



MUM-1 immunohistochemistry has high accuracy and reliability in the diagnosis of chronic endometritis: a multi-centre comparative study with CD-138 immunostaining

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Abstract

Purpose The current gold standard for chronic endometritis (CE) diagnosis is immunohistochemistry (IHC) for CD-138. However, IHC for CD-138 is not exempt from diagnostic limitations. The aim of our study was to evaluate the reliability and accuracy of MUM-1 IHC, as compared with CD-138.

Methods This is a multi-centre, retrospective, observational study, which included three tertiary hysteroscopic centres in university teaching hospitals. One hundred ninety-three consecutive women of reproductive age were referred to our hysteroscopy services due to infertility, recurrent miscarriage, abnormal uterine bleeding, endometrial polyps or myomas. All women underwent hysteroscopy plus endometrial biopsy. Endometrial samples were analysed through histology, CD138 and MUM-1 IHC. The primary outcome was to evaluate the diagnostic accuracy of MUM-1 IHC for CE, as compared with CD-138 IHC.

Results Sensitivity and specificity of CD-138 and MUM-1 IHC were respectively 89.13%, 79.59% versus 93.48% and 85.03%. The overall diagnostic accuracy of MUM-1 and CD-138 IHC were similar (AUC = 0.893 vs AUC = 0.844). The intercorrelation coefficient for single measurements was high between the two techniques (ICC = 0.831, 0.761–0.881 95%CI). However, among CE positive women, MUM-1 allowed the identification of higher number of plasma cells/hpf than CD-138 (6.50 [SD 4.80] vs 5.05 [SD 3.37]; $p = 0.017$). Additionally, MUM-1 showed a higher inter-observer agreement as compared to CD-138.

Conclusion IHC for MUM-1 and CD-138 showed a similar accuracy for detecting endometrial stromal plasma cells. Notably, MUM-1 showed higher reliability in the paired comparison of the individual samples than CD-138. Thus, MUM-1 may represent a novel, promising add-on technique for the diagnosis of CE.

Keywords Chronic endometritis · MUM-1 · CD138 · Diagnostic accuracy · Plasma cells

Introduction

Chronic endometritis (CE) is a subtle pathology characterized by a persistent inflammatory disorder of the endometrial lining [1, 2]. The pathogenesis of CE seems to be sustained by qualitative and quantitative alteration of endometrial microbioma [3]. Additionally, a new theory

about an “impaired inflammatory state of the endometrium (IISE)” at the basis of CE (i.e. involving both infectious and non-infectious factors) was recently formulated [4].

Clinically, CE is commonly overlooked as it is mainly associated with mild and unspecific disturbances, such as abnormal uterine bleeding (AUB), dyspareunia, pelvic discomfort and leucorrhea [5, 6]. Nevertheless, the alterations of endometrial gene expression observed in CE may affect endometrial receptivity, potentially causing female infertility [7]. In this respect, different studies have previously shown that CE is highly prevalent in patients affected by unexplained infertility, repeated embryo implantation

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failure and recurrent pregnancy loss [8–13]. Even if a causal relationship between CE and reproductive failure is still far to be established, CE may theoretically impair embryo implantation due to cytokine dysregulation, leukocyte infiltration, vascular changes, altered decidualization, autophagy and altered uterine contractility [7, 14].

Diagnosis of CE is challenging for different reasons [3, 6, 9, 12]. Many patients with CE lack symptoms and ultrasound signs are non-specific. The microbial examination is not meaningful as most pathogens are not culturable and, additionally, during endometrial sampling, a certain contamination with cervical and vaginal bacteria cannot be prevented. CE may be diagnosed by fluid hysteroscopy based on the detection of specific endometrial signs such as focal or diffuse hyperemia, stromal edema and micropolyps [3, 9]. However, the diagnostic accuracy of hysteroscopy is dependent on operator expertise [15].

Therefore, histopathological identification of plasma cells in endometrial biopsy samples is considered the current diagnostic gold standard for CE [16, 17]. Identification of plasma cells by conventional tissue staining alone is difficult. Plasmacytes typically have a large cell body, high nuclei/cytoplasm ratio, basophilic cytoplasm and nuclei with heterochromatin rearrangement referred to as “spoke-wheel” or “clock-face” pattern [18]. These morphological features of plasmacytes are not always evident under microscopic examination, as plasma cells often exhibit an appearance similar to stromal fibroblasts and mononuclear leukocytes that reside in the human endometrium [15].

Immunohistochemistry for the plasmacyte marker CD-138 (also known as syndecan-1, a transmembrane type heparan sulphate proteoglycan) is currently the most reliable and time-saving diagnostic method for CE [19–21]. It has been shown that CD-138 immunostaining is superior to conventional tissue staining using methyl green pyronin, haematoxylin and eosin in CE detection (odds ratio 2.8; sensitivity, 100% versus 75%; specificity, 100% versus 65%) with less inter-observer (96% versus 68%) and intra-observer variability (93% versus 47%) [22].

Although CD-138 immunostaining is validated for CE diagnosis, it should be used with caution. Other than plasmacytes, also endometrial epithelial cells constitutively express CD-138 on their plasma membrane, potentially leading to a false-positive diagnosis of CE [18, 22]. Consequently, new insights on the staining techniques for plasma cells are warranted.

With regard to this scenario, multiple myeloma antigen 1 (MUM-1) is a protein which is normally expressed in plasma cells, activated B and T cells [23, 24]. Studies have shown that MUM-1 protein is required at several stages of B-cell development, including in the differentiation of mature B cell into antibody-secreting plasma cells [25, 26]. In other

authors' experiences, MUM-1 immunohistochemistry has been successfully used for the diagnosis of lymphomas and plasma cell tumours [27, 28]. Given the need for additional staining techniques in CE, we ought to test the usefulness of MUM-1 immunohistochemistry in the identification of endometrial plasma cells.

The aim of this multi-centre study was to evaluate the accuracy and reliability of MUM-1 immunohistochemistry, as compared with CD-138 immunohistochemistry, for the diagnosis of CE.

Materials and methods

Study design

This was a retrospective multi-centre study conducted at three centres (hysteroscopy units of the University of Bari, Padua and Barcelona) from August 2017 to January 2020. The study received local ethical approval. All patients gave their written consent for the anonymous use of their clinical data for research purposes, as per practice in our centre. The study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [29].

Participants

We included patients referred to our hysteroscopy services due to infertility (i.e. the failure to achieve pregnancy after 12 months or more of unprotected sexual intercourse), recurrent miscarriage (i.e. two or more consecutive miscarriages), abnormal uterine bleeding and suspicion of endometrial polyps or myomas.

Exclusion criteria were post-menopausal age, diagnosis of placental remnants, endometrial cancer or atypical hyperplasia, previous diagnosis of tuberculosis, hormonal or steroid treatment within three months or during the study period, any antibiotic treatments within 3 months or during the study period.

Hysteroscopy and endometrial sampling

All women underwent diagnostic mini-hysteroscopy in the follicular phase of the menstrual cycle. Mini-hysteroscopy was performed using a lens-based 2.7-mm OD mini-tele-scope and 105° angle of visual field equipped with a 3.5-mm OD single-flow diagnostic sheath (Karl Storz, Tuttlingen, Germany). In order to minimize the risk of iatrogenic contamination of the endometrial cavity, all examinations were performed after placing a vaginal speculum and cleaning the external uterine ostium with gauze soaked in iodine solution.

Saline was employed to distend the uterine cavity at a pressure generated by a simple drop from a bag suspended 1 m above the patient. A 300-W light source with a xenon bulb and a HD digital camera (Karl Storz, Tuttlingen, Germany) were used.

The exploration of the uterine cavity consisted of a panoramic view of the cavity followed by a thorough evaluation of the endometrial mucosa as previously described [15]. Diagnosis of CE at hysteroscopy was based on criteria previously published (micropolyps, focal/diffuse hyperemia, “strawberry aspect”, hemorrhagic spots, stromal edema). All hysteroscopies were performed by one author for each centre (A.V, E.C. and S.H.). After hysteroscopy, an endometrial biopsy using a 3 mm Novak’s curette connected to a 20-ml syringe was performed. The tissue samples obtained were placed in formalin for histological and immunohistochemical examinations.

Histology and immunohistochemistry

Endometrial samples were fixed in neutral formalin and later embedded in paraffin. Five microsections were stained with hematoxylin-eosin. Five-micrometre sections were cut and incubated, separately, with mouse anti-human monoclonal CD-138 and MUM-1 antibodies. The clones of anti-CD-138 and anti-MUM-1 monoclonal antibodies used in our study were MI15 Cell Marque (Biocare Medical, Concord, CA) and MRQ-8 Cell Marque (Sigma-Aldrich, St. Louis, USA). CD138-positive plasma cells were identified in the stroma. The biopsies were graded as “negative” for CE if there was less than one plasma cell identified per 20 high-power fields (HPFs) and “positive” when there were one or more plasma cells identified per 20 HPFs, according to published criteria [2, 5]. Initially, histopathological and immunohistochemical analyses were completed by a single pathologist for each centre and served for the assessment of the primary and tertiary endpoints of our study. In a subset of patients, the immunohistochemical analyses with CD-138 and MUM-1 were repeated by two additional pathologists, who were unaware of their respective evaluations. The results of their observations were included in the inter-observer agreement analyses.

Data collection

Clinical data were collected in our clinical registers by two Authors for each centre. Data included patients’ general features (age, BMI, parity), medical history (general health status, pharmacological treatments), the indication for hysteroscopy and the results of each hysteroscopic, histological and immunohistochemical examination with CD-138 and MUM-1. In cases of missing data, the same authors contacted patients by telephone to obtain complete information.

Study outcomes

The primary outcome was to evaluate the accuracy of MUM-1 immunohistochemistry, as compared with CD-138 immunohistochemistry for the diagnosis of CE. The secondary outcome was to assess the inter-observer reliability of MUM-1 and CD-138 immunohistochemistry for the detection of plasma cells. The tertiary outcome was to compare the number of plasma cells identified through using MUM-1 versus CD-138 immunohistochemistry.

Statistical analysis

Statistical analysis was performed by using SPSS v.22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were reported as mean with standard deviation (SD), whereas qualitative variables were presented as absolute frequencies and percentages. Comparisons between categorical variables were tested by using contingency tables and the chi-square test or Fisher’s test when necessary. Comparisons between normally distributed continuous variables were made by using Student’s *t*-test. A value of $p < 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) curves were elaborated to assess the performance of CD-138 and MUM-1 immunohistochemistry in order to detect CE (by using the combination of histological and hysteroscopic diagnoses as reference standard). The agreement was statistically assessed between the two immunohistochemical techniques (i.e. CD-138 and MUM-1 immunostaining), as well as between the evaluation of three pathologists for each single technique (i.e. separately for CD-138 and MUM-1 immunostaining). The measure of inter-observer agreement was expressed as the intraclass correlation coefficient (ICC). An ICC of 0.20 was considered as a poor agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement and 0.61–0.80 substantial agreement, while a value of 0.81–1.00 was considered as perfect agreement. The ICC was calculated by using a linear mixed model.

Results

Descriptive results

The demographic characteristics of the study population are displayed in Table 1.

Two hundred sixty-one patients were initially assessed for eligibility. After the exclusion of 68 women, a total number of 193 patients were included in the study. Of these, $n = 92$ (47.67%) suffered from infertility and $n = 37$ (19.17%) had a history of recurrent miscarriages. The remaining patients were referred to our centres due to abnormal uterine

bleeding ($n = 25$, 12.95%) or the suspicion of endometrial polyps or myomas ($n = 39$, 20.21%).

Hysteroscopy identified CE in 76 women (39.40%), while histology diagnosed CE in 54 women (28.00%). Cumulatively, hysteroscopy and histology diagnosed CE in 84 patients (43.52%), of whom $n = 46$ were positive to both techniques (23.83%). CD-138 immunostaining was positive for CE in 71 cases (36.80%) and MUM-1 immunostaining was positive for CE in 65 cases (33.70%). The total number of patients in whom CD-138 or MUM-1 immunostaining was positive was 76 (39.40%). Both CD-138 and MUM-1 immunohistochemistry revealed the presence of plasma cells in $n = 60$ patients (31.09%). At least one technique among histology, hysteroscopy, CD-138 and MUM-1 immunohistochemistry was positive in 97 cases (50.26%), with a high prevalence in women with recurrent miscarriage ($n = 26/37$, 70.27%) and infertility ($n = 53/92$, 57.61%). Notably, results of CD-138 and MUM-1 immunohistochemistry did not match in 16 cases (Table 2).

CD-138 and MUM-1: diagnostic accuracy and interclass correlation

Using hysteroscopy plus histology as the reference standard, sensitivity and specificity of CD-138 and MUM-1 immunohistochemistry were respectively 89.13%, 79.59% versus 93.48% and 85.03%. The overall diagnostic accuracy of MUM-1 and CD-138 immunohistochemistry were similar (AUC = 0.893 vs AUC = 0.844; Figure 1a).

Using a combination of hysteroscopy, histology and CD-138 immunohistochemistry as the reference standard, sensitivity and specificity of MUM-1 immunohistochemistry were 100% and 84.21%. The overall diagnostic accuracy of MUM-1 immunohistochemistry was AUC = 0.921

(Figure 1b). Conversely, using hysteroscopy plus histology plus MUM-1 immunohistochemistry as the reference standard, sensitivity and specificity of CD-138 immunohistochemistry were 95.35% and 80.00%. The overall diagnostic accuracy of CD-138 immunohistochemistry was AUC = 0.877 (Figure 1c). The intercorrelation coefficient for single measurements between CD-138 and MUM-1 immunohistochemistry was high (ICC = 0.831, 0.761–0.881, 95%CI). Importantly, MUM-1 immunostaining showed similar but higher closeness of agreement with histology (ICC = 0.800 [0.743–0.846, 95%CI]) and hysteroscopy (ICC = 0.592 [0.492–0.677, 95%CI]) when compared to those observed for CD-138 immunohistochemistry (ICC = 0.696 [0.615–0.762, 95%CI] and ICC = 0.463 [0.344–0.567, 95%CI] with histology and hysteroscopy, respectively).

Inter-observer reliability of CD-138 and MUM-1 immunohistochemistry

The inter-observer variability between three pathologists was assessed for 86 patients. Although the agreement was fair with respect to both techniques, MUM-1 immunohistochemistry showed higher inter-observer agreement than CD-138 immunohistochemistry (ICC = 0.902 [0.843–0.943, 95% CI] versus ICC = 0.809 [0.712–0.881, 95% CI]).

Number of plasma cells detected by CD-138 and MUM-1 immunohistochemistry

Among those women in whom MUM-1 and CD-138 immunohistochemistry were both positive for CE, MUM-1 immunohistochemistry allowed the detection of a higher number of plasma cells/20 hpf than CD-138 immunohistochemistry (6.50 [SD 4.80] vs 5.05 [SD 3.37]; $p = 0.017$).

Analysis of cases where CD-138 and MUM-1 immunohistochemistry provided conflicting results

The results of CD-138 and MUM-1 staining did not match in 16 cases (Table 2), of which in 5 women CE was revealed by MUM-1 staining but not by CD-138 staining. In a single patient, both histology and hysteroscopy were negative. In

Table 1 Clinical characteristics of patients included in the study. Data expressed as mean + SD or absolute numbers and percentages

Age, years (mean ± SD)	39.12 ± 4.55
Parity (mean ± SD)	0.86 ± 1.21
BMI (kg/m ²)	23.85 ± 3.17
Indications for hysteroscopy, n (%)	
Infertility	92 (47.67%)
Repeated miscarriage	37 (19.17%)
Abnormal uterine bleeding	25 (12.95%)
Suspicion of uterine polyps or myomas	39 (20.21%)
CE at hysteroscopy, n (%)	76 (39.40%)
CE at histology, n (%)	54 (28.00%)
E at immunohistochemistry with CD-138, n (%)	71 (36.80%)
CE at immunohistochemistry with MUM-1, n (%)	65 (33.70%)

Values are given as mean ± standard deviation or number (%) as appropriate; CE, chronic endometritis

Table 2 2 × 2 table. Immunohistochemical staining (IHC) with MUM-1 as compared with CD-138

		CD-138 IHC		Total
		CE positive	CE negative	
MUM-1 IHC	CE positive	60	5	65
	CE negative	11	117	128
	Total	71	122	193

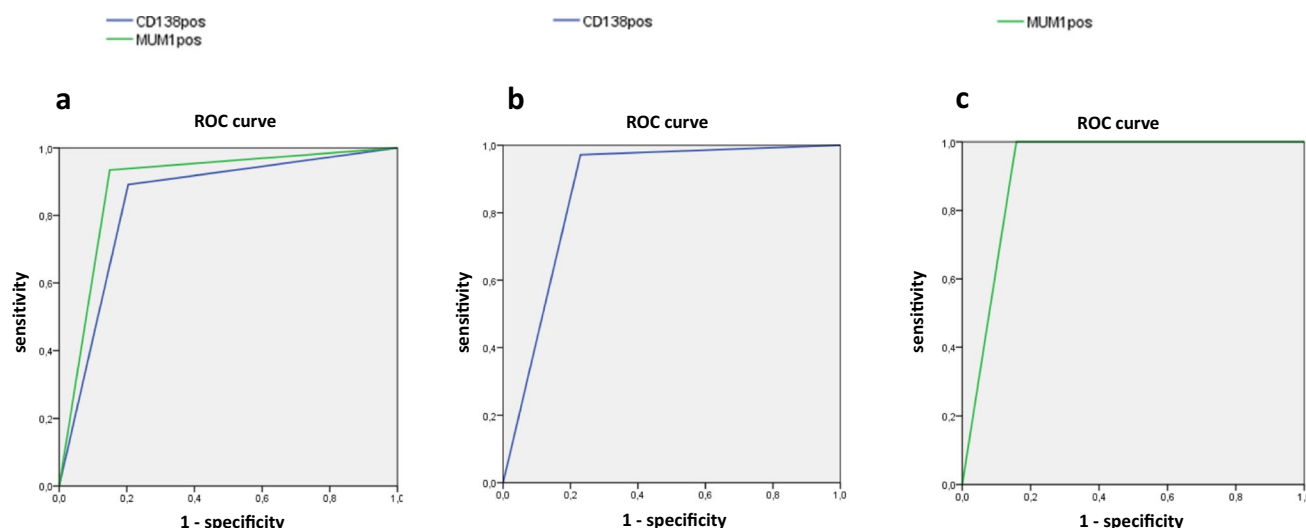


Fig. 1 a–c ROC analyses: **a** diagnostic accuracy of MUM-1 and CD-138 immunohistochemistry for chronic endometritis using histology plus hysteroscopy as a reference standard; **b** diagnostic accuracy of CD-138 immunohistochemistry for chronic endometritis using a combination of MUM-1 immunohistochemistry, histology and hyst-

eroscopy as a reference standard; **c** diagnostic accuracy of MUM-1 immunohistochemistry for chronic endometritis using a combination of CD-138 immunohistochemistry, histology and hysteroscopy as a reference standard

the remaining cases, at least one technique between histology and hysteroscopy was positive. After the re-evaluation of the samples, two patients were considered as false positive CE due to misinterpretation of marked lymphocytes. Contrastingly, in the 11 cases for which CD-138 showed immunoreactivity but MUM-1 did not, both hysteroscopy and histology were negative (Figure 2). After the samples were re-evaluated, nine patients were considered as false positive CE due to the intense reaction of epithelial cells.

Discussion

The current diagnosis of CE relies on the histopathological examination of endometrial biopsy specimens, where the presence of endometrial stromal plasma cells represents the main diagnostic marker. The recent introduction of CD-138 immunohistochemistry has considerably improved the accuracy of the microscopic examination of the endometria in women with CE, thus overcoming the limitations of traditional staining techniques [21, 22]. In this respect, Bayer-Garner and Korourian reported that the diagnosis rate of CE by traditional histopathology was considerably lower than that by CD-138 immunohistochemistry (15% vs 42%, $p < 0.05$) in endometrial curettage samples [30]. Additionally, in a recent study on 100 endometrial preparations (of which 80 were from patients with CE and 20 controls), Kitaya et al. found a higher inter-observer agreement for the detection of endometrial stromal plasma cells using immunohistochemistry for CD138 (ICC 0.968–0.976) compared to the

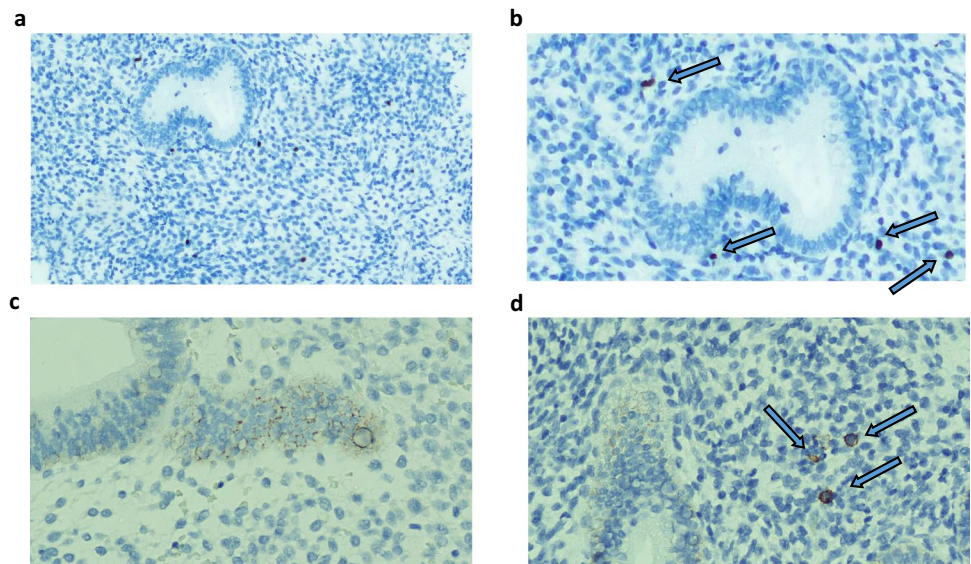
conventional histopathological method (0.687–0.771) [25]. The gap in favour of CD-138 staining was further increased when inexperienced pathologists were involved in the analysis, suggesting a better reproducibility of the immunohistochemical technique. As per the authors' admission, the major concern in the immunohistochemical staining with CD-138 was that anti-human CD138 antibodies detected not only stromal plasmacytes but also endometrial glandular/surface epithelial cells which constitutively express this proteoglycan, potentially leading to false-positive diagnoses of CE. For this reason, the identification of further staining techniques for overcoming diagnostic uncertainty inherent to CE would be important. In this study, we aimed to evaluate the potential usefulness of MUM-1, a protein which is constitutively expressed in plasmacytes, as a novel immunohistochemical marker for endometrial stromal plasma cells in women with CE.

Main findings

Our study included a total number of 193 reproductive-aged patients for whom hysteroscopy plus endometrial biopsy was indicated due to infertility, recurrent miscarriages, abnormal uterine bleeding or the suspicion of polyps or myomas. For all patients, endometrial preparations with CD-138 and MUM-1 staining were realized alongside traditional histology.

By using all the techniques (hysteroscopy, histology, CD-138 and MUM-1 immunohistochemistry), our study revealed a high prevalence of CE in the entire sample of

Fig. 2 a–d MUM-1 (a, b) and CD-138 stains (c, d) demonstrate the presence of plasma cells within the endometrial stroma (blue arrows). Note the endometrial glandular/surface reaction to CD-138 antibodies (2c) but not to MUM-1 antibodies (2a)



patients, especially in those women suffering from reproductive disorders. These findings were in line with our previous data on women suffering from infertility, repeated embryo implantation failure and recurrent miscarriage [4, 8, 31]. On the other hand, when compared to our recent study on women with intrauterine pathologies [5], this present study found a lower prevalence of CE. However, the two studies were different in terms of research design and population characteristics.

Interestingly, in this study, the accuracy for CE diagnosis of MUM-1 immunohistochemistry was similar to CD-138 staining (AUC = 0.893 vs AUC = 0.844 by using a combination of hysteroscopy and histology as reference standard), and the closeness of agreement between the two techniques was high (ICC = 0.831, 0.761–0.881, 95%CI). Notably, sensitivity and specificity of MUM-1 were slightly higher than CD-138 immunohistochemistry (93.48% and 85.03% vs 89.13% and 79.59%, respectively). These findings were further confirmed by individual matching of CE diagnoses achieved by the two immunohistochemical techniques with those obtained by hysteroscopy and histology (ICC = 0.800 and ICC = 0.592 vs ICC = 0.696 and ICC = 0.463 for MUM-1 and CD-138, respectively). These important results deserve consideration concerning the peculiar characteristics of the techniques under comparison. As previously mentioned, CD-138 antigen is constitutively expressed not only by plasma cells, but also by the epithelial cells of the endometrial glandular surface [18, 22]. Therefore, epithelial cells can appear as marked elements in the endometrial staining with anti-CD-138 monoclonal antibodies, generating a focal or diffuse background reaction. In such cases, a correct diagnosis is based on cell morphology-based criteria. Nevertheless, we must stress that some endometrial sections with CD-138 staining may be misleading (e.g.

isolated or fragmented glandular epithelial cells in the sample) and potentially result in misidentification of epithelial cells as stromal plasma cells. Unlike CD-138, MUM-1 is not expressed by epithelial glandular cells but mainly by lymphoid cells, especially plasmacytes and activated B/T lymphocytes [23, 24]. Therefore, this latter staining technique does not display a background reaction, but it should be noted that it marks also other lymphoid lineage cells that can be found in the endometrial stroma. Here, once again differential diagnosis is achieved through morphological criteria, where plasma cells display eccentric nuclei with clock-face chromatin and abundant cytoplasm. Thereby, the inner characteristics of the techniques employed in our study may potentially explain their imperfect agreement in terms of CE diagnoses. Additionally, the lack of background reaction with MUM-1 staining (but not with CD-138 staining) may justify our findings about a higher inter-observer agreement between pathologists, as well as the higher mean number of plasma cells counted in paired samples by using MUM-1 immunohistochemistry. In our opinion, these specific differences between CD-138 and MUM-1 immunohistochemistry emphasize the potential advantages of using both techniques contextually in CE. However, we need to stress that our results, although exciting, need further confirmation in other institutional settings.

Study limitations

To the best of our knowledge, this was the first study investigating the accuracy and reliability of MUM-1 immunohistochemistry for the diagnosis of CE. Originality, rigorous methodology, the multi-centre setting of the study and the relatively large sample size were the points of strength.

The retrospective design was the main point of weakness in our study. Additionally, the choice to make CE diagnosis based on the smallest addressable unit (i.e. one or more plasma cells per 20 hpfs) may have dramatically increased the absolute number of CE in our series, as well as it may have expanded any differences in diagnostic accuracy between comparators. In this regard, recent studies raised important questions about the clinical meaningfulness of < 5 endometrial plasma cells per high-power field in women suffering from infertility. However, these results are still not conclusive and a precise cutoff of plasma cells for performing CE diagnosis has not been established yet [32–34].

Conclusions

Immunohistochemistry for MUM-1 and CD-138 showed similar accuracy for detecting endometrial stromal plasma cells. Notably, MUM-1 staining showed higher reliability in the paired comparison of the individual samples than CD-138 staining. Therefore, MUM-1 immunohistochemistry may represent a novel, promising add-on technique for improving the diagnostic process in women with CE.

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Availability of data and material All data are available upon request.

Declarations

Ethics approval The study received local ethical approval.

Consent to participate All patients gave their written consent for the procedure.

Consent for publication All patients gave their written consent for the anonymous use of their clinical data for research purposes.

Conflict of interest The authors declare no competing interests.

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