

Cytological And Histopathological Aspects Concerning Preinvasive Squamous Cervical Lesions

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ABSTRACT Our study was carried out on a total number of 158 patients, with a mean age of 32, all tested and identified cytologically (Pap-test) as presenting minor cellular abnormalities, respectively ASCUS (10) and LSIL (119), and major cellular abnormalities, respectively SIL-borderline (8) and HSIL (21), and who, either voluntarily or upon cytopathologists' recommendation, were colposcopically examined. Subsequently, they were subjected to cervical biopsy or excision therapy. In patients with ASCUS cytology, 6 cases were morphologically diagnosed with benign cervical lesions, 3 were diagnosed with LSIL, and one patient was diagnosed as HSIL (CIN 2). Out of 119 LSIL smears 108 were confirmed by histopathology, while 11 were diagnosed as HSIL (CIN 2). In SIL-borderline patients, 5 cases were screened as LSIL and 3 as HSIL. In patients with HSIL cytology, 18 were diagnosed histopathologically as HSIL (CIN 2 and CIN 3/CIS), while 3 were diagnosed as invasive squamous carcinoma.

KEY WORDS *Pap-test, colposcopic examination, preinvasive cervical lesions.*

Introduction

Squamous intraepithelial lesions or squamous epithelial dysplasia contain a group of proliferative lesions characterized by abnormal cytological and histological differentiation, maturation and stratification of squamous epithelium: dedifferentiation and disrupted architecture of the malpighian epithelium, increased mitotic activity and the presence of atypical mitoses, cellular pleomorphism, nuclear atypia on all epithelium levels, regardless of the degree of cytoplasm maturation. Their intensity varies in accordance with the severity of the lesion. Because of the potential development of invasive carcinoma, they are viewed as precancerous lesion, presenting, in fact, many of the morphological characteristics of invasive carcinomas. Identification of these entities is the focus of cervical screening programs that aim to discover them and commence their treatment in order to prevent invasive disease.

Progress in understanding preinvasive cervical pathology has led to the need to review the cytology taxonomy methodology (3). As such, Bethesda system has become over a short period of time the internationally standardized method of reporting cytology smears. In 2002, following further reviews, it was accepted that abnormal

squamous epithelial cells be classified in one of the following categories:

- Atypical squamous cells (ASC)
 - of undetermined significance (ASC-US)
 - that cannot exclude HSIL (ASC-H)
- Low grade squamous intraepithelial lesions (LSIL) including HPV/mild dysplasia/CIN 1
 - High-grade squamous intraepithelial lesions (HSIL) including moderate and severe dysplasia, CIN 2 and CIN 3/CIS, with suspicion of invasion (if invasion is suspected)
 - Squamous cell carcinoma

The histopathological taxonomy of precancerous lesions of the cervix has also undergone frequent modifications, alongside the periodical reformulation of concepts pertaining to the pathogenesis and clinical behavior of these lesions: dysplasia – carcinoma in-situ (CIS), Richardt European classification – CIN (cervical intraepithelial neoplasia) and the Bethesda system (TBS), a genuine 100% American innovation.

The traditional grading of CIN is based on the proportion of epithelial thickness occupied by undifferentiated basaloid cells, reflecting the progressive loss of epithelial maturation and glycogenation decrease occurring at the same time as the increase in lesion severity. Based on this criterion, which takes into account the severity of

the lesions (maturation degree, proliferation and atypia), CINs can be grouped into:

- CIN 1 (mild dysplasia): the lesion occupies the lower third of the epithelium
- CIN 2 (moderate dysplasia): the lesion extends over one third up to two thirds of the thickness of the epithelium
- CIN 3 (severe dysplasia and carcinoma in-situ): the lesion overtakes the entire thickness of the epithelium

By adapting the Bethesda System (1991), the three lesion categories are assimilated into two groups (possible with the abandon of the intermediate category CIN 2), representing the currently recommended terminology: low-grade intraepithelial lesions (LGSIL) and high grade ones (HGSIL).

This new taxonomy abandons the concept of lesion continuum and introduces an element of prognosis (17). Instead of a single disease process laid out in different stages, two distinct biological entities were described, one being a productive viral infection and the other a genuine neoplastic process confined to the basal membrane of the epithelium.

The advantages of the binary terminology consist in the fact that this type of terminology reflects our current understanding of the biology of precancerous lesions, it can be used both in cytopathologic diagnosis as well as in the histopathological one, while at the same time reflecting the current clinical attitude according to which patients with LSIL and satisfactory colposcopy may be simply monitored while patients with HSIL must be treated (9).

Sustainers of the European concept argue for the preserving of class CIN 2 within the histological classification (well defined morphological criteria for CIN 2; CIN 2 has a distinct natural history as compared to CIN 1 and CIN 3; CIN2 is involved in the management of women with abnormal Pap cytologies, biomarkers suggest the link between CIN 2 and CIN 1 and not between CIN 2 and CIN 3). They corroborate that the new binary classification would not describe morphology in the most appropriate manner, and what seems of even greater importance, the predictability of the natural history of precancerous cervical lesions. "It is incorrect to label any lesion as high-grade, while more than 40% of them regress spontaneously" (18).

With this foreground in mind and in order to obtain morphological information of cervical biopsies of maximum clinical relevance, the use of the term *CIN 2* is justified since it denotes a distinct category of cervical cancer precursors that

have intermediate potential in between CIN 1 and CIN 3 lesions (18).

Materials and methods

Our study was carried out on a total number of 158 patients, with the mean age of 32, all tested and identified cytologically (via the Pap-test) as presenting minor cellular abnormalities, respectively ASCUS (10) and LSIL (119), as well as major cellular abnormalities, respectively SIL-borderline (8) and HSIL (21), and who, either voluntarily or upon cytopathologists' recommendation, were colposcopically examined. Subsequently, as the colposcopy cartogram hinted more serious lesions either in patients diagnosed with minor pap abnormalities, or in SIL diagnosed patients who had their lesion confirmed by colposcopy, or patients who presented important inflammatory modifications (ectopies, trauma erosions, cervicites, hypertrophic hypersecretive cervix), the gynecologist followed – according to case requirements – a biopsy sample prelevation procedure or an excisional one: pinch biopsy, diathermy excision, loop diathermy conisation, endocervical biopsy curettage, all being examined within histopathology.

Slides obtained via cytology examination were colored by the method Babeş-Papanicolaou and the interpretation of results was performed in descriptive manner in conformity with Bethesda 2001 nomenclature system. The material collected by the gynecologist was processed in the classical histopathological method and, where appropriate, targeted to paraffin and sectioned according to the lesion diagram of the cervix, followed by serial sections and histopathological interpretation. All procedures were carried out within the Pathological Anatomy Laboratory of the Emergency County Hospital of Slatina.

In patients with ASCUS cytology, 6 cases were morphologically diagnosed with benign cervical lesions, 3 were diagnosed with LSIL, and one patient was diagnosed as HSIL (CIN 2). Out of 119 LSIL smears 108 were confirmed by histopathology, and 11 were diagnosed as HSIL (CIN 2). In SIL-borderline patients, 5 cases were screened as LSIL and 3 as HSIL (CIN 2 and CIN 3/CIS). In patients with HSIL cytology, 18 were diagnosed histopathologically as HSIL (CIN 2 and CIN 3/CIS), while 3 were diagnosed as invasive squamous carcinoma.

Results

Concerning cytology concoctions, in ASCUS smears superficial cells (S) or intermediate (I) abnormal ones are visible - the nuclei increased by

two to three times as compared to the normal round-oval - with contour irregularities, dark, but not sufficiently modified as for a diagnosis of LSIL .

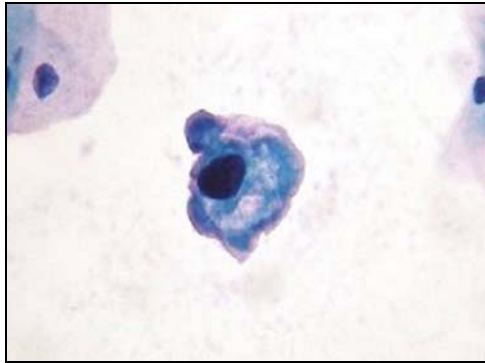


Figure 1 : ASCUS – probably HPV etiology, Pap stain, ob.40

The histopathology of 10 patients with ASCUS cytology, 6 were diagnosed with benign cervical lesions, 3 were diagnosed as having LSIL, while one patient had HSIL (CIN 2). LSIL smears containing cells with size similar to superficial cells (S) and intermediate ones (I), grouped in small or isolated sets, the cytoplasm often transparent, with poorly defined boundaries, with large nuclei (3-6 times larger than normal cells), their nucleo-cytoplasmic ratio below 1/3; the nuclear membrane smooth or slightly invaginate, uniform nuclear chromatin, slightly granular, of Bi- or tri- nuclei, respectively frequent modifications in koilocytosis cells, i.e. the morphological marker of HPV infection (nuclear pleomorphism, nuclear plications, hyperchromasia, perinuclear halo with a clear, distinct area around the nucleus and peripheral densification of the cytoplasm).

On occasion, dyskeratocytes (small size spindle cells with dense eosinophilia cytoplasm or dense orangeophilic, picnotic nucleus).

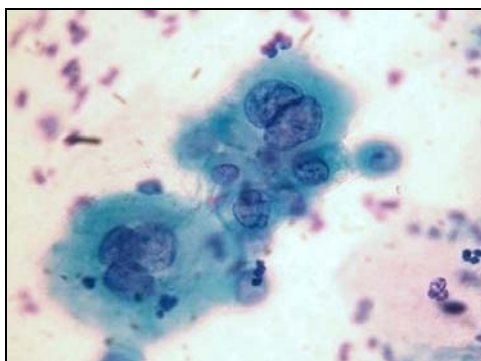


Figure 2 : Multi-nucleated LSIL, Pap stain, ob.10

As regards histopathological samples, frequently taken with loop diathermy, 108 cases were confirmed histologically LSIL/CIN 1 and 11

cases were diagnosed HSIL (CIN 2). The epithelium as a whole is acanthotic (increased thickness in stratum spinosum), with moderate thickening and wavy appearance, proliferation abnormalities, maturation and nuclear atypia are limited to the lower third of the epithelium. In this area cells lose their polarity, being similar to parabasal cells, with increased nuclei and hyperchrome and decreased mitotic activity. In the two upper thirds, cells are mature but their nuclei may be increased, pleomorph; in many a case alterations such as parakeratosis, and, less frequently, hyperkeratosis, with the presence of a granular layer. Quite in a number of cases aspects of atypical koilocytosis, more evident in *condyloma acuminata*, where lesions are composed of a conjunctive-vascular spindle, with papillary ramifications lesion comprising of squamous lesion epithelium.

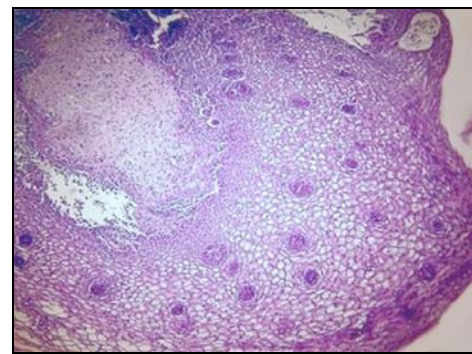


Figure 3 : Condyloma acuminata HE stain, ob. 100

In *keratinized LSIL* versions cell atypia is reduced as opposed to a respectable shallow keratinization . In cases of *reversed condyloma*, cellular proliferation interests glandular clefts, as epithelial islands, concurring in a superficial entophytic aspect.

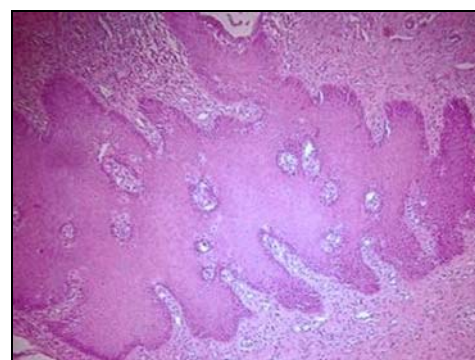


Figure 4 : Particular reverse condyloma aspect LSIL, HE stain, ob. 10

Patients cytologically diagnosed with SIL-borderline exhibit features between LSIL and HSIL cytology: I nuclei, increased approx. 3

times, with uniform or degenerate chromatin, binucleated, increased nucleo-cytoplasmic ratio (LSIL), and parabasal cell (PB) size assortment, irregular nuclear envelope (NE), granular chromatin, obvious cytoplasm, abundant, or quantitatively reduced.

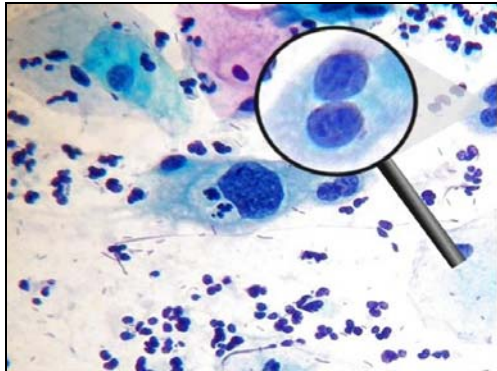


Figure 5 : SIL-borderline, Pap stain, ob. 40

In the histopathology perspective, 5 cases were sorted as LSIL while 3 were defined as HSIL (CIN 2) bearing CIN 1 images. The difference between HSIL and LSIL resides upon the appreciation of the nuclear atypia but also upon the amount in the thickness of the epithelium in which the respective modification is manifest. As far as LSIL is concerned, nuclear atypia is rarely significant, modifications being present exclusively in the inferior third of the epithelium.

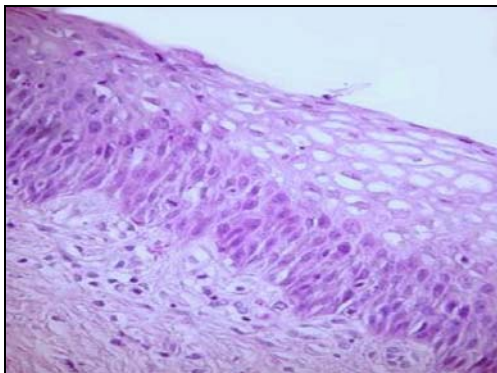


Figure 6 : HGSIL/CIN 2 with CIN 1 images, HE stain, ob. 20

Cytology anomalies in HSIL patients are more prevailing on the level of the genuine I cells or the PB, which have angular or round edges, in a balanced compound. Increased nuclei occupy more than two thirds of the cytoplasm, being hyperchrome with reticulate chromatin and visible empty spaces ('salt-n-pepper' aspect). Irregular nuclear membrane, sometimes multiple abnormal nuclei (CIN 2); severe dyskariotic cells, size B (basal) and PB (para-basal), separately exfoliate, the 'Indian file style, with hyperchrome nuclei,

irregular nuclear membrane, unsteady nucleolus, reticulate chromatin (CIN 3), or round - slightly ovoid cells, monomorphic and anaplastic, of cyanophilic, amphophilic or orangeophilic cytoplasm (the nuclei incurring malignancy criteria), with or without vacuolation, with the nuclei considerably increased, pronounced anisokaryosis, thick irregular chromatin piles, and margination (centrifugal motion). They also include increased triangular nucleus and hyper-nucleasations (CIS) .

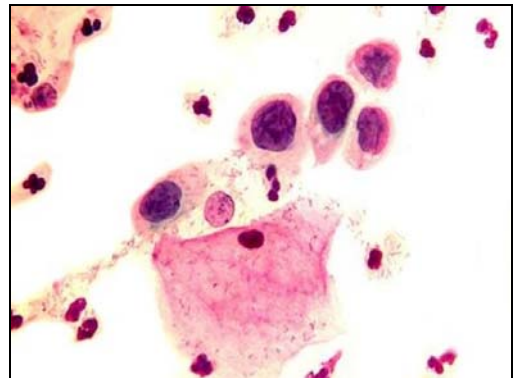


Figure 7 : CIN 2, Pap stain, ob. 40

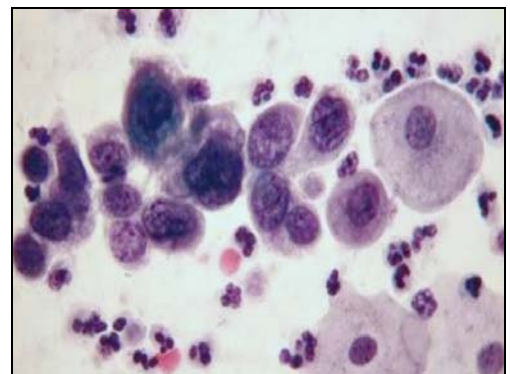


Figure 8 : CIN 3, Pap stain; ob. 40

Out of 21 HSIL cytology smears, 18 were morphologically diagnosed as HGSIL/CIN 2 or CIN3/CIS; another 3 cases were diagnosed via biopsy as invasive squamous carcinoma. Morphologically, the squamous epithelium is replaced in CIN 2 patients by immature abnormal cells, up to the median third of the epithelium, while preserving the upper third virtually unmodified; pleomorph, hyperchrome nuclei of irregular chromatin and abnormal mitoses can be observed up to the median third. Koylocitosis is active up to the superficial and parakeratosis layers. The mucosa adjacent to the CIN 2 lesion frequently manifests CIN 1 lesions . As a peculiar form of CIN 2, I identified one single case of *eosinophilic dysplasia*, with dysplastic and metaplastic characteristics, relatively abundant

eosinophilic cytoplasm and distinct cell walls, moderately increased nucleus/cytoplasm ratio, volume-increased focal nuclei, hyperchrome with evident nucleolus.

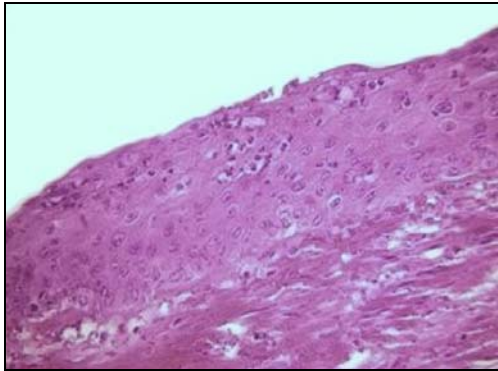


Figure 9 : CIN 2 variant (eosinophilic dysplasia), HE stain, ob. 10

In patients diagnosed CIN 3/CIS we remark immature neoplastic, type PB, cells which replace the entire thickness of the mucosa, by loss of polarity, entailing the tendency to verticalize multiplied nuclei. Atypical mitoses are present up to the surface. Nuclei appear either dark and polymorphic or uniform and vesicular. Epithelial thickness in CIS patients reaches 25-30 hyperchrome cell layers, with high nuclei/cytoplasm ratio, large nuclear atypia, reduced cytoplasm, indistinct cellular bridges. The cells do not reach more depth - basal epithelial membrane. In fact, I have frequently diagnosed *large unkeratinized CIN 3 cells* and a lower proportion of *large keratinized CIN 3 cells*, or even *small anaplastic CIN 3 cells*.

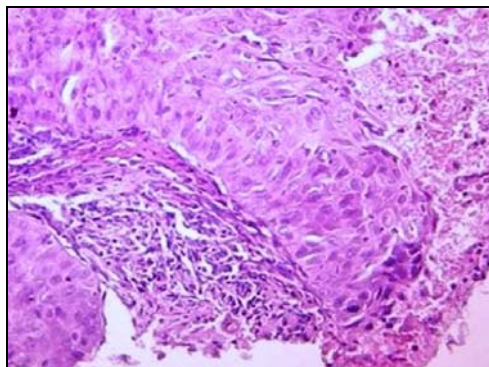


Figure 10: CIS variant with large unkeratinized cells, He stain, ob. 10

Debates

Our study was performed on a total of 158 patients with a mean age of 32, with cervical smears bearing minor and major cytological abnormalities, who were subjected to cervical

biopsy or excision therapy, under colposcopy control.

Relating to the ASCUS results – 4 out of 10 patients had a more severe lesion degree as compared to the cytology appreciation, and the other 6 had benign cervical lesions. Specific literature statistically demonstrates that in patients with ASCUS results there is potential of supra-diagnosis and supra-treatment (post-colposcopy), but which is nevertheless lower than the one pertaining to the possibility of a more severe lesion degree in the cervix as compared to the cytology evaluated one (4). Bethesda Conference proposes in case of ASCUS results, the exclusive "reflex" testing of DNA that may reassure negative HPV patients that they do not present a significant lesion, and, on the other hand, it may prevent between 40 to 60% of unnecessary colposcopies (20). The vast majority of our patients claim that it is expensive and therefore less convenient for viral testing.

The recommended conduct during '90, in approach of LSIL cytology, was to repeat cytological examination or to refer the patients directly to colposcopy examination. The rationale for this repeated and sufficient cytology, developed on the fact that patients may have no cervical change, or a CIN 1 alteration (8), which usually regresses spontaneously without treatment. However, there are CIN 2 or CIN 3 lesions with koilocytosis exfoliating exclusively koilocytes (12, 14). This confirms the need of a colposcope biopsy of all intraepithelial lesions, whether labeled low-grade or high grade, in order to gain the exact degree of histological change. Viral typing for LSIL cytologies is a great deal less specific to a certain level of sensitivity (viral load threshold of 1.0 pg/ml) as compared to the assessment made by a type ASC cytology (2). The desirable conduit option for LSIL cytology patients, explicitly stated in the Bethesda Conference of 2002, is colposcopy (6)

The terms 'SIL-borderline' or 'indeterminate SIL grade', was introduced in 2003, in the second edition of 'The Bethesda System for Reporting Cervical Cytology' publication, as particular to those situations where cytology smears have gross features of LSIL, but, also images consistent with atypical squamous cells that cannot exclude HSIL (Adams KC et al.,2003; Wang SS, et al., 2003). In our experience we have adopted this term from the firm desire of avoiding under- or supra-diagnosing a lesion. Out of a total of 8 SIL borderline cases, 3 were histopathologically diagnosed as LSIL while 5 cases confirmed high-grade lesions.

A HSIL cytology result indicates a significant risk of the patient for preinvasive cervical lesion or invasive cancer. According to a number of trials and studies the chance of a HSIL cytology patient for biopsy confirmation to be CIN 2/CIN 3 is around 70-75%, and their chance of suffering from invasive cancer is 1-2% (13, 16). Traditional recommended conduct viewed as optimal for a HSIL type cytology, is the performing of a colposcopy associated with endo-cervix assessment, which usually easily leads to identify high-grade cervical lesions; the positive predictive value of colposcopy being particularly high in this situation. Diathermy excision stands as immediate response to the case (the 'see and treat' British version) (10, 11).

The clinical situation in which there is a cyto-colpo-histology discrepancy and the biopsy is negative or falling in no more than CIN 1 category, patients' risk of having CIN 2 or unidentified CIN 3, is nevertheless considerable, in some cases proving, subsequent to further investigation, that a proportion of up to 35% of these women received biopsy confirmation of CIN 2, CIN 3 (5). CIN classical classification into types 1, 2 and 3 is consistent with the expansion of the lesion within the epithelium thickness. However, in certain circumstances this criterion may prove insufficient, raising questions of lesion taxonomy. Thus, if there are discrepancies between the degree of maturation expansion and that of cell atypia, the degree to which the mitotic activity extends, since this might lead to changes in lesion classification, respectively outstripping it (7). For example, in the case of cells with more abundant cytoplasm and well shaped cytoplasmic membrane, but with pleomorph hyperchrome nuclei, and atypical mitoses, the lesion should be placed in group CIN 2 even if it only expands over the lower third of the epithelium (15).

We have diagnosed cytologically 21 HSIL smears, out of which 18 were histologically confirmed CIN 2, CIN 3/CIS lesions, while 3 patients were diagnosed with invasive squamous carcinoma.

Conclusions

Conventional Papanicolaou smear cytology is an effective screening test for cancer performed in the past 50 years. However, the limited sensitivity and specificity of cervical cytology in revealing cervical cancer and its precursors, has been the subject of much debate in the past two decades. It is currently clear that the presence of false positives and false negatives cannot be completely prevented, not even in the most advanced

laboratories . False negative results remain a major cytology problem and they justify the imposition of internal and external quality control of smear cytology interpretation. The trend projected for the future could be the use of cytology in liquid media, machine readable, combined with high HPV oncogenic risk testing, both carried out on the same sampling, hoping that this particular combination reduces the number of false positive and false negative smears.

Morphologically, there is a frequent recurrence of HSIL coexisting with LSIL and the majority of SILs actually coexist with cervical condyloma . Generally, it is difficult to evaluate the evolving mode of CIN lesions of various degrees. LSIL are considered more likely to regress, while HSIL will be prone to persistence or progress.

Dialogue between clinicians and cytopathologists (pathologist) is imperative and, in fact, stands as the only means of identification and individualization of the appropriate conduit for each patient suffering from preinvasive cervical pathology

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ABBREVIATIONS:

ASCUS (atypical squamous cells of undetermined significance),
LSIL (low grade squamous intraepithelial lesion),
SIL-borderline (squamous intraepithelial lesion of indeterminate grade),
HSIL (high grade intraepithelial squamous lesion),
CIN (cervical intraepithelial neoplasia),
CIS (carcinoma in-situ).

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