

# THE IMMUNOHISTOCHEMICAL STUDY OF ENDOMETRIAL AND ENDOCERVICAL CARCINOMAS USING ANTI-VIMENTIN IN COEXPRESSION WITH ANTI-ER

Adriana RADU, Corina D. FRANDES, O. POP, Mircea SFERDIAN, L. D. STRETCU

"Vasile Goldiș" Western University of Arad, Romania  
Faculty of Medicine, Pharmacy and Dental Medicine

**ABSTRACT.** The uterus is the organ from which numerous epithelial and mesenchymal tumours originate, some of which arise many difficulties when it comes to differential diagnosis. Immunohistochemistry is an important part of the differential diagnosis between endometrial versus endocervical adenocarcinoma, as well as in identifying precursor lesions. Microscopically the two locations present different histopathological varieties. Within our study we analysed the histological characters and immunohistochemical profiles of the endometrioid type of cancer. In the hereby study, the immunohistochemical marking was made using the following monoclonal antibodies: anti – vimentin, anti-estrogenic receptor and progesteronic anti receptor.

**Keywords:** adenocarcinoma, endometrium, endocervix, vimentin, ER/PR

## INTRODUCTION

Endometrioid adenocarcinoma is the most common type of adenocarcinoma of the uterine corpus. The tumors' degree of histological differentiation assures the microscopical image of the tumor in hematoxylin eosin coloring. The proliferation is represented by glands with various aspects, presenting a thin almost inexistent stroma. Some glands present papillary projections containing connective vascular tissue. The papillae are covered in columnar cells. Cribriform aspects can be spotted. In the stroma we can find foam cells (macrophage) encountered in endometrial hyperplasia.

Vimentin is the most important intermediate filament protein of the mesenchymal tissue. This monoclonal antibody is very useful in the differential diagnosis of weakly differentiated neoplasms.

Vimentin is a marker of the mesenchymal origin of the studied tumor, but there are also certain epithelial tumors (carcinomas) that coexpress this marker.

Isolated analyses of the markers can lead to diagnostical errors. No marker can bring important information per se except those indicating hormonal statuses or the presence of viral infection. Therefore this study presents comparative analyses of vimentin immunoexpression and hormonal antibodies. ER and PR antibodies have been used.

ER, estrogenic receptor, is a 67 kDa protein named the alpha estrogenic receptor. The estrogenic receptor gene consists of 140 kDa DNA divided into 8 exons, C- terminus epitope, SP1 clone and nuclear marker. ER immunomarking of paraffin inclusion tissues need a boiling pretreatment in 10mMl citrate tampon, at a 6.0 pH for 10-20 minutes followed by cooling at room temperature for 20 minutes. After that the tissues are incubated with the primary antibody for 30 minutes.

PR, progesteronic receptor, is a regulating protein of the progesteronic receptor. Along with the ER

expression the immunomarking for PR appreciates the hormonal status of the tumor as well as the effect of the hormonal treatment on patients. PR expression is considered a prognosis parameter: epitope 412-566aa; clone: SP2; nuclear marker. PR immunomarking of paraffin inclusion tissues need a boiling pretreatment in 10mMl citrate tampon, at a 6.0 pH for 10-20 minutes followed by cooling at room temperature for 20 minutes. After that the tissues are incubated with the primary antibody for 30 minutes.

## MATERIALS AND METHODS

The hereby study covers a number of 37 patients presenting endometrioid adenocarcinoma of the uterus and 29 patients previously diagnosed with endocervical adenocarcinoma. We tried including the cases diagnosed by bioptic curettage. The cases have been randomized without any criteria of inclusion or exclusion.

A bioptic diagnosis is imposed from the prospective of the treatment plan, chosen by the physician, which can differ by location: cervical or uterine corpus.

In routine coloring (hematoxylin eosin), the histological aspects of the cervical and uterine corpus adenocarcinomas, are very similar.

Some cases allow an accurate differential diagnosis if premalignant histological lesions can be identified (in situ malignant lesions typical for the endocervix, atypical glandular hyperplasia associated with the endometrium).

Microscopically we can observe the gland formation of various shapes and dimensions, delimited by stratified cylindrical epithelium. The nuclei are oriented perpendicular on the glandular layer. The degree of nucleus variation and its pleomorphism depends on the histological differentiation parameter.

We can see the tendency of "back to back" glandular rearrangement in cervical neoplastic

malignant lesions, its direct consequence being the reduction of the interglandular stroma. The analyses of the aspect of the stroma in hematoxylin eosin coloring, reveals a more lax aspect, far from what we consider a desmoplastic stroma.

Peritumoral immune response can be spotted in many cases (lymphoplasmacytic inflammatory cells).

The detail analyses of the malignant neoplastic proliferation areas reveal nuclei discoloration and deformation that varies with the degree of histological differentiation.

The solution for the bipotic diagnosis is the use of monoclonal antibodies following well established algorithms.

Ab – 2 Vimentin, clone V9, undetermined epitope, cytoplasmatic marker. Vimentin immunomarking of paraffin inclusion tissues need a boiling pretreatment in 10mM citrate tampon, at a 6.0 pH for 10-20 minutes followed by cooling at room temperature for 20 minutes.

ER/PR anti hormonal receptor immunomarking is distinguished in normal uterine structures, benign tumors and malignant proliferative processes.

## RESULTS AND DISCUSSIONS

In the case of endometrial adenocarcinomas of the uterine corpus there is an accentuated expression of the vimentin, and in the case of endocervical carcinomas the expression is really low or lacking, fewer than 10%.

The difficulty in interpreting the coexpression resides in the fact that the tumoral stroma is normally positive to vimentin, containing connective tissue, blood vessels, nerves, inflammatory cells and histiocytes.

Vimentin coexpression is highly important in the differential diagnosis between uterine endometrioid carcinoma and endocervical adenocarcinoma, especially when the diagnosis is made on bioptic peaces. In the case of the endometrium there is a high expression in most of the analyzed cases.

In our study we took into account that anti-vimentin immunomarking in effusion liquid that contains carcinomatous cells lead to false positive results in 100% of the cases.

The presence of vimentin expression in these cells, even in the conditions of an immunomarking with high chromogenic intensity, does not present any significance.

As a general rule, anti-vimentin immunomarking location is diffuse, in the cytoplasm.

Isolated analyses of the markers can lead to diagnostical errors. No marker can bring important information per se except those indicating hormonal statuses or the presence of viral infection. The use of the anti-hormonal receptor markers (anti ER/PR) has been intended in the hereby study in order to avoid compromising a diagnosis that can be possible when using a single marker, cytoskeletal. Therefore, anti-

hormonal receptors immunomarking ER/PR can be observed in both normal uterine structures as well as in malignant and benign neoplasms.

The positivity of the reaction is significantly expressed through a nuclear color reaction that has 3 degrees: low, moderate and high intensity.

As a general rule, in the case of endometrioid adenocarcinomas, ER/PR is highly or moderately expressed compared to clear cell carcinomas where the expression is weak and focal.

Our study has identified a weak immunomarking in the case of anti progesteronic receptor monoclonal antibody, compared to the expression of the anti-estrogenic receptor antibody.

The difference consists in the intensity of the expression as well as in the expression index in 1000 counted cells.

In this study we did not pursue a correlation between the presence of progesteronic receptors and an eventual positive evolution of endometrial adenocarcinomas.

In the microscopic examination with a bigger objective (400X) we can observe the heterogeneity of the nuclei expressivity, even in the case of a single tumoral gland examination.

Our study has revealed a limitation in the glandular structures for both immunomarkings anti ER and anti-PR; stromal endometrial elements do not express hormonal receptors.

The expressivity of the hormonal receptors in endocervical adenocarcinoma, reveals, within our study, a weak presence in the number of positive nuclei as well as in intensity.

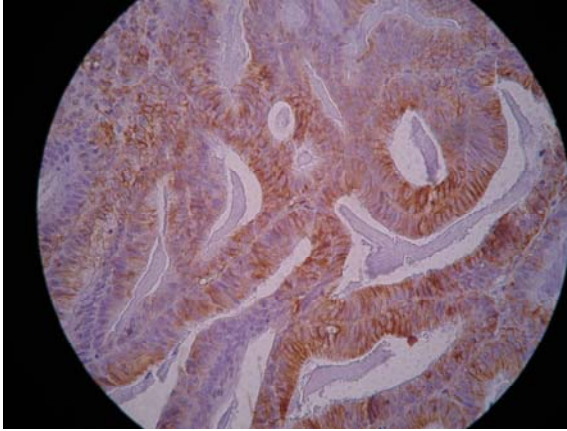
The analyses of endocervical adenocarcinomas revealed a great number of positive hormonal receptor cells in the normal stroma, peritumoral, compared to neoplastic glandular structures.

In the aforementioned case you can see, in the right part of the image, positive nuclei of the stromal cells presenting high chromogenic intensity. According to FIGO (International Federation of Gynecology and Obstetrics) there is a variation in expression according to the histological grading: grade 1 and 2 of endometrioid adenocarcinoma of the uterine corpus express ER/PR and vimentin, whereas most cervical adenocarcinomas are negative for ER/PR.

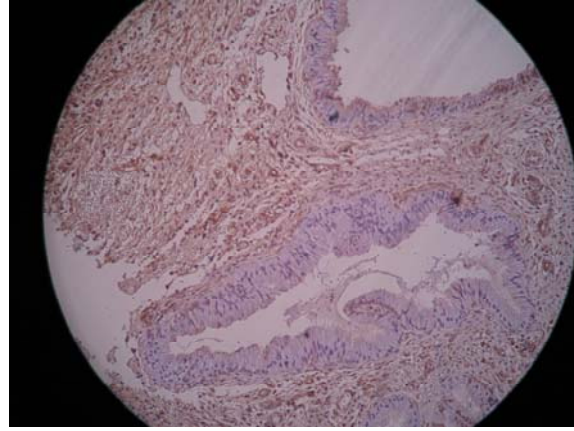
The expression of estrogenic receptors in the case of uterine adenocarcinoma is generalized. ER intensity is high and as a general rule in our study it has been correlated with the immunomarking expressivity for the progesteronic receptor and immunohistochemical expressivity of the vimentin.

ER immunomarking interpretation was realized only for the nuclear expression of the antigen – antibody reaction.

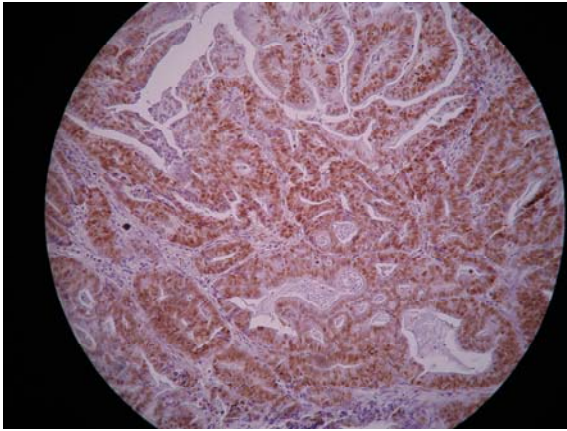
Not all cases in our study or those that presented ER positive reaction expressed progesteronic receptors.



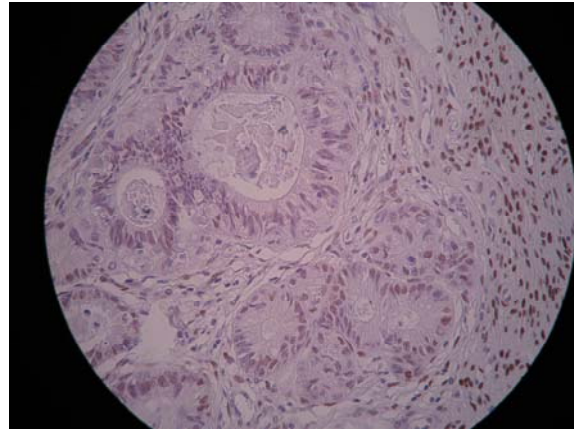
**Fig. 1** Adenocarcinoma of the uterin corpus; vimentin, x200



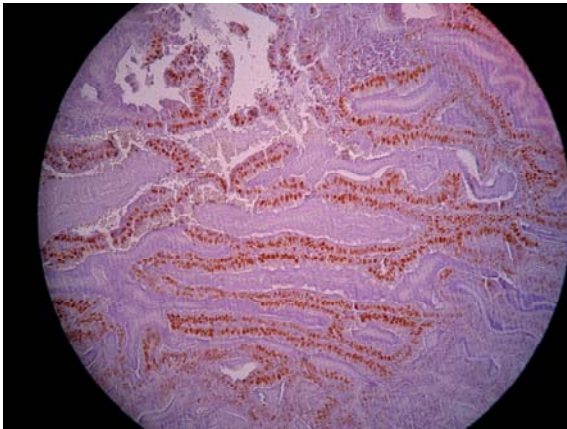
**Fig. 2** Adenocarcinoma of the uterine corpus; vimentin, x100



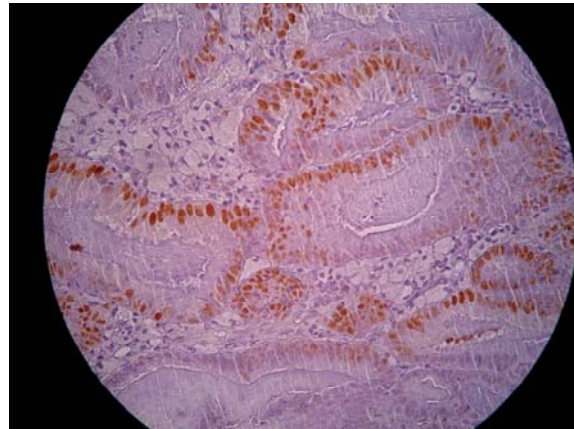
**Fig. 3** Adenocarcinoma of the uterine corpus; ER x100



**Fig. 4** Adenocarcinoma of the uterine corpus; ER x100



**Fig. 5** Adenocarcinoma of the uterine corpus; PR x100



**Fig. 6** Adenocarcinoma of the uterine corpus; PR x400

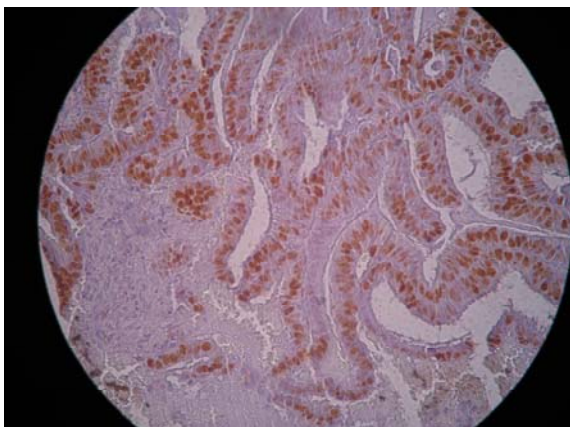


Fig. 7 Adenocarcinoma of the uterine corpus; ER x200

### CONCLUSIONS

Vimentin coexpression is crucial in the differential diagnosis between the endometrial carcinoma of the uterus and endocervical adenocarcinoma especially when the diagnosis is based on cervical biopsies and bioptic material.

Isolated analyses of the markers can lead to diagnostic errors.

The precise differential diagnosis can be reached by using immunohistochemical marker panels (vim, ER, PR).

Our study has revealed the immunohistochemical differences of the tumoral stroma from benign neoplastic lesions compared to the malignant ones.

### REFERENCES

- Soslow R.A., Isacson C., Diagnostic immunohistochemistry of the female genital tract, în: Dabbs DJ: Diagnostic Immunohistochemistry, Churchill Livingstone, Philadelphia: 486-516, 2002.
- Soslow R.A., Isacson C., Zaloudek C., Immunohistopathology of the female genital tract In: Dabbs D, Diagnostic immunohistochemistry, ED DJ Dabbs second edi Churchill Livingstone Elsevier, 2006.
- Sternberg S. S., Diagnostic Surgical Pathology, Raven Press, 1994, vol. 2.
- Frandes D.C., Contributii la corelatiile morfofiziologice la nivel placentar in prematuritate si tulburari de dezvoltare, 2003.
- McCluggage W.G., Recent advances in immunohistochemistry in gynaecological pathology, *Histopathology.*; 40(4):309-26, 2002.
- McCluggage W.G., Sumathi V.P., McBride H.A., Patterson A., A panel of immunohistochemical stains, including carcinoembryonic antigen, vimentin, and estrogen receptor, aids the distinction between primary endometrial and endocervical adenocarcinomas. *Int J Gynecol Pathol* 21:11-15, 2002.