Detection of occult endocervical glandular dysplasia in cervical conization specimens for squamous lesions

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ABSTRACT

The aim of this work was to evaluate the incidence of occult cervical glandular intraepithelial neoplasia (CGIN) and adenocarcinoma of the cervix (AC) in women treated with CO2-laser conization for cervical intraepithelial neoplasia (CIN) or squamocellular cervical cancer (SCC).

The medical records of all women with a histological diagnosis of squamous lesions of the uterine cervix (persistent CIN1, CIN2, CIN3 and SCC) who were subsequently treated with CO2-laser conization at our institution, during the period from January 1991 to December 2014, were analyzed in a retrospective case series.

Among the 1004 women fulfilling the study inclusion/exclusion criteria, 77 cases (7.7%) of occult glandular lesions (CGIN and AC) were detected on the final cone specimen (48 cases of occult low-grade cervical glandular intraepithelial neoplasia (LCGIN), 25 cases of occult high-grade cervical glandular intraepithelial neoplasia (HCGIN), and four cases of occult "usual-type" AC). No difference in the mean age between women diagnosed with occult glandular lesions and women without occult glandular lesions on the final specimen emerged (39.1 ± 9.3 vs 38.4 ± 9.4, $p = 0.5$).

In women with occult LCGIN on cone specimen, mean follow-up of 48 months was reported (range 7–206 months) and no cases of progression to HCGIN or AC were observed.

In conclusion, a relatively high rate of occult glandular lesions was found in women treated for squamous lesions. The natural history of CGIN is still uncertain and, in particular, there are some controversies as to whether LCGIN is a precursor lesion of HCGIN or AC. In this context the role of pathologists become very important since the appropriate diagnosis of these lesions could have potential implications in the clinical management of these patients.

1. Introduction

Similar to the role of cervical intraepithelial neoplasia (CIN) as a precursor of squamocellular cancer (SCC) of the uterine cervix, even adenocarcinoma (AC) is believed to be preceded by dysplastic changes in the glandular epithelium of the cervix. However, it is not easy to define these dysplastic glandular lesions, since different terminologies are currently in use.

Adenocarcinoma in situ (AIS) is considered the real precursor of AC but its development seems to be preceded by the presence of less severe dysplastic lesions. Various diagnostic criteria [1–3] have been proposed for these putative precursor lesions of AIS without widespread acceptance, with some authors [4–9] questioning the existence of this entity.

According to the WHO classification [10], premalignant endocervical glandular lesions have been classified as endocervical glandular dysplasia (EGD) and AIS. One category of glandular atypia has also been included, to encompass atypical glandular epithelial changes, such as those associated with inflammation or previous radiotherapy.

The WHO classification [10] is widely used in the USA, while in the UK and in the other European countries, a different terminology is used [11,12]. Some authors indeed have defined the glandular dysplastic lesions as cervical glandular intraepithelial neoplasia (CGIN) and have divided these lesions into two grades:

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low grade CGIN (LCGIN) and high grade CGIN (HCGIN). The use of CGIN terminology was endorsed in the 2012 NHS Cervical Screening Programme publication "Histopathology reporting in cervical screening" [13]. Compared to WHO terminology [10], in the CGIN terminology, the LCGIN corresponds to EGD and the HCGIN corresponds to AIS [11,12].

According to McCluggage [11,12], the CGIN terminology should be preferable because it is similar to the CIN system used for preinvasive cervical squamous lesions and takes account of the likelihood that there is a continuum of dysplastic lesions from low to high grade.

Malignant and premalignant endocervical glandular lesions are relatively rare and difficult to diagnose. Interestingly, occult CGIN, AIS and early-invasive adenocarcinoma of the cervix (EIAC) are occasionally found in cone biopsy performed for CIN. In this cases, the squamous lesion could been considered as "marker lesion" for the presence of a hidden glandular lesion, with which may occasionally share the expression of the same human papillomavirus (HPV) genotypes [14]. This finding supports the theory that tissue stem cells of the uterine cervix may be the reservoir of a persistent HPV infection [15]. In these cases, the squamous lesions may produce high viral load and shedding of thousands of HPV particles at the squamous-columnar junction (SCJ), that may lead to infection of the adjacent glandular epithelium or undifferentiated stem cells [16]. In fact, the transformation zone between glandular and squamous epithelia is where stem-like cells are thought to reside and their infection may result in the onset of one of both SCC and AC [17].

To date the progression of HCGIN to invasive adenocarcinoma is well known, but the natural history of LCGIN is still uncertain [11]. For this reason, the correct histopathological identification of glandular intraepithelial lesions should be emphasized in order to identify these patients adequately and planning the appropriate treatment and follow up regimen.

The aim of this study was to analyze the women treated with cervical conization for CIN or SCC, evaluating the rate of occult glandular lesions (CGIN or AC).

2. Material and methods

2.1. Patients

The medical records of all the women who underwent a CO2-laser conization at the Gynecologic Oncology unit, National Cancer Institute Aviano, Italy, from January 1991 to December 2014, were retrospectively analyzed. Among all these patients, only the women who underwent conization for a biopsy-diagnosed squamous lesions of the uterine cervix (persistent CIN1, CIN2, CIN3 and cervical SCC) were included in the present analysis.

The initial diagnosis of CIN/SCC was performed with a colposcopy guided biopsy in women with abnormal referral pap smear. Persistent CIN1 was defined as a biopsy-diagnosed CIN1 persisting for 2 years or more in a woman with abnormal pap smear.

All cases included were diagnosed with CIN or SCC for the first time; women with previous diagnosis of CIN/SCC and with one or more previous surgical treatments on the uterine cervix were excluded in order to avoid potential confounding factors.

Women who underwent CO2-laser conization because of atypical glandular cells not otherwise specified (AGC-NOS), atypical glandular cells (AGC), or suspicion of AIS or cancer (AGC-neoplastic) upon pap smear were excluded. Similarly, women who underwent conization for a persistent low-grade SIL on pap smear with unsatisfactory colposcopy and women who underwent conization because of a high grade squamous intraepithelial lesion (HSIL) on pap smear without a previous biopsy diagnosis of CIN/SCC were not included.

Patients were identified by searching our clinical databases, and the medical records of women fulfilling the study inclusion/exclusion criteria were retrospectively analyzed in a retrospective case series. Data obtained included information regarding pertinent medical and surgical history and sociodemographic characteristics of each woman.

All the women with glandular lesions (CGIN or AC) detected on the final histopathological specimen obtained with conization constituted the case series for this report.

2.2. Surgical procedures

All CO2-laser conizations were performed by the same gynecologist with particular expertise in the preinvasive and invasive disease of the lower genital tract (F.S.), with a Surgilase 40 ® CO2-laser, at a power setting of 40 W/cm^2, connected to a micromanipulator mounted on a Zeiss ® colposcope with a focal spot size of 0.2 mm, under local anesthesia (cervical injections of 3.0–5.0 mL of a 2% lidocaine).

2.3. Histopathology

All the specimens collected were reviewed by the same pathologists of the Aviano National Cancer Institute (V.C., L.A.), with particular expertise in gynecological pathology disease. The histopathological examinations of pre-invasive cervical glandular lesions were recorded accordingly to the terminology of the 2012 revised classification of Royal College of Pathologists and the NHS Cervical Screening Program in Britain [13]. The histopathological examinations performed before the introduction of the 2012 terminology were revised accordingly.

The morphological features of CGIN (which are more pronounced in HCGIN than in LCGIN, despite the absence of strict criteria), as described elsewhere [10,11], are as follows: nuclear stratification and loss of polarity, nuclear atypia and hyperchromasia, macronucleoli, loss of intracytoplasmic mucin, increased mitotic activity, apoptotic bodies [Figs. 1 and 2]. Not all of these characteristics need to be present in any individual case. There is often an abrupt transition from normal glands to glands involved by CGIN and this abrupt transition may be seen within individual glands [Fig. 1C]. Both the surface epithelium and the underlying crypts may be involved [Fig. 1A] [11].

2.4. Ancillary techniques

Immunohistochemical (IHC) analysis and in situ hybridization (ISH) technique have been available at our institution and performed since March 2010, in women diagnosed with glandular lesions. In detail, 2.5-μm sections were cut from formalin-fixed paraffin-embedded (FFPE) tissue samples and IHC analysis was performed in an automated system (BenchMark- Ultra, Ventana Medical Systems, Inc., Ventana, Tucson, AZ, USA), using the following antibodies: p16 (monoclonal, clone G175-405, 1:25 dilution, Becton Dickinson Italy) and Ki67 (monoclonal, clone 30.9, prediluted, Ventana,Tucson, AZ).

Moreover, 4-μm sections were cut for In Situ Hybridization (ISH) and stained on the BenchMark Ultra automated system (Ventana) using INFORM HPV III Family probe (B) (Ventana), which contains a cocktail of DNP-labelled HPV genomic DNA probes (High-risk genotypes detected: 16,18,31,33,35,39,45,51,52,56,58,66) and INFORM HPV II Family 6 probe (Ventana), which contains a cocktail of DNP-labelled HPV genomic DNA probes (Low-risk genotypes detected: 6,11).

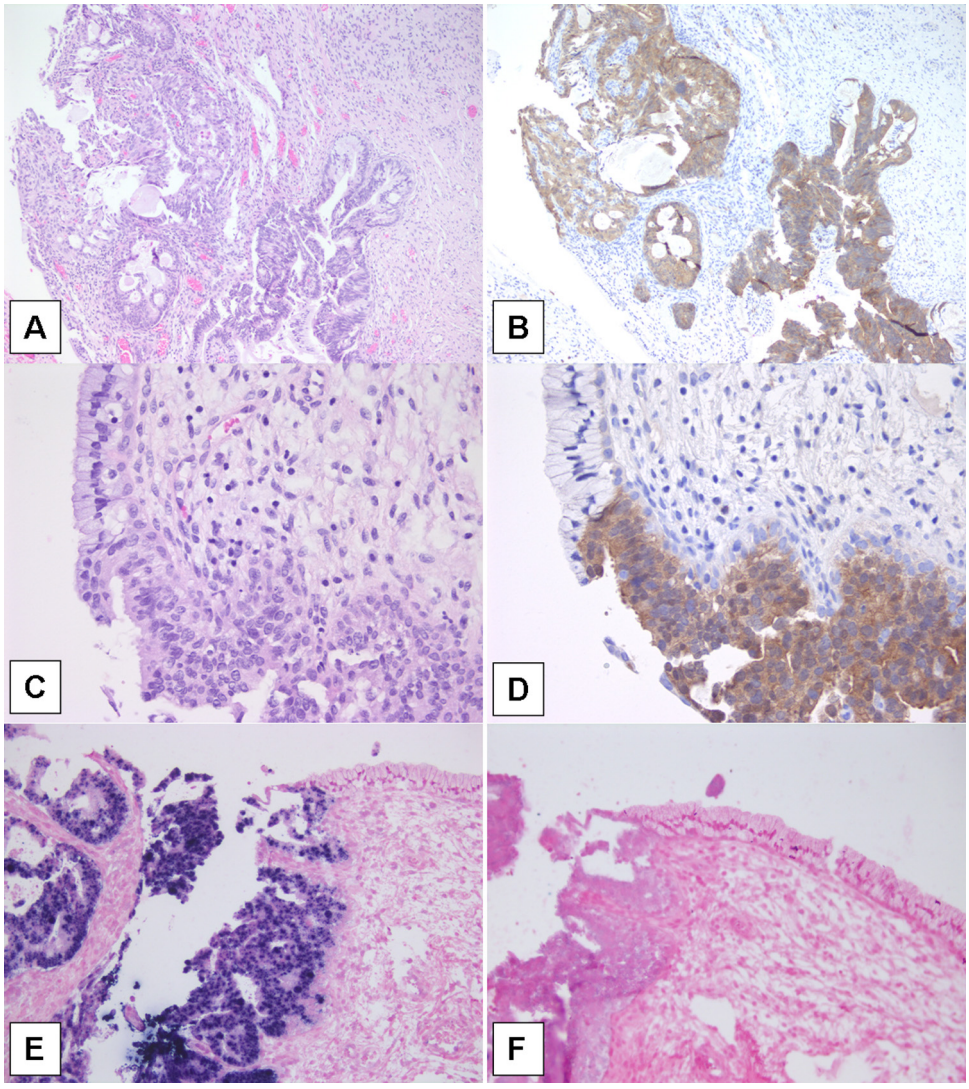

Fig. 1. A, C. High grade dysplastic glandular lesion (HCGIN), showing nuclear stratification and loss of polarity of epithelium, nuclear atypia and hyperchromasia, loss of intracytoplasmic mucin, increased mitotic activity, apoptotic bodies. C. An abrupt transition from normal glands to glands involved by CGIN is particularly evident. B,D. p16 immunohistochemical expression displays a strong and diffuse positivity in dysplastic glands (see for comparison figure LCGIN).
E,F. In Situ Hybridization (ISH) technique was performed on the BenchMark Ultra automated system (Ventana, Tucson, AZ, USA) using INFORM HPV III Family probe (B) (Ventana), which contains a cocktail of DNP-labelled HPV genomic DNA probes (High-risk oncogenic genotypes detected: 16,18,31,33,35,39,45,51,52,56,58,66) (E) and INFORM HPV II Family 6 probe (Ventana), which contains a cocktail of DNP-labelled HPV genomic DNA probes (Low-risk genotypes detected: 6,11) (F).
E.The episomal pattern appears as a large, homogeneous, globular navy-blue precipitate within the epithelial cell nucleus. The integrative pattern is a discrete, stippled navy blue nuclear pattern. Both these patterns of staining, indicating positivity for oncogenic type HPV infection, are evident the images. F. Low-risk HPV genotypes were not detected.
A,B. Original magnification 100x.
C,D,E,F. Original magnification 200x.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS version 22.0 (IBM Corporation- Armonk, New York, 10504-1722, United States). $\chi 2$ testing and *t*-test were used, as appropriate, to evaluate associations. A *p*-value ≤ 0.05 was considered as statistically significant.

2.6. Informed consent

Institutional Review Board approval was not required for the present study because of its retrospective nature and because the study data were constantly managed to exclude subjects' identifying information. Moreover, at the time of admission to our institution, all the women have provided a written informed consent for use of personal and clinical data for future researches, with guarantees of confidentiality.

3. Results

From January 1991 to December 2014, 1280 women underwent CO2-laser conization at the Gynecological Oncology Unit of the Aviano National Cancer Institute (Aviano, Italy).

In 89 cases the cervical conization was performed because of a suspicious glandular disease, thus these patients were excluded from the present analysis. In detail, the conization was performed for AGC on pap smear in 39 cases, and suspicious AC/AIS on biopsy in the remaining 50 cases.

In 136 cases, the conization was performed for a high grade cervical cytology (ASC-H or HSIL) with high-grade colposcopic

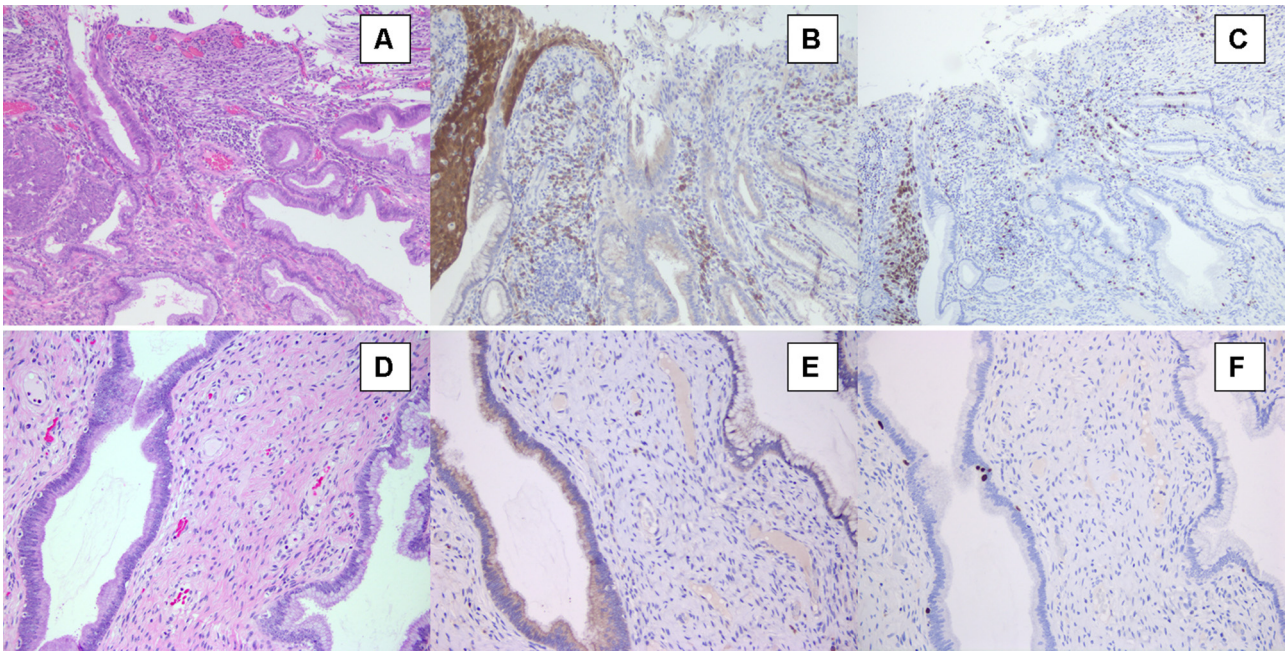

Fig. 2. Low grade dysplastic glandular lesion (LCGIN) at the squamo-columnar junction, which is also involved by a CIN II lesion (left). A,D. Glands show hyperchromatic nuclei, loss of intracytoplasmic mucin resulting in more eosinophilic cytoplasm (D: a dysplastic gland is evident on the left; a normal gland can be seen on the right). B,E. p16 immunohistochemical expression displays a slightly stronger intensity in dysplastic glands than in normal glands. See for comparison a strong and diffuse expression in CIN II lesion- (B, left). C,F. Ki67 immunohistochemical expression displays a slightly increased mitotic index in dysplastic glands than in normal glands. See for comparison a strong and diffuse expression in CIN II lesion- (C, left).
A,B,C. Original magnification 100x.
D,E,F. Original magnification 200x.

impression, without a prior biopsy confirmation of the disease and in other 51 cases one or more previous cervical treatment for previous dysplasia were reported; these patients were not included in the present study. Thus the final cohort consisted of 1004 women, fulfilling the study inclusion/exclusion criteria.

Among these women, 77 cases (7.7%) of occult glandular lesions (LCGIN, HCGIN or AC) were detected on the final specimen.

In these women diagnosed with occult glandular lesions, the average height of the cone specimen was 16.5 mm (SD ± 5.5, range 7.0-30.0 mm). The apex and surgical margins of cervical conizations were free of disease in 84.4% and 98.7% of cases respectively.

The mean age of the women diagnosed with occult glandular lesions was 39.1 years old (SD ± 9.3, range 21–70 years). Eight women were in post-menopausal status and HIV-infection was present only in one case. In the remaining 927 women without occult glandular lesions on the final specimen, the mean age was 38.4 years old (SD ± 9.4). No difference in the mean age between women diagnosed with occult glandular lesions and women without occult glandular lesions on the final specimen emerged (39.1 ± 9.3 vs 38.4 ± 9.4, p = 0.5)

Among the 77 women diagnosed with occult glandular lesions, a total of 48 cases of occult LCGIN, 25 cases of occult HCGIN and four cases of occult usual mucinous type AC were identified (Table 1). The final histopathological evaluation after the cervical conization resulted in detection of glandular lesions (CGIN or AC) not associated with squamous lesions in 13 cases (16.9%).

Considering the whole study cohort of 1004 women fulfilling the study inclusion/exclusion criteria, a total of 26 women (2.6%) underwent conization for persistent CIN1. In this subgroup of patients, four cases of occult glandular disease were found (3 cases of LCGIN and one case of HCGIN, respectively). Thus the rate of occult glandular disease in women who underwent conization for CIN1 was 15.4%. In the whole study cohort, 400 women (39.8%) underwent conization for biopsy diagnosis of CIN2 and in 30 cases (7.5%) an occult glandular lesion was detected on the final specimen. In 538 cases (53.6%) the conization was performed because of biopsy diagnosis of CIN3; among these women, an occult glandular lesion was detected in 41 cases (7.6%).

In the remaining 40 women of the original cohort (4%), the conization was performed because of biopsy diagnosis of SCC; among these women, an occult glandular lesion was detected in 2 cases (5%). Considering the four subgroups of patients (persistent CIN1; CIN2; CIN3; SCC), the rate of occult glandular disease appears to be significantly higher in woman with persistent CIN1 compared to others (p = 0.05).

Of the four women initially diagnosed with persistent CIN1 on biopsy, occult HCGIN was reported in only one case (25%); no invasive AC was detected. Among the 73 women with biopsy diagnosis of high-grade squamous intraepithelial neoplasia (CIN2 and CIN3) or SCC, 28 cases (38.4%) of occult HCGIN or invasive AC were detected in the final specimen. The rate of occult HCGIN or AC was similar in women with previous biopsy diagnosis of persistent CIN1 compared to women with high grade CIN or SCC (25% vs 38.4%, P = 0.54).

Considering only the eight patients in post-menopausal status, an HCGIN or an occult invasive AC was found in three cases (37.5%). In the remaining 69 women, the rate of occult high-grade CGIN or invasive AC was 37.7%. The rate of detection of occult high grade or invasive glandular lesions was similar in menopausal women compared to childbearing-age women (37.5% vs 37.7%, P = 0.70).

From March 2010 to December 2014, 9 women were diagnosed with occult CGIN in cone specimen (5 cases of HCGIN and 4 cases of LCGIN). In these specimens, IHC analysis for p16 and Ki67 and ISH analysis for high-risk HPV were performed. In the 5 women diagnosed with HCGIN, a strong and diffuse immunostaining for p16 [Fig. 1B, D], a high percentage of Ki67 positivity on IHC and a concomitant expression of high-risk HPV on ISH [Fig. 1E] was found. In the 4 women with LCGIN on final specimen [Fig. 2 A, D], in only

Table 1
Histopathological characteristics of the 77 women diagnosed with occult glandular lesions.

Histology on specimen excised	CIN 1 on biopsy (n = 4)	CIN 2 on biopsy (n = 30)	CIN3 on biopsy (n = 41)	SCC on biopsy (n = 2)
L-CGIN	–	6 (20%)	4 (9.7%)	–
L-CGIN/CIN 1	2 (50%)	6 (20%)	2 (4.9%)	–
L-CGIN/CIN 2	1 (25%)	5 (16.6%)	5 (12.2%)	–
L-CGIN/CIN 3	–	5 (16.6%)	7 (17.1%)	–
L-CGIN/CIS	–	–	4 (9.7%)	–
L-CGIN/IA1 SCC	–	1 (3.3%)	–	–
H—CGIN	–	–	1 (2.4%)	–
H—CGIN/CIN 1	1 (25%)	1 (3.3%)	2 (4.9%)	1 (50%)
H—CGIN/CIN 2	–	3 (10%)	5 (12.2%)	–
H—CGIN/CIN 3	–	1 (3.3%)	3 (7.3%)	1 (50%)
H—CGIN/CIS	–	1 (3.3%)	4 (9.7%)	–
H—CGIN/IA1 SCC	–	–	1 (2.4%)	–
IA1 AC	–	1 (3.3%)	–	–
IA1 AC/CIN 2	–	–	1 (2.4%)	–
IA1 AC/CIN 3	–	–	1 (2.4%)	–
IB1 AC	–	–	1 (2.4%)	–

CIN: cervical intraepithelial neoplasia; CIS: carcinoma in situ; SCC: squamocellular cancer; L-CGIN: low grade cervical glandular intraepithelial neoplasia; H-CGIN: high grade cervical glandular intraepithelial neoplasia; IA1 SCC: microinvasive squamous cell carcinoma; IA1 AC: early-invasive adenocarcinoma; IB1 AC: invasive adenocarcinoma.

one case (25%) high-risk HPV on ISH was found, while p16 showed only a faint cytoplasmic staining [Fig. 2B, E] and a slight increase in Ki67 positive nuclei in dysplastic glands was found [Fig. 2C, F].

Among women of childbearing age who had been diagnosed with HCGIN, with involvement of the surgical apex, a further conization was performed to achieve complete excision of the lesion. In women with HCGIN on the cone specimen, who have already completed childbearing (4 cases), a subsequent total hysterectomy was performed, as recommended in the 2012 ASCCP guidelines [18]. Moreover, for all the women diagnosed with occult CGIN after cervical conization, a follow up with cervical cytology and colposcopy (every 6 months for the first two years and annually thereafter) was scheduled.

In the 21 childbearing age women with HCGIN, mean follow-up of 26 months was reported (range 6–76 months) and 1 case of progression to EIAC (after 16 months was observed). All the women diagnosed with HCGIN underwent a total hysterectomy at the end of their childbearing, as recommended [18].

For 5 of the 48 women diagnosed with occult LCGIN, follow up data were not available. In the remaining 43 women with occult LCGIN, mean follow-up of 48 months was reported (range 7–206 months) and no case of progression to HCGIN or AC was observed.

4. Discussion

Compared to squamous lesions, the glandular lesions of the uterine cervix are more difficult to diagnose and relatively infrequent. However, recent reports indicate an increase of these diagnoses approaching about 27% of all cervical cancers and precancers [19]. Anyhow, as perceptively underlined by McCluggage [11], the increase in diagnosis of endocervical glandular lesions is more apparent than real because of the better recognition of premalignant endocervical glandular lesions by histopathologists and the realization that some poorly differentiated cervical carcinomas are glandular in type.

Glandular lesions are believed to develop deep in the endocervical canal, but actually up to 90% of these lesions (especially when an associated high risk HPV infection is documented), can develop close to the transformation zone [20–23].

Interestingly, a previous study [24] reported a 10% rate of endocervical glandular lesions (so-called "AGC"-atypical glandular cells-and AC) coexisting with high grade squamous lesions on Pap smears. The identification of glandular lesions on cervical cytology could help to refine the accuracy of cytological criteria and could contribute to a better patient management.

Moreover, as we have shown in this study, occult CGIN and AC are occasionally found also in cone biopsy performed for CIN.

The potential correlation between human papillomavirus infection and cervical AC is currently under debate [25]. Some authors reported an almost 90% prevalence of HPV DNA in AC [26,27], with higher rate of HPV infection in usual mucinous type AC and neuroendocrine carcinomas compared to other histologic categories [25]. Furthermore, a high frequency of HPV infection in HCGIN is reported [10] and this is consistent with HPV role as an initiating factor in the development of both SCC and most of AC.

In our case series, a relatively high rate (7.7%) of occult glandular lesions (LCGIN, HCGIN or AC) in women who underwent conization for squamous lesions emerged, and all the cases of occult AC detected were usual mucinous type AC.

These findings support the theory that squamous lesions and glandular lesions could share the same HPV-related etiology [14] and in these women, the squamous lesion could be considered as "marker lesion" for the presence of an occult glandular lesion. More in detail, according to previous studies [15–17], it is possible to speculate that tissue stem cells of the uterine cervix may be the reservoir of a persistent HPV infection that may lead to infection of adjacent glandular epithelium or undifferentiated stem cells, determining the development of CGIN and, subsequently, of AC.

To date the progression of HCGIN to invasive AC is well known. However, the management of HCGIN remains controversial, as many assumptions used to justify conservative management for women with CIN2 and CIN3 seem to be not adapt to glandular lesions [18]. For example, colposcopic changes associated with HCGIN can be minimal. HCGIN in the cylindrical epithelium frequently rise close to the SCJ, extending deep inside the cervical canal. These HCGIN can be multifocal and discontinuous, so negative margins on an excision specimen do not ensure complete removal. For these reasons, total hysterectomy remains the treatment of choice in women who have completed childbearing [18]. For women who wish to maintain fertility, conization and subsequent follow up is an option [18], although it carries a less than 10% risk of persistent HCGIN and a small risk of cancer even when excision margins are negative [28–30]. The conservative management of HCGIN is controversial even because of the difficulties in the follow up, since a relapse of the disease can be difficult to diagnose with the conventional cytology and colposcopy, especially in women with SCJ not visible on colposcopy [23].

Even if the natural history of HCGIN is well known, the natural history of LCGIN is still uncertain. Many pathologists and gynecologists do not agree about the potential clinical role of this entity,

and there is also some controversy as to whether LCGIN is a precursor lesion of HCGIN or AC [4,31]. For this reason, currently there is no consensus regarding the appropriate treatment of women with LCGIN.

Moreover, the diagnosis of HCGIN seems to be fairly reproducible, although in some cases the distinction from early invasive adenocarcinoma may be problematic [11]. On the other side, the diagnosis of LCGIN is much more difficult and poorly reproducible and sometimes it can be undiagnosed in absence of HCGIN. However, as underlined by McCluggage, if a diagnosis of LCGIN is made, given our present state of knowledge, the management should be similar to that of HCGIN [11].

However, in our opinion there are no enough data to support the hysterectomy as the treatment of choice in women diagnosed with LCGIN who have completed childbearing, and further studies analyzing the possibility of spontaneous regression and the potential progression of LCGIN to AC are needed. In women diagnosed with LCGIN, especially during the childbearing age, a cervical conization should be performed in order to get a correct and complete diagnosis, and then an appropriate cytological and colposcopic follow up, even with the use of HPV test as suggested by some authors [21], should be scheduled.

In this context, the role of pathologists has become very important. The accurate diagnosis of glandular lesions could have potential implications in the clinical management of these patients, and the carefully exclusion of benign mimics of LCGIN becomes crucial.

This study has some limitations, due to its retrospective nature, and in particular the available data are limited to those already collected in the medical charts. In particular, the IHC for p16/Ki67 and ISH for high risk HPV detection was available only in 9 recent cases.

Notably, in women diagnosed with occult LCGIN on cone specimen, no case of progression to HCGIN or AC was observed during the follow up. However, the number of patients was not big enough to draw conclusions about the natural history of LCGIN and its trend of progression towards more severe disease. Even in this case future studies assessing this question could be very useful to define appropriate follow up strategy in such patients.

5. Conclusions

A relatively high rate of occult glandular lesions was observed in women who had undergone conization for squamous lesions. Further studies are needed to investigate the association between glandular lesions and specific clinical or pathological patient characteristics. Similarly, further studies are needed to delineate the clinical management of women with such lesions and, in particular, women with LCGIN.

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