Immunohistochemical aspects of endometrial glands in dysfunctional uterine hemorrhage

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Abstract
The authors present a specific aspect of the modifications of the endometrium in dysfunctional uterine hemorrhages that is the behavior of the endometrial glands. These glands are studied from a immunohistochemical point of view, regarding both the normal endometrium (inclusively at the age of two years) and the endometrium in dysfunctional uterine hemorrhages. The antigens used were VGEF and PCNA. The result was a different reaction of the glandular structures to these antigens in the cases of patients with dysfunctional uterine hemorrhage.

Keywords: dysfunctional uterine hemorrhage, endometrial glands, VGEF and PCNA antigens.

Introduction
Battistini M (1997) defines dysfunctional uterine bleeding as being an abnormal bleeding, usually in excessive quantity, indicating a disorder of the normal ovulatory function, which is more often associated to anovulation [1].

Another definition of dysfunctional uterine hemorrhages states that they are hemorrhages proceeding from the uterine cavity, which supervene independent of pregnancy, excluding an organic cause of the uterus or annex organs and very often (if not always) being the result of a sex hormone disorder [2].

In order to study the endometrium of patients with DUH, it is necessary to analyze the endometrial glands from an immunohistochemical point of view.

Material and Methods
The antigens I had the occasion to study were VEGF (Vascular Endothelial Growth Factor) and PCNA (Proliferating Cell Nuclear Antigen). Thus, we used anti-VEGF and anti-PCNA antibodies.

The reason for which we used these two antibodies in our study consists of the need to intercept and eventually elucidate the molecular mechanisms that might determine the lesions noticed by means of optical microscopy of the endometrial glands.

VEGF
VEGF is a growth factor, which functions as a powerful mitogen both for the endothelial cells of the capillaries and for the cells of the arterioles, veins and lymphatics, but which does not show a specific mitogenic action regarding other cell types.

VEGF is a growth factor produced by many in vitro cell lines and in vivo tissues, but VEGF-receptors have only one target cell: the endothelial cell. Another biological effect of VEGF is vasodilatation [3].

Because of these biological effects, it is implied in physiological angiogenesis as well as in inflammatory pathological aspects or in those that accompany tumor proliferation [2].

PCNA
PCNA is a 36-kD molecule, which functions as a cofactor for DNA-polymerase delta; present in cell nucleus in S-phase and in late G1-phase, having some levels detectable also in M-phase of nuclear division, it is a long-living protein [2].

Immunohistochemical expression of PCNA can be induced both in vivo and in vitro by growth factors.

The endometrial samples studied in the present chapter were drawn from a group of 31 patients with DUH aged 18- to 45-year-old. These endometrial samples were obtained by means of uterine curettage or biopsy by hysteroscopy.

In order to compare the results we had as control sample the normal adult endometrium without co-affection of another gynecologic pathology, being in one of the phases of physiological evolution (early secretory phase), as well as endometrium drawn from a 2-year-old patient (who was performed total hysterectomy due to another affection which was juxta-uterine).
Working technique

The working method may be schematized as following:

▪ Tissue fixation;
▪ Hydratation;
▪ Paraffin inclusion;
▪ Block sectioning;
▪ Immunohistochemical reaction by means of Avidin–Biotin Complex (ABC) method.

The groups studied were divided into three categories:

▪ One case of a 2-year-old little girl, whose uterus was extirpated due to a juxta-genital affection;
▪ One group of five patients who were performed endometrial biopsy for investigational aim, which were subsequently confirmed to have a normal endometrium;
▪ One group of 20 patients diagnosed and confirmed with DUH.

Results

In the endometrium of the 2-year-old baby, both the reaction to PCNA and that to VEGF were found negative regarding all the endometrial structures: the covering epithelium, stromal cells, blood vessels (Figures 1 and 2).

In the normal endometrium, having histological characteristics that appoint to early secretory period, the anti PCNA antibody presents positivity regarding all the structures, endometrial glands inclusively.

What is notable is the difference of the reaction intensity to anti PCNA between epithelial cells belonging to the same gland or between epithelial cells belonging to different glands (Figures 3 and 4).

Figure 1 – The endometrium of the 2-year-old baby. There can be observed negative reaction to PCNA regarding all the endometrial structures (oc. ×10, ob. ×10).

Figure 2 – The endometrium of the 2-year-old baby. All the endometrial structures are negative to VEGF (oc. ×10, ob. ×20).

Figure 3 – Normal adult endometrium in proliferative period. Epithelial cells in endometrial glands are PCNA+ (oc. ×10, ob. ×20).

Figure 4 – Nuclei in course of division in endometrial glands. There can be observed the difference of intensity of the positive reaction to PCNA, between nuclei that belong to the same gland (oc. ×10, ob. ×100).

In all the cases of DUH patients studied the reaction to PCNA was negative, in endometrial glands.

Histological aspects examined in DUH correspond to the early proliferative phase, or late proliferative one (Figure 5) as well as to the secretory period (Figure 6).

In normal endometrium of fertile women, VEGF has positive reaction of the glandular epithelial cells. Thus, the positivity of glandular epithelial cells is stable during the entire endometrial cycle period, both in the proliferative period (Figure 7) and in the secretory one (Figure 8). Regarding endometrial glandular cells, the reaction is more intense positive and can be observed on
more extended areas than in similar structures of the normal endometrium (Figure 9). There are also stromal cells that have a positive reaction to VEGF in DUH (Figure 10).

![Figure 5 - Endometrial gland in DUH in proliferative period. Both glandular cells and stromal cells are PCNA- (oc. ×10, ob. ×40).](image1)

![Figure 6 - Endometrial stroma and secretory period gland in DUH. Reaction to PCNA is negative (oc. ×10, ob. ×20).](image2)

![Figure 7 - Normal adult endometrium. VEGF+ reaction of glandular epithelial cells in proliferative period (oc. ×10, ob. ×20).](image3)

![Figure 8 - Normal endometrium. Endometrial glands that are in secretory period have VEGF+-reaction (oc. ×10, ob. ×20).](image4)

![Figure 9 - DUH endometrium in proliferative period. There can be observed the intense positivity to VEGF of glandular cells (oc. ×10, ob. ×10).](image5)

![Figure 10 - Secretory endometrium in DUH. Endometrial glandular cells and some stromal cells have a characteristic VEGF+-reaction (oc. ×10, ob. ×40).](image6)

**Discussion**

In the endometrium of the 2-year-old baby, both the reaction to PCNA and the reaction to VEGF were found negative regarding all the endometrial structures: covering epithelium, stromal cells, and blood vessels.

This is a normal situation, which can be explained by the lack of hormone secretion (estrogens-progesterone), which acts on specific receptors in the endometrial structures.

In the normal endometrium, having histological characteristics that appoint to early secretory period, anti PCNA antibody presents positivity regarding all the endometrial structures.
Thus, in endometrial glands there are either isolated nuclei or nuclear stripes belonging to neighboring epithelial cells, with nuclear positivity.

What is remarkable is the difference of reaction intensity to PCNA between epithelial cells belonging to the same gland or epithelial cells belonging to different glands. Besides the process of nuclear multiplication in endometrial glandular cells, the nuclei having positive reaction to PCNA, this was also observed in endometrial stroma.

This fact corresponds to literature data, as it is known that the endometrium in proliferative period of an adult woman with normal menstruation is under the influence of estrogen hormones. Initially, they determine postmenstrual endometrial reconstruction and then, by numerous mitoses, stromal growth by fibroblastic cell multiplication [4].

In all the cases studied regarding patients having DUH, the reaction to PCNA was negative, in endometrial glands.

It is very important the fact that histological aspects examined correspond to early proliferative phase or late proliferative phase, as well as to the secretory period.

Therefore, whether the ovulation is produced or not PCNA is negative in DUH, thus having immunohistochemical aspects similar to the endometrium of the 2-year-old baby, although hormone constellation is different.

Specialty literature (Cameron ST et al., 1996) analyses, from the point of view of immunohistochemical reactions to PCNA and Ki-67 biopsies drawn from women who were administrated mifepristone (RU486 – antiprogestative); in some cases the treatment determined ovulation, while in some other cases it did not [5].

Another group was represented by women who repeatedly had anovulatory cycles because of polycystic ovary syndrome [6].

In the case of women having anovulation due to polycystic ovary syndrome, the existing nuclear proliferation markers (PCNA și Ki-67), as well as endometrial receptors to estrogenos and progesterone, while they lacked in the case of women treated with antiprogestrone. Therefore, antiprogestrone might influence the provenience of uterine hyperplasia, by means of an antiproliferative mechanism, both by blocking mitotic cycle of the cells and by inducing the loss of estrogen receptor in endometrial cycles. The role of the disorder induced by anovulation is partially accepted in DUH etiopathogeny a fact which was also confirmed by the histological aspects that we found in DUH, corresponding both to the proliferative period and the secretory period [7–10].

That is why we consider that the lack of positive reaction to PCNA in DUH cannot be determined only by the presence or absence of ovulatory cycle. Hormone disorder might have an indirect influence over the uterine receptors, deterring hormone-dependent cells to begin the division process [11].

In normal endometrium belonging to fertile women, VEGF has a positive reaction of the glandular epithelial cells.

The results that we obtained correspond to those in specialty literature [12]. In normal endometrium there can be observed an intensity modulation of the immunohistochemical reaction to VEGF in the various endometrial structures [13].

Thus, the positivity in glandular epithelial cells is stable during the entire endometrial cycle period, both in the proliferative period and in the secretory one.

The increase of positive reaction to VEGF from the beginning towards the ending of the proliferative phase is collateral to the increase of the estradiol level [12].

The authors suggest that VEGF might serve as a paracrine mediator for the stimulation of vascular increase produced by the ovarian steroids [14].

The expression of VEGF in DUH endometrium has a different pattern in comparison to that of the normal endometrium.

Regarding endometrial glandular cells, the reaction is more intense positive and can be observed on more extended areas than in similar structures of the normal endometrium.

There are also stromal cells that have a positive reaction to VEGF in DUH. What is interesting is the fact that, unlike the normal endometrium, where antigenic expression is, as previously demonstrated, modulated according to the phases of the endometrial cycle, the reaction to VEGF in DUH is maintained constantly positive in glandular cells [3].

Greb RR et al. (1997) show that the production and expression of VEGF in the case of normally menstruated primates is influenced by steroid hormone receptors of the endometrial cells [15].

The antagonist mifepristone (RU486) of progesterone leads to the abolition of immunohistochemical positive reaction to VEGF [16].

The situation is different in the DUH cases we studied, which pertained both to the proliferative phase and to the endometrial secretory phase. Our results can be explained not only by the modification of the estrogen-progesterone hormone equilibrium in the case of women having DUH [17].

Conclusions

The reaction to PCNA as well as the reaction to VEGF is negative in what concerns the endometrial structures at the age of two years. The reaction to PCNA is positive in what concerns endometrial glands of the normal endometrium and constantly negative on the level of endometrial glands of DUH patients. The reaction to PCNA is positive in what concerns both the glands of the normal endometrium and the glands of DUH patients, in all the phases of the menstrual cycle.

References


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