

The immunoprofile of interstitial Cajal cells within adenomyosis/endometriosis lesions

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Abstract

Adenomyosis and endometriosis are lesions which have aroused the interest for the investigation of antibodies specific to the structures from the composition, but also for the cause behind the appearance of these lesions in completely different structures. The impact they have on fertility is not known entirely, for they are difficult to diagnose. Endometriosis causes infertility and it is a hard to treat lesion. The research performed in the last years has been focused on the so-called linkage analysis, or reverse genetics. It refers to identifying the genes which are prone to developing this affection. We investigated clinically 40 female inpatients ($n=40$) who had underwent genital surgery and received a variegated diagnosis in the "Sf. Ioan" Emergency Hospital, Bucharest, Romania, between January–September 2014 and also their histopathology and immunohistochemistry. We proceeded with the histopathology examination in order to establish a diagnosis in respect to the admission diagnosis and then, using the ABC (Avidin–Biotin complex) method, we analyzed the immunohistochemistry of the following markers: S100 protein (for detection of ganglia and nerve cells), CD117/c-kit (selective detection of interstitial Cajal cells – ICC), desmin and vimentin (intermediary filaments for detecting ICC-like cells, which cohabit with uterine myocytes and are not contractile cells) and CD10 (a sensitive and useful immunomarker in the diagnosis of endometrial stroma and, in some cases, of neoplasia). Our study, regarding the immunoprofile of some markers of adenomyosis/endometriosis lesions, supports the hypothesis that the interstitial Cajal cells are non-reactive, they are not in relationship with investigated lesions, but CD10 is a very useful marker to highlight the endometrial stroma in query cases.

Keywords: histopathology, fertility, immunohistochemistry, marker, diagnosis.

Introduction

Adenomyosis is defined as the presence of islands of endometrial glands and stroma within the myometrium, whereas endometriosis is the term employed for the presence of endometrial tissue outside the uterus. These two disorders are usually regarded as being closely related, but their microscopic appearance, clinical characteristics and probably their pathogenesis are somewhat different. Endometriosis is one of the leading causes of infertility among women at the reproductive age [1]. The eutopic and ectopic endometrium of women with endometriosis differs from normal endometrium in three distinct and important ways: high local estrogen production, high local prostaglandins (PG) production, and resistance to the action of progesterone [2]. The celomic metaplasia theory would explain why most women have some degree of retrograde menstruation but only a small percentage have endometriosis, and the presence of the disease in absence of menses [3, 4]. Anatomic abnormalities are also considered a possible precursor of endometriosis. It was concluded that the depth and volume of the cul-de-sac, differs in patients with endometriosis with or without deep lesions as compared to women with a healthy pelvis [5]. However, adenomyosis is not usually associated with infertility and, furthermore, it is more

common in parous women [6]. Endometriosis can be diagnosed by either laparoscopy or laparotomy, but the mainstay of the precise diagnosis and treatment of adenomyosis remains hysterectomy [7].

The impact adenomyosis has on fertility is not known for certain, because it is difficult to diagnose. As we have underlined, the only method for diagnosing adenomyosis with certainty is through hysterectomy and macroscopic and microscopic investigation of the lesion. Still, there are a 15% of patients which have evident symptoms of adenomyosis and during the macro and microscopic exam of the removed uterus no lesion of adenomyosis is identified. The hysterectomy treats the adenomyosis, but not endometriosis, which should be searched for during the operation. Also, during the surgery, the possibility of ovarian endometriosis should also be investigated, as its presence can bring about oophorectomy.

Aim

We aim to investigate the adenomyosis/endometriosis lesions histopathologically and immunohistochemically, as well as the utility of the CD10 antibody in identifying the endometrial stromal tissue, in the lack of glands, and also the role of CD117/c-kit cells at the level of the lesions.

Materials and Methods

We investigated clinically 40 female inpatients ($n=40$) who had underwent genital surgery and received a varied diagnosis in the “Sf. Ioan” Emergency Hospital, Bucharest, Romania, between January–September 2014, and also their histopathology and immunohistochemistry (IHC). We proceeded with the histopathology examination in order to establish a diagnosis in respect to the admission diagnosis and then, using the ABC (Avidin–Biotin complex) method, we analyzed the immunohistochemistry of the following markers: S100 protein (for detection of ganglia and nerve cells), CD117/c-kit (selective detection of interstitial Cajal cells – ICC), desmin and vimentin (intermediary filaments for detecting ICC-like cells, which cohabit with uterine myocytes and are not contractile cells) and CD10 (a sensitive and useful immunomarker in the diagnosis of endometrial stroma and, in some cases, of neoplasia) (Table 1).

Table 1 – Histopathology examination using the ABC method with the immunohistochemistry of the following markers

Antibodies	Specificity	Dilution	Clone	Source
S100	Ganglionic and nerve cells	1/800	Polyclonal	Dako
CD117 (c-kit)	Interstitial Cajal cells (ICC)	1/400	Polyclonal	Dako
CD10	Endometrial stroma with ectopic localizations	1/30	Monoclonal	Neomarkers
Vimentin	Intermediary filaments for ICC-like non-contractile cells	1/50	Monoclonal	Dako
Desmin	Intermediary filaments for ICC-like non-contractile cells	1/50	Monoclonal	Dako
CD34	Endothelial cells	1/25	Monoclonal	Dako

The 40 patients investigated in our study were admitted for confirming other diagnosis and their treatment; the adenomyosis/endometriosis was established at that time.

Results

Out of the 40 patients group, the age categorization is shown in the table below (Table 2, Figure 1).

Table 2 – Age categorization of adenomyosis patients

Age range [years]	No. of patients ($n=40$)	Percent
31–40	11	27.5%
41–50	20	50%
51–60	9	22.5%

The categorization of patients according to the symptoms prevalent at the time of admittance, and which constituted a reason for doing so, is shown in the table below (Table 3, Figure 2).

We analyzed the histopathological exam and the histological structure of the lesions. We noticed that it can only be present either in the endometrial stroma, or in the glandular epithelium (Figure 3) accompanied by stroma (Table 4, Figure 4), both in adenomyosis and endometriosis.

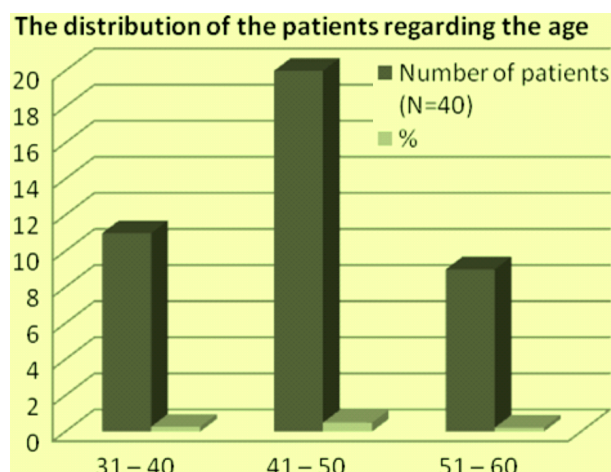


Figure 1 – Age [years] categorization of adenomyosis patients.

Table 3 – The categorization of patients according to the symptoms which lead to admittance

The main symptom	No. of patients	Percent
Bleeding (possible diagnosis of leiomyoma)	30	75%
Bleeding (possible diagnosis of uterine cervix lesion)	10	25%

The main symptoms of the inpatients

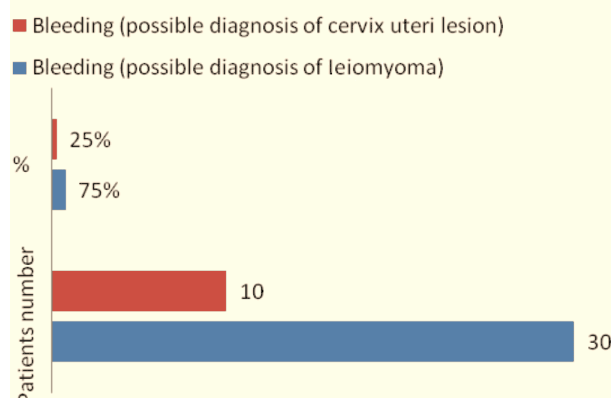


Figure 2 – The categorization of patients according to the symptoms which lead to admittance.

The type of the tissue within the lesions

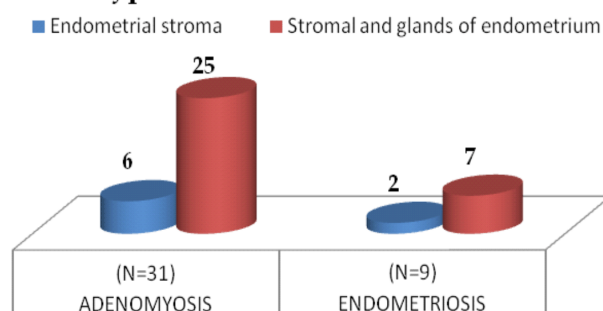


Figure 3 – The histological components of adenomyosis/endometriosis lesions.

Table 4 – The histological compounds of adenomyosis/endometriosis lesions

The tissue type	Adenomyosis ($n=31/77.5\%$)	Endometriosis ($n=9/22.5\%$)
Endometrial stroma	6 (19.35%)	2 (22.22%)
Stromal and glands of endometrium	25 (80.65%)	7 (77.78%)

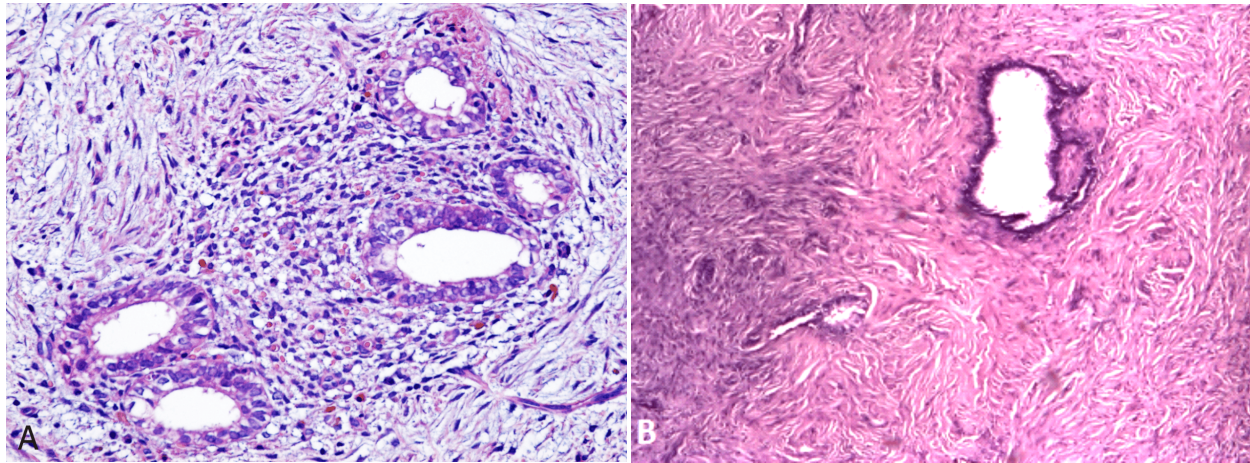


Figure 4 – (A and B) Histopathological pictures from ovarian endometriosis: endometrium glands with stroma (A) and endometrium glands without stroma within the ovarian parenchyma (B). HE staining: (A) $\times 200$; (B) $\times 40$.

The distribution of the investigated markers at the adenomyosis lesion level ($n=31$) was the one represented in Table 5 and Figure 5.

Table 5 – The distribution of the antibodies at the level of the adenomyosis lesions ($n=31/77.5\%$)

Antibodies	Positive immunostaining ($n=31$)	Percent
CD10	20	64.51%
CD117 (c-kit)	17	54.83%
CD34	28	90.32%
S100 protein	27	87.09%
Desmin/vimentin	6	64.51%

The immunostaining profile of adenomyosis lesions ($n=31$)

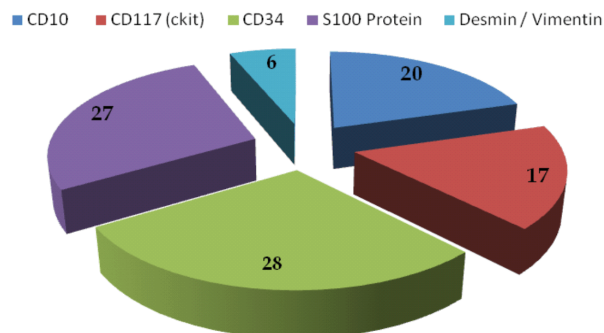


Figure 5 – The distribution of the antibodies at the level of the adenomyosis lesions ($n=31/77.5\%$).

The distribution of the markers at the level of the endometriosis lesions was that represented in Table 6 and Figures 6–7.

Table 6 – The distribution of the antibodies at the level of the endometriosis lesions ($n=9/22.5\%$)

Antibodies	Positive immunostaining ($n=9$)	Percent
CD10	6	66.66%
CD117 (c-kit)	7	77.77%
CD34	8	88.88%
S100 protein	8	88.88%
Desmin/vimentin	2	22.22%

The immunostaining profile of endometriosis lesions ($n=9$)

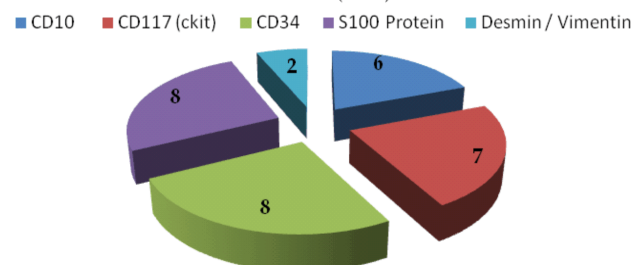


Figure 6 – The distribution of the antibodies at the level of the endometriosis lesions ($n=9/22.5\%$).

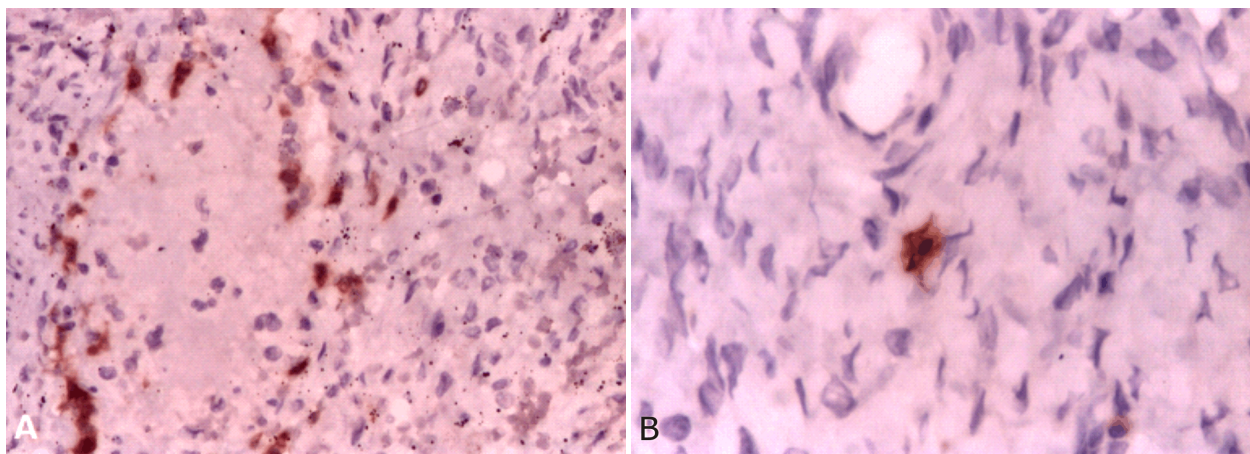


Figure 7 – (A and B) Histopathological aspects from stromal cells positivity for CD10 marker in endometriosis area (A) and CD117/c-kit marker positive in an ovarian endometriosis lesion (B). IHC staining: (A) $\times 200$; (B) $\times 400$.

We have noticed the CD10, an extremely useful marker in the identification of endometriosis lesions in the lack of endometrial glands, was present at the level of the stroma, or periglandular when the glands were present, but negative at the endometrial cysts level, with old hemorrhages. The CD34 antibody, as it was to be expected, became positive at the level of the stroma, of the blood vessels, but also at the periglandular level. The S100 protein was well represented, both at the level of the stroma and in the adenomyosis/endometriosis origin points. The desmin/vimentin were positive in a dissipate manner, predominantly around the blood vessels. CD117 marked rare cells, dissipated, at a distance, or without any topographic link to the adenomyosis/endometriosis lesions.

Discussion

Despite the extensive research performed during the last 25 years, the causes behind endometriosis and adenomyosis are unknown, as well as the reason why they produce pain and infertility. In addition, not least, they are very hard to treat; laparoscopic approach is the first option in the surgical treatment of ovarian endometriosis in adolescents, open surgery is reserved especially for extensive pelvic injuries [8, 9]. Traditionally, adenomyosis is seen as different form endometriosis. It has been said that the two entities come about because of a process of metaplasia. It has also been stated that both affections are the result of the induction of endometrial epithelial cells by the presence of endometrial stroma formed by multipotent pericytes, which can be found intra and extrauterine [10]. There are a few theories [11–20], but none of them explains entirely all of the cases:

- the theory of the retrograde menstruation considers that during the menstruation, the endometrium, stroma and glands, “travels” in other directions: uterine trumps, ovaries, or even pelvic organs (bowel, colon, etc.);
- the theory of the metaplasia considers that even since the fetal stage some abilities are kept in order for the cells to function or act as histological endometrial structures;
- the vascular or lymphatic dissemination considers that the endometrium, because of the blood flow or of the lymphatic system, can get anywhere in the organism;
- the anomalies of the immune system could explain the lack of the destruction process of tissue wrongly situated in the organism;
- and not least, it seems that endometriosis can be inherited.

Endometriosis causes infertility and it is a hard to treat lesion. The research performed in the last years has been focused on the so-called linkage analysis, or reverse genetics [21]. It refers to identifying the genes, which are prone to developing this affection. There are elements, which support the hypothesis of genetic susceptibility in endometriosis: it tends to appear in large families; there are cases of twins with well-rooted endometriosis; it is 6–9 times more frequent in the sisters of the women affected with endometriosis than in the general population; the affected sisters develop the

symptoms at the same age. Thus, the basis of a study project of the endometriosis gene was set (OXEGENE/*Oxford Endometriosis Gene*) in order to identify the gene prone to the development of endometriosis.

It has also been suggested, as ovarian endometriosis is associated to genetic anomalies, and, in many case, with endometrial carcinoma and clear cells, that the bilateral prophylactic oophorectomy in women with a family history of ovarian cancer should be followed by the setting to work of the entire organ, in order to eventually outline the subtle morphological anomalies, which can include ovarian endometriosis [22–26].

Starting from the theory according to which endometriosis is the result of retrograde menstruation, a series of studies have investigated the integrins, the adhesion molecules which fulfill the role of signal molecules in the proliferation and invasion process of endometrial implants. There are three types of peristaltic uterine contractions: cervical-fundus, fundus-cervical, and isthmic, which can change during the menstrual cycle and are controlled by the dominant ovarian structure through the steroid systemic secretion.

The notion of invisible microscopic endometriosis (IME) was created in order to explain the persistence of the symptoms after the surgical intervention for endometriosis. For the experienced surgeons, this notion does not exist. The studies are clear: the presence of IME is closely linked to the laparoscope’s widening power. These criteria are only applied in the case of peritoneal endometriosis. All the endometriosis cases analyzed were at the ovarian level, so that we cannot verify the admission of this notion in the surgical departments from which we chose the study group.

It is well known that endometriosis depends on estrogenic stimulation, especially because of its prevalence during the fertile period [27, 28]. Studies carried out on groups of post-menopausal women, in the absence of endogen and exogenous sexual hormones, have revealed a morphological and immunohistochemical (IHC) profile different in this group, compared to pre-menopausal women [29]. The IHC analysis focused on the estrogen receptor, the progesterone receptor, and CD10. Despite the theory, which claims that the dropping of the estrogen quantity determines, after menopause, the atrophy, regression, and reabsorption of the endometriosis, the studies show that there are no statistical differences regarding the expansion of the lesion in the analyzed age groups [30]. The registered expansion of the disease actually represents the quantity of “lesion” present in the analyzed tissue, and not the macroscopic expansion of the disease, during the surgical act. Also, the density and the intensity of the receptors and of the coloring did not register any statistic differences. This can be interpreted as the fact that the endometriosis deposits of the older patients are still potentially active and can be reactivated, in the presence of an exogenous or ectopic endogen production of hormones. It is possible that the post-menopausal endometrial deposits show proliferative activity and hormonal sensibility, less active than in the case of younger women.

We should keep in mind the fact that ovarian and extraovarian endometriosis present the risk of malignant

transformation, to endometrial adenocarcinoma [31–36]. It is important for clinical doctors to be aware of the fact that endometriosis can also appear post-menopause, given the lack of estrogenic supplements, in smaller quantities than during the pre-menopausal period, but active, with all of the possible serious evolutions, such as becoming malignant.

Endometriosis is categorized according to the severity of the lesions into four degrees. It is considered that women with severe or extensive endometriosis present a high risk of infertility.

It has been stated that the two appear during a process of metaplasia. It has also been said that both affections are the result of the induction of endometrial epithelial cells by the presence of endometrial stroma formed by multipotent pericytes, which can be found intra and extrauterine. Magnetic resonance and ultrasounds have shown anatomical defects in the myometrium of the patients with adenomyosis/endometriosis, which means that the primary defect is a myometric dysfunction [32–35]. Experimental studies carried out have shown that glandular nodules and endometrial stroma do not penetrate the serosa, and that there is at the place of implantation a relative disorder in the arrangement of the muscle fibers, a morphological aspect we too noticed. Thus, it is suggested that the penetration of the endometrial glands succeeds the myometrium's modifications [36–39]. In these areas, one can also notice an infiltrate moderately to abundantly inflammatory for eosinophilia [40]. This presence was noticed even in the endometrial adenocarcinoma cases, and it has been stated that they are involved in the general tissue remodeling [41].

What is interesting is that during the last 20 years, mutations of the tyrosine kinase (KIT) gene were identified. These mutations especially imply the intracellular juxtamembrane domain of the receptor codified by exon 11, followed by the extra cellular domain codified by exon 9. The mutations of exon 11 are heterogeneous, covering especially deletions, amino acid substitutions, or deletions-insertions. The mutations of exon 9 cover especially tandem duplications. In the same context, we find the gene activation mutations, which codify the receptor of the growth factor derived from platelets (PDGFRA), which is a receptor for homologue tyrosine kinase (KIT) [42, 43]. CD10 was shown as being immunopositive only in the cases in which the cytoplasm was colored, and not the nucleus. And only the stromal cells. CD10 is an extremely useful marker for the identification of endometrial stroma, especially in ectopic places. In the presence of endometrial cysts or glands it has no value, being a marker only for stromal cells [44–48].

✉ Conclusions

Our study investigated histopathologically and immunohistochemically a lesion which is not very rare in the gynecology–surgery departments, but which has major implications in pathology: infertility and malignancy. Regarding the immunohistochemical table of the adenomyosis/endometriosis lesions supports, the hypothesis that Cajal cells in this pathology are non-reactive and have no topographic relation to the lesions, and that CD10

is an extremely useful tool in identifying the lesions, when other histological criteria are missing.

Conflict of interests

The authors declare that they have no conflict of interests.

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