Original article

Diagnostic Accuracy of Atypical Glandular Cells in the Pap Test

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Abstract

Objectives: The focus of this study was to determine how much the new type of categorization "Zagreb 2016.", that excluded a certain number of glandular findings as reactive and negative, affected the frequency of Pap Test cytological samples marked as atypical glandular lesions (NOS, probably intraepithelial lesion and probably invasive lesion). In addition, we wanted to determine the diagnostic value of category atypical glandular cells (AGC) for each of the three subtypes of this category.
Participants and methods: This research included respondents who underwent pathohistological examination of the endometrial or endocervical biopsy tissue sample in Clinical Institute for Pathology Clinical Hospital Center Osijek in time frame from year 2018. to 2022. Respondants previously conducted a cytological examination of the same sample and were marked as atypical glandular cells (AGC).
Results: In range from year 2018. to 2022. a decrease in number of patients marked with AGC was noticed. AGC-NOS Pap smear finding has limited diagnostic value for predicting histopathologically confirmed changes. Although there is a significant association, diagnostic concordance is low. In contrast, the AGC – favour invasive lesion category shows high specificity and high positive and negative predictive value.
Conclusion: The research showed that using the classification "Zagreb 2016", which excluded the category atypical glandular cells of undetermined significance, and introduced the category endocervical epithelium reactive and irritated, for epithelium showing only reactive changes, the number of unnecessarily diagnosed and processed patients was reduced. Results of this study confirmed that the diagnostic categories AGC-NOS and AGC – intraepithelial lesion have limited diagnostic value, while the diagnostic category AGC – invasive lesion has significantly better diagnostic features in the prediction of pathohistologically confirmed lesions compared to previous two AGC subgroups.

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KEYWORDS: AGC – favour intraepithelial lesion; AGC – favour invasive lesion; AGC-NOS; atypical glandular cells; diagnostic accuracy

Introduction

Since George Nicholas Papanicolaou developed the technique of collecting, fixing, and staining cervical cells and microscopically evaluating structural abnormalities of the cells in the 1940.-s, this method has become the method of choice in diagnosing precancerous and cancerous changes at the cervix (1).

Classifications of cytologically determined dyskaryotic changes into categories facilitates communication between clinicians gynecologists, and diagnostic cytopathologists. Papanicolaou presented a classification of five degrees of dyskaryosis (2).

Over the years, the initially defined classification of five degrees of dyskaryosis, presented by Papanicolaou, has undergone several modifications. The first modification was presented by Richart in 1966. He introduced the term cervical intraepithelial neoplasia (CIN) and classification with CIN I, CIN II, and CIN III, which is based on the tissue architecture and is related to the pathohistological appearance of the cervical epithelium (3).

After, in 1991, a meeting was held with experts from all over the world at the National Institutes of Health in Bethesda, Maryland, USA, in 1988. During the meeting, a new classification was presented, named " The Bethesda system for reporting cervical cytology " (TBS). The aim of this classification was to provide a uniform system of terminology for reporting with clear guidelines for the management of these lesions (4).

Two more workshops were conducted in 2001 and in 2014. "The Bethesda system" standardized the terminology used in the Pap smear and enabled better communication between gynecologists, pathologists and cytologists, and led to a uniform worldwide recommendation of clinical management for these lesions (5). Based on the TBS classification from 1990 and subsequently from 2001 in Croatia a unique classification of cervical cytological findings is being introduced. It was called "Zagreb 1990" and "Zagreb 2002". It was introduced as the official Croatian version of the classification of cervical cytological findings, in cooperation with three centers for gynecological cytology in Zagreb, Osijek and Rijeka (6).

By combining the unique Croatian classification of cervical cytological findings "Zagreb 2002", in combination with the latest version of the Bethesda system from 2014., the latest Croatian modification called "Zagreb 2016" was developed (7).

The TBS classification from 2014 introduced a number of changes. One of the most significant change was the replacement of the term "atypical glandular cells of undetermined significance" (AGCUS) with the term "atypical glandular cells" (AGC).

The AGC category indicates the finding of atypical glandular cells that suggest cytological changes that are more significant than those typical for reactive or inflammatory processes, but not enough to be classified as malignant (endocervical AIS or invasive). This is a very challenging group of diagnoses, because it carries clinical therapeutic guidelines that include a series of invasive procedures from excochleation of the endocervical canal to curettage and hysterectomy. This is especially significant in the population of younger women, where we strive to preserve a reproductive potential (8) (9).

The new classification defined a new category, "endocervical columnar epithelium- probably reactive changes", which assumes reactive changes in cells in the non-neoplastic range. This new category resulted in a reduction in rate of AGC findings and consequently a reduction in unnecessary work-up procedures (10).

The focus of this study was to determine how much the new type of categorization, that excluded a certain number of glandular findings as reactive and negative, affected the frequency of Pap Test cytological samples marked as atypical glandular lesions (NOS, probably

intraepithelial lesion and probably invasive lesion).

In addition, we wanted to determine the diagnostic value of category atypical glandular cells (AGC) for each of the three subtypes of this category.

Patients and methods

This research included respondents who underwent pathohistological examination of the endometrial or endocervical biopsy tissue sample in Clinical Institute for Pathology and the Clinical Institute for Clinical Cytology, Clinical HospitalCenter Osijek in time frame from year 2018. to 2022. Respondants previously conducted a cytological examination of the same sample and were marked as atypical glandular cells (AGC). The criteria for inclusion in the research were respondents with cytological findings of atypical glandular cells (AGC) of any degree, for which a pathohistological analysis was subsequently performed within a period of one year at the Clinical Institute for Clinical Cytology of KBC Osijek.

The analyzed vaginal, endocervical and cervical samples were processed as conventional Pap smears, stained according to Papanicolaou (11), and microscopically evaluated according to the unique form of cervical cytological findings "Zagreb 2016".

Statistical methods

Categorical data are presented as absolute and relative frequencies. Differences in categorical variables were tested using Fisher's exact test. Normality of distribution of continuous variables was tested using Shapiro-Wilk test. The diagnostic value of AGC compared with histopathological findings was assessed using sensitivity, specificity, positive and negative predictive values, and accuracy. (12).The statistical package MedCalc® Statistical Software version 23.2.1(MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2025) was used for statistical analysis.(13)

Results

The research was conducted with 414 respondents. In range from year 2018. to 2022. a decrease in number of patients marked with AGC was noticed. (Cochran-Armitage test trend, P < 0,001) (Table 1).

Table 1. Distribution of respondents by age

	Number (%) of respondents
2018. year	124 (30,0)
2019. year	96 (23,2)
2020. year	64 (15,5)
2021. year	80 (19,3)
2022. year	50 (12,1)

The analysis of the diagnostic characteristics of the Pap test finding AGC NOS in relation to the pathohistological finding shows that this finding has a limited diagnostic value for the prediction of confirmed pathohistological changes. It has a low specificity (25.9 %) and a negative predictive value (48.6 %), while its sensitivity is extremely low (25.5 %), which indicates a limited ability to detect actually present histological changes. (Fisher's exact test, $P < 0.001$), (Kappa test, $\kappa = 0.346$). (Table 2).

Table 2. Diagnostic characteristics of AGC-NOS Pap findings in relation to PHD findings

	Number (%) of respondents according to PHD findings		*P**	†Kappa test (*P* value)
	Positive	**Negative**		
AGC-NOS				
yes	13 (25,5)	103 (74,1)	< 0,001	0,346 (< 0,001)
no	38 (74,5)	36 (25,9)		
				95% confidence interval
	Sensitivity		25,5 %	14,3 % - 39,7 %
	Specificity		25,9 %	18,8 % - 34,1 %
	Positive predictive value		11,2 %	7,2 % - 16,9 %
	Negative predictive value		48,6 %	40,7 % - 56,7 %
	Accuracy		25,8 %	19,7 % - 32,6 %

AGC-NOS, atypical glandular cells - unspecified; PHD, pathohistological diagnosis.

*Fisher's exact test; †Cohen's Kappa test

The Pap smear for AGC intraepithelial lesion has a moderate specificity (80.6 %) and negative predictive value (74.7 %), while its sensitivity is extremely low (25.5 %), indicating a limited ability to detect the actual histological changes. The positive predictive value is only 32.5 %. The value of Cohen's Kappa coefficient (κ = 0.065; P = 0.36) indicates a very poor agreement between cytological and histological findings, without statistical significance (Table 3).

Table 3. Diagnostic characteristics of the Pap finding AGC intraepithelial in relation to the PHD finding

	Number (%) of respondents according to PHD findings		*P**	†Kappa test (*P* value)
	positive	**negative**		
AGC intraepithelial				
yes	13 (25,5)	27 (19,4)	0,36	0,065 (0,36)
No	38 (74,5)	112 (80,6)		
				95% confidence interval
	Sensitivity		25,5 %	14,3 % - 39,6 %
	Specificity		80,6 %	73,0 % - 86,8 %
	Positive predictive value		32,5 %	21,3 % - 46,2 %
	Negative predictive value		74,7 %	71,1 % - 77,9 %
	Accuracy		65,8 %	58,6 % - 72,5 %

*Fisher's exact test; †Cohen's Kappa test

The Pap smear AGC – probably invasive lesion has significantly better diagnostic features in predicting histologically confirmed lesions compared to previous AGC subtypes. The sensitivity of 49.0 % indicates a moderate ability to detect true positive cases, while the specificity is high (93.5 %), meaning that the test rarely gives false positive results. The positive predictive value of 73.5 % is particularly significant. The negative predictive value (83.3 %) and overall accuracy (81.6 %) confirm the clinical value of this finding. Cohen's Kappa coefficient (κ = 0.476; P < 0.001) indicates a moderate agreement between cytological and histological findings, with statistical significance (Table 4).

Table 4. Diagnostic characteristics of AGC invasive Pap findings compared to PHD findings

	Number (%) of respondents according to PHD findings		*P**	†Kappa test (*P* value)
	Positive	**Negative**		
AGC probably invasive				
yes	25 (49)	9 (6,5)	<0,001	0,476 (<0,001)
no	26 (51)	130 (93,5)		
				95% confidence interval
	Sensitivity		49,0 %	34,7 % - 63,4 %
	Specificity		93,5 %	88,1 % - 96,9 %
	Positive predictive value		73,5 %	58,2 % - 84,7 %
	Negative predictive value		83,3 %	79,2 % - 86,7 %
	Accuracy		81,6 %	75,3 % - 86,8 %

*Fisher's exact test; †Cohen's Kappa test

Discussion

The new way of distribution within the unique pattern of cervical cytology findings in "Zagreb 2016" moved a certain number of atypical glandular cells to negative findings and designated them as endocervical columnar epithelium - reactive and irritated. In this study, we wanted to explore how this change affected the frequency of Pap smear cytology samples designated as atypical glandular cells (NOS, probable intraepithelial lesion and probable invasive lesion (10).

The research included 414 test subjects in the period from year 2018. to 2022. The analyzed samples of vaginal, endocervical and cervical samples were processed with a conventional Pap Test, stained according to the Pap Test protocol, and microscopically evaluated according to the unique pattern of cytological findings of the cervix "Zagreb 2016".

Pathohistological analysis was performed in 190 subjects, of which 31.9 % were positive. The most common pathohistological diagnosis was squamous dysplastic lesions in 11.1 % of subjects, polyps in 9.2 % of subjects, glandular malignant lesions in 8.7 % of subjects, while dysplastic glandular proliferative diagnoses and squamous malignant lesions were the least common.

A comparison of the number of patients identified with atypical glandular cell changes over a five-year period (2018. to 2022.) shows a significant decrease in the number of recorded cases. This can be explained by the fact that over the years, after the introduction of a new categorization within the unique cervical cytology form "Zagreb 2016", in which some

atypical glandular cell findings were identified as reactive changes and classified as negative findings, some patients with milder glandular changes were more actively classified in this negative category and excluded from further follow-up.

The Pap smear is primarily a test for detecting precancerous and cancerous lesions of squamous epithelium, while it is somewhat less sensitive for lesions of glandular epithelium due to the anatomical specificity of this region and closed endocervical canal. (14)

The focus of this study was to determine the diagnostic value of the Pap smear for precancerous lesions of glandular epithelium, namely for the diagnostic categories of atypical glandular cells (AGC) for NOS, probable intraepithelial and probable invasive variants of this category.

The results of the study suggest that AGC NOS has limited diagnostic value for the prediction of histopathologically confirmed changes. The sensitivity, specificity and predictive values for AGC-NOS are very low (diagnostic accuracy is 25.8 %) and suggest that AGC NOS is not a reliable diagnostic indicator. These results can be partly explained by the study design and the fact that both glandular and squamous cell abnormalities were present in same samples. For example, some AGC NOS were later histologically diagnosed as LSIL. According to Shoji T I et al. this occurs due to the similarity of cytological features of glandular HSIL and AGC and the fact that glandular lesions occur together with squamous epithelial lesions (15). The author Sung et al. classified their data in the same way and commented on the possibility of reduced clinical significance with such a classification (16). In other studies, the diagnostic accuracy of AGC-NOS is also lower and amounts to 35.41 % (17).

Analysis of the diagnostic characteristics of the Pap smear AGC – probable intraepithelial lesion in relation to the pathohistological finding shows that this finding has a specificity of 80.6 % and a negative predictive value of 74.7 %, while its sensitivity is extremely low at 25.5 %, indicating a limited ability to detect the actually present histological changes. The low value of the positive predictive value could be increased by HPV genotyping to identify groups of patients at high risk. This was concluded by the group of authors Goetz et al. who found that the presence of human papillomavirus type 6 (HPV16) significantly increased the risk of HSIL and other lesions in subsequent biopsies in patients who had an AGC finding on the Pap smear (18).

The extracted Pap smear finding AGC – probably invasive lesion shows significantly better diagnostic features in predicting histopathologically confirmed lesions compared to previous AGC subgroups. Its sensitivity of 49.0 % indicates a moderate ability to detect true positive cases, while the specificity is high at 93.5 %, meaning that the test rarely gives false positive results, which is in line with previous studies (19). The finding of AGC – probably invasive lesion is significantly more often associated with clinically significant abnormalities compared to the findings of AGC –NOS and AGC intraepithelial lesion, while the findings of AGC-NOS and AGC intraepithelial lesion range from negative and benign to malignant conditions. Similar results were obtained by Izadi-Mood et al. (20). In addition to this finding, we should also take into concern the length of monitoring the progression of the cytological finding, as Chhieng et al. suggest. In our study, we included a period of one year after cytological detection of a glandular lesion, and as Chhieng et al. suggests, glandular lesions should be monitored for a longer period of time, during which the initial lesion could eventually develop into something more serious (21).

Conclusion

The research showed that using the classification "Zagreb 2016", which excluded the category atypical glandular cells of undetermined significance, and introduced the category endocervical epithelium reactive and irritated, for epithelium showing only reactive changes, the number of unnecessarily diagnosed and processed patients was reduced.

Results of this study confirmed that the diagnostic categories AGC-NOS and AGC – intraepithelial lesion have limited diagnostic value, while the diagnostic category AGC – invasive lesion has significantly better diagnostic features in the prediction of pathohistologically confirmed lesions compared to previous two AGC subgroups.

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Disclosure

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Author contribution.

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Analysis and interpretation of data: VJ, MP, KK
Conception and design: VJ, MP, KK
Critical revision of the article for important intellectual content: VJ, MP, KK
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Final approval of the article: VJ, MP
Guarantor of the study: VJ, MP, KK
Provision of study materials or patients: VJ, MP
Statistical expertise: KK

Dijagnostička točnost atipičnih žljezdanih stanica u Papa testu

Sažetak

Ciljevi: Fokus ovog istraživanja bio je utvrditi koliko nova vrsta kategorizacije "Zagreb 2016", koja je isključila određeni broj žljezdanih nalaza kao reaktivne i negativne, utječe na učestalost citoloških uzoraka Papa testa označenih kao atipične žljezdane lezije (NOS, vjerojatno intraepitelna lezija i vjerojatno invazivna lezija). Osim toga, željeli smo utvrditi dijagnostičku vrijednost kategorije atipičnih žljezdanih stanica (AGC) za svaki od tri podtipa ove kategorije.

Sudionici i metode: Ovo istraživanje obuhvatilo je ispitanice koje su podvrgnute patohistološkom pregledu uzorka tkiva endometrija ili endocerviksa u Kliničkom zavodu za patologiju KBC Osijek u vremenskom okviru od 2018. do 2022. godine. Ispitanice su prethodno provele citološki pregled istog uzorka i označene su kao atipične žljezdane stanice (AGC).

Rezultati: U rasponu od 2018. do 2022. godine uočen je pad broja pacijenata označenih AGC-om. AGC-NOS Papa test nalaz ima ograničenu dijagnostičku vrijednost za predviđanje histopatološki potvrđenih promjena. Iako postoji značajna povezanost, dijagnostička podudarnost je niska. Nasuprot tome, kategorija AGC – u korist invazivne lezije pokazuje visoku specifičnost i visoku pozitivnu i negativnu prediktivnu vrijednost.

Zaključak: Istraživanje je pokazalo da je korištenjem klasifikacije "Zagreb 2016", koja je isključila kategoriju atipičnih žljezdanih stanica neodređenog značaja, a uvela kategoriju endocervikalni epitel reaktivni i iritirani, za epitel koji pokazuje samo reaktivne promjene, smanjen broj nepotrebno dijagnosticiranih i obrađenih pacijenata.

Rezultati ove studije potvrdili su da dijagnostičke kategorije AGC-NOS i AGC – intraepitelna lezija imaju ograničenu dijagnostičku vrijednost, dok dijagnostička kategorija AGC – invazivna lezija ima značajno bolje dijagnostičke značajke u predviđanju patohistološki potvrđenih lezija u usporedbi s prethodne dvije AGC podskupine.

Ključne riječi: atipične žljezdane stanice nespecificirane; atipične žljezdane stanice vjerojatno intraepitelna lezija; atipične žljezdane stanice vjerojatno invazivna lezija; dijagnostička točnost