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Cytology of Cervical Intraepithelial Glandular Lesions

Ana Ovanin-Rakić¹, Vesna Mahovlić¹, Silvana Audy-Jurković¹, Ana Barišić¹, Lada Škopljanač-Maćina¹, Danijela Jurić¹, Sanda Rajhvajn¹, Jadranka Ilić-Forko^{2,5}, Damir Babić^{2,5}, Darko Folnović³ and Dubravka Kani⁴

¹ Department of Gynecologic Cytology, University Department of Obstetrics and Gynecology, Zagreb University Hospital Center, Zagreb, Croatia

² University Department of Clinical Pathology, Zagreb University Hospital Center, Zagreb, Croatia

³ University Department of Gynecologic Oncology, Department of Obstetrics and Gynecology, Zagreb University Hospital Center, Zagreb, Croatia

⁴ Children's Hospital Zagreb, Zagreb, Croatia

⁵ Zagreb University, School of Medicine, Zagreb, Croatia

ABSTRACT

Cytological criteria for the identification of glandular intraepithelial lesions (GIL) have not yet been fully described, especially for the precursors of adenocarcinoma in situ (AIS), thus these lesions may frequently remain unrecognized. As most patients diagnosed with AIS or mild to moderate GIL (grades I, II) are free from clinical symptoms, cytology has a very responsible role in the detection of these lesions. The aim of the study was to achieve the most appropriate cytologic diagnosis of intraepithelial lesions of endocervical columnar epithelium, analyzing the cytology findings in patients with histologically verified AIS and GIL (I, II). The value of cytology in the detection and differential diagnosis was assessed in 123 patients with definitive histologic diagnosis of glandular lesions (AIS, n=13; GIL I, n=11; and GIL II, n=7), and glandular lesions associated with squamous component (AIS associated with cervical intraepithelial neoplasia (CIN) or invasive squamous cell carcinoma (SCC), n=58; GIL I or GIL II associated with CIN, n=28; and GIL associated with microinvasive squamous carcinoma (MIC), n=6). In 95.1% of patients, lesions were detected by cytologic analysis that indicated additional diagnostic procedure. In terms of differential diagnosis, cytology showed higher accuracy in predicting lesion severity vs. type of epithelial alteration (75.6% vs. 55.3%) and abnormalities of columnar epithelium (95.7%; vs. 74.2%). The accuracy of cytology was higher in pure (AIS, 61.5% and GIL I, II, 22.2%) than in mixed lesions (25.9% and 20.6%). Continuous improvement in cervical specimens and cytodiagnostic skills, better understanding of intraepithelial adenocarcinoma and precursors, and their inclusion in the classification of cytologic and histologic findings are expected to upgrade the detection of these lesions, and to reduce the invasive cervical adenocarcinoma morbidity and mortality.

Key words: cervix, cytology, glandular intraepithelial lesions, adenocarcinoma in situ

Introduction

The incidence of the cervix uteri adenocarcinoma has been increasing for the last 20 years, especially in young women¹. The reasons for this rising trend include improved diagnosis with more appropriate sampling and better preparation techniques for both cytologic and histologic analysis, better recognition of precursor lesions, changes in nomenclature, evolving methods of treatment and improved understanding of the morpho-

logical features, having led to the development of criteria for the diagnosis of early dysplastic lesions. Another reason is the increasing prevalence of these lesions.

Although endocervical adenocarcinoma in situ (AIS) is a well-known precursor of invasive adenocarcinoma, there is no universally accepted precursor of AIS itself^{2,3}. Cervical cytologic screening has decreased the incidence

of invasive squamous cell carcinomas (SCC) of the cervix by detecting preinvasive lesions⁴. The morphological diagnostic criteria, which are well established for AIS^{5–10}, have not been universally accepted for borderline precursor lesions, which have been termed endocervical dysplasia⁶ or low grade glandular intraepithelial lesion (LGIL)¹¹, or glandular intraepithelial lesion (GIL grade I, II)¹², etc., yet there is an agreement that these lesions should have some but not all features of AIS and need not to be associated with inflammation¹³.

Some authors consider that cytology may not be reliable enough to detect pure AIS¹⁴, whereas others believe that the usefulness of Pap findings in the detection of glandular premalignant lesions may increase with improvement of the sampling technique and strict focusing on glandular lesions during analysis, thus influencing the adenocarcinoma morbidity and mortality¹⁵.

Cytological classification of cervical glandular lesions in Croatia

In the NCI Bethesda 2001 cytologic classification¹⁶ squamous intraepithelial lesions (SIL) with identifiable subgroups (low grade and high grade SIL) are recognized, in contrast to glandular lesions where AIS precursors are not identified. The Australian modification¹⁷ of the 1988 Bethesda System (TBS)¹⁸ for glandular lesions points better to the risk of the presence of high-grade abnormalities, thus resulting in more appropriate recommendations and protocol. A uniform classification of cervical cytology findings named Zagreb 2002¹⁹ (Table 1), i.e. a modification of the Zagreb 1990²⁰ and NCI Bethesda System 2001 classifications¹⁶, is currently used in Croatia. In Zagreb 2002 classification, like NCI Bethesda System 2001, glandular lesions have been divided into three categories: atypical glandular cells (AGC), adenocarcinoma in situ (AIS), and adenocarcinoma. In Zagreb 2002 AGC are divided into three subgroups, similarly like in other studies^{17,21,22}:

- favor reactive – cell alterations that are more pronounced than benign reactive ones but quantitatively and qualitatively less pronounced than those in intraepithelial lesions;
- favor intraepithelial – cell alterations of low to moderate severity, without inflammatory cell changes, and/or suspect of AIS, without definite criteria; and
- favor invasive – cell alterations suspect of invasive lesion, where differential cytologic diagnosis cannot be made, mostly due to poor specimen preparation.

The group of AIS requires definite criteria to be present. The group of adenocarcinoma has not been modified relative to previous classifications. For any group or subgroup of abnormal glandular cells, it is crucial to identify the origin of columnar epithelium whenever possible, as it is of great importance for further diagnostic work-up and therapeutic procedure²³. At the end of the report, the cytologist provides the clinician with instructions on how to improve the quality of cervicovaginal smears, and with guidelines on further procedures for a particular cyto-

logic finding. These instructions are in line with the current diagnostic-therapeutic protocol in use in Croatia²⁴.

In order to reach an as accurate and precise cytologic diagnosis of intraepithelial lesions of endocervical columnar epithelium as possible, the cytologic findings of patients with histologically verified AIS and mild to moderate GIL were analyzed.

Materials and Methods

During the 1993–2007 period, the value of cytology in the detection and differential diagnosis considering lesion severity and/or type of altered epithelium was assessed in 123 patients with definitive histologic diagnosis of glandular lesions (AIS, n=13; GIL I, n=11; GIL II, n=7), glandular lesion associated with a squamous component (AIS+ cervical intraepithelial neoplasm (CIN)/SCC, n=58; GIL I/GIL II+CIN, n=28; and GIL+ microinvasive squamous carcinoma (MIC), n=6).

Cytologic features of intraepithelial glandular lesions of the uterine cervix^{5–13,15,25–29}

The cytomorphological criteria for the diagnosis of AIS refer to changes in the architecture (sheet of cells, strip, rosette, gland opening, feathering), and cells. Cell size is uniform and enlarged. Cytoplasm is cyanophilic, occasionally vacuolated. Nuclear size is uniform and usually enlarged. Nuclear shape is oval or round, uniform. Mitotic figures are occasionally or, according to some authors, regularly present (Figure 1).

However, cellular changes may frequently be less pronounced than those in squamous lesions and are difficult to observe unless architectural alterations call for attention. In mixed lesions, the glandular component may be eclipsed in abnormal cell count and intensity by the squamous component. Mild and moderate glandular intraepithelial lesions (dysplasia) have not been clearly defined, while reproducibility of the cytologic and histologic criteria for their identification has not been fully explored. Cellular alterations in GIL (grade I, II) are simi-

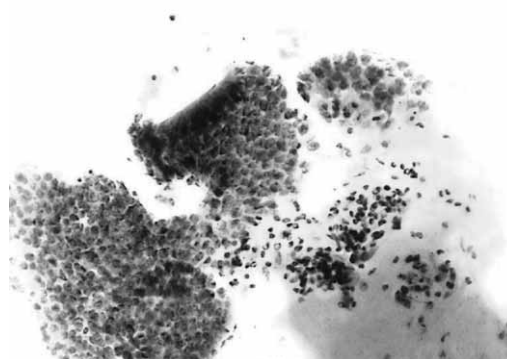


Fig. 1. Large hyperchromatic group of crowded cells typical for Adenocarcinoma in situ. Note the group of normal endocervical cells in the right lower part of the picture. (Papanicolaou-stained cervical scrape, ×100).

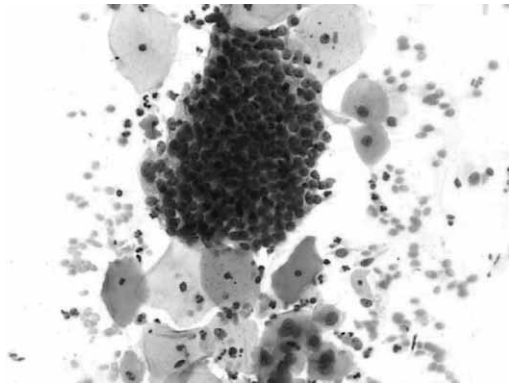


Fig. 2. Crowded sheets of cells from glandular intraepithelial lesion grade 1. (Papanicolaou-stained cervical smear, ×100).

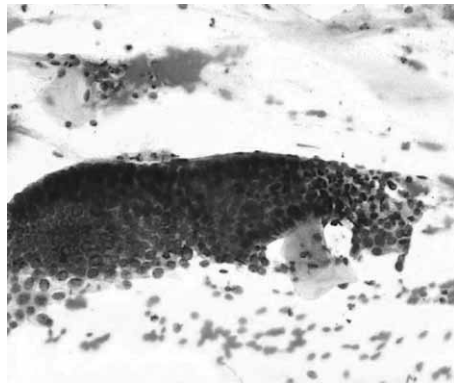


Fig. 3. Hyperchromatic group of crowded cells with scant cytoplasm and pseudostriatification from glandular intraepithelial lesion grade 2. (Papanicolaou-stained cervical smear, ×100).

lar to but less pronounced than those in AIS. The type of desquamation is also similar, only the columnar cells are slightly packed showing a palisading pattern with mild pseudostratification, nuclear overlapping is less pronounced, and also feathering and rosettes are seen. Cell size is like that in normal findings or slightly enlarged. Nuclear size within a cluster varies to a greater extent than in AIS. The nucleus is round or oval, hyperchromasia is less pronounced, chromatin is finely granular and evenly distributed, and nucleoli are small and round. Mitoses are rare. Background is usually clean (Figures 2 and 3).

Results

Intraepithelial endocervical columnar lesions, with or without intraepithelial or invasive squamous component, were diagnosed in histology samples (78 biopsy specimens, 82 excochleation specimens, 70 conization specimens and 24 hysterectomy materials) from 123 patients aged 22–73 (mean 39.89) years. The patients were divided into two categories: group 1 including 71 patients (mean age 39.69) histologically diagnosed as AIS or AIS associated with (CIN) or SCC, and group 2 including 52 patients (mean age 40.15) histologically diagnosed as

TABLE 1
CYTOLOGICAL CLASSIFICATION OF GLANDULAR LESIONS:
ZAGREB 2002 CLASSIFICATION

Glandular cells:	
Atypical glandular cells	Site of origin:
Favor reactive change	Endocervical
Favor intraepithelial lesion	Endometrial
Favor neoplastic lesion	Extrauterine
Adenocarcinoma in situ (AIS)	Not specified
Adenocarcinoma	Other

mild or moderate glandular intraepithelial lesions with squamous component (GIL I/GIL II+CIN, GIL+MIC) or without it (GIL I, GIL II).

In group 1, cytologic findings indicated epithelial abnormality in 98.6% (70/71) of patients. Considering lesion severity, the cytologic and histologic diagnoses were identical in 93% (66/71) of patients. The accuracy of cytologic diagnosis according to lesion severity and type of epithelium was 92.3% (12/13) for glandular lesions and 56.9% (33/58) for mixed lesions. On predicting the type of epithelium involved, agreement between the cytologic and histologic diagnosis was recorded in 61.5% (8/13) of histologically pure (AIS) and 20.7% (12/58) of mixed lesions (AIS+CIN/SCC). The rate of cytologic identification of abnormalities of a particular type of epithelium, histologically diagnosed as either pure or mixed lesions, was 92.3% (12/13) and 96.6% (56/58) for columnar and squamous epithelium, respectively (Table 2).

In group 2, cytologic findings indicated epithelial abnormality in 90.4% (47/52) of patients. Considering lesion severity, the cytologic and histologic diagnoses were identical in 80.8% (42/52) of patients. The accuracy of cytologic diagnosis according to lesion severity and type of epithelium was 61.1% (11/18) for glandular lesions and 35.3% (12/34) for mixed lesions.

On predicting the type of epithelium involved, agreement between the cytologic and histologic diagnosis was recorded in 22.2% (4/18) of histologically pure (GIL I) and 20.6% (7/34) of mixed lesions (GIL I, II+CIN/MIC). The rate of cytologic identification of abnormalities of a particular type of epithelium, histologically diagnosed as either pure or mixed lesions, was 61.1% (11/18) and 100% (34/34) for columnar and squamous epithelium, respectively (Table 3).

Discussion

Most patients diagnosed with GIL (grade I, II) and AIS are free from clinical symptoms⁶, the portio is of normal macroscopic appearance and colposcopic images have long been considered nonspecific⁶. Some authors state that characteristic vascular changes are found in glandular lesions³⁰. The lesion is detected by cytology on routine smear sampling (Pap test)^{31–33} or by histology (on ECC, biopsy specimen, conization specimen, loop excision, hys-

TABLE 2
CYTOHISTOLOGIC CORRELATION OF EITHER PURE ADENOCARCINOMA *IN SITU* (AIS) OR A MIXED AIS AND SQUAMOUS ABNORMALITY

Cytology	n	Histology					
		Pure			Mixed		
		AIS	AIS + CIN	AIS + SCC	total		
		n	(%)	n	n	(%)	
AIS	9	8	(61.5)	1	1	(1.7)	
AIS + CIN	15	1	(7.7)	12	2	14	(24.2)
AC	6	3	(23.1)	3	3	(85.2)	
AC + CIN	2			2	2	(3.4)	
AC + SCC	4			3	1	4	(6.9)
GIL + CIN	9			8	1	9	(15.6)
CIN	21			20	1	21	(36.2)
MIC	2			1	2	(3.4)	
Abnormal	2			2	1	2	(3.4)
Inflammation	1	1	(7.7)				
Total	71	13	(100.0)	52	6	58	(100.0)
%	100.0	18.3				81.7	

AIS – adenocarcinoma in situ, CIN – cervical intraepithelial neoplasia, AC – adenocarcinoma, SCC – invasive squamous cell carcinoma, MIC – microinvasive squamous carcinoma

terectomy material) on examination for SIL or during operative procedure for myoma^{32,33}. Hence, cytology has a very prominent and responsible role in the detection and diagnosis of these lesions.

The cytologic diagnosis of AIS of endocervical columnar epithelium as a separate entity was only included in the NCI Bethesda System 2001 classification¹⁶, whereas dysplasia of endocervical columnar epithelium as an AIS

precursor is still considered as a cytologically and histologically inadequately defined entity¹³ and therefore has not been included in the classification¹⁶. However, the fact that AIS patients are older than women with squamous CIS³⁴ and that the reverse holds true for adenocarcinoma and SCC could imply that the progression of GIL to AIS must be slower than the progression of CIN lesions to CIS; in contrast, AIS should progress to adeno-

TABLE 3
CYTOHISTOLOGIC CORRELATION OF EITHER PURE GLANDULAR INTRAEPITHELIAL LESION (GIL) OR A MIXED GIL/SQUAMOUS ABNORMALITY

Cytology	n	Histology								
		Pure				Mixed				
		GIL I	GIL II	Total	GIL I + CIN	GIL II + CIN	GIL + MIC	Total		
		n	n	n	(%)	n	n	n	n	(%)
GIL I	4	4		4	(22.2)					
GIL I + CIN	8	2	2	4	(22.2)	3		1	4	(11.8)
GIL II + CIN	5	1		1	(5.6)	1	3		4	(1.8)
AIS + CIN	4		2	2	(11.1)	1	1		2	(5.9)
GIL + MIC	2					1		1	2	(5.9)
CIN	24	1	1	2	(11.1)	8	10	4	22	(64.6)
Inflammation	5	3	2	5	(27.8)					
Total	52	11	7	18	(100.0)	14	14	6	34	(100.0)
%	(100.0)			34.6					65.4	

GIL – glandular intraepithelial lesion, CIN – cervical intraepithelial neoplasia, AIS – adenocarcinoma in situ, MIC – microinvasive carcinoma

carcinoma significantly more rapidly than does CIS into SCC, and this seems to be the case indeed³⁵. It would leave ample time for detection of glandular dysplasia, but not necessarily of AIS³⁶.

The prevalence of GIL is unknown; however, it is much lower than that of SIL. Some authors report on the AIS to SIL ratio of 1:105³⁴. The rate of dysplasia of endocervical columnar epithelium is 16-fold that of AIS³⁴. The ratio of in situ and invasive lesions is 1:3 for glandular and 5.25:1 for squamous lesions³⁵. The latency period between AIS and invasive adenocarcinoma is 13 years compared with 18 years latency period between CIN and invasive SCC³⁵, allowing for ample time for screening for preinvasive lesion.

Patients diagnosed with mild glandular lesions are by some 10 years younger than those with invasive disease³⁷. The median age of patients in our study was 39.89 years (GIL-40.15; AIS-39.69), which is comparable to 41 years reported in the literature³⁸ and slightly older than the averages from other studies^{39,40}. In a series of initial cervical smear, minimal to severe atypia of columnar epithelium was detected in 50% of cases with squamous epithelial lesions¹⁰, pointing to common etiologic factors, among them human papillomavirus (HPV) at the first place.

A coexisting SIL may obscure glandular lesion because abnormalities involving exclusively the squamous component were quite frequently observed in the latter, either because of the more distinct criteria and easier recognition, or due to more pronounced cellular lesions, or because of the predominant population of abnormal squamous cells, especially when extensive or high grade. In our study, the incidence of coexisting squamous lesions was 74.8%, which is consistent with literature reports on 41%–76.7%^{39,41}.

The pre-conization diagnosis of AIS and GIL (I, II) is challenging. Historically, only sporadic cases of AIS have been reported since it was first defined by Friedell and McKay in 1953⁴², when they only described its histologic appearance. In the 1970s and 1980s, descriptive studies detailing the cytologic criteria necessary for the prospec-

tive cytologic diagnosis of AIS of uterine cervix were published^{5,6,8}, increasing the awareness and diagnostic skill of cytologists. Papanicolaou smears may be less sensitive than they are for squamous precursors because AIS may mimic endometrial cells or reactive endocervical cells^{11,43}. Also, benign conditions such as tubal metaplasia and cervical endometriosis⁹ may cytologically mimic AIS. There is overlap between the cytologic criteria for various glandular lesions of the cervix, and there is an increasing need for defining and recognizing both benign and malignant cervical glandular lesions. In a number of retrospective case series published within the last 15 years, screening of Papanicolaou smears detected glandular abnormality before confirmation of AIS on cone biopsy or hysterectomy in 32%–69% of cases^{8,32,39,44–47}.

In our experience, the number of AIS cases we identified has increased with time after our first identification in 1986. In our patients, Papanicolaou smear had a sensitivity of 74.2% in detecting glandular abnormality preoperatively, while cytologic differential diagnosis of AIS and GIL yielded a 61.5% and 22.2% accuracy, respectively, similar to other reports^{3,8,39}. Ioffe et al.³ demonstrated the use of a semiquantitative system for the diagnosis of noninvasive endocervical glandular lesions to result in better diagnostic reproducibility even in diagnostically problematic cases.

Conclusion

The cytodiagnosis of cervical columnar epithelial lesions lags behind the cytodiagnosis of squamous epithelial lesions both in terms of screening and differential diagnosis. We believe that Papanicolaou smear that includes adequate material from the transformation zone and endocervix can be useful method for detecting precursor lesions of adenocarcinoma of the cervix. As our understanding of glandular lesions continues to expand and cervical sampling techniques continue to improve, we may expect constant enhancement in our ability to detect and treat intraepithelial glandular lesions and thus help reduce the morbidity and mortality of cervical adenocarcinoma.

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A. Ovanin-Rakić

Department of Gynecologic Cytology, University Department of Obstetrics and Gynecology, Zagreb University Hospital Center, Petrova 13, Zagreb, Croatia
e-mail: ana.ovanin@gmail.com

CITOLOGIJA CERVICALNIH INTRAEPITELNIH LEZIJA CILINDRIČNOG EPITELA

SAŽETAK

Citološki kriteriji za identifikaciju glandularnih intraepitelnih lezija još nisu detaljno opisani, posebice za prekursor adenokarcinoma in situ (AIS) te ove lezije često nisu prepoznate. Većina bolesnica u kojih je dijagnosticiran AIS ili glandularna intraepitelna lezija (GIL) lakog i srednjeg stupnja su bez kliničkih simptoma te citologija ima vrlo odgovorno mjesto u otkrivanju ovih lezija. U svrhu postizanja što adekvatnije i točnije citološke dijagnoze intraepitelnih lezija endocervikalnog cilindričnog epitela, analizirani su citološki nalazi bolesnica u kojih su histološki verificirane glandularne intraepitelne lezije. Vrijednost citologije u otkrivanju i u diferencijalnoj dijagnozi, uzimajući u obzir težinu lezije i/ili vrstu promjenjenog epitela, testirana je u 123 bolesnice s konačnom histološkom dijagnozom intraepitelne glandularne lezije AIS (n=13), GIL I (n=11), GIL II (n=7), odnosno glandularne lezije udružene s pločastom komponentom AIS uz cervikalnu intraepitelnu leziju (CIN) i/ili invazivni karcinom pločastog epitela (IC) (n=58), GIL I ili GIL II+CIN (n=28), GIL uz mikroinvazivni pločasti karcinom (MIC) (n=6) U 95,1% lezija je otkrivena citološkom analizom koja je indicirala daljnju dijagnostičku obradu. Diferencijalno dijagnostička točnost citologije bila je viša u predviđanju težine lezije 75,6% nego vrste promjenjenog epitela 55,3% te u predskazivanju abnormalnosti pločastog 95,7% za razliku od cilindričnog epitela 74,2%. Točnost citologije bila je veća za čiste lezije (AIS – 61,5%; GIL I/GIL II – 22,2%) nego za miješane lezije (25,9% i 20,6%). Kontinuirano poboljšanje cervikalnih razmaza i citodijagnostičkih vještina, bolje razumijevanje i definiranje predstadija intraepitelnog adenokarcinoma i njihovo uključivanje u citološku i patohistološku klasifikaciju lezija vrata maternice, moralo bi rezultirati boljom detekcijom i dijagnostikom glandularnih intraepitelnih lezija te smanjenjem morbiditeta i mortaliteta od invazivnog cervikalnog adenokarcinoma.