A study regarding the treatment of cervico-vaginal infections and precancerous conditions of the uterine cervix with Cervugid ovoules.

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Overview

The cervico-vaginal infections are one of the female morbid conditions that account for the most frequent gynecologic visits and have deep implications in the health status and social life. Thus, vaginitis may cause serious shortcomings through local manifestations (discomfort, burns, pricks, pain, smelly leaks a.s.o.), sexual disturbances, missing days from work or school.

Also, genital infections often alter the functional status of other parts of the reproductive apparatus (pelvic and parietal viscera), and in the pregnant woman they are frequently involved in premature rupture of the membranes, amniotic infection, premature birth, low weight newborn at delivery; septic post-operative complications are much more frequent, both in pregnant and non-pregnant women, should the patient exhibit an untreated cervico-vaginal infection (1).

Heavy progress has been recorded lately in the treatment of cervico-vaginal infections, by the issuance of numerous pharmaceutically active substances, as well as by broadening the knowledge on the pathogenic and physiopathologic mechanisms of infections generally, and genital infections specifically.

This work presents a study on a new drug, Cervugid Ova, endorsed by tge National Drug Agency in 2001, which combines the anti-inflammatory action of chloramphenicol, metronidazole and nistatin with the action of acetate hydrocortisone, unspecific anti-inflammatory drug, all of them included in a synthetic fat of Lipex-403. All these three anti-inflammatory substances of this drug - chloramphenicol, metronidazole and nistatin – cover the entire vaginal pathogenic flora: germs, protozoa, fungi, as well as a group of infra-microbial agents – chlamydia, mycoplasma and rickettsia, constantly sensitive to chloramphenicol.

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Because the cytological test is currently expressed in the Bethesda system, in view of the correct understanding and interpretation of a cytological outcome in the sight of the new terms and data of the Bethesda system, and also in view of a proper, effective and responsible approach, we thought necessary that in this introductory part one also presents items on reporting a cytological test in the Bethesda system; these items shall better identify the place of the above mentioned drug, for the treatment of the inflammatory processes where the Human Papilloma Virus (HPV) is involved.

One has also overviewed a series of essential data regarding the vulvo-vaginal inflammations, from the present dedicated literature, outlining that local infections caused by miscellaneous agents certainly have an important role in easing the HPV infection, a fact that is distinctly treated by some authors (2, 3, 4).

The data on the colposcopic examination of the uterine cervix make an end to the overview in this part of the work.

I. Since the performing in 1926 of the first cytological test by Aurel Babeş, its standardization in the 5 classes by G. Papanicolaou in 1928, and until today, the nomenclature, interpretation, the means of reporting and analyzing of the cytological test have undergone a series of modifications. Thus, we remind the classifications of the German and British cytology schools, followed by the adoption of the Bethesda system in 1989 and its revision in 2001 (5). It is here one should tell about the classification of the potentially invasive intraepithelial histological lesions under the name of cervical intraepithelial neoplasia CIN I, II and III (CIN comes as an acronym from Cervical Intraepithelial Neoplasia) by Richart in 1967, reviewed in 1990.

But, regardless the school one interprets and reports the cytological test by, regardless the fore and aft opinions on the efficiency of one or another of the above mentioned classifications, what is important to keep in mind is that a properly sampled and correctly interpreted cytological test may indicate in an overwhelming proportion the presence of a precancerous or cancerous development at the level of the uterine cervix.

Most of the world's medical schools has adopted the interpretation of the cytological test in the Bethesda system, a system by which the test is clearly expressed, standardized, in definite terms, without any ambiguity or confusion, which allows for the optimization of the diagnosis in view of an adequate treatment should the cytology rate positively.

The Bethesda terminology, in its last and final review, has been adopted in 2001, when the National Cancer Institute of the US has conducted a study regarding the quality of the HPV testing in patients with ASCUS and LSIL cytology ("The atypical Squamous Cells of undetermined Significance – Low grade intraepithelial Lesion Triage Study"), also known in the literature as ALTS. Some of the recommendations from this study have been used in this paper as well. The 2001 Bethesda terminology set up after the above mentioned ALTS study was the result of a fruitful cooperation of clinical, forensic and cytology experts, which allowed the enhancement of presenting the cytological outcome that allowed for ambiguity or equivocal interpretation.

Thus the term of atypical squamous cells that cannot exclude a high grade intraepithelial lesion has been introduced (Atypical Squamous Cells – cannot exclude HSIL), expressed by the acronym ASC-H, synonymous with Atypical squamous cells of undetermined significance (ASC-US). This includes atypical metaplasia, thick tissue fragments, nuclear irregularities, hyperchromia, and scarce cells with a high nucleus/cytoplasm ratio.

Below there is a classification based on this system, which includes anomalies of the squamous cells on the exo-cervix and of the glandular cells on the endo-cervix (44).

<u>The anomalies of the squamous cells include</u>:

1. ASC – atypical squamous cells:
    - Of undetermined significance ASC-US
    - Without the possibility of ruling out a high grade intraepithelial lesion ASC-H
2. Low grade squamous intraepithelial lesions LSIL, including HPV infection / light dysplasia / CIN 1.
3. High grade squamous intraepithelial lesion HSIL, including moderate and severe dysplasia, CIS, CIN 2 and CIN 3.
4. Squamo-cellular carcinoma.

<u>The anomalies of the glandular cells include</u>:

1. AGC – atypical glandular cells:
    - Endocervical (NOS or context specified)
    - Endometrial (NOS or context specified)

- Glandular cells (NOS or context specified)

2. Atypical glandular cells in favor of an endocervical neoplasm or with no other specification.
3. In situ endocervical adenocarcinoma (AIS)
4. Adenocarcinoma:
   - Endocervical
   - Endometrial
   - Extrauterine
   - With no other specification (NOS)

It is today unanimously accepted by the international scientific community, based on many epidemiologic, morphologic and molecular biology evidence, that the etiological agent of the uterine cervix cancer and its precancerous conditions is the Human Papilloma Virus (HPV), but only those breeds of high oncogenic risk, as seen below.

The evidence that sustain the positive correlation between the HPV infection and the uterine cervix cancer (3) are as follows:

1. The DNA of the HPV is almost 100% present in both primary cervix cancer and its metastases (6).
2. Te cases where biopsies show cytological modifications type CIN I, II and III feature the DNA of the HPV in more than 75% (7, 8).
3. The HPV DNA shows negative in those cases where the cytological test or the histological section features no cellular anomalies.
4. The existence of a chronologic sequence, meaning the infection of high oncogenic types of HPV is followed by histological alterations on the squamous epithelium (9, 10, and 11) that may be outlined through different stages of cervical intraepithelial neoplasia (CIN).
5. The patients that show positive on the HPV tests have a 3.8 higher chance to develop a CIN I and a 12.7 higher chance to develop a CIN II or III than HPV negative women (11). In the same spirit, the women infected with types 16 or 18 have a 38% chance to develop a CIN II or III in the next 2 years (10). In patients with a LSIL histological diagnosis, the HPV testing proved positive in more than 82.9% of all cases. Also, the association of ASC-H as cytological substrate and CIN

II as histological substrate is lower than the association with HSIL, but higher than the association within the ASCUS category, which recommends an increased attention for the ASC-H cyto-test regarding case surveillance and test repeats (5). One reckons that almost 5 – 10% of the ASCUS cytological tests can be included in the ASC-H category, which comes as guidance that in this group one can include a series of high degree lesions that may have a histological correspondence of CIN II or CIN III (5).

6. The HPV mostly causes squamous cancer, to which it is most often associated, and much rarely with cervical glandular cancer (adenocarcinoma).

Among over 100 types of HPV, the viral typing showed that, according to the association with the squamous cancer, only the breeds 16, 18, 31 and 45 are thought to be of high oncogenic risk, and the breeds 33, 35, 39, 51, 52, 56, 58, 59 and 68 are considered to be of an intermediate risk, followed by low oncogenic risk breeds 6 and 11, and others.

Only 5% of the infections with high oncogenic risk breeds can cause cervical cancer, while for the intermediate risk breeds the percentage varies from 1 to 5% (12).

As suite of the HPV specificity, the literature data show that the high grade lesions are determined by the types of viruses with high oncogenic risk; therefore they might appear de novo and are not a consequence of a successive evolution of low grade lesions caused by intermediate risk virus breeds.

Here one spots a discrepancy in that that if in the case of CIN histological lesions one admits a progressive evolution from CIN I to CIN III, and they are not necessarily caused by virus breeds with high oncogenic risk, it is then possible that low grade lesions feature an evolution to high grade lesions.

Low grade squamous intraepithelial lesions (LSIL) are represented by the cellular alterations characterized by the HPV's cytopathic effect of koilocytosis, and they correspond histologically to light dysplasia or CIN I. These lesions are the outcome of the infections with HPV of different oncogenic potentials. The morphological expression features a productive inflammation with HPV that has as main characteristic the expression of its own cytopathic effect. This effect presents itself with vacuolization of perinuclear cytoplasm, thickening of the cell membrane, nuclear atypia (nucleus of increased volume, increase of nucleus / cytoplasm ratio,

irregularly shaped nuclear membrane, hyperchromia) and anisocytosis. The cytoplasm vacuolization and nuclear atypia, when they appear combined, are known under the name of koilocytosis.

Along with these cellular alterations one also describes architectural alterations of the epithelium, characterized by hyperplasia of its own basal and para-basal cells. Hyperplasia may take different aspects, among which acanthosis and papillomatosis are the most common. Acanthosis (multiplication of the cell rows in the malpighian layer) leads to a moderate thickening of the squamous epithelium, with a waved and slightly bounced surface, which Meisels describes as flat condiloma. When the cytopathic effect of acanthosis of the HPV infection begins to involve the endocervical glands, the aspect becomes that of an inversed condiloma, which is neophytic.

These cytopathic effects of the HPV infection are the same for CIN I and for plane condiloma, and subsequently these notions have been integrated into LSIL.

It is unanimously acknowledged that the HPV infection is got through the sexual way. Considering the viral types involved in carcinogenesis, as well as the receptivity degree of the host cells, influenced on its turn by a series of factors (the co-carcinogenetic factors), the literature data (14) showed that the HPV infection may evolve in one of the following situations:

- The virus persists in the host cell in an episomal form, without getting integrated into the cell's genome; in this situation the objective cytological, colposcopic or histological alterations are not present, yet the woman is positive to the HPV testing.
- The virus replicates within the cell, yet it is not integrated into the genome of the host cell, but realizes a productive infection expressed by low grade squamous intraepithelial lesions and / or formation of benign condiloma.
- The virus gets integrated in the genome of the host cell and leads to the inactivation of tumor suppressing mechanisms, and then manifests itself by the malignant transformation outlined cytologically by high grade squamous intraepithelial lesions (HSIL), and histologically by CIN II or CIN III.

Actually, in this last situation, the phenomena have the following evolutionary succession: after the infection with a type of virus of high oncogenic risk, there is an

incubation period between 4 to 6 weeks and 8 months. As suite of the immune response, most of the women heal without developing cytological (HSIL) or histological (CIN) alterations. The other patients have been classified in two groups: one group that remains with a latent infection, thus being potentially contagious to new sexual partners, and a second group with persistent infection, that after an apparent clinical remission or healing, evolve cytologically to HSIL or histologically to CIN III.

II. Vaginites manifest by a series of unspecific symptoms, such as local discomfort, pain, pricks, dysuria, itching, abnormal and bad-smelling leaks. They may associate with other sexually transmitted diseases among which the HIV, and may determine complications in the normal evolution of a carriage, or post-partum and post-abortum complications, or postoperative septic complications in the genital area.

By ACOG (American College of Obstetricians and Gynecologists – Clinical management Guidelines for Obstetricians-Gynecologists, Practice Bulletin, May 2006), vaginites are determined by three major causes:

- Bacterial vaginosis, known until recently as unspecific vaginitis (22 – 50%);
- Vulvo-vaginal candidosis (17 – 39%);
- Genital trichomoniasis (4 – 35%).

The bacterial vaginosis, mainly determined by *Gardnerella vaginalis* (anaerobic gram-negative coco-bacillus also called *Corynebacterium vaginalis* or *Haemophylus vaginalis*), comes to illustrate a syndrome caused by a mixed aerobic and anaerobic flora, accompanied by a minimal response of the vagina. This syndrome is characterized by a rupture in the balance between the normal prevailing flora of Lactobacillus species and the displacement of this balance to a mixed pathogenic vaginal flora dominated by *Gardnerella vaginalis*, Bacteroides, Mobilunculus, Mycoplasma and peptococci (29, 30). As suite of a very high incidence of the germ *Gardnerella vaginalis* in the etiology of bacterial vaginosis, this is by some authors synonymous to the actual infection with *Gardnerella vaginalis* (15).

The diagnosis of bacterial vaginosis is established on the following specific criteria, proposed by Amsel (17, 18):

- Homogenous, white-grey vaginal leak;
- Vaginal pH greater than 4.5;

- Emanation of a bad fishy smell from the vaginal secretion, when it is treated with an alkaline solution of KOH 10%, due to the presence of abnormal amines in the vaginal fluid;
- Presence of cells with a specific morphological aspect, known as indicator cells or 'clue cells'.

Due to the fact that, by Amsel's criteria, one can reach to an overbid of the diagnosis of bacterial vaginosis, one simpler considers that the diagnosis of bacterial vaginosis could be determined by the criteria of Nugent and Spiegel on Gram stained smears, method which proves more practical and useful for perambulatory diagnosis and treatment (31, 32).

By Nugent and Spiegel criteria, the bacterial vaginosis is confirmed if, on a microscope field x1000 with immersion, the number of lactobacilli is lower than 5, along with 5 or more *Gardnerella vaginalis* and with 5 or more other morphological types (Gram-positive cocci, Gram-negative bacilli, Gram fusiform bacilli).

If the microscope field x1000 with immersion features more than 5 lactobacilli, and the number of the other morphological types is 5 or smaller, the Gram staining may be looked at as normal (31, 32).

The vulvo-vaginal candidoses mainly determined by *Candida albicans*, but also by other types of fungi, have been classified according to the occurrence circumstances in uncomplicated and complicated vulvo-vaginal candidoses (1).

The uncomplicated vulvo-vaginal candidoses include candidoses with moderate manifestations, rare or seldom infection, without medical complications, candidosic infection suspected.

The complicated vulvo-vaginal candidoses include severe symptom candidosis, recurrent candidoses with 4 or more annual episodes, proven candida infection, women with diabetes or other severe medical conditions, immune suppression a.s.o. This classification is important in view of establishing an individualized treatment.

The vaginal trichomoniasis is a sexually transmitted disease, having as specific etiological agent the *Trichomonas vaginalis* protozoon, and its clinical signs range from asymptomatic conditions to severe local manifestations.

The incidence of different kinds of vaginites has altered during the last years; thus, the trichomoniasis is less frequent than times past (15), and the most common causes remain the bacterial vaginoses and candidosis (16). It is thought that almost 50

– 75% of women have had during their life at least one candidosic vaginites, either by the diminishment of the local defense capacity, or by the alteration of proportions of the normal constituents of the vaginal flora. During the reproductive period, the bacterial vaginosis is the most frequent.

III. It is known that colposcopic data that may suggest a high grade lesion (major modifications) are concurrent for a high grade lesion outlined cytologically or histologically under 70 – 80% of the cases, which indicates a specificity similar to that of the high grade cytology for the CIN II or CIN III lesions. This is the source for the essential grounding to maintain the colposcopy as "essential technique for depicting high grade CIN lesions" (5). Minor modifications are not specific and they may correspond equally to a low grade lesion or a metaplasia.

The colposcopy has the role to check the presence or absence at the level of the uterine cervix of precancerous conditions, by revealing major colposcopic modifications, suggestive for a high-grade lesion: Lugol test negative on a former high acetic-white surface, a smooth surface with regular exterior contour, intense acetic-white reaction that appears fast and disappears slow, coarse or fine pointing, irregular mosaic, coarse with different mesh size (atypies of the vascular design), partially iodine-positive areas, acetic-white area on the cylindrical epithelium zone.

Colposcopic, too, one evidences suggestive images for the invasive carcinoma: irregular surface with erosions or ulcerations, area with an intense acetic-white reaction, with pointing or mosaic, highly irregular, and atypical vessels.

Since the cytological outcome may be discrepant with the data coming from the colposcopy, in that that the cytology gives a result classified as being one that raises serious questions, while a good result in colposcopy has a very strong negative predictive value on the cytological exam, the patient needing only annual monitoring by cytology checks.

So, only by combining cytological, colposcopic and histological data, one can establish a final diagnosis and an appropriate conduct.

Work material and method

Among several hundred thousand cases that were treated with Cervugid Ovules since the market release of the drug, this study only reports to a set of 500 patients aged between 18 and 60 years, diagnosed with vaginitis in the specialty practice in Municipal Polyclinic #1 Iasi.

The patients featuring intolerance to one of the product's components have been excluded from the lot, as well as all pregnant and milking women, and those with systemic conditions where the treatment was incompatible with the general status.

The treatment consisted of the intravaginal administration of a Cervugid ovule, at evening before bed, for 12 days, after which another two packs (according to the local conditions – aspect of the cervix, intensity of the inflammatory reaction etc.) have been given, each of 12 ovules, with a break of 7 days in between. The data below come to give extra explanation on the duration and doses used.

Sample taking from the patients considered:

1. Assessment of the cyto-test and of the precancerous lesions of the cervix
2. Microbiological assessment
3. Colposcopic assessment of the uterine cervix.

1. Assessment of the cyto-test and of the precancerous lesions of the uterine cervix

The obtaining of a quality smear in view of an optimal cytological assessment is extremely important, considering that almost 50 – 70% of the false negative results are owed to sampling errors. This is why one took into account the necessity that the smear contains all types of cells: exo-cervical squamous cells, endo-cervical cylindrical cells, and squamous metaplasic cells.

Besides, the presence in the smear of the cells in the transition area (situated at the limit between exo- and endo-cervix, where the squamous epithelium of the exo-cervix continues with the cylindrical epithelium of the endo-cervix), assures us the fact that this area has been sampled, and constitutes an important indicator of the quality of the sampling process. For this reason, when the performance of such investigation was decided, a series of recommendations has been set forward to the patients, being of an extreme importance for having a good quality cyto-test: avoidance of sexual contact for 48 hours prior to the sampling, vaginal lavage, application of the medication intravaginal exclusively, intravaginal shower or the use of tampons.

The sampling was performed with the observance of the following technical details:

1) Visualization of the uterine cervix, so that it got better exposed and the entire transition area to be well razed for the smear;
2) After the insertion of vaginal speculum or of a pair of non-lubricated valves of suitable size, the cervical mucus was eliminated by the use of a sterile cotton wool tampon, and then one proceeded to the sampling of the cyto-test before any other maneuver (such as a Schiller test or a vaginal digital examination).
3) One considered the fact that, for the sampling to be correct, one needs to sample cells from the exo-cervix, endo-cervix and cells between these two areas, that is the transition area, therefore the area with a maximum risk for the development of uterine cervix cancer.
4) As sampling devices, the following have been used:

- Ayre plastic spatula for large cervices of the multi-pregnant women;
- Aylesbury plastic spatula with a sharpened prolongation on one end, for the endo-cervix (19, 20);
- The Cervex brush for the narrow endo-cervix at menopause, the post-conization cervix, situations where the Squamo-cylindrical junction is not visible (21).
- The Rovers type Cervex brush with longer central bristles for the endo-cervix and shorter peripheral bristles for the exo-cervix.

During the execution of the sampling operation, both brushes and spatulas were completely rotated in the same direction, several times, after being placed with the central bristles or the sharpened end in the endo-cervix.

5) After sampling, the sample material was laid on a blade or two by rotating the endo-cervical brush or by application of the cellular material from the both sides of the spatula. Smearing was done on three distinct transversal areas, for the cells sampled separately from the endo-cervix, exo-cervix and the upper third of the vagina. One considered the execution of a thin and uniform layer, so that fixation and staining could be done appropriately.

6) Immediately after the above operations were performed, one proceeded to the fixation of the blades in 95% alcohol for 30 minutes, or with a fixating aerosol of polyethylene-glycol applied from 15-20 cm to the blade, at an angle of 45 degrees towards the blade. The blades were separately laid in the alcohol bath so that they did not touch one another and to ensure proper circulation of the fixation agent.
7) After the fixation of the blades in alcohol, one proceeded to their marking by writing the identification data of the patient in the sanded part of the blade. The blades were accompanied by an analysis sheet where one wrote the patient's identification data as well as some data from her personal pathological and physiological antecedents, which have the role to provide the cytologist with valuable information regarding the patient.
8) Staining of the smears was done by the Papanicolaou method, which gives an increased cell transparency, clear visualization of fine structures, including areas with overlapped cells, mucus or cell residues.

2. Assessment of the vaginal flora.

The study of cervico-vaginites addressed their three main causes: bacterial vaginosis, candidosis and genital trichomoniasis.

2.1. Assessment of the bacterial vaginosis was performed both by Nugent & Spiegel and Amsel criteria.

2.1.1. By Nugent and Spiegel criteria

One proceeded to the sampling of secretions from the middle section of the vagina, in order to avoid false results caused by cervical mucus, blood, sperm remains or other substances. Sampling was performed after opening the vagina with a speculum or a pair of non-lubricated valves, after which, with a spatula, one sampled a drop of secretion from the vaginal wall, that shall be smeared on a couple of blades in view of Gram staining.

After sampling, one filled an analysis sheet for the laboratory physician, where some succinct data regarding the patient, the aspect of leucorrhea (amount, appearance, smell, color that might orient a diagnosis), local symptoms, circumstances of occurrence (contact with a known or unknown partner,

convalescence after some disease, after antibiotic or hormonal treatment), age of the symptoms, information on the partner (urethritis, irritations of the prepuce, rashes), control after recurrence etc. are written down. One also wrote down the aspect of the external genitalia, as well as all data obtained after inspection, valve examination and palpation.

Once stained, each blade was examined on the immersion microscope (x1000), aiming the outlining by the type of morphological aspect of the Gram-positive lactobacillus, of the Gram-variable coccobacillus *Gardnerella vaginalis*, of the Gram-negative species of Bacteroides, Gram-variable species of Mobilunculus and Gram-positive cocci.

2.1.2. By Amsel's criteria

1. One outlined the homogenous, thin appearance and the white-grey color of the vaginal secretion, proceeding afterwards to the qualitative and quantitative assessment of the species of aerobic and anaerobic microorganisms (bacteria), after ruling out the trichomoniasis, the candidosis and gonococcia.

One performed the May-Grünwald-Giemsa staining, which outlined, besides the normal bacterial flora, a series of cells, especially leucocytes, whose number was according to the intensity of the inflammatory reaction, red blood cells, mucus. The neutrophyllic polynucleates were more or less altered; the eosynophyles were seldom observed. One also evidences hystiocytes, and their presence was a clue for the diagnosis of Mycoplasma. The most frequently seen was the Gram-negative *Gardnerella vaginalis* coccobacillus, along with a series of other anaerobic germs (Bacteroides, Peptococcus etc.).

Afterwards, the other criteria for the diagnosis of bacterial vaginosis were checked.

2. The vaginal pH was studied by pressing a length of indicator paper on the vaginal mucosa, taking care to avoid the cervical mucus, since it has an alkaline reaction.
3. The smell of rotten fish was evidenced after mixing the vaginal exudates with a 10% solution of KOH.
4. Outlining of the "clue cells" was done by mixing a drop of the vaginal fluid with physiological serum, and then observing it on a microscope; one spotted epithelial cells with undefined margins, with granulations

or ‘dusty’, because of the fixation of a large number of germs on their surface (*Gardnerella vaginalis* coccobacilli, streptococci, dyphteroids, lactobacilli). These are the ‘clue cells’, so named by Gardner and Dukes, considered as ‘indicator’ or pathognomonic cells for the bacterial vaginosis.

2.2. Assessment of fungal infections. Where the clinical signs and the local examination suggested the presence of a vaginitis with fungal origin, one proceeded to the revealing of the candidosis.

The product was sent to the laboratory, where one evidenced the presence of fungal blastopores or pseudo-hyfae by microscopic examination of the secretion in solution with physiological serum or 10% KOH (instant examination), or by fixation and staining of blades (methylene blue, Giemsa or Gram), and by spreading into the Sabouraud culture medium.

2.3. Assessment of genital trichomoniasis. The sampling for the revelation of the *Trichomonas vaginalis* parasite requires a series of supplementary measures towards the bacterial vaginosis or candidosis; thus, for assuring the accuracy of the diagnosis, the sampling was performed at three – five days of the end of the menstruation, from the vaginal exudates, the patient being advised to avoid vaginal lavage and sexual contact for the previous 48 hours, and any other local treatment a week before.

Also, in order to have a result as conclusive as possible, the sampling was mainly performed from the vaginal ends of bag. In the laboratory, the identification was done by instant examination (evidence of flagellates’ motility on the blade, in a drop of vaginal fluid mixed with physiological serum), Giemsa staining, and only seldom on culture. By Ciuca T, the antibiogram is performed only after the isolation and identification of the pathogenic germ; global antibiogram, performed for all of the germs in the vagina, has a limited value, because if more samples are taken, from the same vaginal contents, the results are different.

3. Colposcopic assessment of the uterine cervix.

For the colposcopic study of the uterine cervix one used video-colposcopy and computer recording of the images obtained, fact that allowed the storage of those images, case follow-up, consulting with other specialists a.s.o.

The reason for the colposcopic examination was the identification of cervico-vaginal inflammatory processes, alteration of squamous or glandular cells, presence of

keratinized cells as characteristic for the keratosis or leukoplakia, precancerous or cancerous lesions of the cervix.

Colposcopy was performed on all cases with altered cyto-test, in order to differentiate images that were suggestive for a high grade lesion (major alterations) from those with minor alterations, characteristic for low grade lesions.

Colposcopy was performed ahead of the treatment, the data obtained having an informative role, and after the treatment with Cervugid for the inflammatory bacterial and parasitic cervico-vaginal processes, that effects the removal of different side aspects of the inflammation (epithelial scaling, ulcerations, erosions, false membranes, mucus) one proceeded to the repeating of colposcopy, expecting conclusive data this time.

For this reasons, the patients were scheduled between days 8 and 12 of their monthlies (those with a normal cycle), because in this period the uterine cervix is ajar, and the abundant and transparent mucus eases the investigation. In patients with subtotal hysterectomia, or at menopause, one gave a short estrogen treatment, which by their trophic role allow an easier fitting of the speculum, ease the exploration of the transition area, the reactions on acetic acid and Lugol are more obvious, and their results are more conclusive.

Once the colposcopy began, the investigation was done with acetic acid and Lugol. After the insertion of the vaginal speculum, the uterine cervix was wiped with physiological serum, and then one proceeded to the examination of the cervix after the application of 3 – 5% solution of acetic acid. The acetic acid has a coagulant effect on the proteins at the level of the uterine cervix. Since they are normally in a low amount, the effect of applying acetic acid is rather imperceptible (only the surface proteins of the epithelium shall get coagulated), the epithelium becoming thus translucent, and the connective tissue underneath becoming visible. But if there is a local pathological process, the epithelium usually thickens, the amount of protein increases, and the acetic acid coagulates them by penetrating in the depth, giving a characteristic acetic-white reaction, that masks the underlying connective tissue.

One always sought after the visualization of the squamous-cylindrical junction (the transition area), found at the limit between the exo-cervical squamous epithelium and the endo-cervical cylindrical epithelium.

The Schiller testis based on the presence of glycogen in the upper and intermediate cells of the squamous epithelium, that make with the iodine a dark brown

reaction. The coloration of the epithelium changes in the presence of an epithelium with different lesions, and of an insufficient degree of epithelial maturation. Thus, if an acetic-white area gets partially stained with Lugol, this suggests a low grade lesion; if the area becomes iodine negative, yellowish, then this is probably a high grade lesion.

1 Results

1. Analysis of the cyto-test.

The cyto-test reported in the Bethesda system was in most of the cases of the inflammatory type, and in 26 cases one found modifications of the cyto-test that were assigned as follows: 14 ASCUS, 9 LSIL and 3 HSIL. The figures below come to illustrate these types of cyto-test:

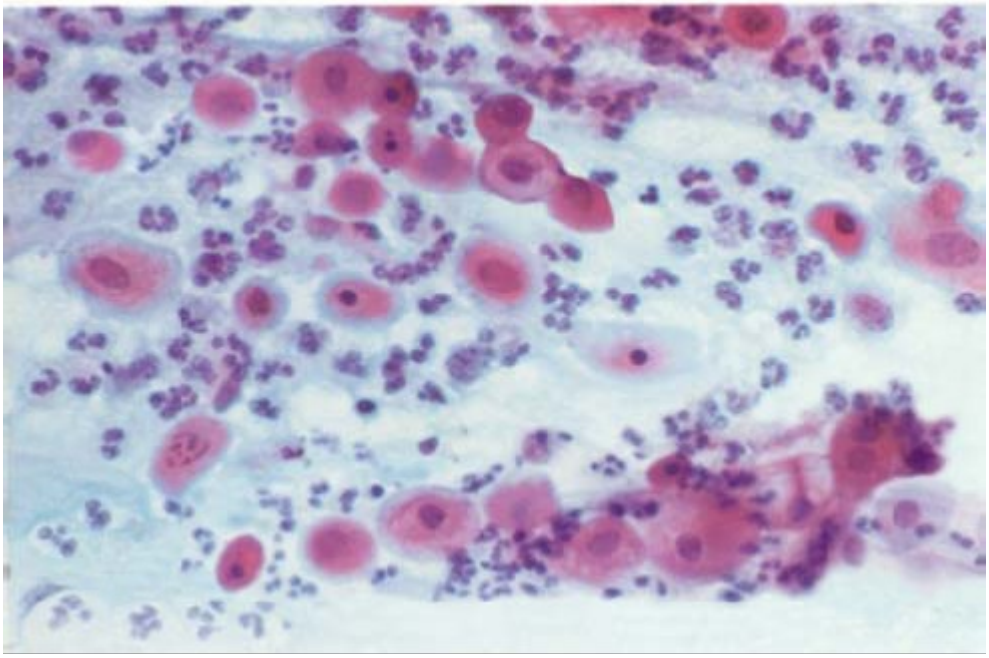

Figure 1: Cyto-test: acute inflammation; picnosis and cariorexis processes, eosinophyllic cytoplasm; numerous polynucleate leucocytes.

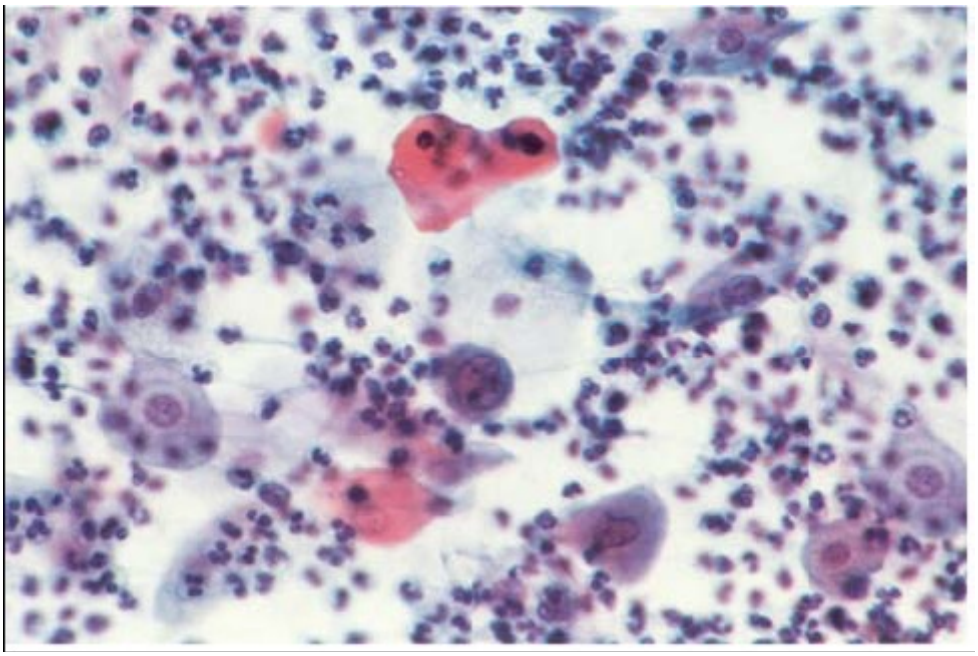

Figure 2: Cyto-test: chronic inflammation: polynucleate leucocytes with lymphocytes and degenerative inflammatory alterations; swollen hyperchromic nuclei.

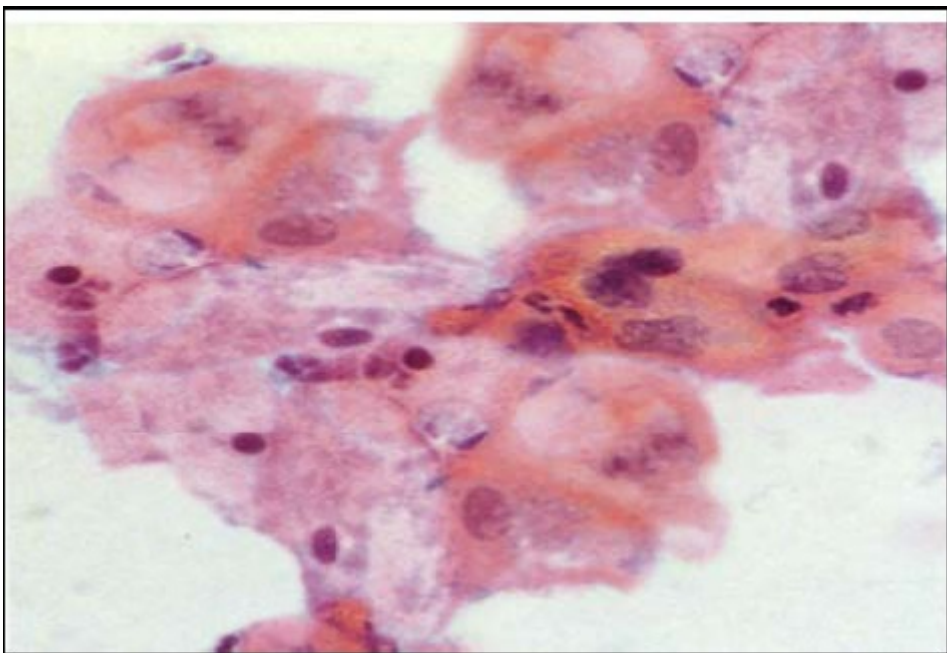

Figure 3: Cyto-test: HPV infection: koilocytes and multinucleate cells.

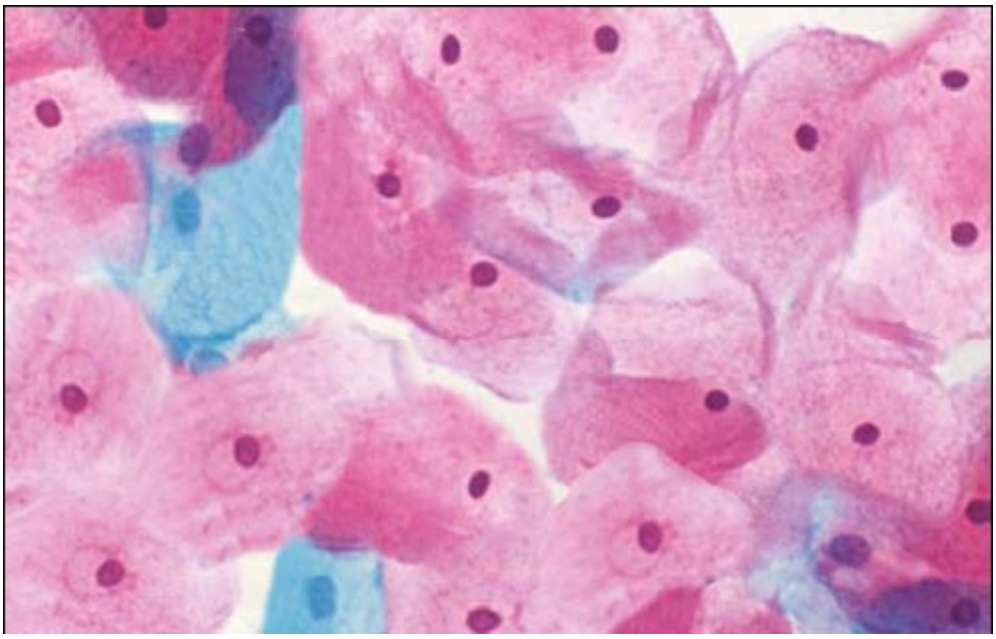

Figure 4: Normal cyto-test after treatment: superficial squamous cells at the mid-cycle.

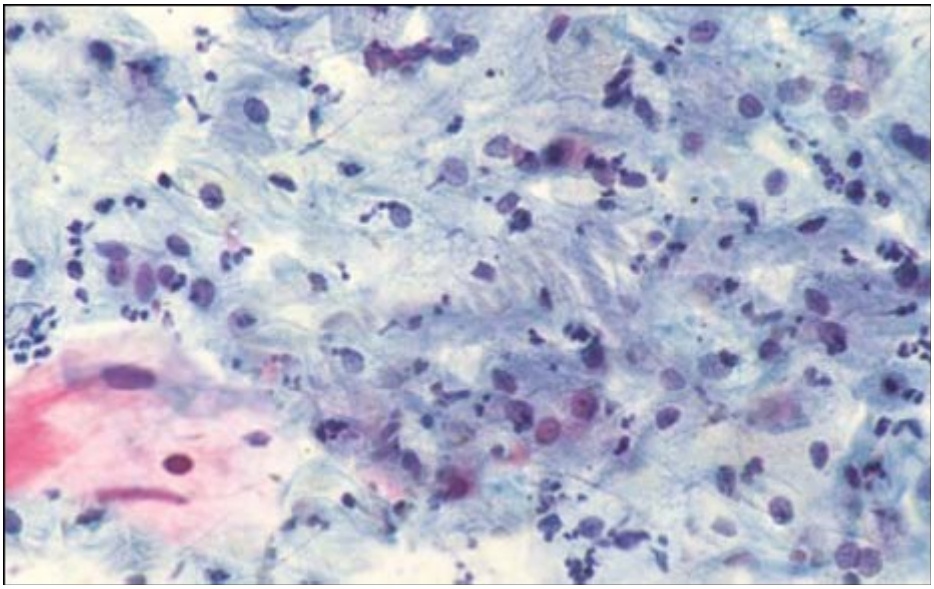

Figure 5: Normal cyto-test after treatment: intermediate cells and scarce glycogen-loaded cells in day 19 of the cycle.

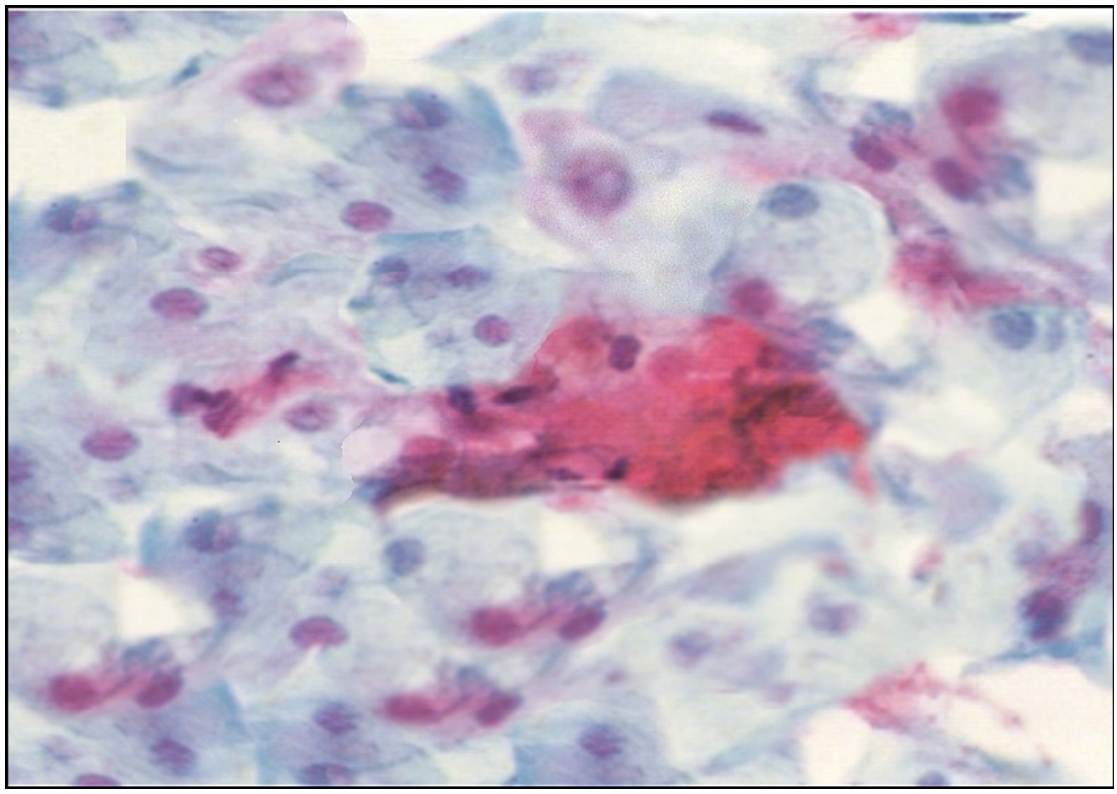

Figure 6: Normal cyto-test in day 26 of the cycle: folded intermediate cells, polynucleates and cell residues.

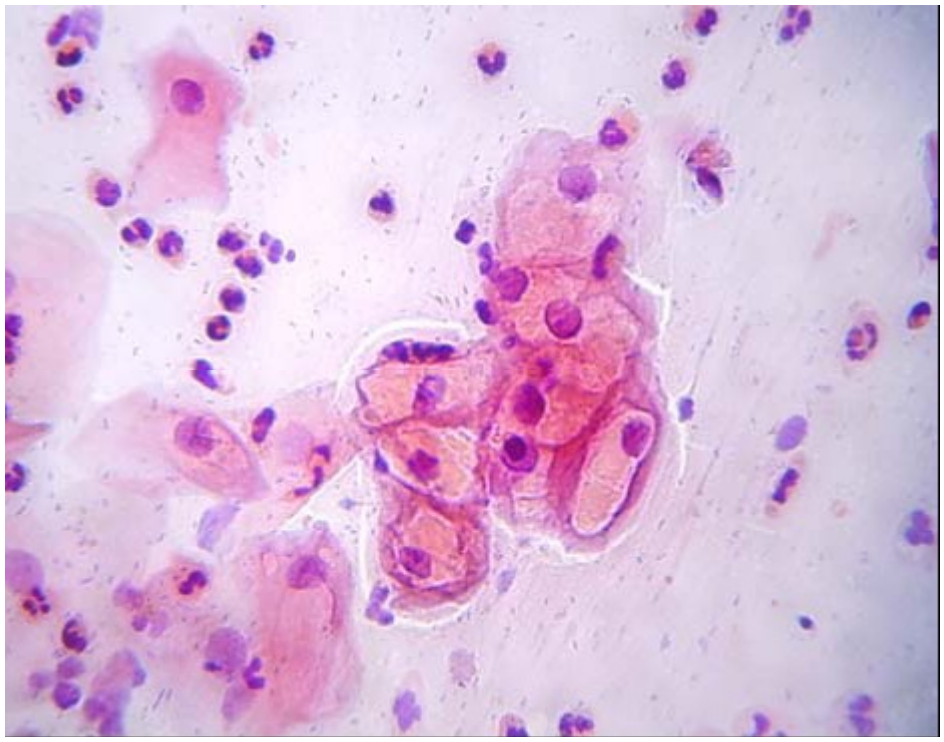

Figure 7: group of koilocytes.

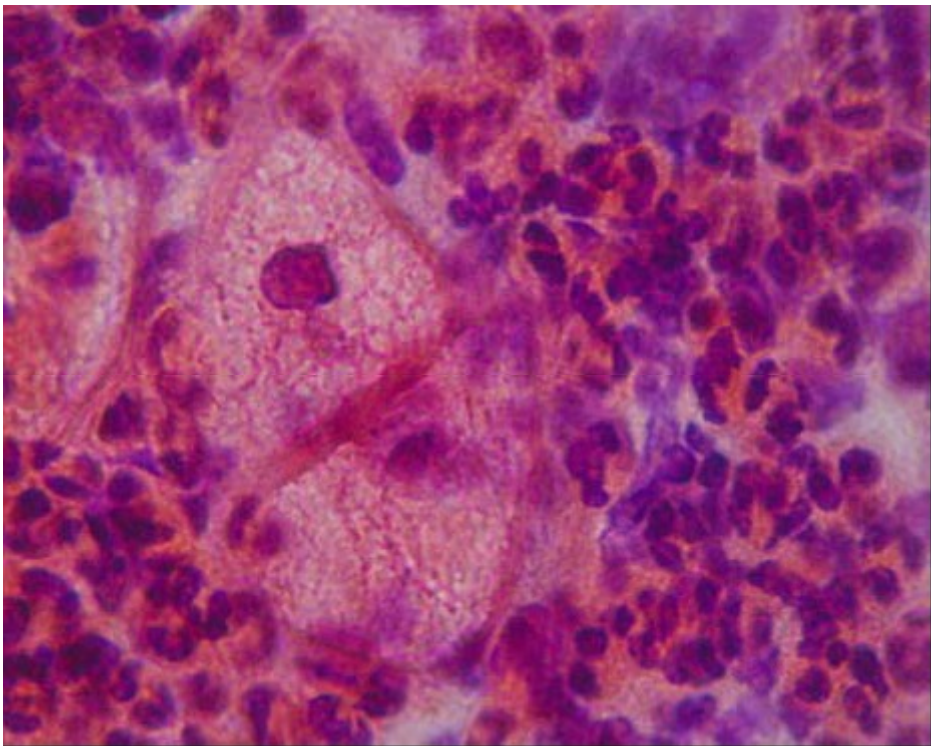

Figure 8: koilocytosis masked by inflammation.

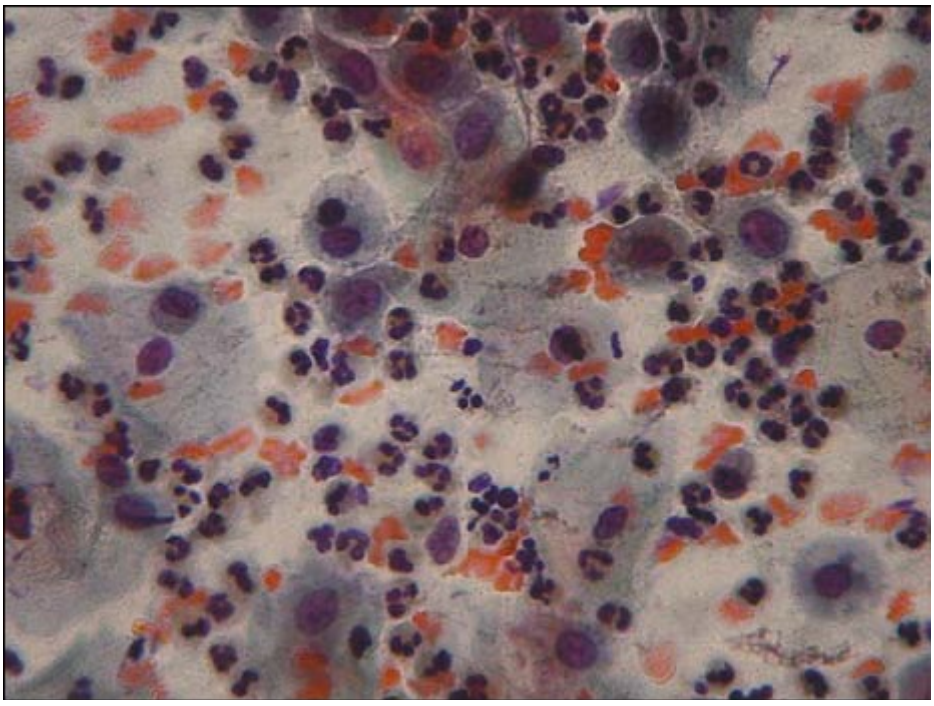

Figure 9: ASCUS-H: swollen hyperchromic nuclei, irregular contour, and inflammation.

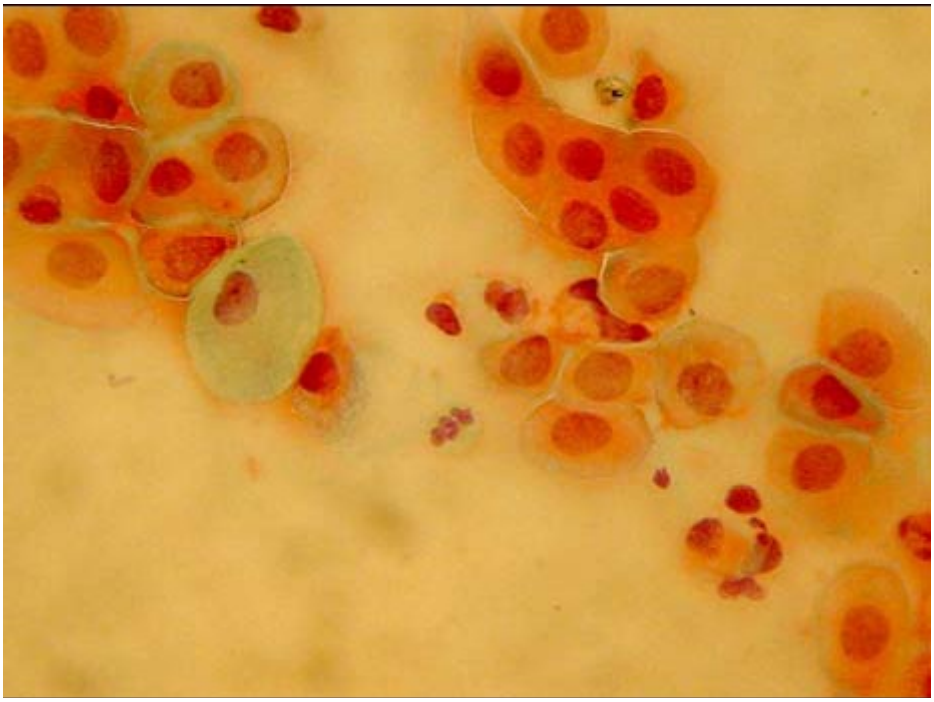

Figure 10: ASCUS-NOS: slight atypia of metaplasic cells; atrophic background.

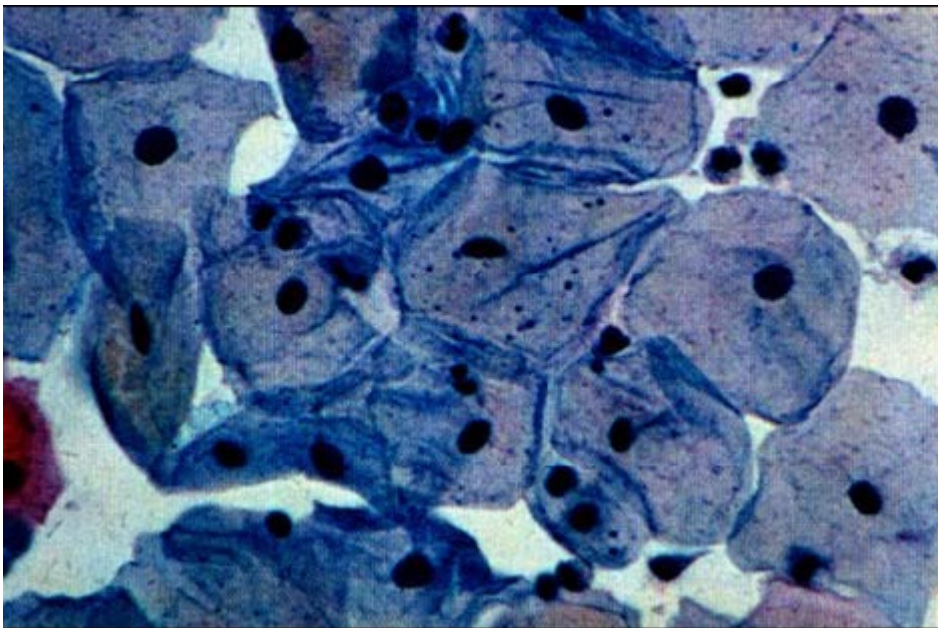

Figure 11: The cases above after treatment: squamous and polygonal cells with no inflammatory infiltrate.

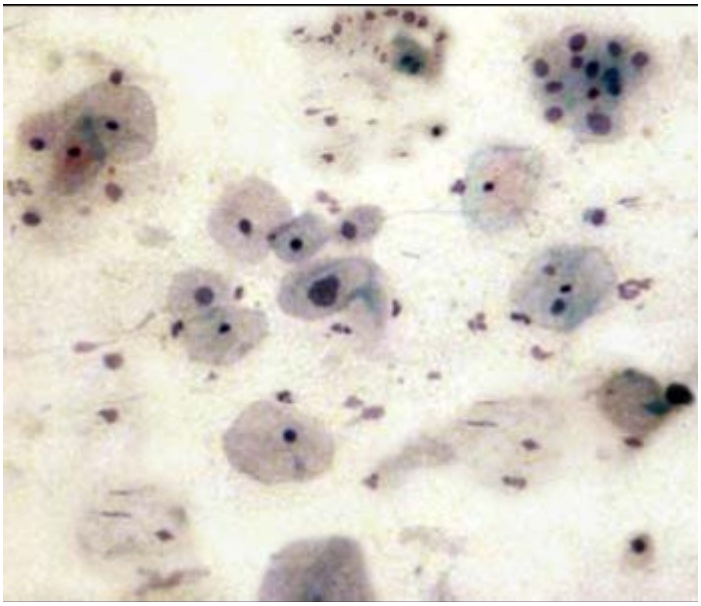

Figure 12: Cyto-test: intermediate squamous cells with swollen, hyperchromic and eccentric nucleus; perinuclear halo; observable condensation of the perinuclear cytoplasm (koilocyte). Low grade squamous intraepithelial lesion: LSIL.

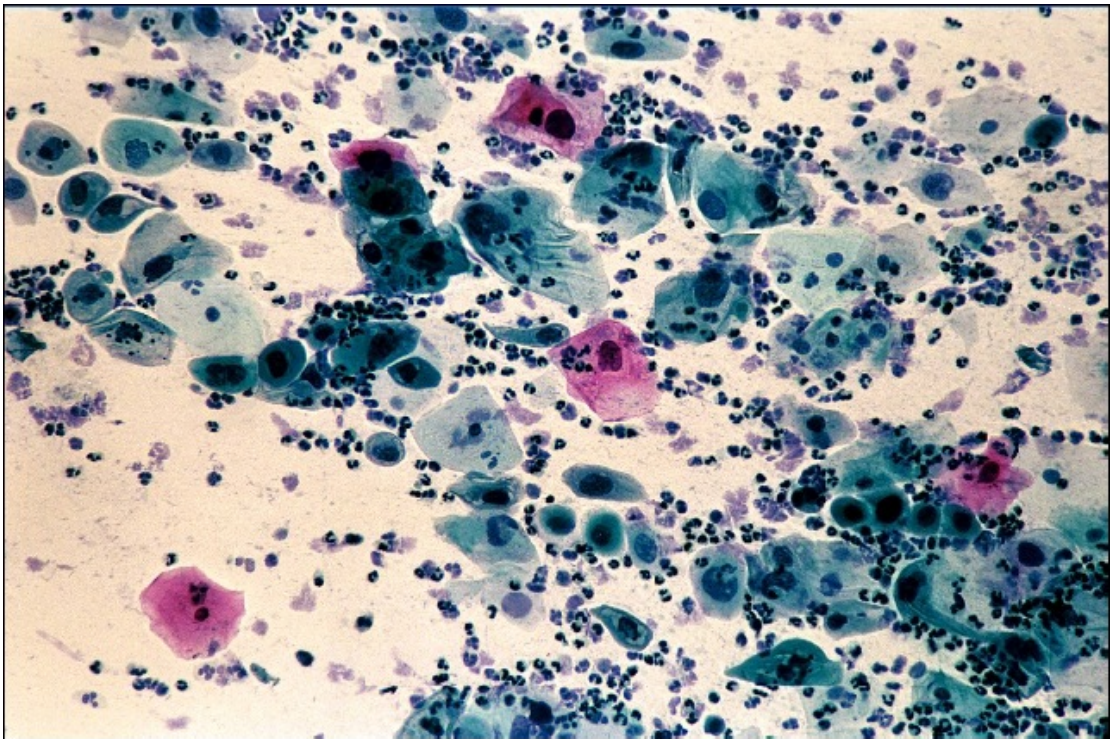

Figure 13: Cyto-test: basal cells with hyperchromia and anisocariochromia: HSIL.

2. Analysis of the cervico-vaginitis

500 patients were taken into observation, that were followed up during the entire investigation, and in which one studied the evolution of the inflammatory

processes, the evolution of the cyto-test and the control of the cervix by colposcopy after the treatment with Cervugid – ovules. In whole there were four medical visits: at the beginning of the treatment, immediately after the end of the treatment, and then at one and three months from the end of the treatment respectively. Among the 500 patients considered in this study, 3 missed the second visit and 7 the third visit, but this does not change the results of our investigation. The age ranged between 18 and 60 years, and the average age was 34.

Bacterial vaginosis was assessed both by determination of the Nugent & Spiegel index, and by assessment of Amsel's criteria.

Determination of the Nugent & Spiegel index

The study of the vaginal secretion was done by quantitative assessment of the different types of bacteria, by their morphological aspect or morph-type obtained after the Gram staining. The types of bacteria counted in the immersion microscopic field (x1000) were recorded on a scale from 1+ to 4+, where 0 means no bacteria in the microscopic field, 1+ means no less than one type of bacteria in the field, 2+ means one to four different types, 3+ means from five to thirty types, and 4+ means more than 30 types of bacteria.

The distribution of the bacterial types in the 500 patients according to their observed specific morphological aspect, after the Gram staining, was the following: small Gram-variable bacilli in 489 cases (97.6%) = 3+; small Gram-negative bacilli in 452 cases (90.5%) = +3; curved Gram-variable in 121 cases (24.2%) = +3; Gram-positive cocci in 100 cases (20%) = +3; large Gram-positive bacilli in 167 cases (33.4%) = 1+ and 2+; absence of large Gram-positive bacilli (lactobacilli) in 334 cases (66.8%).

After the end of the Cervugid treatment, the analysis of the secretion revealed an almost complete diminishment of different types of bacteria, as follows: small Gram-variable bacilli from 97.6 to 1.3%, small Gram-negative bacilli from 90.5% to 0, curved Gram-variable bacilli from 24.2 to 1.5%, Gram-positive cocci from 20% to 0, and large Gram-positive bacilli (lactobacilli) have developed freely, being present in an overwhelming proportion in 484 cases, that is 96.8%.

There are two aspects that strike on a first analysis of the types of bacteria by their morphological aspect, that is that the microbial flora is mixed (formed of several types for the same subject), a total absence of large Gram-positive bacilli in a proportion of 66.8% or their presence in an insignificant number (1+ or 2+) in 33.4%.

The analysis of the bacterial types after the treatment shows a clear prevalence of the lactic flora (large Gram-positive bacilli) in the vaginal secretions.

With little difference, these aspects maintain in the other two examinations of the vaginal flora, at one and three months respectively.

Assessment of Amsel's criteria

1. The bacteria have been microscopically assessed both qualitatively and quantitatively by Gram and May-Grünwald-Giemsa stainings, the results being almost identical to those obtained by Gram staining and determination of Nugent & Spiegel index.
2. The bad fishy smell specific to the bacterial vaginosis (provoked by mixing a drop of vaginal secretion with a 10% solution of KOH) was evidenced in all analyzed cases.
3. The 'clue cells' were present in more than 95% of the cases.
4. The vaginal pH, revealed by means of a shim of indicator paper, was over 4.5 in 97.6% of the cases.

The distribution of the microbiological data for the 500 patients is given in table 1

Bacterial vaginosis	390	58.0%
Vulvo-vaginal candidosis	48	29.6%
Vaginal trichomoniasis	62	12.4%
Total	500	100%

In 180 one revealed mixed inflammatory processes, produced by two or more causes, according to table 2.

Bacterial vaginosis, candidosis and trichomoniasis	21	11.67%
Bacterial vaginosis and trichomoniasis	85	47.22%
Bacterial vaginosis and candidosis	74	41.11%

In 2% of the cases the secretion persisted after the Cervugid treatment, but in a smaller amount. In these cases one proceeded to the identification by cultivation, and the treatment has been conducted by antibiogram.

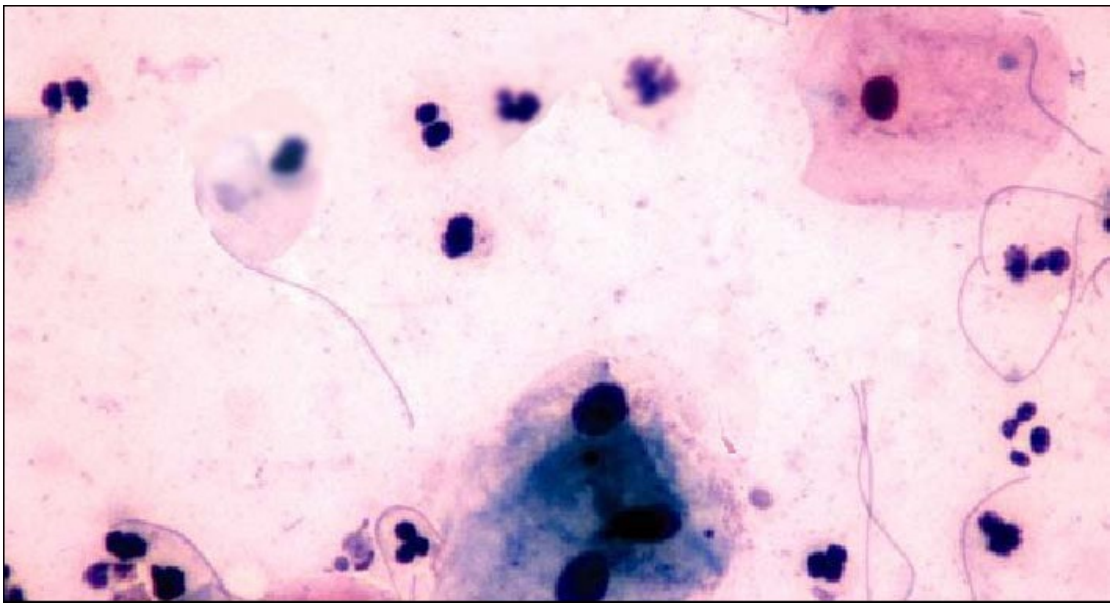

Figure 14: Smear with *Trichomonas vaginalis*.

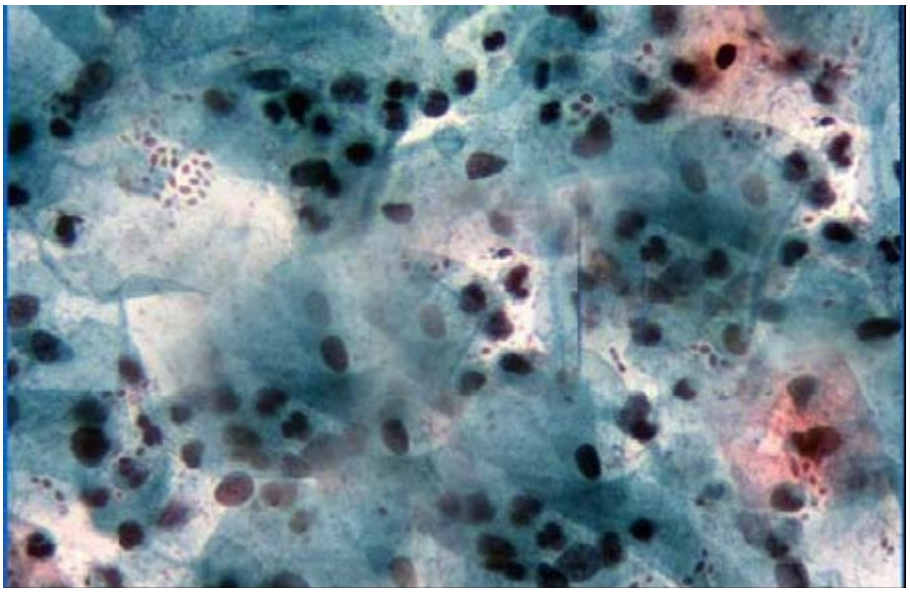

Figure 15: Smear with *Candida albicans* fungi.

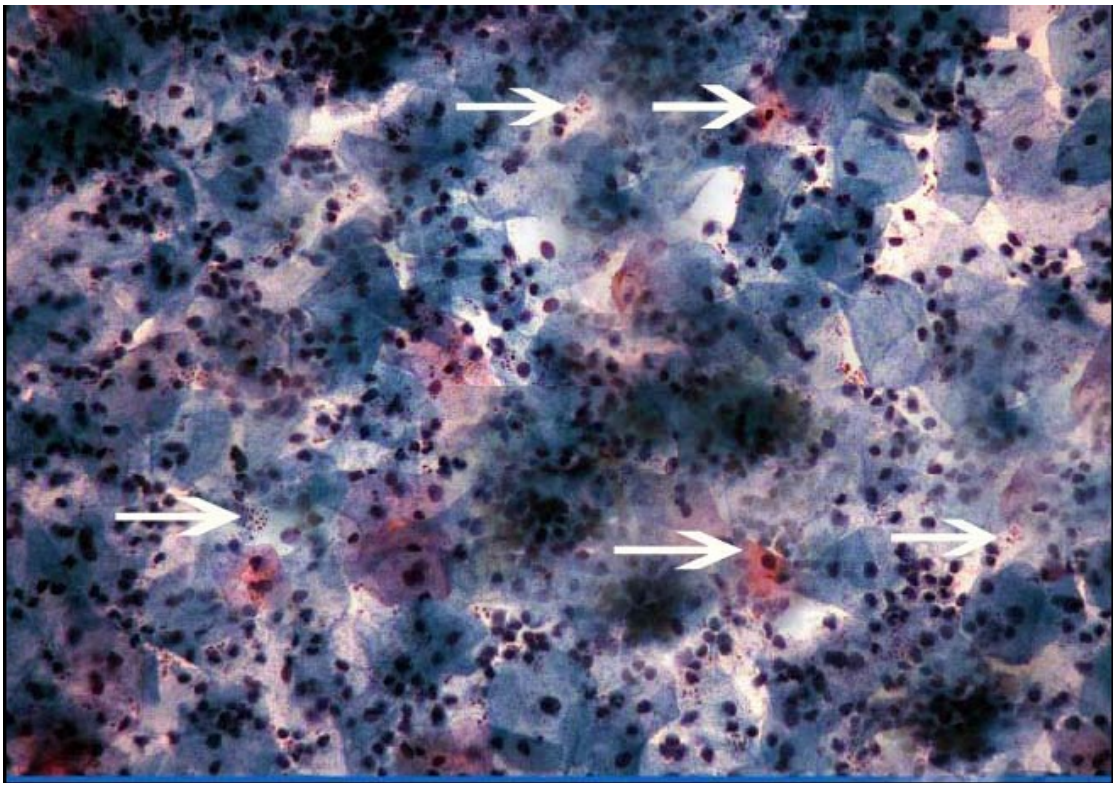

Figure 16: Smear with *Candida albicans* fungi and bacterial vaginosis.

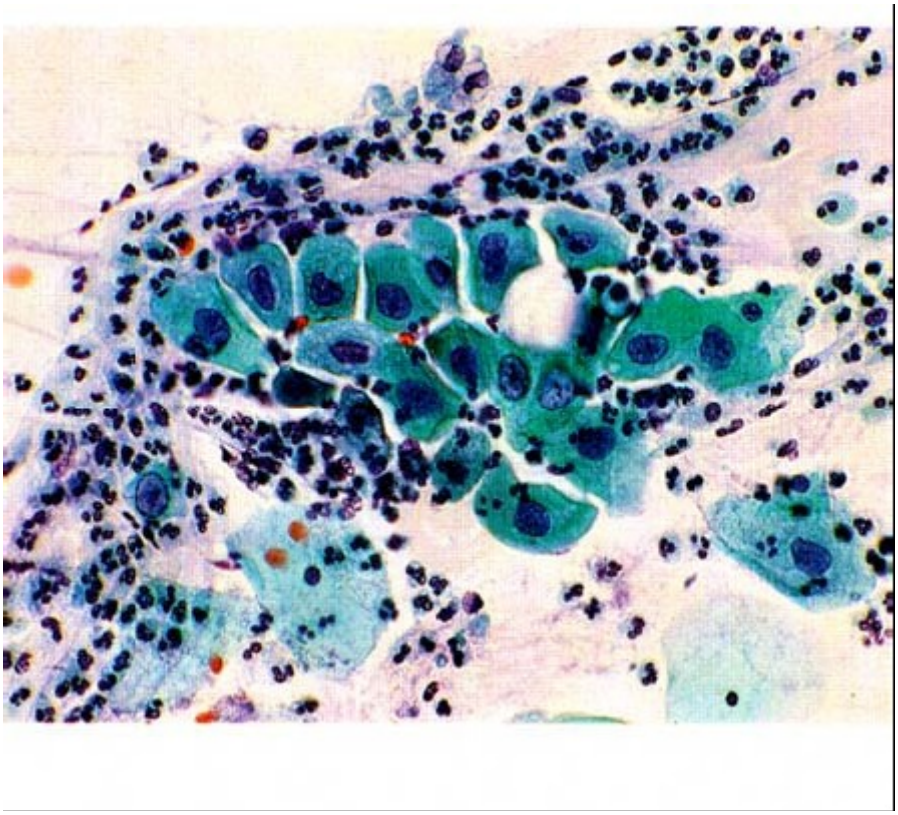

Figure 17: Bacterial vaginosis, before treatment; small conglomerates of intermediate cells, along with intense leucocitary inflammatory reaction.

Figure 18: Bacterial vaginosis, after treatment; presence of reparatory tissue.

Analysis of colposcopic data

In those situations where the cyto-test revealed specific modifications for the HPV, one performed the colposcopic examination in order to see whether the observed colposcopic aspects are suggestive for low grade lesions (minor alterations) or they are suggestive for high grade lesions (major alterations).

The colposcopic aspects specific for the low grade lesions that could be observed were: fine pointing and fine irregular mosaic (figures 19 and 20), acetic-white epithelium with slight reaction that appeared slow and disappeared fast (figure 21), epithelium with positive Lugol test (figure 22), smooth surface with irregular exterior contour (figure 23).

Figure 19: Fine pointing.

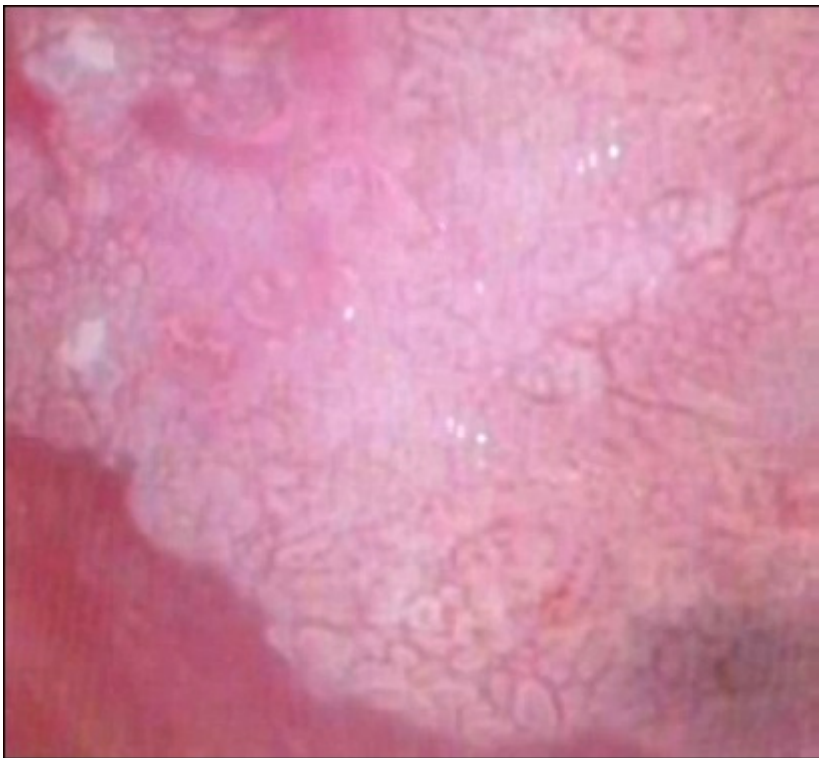

Figure 20: Fine irregular mosaic.

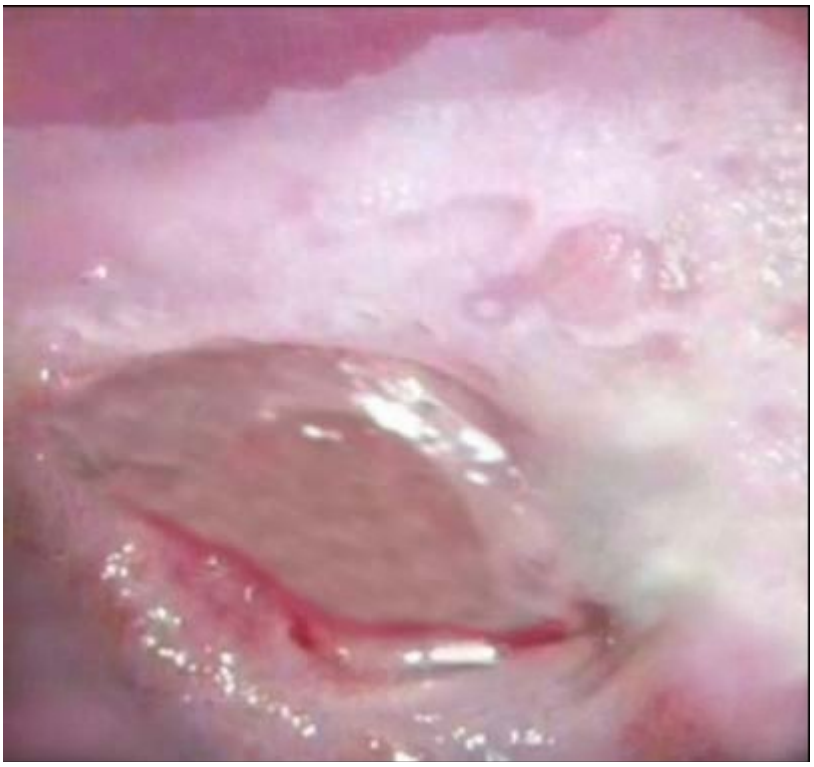

Figure 21: Acetic-white epithelium with slight reaction.

Figure 22: Cervix after treatment – positive Lugol test.

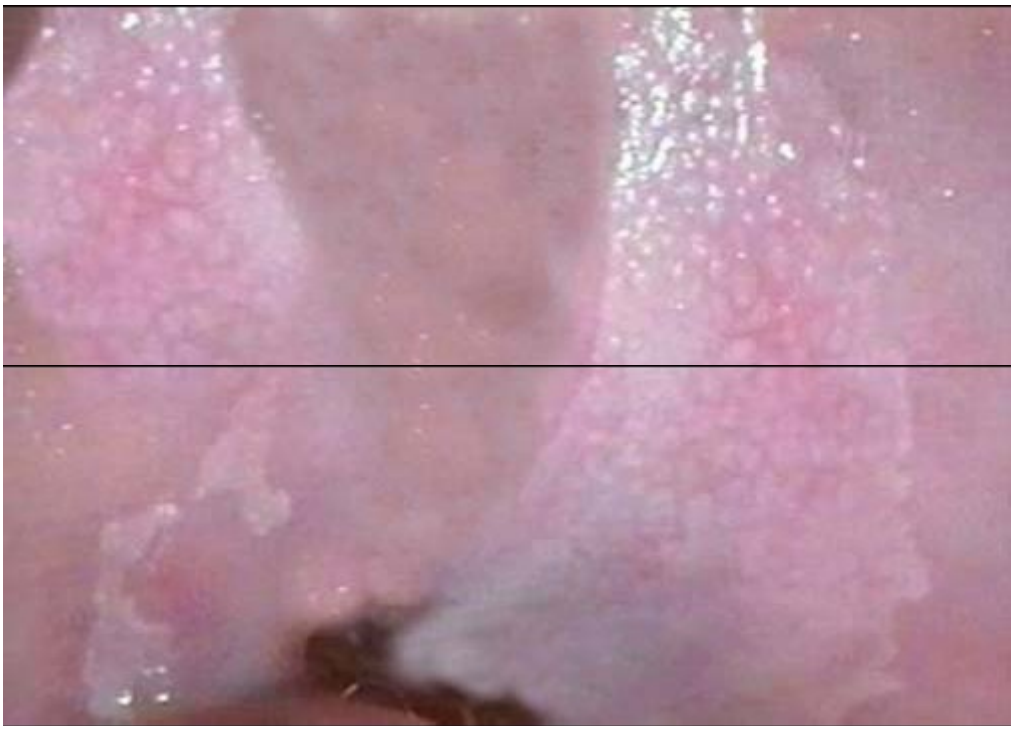

Figure 23: Colposcopic aspect with slightly positive Lugol test, epithelium with acetic-white reaction and mosaic. Smooth surface with irregular exterior contour.

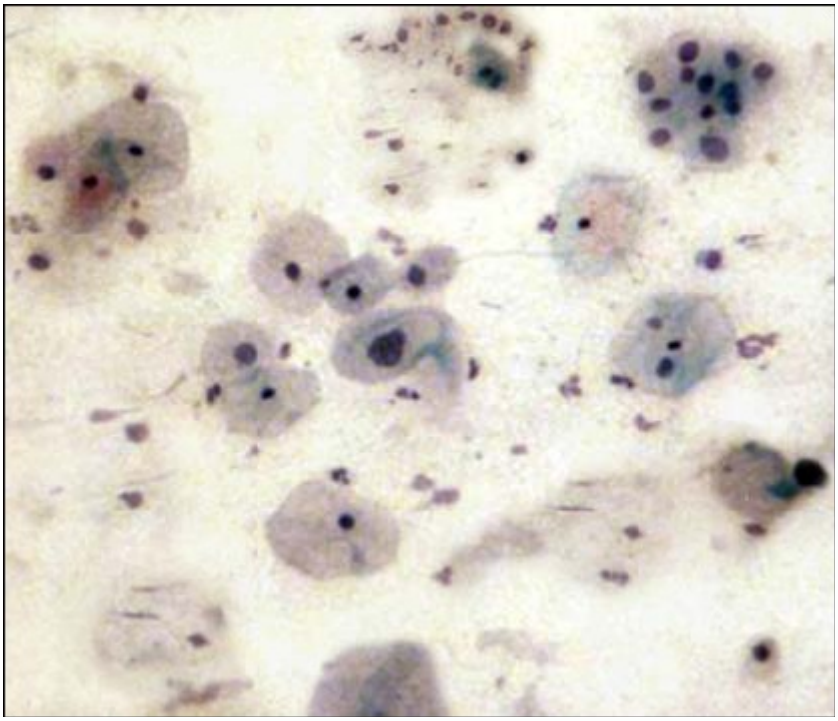

Figure 24: Cyto-test: intermediate squamous cells with swollen hyperchromic eccentric nucleus, and perinuclear halo; obvious condensation of the perinuclear cytoplasm (koilocyte) - low grade squamous intraepithelial lesion (LSIL).

As colposcopic images suggestive for a high grade lesion we present figures 25, 26, 27 and 28, which include colposcopic, cytological and histological examination.

Figure 25: Colposcopy – acetic acid test: bounced area situated on the anterior base of the cervix.

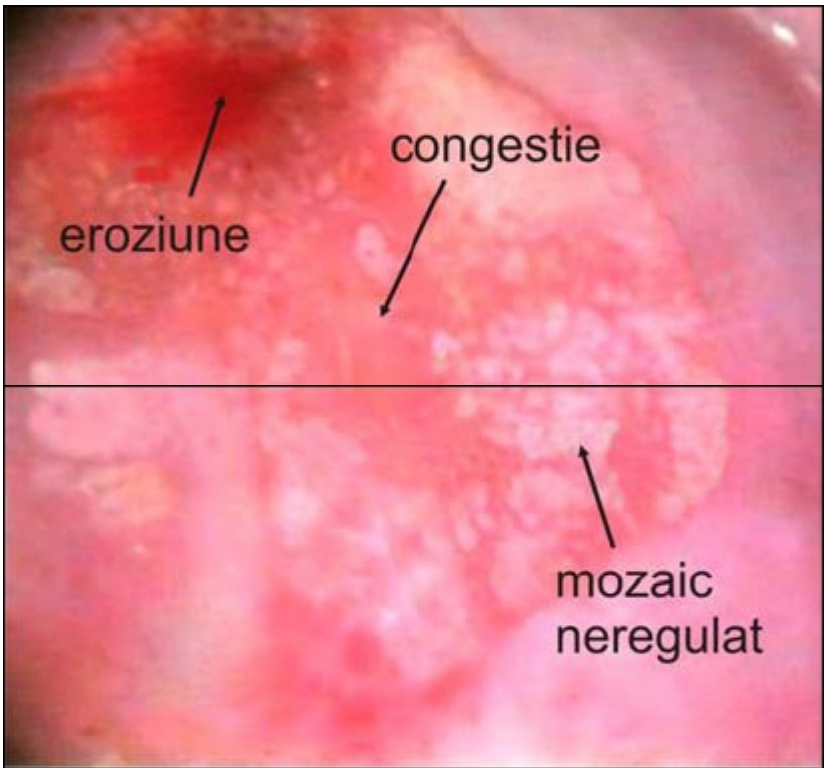

Figure 26: Colposcopy (previous case) with the lesion area enhanced: erosive and congestive areas and irregular mosaic (on the picture: erosion, congestion, irregular mosaic).

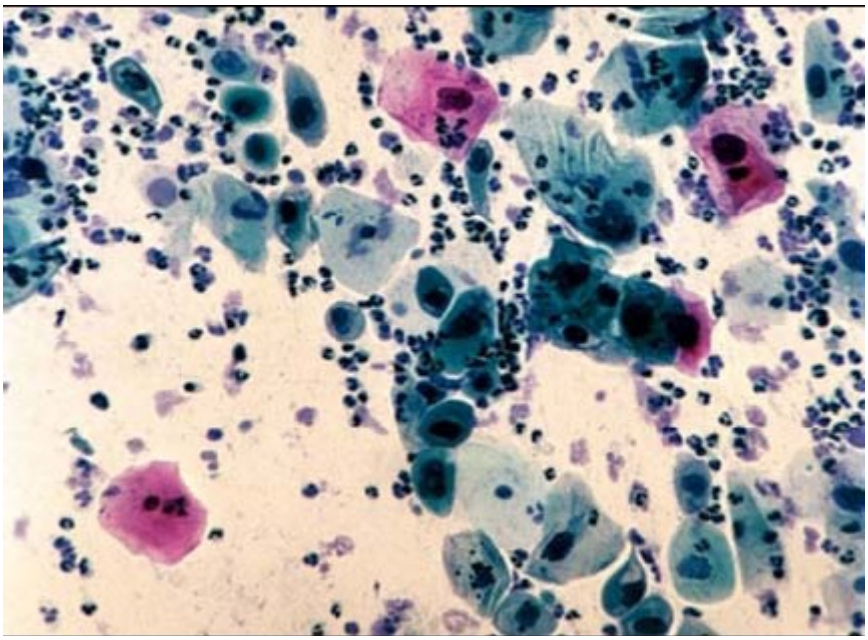

Figure 27: Cyto-test: HSIL – basal cells with hyperchromia and anisocariochromia.

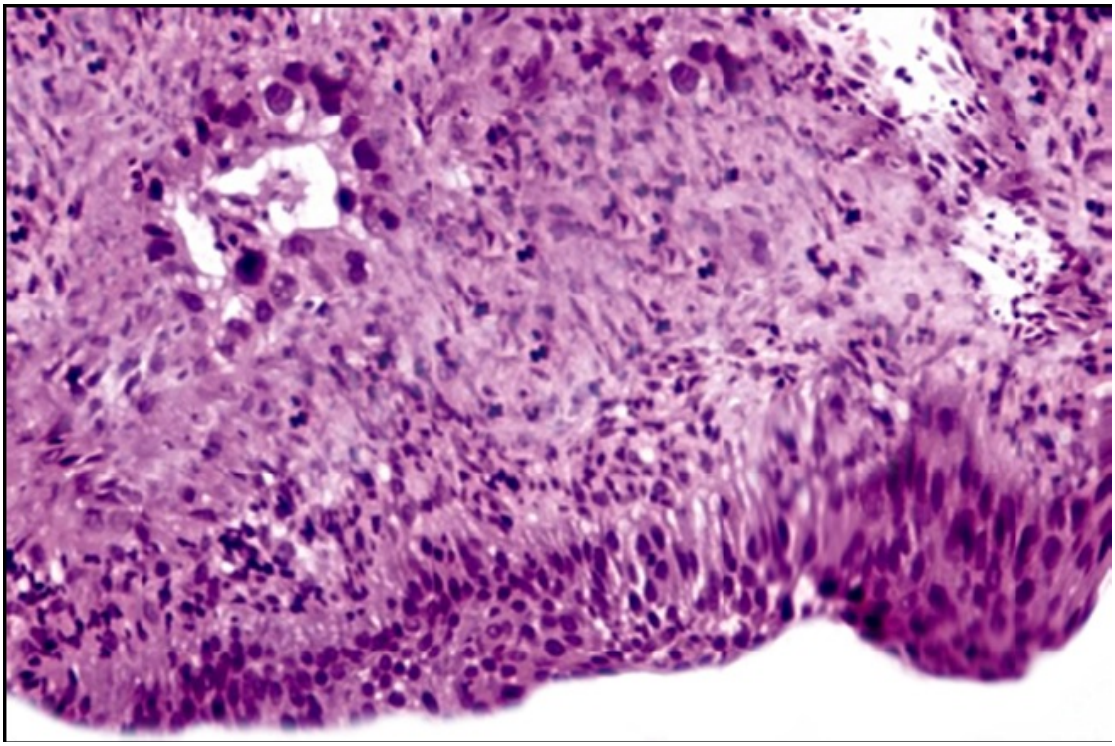

Figure 28: Histo-pathological view from the mosaic area: atypical cells in the superficial layer, absence of stratification and differentiation, absence of squamous epithelium maturation for its entire thickness, atypical cells in the superficial layer, diffuse lymphocitary infiltrate. Diagnosis: high grade intraepithelial lesion – CIN III. In this situation, one resorted to the excision with the wire loop (LLETZ) and follow-up.

Discussions

This study is an open, non-comparative study, and had as main objective the assessment of:

1. Indications, counter-indications, efficiency, tolerance and adverse reactions of the Cervugid treatment.
2. Local subjective manifestations (pricks, burns, local discomfort, pelvic pain, irritations, dysuria and urinary burns, and sexual uneasiness).
3. The evolution of cervico-vaginal inflammatory processes, under treatment.
4. Evolution of the cyto-test (in those cases where the cytological examination outlines pathognomonic modifications for the HPV) and of the precancerous alterations of the uterine cervix, together with the colposcopic aspect of the cervix.
5. Analysis of recurrences and their elimination.

1. Indications, counter-indications, efficiency, tolerance and adverse reactions of the treatment with Cervugid – ovules.

The indications are represented by microbial vaginites and cervicites caused by the Gram-variable bacillus *Gardnerella vaginalis* (*Haemophylus vaginalis* or *Corynebacterium vaginalis*), the Gram-negative species of Bacteroides, Gram-variable species of Mobilunculus and Gram-positive cocci, streptococci and other pyogenic germs, either aerobic or anaerobic, vaginal trichomoniasis (in which case oral treatment shall be added and the partner shall be treated as well). The first trimester of the carriage shall be avoided, since metronidazole is forbidden. It is also indicated in vaginites caused by fungi, especially *Candida albicans*, alone or in association with other species of fungi, vaginites caused by Mycoplasma and Chlamydia (constantly sensible to chloramphenicol), mixed vaginites caused by the association of bacteria, fungi and Trichomonas, atrophic vaginites caused by estrogen starvation at menopause.

Before the cyto-test is performed, the cervico-vaginal infections must always be treated, since the treatment comes to clear the microscopic field as seen above, allowing a clear and certain diagnosis, ruling out the false positive results given by the inflammatory processes, especially chronic, which could impersonate a degenerative process.

It is also indicated within the local treatment before surgical interventions in the women's genital area, the postoperative evolution being significantly enhanced, especially with no septic complications.

The counter-indications are represented by those patients who exhibit intolerance to one of the drug's components, pregnant women in the first trimester of the carriage, and as a precaution the breastfeeding period, though there is no major counter-indication.

The efficacy of the treatment with Cervugid ovules had proved very good, because of several factors:

- Broad action spectrum, including practically all pathogenic germs involved in the appearance of cervico-vaginites (bacteria, fungi, protozoa, mycoplasma, Chlamydia and rickettsia).
- The intravaginal administration has the advantage of the highest outcome in active substance concentrations locally; thus, the

pharmacokinetics studies performed by Dr. T. Ciuca showed that in local administration, the concentration in the vaginal walls and in the mucosa of the cervix are 10 times higher than in the parenteral, oral or rectal administration.

- These higher local concentrations are obtained with relatively small doses of active substances, for which reason the accidents or adverse reactions and counter-indications are very rare. When they occur, it is a consequence of the intolerance or hypersensitivity to one of the drug's components, and they are specific for each person apart.
- By the simultaneous treatment of the four classes of pathogen agents involved in the etiology of the cervico-vaginites, one prevents the rebounds that occur in treatments that aim only one or two of these classes of pathogen agents. This is explained by that that in such situation the saprophytic forms (potentially pathogen) are stimulated, and by disturbing the balance between them they become pathogen and cause rebounds (42, 43).
- The Lipex 403, the mass that incorporates the active principles, has an important therapeutic role in itself: lipex is a semi synthetic fat, extracted from the coconut, and has a trophic role on the cervico-vaginal mucosa, disturbed in the inflammatory processes of different origins, and also in the trophic disturbances of the menopause.

2. Local subjective manifestations (pricks, burns, local discomfort, pelvic pain, irritations, dysuria and urinary burns, and sexual uneasiness).

The subjective symptoms: pelvic pain, local discomfort, itching, burns, draught, sexual uneasiness have totally disappeared after treatment in more than 96% of cases. Also, many patients confirmed us that even from the first or second ovule the local tension, moisture and discomfort began to disappear.

3. The evolution of cervico-vaginal inflammatory processes

Bacterial vaginosis represents a complex infection caused by the association of an anaerobic-aerobic flora, the microorganisms being most often the components of a

normal vaginal flora, yet under certain conditions they leave their saprophytic statute and become pathogenic.

In an adult woman with normal menstrual cycle, the vagina is usually resistant to different invasive microorganisms, the complex defense mechanism being mainly ruled by three factors: local acidity (pH=4.5), presence of the saprophytic vaginal flora mainly represented by the Doderlein lactobacillus, and a normal function of the ovaries that assure a proper estrogen concentration, these being responsible of a normal glycogen contents in the vaginal epithelium.

The Cervugid drug helps to repopulate the flora with lactic bacilli, as we shall see below.

Numerous authors have shown a long time ago the important role that the local and general defense capability has in the pathogeny of vulvo-vaginites, and it can be assessed by the level of immune-globulins in the cervical mucus and in the vaginal fluid. Thus, the studies have shown that in patients with vulvo-vaginal inflammatory conditions there is a decrease of IgA, IgM and IgG (25), a fact that leads to a decrease in local immunity. It has to be mentioned that other general somatic illnesses or organ-specific illnesses of the genital apparatus lead to a decrease in general and local immunity.

The inflammatory conditions at the level of cervix and vagina determine morphological alterations of the epithelium of the exo-cervix and endo-cervix (squamous and cylindrical epithelium respectively), which also manifest in their functional activity (reaction to acetic acid, iodine, or the secretor function of the endocervical glands).

At the same time, the inflammatory conditions bring important alterations in the quality of the cyto-test and of the colposcopic images, rendering their proper interpretation almost impossible, and limit (if not cancel) their predictive value. Cervico-vaginitis is characterized anatomo-pathologically by an acute process of cytolysis with scaling of the vaginal epithelium, sometimes down to the deep layers, a diffuse congestion of the superficial connective tissue, with leukocytes' extrusion, increase of the conjunctive-vascular areas and formation of an inflammatory infiltrate whose intensity is determined by the severity of the infection.

When the inflammatory condition, followed by the scaling phenomenon begins to involve the deep layers of the epithelium of the exo-cervix, ulcerations

occur, and if it extends to the endo-cervical glands, a purulent secretion at the exterior aperture of the exo-cervix shall appear.

As suite of the elimination of the superficial layers by scaling, the acetic-white reaction proper to the intraepithelial lesions shall be much less visible or even absent, so the image shall have the aspect of an obvious congestion of the connective tissue. Also, due to the removal of the superficial layers which contain glycogen, the Schiller test shall become slightly positive or less at the level of the epithelial surface, the reaction is negative in the areas of conjunctive-vascular peaks which approach the surface of the epithelium, given the characteristic aspect of the colposcopic images of diffuse colpitis, either focal or macular.

When the inflammatory condition begins to involve the mucosa of the endo-cervix (endo-cervicitis), the glandular cells modify by transforming from cylindrical cells into cube-like shaped cells; also, their secretion modifies, and the connective tissue gets surrounded by an inflammatory infiltrate, rich in leukocytes. The colposcopic images are characterized by the disappearance of the specific papillary aspect, and the presence of adherent, viscous and opaque mucus, that hinders the view of the Squamo-cylindrical area.

All this data force the repeating of the cyto-test, aiming that conclusive data is only obtained after the treatment of cervico-vaginal infections, a treatment that promotes the morphological and functional remake of the squamous and cylindrical epithelium of the uterine cervix. According to the results of the cyto-test, a colposcopic examination shall then be performed on the uterine cervix, which shall also provide decisive data.

In what the formulation of the Cervugid drug and its means of administration are concerned, the following specifications must be stated:

- Metronidazole was considered, since 1978, the election medication for the treatment of bacterial vaginosis, by being active on anaerobic Gram-positive bacilli, anaerobic Gram-negative bacilli, anaerobic cocci and protozoa, having an efficiency of 70 – 90% (33). Authors in the specialty literature unanimously recommend metronidazole as being the election treatment in the bacterial vaginosis and genital trichomoniasis (1, 4, 17, 22, 24, 29, 30, 32, and 33). The same unanimity is met in the descriptions of treatment failures, rebounds being mentioned in one to two or three months.

Yet metronidazole is not active towards Gram-positive cocci; for this reason, if a treatment is instituted with metronidazole alone, and the smear made at the beginning of the treatment shows Gram-positive cocci (by Nugent & Spiegel score), then one could say the smear has predictive value on the rebound in a proportion of 79.9% (33) for the very first month. The benefic action of metronidazole also manifests by not having any effect whatsoever on lactobacilli, furthermore, as we observed in most of our studied cases, metronidazole helps the repopulation of the flora with lactobacilli, fact that is confirmed by the data in the literature (33).

- Chloramphenicol, another component of the Cervugid drug, includes in its action spectrum those Gram-positive cocci, thus happily completing the action of metronidazole, explaining this way the rareness or absence of the rebounds, when other conditions of the treatment, which shall be explained below, are strictly observed.
- Besides, chloramphenicol is active on Chlamydia, Mycoplasma and Rickettsia, which are constantly sensible to chloramphenicol. For the revealing of Chlamydia, Mycoplasma and Rickettsia, one needs expensive and arduous laboratory tests (immunological reactions, cell cultures etc.), and their incidence in genital infections is quite high, reaching 30 – 40% of the cases, including men with non-gonococcal urethritis (22). This last issue regarding chloramphenicol explains the efficiency of the treatment with Cervugid – ovules in a series of cases refractory to other treatments.
- Nistatin is active on *Candida albicans* and other species of Candida, thus acting on present candidoses, but mostly preventing the installation of the fungi as a side consequence of the antibiotic treatment. The treatment of infections caused by fungi raises extremely serious problems because of frequent rebounds and treatment resistance.
- Hydrocortisone acetate has an unspecific anti-inflammatory action, which explains the benefic action of Cervugid in unspecific pelvic inflammatory conditions.

Though the effect of antifungal medication is comparatively the same, the individualization of the treatment is a must, according to the adverse reactions to the

previous treatments, if any, to the cost price and presence of a complicated or uncomplicated candidosis, as we have previously seen.

The common element in the approach of different authors in the specialty literature for all cases refractory to antifungal treatments is the prolongation of the treatment period. Thus, after an initial period of 7 to 14 days for a recurrent vulvo-vaginal candidosis caused by *Candida albicans*, some authors recommend the prolongation of the treatment with ketoconazole 150 mg per week for another 6 months, to gain a treatment success of over 90% (34).; for the recurrent cases one also recommends fluconzaole weekly for the same period of time (35), this one having less toxic effects on the liver. Topic therapy with clotrimazole 500 mg weekly has also been recommended, or capsules with 600 boric acid intravaginally for at least 14 days (36).

The Cervugid drug, with 12 ovules per pack, is recommended in three one-pack cures with 7 days break in between, and comes to join this conduct of prolonged antifungal treatment.

Cervicites and endo-cervicites could sometimes be caused by *Chlamydia trachomatis*, species of Mycoplasma, herpes simplex virus and *Neisseria gonorrhoeae*. Isolation of the *C. trachomatis* microorganism is made on tissue cultures, and identification of the mycoplasma is done by immune-fluorescence reactions. If the possibilities do not allow for their revelation, and if *N. gonorrhoeae* was not evidenced on cultures, the treatment with chloramphenicol is the first choice for mycoplasma and Chlamydia, since they are invariably sensible to chloramphenicol.

The cervico-vaginal infections are tightly connected to pelvic inflammations, the studies and observations showing that there is actually no pelvic inflammation without vulvo-vaginal inflammatory conditions (37). This aspect justifies the treatment with Cervugid in pelvic inflammatory conditions (parametrites, annexites etc.).

Vulvo-vaginitis may be one of the causes of a urinary infection, the uro-pathogenic germs being present in a much higher extent at the level of vagina and vulva (38).

The first cause of maternal-fetal infections is represented by cervico-vaginites (39). The infectious complications of abortion are much frequent in women with vaginal infection, a special risk being represented by the infection with *Clostridium*

perfringens. The amniotic infection, premature rupture of membranes, the low weight newborn are highly associated with cervico-vaginal infections, the contamination occurring in most cases with germs from the vagina; the cultures spread with amniotic and vaginal fluid showed in cases of amniotic infection a perfect correspondence between the vaginal microbial flora and the germs in the cultures (40).

A pathological vaginal flora can be considered as having a predictive value on the possibility of premature birth, if investigated in the very first trimester of the carriage (41).

These last elements come to strengthen the usage recommendation of the Cervugid drug, especially in the last trimester of carriage, for the treatment of cervico-vaginal infections and prevention of the carriage-associated conditions presented above.

4. Evolution of the cyto-test under treatment

If one considers that, even though the incidence of high oncogenic risk HPV is over 29%, and only 10 – 20% of the genital infections with HPV would develop a condiloma or a CIN of which less than 1% of the women with high oncogenic HPV develop a uterine cervix cancer, there is a logic conclusion that a series of factor concur in the malignant cellular transformation, the so-called co-carcinogenetic factors.

These factors include smoking, immune suppression (secondary to organ transplant, HIV infection), nutritional starvation (especially vitamins C and A) occurring in economically deprived people, progesterone stimulation secondary to administration of oral hormonal contraceptives, local bacterial and viral infections.

In case of local infections, *Trichomonas vaginalis, Chlamydia trachomatis,* human herpes virus type 2 (HSV-2), *Neisseria gonorrhoeae* etc. have been involved (24), but we think that a role of similar importance is taken by unspecific infections, presently included in the term of bacterial vaginosis (unspecific vaginitis) that diminish the local defense capability of the organism by the diminishment of IgA, IgM and IgG as shown even since 1985 by Letucih (25).

In this respect, the treatment with Cervugid – ovules is fully justified in that that by treating the local co-carcinogenetic factors (genital trichomoniasis, infections with Chlamydia, mycoplasma, rickettsia or mere bacterial vaginosis) the local defense

capability increases and prevents the grafting of the HPV infection, including those HPV with high oncogenic risk.

If we take into account that the cytological smear does not provide for analysis but the cells of the superficial and not the deep layer of the malpighian epithelium, the presence in the smear of koilocytes alone cannot sustain the statement that the lesion belongs to a condiloma with normal basal layers or to a CIN I with koilocytosis. The CIN I histological lesions correspond in the Bethesda system with the LSIL cytological category, where the viral HPV infection is expressed by the pathognomonic cytopathic effect – the koilocyte – produced during the cellular differentiation of the squamous epithelium infected with HPV (figures 7, 8, 9 and 12).

The follow-up control after the treatment with Cervugid – ovules has always normalized the aspect of the smear above and avoided the histopathological examination (biopsy) which settles the difference between condiloma and CIN.

Also, considering that "about 15 – 30% of the cases labeled LSIL have as histological substrate a CIN II or III" (5), and the technical possibility in many sanitary units do not allow further investigation (DNA typing of HPV), which are otherwise very expensive, we thought the treatment with Cervugid – ovules as a proof for cyto-test validation: if it gets normal, and the colposcopic control comes to find a normal aspect, there is an overwhelming outcome in using this therapy, and the cyto-test needs repeating in order to avoid an eventual error – either in sampling or in interpretation.

Obviously, one can think that facing an ASCUS or LSIL cytology, one can talk about the risk of an over-treatment, but this risk is a minor one if we are to take into account the possibilities, otherwise statistically proven, of other lesions, more severe, hidden at the cervical level in respect to the cytological diagnosis.

If, after the administration of three packs of Cervugid – ovules one finds a re-conversion of the cyto-test, that is in the place of LSIL one finds ASCUS or in the place of HSIL one finds LSIL, then other three packs of Cervugid were given after a break of several weeks. In such cases, the follow-up cyto-test was always normal, a fact confirmed by the colposcopic examination (23).

Though, if the cytological alterations persist after the second treatment series, one proceeded to more aggressive treatments: thermo-coagulation and conization with the diathermal loop.

If the cytological result shows HSIL, there is an increased risk for the patient to develop an invasive cervical lesion or an invasive cancer. In such case, the biopsy may reveal a CIN II or III in 70 – 75% of the cases, but the possibility for an invasive cancer to be there already is only of 1 – 2%. In this situation, the follow-up control comes to confirm whether the cytology maintains or not.

If this is the case, the treatment comes to clarify the microscopic field by eliminating the associated inflammatory conditions, and thus eases the precise stating of the diagnosis (26). Also, it is now that colposcopy needs to reveal the specific alterations that justify the HSIL cytology (27, 28).

In order to sustain the LSIL diagnosis, one needs to eliminate de reactive squamous alterations given by different types of cervico-vaginal inflammations; for this, it is absolutely necessary that besides the elements of koilocytosis (perinuclear halo or cytoplasm vacuolization and nuclear atypies – meaning significant alterations of the nucleus) there are aspects of cellular disorganization manifested by altering the normal epithelial stratification and maturation due to basal and parabasal cell proliferation.

These morphological aspects are defining for the differential diagnosis of cellular alterations induced by cervico-vaginal inflammations caused by bacterial vaginosis, trichomoniasis and candidosis. In this case, cytoplasm vacuolization is not accompanied by nuclear atypies and the normal epithelial stratification and maturation is maintained.

In view of differentiation the cytological and histological effects induced by the HPV or different causes of vaginitis discussed earlier, the anti-inflammatory treatment with Cervugid has a decisive role because of its broad action spectrum, its top local concentrations of active substances, its corresponding local and general tolerance and its adverse effects, practically null. We also outline that vaginitis given by the three major causes (bacterial vaginosis, candidosis and trichomoniasis) are quite frequently associated with the HPV infection, that is revealed by the cytopathic effect characteristic for HPV.

5. Analysis of the rebounds and their cure

One of the most complex and most unpleasant issues of the treatment of cervico-vaginal infections is the rebound.

The analysis of rebounds and especially their cure is extremely difficult because a series of causes pertain to the intimate life, the educational degree and the functional status of the ovaries that need to assure a proper hormonal estro-progesterone balance. A woman with a normal menstrual cycle presents a rather normal defense against a series of factors, through the local defense mechanisms.

The issue gets nonetheless complicated when we get to analyze the causes of an ovary dysfunction, be it even minor. In this respect we have to think that the ovary is the final link of the hypothalamo-hypophyso-ovarian axis, and its proper functioning assumes the normal function of an entire gear, nervous, hormonal, synaptic transmission mediators (stimulators and inhibitors), of the secondary action of other endocrine glands that affect the correct functionality of the gonads (thyroid and suprarenal).

We shall briefly analyze the role of the nervous factor, of the hypophysis, thyroid, suprarenal, and of course of the ovary. We shall furthermore analyze the role of men as sexual partners, self-contamination and other factors.

The nervous factor

Synthetically speaking, all information coming to the encephalon level from the external or internal environment are decoded information as nervous impulse, which shall determine at the level of terminal extremities of a certain type of mediation (excitatory – dopamine and its analogues, or inhibitory – serotonin and its analogues) according to the type of peripheral excitation: positive or negative. In this category, too, there are the harmony statuses, which shall stimulate de dopaminergic system, or conflicting statuses, which shall stimulate de serotoninergic system. At the level of the hypothalamus, the dopamine and its analogues (l-Dopa, 2-alpha-brome-ergocryptine etc.) have the role of stimulating the secretion of PIF (prolactin – inhibiting factor), which on its turn shall inhibit the secretion of prolactin at the hypophysary level. The serotonin and its analogues have the role of inhibiting the secretion of PIF, which has as consequence on the hypophysis the increase in prolactin secretion (the prolactin release mechanism is by inhibition, thus inhibiting the inhibition means excitation).

This direct intervention of the nervous system in the regulation of prolactin secretion according to the nature of stimuli that reach its level has a decisive role on the health status of the mammary gland, but also on the endocrine function of the ovary, by the following mechanism: high levels of prolactin do not alter the FSH

secretion, but inhibit the LH secretion, giving raise to an estro-progestative balance disruption with relative hyperestrogeny, which by increasing the glycogen deposits at the level of the vaginal mucosa, promotes the setup of candidosis. In these situations, the administrative of a synthetic progestative during the days 16 to 25 of the cycle, for 2 or 3 cycles, may prove salutary.

The thyroid gland

Hypothyroidism, even sub clinical, leads by a feed-back mechanism to the increase of TRH, which on its turn, by its inhibitory action on the hypophysary PIF, leads to the increase of the prolactin secretion. Increased prolactin secretion leads to alterations in the production of ovarian steroids, which manifests peripherally by menstrual disturbances that may reach amenorrhea (the amenorrhea – galactorrhea syndrome).

The cortico-suprarenal gland, by secretion of suprarenal steroids, may interfere at central level with the release of hypophysary gonadotrophins, from where the alteration of the ovarian function and therefore of the secretion level of ovarian steroids.

The ovary as peripheral organ of the hypothalamo-hypophyso-ovarian axis, may be involved in a series of own illnesses (inflammatory, degenerative, genetic etc.), which qualitatively and quantitatively influences the estro-progesteronic balance. All these factors must be depicted by anamnesis, proper clinical and paraclinical examination.

Besides these causes, during the treatment and after treatment, one shall give a distinct attention to the prevention of re-contamination.

For this purpose, one shall depict and cure, in the patient, partner or any other family member any other location of candidosis (inguinal, oral, thigh intertrigo, hip, ano-rectal, inter-digital or nail candidosis).

The local hygiene shall be undertaken only after a hand wash with soap and water, and the vessels used for this shall be washed or scalded, or disinfected with alcohol. The vaginal shower is not indicated since it alters the environmental conditions of the saprophytic vaginal flora, removes the lactic bacilli and promotes other populations of microorganisms. The underwear (personal, non-transmittable and kept in separate bags) is to be boiled, hot ironed, and changed daily.

It is a must that the partner is treated, either if he is ill or a healthy carrier (some gonococcal urethritis are asymptomatic and this is why they have to be

depicted and treated); before sexual intercourse, the partner shall wash his genitalia with soap and water. In order to generally avoid sexually transmitted diseases, the local hygiene and fidelity ought to come first.

Cold and moisture shall be avoided using proper shoes and clothing. A proper intake of liquids shall be assured (no less than 1.5 liters per day) in order to prevent constipation, that promotes intestinal bacterial stalling and promotes bacteriemia. 30 to 50% of the vaginoses are of bacterial origin. Different inmate microorganisms of the digestive tract, such as *Escherichia coli*, Klebsiella, Enterobacter, Pseudomonas, several Bacteroides species, Serratia, may cause bacterial vaginosis due to an improper hygiene, by getting from the feces to the perineum, and then to the vagina and urethra, through lack of hygiene.

One shall avoid alcohol, spicy food (garlic, onion, pepper) and canned food, since they cause pelvic congestion. Refined and concentrated sweets shall be avoided as possible since sugars in large amounts decrease immunity and promote the development of candidosis; a regime rich in vegetal and animal proteins (meat, dairy, butter, eggs) shall be preferred. One shall avoid or quit smoking and shall check for sub-clinical diabetes by the proof of provoked hyperglycemia. The medical examination shall eliminate an organic cause (cervical polyp, uterine polyp, chronic endometritis, uterine fibroma, salpingo-ovarian tumors etc.).

Conclusions

1) The drug Cervugid – ovules has proven to be the first choice treatment for the cervico-vaginal infections caused by pathogenic microbial germs, mycoses, trichomoniasis, Chlamydia, mycoplasma and rickettsia.
2) When the cyto-test reads ASCUS, LSIL or AGUS, this gets totally cured.
3) When the cyto-test reads HSIL, the treatment with Cervugid comes to clarify the eventual false-positive results. There are cases that may convert under treatment from HSIL to LSIL and then to normal cyto-test.
4) The treatment with Cervugid administered preoperative for interventions in the genital area assures a favorable evolution with no septic complications.

5) Given in pregnant women with cervico-vaginal infections, it prevents certain major complications of the pregnancy: amniotic infections, premature rupture of the membranes, premature birth, and low-weight newborn.
6) It is a well tolerated drug, with minimal adverse effects and counter-indications.

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