Received: 11 February 2024 | Revised: 10 April 2024 | Accepted: 18 April 2024

DOI: 10.1111/cyt.13383

ORIGINAL ARTICLE

WILEY

Atypical glandular cells and predictive features of malignancy in Pap smears: A retrospective monocentric study

Federica Cianfrini[1] | Antonio d'Amati[1] | Damiano Arciuolo[1] | Antonio Travaglino[2] | Nicoletta D'Alessandris[1] | Giulia Scaglione[1] | Michele Valente[1] | Belen Padial Urtueta[1] | Francesca Addante[1] | Nadine Narducci[1] | Giuseppe Angelico[3] | Alessia Piermattei[1] | Antonino Mulè[1] | Angela Santoro[1,4] | Esther Diana Rossi[1,4] | Gian Franco Zannoni[1,4]

[1]Unità di Ginecopatologia e Patologia Mammaria, Dipartimento Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Fondazione Policlinico Universitario A. Gemelli Istituto di Ricerca e Cura a Carattere Scientifico (IRCCS), Rome, Italy

[2]Pathology Unit, Department of Medicine and Technological Innovation, University of Insubria, Varese, Italy

[3]Department of Medical, Surgical Sciences and Advanced Technologies G.F. Ingrassia, Anatomic Pathology, University of Catania, Catania, Italy

[4]Istituto di Anatomia Patologica, Università Cattolica del Sacro Cuore, Rome, Italy

Correspondence
Gian Franco Zannoni, Fondazione Policlinico Universitario A. Gemelli IRCCS, Dipartimento Scienze della Salute della Donna, del Bambino e di Sanità Pubblica, Unità di Gineco-patologia e Patologia Mammaria, Largo A. Gemelli 8, 00168 Rome, Italy.
Email: gianfranco.zannoni@unicatt.it

Abstract

Objective: The introduction of cytological screening with the Papanicolau smear significantly reduced cervical cancer mortality. However, Pap smear examination can be challenging, being based on the observer ability to decode different cytological and architectural features. This study aims to evaluate the malignancy rate of AGC (atypical glandular cells) category, investigating the relationships between cytological and histological diagnosis.

Methods: Eighty-nine patients, diagnosed as AGC at cytological evaluation and followed up with biopsy or surgical procedure at Policlinico Gemelli Hospital, Rome, Italy, were included in the study. The cytopathological architectural (feathering, rosette formation, overlapping, loss of polarity, papillary formation, three-dimensional formation) and nuclear (N/C ratio, nuclear enlargement and hyperchromasia, mitoses, nuclei irregularity, evident nucleoli) features of AGC were evaluated. Statistical analyses were performed to assess cyto-histological correlation and determine the relevance of architectural and nuclear features in the diagnosis of malignancy.

Results: Of the 89 AGC patients, 48 cases (53.93%) were diagnosed as AGC-NOS and 41 (46.07%) were diagnosed as AGC-FN, according to the Bethesda classification system. The follow-up biopsies or surgical resections revealed malignancy in 46 patients (51.69%). The rates of malignancy for AGC-NOS and AGC-FN were 35.41% and 70.73% respectively. Furthermore, analysing cytopathological features, we found that both architectural and nuclear criteria were statistically significant ($p < 0.05$). Only overlapping, nuclear irregularity and increased N/C ratio were not found to be statistically significant for detecting malignancy.

Conclusions: Cytological diagnosis of glandular lesions remains a valid tool, when appropriate clinical correlation and expert evaluation are available.

Angela Santoro, Esther Diana Rossi and Gian Franco Zannoni contributed equally and share senior authorship.

KEYWORDS
atypical glandular cells, cervical cancer, cyto-histological correlation, endocervical adenocarcinoma, Papanicolaou smear

1 | INTRODUCTION

Cervical cancer represents one of the most frequent cause of death in women worldwide, ranking as fourth for both incidence and mortality among all gynaecological neoplasms. However, incidence and mortality related to cervical cancer have been significantly reduced after the introduction of cytological screening with Papanicolau (Pap) smear, which still now represents the worldwide accepted method for the early diagnosis of cervical cancer. The Pap smear allows to detect both squamous and glandular lesion, with the latter often representing a more difficult challenge, in particular for less experienced 'eyes'. Actually, in Pap smears, it is not uncommon to observe the presence of just few glandular cells, characterized by features of mild atypia, which is sometimes difficult to certainly relate with a neoplastic or reactive process.

The 1988 Bethesda system defined the finding of glandular cells with changes beyond reactive or reparative changes, but without unequivocal features of invasive adenocarcinoma, as 'atypical glandular cells of undetermined significance (AGUS)'.[1] After many years, the 2001 Bethesda system modified the AGUS category, reclassifying it as 'atypical glandular cells (AGC)', subdivided it into two subcategories, 'favour neoplastic' and 'not otherwise specified'.[2] This category, together with its definition, remained unchanged over time, even though its real clinical relevance still needs to be fully elucidated. The incidence of Pap smears receiving a diagnosis of AGC ranges from 0.08% to 2.1%, according to the literature.[3,4] In this study, we aimed to evaluate the incidence of AGC in Pap smears that have been examined at our centre, in particular investigating the relationships between a cytological diagnosis of AGC in Pap smears and a histological diagnosis of malignancy in bioptic or surgical specimens, and describing the morphological features possibly representing more specific criteria of glandular neoplasia.

2 | MATERIALS AND METHODS

Over a 4-year period, from January 2017 to December 2021, Pap smears of 23,864 patients were reviewed in the Unit of Gynecological and Breast Pathology of Policlinico Gemelli, Rome, Italy, of whom 113 (0.47%) were diagnosed as having AGC. Among these 113 patients with a diagnosis of AGC, 89 (78.76%) patients had a follow-up biopsy or surgical resection and were included in the study. Figure 1 shows the flowchart of this study and the selection criteria.

The patients' clinical findings, including age, symptom, parity and menopausal status, were recorded from the hospital database, and only patients who had undergone a bioptic or surgical procedure

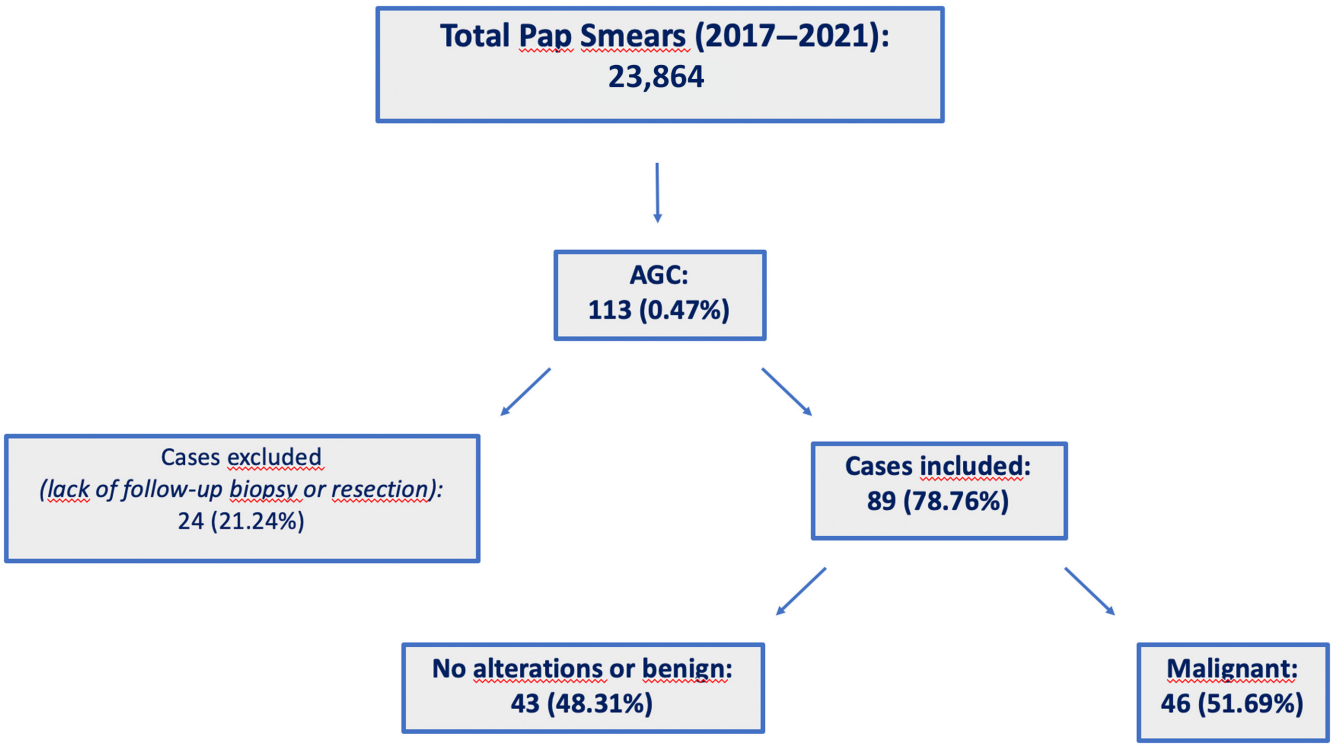

FIGURE 1 Schematic diagram representing the study flowchart.

during follow-up were included in the study. The remaining 24 patients who had not undergone biopsy for histopathological diagnosis in our institution were excluded. Patients with a previous history of cervical intraepithelial neoplasia or any gynaecological cancer were also excluded.

The ethics committee of the Fondazione Policlinico Gemelli, Rome, Italy, approved our study. All slides were retrospectively reviewed and subclassified according to the Bethesda classification system (2001) as 'atypical glandular cells' (AGC), not otherwise specified (AGC-NOS), and atypical glandular cells, favour neoplastic (AGC-FN). Cases showing cells with nuclear atypia surpassing evident reactive or reparative alterations, but not exhibiting apparent characteristics of either invasive or in situ adenocarcinoma, were designated as AGC-NOS. Cases showing cells with more evident nuclear atypia compared to AGC-NOS, but still quantitatively or qualitatively insufficient to be diagnosed as adenocarcinoma, were designated as AGC-FN.[2] Both conventional and liquid-based (ThinPrep, Hologic) Pap smears were included in the study. The cytopathological features of AGC, including architectural and nuclear features, were evaluated. The architectural features that have been evaluated were as follows: feathering, rosette formation, overlapping, loss of polarity, papillary formation and three-dimensional formation. The nuclear features evaluated were as follows: increased nucleus-to-cytoplasm (N/C) ratio, nuclear enlargement and hyperchromasia, mitoses, irregularity in shape and size of nuclei and presence of evident nucleoli.

Statistical analysis was performed using GraphPad Prism, version 10.0.0. The chi-square and Fisher exact tests were used to assess cyto-histological correlation and determine the relevance of architectural and nuclear features in the diagnosis of malignancy. Furthermore, these tests were also used to identify the malignancy ratio. A p-value of <0.05 was considered statistically significant.

3 | RESULTS

Among the 89 smears followed up with biopsy and included in the study, 77 (86.52%) were conventional and 12 (13.48%) were liquid-based Pap smears. Follow-up specimens included 39 (43.82%) obtained through a cervical biopsy/loop electrosurgical excision procedure, 26 (29.21%) by endometrial biopsy, 8 (8.99%) by vaginal cuff biopsy and 16 (17.98%) were surgical resections. The mean age of the patients was 38 years (range: 15–79 years). Fifty-two patients (58.43%) were pre-menopausal, while 37 (41.57%) were post-menopausal. Fifty-six women (62.92%) were uniparous or multiparous and 24 (37.08%) were nulliparous. Fifty-nine (66.29%) patients reported complaints of vaginal bleeding, while the remaining 30 were asymptomatic (33.71%). In the cytological evaluation of the 89 AGC patients, 48 cases (53.93%) were diagnosed as AGC-NOS and 41 (46.07%) were diagnosed as AGC-FN, according to the Bethesda classification system. The follow-up biopsies or surgical resections of the selected 89 patients revealed the absence of alterations or a benign pathology in 43 patients (48.31%) and a malignant pathology in 46 (51.69%). Table 1 shows the cyto-histopathological correlations of the patients.

In our group, there was no significant difference in terms of Pap smear technique, age, parity status or menopause status between the women with AGC-NOS and those with AGC-FN. The rates of malignancy for AGC-NOS and AGC-FN were 35.41% and 70.73%, respectively, with a statistically significant difference ($p=0.00089$). Compared to women with AGC-NOS smears, the frequency of malignant lesions was considerably higher in women with AGC-FN smears. Cervix was the most common localization among the malignant cases in both groups, but the AGC-FN group also showed three cases (7.32%) of endometrial cancer, all represented by endometrioid histological type. However, 31 patients (64.59%) patients of the AGC-NOS group and 12 (29.27%) patients of the AGC-FN group did not have any malignancy. Among them, eight (16.67%) AGC-NOS cases and two (4.88%) AGC-FN cases had no significant alterations at histological examination. On the other hand, 23 (47.92%) AGC-NOS cases and 10 (24.39%) revealed benign alterations, such as squamous metaplasia (4 AGC-NOS and 2 AGC-FN), tubo-endometrioid metaplasia (11 AGC-NOS and 5 AGC-FN), endometrial polyp (5 AGC-NOS and 2 AGC-FN) and endocervical polyp (3 AGC-NOS and 1 AGC-FN). In both groups, tubo-endometrioid metaplasia constituted the most common lesion.

Furthermore, we analysed cytomorphological features of glandular cells and we found that both architectural and nuclear criteria were statistically significant. In particular, among architectural features, we observed a statistical significance in the presence of feathering, three-dimensional formations, papillary pattern, rosette formation and loss of polarity ($p<0.05$). Only overlapping was not found to be statistically significant for detecting malignancy ($p>0.05$). Nuclear features, such as hyperchromasia, mitoses and presence of macronucleoli, were statistically significant for detecting malignancy ($p<0.05$), differently from nuclear irregularity and increased N/C ratio ($p>0.05$). Figure 2 shows representative images from some AGC cases with typical architectural and nuclear features.

4 | DISCUSSION

4.1 | AGC incidence and malignancy rate

'Atypical glandular cells (AGC)' is the term adopted by The Bethesda system of reporting cervicovaginal cytology.[1,2] One of the major revisions of the Bethesda system has been the substitution of 'atypical glandular cells of unknown significance (AGUS)' with 'atypical glandular cells (AGC).' AGC is the term used to categorize glandular cells with cytological features that are too pronounced to be called inflammatory or reactive but cannot be classified as malignant in endocervical or endometrial settings. AGC is a significant cytological diagnosis, including conditions that range from reactive and inflammatory conditions to dysplasia and malignancy. This high pathological variety carries a great deal of clinical significance, in particular in

TABLE 1 Histological diagnosis, according to AGC subgroups.

Histological diagnosis	AGC-NOS *n* (%)	AGC-FN *n* (%)	AGC *n* (%)
No significant alterations (negative)	**8 (16.67%)**	**2 (4.88%)**	**10 (11.24%)**
Benign alterations	**23 (47.92%)**	**10 (24.39%)**	**33 (37.08%)**
Squamous metaplasia	4 (8.33%)	2 (4.88%)	8 (8.99%)
Tuboendometrioid metaplasia	11 (22.92%)	5 (12.19%)	7 (7.87%)
Endometrial polyp	5 (10.42%)	2 (4.88%)	15 (16.85%)
Endocervical polyp	3 (6.25%)	1 (2.44%)	3 (3.37%)
Squamous neoplasia	**13 (27.08%)**	**10 (24.39%)**	**23 (25.84%)**
L-SIL	9 (18.75%)	1 (2.44%)	10 (11.24%)
H-SIL	4 (8.33%)	9 (21.95%)	13 (14.61%)
Glandular neoplasia	**4 (8.33%)**	**19 (46.34%)**	**23 (25.84%)**
Cervical cancer	4 (8.33%)	16 (39.02%)	20 (22.47%)
AIS, HPV-associated	2 (4.17%)	2 (4.88%)	4 (4.49%)
AIS, HPV-independent	–	–	–
Adenocarcinoma, HPV-associated, usual type	2 (4.17%)	10 (24,39%)	12 (13.48%)
Adenocarcinoma, HPV-associated, mucinous type	–	2 (4.88%)	2 (2.25%)
Adenocarcinoma, HPV-independent, gastric type	–	1 (2.44%)	1 (1.12%)
Adenocarcinoma, HPV-independent, clear cell type	–	1 (2.44%)	1 (1.12%)
Endometrial cancer	–	3 (7.32%)	3 (3.37%)
Endometrioid	–	3 (7.32%)	3 (3.37%)
Total of cases	48 (53.93%)	41 (46.07%)	89 (100%)

Bold values represent the total % value for each category.

Abbreviations: AGC, atypical glandular cells; AGC-FN, atypical glandular cells, favour neoplasia; AGC-NOS, atypical glandular cells, not otherwise specified; L-SIL, low-grade squamous lesion; H-SIL, high-grade squamous lesion; AIS: adenocarcinoma in situ; HPV: human papilloma virus.

younger women, for whom the management is predominantly based on the cytological diagnosis.

The identification of atypical glandular cells is one of the main diagnostic difficulties in Pap smears, because of lack of precise morphological criteria and the presence of a low interobserver reproducibility. In fact, the incidence of AGC varies significantly among the several studies currently published in literature, ranging from 0.08% to 2.1%.[3–10] In our study, the incidence was 0.47%. The malignancy rate ranges from 15% to 59% in literature, being higher in AGC-FN cases compared to AGC-NOS cases.[4–6,8,9,11–14] In our study, the overall malignancy rate of AGC category was 51.69%, being respectively 35.41% for AGC-NOS and 70.73% for AGC-FN, highlighting the higher predictive value of AGC-FN, as previously demonstrated in other studies. Scheiden et al. reported a malignancy rate of 56.3% in their study, distributed in 44% of endocervical and 56% of endometrial origin, but did not subdivide the cases into AGC-NOS and AGC-FN, because it was published in 2004.[15] In a more recent study, Daniel et al. reported a 45.2% overall malignancy rate, that was of 48% and 73% for AGC-NOS and AGC-FN cases, respectively,[14] demonstrating the higher predictive value of AGC-FN category compared to AGC-NOS. The largest study in literature by Toyoda et al.[16] reported an overall malignancy rate of 43.6%. Other studies described also higher malignancy rates, compared to these reported below and in our study. A recent study by Yucel Polat et al.[4] reported a malignancy rate of 68.6%, with a 48.8% rate for AGC-NOS and a 89.5% rate for AGC-FN cases. The malignancy rate recorded by Suresh et al. was 66.7%,[17] whereas the rates reported by Selvaggi et al. and Kirwan et al. were 78% and 81.6% respectively.[18,19]

Table 2 summarizes the rate of malignancies reported in the different studies, subdivided into AGC subgroups. According to the 2015 Bethesda system, AGC 'should be categorized according to the site of origin as endocervical or endometrial whenever possible'. If the site of origin cannot be identified, then the general term 'atypical glandular cells' can be used. On the basis of these considerations, and in order to focus our attention exclusively on the macro-category of AGC, we decided not to subdivide AGC according to the site of origin. Instead, we consequently analysed and compared the origin of malignancy according to the histological examination. In most of these studies, endometrial cancer was the most common malignancy. Differently from them, in our study, we observed only three AGC cases with endometrial cancer, all belonging to the AGC-FN category, while cervical cancer was the most common malignancy with 43 cases. This difference observed in our study may be explained by the lower mean age (38 years) of our patients. In fact, as reported

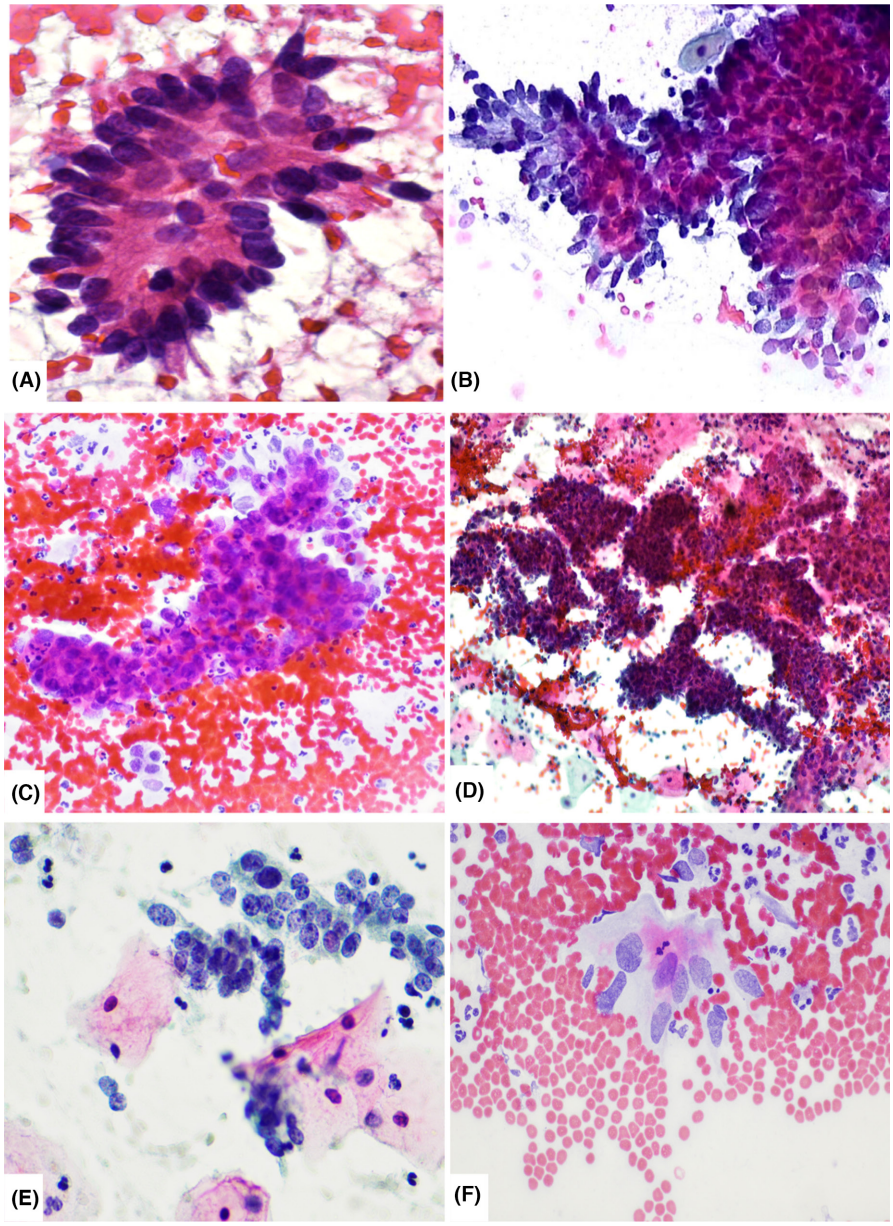

FIGURE 2 (A) AGC-NOS. Groups of cells showing a single rosette formation with overlapping and hyperchromatic nuclei; conventional Pap smear, ×400. Histological diagnosis was AIS. (B) AGC-FN. Glandular cell clusters and papillary formation, with increased N/C ratio, overlapping and nuclear hyperchromasia; conventional Pap smear, ×200. Histological diagnosis was endocervical adenocarcinoma, HPV-associated, usual type. (C) AGC-FN. Glandular cell clusters and single cells, with nuclear enlargement, macronucleoli and overlapping, in a red blood cell-rich background; conventional Pap smear, ×200. Histological diagnosis was endocervical adenocarcinoma, HPV-independent, clear cell type. (D) AGC-FN. Numerous glandular cell clusters and three-dimensional formations, with increased N/C ratio, overlapping and nuclear hyperchromasia; conventional Pap smear, ×100. Histological diagnosis was endometrial endometrioid cancer. (E) AGC-FN. Clusters and single atypical glandular cells, with overlapping, nuclear enlargement, and some macronucleoli; conventional Pap smear, ×400. Histological diagnosis was endocervical adenocarcinoma, HPV-associated, usual type. (F) AGC-FN. Few single atypical glandular cells, showing nuclear irregularity, nuclear enlargement and increased N/C ratio, in a background rich of red blood cells and neutrophils; conventional Pap smear, ×400. Histological diagnosis was endocervical adenocarcinoma, HPV-associated, mucinous type.

in other studies,[4,14–19] patients under <40 years were significantly associated with cervical squamous lesions, while women >40 years were associated with glandular lesions, predominantly of endometrial origin. The prevalence of glandular lesions of endometrial origin in other studies was probably related to the higher mean age of their patients. It is well known that cervical malignancies, both squamous and glandular, particularly if HPV-related, occur more frequently in the reproductive age, while endometrial cancer more commonly occurs in peri−/post-menopausal age.[20] In particular, in our AGC-FN group, although the most prevalent detected cervical cancer has been represented by HPV-related Adenocarcinoma, also rare cases of HPV-independent Adenocarcinoma have been diagnosed. They

TABLE 2 Comparison of studies regarding rate of malignancy (ROM) in AGC-NOS and AGC-FN groups, and total.

Author and Year	AGC-NOS	AGC-FN	AGC (overall)
Scheiden et al. (2004)[15]	/	/	56.3%
Kirwan et al. (2004)[19]	/	/	81.6%
Daniel et al. (2005)[14]	48%	73%	45.2%
Selvaggi et al. (2016)[18]	/	/	78%
Suresh et al. (2017)[17]	/	/	66.7%
Toyoda et al. (2019)[16]	32.7%	87.9%	43.6%
Yucel Polat et al. (2021)[4]	48.8%	89.5%	68.6%
Our study (2023)	35.41%	70.73%	51.69%

are rare entities, but clinically significant subtypes, that can represent diagnostic challenges. Given their relative rarity and the importance of early diagnosis, the awareness of the tumour type and their proper cytomorphology is critical for early detection. In the single case of gastric type adenocarcinoma (GAS), honeycomb-like sheets, nuclear enlargement, anisonucleosis, prominent nucleoli and microvesicular cytoplasm were the most common architectural, nuclear and cytoplasmic features, respectively, discriminatory for GAS versus usual type endocervical adenocarcinoma (UEA) respectively. On the other hand, in the single case of clear cell carcinoma (CCC), the presence of dense clear /eosinophilic cytoplasm, deep nuclear membrane irregularities and coarse chromatin have been observed, indicating an aggressive malignancy with poor prognosis. GAS and CCC are aggressive malignancies with poor prognosis. The described cytomorphological features could be considered important predictors of malignancy in cervical cytology, compared to their common cytological benign add malignant mimics. Another difference between our and other studies was the absence of other gynaecological malignancies (e.g. ovary) and metastatic tumours. This may be due to our institute being a reference centre for gynaecological oncology. In our centre, patients >40 years are carefully evaluated for possible endometrial, ovarian or metastatic tumours, also with routine pelvic ultrasound imaging, performed by expert gynaecologists. Most of the patients undergoing Pap test in our centre are represented by women <40 years, in which endometrial, ovarian and metastatic glandular neoplasms are less likely to occur.

In the literature, some studies compared conventional and liquid-based smear techniques in terms of detecting glandular lesions, reporting a higher sensitivity for the liquid-based technique.[21–23] In our studies, we did not find any statistically significant difference between the two techniques. This could be due to the fact that most cases (86.52%) were conventional smears and also to the fact that in our centre the cases are diagnosed by expert gynaecological cytopathologists only. Benign pathologies are very common causes of potential pitfalls in the cytological diagnosis of AGC, consisting of many different lesions or conditions, such as endocervical/endometrial polyps, squamous metaplasia, tubo-endometrioid metaplasia and inflammation (i.e. cervicitis). These benign pathologies may induce modifications in glandular cell morphology that may be mistaken for AGC at Pap smear evaluation. In our study, nonetheless the experience of our cytopathologists, we had 33 cases (37.08%) of AGC that at follow-up biopsy were demonstrated to be false-positive, related with benign pathologies, and 10 cases (11.24%) that revealed no significant alterations. However, most cases were diagnosed as AGC-NOS (23 benign +8 negative cases), compared to AGC-FN (10 benign +2 negative cases). In the literature, the incidence of benign pathologies is highly variable, ranging from 20% to 80%.[4,6,24,25] This high variability may be due to various factors that are often difficult to be identified and compared among different studies. Among them, probably the main factor is represented by the experience of the pathologist, but as also demonstrated by our study, this is not the only one, because many factors may co-occur and contribute to false-positive results in AGC.

4.2 | Pap test as the fundamental screening test

Pap test remains fundamental in providing an early detection of possible glandular malignancies, and, according to the latest Bethesda classification, there are precise cytomorphological criteria to identify AGC cases. Based on this, as already done in previous studies, we investigated whether these cytomorphological features could be reliable in the detection of malignancies.[26–31] N/C ratio and loss of polarity were reported as indicative features by Goff et al. in 1992.[31] In 1995, Raab et al.[32] reported irregular nuclear borders, atypical single cells and decreased cytoplasm as predictive features of malignancy, while Lee et al.[27] identified nuclear rosettes and feathering in the same year. After these first reports, many other studies described additional cytomorphological features significantly associated with glandular malignancy, that have been included as nuclear and architectural criteria for AGC diagnosis.[33–36] However, some studies found that nuclear enlargement, nuclear irregularity, increased N/C ratio and overlapping, may be also observed in reactive conditions, hence they should not be considered as reliable indicators.[37–39] Yucel Polat et al.[4] reported only nuclear irregularity and macronucleoli to be significant nuclear features, and only loss of polarity, papillary pattern, three-dimensional formation and feathering as significant architectural features. As described below, in literature there are certain controversies regarding the current cytomorphological criteria for AGC, probably explaining why this category shows a low interobserver reproducibility, particularly for unexperienced cytopathologists. In our study, among architectural features, we observed a statistical significance for feathering, three-dimensional formations, papillary pattern, rosette formation and loss of polarity. Overlapping was the only architectural criteria found to be not significant. Among nuclear features, hyperchromasia, mitoses and presence of macronucleoli, were significant for detecting malignancy, while nuclear irregularity and increased N/C ratio were not reliable criteria. Considering our results and comparing them with the literature, we may say

that hyperchromasia, mitoses and macronucleoli are the most reliable nuclear criteria for the diagnosis of AGC. Nuclear irregularity is a doubt and controversial feature, probably due to the subjectiveness of what 'irregular' and 'regular' mean. Nuclear enlargement and increased N/C ratio are the less significant features, because they may be frequently observed in benign/inflammatory conditions also, such as cervicitis, endometrial or endocervical polyps, microglandular hyperplasia, tubo-endometrioid metaplasia.[40–42] As regards architectural criteria, it could be said that overlapping is the most unreliable criteria, reported to be not significant in many different studies, including ours. Feathering, three-dimensional formations, papillary pattern and loss of polarity have been demonstrated to be significant in several studies, and hence should be considered as reliable architectural criteria for AGC diagnosis. Rosette formation has been reported to be not significant only in a single study,[4] probably due to the low number of cases presenting rosettes, as also stated by its authors. Therefore, rosette formation could be also considered as a reliable architectural criterion. However, it should be always kept in mind that, in order reduce the rate of false-positive results, the Pap smear cytopathological evaluation necessarily needs appropriate clinical correlation (age-related pathologies) and expert cytopathologists. In fact, the higher is the number of cases observed, the higher will be the ability in recognizing each possible pitfall and distinguish reactive from neoplastic atypical cytological features.

5 | CONCLUSION

In the present work, we have observed rates of malignancy for AGC-NOS and AGC-FN of 35.41% and 70.73% respectively. Furthermore, we analysed cytomorphological features of glandular cells and we found that both architectural (three-dimensional formations, papillary pattern, rosette formation and loss of polarity) and nuclear criteria (hyperchromasia, mitoses and presence of macronucleoli) were statistically significant. We retain that cytological diagnosis of glandular lesions, even though difficult and still improvable, remains a valid tool when appropriate clinical correlation and expert evaluation are available. Finally, with a more than 50% malignant outcome, we would suggest that AGC diagnosis must be considered as a 'high grade' cytological finding which has to be taken seriously by clinicians and warrants immediate work-up.

AUTHOR CONTRIBUTIONS

FC, AS, EDR and GFZ conceived and designed the study. ND, GS, FA, NN and GA provided administrative support. FC, DA, MV, BPU, AP and AM collected and assembled the data. AD wrote the manuscript. All authors, collectively accountable for all aspects of this work, participated in data analysis and interpretation and gave final approval for the manuscript.

ACKNOWLEDGEMENTS

Not applicable.

FUNDING INFORMATION

The present paper received no funding.

CONFLICT OF INTEREST STATEMENT

The authors have no financial or personal conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

Not applicable.

ORCID

Antonio d'Amati https://orcid.org/0000-0002-4810-1201
Esther Diana Rossi https://orcid.org/0000-0003-3819-4229
Gian Franco Zannoni https://orcid.org/0000-0002-4473-7560

REFERENCES

1. The 1988 Bethesda system for reporting cervical/vaginal cytological diagnoses. *JAMA*. 1989;262(7):931-934.
2. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda SystemTerminology for reporting results of cervical cytology. *JAMA*. 2002;287(16):2114-2119.
3. Cangiarella JF, Chhieng DC. Atypical glandular cells—an update. *Diagn Cytopathol*. 2003;29(5):271-279.
4. Yucel Polat A, Tepeoglu M, Tunca MZ, Ayva ES, Ozen O. Atypical glandular cells in Papanicolaou test: which is more important in the detection of malignancy, architectural or nuclear features? *Cytopathology*. 2021;32(3):344-352.
5. Boyraz G, Basaran D, Salman MC, et al. Histological follow-up in patients with atypical glandular cells on pap smears. *J Cytol*. 2017;34(4):203-207.
6. Marques JP, Costa LB, Pinto AP, et al. Atypical glandular cells and cervical cancer: systematic review. *Rev Assoc Medica Bras (1992)*. 2011;57(2):234-238.
7. Jeng CJ, Liang HS, Wang TY, Shen J, Yang YC, Tzeng CR. Cytologic and histologic review of atypical glandular cells (AGC) detected during cervical cytology screening. *Int J Gynecol Cancer*. 2003;13(4):518-521.
8. Westin MC, Derchain SF, Rabelo-Santos SH, et al. Atypical glandular cells and adenocarcinoma in situ according to the Bethesda 2001 classification: cytohistological correlation and clinical implications. *Eur J Obstet Gynecol Reprod Biol*. 2008;139(1):79-85.
9. Kim SS, Suh DS, Kim KH, Yoon MS, Choi KU. Clinicopathological significance of atypical glandular cells on pap smear. *Obstet Gynecol Sci*. 2013;56(2):76-83.
10. Zhao C, Austin RM, Pan J, et al. Clinical significance of atypical glandular cells in conventional pap smears in a large, high-risk U.S. west coast minority population. *Acta Cytol*. 2009;53(2):153-159.
11. Sawangsang P, Sae-Teng C, Suprasert P, Srisomboon J, Khunamornpong S, Kietpeerakool C. Clinical significance of atypical glandular cells on pap smears: experience from a region with a high incidence of cervical cancer. *J Obstet Gynaecol Res*. 2011;37(6):496-500.
12. DeSimone CP, Day ME, Tovar MM, Dietrich CS, Eastham ML, Modesitt SC. Rate of pathology from atypical glandular cell pap tests classified by the Bethesda 2001 nomenclature. *Obstet Gynecol*. 2006;107(6):1285-1291.
13. Chatchotikawong U, Ruengkhachorn I, Laiwejpithaya S. Factors predicting pathologic significance among women with atypical glandular cells on liquid-based cytology. *Int J Gynaecol Obstet*. 2012;119(1):30-34.

14. Daniel A, Barreth D, Schepansky A, Johnson G, Capstick V, Faught W. Histologic and clinical significance of atypical glandular cells on pap smears. *Int J Gynaecol Obstet*. 2005;91(3):238-242.
15. Scheiden R, Wagener C, Knolle U, Dippel W, Capesius C. Atypical glandular cells in conventional cervical smears: incidence and follow-up. *BMC Cancer*. 2004;4(1):37.
16. Toyoda S, Kawaguchi R, Kobayashi H. Clinicopathological characteristics of atypical glandular cells determined by cervical cytology in Japan: survey of gynecologic oncology data from the obstetrical gynecological Society of Kinki District. *Japan Acta Cytol*. 2019;63(5):361-370.
17. Suresh PK, Kini H, Minal J, et al. The significance of glandular cells on conventional cervicovaginal smears: experience from a tertiary Care Hospital in Coastal India. *Acta Cytol*. 2017;61(3):199-206.
18. Selvaggi SM. Glandular epithelial abnormalities on thinprep® pap tests: clinical and cytohistologic correlation. *Diagn Cytopathol*. 2016;44(5):389-393.
19. Kirwan JM, Herrington CS, Smith PA, Turnbull LS, Herod JJO. A retrospective clinical audit of cervical smears reported as 'glandular neoplasia'. *Cytopathology*. 2004;15(4):188-194.
20. Ledford LRC, Lockwood S. Scope and epidemiology of gynecologic cancers: an overview. *Semin Oncol Nurs*. 2019;35(2):147-150.
21. Hecht JL, Sheets EE, Lee KR. Atypical glandular cells of undetermined significance in conventional cervical/vaginal smears and thin-layer preparations. *Cancer*. 2002;96(1):1-4.
22. Schorge JO, Hossein Saboorian M, Hynan L, Ashfaq R. ThinPrep detection of cervical and endometrial adenocarcinoma: a retrospective cohort study. *Cancer*. 2002;96(6):338-343.
23. Ashfaq R, Gibbons D, Vela C, Saboorian MH, Iliya F. ThinPrep pap test. *Accuracy for Glandular Disease Acta Cytol*. 1999;43(1):81-85.
24. Bose S, Kannan V, Kline TS. Abnormal endocervical cells. Really abnormal? Really endocervical? *Am J Clin Pathol*. 1994;101(6):708-713.
25. Ronnett BM, Manos MM, Ransley JE, et al. Atypical glandular cells of undetermined significance (AGUS): cytopathologic features, histopathologic results, and human papillomavirus DNA detection. *Hum Pathol*. 1999;30(7):816-825.
26. Torres JCC, Derchain SFM, Gontijo RC, et al. Atypical glandular cells: criteria to discriminate benign from neoplastic lesions and squamous from glandular neoplasia. *Cytopathology*. 2005;16(6):295-302.
27. Lee KR, Manna EA, St John T. Atypical endocervical glandular cells: accuracy of cytologic diagnosis. *Diagn Cytopathol*. 1995;13(3):202-208.
28. Dalla Nora LC, Azara CZS, Pace EL, et al. Cytomorphological criteria, subclassifications of endocervical glandular cell abnormalities, and histopathological outcome: a frequency study. *Diagn Cytopathol*. 2010;38(11):806-810.
29. Tam KF, Cheung ANY, Liu KL, et al. A retrospective review on atypical glandular cells of undetermined significance (AGUS) using the Bethesda 2001 classification. *Gynecol Oncol*. 2003;91(3):603-607.
30. Raab SS, Snider TE, Potts SA, et al. Atypical glandular cells of undetermined significance. Diagnostic accuracy and interobserver variability using select cytologic criteria. *Am J Clin Pathol*. 1997;107(3):299-307.
31. Goff BA, Atanasoff P, Brown E, Muntz HG, Bell DA, Rice LW. Endocervical glandular atypia in Papanicolaou smears. *Obstet Gynecol*. 1992;79(1):101-104.
32. Raab SS, Isacson C, Layfield LJ, Lenel JC, Slagel DD, Thomas PA. Atypical glandular cells of undetermined significance. Cytologic criteria to separate clinically significant from benign lesions. *Am J Clin Pathol*. 1995;104(5):574-582.
33. Reynolds JP, Salih ZT, Smith AL, Dairi M, Kigen OJ, Nassar A. Cytologic parameters predicting neoplasia in Papanicolaou smears with atypical glandular cells and histologic follow-up: a single-institution experience. *J Am Soc Cytopathol*. 2018;7(1):7-15.
34. Stoll LM, Johnson MW, Ali SZ. Papillary tissue fragments in cervicovaginal (pap) smears: cytomorphologic characteristics and clinicopathologic significance. *Diagn Cytopathol*. 2011;39(9):657-662.
35. Mariani R, Grace C, Hughes K, Dietrich RM, Cabay RJ, David O. Can we improve the positive predictive value of atypical glandular cells not otherwise specified? *Diagn Cytopathol*. 2014;42(3):200-204.
36. Rabelo-Santos SH, Derchain SFM, do Amaral Westin MC, et al. Endocervical glandular cell abnormalities in conventional cervical smears: evaluation of the performance of cytomorphological criteria and HPV testing in predicting neoplasia. *Cytopathology*. 2008;19(1):34-43.
37. Levine L, Lucci JA, Dinh TV. Atypical glandular cells: new Bethesda terminology and management guidelines. *Obstet Gynecol Surv*. 2003;58(6):399-406.
38. Babkowski RC, Wilbur DC, Rutkowski MA, Facik MS, Bonfiglio TA. The effects of endocervical canal topography, tubal metaplasia, and high canal sampling on the cytologic presentation of nonneoplastic endocervical cells. *Am J Clin Pathol*. 1996;105(4):403-410.
39. Ghorab Z, Mahmood S, Schinella R. Endocervical reactive atypia: a histologic-cytologic study. *Diagn Cytopathol*. 2000;22(6):342-346.
40. Novotny DB, Maygarden SJ, Johnson DE, Frable WJ. Tubal metaplasia. A frequent potential pitfall in the cytologic diagnosis of endocervical glandular dysplasia on cervical smears. *Acta Cytol*. 1992;36(1):1-10.
41. Yahr LJ, Lee KR. Cytologic findings in microglandular hyperplasia of the cervix. *Diagn Cytopathol*. 1991;7(3):248-251.
42. Bansal B, Gupta P, Gupta N, Rajwanshi A, Suri V. Detecting uterine glandular lesions: role of cervical cytology. *Cytojournal*. 2016;13:3.

How to cite this article: Cianfrini F, d'Amati A, Arciuolo D, et al. Atypical glandular cells and predictive features of malignancy in Pap smears: A retrospective monocentric study. *Cytopathology*. 2024;35:473-480. doi:10.1111/cyt.13383