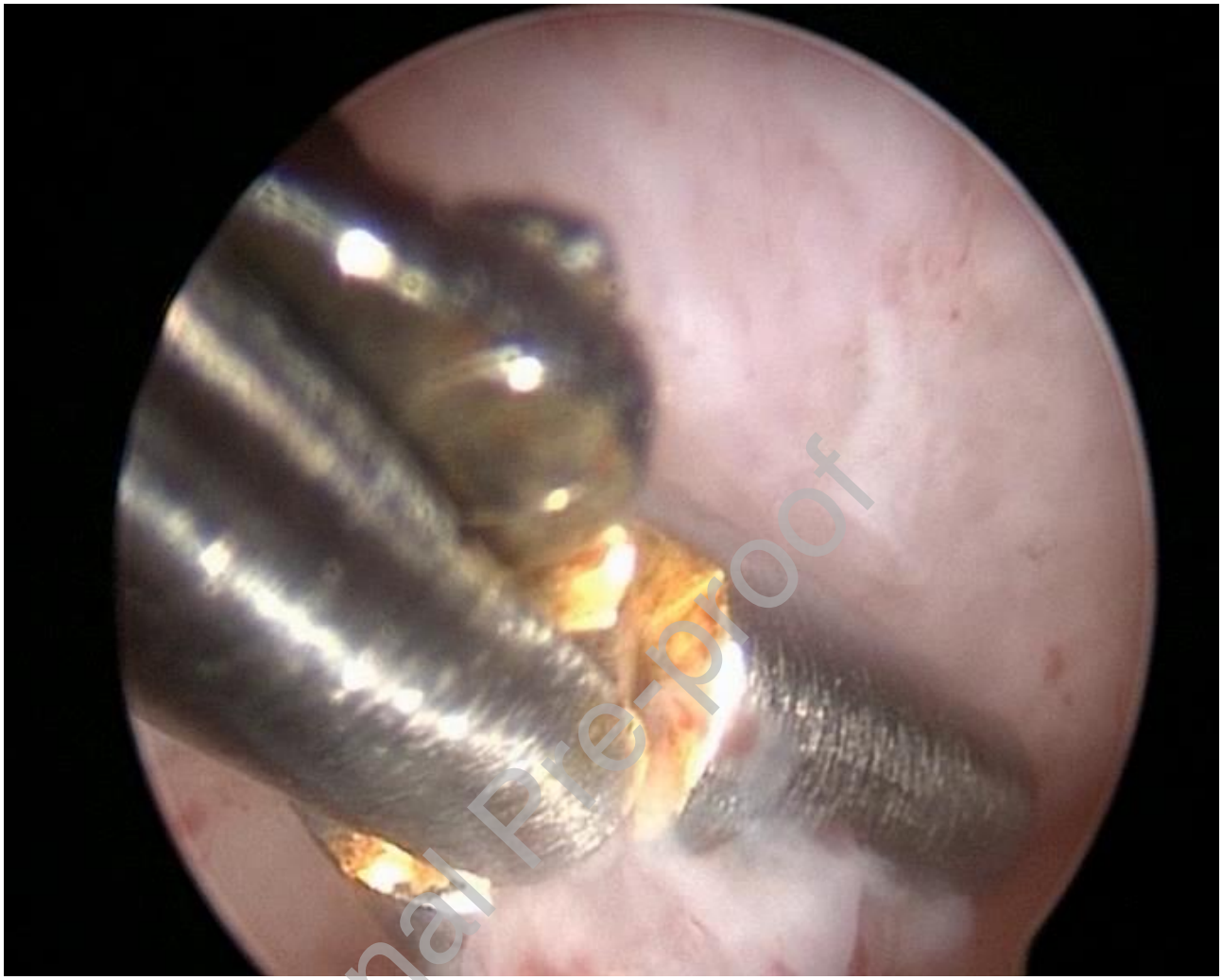

Figure 2a

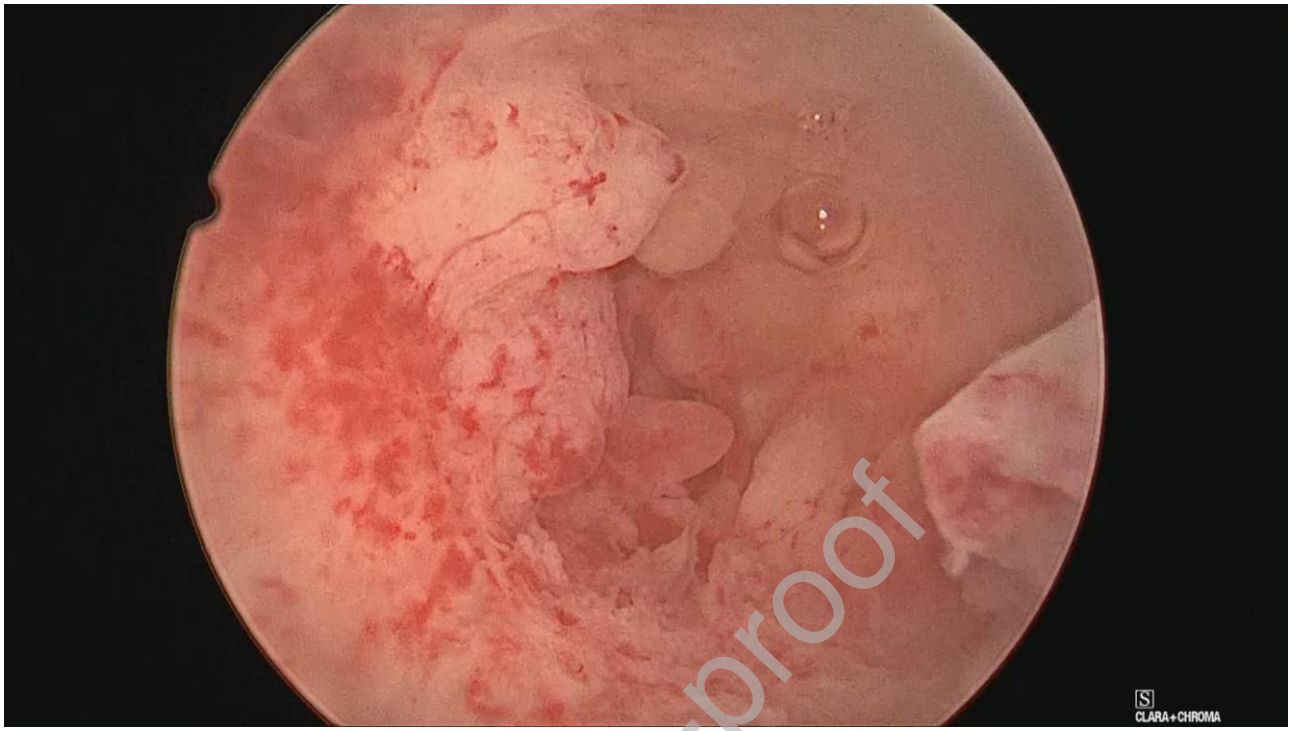

Figure 2b

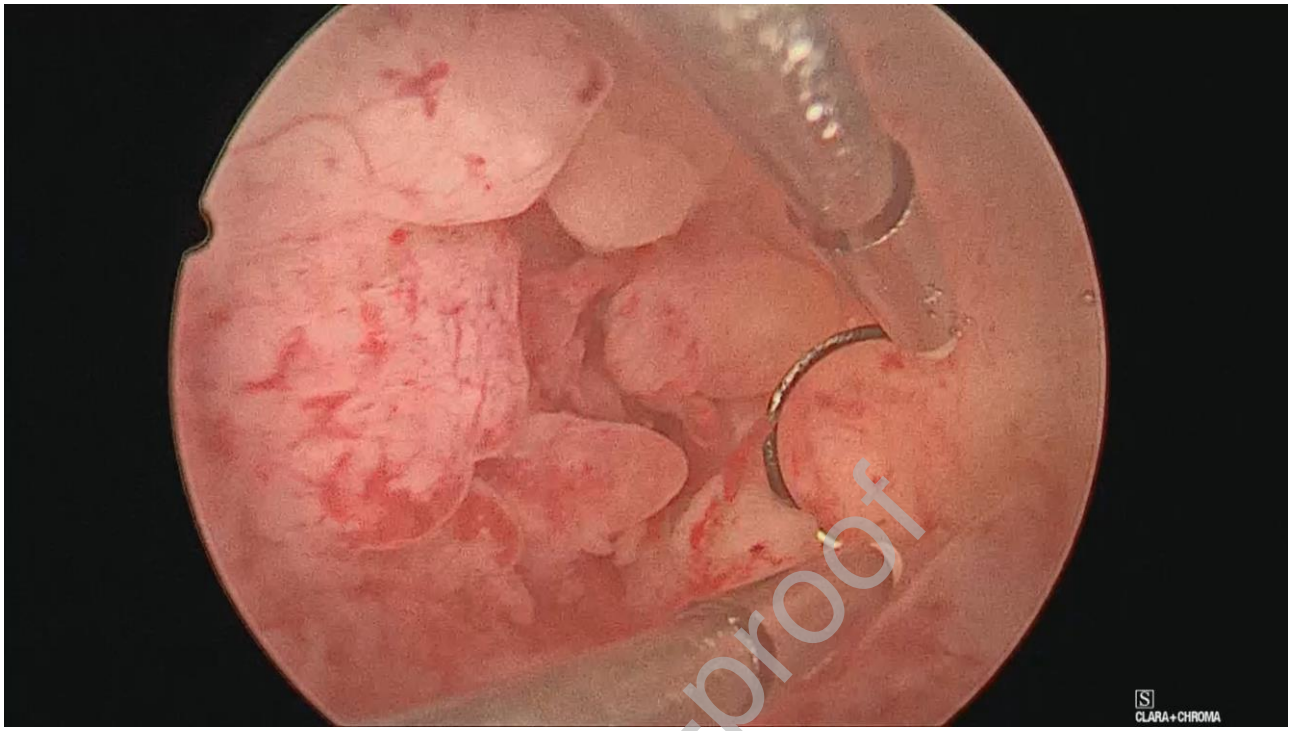

Figure 2c

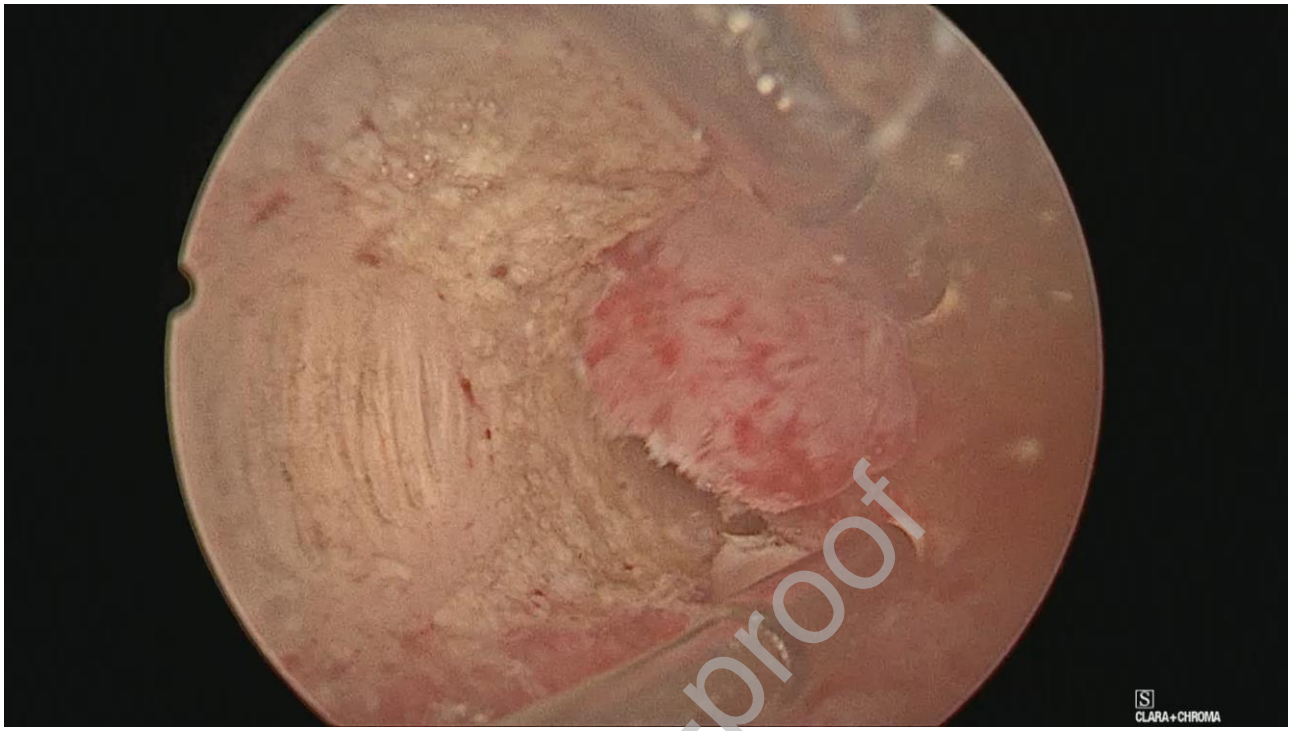

Figure 2d

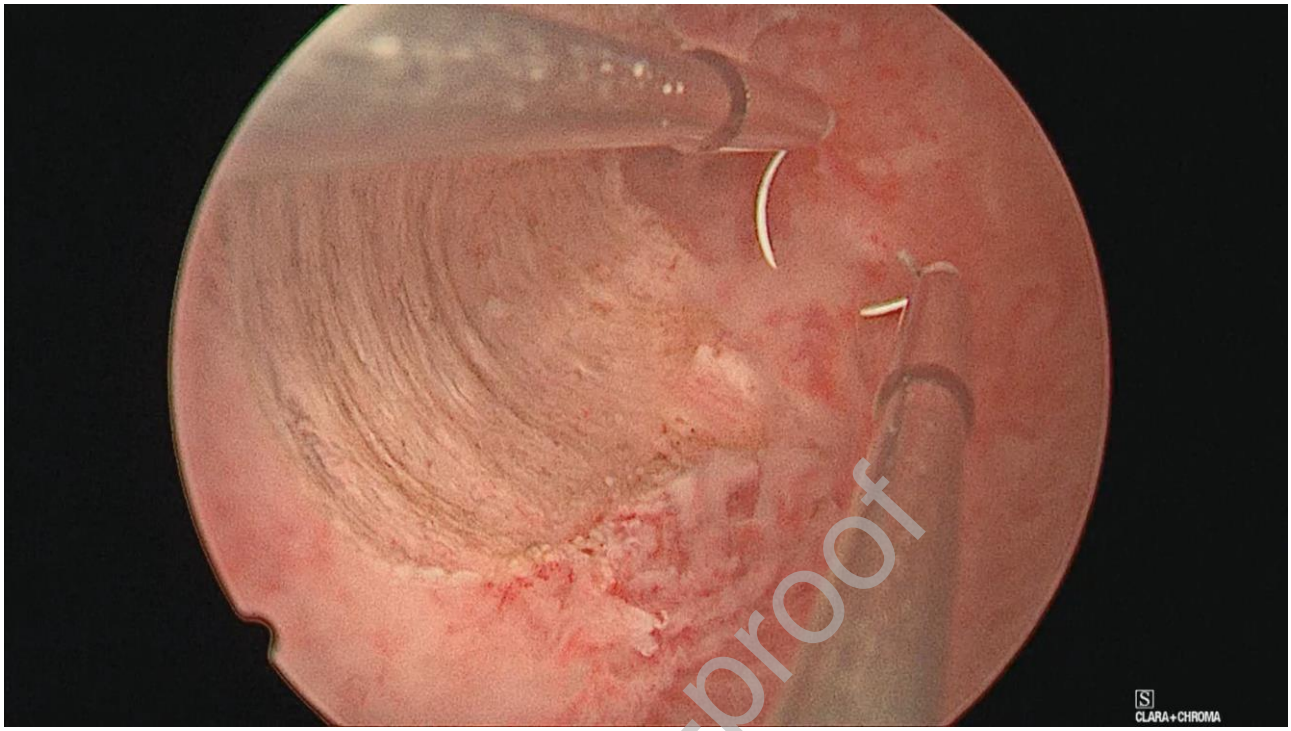

Figure 2e

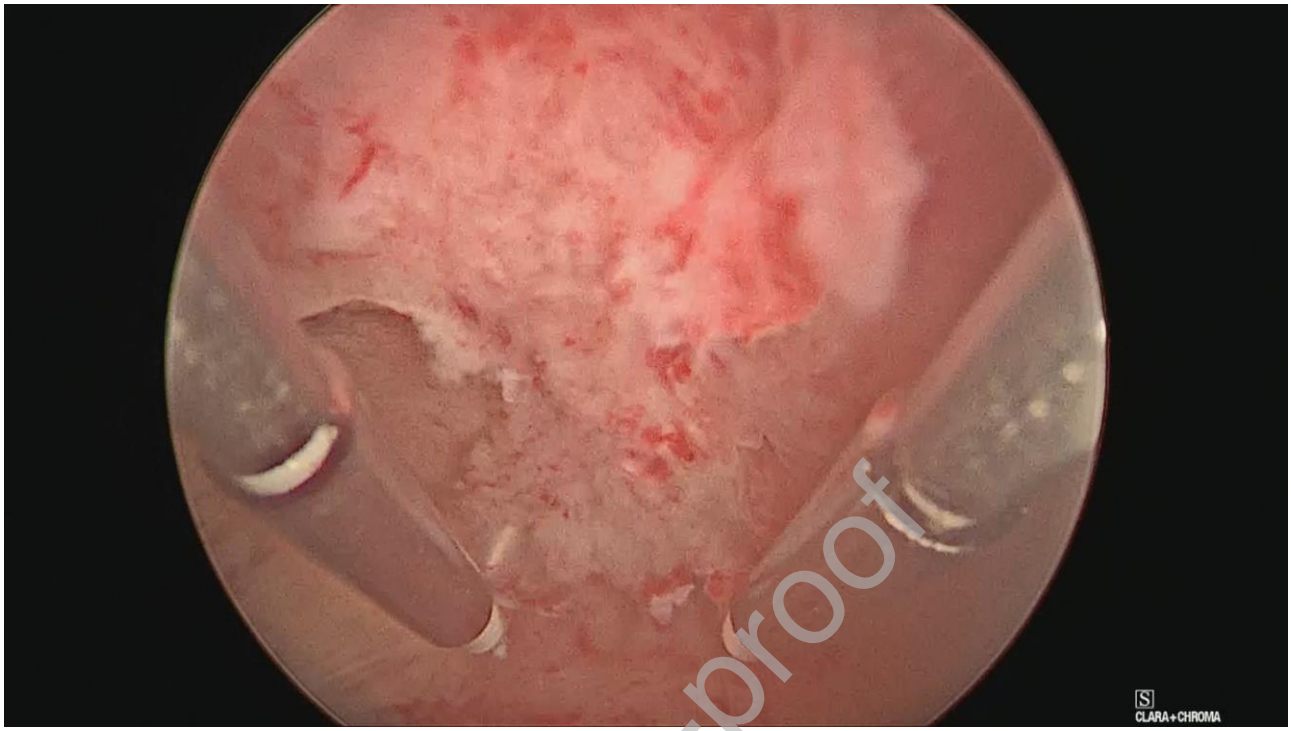

Figure 2f

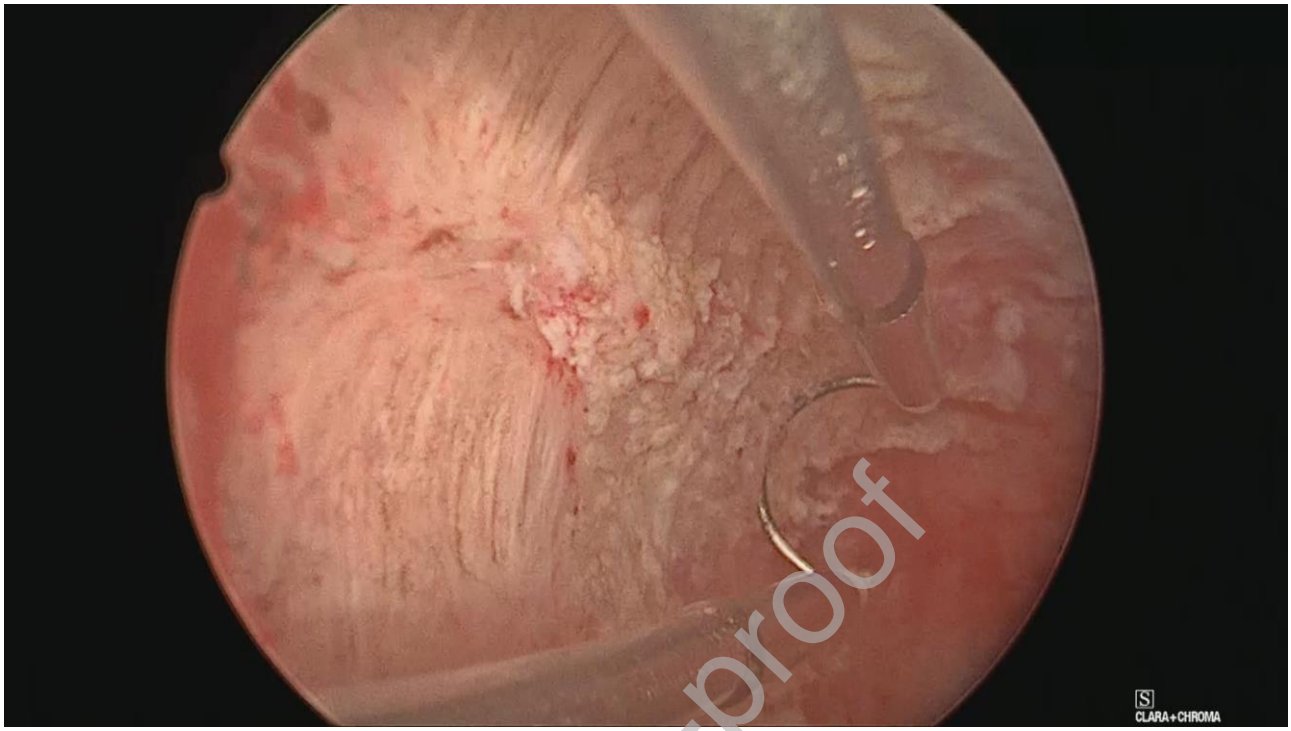

Figure 2g

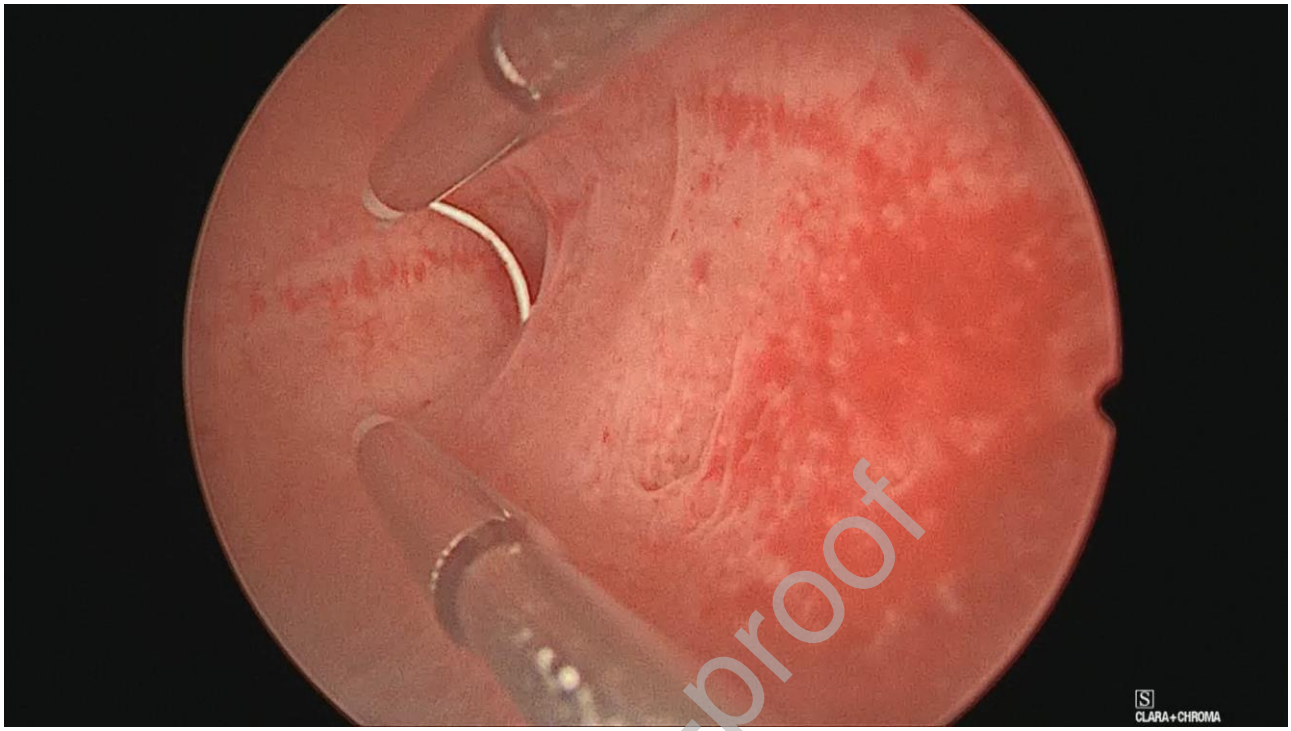

Figure 2h

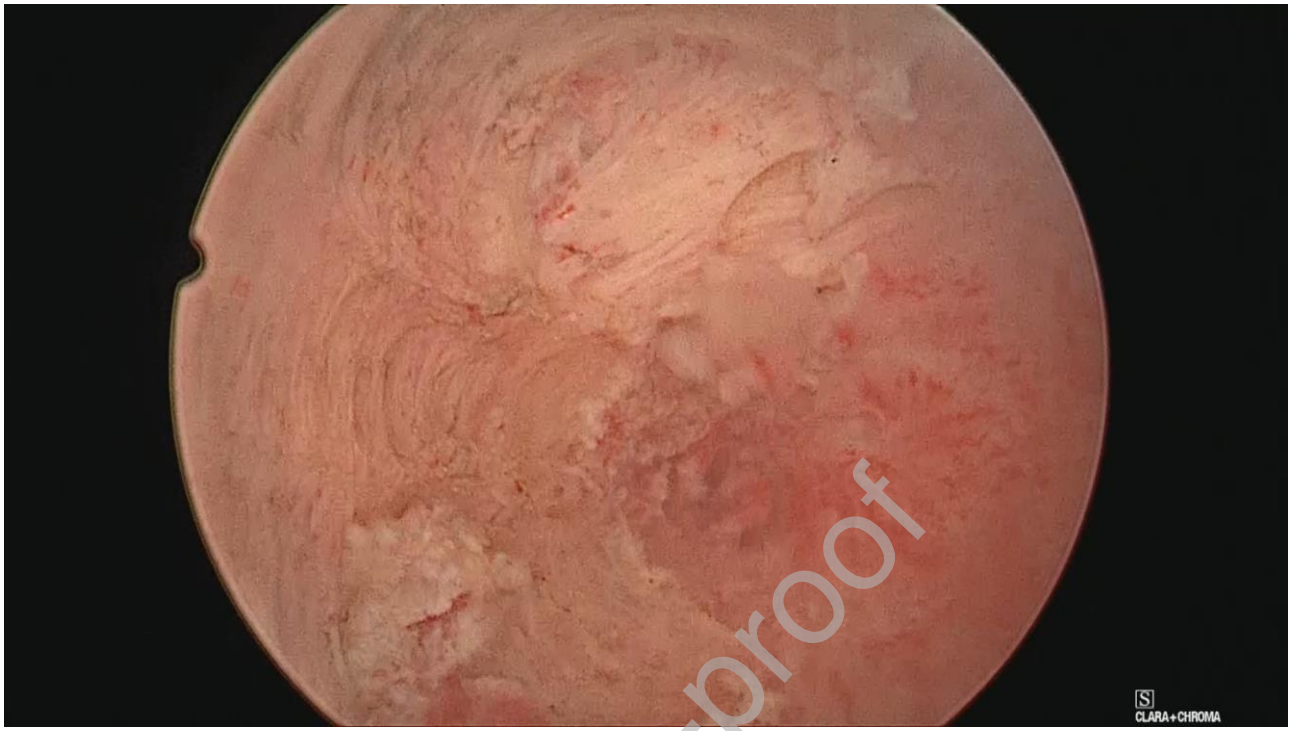

Figure 3a

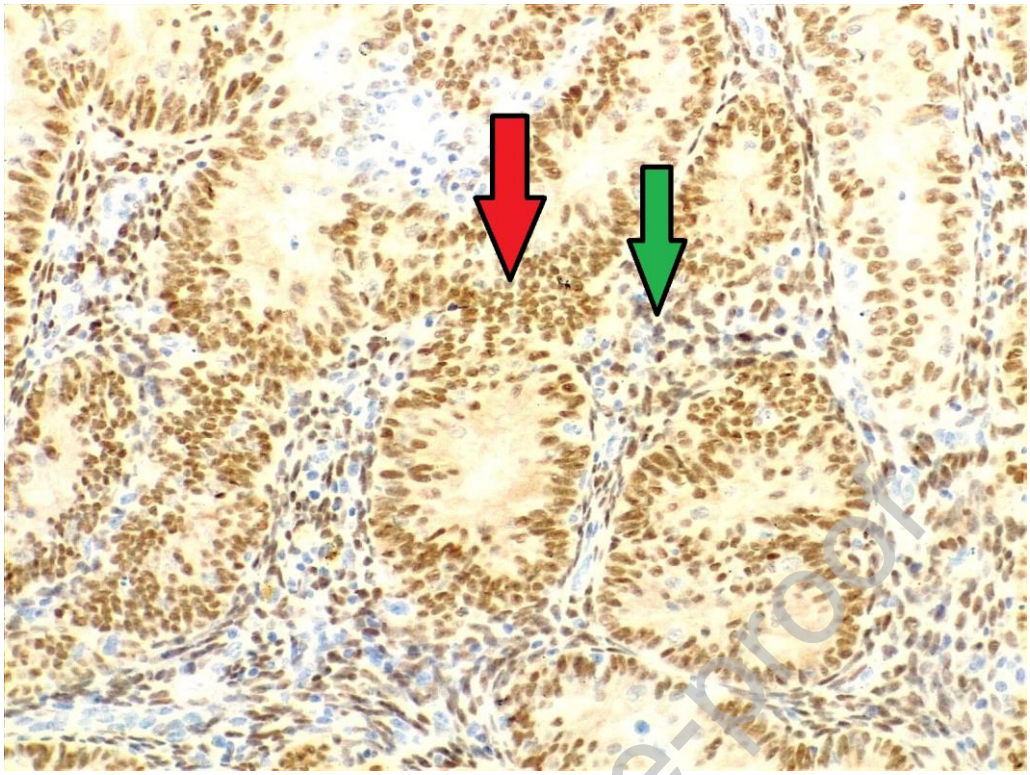

Figure 3b

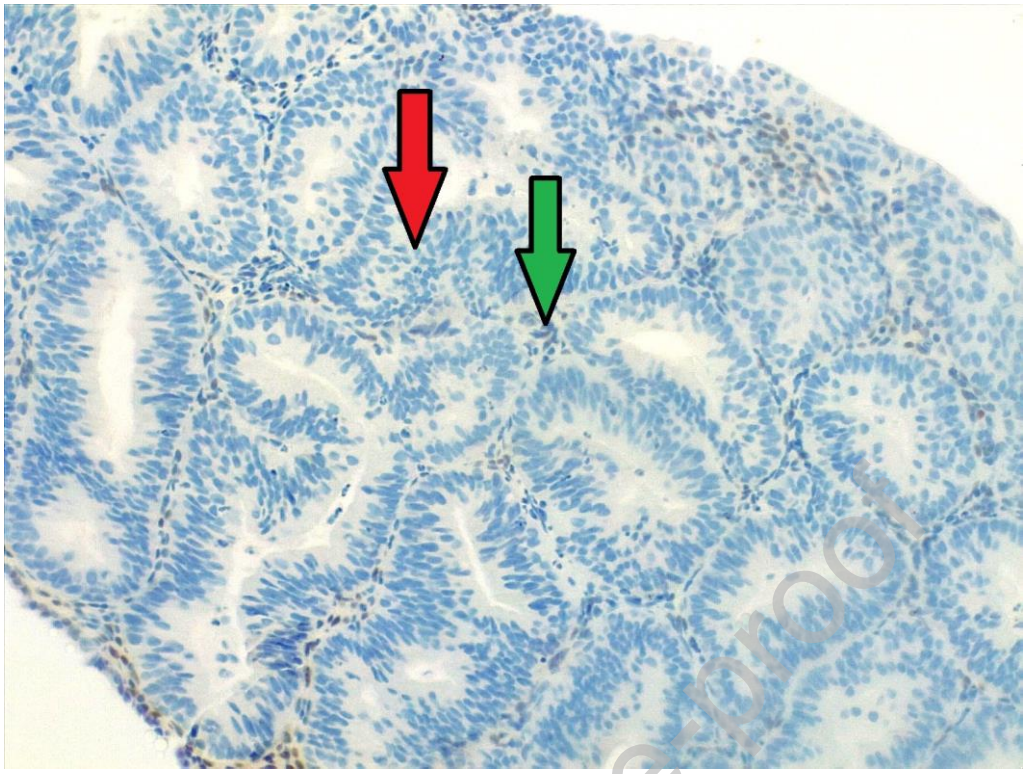

Figure 3c

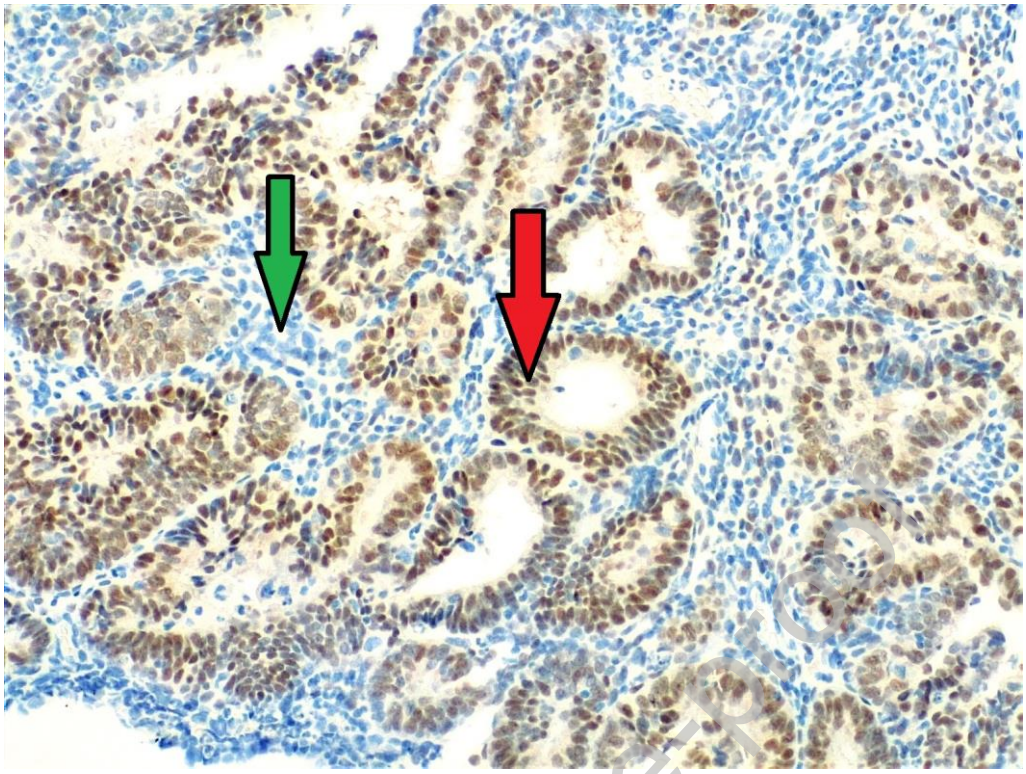

Figure 3d

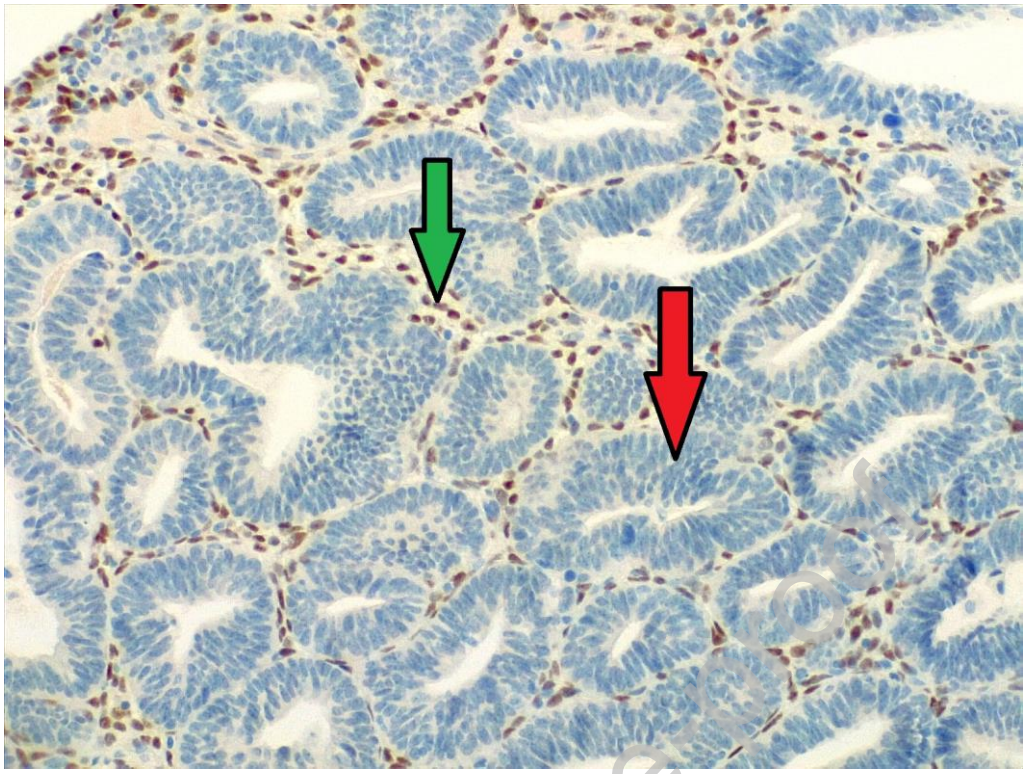

Figure 4

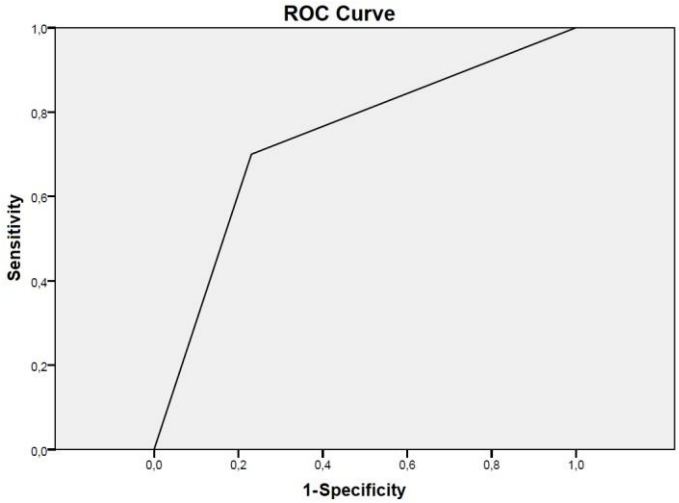

Figure 5

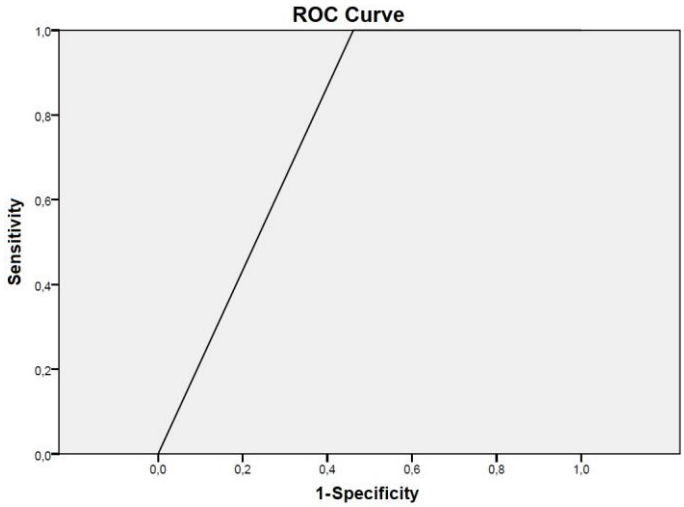

Figure 6

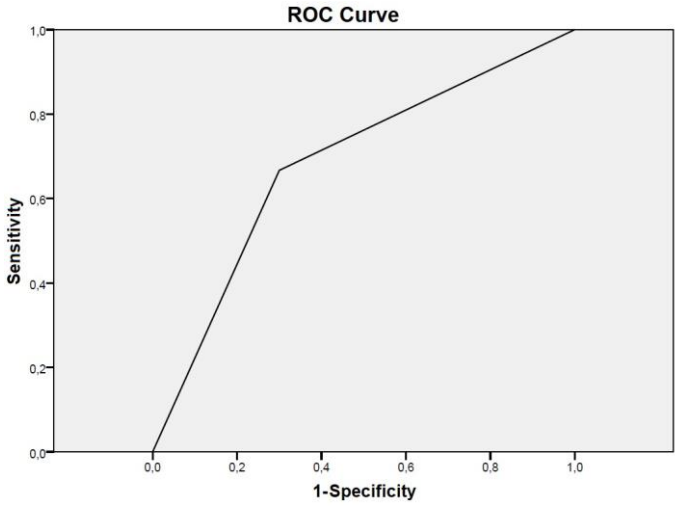

Figure 7

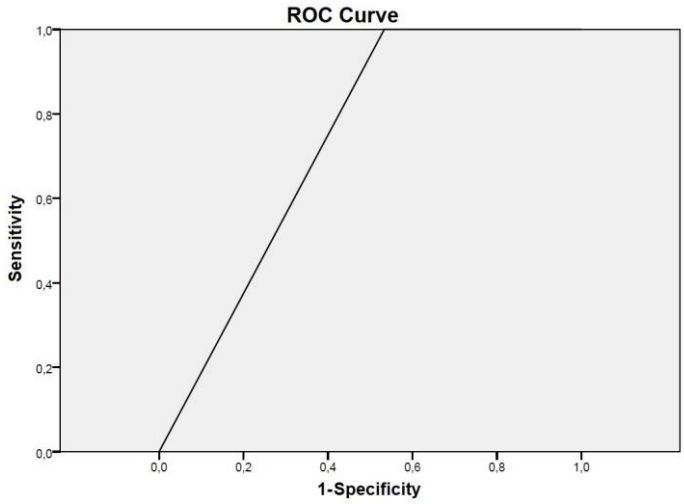

Figure 8

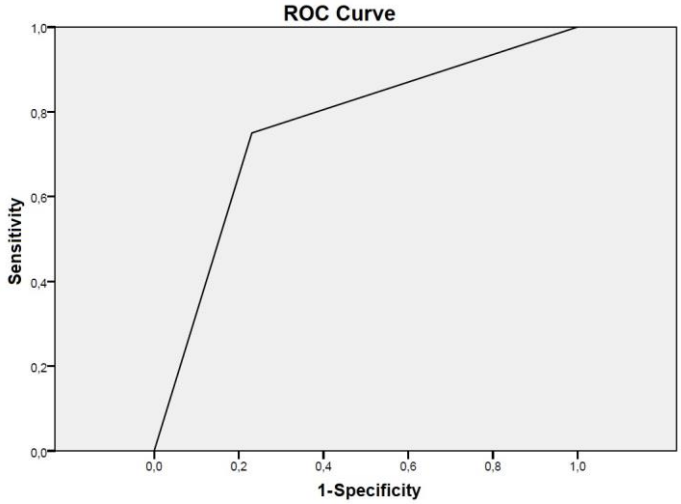

Figure 9