VAGINAL INTRAEPITHELIAL NEOPLASIA (VAIN)
1. RATIONALE AND OBJECTIVES

The principal objective of the Spanish Association of Cervical Pathology and Colposcopy (AEPCC) is to “promote knowledge and investigation of the lower genital tract in women”. The “AEPCC-Guidelines” were developed to fulfill this objective and to respond to the demand of professionals devoted to disease of the lower genital tract and colposcopy.

The AEPCC-Guidelines cover specific areas of knowledge of disease of the lower genital tract which are characterized by their relevance and important repercussion in clinical practice. The AEPCC-Guidelines are scientific evidence-based documents which have been systematically developed and aim to help professionals achieve consensus in decision making in clinical practice regarding the most adequate diagnostic and therapeutic options for a determined health problem.

The specific objectives pursued by the AEPCC-Guidelines are:

- To promote standardized lines of action based on the current scientific evidence and reliable consensus information.
- Guarantee patient equality when attended, independently of their location of residence, promoting good praxis.
- Improve the effectiveness of interventions and the quality of health care.
- Favor the implementation of indicators of quality control or of clinical efficacy.
- Facilitate decision making within the administrative setting for managers or health care resources planners.

The methodological rigor established for the development of the AEPCC-Guidelines pursues the elaboration of documents of excellent scientific quality with which better clinical practice and greater knowledge of lower genital tract disease are achieved.

2. METHODOLOGY

The specific methodology followed for the elaboration of the AEPCC-Guidelines includes the following aspects:

- The AEPCC Steering Committee appoints a Coordinator who is responsible for the elaboration of the AEPCC-Guidelines. In accordance with the Steering Committee, the Coordinator appoints the Writing Committee consisting of him/herself, a Secretary and the participants. The members are professional experts who are members of the AEPCC or other scientific societies with recognized prestige in this topic.
- Consensual development of the index.
- Critical review of the available literature and assignment of levels of evidence.
- Discussion and consensus among the members of the Committee for assigning the grade of recommendation.
- Elaboration of the document.
- Final analysis of the document on behalf of the Review and Editing Committee.
- Printed and online format of the final version.
- Diffusion of the AEPCC-Guidelines in congresses, courses and seminars organized by the AEPCC.
- Elaboration of online Courses of Continuing Education on the content of the AEPCC-Guidelines to provide in depth knowledge of the guidelines and facilitate their application in daily clinical practice (training credits).
- Translation of the AEPCC-Guidelines to English (online edition).
- Update of the AEPCC-Guidelines.
Assessment of the scientific evidence and extent and strength of the recommendations. The GRADE System.

The “Guidelines of Clinical Practice” consist of recommendations aimed at health professionals to help them with patient care related to a determined clinical condition. They are based on the most important bibliographic evidence of a determined subject (systematic reviews of the medical literature and identification of studies with the greatest scientific evidence available) and on clinical practice. In general, the highest level of classification is given to prospective studies to which patients are randomly assigned and the minimum levels are given to data related to expert opinion. In this way it is possible to assess the quality of evidence associated with the results obtained of a determined strategy. For the elaboration of the AEPCC-Guidelines all the recommendations made have taken into account the quality of the current scientific documents. The strength of the recommendations is agreed upon by the Committee of the AEPCC-Guidelines based on the quality of the studies available.

The GRADE system is used to classify the scientific evidence and the grade and strength of the recommendations. (Grading of Recommendations Assessment, Development and Evaluation Working Group) (http://www.gradeworkinggroup.org/).

To do this the following steps are followed:

1. **Formulation of the PICO questions** (patient, intervention, comparison, outcomes”) and definition of the outcome variables (in relation to benefits and risk) for each of the intervention questions made.
2. **Scoring of the outcome variables from 1 a 9.** Variables which are key for decision making were assigned a score from 7 to 9; for important variables (but not key) from 4 to 6 and from 1 to 3 for less important variables. The working group identified, assessed and agreed upon the importance of the outcome variables.
3. **Evaluation of the quality of evidence** of each of the key outcome variables. Searches were designed to identify systematic reviews, randomized clinical studies and other published studies. The quality of evidence of each of the variables in the GRADE system was assessed as high, moderate, low or very low. Randomized clinical trials and systematic reviews of randomized clinical trials start with a high level of evidence, and observational studies and systematic review of observational studies have a low quality of evidence. Table 1 shows the previously described aspects which increase or decrease the quality of evidence.
4. **Evaluation of the global quality of the evidence.** The global quality of the evidence is considered according to the lowest level of quality obtained by the key outcome variables. In cases in which the evidence for all the key variables favors the same alternative and there is evidence of quality for some, although not for all the variables, the global quality is considered to be high. Low quality evidence related to benefits and risks of little importance do not reduce the grade of global evidence.
5. **Assignment of the strength of the recommendation.** The GRADE system distinguishes between strong and weak recommendations and makes explicit judgments of the factors that can affect the strength of the recommendation: balance between benefits and risks, global quality of evidence, values and preferences of the population and costs. Both the strong and the weak categories can favor or not favor a determined intervention. The importance of informing people of the benefits and risks of a determined procedure is highlighted. Table 2 shows the significance of the strong and weak categories.
### QUALITY OF THE EVIDENCE

**Table 1.- GRADING SYSTEM FOR ASSIGNING THE QUALITY OF THE EVIDENCE**

<table>
<thead>
<tr>
<th>Study design</th>
<th>Initial quality of evidence</th>
<th>In clinical trials, decrease if *</th>
<th>In observational studies increase if</th>
<th>Final quality of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized clinical trials</td>
<td>High</td>
<td>Critical (-1) or very important (-2) limitation to study quality</td>
<td>Strong association, without confounding factors, consistent and direct (+1)</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Important inconsistency (-1) Some (-1) or great (-2) uncertainty as to whether the evidence is direct</td>
<td>Very strong association***, without important threats to validity (no biases) and direct evidence (+2)</td>
<td>Moderate</td>
</tr>
<tr>
<td>Observational study</td>
<td>Low</td>
<td>Inaccurate or scarce data</td>
<td>Dose-response gradient (+1)</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High probability of notification bias (-1)</td>
<td>All the possible confounding factors may have reduced the effect observed (+1)</td>
<td>Very low</td>
</tr>
</tbody>
</table>

* Raise (+1) or lower (-1) one level (i.e from high to moderate); 2: raise (+2) or lower (-2) two levels (i.e. from high to low);
** A statistically significant relative risk > 2 (< 0.5) based on consistent evidence in two or more observational studies without plausible confounding factors.
*** A statistically significant relative risk > 5 (< 0.2) based on direct evidence without important threats to validity.


### STRENGTH OF RECOMMENDATIONS

**Table 2.- GRADING SYSTEM FOR ASSIGNING THE STRENGTH OF THE RECOMMENDATIONS**

<table>
<thead>
<tr>
<th></th>
<th>Strong</th>
<th>Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>The vast majority of people agree with the action recommended and only a small part do not.</td>
<td>The vast majority of people agree with the action recommended but an important number do not.</td>
</tr>
<tr>
<td>Clinicians</td>
<td>Most of the patients should receive the recommended intervention.</td>
<td>Recognizes that different options would be appropriate for different patients and that the health care professional should help each patient adopt the decision which is most consistent with their values and preferences.</td>
</tr>
<tr>
<td>Managers/Planners</td>
<td>The recommendations may be adopted as a health care policy in most situations.</td>
<td>There is need for important debate and the participation of interest groups.</td>
</tr>
</tbody>
</table>

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VAGINAL INTRAEPITHELIAL NEOPLASM (VAIN)

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1. Introduction

Vaginal intraepithelial neoplasia (VAIN) is considered to be the precursor lesion of cancer of the vagina. VAIN is infrequent and asymptomatic and may easily remain unnoticed on examination of the lower genital tract. The diagnosis of VAIN represents 1.2% of all premalignant lesions of the lower genital tract; however, these values likely underestimate the real prevalence of this disease. Although the exact prevalence of these lesions is unknown, different studies have reported an increase in the diagnosis of VAIN in the last years, in part favored by the increase in cytological screening and greater systematization of colposcopic examinations1.

VAIN lesions are classified based on the grade of involvement of epithelial maturation in high grade or HSIL (VAIN) or low grade intraepithelial or LSIL (VAIN) lesions. HSIL (VAIN) is considered the true precursor of cancer of the vagina2.

Vaginal cancer is a very infrequent neoplasia, representing 1.2% of gynecological neoplasias3. Squamous cell vaginal carcinoma is the most frequent histological variety and represents 6-95% of all vaginal cancers4,5. Due to the low prevalence of this neoplasia there are few studies in the literature providing in depth knowledge of its etiopathogenesis and natural history, and the studies available often present controversial data. It is likely that, similar to what occurs in vulvar carcinoma, the etiological factors involved in carcinoma of the vagina are different in younger patients compared to women of older age6. In this regard, different studies have shown that the etiology of vaginal cancer in younger women is related to human papilloma virus infection (HPV) and the development and progression by precursor lesions (VAIN). On the other hand, cancer of the vagina is older women is not related to HPV infection and is probably associated with hormonal factors and chronic trauma.

HPV infection is causally involved in up to 90% of the cases of VAIN. This justifies the association of VAIN lesions with multicentric lesions of the anogenital tract such as cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN) or anal intraepithelial neoplasia (AIN)7. Specifically, VAIN is associated with CIN in up to 40% of the cases, and it is estimated that around 20-30% of patients with VAIN have been previously treated for cervical cancer8.

There is no evidence regarding the most adequate therapeutic method to be used in all cases of VAIN. However, excisional treatments such as the classically used partial or total colpectomy present important morbidity. In the last years, this circumstance has favored greater use of destructive treatments, which have fewer adverse effects and acceptable cure rates.

For gynecologists, the clinical approach in patients with VAIN constitutes a real challenge in health care practice. The low frequency of VAIN, the difficulty in diagnosing this disease, the scarce knowledge of its natural history and the lack of evidence and guidelines supporting a specific treatment and defining follow-up explain the complexity in determining the most adequate clinical approach to VAIN.

The aim of this AEPCC-Guideline is to provide an updated and in depth review of the most relevant aspects of VAIN to support and help in decision making and enable the adoption of a protocolized approach to this disease based on the scientific evidence currently available and the most up to data knowledge.
The real prevalence of VAIN remains largely unknown and the data available at an international level or in our country are very scarce. The incidence of VAIN is difficult to discern since most lesions are asymptomatic, and there are no protocols standardizing examination of the lower genital tract aimed at the detection of VAIN. The Nordic countries have very good populational registries which are directly linked to histological databases, reporting an incident rate of HSIL (VAIN) of 0.5-1.3 cases per 100,000 women-year, with a higher incidence in women over 60 years of age. In a series of 189 cases, the mean age at diagnosis was 50 years. In Spain, a multicenter study of 5,665 women attended by 385 gynecologists showed that VAIN constituted 2% of all the lower genital tract diseases associated with HPV. This study suggested that this proportion may be overestimated by the preferential inclusion of lower genital tract reference centers. This study also highlighted that 63% of the cases of HSIL (VAIN) were diagnosed in women over 45 years of age versus 29% of LSIL (VAIN) (figure1). It was estimated that at the level of hospitalizations in Spain, vulvar and vaginal intraepithelial lesions together represented 182 annual admissions during the period 1997-2008.
3. Etiopathogenesis

The real precursor of cancer of the vagina is HSIL (VAIN)\(^2\). It is accepted that HPV is the causal agent of vaginal cancer and HSIL (VAIN) in approximately 70% and 90% of the cases, respectively\(^{10,13,14,15}\). However, the prevalence of HPV in patients with VAIN reported in the literature varies greatly. In a Spanish study including 152 cases, the prevalence of HPV was 51.1% (63.4-77.7%)\(^{11}\).

At a world level the most frequent phenotype of HPV in VAIN in HPV 16 which is isolated in 56.1% of the cases. The remaining 9 most frequent phenotypes are: HPV 18 (5.3%), HPV 52 (5.3%), HPV 73 (4.8%), HPV 33 (4.2%), HPV 59 (3.7%), HPV 56 (2.6%), HPV 51 (2.1%), HPV 6 (1.6%) and HPV 35 (1.6%)\(^{10,16,17}\). In the study by Alemany et al, the 5 most frequent phenotypes among patients with VAIN in Spain were HPV 16: 47.4%, HPV 18: 3.3%, HPV 73: 3.3%, HPV 33: 2.6% and HPV 56: 26%\(^{10}\).

The concept of HPV infection “en masse” with involvement of the lower genital tract and the risk of neoplastic transformation is a hypothesis that bases the susceptibility for HPV infection of this anatomical region on the embryologic common origin of the upper third of the vagina and cervix. This hypothesis would explain that the upper third of the vagina is the most frequent localization of VAIN and that many of these lesions are multifocal or multicenter and metachronically or synchronically develop with cervical intraepithelial neoplasias (CIN)\(^{16,17}\). Piovano et al\(^{18}\) found that 29% of VAIN are associated with previous CIN, 23% present concomitantly with CIN, and 3% are associated with VIN. On the other hand, the incidence of VAIN in patients with hysterectomy by benign disease is minimum compared to that observed in patients with hysterectomy for CIN (around 1% vs. 40% at 10 years of follow-up)\(^{19,20}\).

Although HPV is the principal etiological factor, the incidence of VAIN is significantly lower than that of CIN. Silman et al\(^{21}\) described an 100-fold lower incidence in a 10-year follow-up study. Some studies attribute the squamocolumnar junction of the cervix, inexistent in the vagina, as the area that justifies greater susceptibility of the cervix in the development of premalignant lesions. Other authors have speculated that although HPV infection in the vagina is as frequent as that in the cervix, the innate and adaptative immunity at a vaginal level is able to promote regression of vaginal lesions\(^{22}\).

There are no significant differences between HSIL (VAIN) and LSIL (VAIN) with regard to age, parity, smoking, previous history of cervical dysplasia, previous history of cervical cancer or previous hysterectomy\(^{20}\).

Different co-factors have been related to the development of VAIN and cancer of the vagina: number of sexual partners, smoking habit, previous radiotherapeutic treatments, exposure to diethylstilbestrol (DES) and immunosupression\(^{23,24,25}\).
4. Histology. Terminology And Classification

Histologically, lesions of the squamous epithelium of the vagina are closely related to those of the cervix. The first description of VAIN was reported in the 1930s and for many years was denominated carcinoma in situ of the vagina. In the 1950s the term “dysplasia” was used to refer to intraepithelial lesions of the vagina. Later, it was causally associated with HPV, and vaginal lesions were graded according to the alteration in maturation of the epithelium in analogy with the classification of Richart for cervical lesions. Three grades of lesions were established: mild dysplasia (VAIN 1), moderate (VAIN 2) and severe or in situ carcinoma (VAIN 3). In 2012 the American College of Pathologists and the American Society of Cervical Pathology and Colposcopy achieved consensus of a new terminology “Lower Anogenital Squamous Terminology Standardization Project for HPV Associated Lesions”, thereafter called the LAST terminology. This terminology has been redefined and the diagnostic categories have been fixed for all the squamous lesions related to HPV infection in the anogenital area. The objective of this consensus terminology is to improve the clinical approach to these patients and communication between pathologists and clinicians.

The LAST terminology established a dichotomic classification for squamous lesions of the lower genital tract according to the biology of HPV. Active and transitory infections are related to low grade intraepithelial lesions (LSIL), and persistent infections with the capacity to progress to neoplasia are related to high grade intraepithelial lesions (HSIL) considered to be true premalignant lesions. The LAST terminology recommends the use of LSIL and HSIL, specifying the term VAIN in brackets to refer to intraepithelial lesions of the vagina. This terminology was recently accepted as the official classification of the International Agency for Research on Cancer/World Health Organization (IARC/WHO) for tumors of the female genital tract.

The histological characteristics of LSIL are:
1. Nuclear alterations: increase in nuclear size, irregularity of the nuclear membrane, increase in the nucleus-cytoplasm ratio.
2. Absence of cytoplasmatic maturation of the lower third of the squamous epithelium.
3. Mitosis limited to the lower third.
4. Presence of multinucleated cells with increased nuclear size and pleomorphism accompanied by perinuclear halos (koilocytes) in the absence of characteristics of high grade lesions.
5. Condyloma acuminatum or papillary lesion with cytopathic characteristics of HPV.

The histological characteristics of HSIL are:
1. Nuclear alterations: increase in nuclear size, irregularity of the nuclear membrane, increase in the nucleus-cytoplasm ratio.
2. Absence of cytoplasmatic maturation reaching the most superficial two thirds.
3. Mitosis in the upper two thirds.
4. Special situations:
   a. Anomalous mitosis and important nuclear atypia, referring to apparently low grade lesions presenting very marked nuclear atypia in the lower third of the epithelium with mitosis at any level of the epithelium. These lesions are considered HSIL.
   b. “Thin” intraepithelial lesion: This refers to intraepithelial lesions with less than 10 cells of thickness and which present important immature basal proliferation or mitosis above this basal stratum.
   c. Keratinizing intraepithelial lesion. Lesions with important atypical proliferation, marked cytoplasmatic eosinophilia, and abnormal superficial keratinization. These lesions are more common in the vulvar and perianal region but are occasionally observed in the vagina.
4.1 USE OF BIOMARKERS IN HISTOLOGICAL DIAGNOSIS.

The LAST terminology recognizes that only immunohistochemical detection of the overexpression of p16 presents sufficient scientific evidence to be selectively used in the histological diagnosis of intraepithelial lesions.

A sample is considered to be p16 positive with strong continuous cytoplasmatic and nuclear staining of the basal layer of the epithelium which may extend to the most superficial strata with staining of the whole area.

**p16 staining is indicated in the following situations:**
1. In the differential diagnosis between HSIL and non neoplastic lesions such as atrophy, reparative changes or defects in sample orientation (tangential slices).
2. In the dichotomic categorization of lesions previously considered as moderate dysplasia (VAIN 2). In these cases intense or diffuse staining is considered to be HSIL (VAIN) while negative staining is considered to be LSIL (VAIN).
3. In diagnostic discrepancies of HSIL (VAIN) p16 increases the accuracy and the diagnostic sensitivity with a slight reduction in specificity since a percentage of LSIL are positive for p16.

**To the contrary its use is not recommended in the following situations:**
1. In the differential diagnosis between LSIL and normal epithelium since a percentage of LSIL is negative for p16.
2. To condition the clinical approach to LSIL according to the expression of p16. Although some studies of the cervix show that p16 positive LSIL (CIN1) presents an increased risk of progression to HSIL and p16 negative results have a lower rate of progression to HSIL, these findings have not been confirmed in other series.
5. Natural History

The natural history of VAIN is largely unknown, and there are very few studies on this subject in the literature. This can be explained, in part, by the fact that it is a very infrequent asymptomatic entity, and it is usually diagnosed synchronically or metachronically with intraepithelial lesions of the cervix and/or vulva.

VAIN has several forms of presentation: 1) de novo, not associated with other lesions of the genital tract; 2) associated with CIN or cervical cancer; 3) associated with vulvar intraepithelial (VIN) lesions or cancer of the vulva; or 4) associated with CIN and VIN or their respective invasive homologs.

One of the largest series published included 127 cases of VAIN followed over a mean of 34 months (range: 12 to 169 months). Lesional regression of 89% was described with no cases of progression to cancer and with no influence of the grade or type of treatment on disease evolution. In this study, 11% of the lesions presented recurrence. In another study including 163 cases with a mean follow-up of 18 months (range 1 to 194 months), LSIL (VAIN1) presented a similar evolution independently of whether the patients received treatment or not. The same study reported a higher percentage of recurrence in HSIL (VAIN) than LSIL (VAIN), and the appearance of 6 cases of vaginal cancer diagnosed in women with HSIL (VAIN 2-3).

Studies evaluating the risk of recurrence after treatment for VAIN are heterogeneous and are limited by: 1) the reduced number of patients included; 2) the difference with regard to the criteria of treatment of VAIN according to histological grade; 3) the characteristics of the lesions included in the series published; 4) the type of treatment administered; and 5) the disparity in the length of follow-up.

In general, after treatment of VAIN it is estimated that the percentage of recurrence is approximately 30%.

The risk of progression of LSIL (VAIN) to HSIL (VAIN) is estimated to be 5-30%. The risk of progression to cancer of VAIN is generally estimated to be around 3% or 5-6% if we refer to only HSIL (VAIN), with a time of progression of between 8 months and 20 years according to the series consulted. It is likely that the cases in which progression is reported within a period of less than 12 months are actually invasive lesions of the vagina not initially diagnosed.

The natural history of VAIN is very different in the subgroup of patients with previous hysterectomy for CIN or cervical or uterine cancer. A higher frequency of VAIN has been described in this subgroup of women. It is estimated that up to 20% of women treated for cervical cancer develop vaginal dysplasia, especially in women who have undergone treatment with radiotherapy.

In these cases VAIN presents a greater risk of progression and occult invasion and a higher tendency to relapse after treatment, and this should be taken into account when evaluating the most adequate therapeutic approach in these patients.

Women who have undergone hysterectomy for CIN or have a history of cervical cancer require long-term gynecological controls (up to 20 years) since the interval between surgery and the appearance of VAIN ranges from 4 to 13 years.

The principal factor of risk of recurrence is persistence of HPV infection. However, there are discrepancies in the literature in relation to other factors of risk of recurrence. Some authors have described a higher percentage of recurrence in HSIL (VAIN), multicentric lesions of the lower genital tract (CIN, VIN, AIN, among others) and extensive or multifocal lesions. Other authors conclude that the type of treatment is the main factor which modifies the risk of recurrence, although not all the studies have found significant differences in this aspect.
6. Clinical manifestations and diagnosis

6.1 CLINICAL MANIFESTATIONS

Patients with VAIN are usually asymptomatic, although they may occasionally refer pruritis, dysparenuia or leukorrhea. The appearance of bleeding or bloody leukorrhea is a sign of suspicion of invasion.

VAIN may present as a single lesion, but in most cases it appears in association with a cervical lesion, or it may appear in the vaginal vault after hysterectomy in patients with a previous cervical lesion. Thus, an abnormal cytology is a woman diagnosed with CIN who has undergone hysterectomy is often the first indication of a possible vaginal lesion.

If the colposcopy in non hysterectomized patients with an abnormal cytology does not show an exocervical or endocervical lesion, it is recommended to perform a vaginoscopy in order to rule out the presence of VAIN. This situation is infrequent in clinical practice.

6.2 DIAGNOSIS

6.2.1 Clinical examination and vaginoscopy

Vaginoscopy should be meticulously performed and should follow a rigorous order in order not to miss any lesion. The large surface area of this anatomical cavity, its folds, the presence of the cervix which hinders access to the fornices of the vaginal pouch, the valves of the speculum itself which might block the view of an important part of the vagina and the tangential view of the mucosa generally make this examination difficult to perform. It is therefore important to follow a rigorous system which can be summarized in the following 5 steps:

1. Before the introduction of the speculum make a direct inspection of the urethral meatus, introitus and proximal mucosa of the vagina in search of lesions requiring greater attention.
2. Careful introduction of the speculum.
3. Evaluation of vaginal fluid (take a sample for culture if indicated), the mucosa and its vascularization before application of the acetic acid. Colpitis produces congestion, erythema and diffuse vascular changes in contrast to VAIN.
4. Application of acetic acid and colposcopic examination to identify lesions at the level of the cervix. Afterwards, examine the fornices of the vaginal pouch by lateral mobilization of the cervix with a gauze pad or spatula. In patients with previous hysterectomy carefully examine the angles of the vaginal vault since this is the area most frequently presenting recurrence. It may be useful to use a hook or retractor, an endocervical speculum or a polyp forceps to order to achieve access to these angles.
5. Examination of the middle and external thirds of the vagina and afterwards, after careful rotation of the speculum, examine the anterior and posterior walls. Before returning the speculum to its initial position, the Lugol solution should be applied and examination of the cervix and the vagina should be repeated. Lugol staining during vaginoscopy is especially important since it enables the detection of small lesions which may remain unnoticed with acetic acid. The presence of weak or negative Lugol staining of defined borders is very suggestive of the presence of VAIN. A homogeneous mustard yellow color is characteristic of HSIL.
VAGINAL INTRAEPITHELIAL NEOPLASIA (VAIN), and clear differentiation from the mahogany color of the healthy surrounding mucosa allows a direct biopsy to be performed and localize the lesion to be treated.

During menopause and in patients taking contraception with gestagens, the deficit of estrogens reduces the glucogenesis of the squamous cells and reduces Lugol uptake. In these cases the administration of local estrogens during 3 weeks improves the trophism and facilitates visualization of abnormal areas.

The colposcopic images obtained by vaginoscopy should be described according to the Classification of the International Federation of Cervical Pathology and Colposcopy (IFCCP) of 2011. Similar to what occurs in the cervix, abnormal images of the vagina include acetowhite, mosaic, and spotted epithelium classified as grade 1 and 2. However, the mosaic pattern is more infrequent in the vagina than in the cervix and its presence is usually related to metaplasia of a congenital transformation zone, vaginal adenosis or vaginal extension of a cervical lesion.

As occurs in the cervix, there is a certain correlation between the characteristics of the lesions observed with vaginoscopy and the grade of histological lesion. However, the objective of this examination is to direct biopsy sampling of the most altered lesional area. Colposcopic patterns suggesting the presence of a LSIL (VAIN) are a flat acetowhite or slightly elevated acetowhite epithelium with a smooth or micropapillary surface of clear or undefined borders, varying in size from a few millimeters to several centimeters. Scarce whitening with acetic acid make its distinction difficult with respect to the normal vaginal mucosa. The vascular pattern is infrequent and usually involves a fine, uniform spotting with a small intercapillary distance. The absence or weak uptake of iodine allows easy detection with this procedure. A heterogeneous staining pattern with Lugol is typical of LSIL (VAIN) while a homogeneous mustard yellow color is associated with both low and high grade lesions. LSIL (VAIN) lesions are generally multifocal and may be diffuse throughout the vagina.

The colposcopic characteristics of HSIL (VAIN) are similar to those observed in cervical HSIL, except that mosaic areas are rarely found in the vagina. The areas of HSIL (VAIN) are usually elevated with a flat or rough surface or with hyperkeratosis or erosive areas. The borders are usually clear. The most notable characteristic of HSIL (VAIN) is its acetwhite coloring which become more opaque and dense according to the severity of the lesion. This characteristics impedes the visualization of the underlying vascularization. On increasing the magnification, and when the effect of the acetic acid dissipates, it is sometimes possible to see a more or less thick localized spotting of the zone of the lesion suggesting the presence of vascular changes. These changes appear late in the carcinogenic process, and if they are very apparant, they indicate suspicion of invasion. In contrast with low grade lesions which are usually multifocal, HSIL (VAIN) are usually unifocal.

Colposcopic characteristics of suspicion of invasion: the presence of atypical vessels are the principal sign of suspicion of invasion. Ulceration, the presence of necrotic or very exophytic areas, and the presence of nodules and friability are other signs suggestive of the presence of an invasive lesion. The presence of any of these signs requires the performance of a biopsy or complete excision of the lesion in order to rule out invasion and contraindicates any destructive treatment.

6.2.2 Indication and biopsy technique

Directed vaginal biopsy by vaginoscopy is the only method to confirm the diagnosis of VAIN.

Biopsy is performed using punch forceps on the mucosa in the most perpendicular way possible. This procedure can most often be performed without anesthesia, especially in the upper two thirds of the vagina which have less sensitivity. A depth of 1.5 – 3 mm is sufficient since there is no glandular tissue in the vagina. Bleeding is scarce and can be easily solved with Monsel solution, silver nitrate or iron perchlorate. In some cases with important vaginal atrophy, it may be necessary to do the examination and biopsy under anesthesia.

6.2.3 Differential diagnosis

There are a series of entities which may be confounded in the examination of VAIN (Table 2).

1. Vaginal papillomatosis: this is characterized by the presence of physiological papillas which may be
confounded with HPV infection. The color is similar to the rest of the mucosa. It lacks the vascular axis and presents Lugol uptake since the covering epithelium is normal. There is not always a history of DES exposure. Does not require biopsy sample.

2. Congenital transformation zone, this is normally situated in the cervix, but it may extend to the fundus of the anterior and posterior pouch making a rhomboidal image. It presents a light acetowhite color and a fine spotted mosaic. Lugol uptake is weak or heterogeneous. Differential diagnosis with an extensive cervical lesion which extends to the vagina or with a lesion located in a congenital transformation zone may be very difficult. Biopsy in this case may rule out the presence of an intraepithelial lesion.

3. Vaginal adenosis. This is the presence of glandular cells in the vagina. The first impression is that of a red area which, after applying acetic acid, shows images similar to VAIN (light acetowhite area, mosaic and fine spotted). It may be associated with changes of the glandular epithelium at the cervical level as ectopy or hypertrrophic or polypoid cylindrical cervical epithelium. It is found in a small percentage of women either spontaneously or associated with intra-uterine exposure to DES. It has also been described after vaginal treatments with CO2 laser or 5-flouracyl.

4. Vaginal candidiasis. With the Schiller test, the diffuse erythema may reproduce a spotty light Lugol similar to condylomatous vaginitis or lead to the appearance of light areas of Lugol.

5. Infection by trichomonas. This produces intense inflammation which impedes correct examination. The changes may be diffuse or appear in the form of Lugol negative, red stains of several millimeters in diameter denominated “strawberry cervix”. Vaginoscopy may be not be considered adequate due to inflammation, and the patient should be re-evaluated after treatment.

6. Vaginal Lichen planus. More or less diffuse erythematous lesions are observed, with thinning of the vaginal mucosa and erosions which produce irregular Lugol staining. The presence of an abundant yellow-grey secretion is frequent.

7. Changes due to radiotherapy. The vagina has a pale, fibrous appearance with a very thin smooth epithelium and abundant vessels with atypical characteristics (vessels presenting changes in direction and which brusquely appear or disappear) and which often bleed on touch. They should not be confounded with atypical vessels intrinsic to intraepithelial or malignant lesions (possible relapse after radiotherapy).

8. Vaginal atrophy. This is associated with a deficit in estrogen which can make identification of VAIN difficult in menopausal patients. The vagina is usually stenotic, with scarce folds, is pale in color and shows an absence of acetowhite reaction or Lugol staining. The fragility of the mucosa produces erosions and bleeding with manipulation. All of this makes vaginoscopy and biopsy sampling difficult in the case of suspicion of VAIN.

<table>
<thead>
<tr>
<th>Table 2: Differential diagnosis of VAIN. Situations which may simulate or occult VAIN.</th>
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<tbody>
<tr>
<td>Inflammation by candidiasis, trichomonas, vaginal Lichen planus, vaginal atrophy</td>
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<tr>
<td>Vaginal papillomatosis</td>
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<tr>
<td>Congenital transformation zone (CTZ)</td>
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<tr>
<td>Vaginal adenosis</td>
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<td>Changes due to radiotherapy</td>
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7. Treatment

7.1 GENERAL PRINCIPLES

The objective of the treatment of VAIN is to avoid progression to an invasive cancer of the vagina. At present, there are different therapeutic options, albeit with no unanimous agreement as to which is the best treatment.

In order to choose a determined treatment the following should be taken into account: 1) the characteristics of the lesion (size, number, localization, multifocality and multicentricity, history of treatment for CIN or cervical cancer) and 2) the characteristics of the patient (age, comorbidity, possibility of follow-up). The technical knowledge, personal experience and the means available are also important when selecting the best therapeutic option.

Most of the information available in the literature on the efficacy of the treatments in patients with VAIN correspond to retrospective studies, including a reduced number of cases, and their analyses present limitations since they often evaluate cases of LSIL or HSIL (VAIN) together or different therapeutic modalities alone or in combination. Therefore, the conclusions of these studies have a relatively low quality of evidence.

7.2 WAIT AND SEE TREATMENT

The schedule recommended in patients with LSIL (VAIN) is follow-up without treatment. Quality of evidence: low; Grade of recommendation: strongly in favor.

**Schedule**

Annual control with cytology, HPV test and vaginoscopy. If changes in the number of lesions or increase in size is observed, consider biopsy.

**Justification**

Most of these lesions regress spontaneously. In fact, it has not been established that they present a risk of progression to vaginal cancer. However, these lesions often present a recurrent course. One exception corresponds to patients with immunosuppression in whom systematic treatment is generally recommended. Follow-up without treatment can be considered in selected patients with HSIL (VAIN). The criteria of inclusion for this option are: 1) no previous history of cancer of the lower genital tract, 2) no immunosuppression, 3) single vaginal lesions or lesions of less than 2 cm, and 4) vaginoscopy and biopsy results ruling out invasion.

**Schedule**

Control every 6 months with vaginoscopy and cytology. If any change such as an increase in size or in the number of lesions is observed, treatment is recommended.

**Justification**

It has been described that this selected subgroup of lesions evolves more slowly and presents a lower risk of progression. Indeed, with the application of these criteria, the rate of spontaneous regression of HSIL (VAIN) reported is of 70-80%.43,48

7.3 TOPICAL TREATMENTS

Topical treatments have the principal advantage of being able to apply them to all the vaginal mucosa. They are therefore especially useful in multifocal lesions and are a good alternative to destructive or excisional treatments, and they can also be combined with these. Quality of evidence: low; Grade of recommendation: weakly in favor.

7.3.1 5-Fluorouracil (5-FU)

**Mechanism of action**

5-FU is a antagonist cytotoxic agent of pyrimidine which blocks the reaction of methylation of deoxyuridylic acid impeding its conversion to thymidylic acid. The efficacy of 5-FU lies is that it irreversibly binds to the thymidylate synthase enzyme which is essential for the synthesis of thymine nucleotides. The lack of thymine blocks DNA replication, impeding cell division. 5-FU was one of the first cytostatic drugs and is frequently used intravenously in different neoplasias.
Posology

Cream at 5% applied by the gynecologist once a week during 6-10 weeks. It is recommended to insert a vaginal tampon after the application of 5-FU and withdraw the tampon at 24 hours or protect the vulva with vaseline in order to avoid vulvitis due to contact with the cream.

Indication

Very extensive, multifocal lesions without suspicion of invasion. **Quality of evidence: moderate; Grade of recommendation: weakly in favor.**

Secondary effects and contraindications

Vaginal irritation, dyspareunia, abnormal fluid and ulcers are relatively frequent. Prolonged application of 5-FU (more than 10 weeks) may lead to cronification of the vaginal ulcers secondary to treatment in around 10% of the patients. In very severe cases, resolution of these ulcers may require excision and surgical closure\(^{55}\). The appearance of columnar metasplasia or vaginal adenosis has been described similar to what is produced by exposure to DES with no precise knowledge of the clinical repercussions\(^{56,57}\). Reactions of photosensitivity with maculopapillary rash and pruritis have also been described, and therefore, adequate photoprotection is recommended as well as avoidance of exposure to the sun during treatment. 5-FU is contraindicated during pregnancy, being classified as category X. Miscarriages and septal defects have been described following the application of 5-FU during gestation.

Justification

For several years 5-FU was an alternative to partial or total vaginectomy for the treatment of HSIL (VAIN) lesions. After the 1990s the use of CO2 laser and the development of local destructive treatments with scarce secondary effects was implemented, replacing the use of this drug. There are few studies on the use of 5-FU after the 1990s. The data published are short, heterogeneous series with varying efficacies and with a rate of recurrence ranging from 11% to 38%\(^{58}\).

7.3.2 Trichloroacetic acid (TCA)

**Mechanism of action**

This is an organic acid obtained synthetically which causes a caustic reaction on contact with the skin and/or mucosas.

**Posology**

Preparation according to a master formula (solution of trichloroacetic acid at 80% in 70 % alcohol). It is applied in the medical consulting office by repeated application with an impregnated cotton swab or small pad to the zone to be treated during several minutes. It is applied in a weekly session during 4 consecutive weeks. It is recommended to change the TCA solution every two weeks since the solution is very volatile and loses effectiveness even when the recipient is closed.

**Indication**

Patients with localized VAIN accessible to direct application (not indicated in areas near the urethra or fornice of the vaginal pouch post-hysterectomy). **Quality of evidence: low; Grade of recommendation: weakly in favor.**

Secondary effects and contraindications: it has no systemic secondary effects and only local damage has been described. Therefore, its use is not contraindicated during pregnancy. Special care should be taken in lesions near the urethral meatus due to the possibility of cicatricial retraction. In sites with poor access (fornices of vaginal pouch, invaginations of the vaginal vault after hysterectomy, etc.) its application may injure zones adjacent to the vagina, and therefore, its use should be very limited.

**Justification**

The success rate described is lower of 5-FU, and with a higher rate of recurrence\(^{59}\).

7.3.3 Imiquimod

**Mechanism of action**

Imiquimod is a molecule which belongs to the group of imidazquinolines which act as a potent modifier of immune response and induce the activation of natural killer (NK) cells, Langerhans cells, macrophages, and stimulate the production of immunoferon alpha, beta and gamma, tumoral necrosis factor and interleukiings\(^{1,6,8,10,12}\).
### Posology

Cream of imiquimod at 5% applied by the gynecologist or self-applied with the fingers. The schedules published are: 6.25 mg 3 times a week during 16 weeks or 50 mg twice a week for at least 5 doses or 250 mg weekly during 12 sessions. After application, the use of an intravaginal tampon can be recommended. Vaginal use for the treatment of VAIN is not accepted in the Summaries of Product Characteristics. However, different studies have demonstrated its efficacy.

### Indication

Extensive multifocal or single vaginal HSIL (VaIN) lesions with no suspicion of invasion. Quality of evidence: low; Grade of recommendation: weakly in favor.

Secondary effects and contraindications: in general this drug is well tolerated with scarce secondary effects at a vaginal level. The most common adverse effects are local irritation (stinging and pain), but in general they are not sufficiently severe to suspend treatment. Systemic effects have been described (pseudoinfluenza picture, general discomfort, fever, headache) in a small percentage of cases and may make treatment difficult to continue.

Imiquimod is classified as category B in pregnancy. No teratogenic effects have been reported in laboratory animals, but controlled studies in humans are lacking.

### Justification

Different studies have demonstrated the efficacy of vaginal imiquimod in patients with VAIN. The rate of response in HSIL (VAIN) is approximately 70% for self-application of 250 mg of imiquimod at 5% at least twice a week during 12 weeks.

### 7.3.4 Polygammaglutamic acid (γ-PGA)

**Mechanism of action**

γ-PGA is a natural polymer synthesized by determined species of bacilli which have shown certain antitumoral activity.

**Indication**

The use of γ-PGA in limited to the field of investigation. Quality of evidence: very low; Grade of recommendation: strongly against.

**Justification**

A single study on the use of γ-PGA for the treatment of VAIN has recently been published. This study analyzed 17 patients and described a rate of complete response of 66.7%.

### 7.4 EXCISIONAL TREATMENT

Surgical treatment by lesion excision is the treatment of choice for HSIL (VAIN), especially in patients with an elevated risk of occult invasion or progression. Quality of evidence: moderate; Grade of recommendation: strongly in favor.

Patients with HSIL (VaIN) after hysterectomy for intraepithelial lesions localized in the vaginal fundus and in cicatrical recesses constitute the principal indication for this treatment. The probability of occult invasion in these patients is estimated to be of around 12%. In these cases, excisional treatment allows histological study of the piece, confirming complete excision of the lesion and ruling out occult invasion.

#### 7.4.1 Excisional treatment with loop electrosurgical excision (LEEP) or CO2 laser

**Procedure**

Lesion excision with CO2 laser or electrosurgery. The greatest difficulty of these methods is to calculate the depth of the tissue resected and this carries an important risk of injuring underlying anatomical structures (bladder, rectum).
This risk may be reduced by the instillation of a submucosal anesthesia solution (creating a wheal) under the lesion to be excised.

**Indication**

Unifocal or multifocal VAIN (HSIL) lesions of little extension, especially in immunosuppressed patients in whom treatment should be as conservative as possible due to the elevated risk of recurrence and need for re-treatment. It is not recommended in extensive and multifocal lesions.

**Quality of evidence:** low; **Grade of recommendation:** weakly in favor.

Secondary effects and contraindications: the main risk is intraoperative damage to the bladder, rectum or intestinal loops.

**Justification**

Treatment with simple local excision can confirm the diagnosis of VAIN, rule out the presence of invasion and assess the status of the margins of the lesion treated. After local excision, one study reported recurrence in 13% of the cases during the first year and 25% at two years[^40].

### 7.5 DESTRUCTIVE TREATMENT

This consists in total destruction of the lesion by cauterization or vaporization. The principal advantage of these methods is the greater radicality and better anatomical and functional preservation of the vagina. However, it does not enable the collection of material for histological study. Therefore, it is strictly necessary to perform multiple biopsies which can rule out the presence of occult invasion.

In cases with suspicion or elevated risk of occult invasion, destructive treatment should not be used. Therefore, when the decision is to use destructive treatment it is important to guarantee complete visualization of the lesions.

#### 7.5.1 CO2 laser

**Procedure**

Destruction of tissue by vaporization with CO2 laser. The laser (Light Amplification by Stimulated Emission or Radiation) is a light amplified by radiation stimulated emission in which all the photons have the same quantity of energy, present scarce dispersion and generate a beam of light which concentrates all the photonic energy.

This property allows the vaporization of tissues with a considerable potency, thereby eliminating tissue lesions. Among the different types of laser, the most commonly used in gynecology is the CO2 laser. This emits a wavelength of 10600 nm (invisible spectrum) and requires a helium beam (red) as a guiding ray. It is a laser of gaseous state in which the conductor is CO2. The light beam is transmitted through the air and is absorbed by water and tissues. It has...
VAGINAL INTRAEPITHELIAL NEOPLASIA (VAIN)

properties for slicing, ablation and vaporization. Ideally, the procedure should be performed under colposcopic vision, directing the laser beam under magnified view which enables more accurate ablation with respect to both depth and extension.

**Indication**

Treatment of choice for HSIL (VAIN) lesions which are totally visible, uni or multifocal and without risk of occult invasion. **Quality of evidence: moderate; Grade of recommendation: strongly in favor.**

**Secondary effects and contraindications**

In general, this is a well tolerated treatment with a low intra- and postoperative morbidity. It is not indicated in patients who have undergone hysterectomy, with vaginal lesions localized in the colpotomy angles, where the border remains occult and the treatment may be incomplete. Neither is its application recommended on thick or distorted scars of the vaginal vault.

**Justification**

A wide range of effectiveness has been described for this procedure (42-90%)18,44,74,75,76. In a series with a mean follow-up of 26 months, Yalcin et al, reported a rate of complete resolution of 71% in the first treatment and of 79.2% after multiple ablations77. A higher rate of recurrence has been described in hysterectomized women with vaginal intraepithelial lesions localized in the angle of the vagina treated by CO2 laser, justified by the difficulty in treating the buried areas of the scar.

7.5.2 Cavitative Ultrasonic Surgical Aspirator (CUSA)

**Procedure**

This is based on the emission of a ray able to eliminate lesional tissue very selectively while preserving the surrounding tissue and with little repercussion on neighboring structures. The equipment is made up of a suction tube connected to an ultrasound generator and a source of serum which allows the destruction and emulsion of the tissue followed by aspiration of the fragments. Its radius of action is 2 mm, and while the tissue is being destroyed, hemostasis of the small vessels of the lesional area is performed. The parameters of ultrasound potency, irrigation and aspiration are variable and are adapted to the characteristics of the tissue to be eliminated.

**Indication**

At present, its use is limited to investigation. **Quality of evidence: low; Grade of recommendation: strongly against.**

**Justification**

A retrospective study in 92 women treated for VAIN with CUSA reported complete resolution in 80% of the patients, with a rate of recurrence of 19.6% at a mean follow-up of 4.5 years. The rate of recurrence in the cases of HSIL (VAIN) was 32.3% and 13.1% in LSIL (VAIN). There were no cases of progression to cancer or surgical complications78. A randomized controlled study comparing treatment with CUSA and CO2 laser vaporization in VAIN and VIN lesions showed no differences between the two procedures (25% rate of recurrence with a mean follow-up of 12 months)79.

7.5.3 Photodynamic therapy

**Procedure**

This involves the application of a laser beam with a wavelength of 635 nanometers and a power of 80 to 125 joules/cm2 after the intravenous administration of a photosensitizer (5-aminolevulinic acid), which is selectively deposited in the dysplastic cells.

**Indication**

At present, the use of this therapy is limited to investigative studies. **Quality of evidence: low; Grade of recommendation: strongly against.**

**Justification**

Photodynamic therapy is fundamentally used in dermatology for the treatment of benign processes and cutaneous neoplasias. In 2000 it was used for the first time in the treatment of VAIN79. Later studies performed in small series of patients showed an efficacy similar to CO2 laser80. A recent study in 15 patients reported a complete response rate of 71.4% after one year of treatment and good tolerance.
7.6 BRACHYTHERAPY

**Procedure**

Ionizing radiation applied by a radioactive substrate directly in contact with or in the interior of the tumor. The most commonly used irradiation is with photons (X and gamma rays) and accelerated electrons.

**Indication**

Brachytherapy is used in exceptional cases (older women with extensive high grade lesions or lesions localized in the vaginal fundus of patients who have undergone hysterectomy for CIN, or recurrent lesions with no other therapeutic option or with elevated surgical risk) in which other treatments are not possible. **Quality of evidence: low; Grade of recommendation: weakly against.**

Secondary effects and contraindications: there is a high risk of adverse effects such as vaginitis, dyspareunia, vaginal stenosis, proctitis with mucositis and/or rectorrhagia and radiation cystitis. According to the dose administered, Agnieszka et al. found a rate of mild-moderate adverse effects of 16.7% (95%CI 0%-47%) at doses between 47.3-63 Gy and of 71.4% (CI 95% 48%-95%) when doses higher than 70 Gy were used.

**Justification**

The efficacy of radiotherapy ranges from 80% to 100% with respect to complete resolution of the lesion. However, the most adequate therapeutic doses are not established. The current studies show good therapeutic results at both low and high doses of brachytherapy. Despite the aggressiveness of this treatment, recurrence occurs in 5-14% of the cases treated.

7.7 COMBINED TREATMENTS

**Procedure**

This consists in the combination of more than one primary treatment.

Initial topical treatment is often combined with destructive or excisional treatment. In these cases the following approaches may be considered: 1) administer an initial topical treatment of the vaginal lesion with the aim of reducing the number and size of the lesions and perform other destructive or excisional treatments in the residual lesions, or 2) first carry out destructive or excisional treatment and later applying topical treatments in the residual lesions or recurrences.

Another option is excisional treatment followed by vaporization of small residual lesions. The combination of different therapeutic modalities can reduce the morbidity and recurrences of the treatments, being an especially relevant factor in younger patients.

**Indication**

Multifocal and extensive VAIN, especially if in difficult to treat anatomical areas. **Quality of evidence: low; Grade of recommendation: weakly in favor.**

**Justification**

Sillman et al. demonstrated that the use of 5-FU as part of a combined treatment with other therapeutic modalities presents an efficacy of 75% versus 29% when used alone. The application of 5-FU after partial colpectomy with loop diathermy does not present recurrence and is able to significantly reduce the secondary effects to topical treatment. Combined treatment can also reduce the risk of recurrence/persistence of VAIN. Gunderson et al. reported a risk of recurrence/persistence of VAIN of 25% with the exclusive use of excisional treatment, 61% with destructive treatment and 17% with the combination of the two treatments.

7.8 TREATMENT IN SPECIAL SITUATIONS

7.8.1 Population with immunosuppression

**Indication**

In patients with single, well delimited lesions, both topical (topical application of imiquimod) and destructive treatment with CO2 laser can be used, or lesion resection can be considered. When there are multiple non extensive lesions, the application of CO2 laser is the most recommendable option, while in multiple extensive or multicentric lesions, combined therapies are recommended (Imiquimod and CO2 laser or resection of major lesion with posterior destructive treatment). **Quality of evidence: low; Grade of recommendation: weakly in favor.**
Justification

Women with human immunodeficiency virus (HIV) infection present a higher risk of infection and persistence of HPV and are therefore more susceptible than the general population to the development of intraepithelial lesions secondary to viral infection as well as lesion persistence and recurrence.

The severity and behavior of these lesions is correlated with the immunosuppressive status of the woman. In a prospective study, Massad et al. described an increase in the prevalence of HSIL (VAIN) and cancer of the vagina in HIV positive women versus HIV negative women. Smoking, a low CD4 count, an elevated viral load and non adherence to the antiretroviral treatment are associated with a higher risk of VAIN.

7.8.2 Pregnant women

As in HSIL (CIN), if no findings suggestive of invasion are seen in the vaginoscopy of pregnant women, neither biopsy nor treatment is recommended and may be delayed 6 weeks.

7.9 THERAPEUTIC ALGORITHM

The schedules established in the following algorithms are the result of retrospective studies and recommendations by experts. Although algorithms may help in the general decision making in clinical practice, it is important to consider each case and the different aspects of the patient and the lesion individually as well as the experience and means available when making therapeutic decisions.

In lesions suggestive of VAIN the whole lower genital tract should be examined, and lesions suggesting greater severity should be biopsied. Whenever hysterectomy for CIN is indicated, it is important to perform an exhaustive study of the upper third of the vagina.

7.10 THERAPEUTIC APPROACH TO PERSISTENCE FOLLOWING TREATMENT

Persistence following treatment is understood to be evidence of the same grade of vaginal lesion after treatment or wait and see follow-up. This situation is quite infrequent, except in immunosuppressed patients who usually present extensive, multifocal and multicentric disease.

Indication

When using a new treatment the balance between the importance of eliminating the vaginal lesion and the need to preserve vaginal function and minimize morbidity induced by the sum of treatments must be evaluated. After a patient recovery period of about 3 months, it is recommended to undertake a new treatment. Based on the characteristics of the lesion and the patient several strategies may be considered.

- CO2 laser vaporization: this treatment may be repeatedly applied in cases of lesion recurrence/ persistence with acceptable results. Remission of VAIN is observed in approximately 45% of the cases after one treatment, and up to 80% of the cases require repeating 2 treatments. Other authors have confirmed these results, obtaining up to 100% of remission after multiple applications. Quality of evidence: low; Grade of recommendation: weakly in favor.
- Imiquimod: this treatment should be assessed in the case of therapeutic failure after a first destructive or excisional treatment or in the case of persistence/recurrence of lesions in zones of difficult access. Quality of evidence: low; Grade of recommendation: weakly in favor.
- Excisional treatments: in very selected cases of persistence-progression, excision may be necessary to rule out possible occult invasion. Quality of evidence: low; Grade of recommendation: weakly in favor.
HSIL (VaIN)

Older patients with elevated surgical risk
Disease recurrence
Extensive lesions
Option: Brachytherapy

Hysterectomized patients with lesion not totally visible in colpocopy
Partial colpectomy

HSIL (VaIN)

Single lesion
Observation (selected cases)
1. Young patients
2. No history of cancer of the lower genital tract
3. No immunosuppression
4. Small lesions
5. Absence of vaginoscopic findings of invasion
Topical treatment (local imiquimod) or CO2 laser or Resection (preferentially in older women)

Multiple lesions
Not extensive
Láser CO2

Extensive
Young women
Combined therapy (Imiquimod and CO2 laser)

Older women
Combined therapy (Imiquimod and CO2 laser) or Resection and CO2 laser
8. Follow-Up

The principal objective of post-treatment follow-up of VAIN is early detection of recurrence and the prevention of progression to invasive cancer. There is no evidence or consensus as to the most adequate follow-up schedule in women diagnosed with or treated for VAIN. The guidelines of the different scientific societies do not define how to carry out post-treatment follow-up of VAIN.

Schedule

Perform the first control at 6 months with cytology and HPV test in order to avoid confounding elements with reparative phenomena.

- If the control is negative make a new control at one year.
  After two consecutive annual controls with negative cytology and HPV test results continue with routine screening.
- Positive HPV test and/or abnormal cytology.
  Perform vaginoscopy

Justification

There is no evidence as to the most adequate approach to carry out in the follow-up of lesions treated in the vagina. In general, it has been suggested to carry out follow-up schedules in patients with VAIN that are similar to those of patients with CIN. However, the lower risk of progression of VAIN justifies a longer follow-up time than in CIN. VAIN may recur after several years, and therefore, long-term controls are recommended.
Prophylactic vaccination against HPV is the fundamental pillar of primary prevention of these lesions. The vaccines currently available in the European Union are: Gardasil/Silgard, Gardasil 9, and Cervarix. Gardasil was authorized for men and women in September 2006, while Cervarix was authorized in September 2007 and Gardasil 9 in June 2015.

At present, 58 countries (30%) have introduced these vaccines into their national vaccination calendars. Although the studies performed were designed to evaluate their efficacy in CIN lesions, they present data which support their utility in the prevention of diseases related to HPV infection in all of the lower genital tract (cervix, vagina, vulva and anus).

The maximum efficacy of these vaccines is observed in subjects not exposed to HPV infection. However, vaccination is considered recommendable up to the age of 26 years and then individualized from 26 to 45 years of age. The results of studies on the safety and efficacy of these vaccines in adult women (up to 45 years of age with the quadrivalent vaccine and up to 55 years with the bivalent vaccine) recommend their use beyond the age of 26 years\textsuperscript{96}. Vaccination after 16 years of age is associated with a reduction in HSIL (VAIN)\textsuperscript{97}.

In a document published in August 2014, the WHO endorsed the safety of these vaccines and recommended vaccination against HPV\textsuperscript{98}. The Morbidity and Mortality Weekly Reports of 2014 and 2015 support these recommendations\textsuperscript{99,100}. The three vaccines available have also demonstrated their immunogenicity and safety in women with HIV infection\textsuperscript{101,102}.

### 9.1 Quadrivalent Vaccine: Gardasil

In the naïve population, the efficacy of Gardasil versus the combined incidence of persistent infection, genital warts, vulvar and vaginal lesions, CIN of any grade, adenoma in situ (AIS) and cervical cancer related to the viral genotypes included in the vaccine was 84.7% (CI 95%: 67.5; 93.7)\textsuperscript{103}.

The efficacy versus HSIL (VAIN) related to the viral vaccines was 85.7% (CI 95%: 37.6; 98.4)\textsuperscript{103}.

In the intention to treat population, that is, the population including women independently of their basal status with respect to HPV infection who received at least one dose of the vaccine at the time of recruitment, the efficacy of the quadrivalent vaccine against the combined incidence of persistent infection, genital warts, vulvar and vaginal lesions, CIN of any grade, AIS and cervical cancers related to HPV 6, 11, 16 or 18 was 47.2% (CI 95%: 33.5; 58.2).

In the population of women between 16 and 26 years of age with evidence of previous resolved infection by any of the HPV genotypes included in the vaccine (seropositive women but polymerase chain reaction negative for the virus), the efficacy of Gardasil to prevent high grade lesion recurrence by the same viral genotype was 100% (CI 95%: 62.8%-100.0%) versus not only cervical but also vulvar or vaginal lesions as well as genital warts.

### 9.2 Bivalent Vaccine: Cervarix

The efficacy of the bivalent vaccine versus VIN and VAIN evaluated together associated with the viral genotypes included in the vaccine (HPV 16 and 18) was 7.1% (CI 95% 36.3%–90.1%) in the total vaccination cohort and was 75.1% (CI 95% 7.9%–95.5%) in the naïve cohort\textsuperscript{105}.

#### 9.3 Nonavalent Vaccine: Gardasil 9

This vaccine contains HPV serotypes 6,11, 16, 18, 31, 33, 45, 52 and 58. The efficacy in the naïve population versus HSIL (VIN or VaIN) reported in the studies was 100% (CI 95%: <0, 100). In the intention to treat population, the efficacy versus HSIL (CIN, VIN or VAIN) related to HPV 31, 33, 45, 52, or 58 was 96.7% (CI 95% 80.9% - 99.8%), and versus HSIL related to HPV 6, 11, 16, or 18 it was 66.6% (CI 95% 53% - 98.7)\textsuperscript{104}.
VAIN is an infrequent preneoplastic entity, although its real prevalence is unknown. The incidence of VAIN has been estimated to be approximately 0.2 cases per 100,000 women/year and represents 0.4% of intraepithelial neoplasias of the lower genital tract. Little is know about its natural history, but due to embryogenesis, etiology, risk factors and coexistence with CIN, it is likely that it presents multiple similarities with the natural history of cervical lesions.

The presentation of VAIN is asymptomatic, and it is mainly diagnosed on performing a colposcopy for an abnormal cytology result. There are no guidelines with a consensus defining the most adequate management of VAIN. When evaluating the best approach or treatment of the lesion, multiple aspects of the lesion, patient, means available and experience must be taken into account.

With the appearance of new therapeutic modalities, extensive excisional treatments and mutilating treatments such as partial vaginectomy are being increasingly replaced by destructive treatments or by combinations of these with topical vaginal applications.

The implementation of vaccination versus HPV will contribute to the prevention of VAIN, and thus, cancer of the vagina.
11. Recommendations

The recommendations present a low quality of evidence due to the nature of the studies published. The following are the most consensuated points related to the findings of lesions suspected of being VAIN.

1. Examination of the vagina should be performed during colposcopy as part of the study of the lower genital tract. Most vaginal lesions are located in the upper third of the vagina.

2. Biopsy of larger sized lesions suspected of being high grade is recommended. Histological study of all the lesions is not necessary.

3. When evaluating the application of a treatment, individualize the case, assessing the following aspects: size, localization, number and appearance of the lesion(s), patient age, reproductive desire, concomitant pathology, immunological status, follow-up capacity, lesions at another level of the lower genital tract and means available and experience.

4. A wait and see approach should be considered for low grade lesions, except for extensive lesions and in women with immunosuppression.

5. In the case of HSIL (VAIN) in young women with localized lesions and without risk factors, wait and see treatment may be considered.

6 In patients with visible localized lesions, both destructive and topical treatment may be considered.

7. Surgical treatment with lesion excision or partial colpectomy is the choice in patients with HSIL (VAIN) and a history of hysterectomy for CIN. These cases have an elevated risk of occult invasion in the colpotomy scar or progression to vaginal cancer.

8. In general, it is recommended to initiate treatment with one therapeutic modality in cases with single or scarce and localized lesions, reserving combined treatments for multiple lesions, not completely visible or of difficult access.
12. References

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