

APPLICATION OF THE SUB-CLASSIFICATION AGC NOS AND AGC FN IN PAP SMEARS AGC: UTILITY IN CLINICAL PRACTICE

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ABSTRACT

With the use of the pap smear in screening programs there has been a decrease in cervical cancer but the pap smear is also a tool for early diagnosis of endocervical adenocarcinoma. Endocervical adenocarcinoma is a delicate disease because, even though it is a rare disease, it is becoming more and more widespread, affecting young women of childbearing age. Furthermore, it is asymptomatic, hardly visible colposcopically and is also a dangerous disease whose risk does not diminish after diagnosis of AGC but remains high over time.

The study aims to evaluate the usefulness of the subcategory of the AGC in AGC NOS and AGC FN to assess whether the use can give advantages in clinical practice

93248 Pap Smears were considered in this study, of which only 50 were suitable for the study (diagnosis of AGC and histological examination on the endocervix), these were reclassified according to the indications of Bethesda in AGC NOS and AGC FN

In the reclassified Pap Smears as AGC NOS we obtained, on the histological examination, 79% of lesions of low clinical-evolutionary impact and only the 21% of lesions with a high clinical-evolutionary impact. on the other hand, in the reclassified Pap Smears as AGC FN we obtained 23% of lesions of low clinical-evolutionary impact and the 77% of lesions with a high clinical-evolutionary impact. In addition, in patients over 50 with the diagnosis of AGC FN, extracervical diseases prevail

The sub-classification in AGC NOS and AGC FN has proved useful to guide the clinician in carrying out more detailed examinations in women diagnosed with AGC FN and moreover in a patient over 50 years the diagnosis of AGC FN must orientate the clinician to an extension of the investigations also, and above all, at the level of the remaining parts of the genital apparatus (in particular the endometrium) or of the neighboring organs (bladder, rectum).

INTRODUCTION

The Papanicolaou test has substantially helped in decreasing cervical cancer rates and mortality since its widespread implementation in the early 1970s. In 1975 the incidence of cervical cancer was 14.8 per 100 000 women in the United States and in 2011 the incidence was 6.2 cases per 100 000 women¹⁻². It has been shown that combining the Pap smear with appropriate screening program and follow-up can result in a reduction of cervical cancer deaths by up to 80%³⁻⁴. These satisfactory results make the Pap smear the most effective cancer screening and prevention test in medical history. The aim of Pap smear is to early detect squamous lesion but it can be useful to detect precursor of glandular lesions. It has to be noted, however that the overall sensitivity of Pap smear for detection of glandular cell abnormalities was found to be low (41.8%)⁵ and only 0.05% of Pap smear show these alterations⁶. The main difficulties in studying glandular pathologies are both in the adequacy of the sample, that could have an unrepresentative numbers of cells, and in the interpretation of the cytologist due to the rarity of these pathologies. Cervical adenocarcinoma is a rising condition⁷⁻⁸ that mainly affects young women and can be asymptomatic for a long time, to

the point that the adenocarcinoma in situ can precede the infiltrating form even of 10-15 years. These lesions are also difficult to be observed with colposcopy because they are into the cavity of the cervix, sometimes near the internal orifice, where the colposcopist is not always able to see clearly. The definitive treatment for this disease is the hysterectomy, however for the adenocarcinoma in situ, if women haven't finished the reproductive cycle yet, the conization can be considered as an option with negative margins for neoplasia; even if there is a risk less of 10% for persisting AIS and lower of occult invasive carcinoma⁹. For its biological characteristics and for its location, **the only test that can make an early diagnosis of adenocarcinoma is the Pap Smear**. However, not always in a Pap smear the modifications of the glandular cells have all the characteristics necessary to make a unequivocal diagnosis of adenocarcinoma; to solve this problem, the Bethesda Reporting System provide the category called AGC (Atypical Glandular Cells of undetermined significance) that includes a large range of modifications that can be associated with pathologies that go from the simple inflammatory reactivity to the malignant pathologies of the endocervix, but also of the exocervix or of the endometrium. This

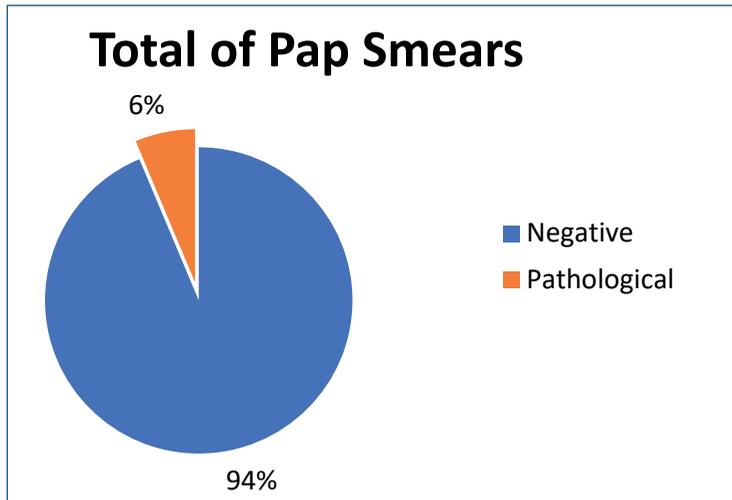


Fig. 5a: Percentage of pathological pap smears on the total analyzed

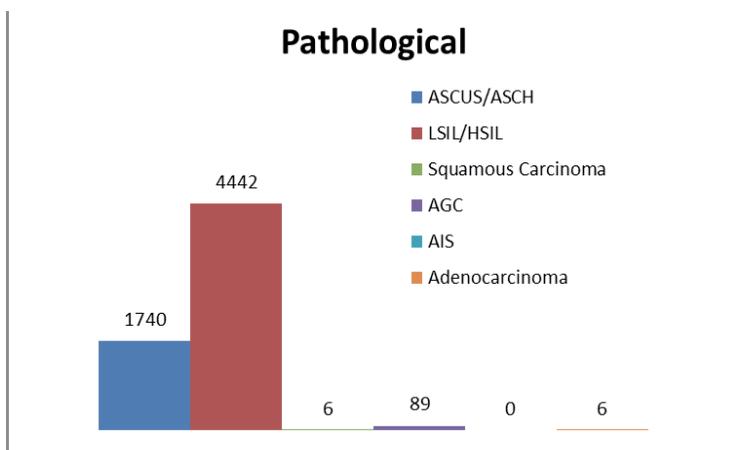


Fig. 5b: Distribution of diagnoses.

category, in the edition of Bethesda 2014, is further divided into ACG FN (Favor Neoplastic) and ACG NOS (Not Otherwise Specified) to try to provide the clinician with more detailed information that allows to perform the most effective treatment.

In 2016 a research of the Karolinska Institute of Sweden studied more than 3 million women to investigate the risks of invasive cervical cancer after detection of atypical glandular cells (AGC) during cervical screen-

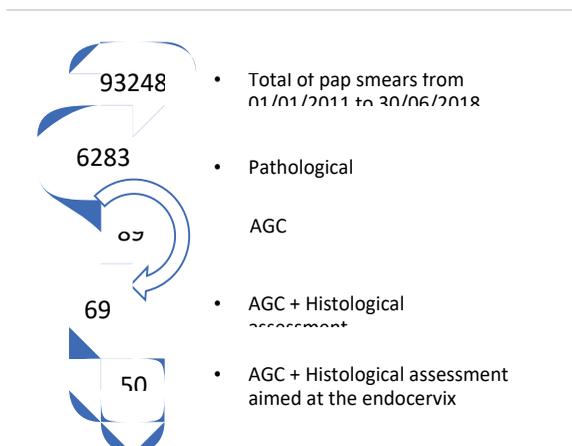


Fig.6. - Selection process of cases to be analyzed in the study.

ing. Cumulative incidence of invasive cervical cancer among women who had AGC increased drastically in the first six months and continued to increase steadily during follow-up, reaching 2.6% at 15.5 years. Moreover, if we divided women according to their age, the women diagnosed with AGC at ages 30-39 had the highest incidence rate and incidence rate ratio compared with those with normal cytology results for all types of cervical cancer, while risk of cervical cancer after an HSIL was shifted towards higher ages. This study shows the **dangerousness of this pathology** in which risk does not diminish after diagnosis but it remains high for a long time. The risk of prevalent cancer remains so high also because only 54% of women with AGC were followed up with histology within six months while 86% of HSIL were10.

In another study published on Cancer Cytopathology in 201611 out of 589830 Pap Test, performed between 2008 and 2013, 3709 Pap tests were reported as AGC of these only 3007 (0.5%) cases have an histologic follow-up. Among the 886 patients (29.5%) with non-negative histologic follow-up, 70.0% had cervical squamous lesions, 6.4% had cervical glandular neoplasia, 22.8% had endometrial neoplasia and 1.6% had metastatic carcinoma. In conclusion, out of 3007 lesions classified as AGC, 20.7% were squamous lesions, 6.7% endometrial lesions, 0.5% metastatic lesions while only 1.9% glandular lesions, that represent 1.9% of 0.5% of all the Pap Test examined. This makes us understand how the endocervical adenocarcinoma is a very **rare pathology**.

Aim of the Study

We have seen how the pathologies of the cervical canal are very rare in the Pap smears and how, even in the presence of a diagnosis of AGC, it is not possible to obtain a rapid decrease in the incidence rate of adenocarcinoma, especially in the age group 30-39 that nowadays is the one with the greatest reproductive activity. If on the one hand they are therefore very rare tumors, on the other they require a continuous improvement in our ability to diagnose and especially early diagnose because of their peculiarities:

1. They are increasing;
2. They affect young women in fertility age;
3. They are not visible with colposcopy;
4. They are asymptomatic at an early stage;
5. Their treatment does not allow to preserve fertility if diagnosed when they are not infiltrating;
6. If not treated they don't regress;
7. They are not always successfully studied from a clinical point of view because they require accurate, targeted and more invasive treatments than squamous lesions.

Up to now, in the Cytopathology laboratory of the Busto Arsizio Hospital the diagnosis of AGC is not sub-categorized. Given the numerous considerations illustrated above, we have decided to organize a review of the cases AGC of the last seven years by applying the sub-classification of the AGC, collecting cyto-histological correlations and evaluating any differences.

MATERIALS AND METHOD

93248 Pap Smears performed from 01/01/2011 to 30/06/2018 were analysed and, among the 6283 pathological one, the 89 interpreted as Atypical Glandular Cells (AGC) were retrieved from the database at the "ASST Valle Olona" Hospital of Busto Arsizio and

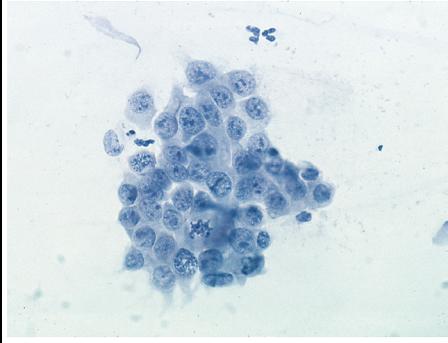
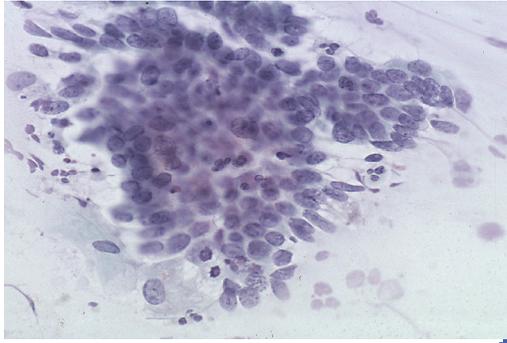
	<i>ATYPICAL GLANDULAR CELLS NOT OTHERWISE SPECIFIED (NOS)</i>	<i>ATYPICAL GLANDULAR CELLS FAVOR NEOPLASTIC (FN)</i>
Structure	Crowded groups with pseudostratifications	Crowded groups with pseudostratifications, rosettes or "Fathering". Loss of polarity
Nucleus	Overlapped, enlarged 3/4 times with slight hyperchromasia	Very enlarged, elongated and hyperchromatic
Chromatin	Slightly irregular	Coarse
Nucleoli	Occasional	Occasional
Mitosis	Absent	Present
N/C	Increased	Increased
Cytoplasm	Indistinct edges	Indistinct edges
		

Fig. 7: characteristics of AGC NOS and AGC FN according to Bethesda 201412.

Saronno (Lombardia, Italy). Analyzing the distribution we found that, similar to the literature review, in our experience the diagnosis of AGC is very rare and covers 1% of the total pathological Pap smears (Fig.5a/b).

Only the 69 cases followed by a traceable histological assessment were considered in the study. However, further 17 cases were subtracted from these cases because this assessment had not been aimed at the endocervix but on the exocervix, thus making the histological examination performed not pertinent to the diagnostic doubt. We therefore obtained 50 cases (Fig.6) that were recovered from the archive and reclassified according to the indications of the Bethesda system 2014

The Bethesda System for reporting Cervical Cytology 201412 describes the AGC category as the set of modifications related to glandular cells that presents atypia that goes beyond the reactive and reparative processes but does not have all the characteristics typical of in situ or invasive adenocarcinoma.

For this category, about endocervical cells, the Bethesda shows two subcategories (Fig.7):

- Atypical Glandular Cells Not Otherwise Specified (NOS);
- Atypical Glandular Cells Favor Neoplastic (FN);

Using the indications of the Bethesda 2014 in the reclassification of the samples, we obtained 33 diagnosis of AGC Not Otherwise Specified and 17 diagnosis of AGC Favor Neoplastic (Fig.8).

. 1: Cyto-histological associations of the 50 cases considered (Synthetic Diagnosis)

We then associated cytological and histological diagnosis (Tab.1).

RESULT

The 33 cases reclassified as AGC Not Otherwise Specified had as histological outcome:

- 22 Inflammatory / hyperplastic disorders;
- 4 Lsil;
- 6 Hsil mainly above the squamocolunar junction;
- 1 usual microinvasive endocervical adenocarcinoma.

to sum up, we obtained a predominance (79%) of lesions with low clinical-evolutionary impact and a minority (21%) high impact lesions (Fig.9).

The 17 cases reclassified as AGC Favor Neoplastic had as histological outcome:

- 10 adenocarcinomas (6 cervical, 3 endometrial, 1 metastatic);

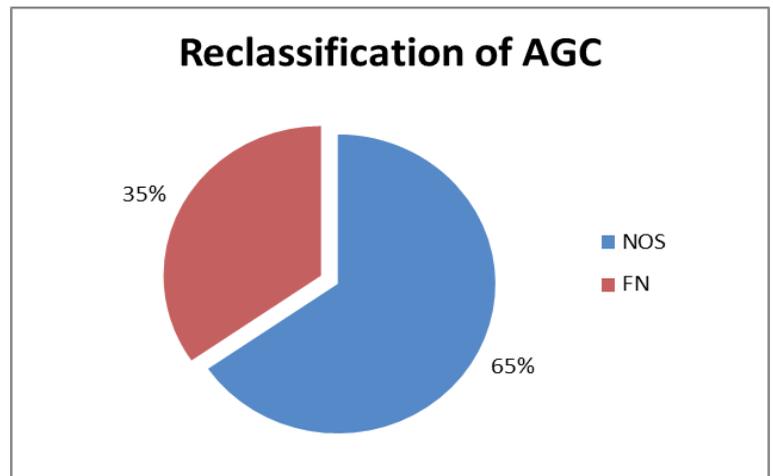


Fig.8: Results of reclassification.

AGC NOS				AGC FN			
Patient	Age	Pap Smear	Histologic	Patient	Age	Pap Smear	Histologic
B.C.	47	Hsil/AGC	Hsil	F.E.	73	AGC	Usual endocervical adenocarcinoma p16 +
B.C.	36	AGC	Endocervicitis	F.A.	36	AGC	Vaginal metastasis of mucinous adenocarcinoma of the large intestine
C.DV.	32	AGC	Squamous metaplasia	L.M.	78	AGC	Clear cell carcinoma of the endometrium
C.AM.	60	AGC	Lsil	O.G.	67	AGC	Endometrial polyp
C.ML	54	AGC	Endocervical polyp	P.D.	72	AGC	Endometrioid adenocarcinoma of the endometrium
P.F.	47	AGC	Endocervicitis ed endometrium	R.I.	38	Asc H/ AGC	First biopsy assessment: Lsil, Second biopsy assessment: Hsil
A.M.	50	AGC	Hsil	V.I.	36	Hsil/AGC	Hsil
C.M.	63	AGC	Endocervical polyp	AM.	43	AGC	Adenocarcinoma of the rectum radio-chemio treated. Subsequently, negative Pap smears
G.S.	42	AGC	Endocervicitis	G.I.	30	AGC	Cervicitis and squamous metaplasia
C.E.	34	Hsil/AGC	Lsil	I.E.	37	AGC	AIS usual type
F.S.	31	AGC	Endocervicitis	MAN.	43	AGC	Infiltrating spinocellular carcinoma
L.FL.	33	AGC	Endocervicitis	C.R.	63	AGC	Endocervical polyp
P.R.I.	36	Hsil/AGC	Lsil	P.L.	40	AGC	Usual endocervical adenocarcinoma p16 +
R.L.	55	AGC	Endocervical polyp	M.M.D.	46	AGC	Usual endocervical adenocarcinoma p16 +
C.C.L.P.	56	AGC	Hsil	G.A.	36	AGC	Adenosquamous infiltrating carcinoma
C.A.	64	AGC	Endocervicitis	E.M.	63	AGC and RX Alterations	Endocervical adenocarcinoma
C.M.	38	AGC	Endocervicitis	C.G.	64	AGC	Endometrial carcinosarcoma
C.M.	51	AGC	Endocervicitis				
C.M.	38	AGC	Endocervicitis ed endometrium				
D.R.	55	AGC	Negative				
D.A.	36	AGC	Hsil				
D.S.	40	AGC	Endocervicitis ed endometrium				
G.P.	44	AGC	Endocervicitis ed endometrium				
L.I.	35	AGC	Endocervicitis ed endometrium				
G.G.L.	47	Ascus/AGC	Micro-invasive adenocarcinoma				
L.G.L.A.	49	AGC	Endocervicitis				
P.M.	43	Ascus/AGC	Lsil				
L.D.	49	AGC	Endocervicitis				
V.M.A.	43	AGC	Endocervicitis ed endometrium				
V.B.	42	AGC	Endocervicitis				
B.R.	32	AGC	Endocervicitis ed endometrium				
M.M.	58	Asc H/ AGC	Hsil				
AA.	56	AGC	Hsil				

Tab. 1: Cyto-histological associations of the 50 cases considered (Synthetic Diagnosis).

- 3 squamous lesions (2 Hsil and 1 infiltrating spinocellular carcinoma);
- 3 inflammatory / hyperplastic disorders;
- 1 alteration from RX.

to sum up, 77% were clinically-evolutive lesions and 23% low impact lesions (Fig.10).

We have also observed how diseases are distributed within two large age groups: younger and older than 50. The AGN NOS cases spread unevenly while the AGC FN cases showed differences depending on the type of pathology: in women > 50 years we only had glandular lesions, mostly extracervical (3 out of 5); in women < 50 years we also had squamous lesions and the glandular were predominantly cervical (4 out of 5) (Fig.11).

DISCUSSION AND CONCLUSION

These results, although statistically not significant because numerically not relevant, have allowed us to highlight the extreme utility of the sub-classification of the AGC proposed by the Bethesda reporting sys-

tem 2014¹². In fact, if it is true that the recognition of glandular alterations is difficult both due to the difficulty in sampling, and to the rarity and the asymptomatic nature of the pathologies, it is also true that the effort to improve their recognition is very important because the increase of endocervical adenocarcinoma, especially in young women of childbearing age. This evolutionary pathology is, due to its location, difficult to see colposcopically and asymptomatic in the early phase and is, finally, difficult to study from a clinical point of view because it requires accurate, targeted, more invasive investigations, compared to those for squamous lesions, and persistent over time for years even after repeated negative outcomes. Thanks to the sub-categorization, the clinician, being faced with a diagnosis of AGC FN in a young patient, **is alerted on the need to promptly perform the targeted**, even if invasive, investigations necessary to exclude a glandular endocervical neoplasia or pre neoplasia and to repeat them over time. On the other hand, the same diagnosis in a patient > 50 years should orient the cli-

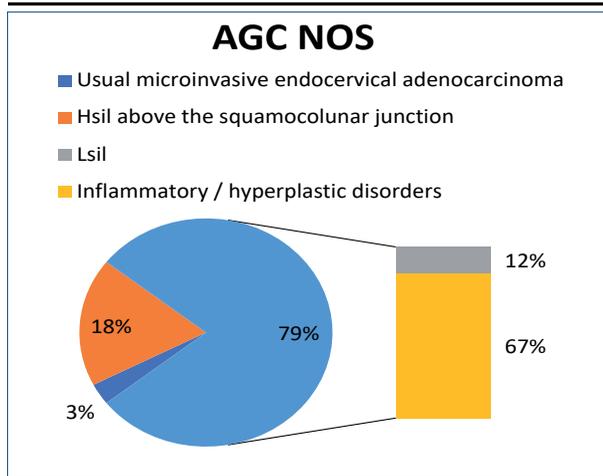


Fig.9: Cyto-histological association in AGC NOS cases.

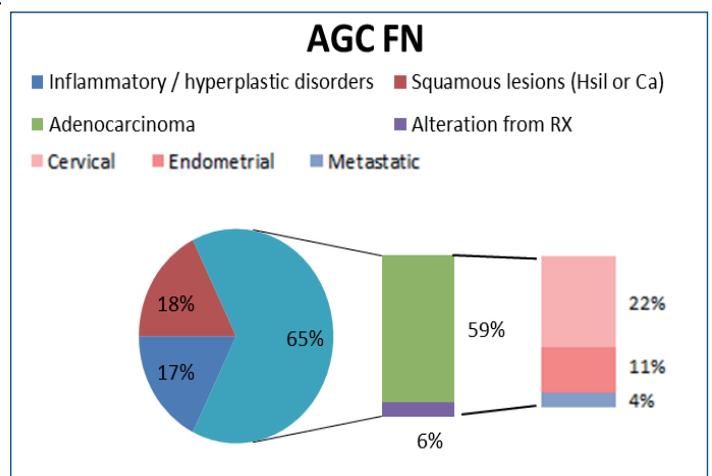


Fig.10: Cyto-histological association in AGC FN cases.

nician to an extension of the investigations also, and above all, to the level of the remaining parts of the genital apparatus (in particular the endometrium) or of the neighboring organs (bladder, rectum). However, it can be questioned whether the effort for morphological recognition of adenocarcinoma and / or its precursors is still justified in the age of HPV Test as a first-level screening. In fact, HPV DNA is present in almost all the Usual endocervical adenocarcinoma and in Mucinous Intestinal type endocervical adenocarcinoma . However, viral DNA is present only in a part of Clear Cell endocervical adenocarcinomas, Endometrioid adenocarcinoma and Serous adenocarcinoma, and is ultimately **absent in Gastric type endocervical adenocarcinoma, in the Mesonephric and in extracervical neoplasms**13. In the latter cases, the negativity of the HPV Test makes the screening ineffective. In the extracervical or metastatic gynecological neoplasms, despite the negativity of the HPV Test, the secondary cervical involvement causes specific symptoms of the site that induce the

execution of targeted clinical investigations. In contrast, in primitive non-HPV-related endocervical adenocarcinomas, the appearance of specific symptoms is late and usually related to an advanced stage of disease. These are neoplasms of the young woman of childbearing age, which have a worse prognosis than the histotypes associated with HPV (lymph node, adnexal and peritoneal metastases already in the first diagnosis) 14. In these neoplasms the cytological examination becomes the only tool to recognize the injuries in the initial stage, or better still the precursors, and implement all the strategies necessary for the preservation of the health of the woman and, if possible, of the fertility. The most common non-HPV related histotype (1% -1.3% of the total) 15 is the gastric type mucinous adenocarcinoma (also referred to as minimal deviation adenocarcinoma or malignant adenoma) whose growth is clinically characterized by a profuse production of fluid mucus escaping from the vagina13. As can be inferred from its name it is a neoplasm usu-

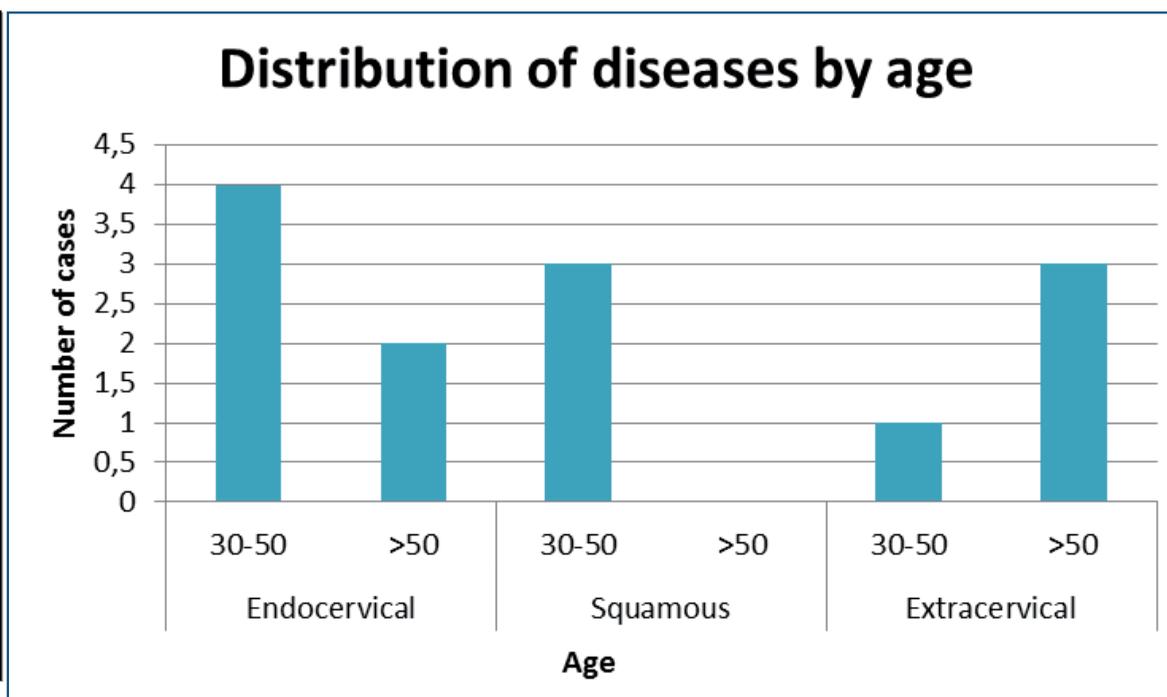


Fig.11: Age-pathology correlation in AGC FN cases.

ally well differentiated and therefore of difficult recognition on the cervico-vaginal smear because of the small atypical presents. Some studies in the literature, talking about this particular histotype, describe how this mucin, which expresses the gastric immunophenotype, takes on the coloring of Papanicolaou a gold-

en/orange-yellow color in contrast with the mucus of normal endocervical cells which appears pink. In negative HPV cases, therefore, it could be useful to select patients aged between 30 and 40 with abundant mucosal losses to be cytologically examined looking for the gold-yellow mucin.

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