Colposcopic patterns of vaginal intraepithelial neoplasia: a study from the Italian Society of Colposcopy and Cervico-Vaginal Pathology
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The aim of this study was to evaluate the colposcopic patterns observed in women with a histopathological diagnosis of vaginal intraepithelial neoplasia (VaIN). The medical charts and the colposcopy records of women diagnosed with VaIN from January 1995 to December 2013 were analysed in a multicentre retrospective case series. The abnormal colposcopic patterns observed in women with VaIN, VaN2 and VaIN3 were compared. The vascular patterns and micropapillary pattern were considered separately. A grade II abnormal colposcopic pattern was more commonly observed in women with a biopsy diagnosis of VaIN3 rather than with VaIN1 or VaIN2 ($P < 0.001$). Vascular patterns were also more common in women with VaIN3 rather than with VaIN1 or VaIN2 ($P < 0.001$). Moreover, in women with grade I colposcopy, the rate of VaIN3 was significantly higher when a vascular pattern was observed (62.5 vs. 37.5%; $P = 0.04$). The micropapillary pattern was more common in women with grade I colposcopy and it was more frequently observed in women with VaIN1 rather than in those with VaIN2 or VaIN3 ($P < 0.001$). Grade II abnormal colposcopic pattern was more commonly observed in women with VaIN3. Moreover, the detection of vascular patterns appeared to be associated with more severe disease (VaIN3) even in women with grade I colposcopy, whereas the micropapillary pattern should be considered an expression of a less severe disease (VaIN1 and VaIN2).

Keywords: colposcopic pattern, colposcopic picture, colposcopy, vagina, vaginal vaginal high grade squamous intraepithelial lesion, vaginal intraepithelial neoplasia

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Received 17 April 2016 Accepted 29 June 2016

Introduction
Vaginal intraepithelial neoplasia (VaIN) is usually classified, according to the depth of the tissue involved by the dysplasia, as VaIN1 (mild dysplasia), VaIN2 (moderate dysplasia) and VaIN3 (severe dysplasia/carcinoma in situ) (Sopracredevole et al., 2016b). VaIN1 can be properly considered as the transient expression of human papillomavirus (HPV) infection, and is reported to have a high rate of spontaneous regression (Rome and England, 2000; Massad, 2008); therefore, it is defined as ‘low-grade vaginal intraepithelial neoplasia’ (LG-VaIN). Instead, VaIN3 is considered as ‘high-grade vaginal intraepithelial neoplasia’ (HG-VaIN) because of its potential progression towards vaginal cancer (Sopracredevole et al., 2016a).

The VaIN2 category is not a reproducible histopathological category among pathologists (Darragh et al., 2012; Frega et al., 2013; Sopracredevole et al., 2016b), and its risk of progression should be intermediate between VaIN1 and VaIN3 (Darragh et al., 2012). However, as the real potential of progression of VaIN2 to invasive cancer is still under discussion, some authors encompass VaIN2 in the HG-VaIN category (Rome and England, 2000; Gunderson et al., 2013; Ratnavelu et al., 2013; Zeligs et al., 2013; Sopracredevole et al., 2015).

VaIN is diagnosed through colposcopy-guided biopsies of suspicious areas after an abnormal Pap smear (Sopracredevole et al., 2015). However, vaginal colposcopy is more difficult
than cervical colposcopy, and the ability to reliably predict the histology of lesions is a challenge for most colposcopists (Boonlikit and Noiunual, 2010). The colposcopic pictures of VaIn are extremely variable and nonspecific; the lesion can present itself as an area with varying degrees of acetowhite epithelium, sometimes with vascular patterns, and with varying degrees of staining with Lugol’s solution (Indraccolo and Baldoni, 2012). Moreover, the colposcopic patterns of VaIn seem to be less specific than those of cervical intraepithelial lesions (CINs), and the histology of lesions can be more severe than their colposcopic appearance (Boonlikit and Noiunual, 2010).

To our knowledge, very few studies have analysed the colposcopic patterns observed in women with a histopathological diagnosis of VaIn (Boonlikit and Noiunual, 2010; Indraccolo and Baldoni, 2012), substantially confirming the lack of a clear correlation between colposcopy and histology. However, the opportunity to detect a potential link between colposcopic patterns and histopathological findings on the vaginal epithelium is of particular clinical relevance for the colposcopist to better choose the site at which to perform the biopsy, increasing the probability that the most severe area of disease is sampled for histological diagnosis. Moreover, a correct histopathological diagnosis of VaIn is crucial, as the subsequent therapeutic management depends on the grade of the lesion.

The aim of this study was to evaluate the colposcopic patterns observed in women with a histopathological diagnosis of VaIn, with particular interest in additional colposcopic features such as vascular patterns and micropapillary pattern.

Methods
This study was sponsored by the Italian Society of Colposcopy and Cervico-Vaginal Pathology (SICPCV), and seven hospitals in central and northern Italy participated in the data collection.

All women with a histological diagnosis of VaIn (VaIn1, VaIn2 and VaIn3) consecutively referred to the institutions involved, from January 1995 to December 2013, were considered. These women were diagnosed with VaIn through biopsies of suspicious areas detected on colposcopy after an abnormal Pap smear. Colposcopic examinations were recorded according to the 2011 revised colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy (IFCPC) (Bornstein et al., 2012). The colposcopies performed before the introduction of the 2011 IFCPC terminology were revised accordingly, through the revision of colposcopic charts and images. The revision of all colposcopic charts and images was carried out by the same experienced colposcopist (certified by the SICPCV), avoiding potential interobserver variability.

More thoroughly, according to the 2011 IFCPC terminology (Bornstein et al., 2012), we considered thin acetowhite epithelium, a fine punctuation and a fine mosaic as a grade I abnormal colposcopic pattern, whereas dense acetowhite epithelium, a coarse punctuation and a coarse mosaic were considered as a grade II abnormal colposcopic pattern.

In the 2011 IFCPC terminology, Lugol’s nonstaining areas were considered as nonspecific colposcopic findings. However, as VaIn may sometimes appear only as a Lugol’s nonstaining area (Indraccolo and Baldoni, 2012), for this study we have considered the acetonegative Lugol’s nonstaining areas as a grade I abnormal colposcopic pattern.

Furthermore, vascular patterns (fine/coarse punctuation and fine/coarse mosaic) and micropapillary pattern (defined as an acetowhite area with irregular micropapillary surface) were considered separately.

Colposcopic examinations were performed after the treatment of cervical/vaginal infection or oestrogenic treatment of postmenopausal dystrophy when necessary (and if not contraindicated). All colposcopies were performed by staining with a 5% acetic solution and a 3% Lugol’s solution (Schiller test). In each case, the biopsy was taken from the site with the worst colposcopic pattern, with the goal of sampling the area most likely to contain precancer or cancer cells.

In some cases of multifocal or extremely wide lesions, multiple biopsies were performed in the same patient. In these cases, if different grades of VaIn coexisted in the same woman, we considered the worst histopathological diagnosis and the related colposcopic pattern of the specific site in which such a biopsy had been performed.

All colposcopies and vaginal biopsies were performed by gynecologic oncologists with particular expertise in the diagnosis and management of preinvasive and invasive lesions of the female lower genital tract. Similarly, all vaginal biopsies were analysed by pathologists with particular expertise in the preinvasive and invasive lesions of the female lower genital tract.

All the women considered were diagnosed with VaIn for the first time; thus, women with a previous diagnosis and/or who had undergone treatments for VaIn were excluded, to avoid potential confounders. Similarly, women with a histological diagnosis of invasive vaginal cancer were excluded. In addition, women with an incomplete colposcopic description of the vaginal lesions or for whom colposcopic images were not available were excluded.

Patients were identified by searching the clinical databases of the institutions involved, and the medical records of women fulfilling the study inclusion criteria were analysed in a retrospective case series. Data collected included information regarding sociodemographic characteristics of each woman, colposcopic pictures and histopathologic diagnosis.
Statistical analysis
Statistical analysis was performed using IBM SPSS (version 22.0; IBM Corporation, Armonk, New York, USA). The $\chi^2$-test was used for the statistical evaluation and a $P$-value less than 0.05 was considered statistically significant. The $K$ coefficient was used to evaluate the potential correlation between the grade of colposcopic abnormalities and the histopathologic grading of VaIN.

Institutional Internal Review Board approval (CRO IRB n. 17/2013) was obtained.

Results
From January 1995 to December 2013, 395 women were diagnosed with VaIN (VaIN1, VaIN2 or VaIN3) for the first time in the institutions involved. Thirty-four women were not included in the present analysis because of an incomplete colposcopic description of vaginal lesions or because of the absence of images in the colposcopic reports. The remaining 361 women, fulfilling the study inclusion criteria, constituted the study cohort.

The mean±SD age of these women was 46.6±13.6 years (range: 18–79 years) and, in particular, 164 (45.4%) women were in postmenopausal status. HIV infection was reported in 14 (3.9%) women, whereas data on tobacco usage were available only for 263 women, with 82 (31.2%) being smokers. Previous diagnosis of HPV-related cervical disease (CIN or invasive cervical cancer) was reported in 95 (26.3%) women. Seventy (19.4%) women had undergone hysterectomy earlier; hysterectomy had been performed because of CIN or invasive cervical cancer in 55 women and because of benign conditions or non HPV-related malignancies in the remaining 15 women.

In the whole study cohort, 97 (26.9%) women were diagnosed with VaIN1 on biopsy, 182 (50.4%) women were diagnosed with VaIN2, and the remaining 82 (22.7%) women were diagnosed with VaIN3.

In the whole study cohort, grade I abnormal colposcopic patterns were recorded in 279 (77.3%) women, whereas grade II patterns were recorded in the remaining 82 (22.7%) women. In detail, grade I abnormal colposcopic patterns were recorded in 90 (92.8%) women with a biopsy diagnosis of VaIN1, 149 (81.9%) women with VaIN2 and 40 (48.8%) women with VaIN3. Conversely, grade II abnormal colposcopic patterns were recorded in seven (7.2%) women with a biopsy diagnosis of VaIN1, 33 (18.1%) women with VaIN2 and 42 (51.2%) women with VaIN3. Therefore, a grade II colposcopy was more commonly observed in women with a biopsy diagnosis of VaIN3 rather than with VaIN1 or VaIN2 ($P<0.001$).

For this study, we considered the acetonegative Lugol’s nonstaining areas as a grade I abnormal colposcopy, and this pattern was observed as a unique colposcopic finding in 32 (8.1%) women. In detail, this pattern was observed in 12 (12.4%) women among the 97 with a biopsy diagnosis of VaIN1, in 18 (9.9%) women among the 182 with VaIN2 and in one (1.2%) among the 82 women with a biopsy diagnosis of VaIN3. Thus, the detection of an acetonegative Lugol’s nonstaining area as a unique colposcopic finding was more common in women with VaIN1, rather than with VaIN2 or VaIN3 ($P=0.02$).

Table 1 shows the colposcopic pictures of the women in the study group.

On comparing women with LG-VaIN (VaIN1) and HG-VaIN (VaIN2 and VaIN3 considered together), we found that the $K$ coefficient, used to evaluate the potential correlation between the grade of colposcopic abnormalities and the histopathologic grading of VaIN, showed a poor correlation ($K<2$; SE: 0.03; 95% CI: 0.08–0.18).

Subsequently, the vascular patterns (fine/coarse punctuation and fine/coarse mosaic) were considered separately. These patterns were found in eight (8.2%) among the 97 women with a biopsy diagnosis of VaIN1, in 17 (9.3%) among the 182 women with VaIN2 and in 27 (32.9%) among the 82 women with a biopsy diagnosis of VaIN3. Thus, vascular patterns were observed more frequently in women diagnosed with VaIN3 rather than in those with VaIN2 or VaIN1 ($P<0.001$).

The micropapillary pattern was also considered separately. This colposcopic pattern was found in 46 (47.4%) among the 97 women with a biopsy diagnosis of VaIN1, in 62 (34%) among the 182 women with a biopsy diagnosis of VaIN2 and in 17 (20.7%) among the 82 women with a biopsy diagnosis of VaIN3. The micropapillary pattern was observed more frequently in women diagnosed with VaIN1 rather than in those with VaIN2 or VaIN3 ($P<0.001$). This colposcopic pattern was found more frequently in women of childbearing age than in those in menopause (40.9 vs. 16.6%; $P<0.001$). Among the 125 women with a micropapillary pattern, 92 (73.6%) had a grade I abnormal colposcopy, whereas the remaining 33 (26.4%) had a grade II abnormal colposcopy. Thus, the detection of micropapillary pattern was significantly higher in women with a grade I colposcopic picture (73.6 vs. 26.4%; $P<0.001$).

### Table 1 Colposcopic pictures in women with a histopathological diagnosis of vaginal intraepithelial neoplasia on biopsy (study cohort n=361)

<table>
<thead>
<tr>
<th></th>
<th>VaIN1</th>
<th>VaIN2</th>
<th>VaIN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I colposcope pattern</td>
<td>90 (92.8)</td>
<td>149 (81.9)</td>
<td>40 (48.8)</td>
</tr>
<tr>
<td>Grade II colposcope pattern</td>
<td>7 (7.2)</td>
<td>33 (18.1)</td>
<td>42 (51.2)</td>
</tr>
<tr>
<td>Total</td>
<td>97 (100)</td>
<td>182 (100)</td>
<td>82 (100)</td>
</tr>
</tbody>
</table>

$\chi^2 = 53.402; P<0.001$

Data are expressed as n (%).

Grade I colposcopic pattern: thin acetowhite epithelium, fine punctuation and/or fine mosaic, acetonegative Lugol’s nonstaining areas.

Grade II colposcopic pattern: dense acetowhite epithelium, coarse punctuation and/or coarse mosaic.

VaIN2, vaginal intraepithelial neoplasia grade 2; VaIN3, vaginal intraepithelial neoplasia grade 3.
As a secondary analysis, we focused only on the 264 women with a biopsy diagnosis of HG-ValN (ValN2 and ValN3). Among these 264 women, grade I abnormal colposcopic patterns were recorded in 189 (71.6%), whereas grade II patterns were recorded in the remaining 75 (28.4%). Considering the women diagnosed with HG-ValN, grade I abnormal colposcopic patterns were significantly more frequent than grade II patterns (71.6 vs. 28.4%; \( P < 0.001 \)).

More specifically, in the 182 women diagnosed with ValN2, a grade I colposcopy was recorded in 149 (81.9%), whereas the remaining 33 (18.1%) had a grade II colposcopy. Out of the 82 women diagnosed with ValN3 on biopsy, a grade I colposcopy was reported in 40 (48.8%) women, whereas a grade II colposcopy was reported in the remaining 42 (51.2%).

Therefore, a grade I colposcopy was more commonly observed in women with a biopsy diagnosis of ValN2 rather than with ValN3 (81.9 vs. 48.8%; \( P < 0.001 \)), whereas a grade II colposcopy was more common in women with a biopsy diagnosis of ValN3 rather than ValN2 (51.2 vs. 18.1%; \( P < 0.001 \)).

However, considering only the 75 women with an initial grade II abnormal colposcopic pattern, a similar rate of ValN2 and ValN3 on biopsy emerged (44 vs. 56%; \( P = 0.19 \)). Similarly, considering only the women with ValN3, similar rates of grade I and grade II colposcopic pictures were found (51.2 vs. 48.8%; \( P = 0.88 \)).

In women with HG-ValN, the \( K \) coefficient, used to evaluate the potential correlation between the grade of colposcopy and the histopathologic grading of ValN, showed a fair correlation (\( K = 0.339 \); SE: 0.06; 95% CI: 0.217–0.461).

Subsequently, in women with HG-ValN, the vascular patterns (fine/coarse punctuation and fine/coarse mosaic) were considered separately. These patterns were observed more frequently in women diagnosed with ValN3 rather than in those with ValN2 (32.9 vs. 9.3%; \( P < 0.001 \)). In more detail, the vascular pattern was classified as grade I abnormal colposcopy (fine punctuation or fine mosaic) in 35 women, and among them a final diagnosis of ValN3 was made in 25. Considering only the 40 women with grade I colposcopy and a biopsy diagnosis of ValN3, a ‘fine’ vascular pattern was detected in 25 women, whereas the remaining 15 women had no vascular pattern. Therefore, the rate of ValN3 in women with grade I colposcopy was significantly higher when a vascular pattern was observed (62.5 vs. 37.5%; \( P = 0.04 \)).

Further, the micropapillary pattern was considered separately. This colposcopic pattern was found in 62 (34%) women among the 182 with a biopsy-proven diagnosis of ValN2 and in 17 (20.7%) women among the 82 with a biopsy-proven diagnosis of ValN3. Hence, the micropapillary pattern was more frequently observed in women diagnosed with ValN2 rather than with ValN3 (34.1 vs. 20.7%; \( P = 0.04 \)).

**Discussion**

A correct biopsy diagnosis of ValN is crucial, as subsequent management depends on the grade of the lesion. In case of ValN1, no controversy about treatment options exists and, as a high rate of spontaneous regression is reported (Rome and England, 2000; Massad, 2008), observation alone with cytology and colposcopy seems to be the best choice (Gunderson et al., 2013; Chen et al., 2016). In contrast, the optimal management of HG-ValN (ValN2 and ValN3) actually remains a ‘therapeutic dilemma’ (Frega et al., 2013), and several therapeutic strategies have been proposed, including surgical excisions and ablative procedures (Sopracoerdele et al., 2016b). Furthermore, some authors have proposed a conservative surveillance with cytology and colposcopy even in women with HG-ValN, reporting encouraging results (Ratnavelu et al., 2013).

In our opinion, considering the risk of progression of HG-ValN to invasive cancer (Sopracoerdele et al., 2016a), the risk for histopathological upgrading of vaginal dysplastic lesions, and the possibility of finding occult invasive cancer in specimens excised for HG-ValN (Sopracoerdele et al., 2016b), both ValN2 and ValN3 should undergo surgical treatment. However, the treatment can be tailored to the characteristics of the patient and to the grade of the lesion, choosing the more complex excisional procedures for women with a high risk of occult invasive disease or progression to invasive cancer, whereas the easier and more feasible ablative procedures could be used in the other cases of HG-ValN.

For this reason, to plan the appropriate management for these women, not only a distinction between LG-ValN and HG-ValN but also a distinction between ValN2 and ValN3 would be worthwhile. Indeed, both the rate of progression to vaginal cancer and the incidence of occult invasive lesions seem to be significantly higher for ValN3 compared with ValN2 (Sopracoerdele et al., 2016a). Hence, women diagnosed with ValN3 should undergo excisional treatments, whereas for women with ValN2 an ablative procedure could be proposed, especially if specific risk factors for the presence of occult lesions, such as a previous hysterectomy for CIN, are absent (Sopracoerdele et al., 2016b).

The chance of identifying the colposcopic patterns indicative of ValN3 could be of particular relevance in case of large or multifocal lesions, in which different grades of ValN can coexist. In this case, it is important for the colposcopist to choose the site for biopsy carefully, with the goal of sampling the area most likely to contain ValN3 (or cancer), for correct diagnosis, which is necessary for appropriate management of the patient.
Despite the absolute importance of colposcopy in diagnosing vaginal lesions, its capability for detecting VaIN and predicting the grade has not been extensively assessed in the literature. To our knowledge, only two studies have assessed this topic (Boonlikit and Noinual, 2010; Indraccolo and Baldoni, 2012), substantially affirming the lack of a clear correlation between colposcopic features and histopathological findings of VaIN.

In our cohort, we found an overall higher prevalence of grade I abnormal colposcopic patterns, whereas grade II patterns were found in only 22.7% of the women. Furthermore, on comparing the women with LG-VaIN and HG-VaIN, we found that the K coefficient (used to evaluate the potential correlation between the grade of colposcopic abnormalities and the histopathologic grading of VaIN) showed only a poor correlation, substantially confirming the findings of previous studies (Boonlikit and Noinual, 2010; Indraccolo and Baldoni, 2012).

However, in our cohort, grade II colposcopic patterns were more commonly observed in women with a biopsy diagnosis of VaIN3 rather than with VaIN2 or VaIN1.

Interestingly, considering only the women with HG-VaIN and on comparing women with VaIN2 and VaIN3, we found that the K coefficient showed a better correlation between the grade of colposcopic abnormalities and the histopathologic grading of VaIN.

Moreover, the presence of vascular patterns appeared to be associated with more severe histopathological disease (VaIN3). Furthermore, on considering only women with HG-VaIN and a grade I colposcopy, we found that the rate of biopsy diagnosis of VaIN3 was significantly higher when vascular patterns were observed. A possible explanation of the association between vascular pattern and high-grade vaginal disease has been already provided by other authors (Boonlikit and Noinual, 2010). They argued that, in the uterine cervix, the vascular patterns can be detected early in the dysplastic process, as the vascular patterns are the result of exaggeration of the vasculature of immature squamous metaplasia in the transformation zone. In contrast, VaIN develops on very mature squamous epithelium, which lacks an underlying vascular structure, and abnormal vascular patterns therefore, develop late in the neoplastic process (Boonlikit and Noinual, 2010).

In addition, we detected a relatively high rate of micro papillary pattern in the women of our cohort, especially in women with a biopsy diagnosis of VaIN1 and VaIN2. This colposcopic pattern, not specifically considered in the 2011 IFCPC terminology (Bornstein et al., 2012), is quite rare on the cervix but seems to be relatively common on the vaginal epithelium, especially in women of childbearing age. The specific significance of this pattern is currently unknown, but it seems to be associated with less severe disease, and, in our opinion, it could probably be an expression of a persistent HPV infection, reflecting the shift from a VaIN1 to a VaIN2 lesion. However, further studies clarifying this aspect are required.

In the present case series, we were able to collect data from a large number of patients, and, even though the retrospective nature of this study limited the available clinical data to those already collected in the medical charts, to our knowledge, this is the largest cohort of patients with a histopathological diagnosis of VaIN in which the colposcopic pictures were extensively evaluated. Unfortunately, because of the retrospective nature of the study, data on colposcopic patterns observed in women with negative biopsy or colpitis were not available.

Furthermore, in the present study, 97 women with VaIN1 were included; considering the 264 women with HG-VaIN included in the analysis, a higher number of VaIN1 was expected. However, most of the institutions involved in the present study are highly specialized in the management of HG-VaIN, whereas women with VaIN1 (which needs only cytological and colposcopic observation) are often managed in smaller institutions. Thus, the number of women with HG-VaIN was relatively higher than those with VaIN1 in our study.

In conclusion, our findings confirmed the substantial heterogeneity of colposcopic patterns in women with VaIN. Globally, grade I abnormal colposcopic patterns are more frequently observed, but grade II colposcopic patterns are more common in women with VaIN3 rather than in those with VaIN2 or VaIN1. Moreover, the detection of vascular patterns appears to be associated with more severe disease (VaIN3) even in women with grade I colposcopy, whereas the micropapillary pattern (quite common in women of childbearing age) should be considered an expression of less severe disease.

This finding could be useful for colposcopists to choose appropriately the site for biopsy, in order to increase the probability that the most severe area of disease is sampled for histological diagnosis. Moreover, a correct diagnosis could be useful to determine the appropriate therapeutic management for these women.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References


College of American Pathologists and the American Society for Colposcopy
Frega A, Sopracordevole F, Assorgi C, Lombardi D, DE Sanctis V, Catalano A,