CIRUGÍA ESPAÑOLA

www.elsevier.es/cirugia

Original article

Anal intraepithelial neoplasia: application of a diagnostic protocol in risk patients using anal cytology☆

Estela Membrilla-Fernández,[a] David Parés,[a,] Francisco Alameda,[b] Marta Pascual,[a] Ricard Courtier,[a] María José Gil,[a] Gabriel Vallecillo,[c] Pere Fusté,[d] Miguel Pera,[a] and Luis Grande[a]*

[a]*Servicio de Cirugía General y Digestiva, Unidad de Cirugía Colorrectal, Hospital Universitario del Mar, Barcelona, Spain*
[b]*Servicio de Anatomía Patológica, Hospital Universitario del Mar, Barcelona, Spain*
[c]*Servicio de Medicina Interna, Hospital Universitario del Mar, Barcelona, Spain*
[d]*Servicio de Ginecología y Obstetricia, Hospital Universitario del Mar, Barcelona, Spain*

ARTICLE INFORMATION

Article history:
Received October 16, 2008
Accepted December 5, 2008
Online March 20, 2009

Keywords:
Anal intraepithelial neoplasia
Diagnosis
Cytology
Risk factors

A B S T R A C T

Introduction: Anal intraepithelial neoplasia is a precursor condition of squamous anal carcinoma. The groups at risk of this lesion are patients with anogenital condylomata, cervical dysplasia, human immunodeficiency virus infection and, in general, patients with HPV infection. The aim of this study was to analyse the results of a diagnostics protocol of anal intraepithelial neoplasia in high risk population using anal cytology.

Patients and method: The protocol is based on a visit in the outpatient department, clinical interview, physical examination, and anal cytology evaluated by Bethesda criteria. The cross-sectional observational study was designed to study the anal smear results and their relationship with risk factors.

Results: A total of 64 patients were included from January 2005 to December 2006. In the overall series, 25 patients have been diagnosed with abnormal anal cytology: 9 atypical squamous cells of undetermined significance (ASCUS), 15 low-grade, and 1 high-grade squamous intraepithelial lesions. There were no significant associations between abnormal cytology results and the presence of anal condyloma (P=.22). Neither were there statistical associations found with high risk-HPV infection (P=.84), HIV infection (P=.98), or tobacco use (P=.14).

Conclusions: Our diagnostic protocol of anal intraepithelial neoplasia revealed 25% of patients with pre-invasive lesions of squamous anal cancer.

☆Presented in the 6th Catalan Conference of Surgery. October 2007.

*Author for correspondence.
E-mail address: Dpares@imas.imim.es (D. Parés).

Neoplasia intraepitelial anal: resultados de la aplicación de un protocolo diagnóstico en pacientes de riesgo mediante el uso de citología anal

Palabras clave:
Neoplasia intraepitelial anal
Diagnóstico
Citología
Factores de riesgo

RESUMEN

Introducción: La neoplasia intraepitelial anal es una lesión precursora del carcinoma escamoso anal. Se considera población en riesgo de padecer esta lesión a los pacientes con condilomas anogenitales, historia previa de displasia de cérvix, infección por VIH y en general los pacientes con infección por el VPH. El objetivo de este estudio es analizar los resultados de la aplicación de un protocolo diagnóstico de neoplasia intraepitelial anal en población de riesgo mediante el empleo de citología anal.

Material y método: El protocolo diagnóstico de neoplasia intraepitelial anal consistió en realizar una anamnesis estructurada, exploración física y citología anal, la cual se interpretó mediante los criterios de Bethesda. En este estudio observacional de corte transversal se analizan los resultados de diagnóstico de neoplasia intraepitelial anal y su asociación con factores de riesgo.

Resultados: Se incluyó a 64 pacientes en los que se diagnosticaron 25 alteraciones citológicas: 9 alteraciones citológicas de significado incierto o ASCUS, 15 casos de neoplasia intraepitelial anal de bajo grado y 1 de alto grado. Al relacionar la presencia de alteraciones en la citología anal con los factores de riesgo conocidos, no hubo asociación estadísticamente significativa con la presencia de condilomas (p = 0,22), infección por VPH de alto riesgo (p = 0,84), infección por VIH (p = 0,98) o tabaquismo (p = 0,14).

Conclusiones: La aplicación de un protocolo de detección de neoplasia intraepitelial anal en población de riesgo ha permitido detectar un 25% de pacientes con lesiones precursoras de carcinoma anal.

Introduction

Anal intraepithelial neoplasia (AIN) is a precursor lesion to squamous anal carcinoma, just as cervical dysplasia or CIN is with respect to cervical squamous carcinoma.[1] This lesion has received other names such as anal carcinoma in situ or Bowen's disease, when it only affected the perianal skin. Recently the name AIN has been proposed, which may be divided into grade I, grade II, and grade III, depending on the depth extension of these lesions in the affected epithelium, or in low and high-grade AIN in the anal cytology samples according to the Bethesda classification[2]. Just as with cervical dysplasia, the human papillomavirus (HPV) is mainly responsible for these lesions, especially the high risk types.[3]

Interest in this lesion is relatively recent due to an increased incidence of squamous anal carcinoma detected in recent years.[4] The individuals who have a higher risk of contracting this type of disease when compared to the general population, and therefore, the at-risk population which would benefit most if a diagnosis campaign and early treatment of these lesions were applied, have been studied.[3] In epidemiological studies, it has been observed that the population with greater prevalence of AIN corresponds to patients with human immunodeficiency virus (HIV) infection, patients with antecedents of anal or genital condylomas, patients with a history of cervical intraepithelial neoplasia (CIN) and in general, groups with a greater prevalence of HPV infection, including persons with anal-receptive intercourse.[5-7] Recently, the relationship with certain social habits such as tobacco smoking has also been suggested.[8]

The main objective of this study is to analyze results in the detection of AIN cases by anal cytology after the implementation of a standardized diagnostic protocol of this disease in an at-risk population. Secondarily, the relationship between the detection of alterations in anal cytology and each of the different risk factors in the group of patients to whom the protocol was applied are studied.

Patients and method

Cross-sectional, observational study of a consecutive series of cases for which a standardized diagnostic protocol of AIN was applied (Figure). The protocol was presented and approved by the ethical committee of clinical research from our hospital (code 2289).

Inclusion criteria

All patients considered as at-risk population who presented with one of the following antecedents were included: anal or genital condylomas, cervical dysplasia or CIN, or HIV infection.

Patients came from outpatient services of general surgery, internal medicine or gynecology, and in our hospital, obstetrics.

Structured anamnesis, physical examination, anorectoscopy, and anal cytology were carried out on all

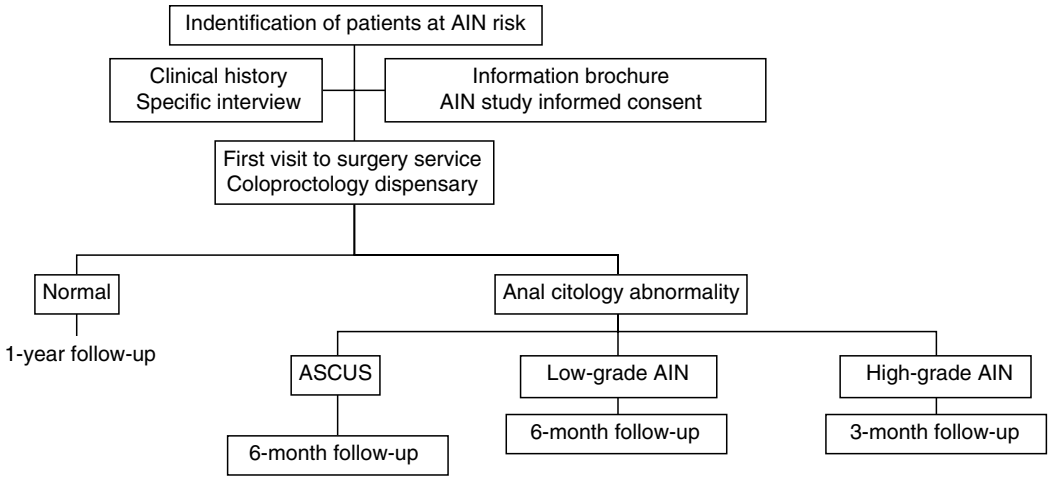

Figure – Diagnostic protocol of anal intraepithelial neoplasia in a selected population.

patients. This protocol was agreed upon by specialists from the involved clinical departments and was based on existing literature.

Anal cytology

Anal cytology was carried out from material obtained by brush biopsy in outpatient service. The brush biopsy used is the same as that used for the cervical cytology. The brush is inserted into the anal canal, scraping from the perianal skin up to 4 cm above the anal margin. The material for cytology is obtained after submerging the brush in a liquid (ThinPrep Pap Test. PreservCyt®, Marlborough, United States). Interpretation of results was carried out by a single anatomopathologist specialized in diagnosis of these lesions, using the Bethesda criteria.[2,10]

In all cytology samples the presence of high-risk HPV infection was also analyzed by the second-generation hybrid capture techniques (Digene Hybrid Capture®, Gaithersburg, United States).[11] This technique allows for detection of the presence of any of the 13 high-risk viral types in a single reaction (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).[12]

Risk factors

As in other published studies, it was evaluated whether the following factors were considered AIN risk factors. Each one was related to the detection of alterations in anal cytology in samples from patients: presence of anal condylomas, detection of high-risk HPV infection, HIV infection and finally, active tobacco consumption, considering 20 cigarettes a week as the minimum consumption.[13]

Statistical analysis

Quantitative variables are presented with absolute numbers, the average, and range between parentheses. Categorical variables are presented with absolute numbers or percentages. Qualitative variables were compared with the χ^2 test (or Fisher's exact test when it was precise). For statistical analysis, the SPSS program version 10.0 (SPSS, Chicago, USA) was used. A *P* less than .05 was considered statistically significant.

Table 1 – Results obtained in anal cytology in 64 patients included in the detection protocol for anal intraepithelial neoplasia in a selected population according to the Bethesda classification[2]

	No. (%)
Normal	39 (60.9)
ASCUS	9 (14)
Low-grade intraepithelial neoplasia	15 (23.4)
High-grade intraepithelial neoplasia	1 (1.5)

ASCUS indicates atypical cytological results of undetermined significance.

Results

Patients included

From January 2005 to December 2006, in this AIN diagnostic protocol, 64 patients (76.5% men) were included: forty-four presented with anal condylomas, 14 cases of HIV infection, and 6 patients with CIN antecedents. Average age was 33 (interval, 22–82) years, and in the overall study, 35 (48%) individuals were active smokers.

Results from anal cytology

As observed in Table 1, alterations in anal cytology were detected in 25 (39%) cases: of them, 9 presented with cytological alterations of uncertain significance or ASCUS, and 16 (25%) had lesions compatible with AIN. High-risk HPV infection was detected in 31 (48.4%).

Table 2 – Results from the study of the relationship between the presence of risk factors and anal intraepithelial neoplasia findings in anal cytology in patients from the study

	Patients with abnormalities in anal cytology (n=25), No. (%)	Patients with a normal study (n=39), No. (%)	P[a]
Anal condylomas	17 (68)	27 (69.2)	.22
High-risk HPV	13 (52)	18 (46.1)	.84
HIV infection	5 (20)	9 (23)	.98
Active smoker	17 (68)	18 (46.1)	.14

[a] χ^2 test.

Study of risk factors

As observed in Table 2, there was no statistically significant association between the presence of alterations in the anal cytology results and the presence of anal condylomas, high-risk HPV infection, being an HIV carrier or being an active smoker.

Discussion

Incidence of squamous anal carcinoma has increased in the last 30 years, especially in HIV infected patients, and these figures are comparable to the prevalence of cervical carcinoma when population screening methods had not yet been introduced.[8] Even more concerning is the fact that anal carcinoma is normally diagnosed late with significant local or distant extension.[4] Because of this, the implementation of early diagnosis campaigns of squamous anal carcinoma or of its precursor lesions in the population with risk factors has been of interest in recent years.[9] In this study, 25% of AIN cases were detected after applying a standardized detection protocol in the selected population through the use of anal cytology. This percentage of at-risk patients is similar to the only European study published on this until now, which establishes the need for and normalization of screening methods of specific population groups.[13]

High-grade AIN is considered to be premalignant, similar to precursor lesions of cervical carcinoma, whose incidence and prevalence seems to have increased in recent years.[7,14] From a histopathological perspective, AIN is defined as the presence of a progressive change of normal epithelium of the anal canal with immature cells which have characteristics of basal cells.[15] Through anal cytology, lesions of the uterine cervix may be defined in a similar manner, and therefore, the Bethesda classification may be used, which would differentiate between low and high-grade lesions.[2,3] Even though diagnostic results by multiple anal biopsies seem to be more exact because of the quantity of material obtained and because it allows for the exact location of the lesions to be known, the need to subject the patient to a more invasive examination under anesthesia makes cytology obtained by swab an attractive option.[13] This is a minimally invasive method which may be undertaken in an outpatient office in the patient's first visit without previous preparation or anesthesia, and we should understand that the overall goal is early diagnosis of premalignant lesions in order to carry out a suitable follow-up of the patient.[16] This reasoning indicates that precise anatomical determination of the lesion's location in the anal canal is not as necessary as its diagnosis. Through this technique, the presence of infection from the high-risk types of HPV may also be determined. The presence of this infection is used in CIN patients to evaluate the presence of a high risk of invasive carcinoma, a determining factor in monitoring regimen. This data might also be useful in monitoring after AIN diagnosis.[10,13]

The first studies published determined that anal cytology detected around 35% of AIN lesions compared to the biopsies.[4] This low sensitivity was mainly due to the difficulty of obtaining a good sample in consultation because of fecal contamination. Today cytological techniques have improved significantly, and sensitivity for detecting AIN is up to 80% in patients with HIV infection and surpasses 50% in non-infected patients.[7,17]

Although it was not the objective of this study, during the monitoring of these patients, no case of invasive anal carcinoma in AIN diagnosed cases was detected or in the rest of the patients subjected to this protocol. Nonetheless, it is worth mentioning that our strategy, once an AIN case was diagnosed and in agreement with other groups, consisted of carrying out a follow-up schedule with another visit in outpatient offices with an anorectoscopy and another anal cytology. This visit took place at 6 months for the low-grade AIN cases, or after 3 months for patients with high-grade AIN or with antecedents of immunosuppression or HIV infection (Figure).[7] In cases where the patient presented with any suspicious symptom of invasive anal carcinoma during the follow-up—for example changes in the physical examination or rectal bleeding—or progression of the grade of dysplasia was detected in the anal brush biopsy cytologies, surgical intervention should be recommended to carry out surgical examination with serial anal biopsies or when there are lesions suspicious of invasive anal carcinoma. In our study, this was not necessary in any of the cases of the group of patients included.

The relationship between squamous anal carcinoma, AIN, and HPV has been known for years.[18] Of the more than 200 types of HPV, the oncogenic types or high-risk types cause high-grade dysplastic lesions and squamous cell carcinoma of the uterus and anus.[8] The use of high-risk probes for detecting the DNA of these types of HPV has had an influence in the high degree of detection of the virus in samples obtained in recent years. Hybrid capture seems to have a certain benefit with respect to the use of the PCR technique, because it decreases interferences due to the high degree of contamination, and because it is a technique with greater simplicity for laboratory use.[12] Our percentage of high-risk HPV identification in samples was 52% in patients with cytology alterations, versus 46.1% in the group of patients without lesions; these figures are somewhat lower than others published.[13]

The incidence published of AIN lesions is higher in the group of patients with condylomas, those with HIV infections and especially in those with anoreceptive intercourse.[14,19] In spite of this and probably due to the fact that the sample is still insufficient, the relationship between alterations in anal cytology and the presence of condylomas or HIV infection could not be demonstrated in our results. A few meta-analyses showed a relationship with HIV infection and cervical carcinoma, and the same seems to occur with AIN and squamous anal carcinoma.[20] In addition, it is also known that AIN is closely related to the presence of different grades of immunosuppression.[14,21] These observations have allowed for the hypothesis to be postulated that immunodeficiency would favor HPV replication, and this would act as a promoter factor for carcinogenesis synergistic with other factors, the majority still unknown[7]. Consequently, it is considered that HIV could favor the replication of the types associated with a greater risk of dysplasia progression or synergistically increase its detrimental action.[20] Regarding this, we think it would be interesting to study the relationship between the grades of AIN and the grade of infection in individuals with HIV infection (viral load and level of CD4 lymphocytes).

The relationship between tobacco consumption and AIN detected by anal cytology has only been discussed by one study in the literature. As done in our study with a selected population, Etienney et al[13] also detected an increase of AIN lesions in patients who were active smokers after the application of an anal cytology protocol. The relationship of tobacco with squamous anal carcinoma was discussed in the literature without knowing tobacco's effect or its connection to other associated risk factors such as risky sexual behaviors or HPV infection.[8] Nevertheless, it has been indicated that certain chemical substances in tobacco could cause toxic changes in the epithelial DNA of the anal canal, and this could in part explain these findings.[22]

One aspect worth highlighting is that to evaluate alterations in the transitioning anal epithelium, much experience is needed, because there may be a significant number of false positives. Indeed, it is necessary that the pathologists involved in diagnosis and treatment programs of these lesions be particularly dedicated to the interpretation of these lesions and show special attention to the high variation among observers in the determination of different grades of dysplasia.[23] In our group, a single pathologist specialized in cervical squamous and anal lesions interpreted all samples.

To conclude, the application of an AIN diagnostic protocol in the at-risk population has allowed for an increased diagnosis of these lesions. Nonetheless, this is a preliminary study, and more prospective studies are necessary, and if possible, multi-centre studies to determine the clinical benefit of detecting these lesions.

REFERENCES

1. Scholefield JH, Castle MT, Watson NF. Malignant transformation of high-grade anal intraepithelial neoplasia. Br J Surg. 2005;92:1133–6.
2. Smith JH. Bethesda 2001. Cytopathology. 2002;13:4–10.
3. Zbar AP, Fenger C, Efron J, Beer-Gabel M, Wexner SD. The pathology and molecular biology of anal intraepithelial neoplasia: comparisons with cervical and vulvar intraepithelial carcinoma. Int J Colorectal Dis. 2002;17: 203–15.
4. Abbasakoor F, Boulos PB. Anal intraepithelial neoplasia. Br J Surg. 2005;92:277–90.
5. Marchesa P, Fazio VW, Oliart S, Goldblum JR, Lavery IC. Perianal Bowen's disease: a clinicopathologic study of 47 patients. Dis Colon Rectum. 1997;40:1286–93.
6. Devaraj B, Cosman BC. Expectant management of anal squamous dysplasia in patients with HIV. Dis Colon Rectum. 2006;49:36–40.
7. Parés D, Mullerat J, Pera M. Anal intraepithelial neoplasia. Med Clin (Barc). 2006;127:749–55.
8. Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. Cancer. 2004;101:270–80.
9. Anderson JS, Vajdic C, Grulich AE. Is screening for anal cancer warranted in homosexual men? Sex Health. 2004;1:137–40.
10. Fox PA, Seet JE, Stebbing J, Francis N, Barton SE, Strauss S, et al. The value of anal cytology and human papillomavirus typing in the detection of anal intraepithelial neoplasia: a review of cases from an anoscopy clinic. Sex Transm Infect. 2005;81:142–6.
11. Carvalho MO, Almeida RW, Leite FM, Fellows IB, Teixeira MH, Oliveira LH, et al. Detection of human papillomavirus DNA by the hybrid capture assay. Braz J Infect Dis. 2003;7:121–5.
12. Sirera G, Videla S, Castella E, Cavalle L, Grane N, Llatjos M, et al. Contribution of human papillomavirus second-generation hybrid capture test for the diagnosis of cervical pathology in HIV-infected outpatients. Med Clin (Barc). 2005;125:127–31.
13. Etienney I, Vuong S, Daniel F, Mory B, Taouk M, Sultan S, et al. Prevalence of anal cytologic abnormalities in a French referral population: a prospective study with special emphasis on HIV, HPV, and smoking. Dis Colon Rectum. 2008;51:67–72.
14. Pera M, Sugranes G, Ordi J, Trias M. Association between human papillomavirus infection, premalignant lesions of anal cancer, and the human immunodeficiency virus: prospective study on subjects with condylomata acuminata. Med Clin (Barc). 1999;113:13–4.
15. Scholefield JH, Sonnex C, Talbot IC, Palmer JG, Whatrup C, Mindel A, et al. Anal and cervical intraepithelial neoplasia: possible parallel. Lancet. 1989;2:765–9.

16. Friedlander MA, Stier E, Lin O. Anorectal cytology as a screening tool for anal squamous lesions: cytologic, anoscopic, and histologic correlation. Cancer. 2004;102:19–26.
17. Vajdic CM, Anderson JS, Hillman RJ, Medley G, Grulich AE. Blind sampling is superior to anoscope guided sampling for screening for anal intraepithelial neoplasia. Sex Transm Infect. 2005;81:415–8.
18. Palefsky J. Editorial comment: screening and treatment of AIN to prevent anal cancer —where do we stand? AIDS Read. 2005;15:87.
19. Palefsky JM, Holly EA, Efirdc JT, Da Costa M, Jay N, Berry JM, et al. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. AIDS. 2005;19:1407–14.
20. Mandelblatt JS, Kanetsky P, Eggert L, Gold K. Is HIV infection a cofactor for cervical squamous cell neoplasia? Cancer Epidemiol Biomarkers Prev. 1999; 8:97–106.
21. Mullerat J, Deroide F, Winslet MC, Perrett CW. Proliferation and p53 expression in anal cancer precursor lesions. Anticancer Res. 2003;23:2995–9.
22. Phillips DH, Hewer A, Scholefield JH, Skinner P. Smoking-related DNA adducts in anal epithelium. Mutat Res. 2004;560:167–72.
23. Lytwyn A, Salit IE, Raboud J, Chapman W, Darragh T, Winkler B, et al. Interobserver agreement in the interpretation of anal intraepithelial neoplasia. Cancer. 2005;103: 1447–56.