Interobserver variability in the diagnosis of anal cancer precursor lesions: study of the usual scenario

Variabilidade interobservadores no diagnóstico de lesões precursoras do câncer anal: estudo do cenário habitual

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A B S T R A C T

Objective: To assess interobserver variability in the diagnosis of anal cancer precursor lesions in the usual scenario of a service consisting of pathologists without previous experience in the diagnosis of these lesions. **Methods**: Five hundred and two anal specimens taken from 372 HIV-positive and HIV-negative patients were analyzed at the Pathology Department of the Tropical Medicine Foundation of Amazonas by three pathologists with extensive experience in the diagnosis of infectious and tropical diseases, but without significant prior experience in the diagnosis of anal cancer precursor lesions. The individual readings of each pathologist were compared to the one following the consensus diagnosis in shared optical microscope by kappa statistics. **Results**: The absolute agreement between each individual diagnosis and corresponding consensus was poor (kappa = -0.002). Considering only the positive or negative results for anal squamous intraepithelial lesions, we obtained a fair agreement between observers (kappa = 0.35), while the agreement was moderate when the histopathological findings were considered positive or negative for high-grade squamous intraepithelial lesion or cancer (kappa = 0.52). **Conclusion**: The interobserver variability in histopathologic diagnosis of anal cancer and its precursor lesions among pathologists with little experience in the area is such that the diagnoses in this field and this scenario should always be a consensus.

Key words: Anal canal. Anus neoplasms. Observer variation. Pathology.

INTRODUCTION

Anal cancer is still considered a rare disease in the general population, despite reports that its incidence is increasing in recent years in certain groups recognized as in risk for its development[1-4]. In the general population, the incidence of this cancer varies from 0.8 to two cases per 100,000, but in risk groups the incidence reaches figures 70 to 120 times higher[5-7].

Because of its recognized association with ongoing infection caused by human papillomavirus in people who have some degree of immunologic compromise[8], it is considered that the anal cancer presents a behavior similar to cervical cancer, a much more studied condition[9,10].

As in cervical cancer, anal cancer is preceded by precursor lesions, the anal intraepithelial neoplasias (AIN), which are classified into three ascending categories according to their known potential for malignant transformation: AIN I, AIN-II and AIN-III[11]. Due to the considerable degree of inter-and intraobserver disagreement in diagnostic analysis of cervical intraepithelial lesions (CIN)[12], the current trend is to condense the original threefold classification (CIN I, CIN II, CIN-III) in the binary one proposed by Consensus Bethesda, 2001[13]. Thus, similarly, the AIN-I has been called low grade squamous intraepithelial lesions (LSIL), while AIN-II and AIN III form a single category called high-grade squamous intraepithelial lesion (HSIL)[3,14-17].

Despite this effort there are still some diagnostic difficulties in distinguishing between LSIL and HSIL, and especially in the anal canal some interpretive difficulties may occur in differentiating inflammatory reaction of the anal transition zone[18] and those that should be considered anal squamous intraepithelial lesions (ASIL), either low or high-grade[3].

Study conducted at the Tropical Medicine Foundation of Amazonas.
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Studies addressing this issue are usually conducted by pathologists with recognized degrees of experience in the diagnosis of anal cancer and its precursor lesions and do not reflect what is most likely observed in the daily practice of pathology services in general. Because of the low incidence and prevalence of anal cancer in all its phases in the general population, the disease is rarely observed in clinical practice in these services[15,17,19].

The Foundation for Tropical Medicine of Amazonas (FMT-AM) is an institution of the state of Amazonas, Brazil, specialized in the diagnosis and treatment of infectious and tropical diseases. It concentrates the treatment of most cases of AIDS in the Amazon and promotes the following of a significant portion of HIV-positive patients. By 2006, the FMT-AM had no policy of routine screening of patients enrolled in the institution at risk of developing anal cancer and its precursor lesions. With the inauguration of the Clinic of Coloproctology of the institution in January 2007, there was the sudden advent of a new demand on its Department of Pathology: the processing and interpretation of histopathology specimens of anal biopsies performed in patients at risk of developing anal cancer. Pathologists with extensive experience in the histopathological manifestations of tropical diseases and infectious diseases were then faced with a new challenge: to accurately identify lesions that, until then, were rarely observed in the institution.

Since the action to be taken against a lesion associated with anal cancer is directly dependent on its precise histological diagnosis, it is of utmost importance that decisions made are based on solid evidence as for the presence or absence of ASIL or cancer[17].

This study was designed to assess the interobserver variability in the early diagnosis of anal cancer in a pathology service specialized in the diagnosis of infectious diseases, but no specific prior experience in the diagnosis of anal cancer and its precursor lesions. It was designed to reproduce what is probably the most commonly observed in most pathology services worldwide.

METHODS

This is an observational study of diagnostic agreement between pathologists, the histopathology of anal biopsies performed in HIV-positive and HIV-negative patients, with or without conditions or risk factors for the development of anal cancer. There was blinding of pathologists in relation to patients' clinical and epidemiological data, as well as the macroscopic characteristics of anal specimens.

The project was approved by the Ethics Committee of the FMT-AM (CEP / FMT-AM 1768 / 2006) and is part of a project to evaluate the diagnostic methods most commonly used to detect anal cancer and its precursor lesions in patients followed at the Clinic of Coloproctology of the institution between January 2007 and December 2008.

All fragments sent to biopsy were removed from the anal transition zone (ATZ) by the proctologist through the use of anoscopy with magnification image, according to the protocol previously described[20]. The procedures were preceded by clarification of the patient and signing an informed consent.

The specimens were fixed in a solution of 10% buffered formalin and sent to the laboratories of the Department of Pathology at FMT-AM for processing and analysis. Four-micrometer cuts of the specimens included in paraffin blocks were prepared and stained with hematoxylin-eosin. Each slide produced was identified by a random number that gave no hint about the origin of the specimen. The possible diagnoses were: INS (insufficient for analysis), ASE (absence of squamous epithelium), NEG (negative for cancer or ASIL), BCA/I (benign cellular alterations / inflammation), ACU (condyloma acuminata), LSIL, HSIL, CECIS (squamous cell carcinoma in situ), SCCin (invasive SCC), ADCis (adenocarcinoma in situ) ADCin (invasive ADC). The diagnostic criteria for the definition of SCA, LSIL, HSIL and SCC have been described[21].

Three senior pathologists, with 35, 28 and 13 years of experience in general pathology, participated in the first study, individually examining the slides, which had random numbers, without clinical and epidemiological data of patients or macroscopic descriptions of where the tissue specimens were taken. During a period of nine months after the accomplishment of the anal biopsies, several meetings were held for the consensus reading of the previously diagnosed slides. At those meetings, the slides were jointly re-examined by three pathologists sharing an optical microscope.

All histopathological findings were compiled by one of the researchers, who did not participate in reading sessions of the slides.

Statistical analysis of categorical variables represented by the microscopic diagnoses of individual readings and the consensus reading was performed by studying the frequency with which diagnoses issued agreed with the consensus reading, taking into account the results of the 95 % confidence intervals and the chi-square or "G" for contingency tables. The concordance between the diagnoses of individual pathologists and a consensus was also studied by calculating the kappa coefficient. The BioEstat 5.0 was used for the calculation of frequencies, confidence intervals and the kappa coefficient[22]. The degree of interobserver agreement was assessed using the criteria proposed by Landis and Koch[23] (Table 1). The significance p values were considered <0.05.

RESULTS

Of a total of 372 patients studied, 1643 anal biopsy histopathologic interpretations were performed. Of these, 502 were randomly selected for reading consensus.

Table 2 shows the relationship between all individual diagnoses initially issued with the consensus. The gray-shaded cells indicate complete agreement between the individual initial diagnosis and consensus diagnosis. Nine of the 11 possible diagnoses have been effectively observed.

Table 1 - Interpretation of kappa according to Landis & Koch.[23]

Degree of concordance (kappa)	Interpretation
< 0	bad
0.01 - 0.20	poor
0.21 - 0.40	fair
0.41 - 0.60	moderate
0.61 - 0.80	strong
0.81 - 1.00	almost perfect

Table 2 - Of individual Diagnoses of pathologists versus consensus diagnoses.

Individual	Consensus									
P1	INS	ASE	NEG	BCAI	ACU	LSIL	HSIL	SCCis	ADCin	TOTAL
INS										0
ASE							1			1
NEG			2							2
BCAI										0
ACU										0
LSIL						1				1
HSIL					2		1			3
SCCis										0
ADCin										0
Total P1	0	0	2	0	2	1	2	0	0	7
P2	INS	ASE	NEG	BCAI	ACU	LSIL	HSIL	SCCis	ADCin	TOTAL
INS	3		2	3	1	3				12
ASE	1	2	11	30		5	3			52
NEG	3		37	21		89	7			157
BCAI			6	25		34	6			71
ACU					5	15	1			21
LSIL				3	2	45	4			54
HSIL				1		14	25			40
SCCis										0
ADCin							1		1	2
Total P2	7	2	56	83	8	205	47	0	1	409
P3	INS	ASE	NEG	BCAI	ACU	LSIL	HSIL	SCCis	ADCin	TOTAL
INS	2									2
ASE		1		9		3				13
NEG			2	17		9	1			29
BCAI				15		5	1			21
ACU				1		1	1			3
LSIL				3	1	4	2			10
HSIL						3	2	2		7
SCCis										0
ADCin	1									1
Total P3	3	1	2	45	1	25	7	2	0	86
Total Global	**10**	**3**	**60**	**128**	**11**	**231**	**56**	**2**	**1**	**502**

Individual = Diagnoses of individual pathologist (P1, P2, P3) = INS unsatisfactory for analysis; ASE = absence of squamous epithelium, NEG = negative for squamous intraepithelial lesions or cancer, BCAI = benign cellular changes / inflammation; ACU = *condyloma acuminatum*; LSIL = low-grade squamous intraepithelial lesion, HSIL = high-grade squamous intraepithelial lesion; SCCis = squamous cell carcinoma in situ; ADCin = invasive adenocarcinoma.

The distribution of individual frequencies of concordant (sum of the values existing in the gray cells of table 2) or discordant (sum of the values existing in the non-shaded cells of table 2) diagnoses in relation to the reading of consensus is shown in table 3. One can note that absolute agreement between each individual diagnosis and corresponding consensus was seen in only 34.5% of histopathological readings. It was also observed that, although the numerical quantities of each pathologist readings have been very different, there was no statistical difference between them when we analyzed the proportion of positive and negative concordant results. However, findings of pathologist 1 are be interpreted with caution due to the small number of slides that he individually appraised, which was reflected in wide confidence intervals of 95%. The Kappa analysis of agreement between the different initial diagnoses and the corresponding consensus returned a poor index.

For tables 4, 5 and 6, the INS results, ASE and BCA/I were included among the NEG results. The INS and ASE diagnostic categories were considered NEG because, in doing so, 80% of the observed diagnostic combinations were covered. In table 5, ACU results were included among the LSIL.

Table 4 contrasts the readings with the initial histopathological consensus, considering the results either positive or negative for ASIL or cancer. In this approach, the initial readings of the three pathologists showed greater concordance with the results of the consensus reading. With the exception of pathologist 1, who, despite strong agreement with the consensus, examined very few individual slides, the best observed performance was over of pathologist 3, who tended to agree more with the consensus reading, although the kappa obtained has indicated only fair agreement. The highest percentage of diagnostic concordance for positive results for ASIL or cancer of pathologist 2 (compared with the pathologist 3) reflected

Table 3 - Absolute concordance between the diagnoses of individual pathologists and consensus diagnoses.

	Consensus						
Pathologist	**Yes**	**%**	**95% CI**	**No**	**%**	**95% CI**	**Total**
P1	**4**	57.14	18.41 - 90.10	**3**	42.86	9.90 - 81.59	**7**
P2	**143**	34.96	30.34 - 39.80	**266**	65.04	60.20 - 69.66	**409**
P3	**26**	30.23	20.79 - 41.08	**60**	69.77	58.92 - 79.21	**86**
Total	**173**	34.46	30.31 - 38.80	**329**	65.54	61.20 - 69.69	**502**

95% CI = 95% confidence interval; G Test (Williams) = 2.14, p = 0.34, kappa = -0.002 (95% CI = -0.10 to 0.10).

Table 4 - Concordance between each individual pathologist diagnoses and consensus considering the positive or negative histopathological results for cancer or ASIL.

Pathologist			**Consensus**						
P1	POS	%	95% CI	NEG	%	95% CI	TOTAL	*Kappa*	p-value
POS	4	100.00	47.29 – 100.00	0	0.00	0.00 – 52.71	4		
NEG	1	33.33	0.84 – 90.57	2	66.67	9.43 – 99.16	3		
-	-	-	-	-	-	Total P1 =	7	0.70	0.03
P2	-	-	-	-	-	-	-		
POS	113	96.58	91.48 – 99.06	4	3.42	0.94 – 8.52	117		
NEG	148	50.68	44.80 – 56.56	144	49.32	43.44 – 55.20	292		
-	-	-	-	-	-	Total P2 =	409	0.34	<0.01
P3	-	-	-	-	-	-	-		
POS	16	76.19	52.83 – 91.78	5	23.81	8.22 – 47.17	21		
NEG	19	29.23	18.60 – 41.83	46	70.77	58.17 – 81.40	65		
-	-	-	-	-	-	Total P3 =	86	0.38	<0.01
Pathologists	-	-	-	-	-	-	-		
POS	133	93.66	88.31 – 97.06	9	6.34	2.94 – 11.69	142		
NEG	168	46.67	41.42 – 51.97	192	53.33	48.03 – 58.58	360		
-	-	-	-	-	-	Total Global =	502	0.35	<0.01

ASIL = anal squamous intraepithelial lesions; Consensus = consensus diagnoses; P1, P2 and P3 = Pathologists 1, 2 e 3; POS = positive diagnosis for ASIL or cancer; NEG = negative diagnosis for ASIL or cancer ; 95% CI = 95% confidence interval. Kappa = *unweighted* kappa *index.*

in a lower kappa index, since pathologist 2 tended to assign diagnoses inferior than those achieved by the consensus reading.

Table 5 shows the histopathological findings in three categories: LSIL (including ACU), HSIL or higher (including cancer) and NEG (negative for intraepithelial lesion or cancer). The analysis of interobserver agreement in this situation should be performed using the linear weighted kappa index, which assigns different weights to each outcome in order to measure the degree of disagreement between two observations[24]. The index considers that the difference between the ratings of two observers who interpret a specific slide NEG (pathologist 1) and HSIL (pathologist 2) is considerably larger than the difference that would exist should pathologist 1 interpreted the slide as LSIL and pathologist 2 as HSIL. The linear weighted kappa of these two pathologists with greater individual production of diagnostic interpretations was only fair due to the higher trend presented by pathologist 2 to consider NEG results that have been interpreted as LSIL in the consensus readings. Results were also affected by the misinterpretation related to the LSIL results from pathologist 3 compared with the consensus readings.

Table 6 shows the results of the individual diagnoses of each pathologist compared with the consensus, considering only the presence or absence of signs of severe dysplasia or cancer (□HSIL). In this type of analysis, replication of cobined diagnoses of the three pathologists was moderate, despite the great difference observed between pathologist 1 and the others. Pathologists 2 and 3 showed greater concordance with the consensus readings. There was no statistical difference between the individual readings and the consensus results as for severe dysplasia or worse conditions, according to the analysis of the confidence intervals of 95%.

DISCUSSION

This study was conducted in an institution in which the pathologists did not have considerable previous experience in the diagnosis of anal cancer or anal squamous intraepithelial lesions, although they are experts in other areas of general pathology. The study certainly reproduced what is more commonly observed in most centers that do not routinely deal with anal cancer screening in populations at risk.

Besides the absence of an expert in detecting histopathological anal cancer precursor lesions, this study also reproduces what is probably seen in many

Tabela 5 - Comparison between individual diagnoses of each pathologist with consensus diagnoses, considering two diagnostic classes of displasia/anal cancer.

Ind.	Consenso											
P1	>HSIL	%	95% CI	LSIL	%	95% CI	NEG	%	IC95%	Total	*K*	*p*
>HSIL	1	33.33	0.84 – 90.57	2	66.67	9.43 – 99.16	0	0	0.00 – 63.16	3	-	-
LSIL	0	0	0.00 – 95.00	1	100	50.00 – 100.00	0	0	0.00 – 95.00	1	-	-
NEG	1	33.33	0.84 – 90.57	0	0	0.00 – 63.16	2	66.67	9.43 – 99.16	3	-	-
-	-	-	-	-	-	-	-	-	Total P1 =	70.53		0.3
P2	-	-	-	-	-	-	-	-	-	-	-	-
>HSIL	27	64.29	48.03 – 78.45	14	33.33	19.57 – 49.55	1	2.38	0.06 – 12.57	42	-	-
LSIL	5	6.67	2.20 – 14.88	67	89.33	80.06 – 95.28	3	4	0.83 – 11.25	75	-	-
NEG	16	5.48	3.16 – 8.75	132	45.21	39.40 – 51.11	144	49.32	43.44 – 55.20	292	-	-
-	-	-	-	-	-	-	-	-	Total P2 =	4090.39		<0.01
P3	-	-	-	-	-	-	-	-	-	-	-	-
>HSIL	4	50	15.70 – 84.30	3	37.5	8.52 – 75.51	1	12.5	0.32 – 52.65	8	-	-
LSIL	3	23.08	5.04 – 53.81	6	46.15	19.22 – 74.87	4	30.77	9.09 – 61.43	13	-	-
NEG	2	3.08	0.37 – 10.68	17	26.15	16.03 – 38.54	46	70.77	58.17 – 81.40	65	-	-
-	-	-	-	-	-	-	-	-	Total P3 =	860.39		<0.01
Pathologists	-	-	-	-	-	-	-	-	-	-		
>HSIL	32	60.38	46.00 – 73.55	19	35.85	23.14 – 50.20	2	3.77	0.46 – 12.98	53	-	-
LSIL	8	8.99	3.96 – 16.95	74	83.15	73.73 – 90.25	7	7.87	3.22 – 15.54	89	-	-
NEG	19	5.28	3.21 – 8.12	149	41.39	36.25 – 46.67	192	53.33	48.03 – 58.58	360	-	-
-	-	-	-	-	-	-	-	-	Total =	502	0.4	<0.01

Ind. = individual results of the pathologists 1 (P1), 2 (P2) and 3 (P3); Consensus = results of consensus readings ; Patol. = consolidated results of P1, P2 and P3; LSIL = low-grade squamous intraepithelial lesion or condiloma acuminatum*; HSIL = high-grade squamous intraepithelial lesion or cancer; NEG = negative for squamous intraepithelial lesion or cancer, including absence of squamous epithelium and unsatisfactory results; 95% IC= 95% confidence interval;* K *= linear weighted* kappa *index;* p *=* p*-value of the linear weighted* kappa *index.*

Table 6 - Association between individual diagnoses of each pathologist and consensus diagnoses considering the results with or without the presence of severe dysplasia / cancer.

Individual	Consensus								
P1	<HSIL	%	95% CI	>HSIL	%	95% CI	TOTAL	*Kappa*	*p*
<HSIL	3	75.00	19.41 – 99.37	1	25.00	0.63 – 80.59	4		
>HSIL	2	66.67	9.43 – 99.16	1	33.33	0.84 – 90.57	3		
-	-	-	-	-	-	Total P1 =	7	0.09	0.40
P2	-	-	-	-	-	-	-		
<HSIL	346	94.28	91.39 – 96.42	21	5.72	3.58 – 8.61	367		
>HSIL	15	35.71	21.55 – 51.97	27	64.29	48.03 – 78.45	42		
-	-	-	-	-	-	Total P2 =	409	0.55	<0.01
P3	-	-	-	-	-	-	-		
<HSIL	73	93.59	85.67 – 97.89	5	6.41	2.11 – 14.13	78		
>HSIL	4	50.00	15.70 – 84.30	4	50.00	15.70 – 84.30	8		
-	-	-	-	-	-	Total P3 =	86	0.41	<0.01
Pathologists	-	-	-	-	-	-	-		
<HSIL	422	93.99	91.37 – 96.00	27	6.01	4.00 – 8.63	449		
>HSIL	21	39.62	26.45 – 54.00	32	60.38	46.00 – 73.55	53		
-	-	-	-	-	-	Total =	502	0.52	<0.01

Individual = diagnoses of individual pathologist (P1, P2, P3); < HSIL= lesions of lower grade than high grade squamous intraepithelial lesions; >HSIL = lesions of grade equal to, or worse than, HSIL; Pathologists = consolidated results for all pathologists; 95% CI = 95% confidence interval; Kappa = unweighted kappa; p = p-value of the unweighted kappa.

services, in which pathologists have different workloads according to their areas of interest and excellence or because of administrative requirements. This research was then designed to analyze, without any undue interference, the routine diagnostic production of pathologists during their work shifts. There was no pressure exerted on the diagnostic observers before consensus reading sessions.

Anal cancer is currently considered a disease amenable to cure and, moreover, a preventable malignancy. However, in order to be controlled, this disease must be accurately diagnosed at an early stage, if possible before malignant transformation[2,25,26].

The gold standard test for anal cancer and its precursor lesions is the conventional histopathological study[27], the same for cervical cancer, a much more studied condition. For cervical cancer, interobserver variability between experienced pathologists ranges from moderate to almost perfect[12,13,24]. For anal cancer, on the other hand, there are several studies in the literature pointing out the imperfections of the diagnostic histopathological analysis of anal specimens, even among pathologists with reputable experience in the field[15,17,19,28].

Carter *et al.*[28] conducted a study of diagnostic agreement in 100 archived histological slides from biopsies of the anal canal. The slides were examined by five pathologists, three of them with experience in the interpretation of anal dysplasia and two with extensive experience in the diagnosis of cervical cancer precursor lesions (CIN-I, CIN II and CIN-III). The diagnostic categorization was based on a similar classification of AIN proposed by Fenger and Nielsen[11]. The authors observed that the pathologists in the study tended to agree on the diagnosis of normal anal epithelium and in cases of invasive cancer, but for intermediate lesions the concordance was only moderate. The authors said they need to re-examine the issue using the binary classification of AIN[28].

Colquhoun *et al.*[15] published a study of 190 surgical specimens with all evolutionary degrees of anal dysplasia, from normal to invasive anal cancer, according to the classification of Fenger and Nielsen[11]. The slides were reviewed by three pathologists with experience in anal pathology. Only moderate interobserver agreement was achieved in accordance with the evaluation criteria of the Kappa index used in this study. Nevertheless, when the interpretations of the three pathologists were compared with a prior consensus reading performed by nine other pathologists, the kappa index ranged from 0.38 to 0.60. The authors concluded that for higher levels of interobserver agreement, it would probably be better to base the assessment of anal specimens in the binary definition of AIN (high-grade dysplasia and low grade). They also suggested that the use of molecular markers could facilitate the identification of dysplastic lesions[15].

Lytwyn *et al.*[17] published an analysis of diagnostic agreement among four experienced pathologists in the interpretation of cervical and anal cytopathology and histopathology specimens. The pathologists evaluated the slides of 155 specimens taken from the anuses of 93 HIV-positive patients with receptive

anal sexual habits. Anal dysplasia and cancer were analyzed in two levels (LSIL or □HSIL). The kappa index of agreement between diagnoses of the four pathologists was 0.59 (moderate agreement according to the criteria employed by the authors to interpret kappa). When they analyzed the average correlation between each of the two pairs of pathologists, the kappa was 0.66 (strong), while the kappa index of agreement with the consensus reading was 0.75 (strong). The authors concluded by recognizing that, even among experienced pathologists, interobserver agreement was moderate, and that it would be desirable that new gold standards for diagnosis of anal cancer and its precursor lesions were investigated[17].

Kreuter *et al.*[26] studied the sensitivity and specificity of various surrogate markers for the diagnosis of AIN and found that both the p16 and Ki67 protein, or the ones of minichromosome maintenance 3, 4, 6 and 7, showed 100% sensitivity and 100% specificity in the diagnosis of HSIL, as assessed by two highly experienced pathologists. The authors concluded that the used markers are effective as additional tools to be used in the routine evaluation of anal pathology to optimize the diagnosis of AIN, especially in borderline cases[26].

In the present study, the correlation between the initial diagnosis of each of the three pathologists and the diagnostic consensus, taking into account all the different diagnoses observed, resulted in a negative kappa, reflecting a greater discrepancy in the exact interpretation of each diagnoses observed. This is an expected finding when considering the type of analysis that was done on nine different diagnostic categories. If the exact interpretation of a specific degree of AIN may be subject of considerable disagreement among experienced pathologists[28], it should not be surprising that the disagreement about many other diagnostic categories could be higher, mainly because it was established among pathologists without large previous experience in the diagnosis of anal cancer precursor lesions.

Regarding the analysis of the presence or absence of ASIL or cancer, the correlation between the initial diagnosis of the two more productive pathologists and the results of consensus was just regular. This observation can be explained because there has been a tendency to underestimate lesions in the initial reading, meaning that if it were not for reading consensus, a considerable number of lesions with a higher degree of dysplasia would not be detected.

In order to avoid the described potential for diagnostic discordance involving the classification of AIN in three degrees [15,28], this study interpreted the pre-cancerous anal lesions in two categories: LSIL and HSIL. However, considerable disagreement with the consensus diagnosis was observed for LSIL results (71.2% and 72.0% of the interpretations of pathologists 2 and 3 were, respectively, below the consensus LSIL) and HSIL (44.7% and 71.4% of the readings of pathologists 2 and 3 were, respectively, below the consensus HSIL). Plausible explanations for this diagnostic disagreement were the biases that probably arise due to the higher frequency of anal canal condyloma acuminata in patients at risk of developing anal cancer and crushing artifacts observed in biopsies performed in the ATZ region, noting that the diagnostic quality of anal cancer precursor lesions depends on the appropriate collection of specimens.

The action to be taken when dealing with diagnosed high-grade anal lesions remains controversial. Some advocate immediate treatment of any detected HSIL[29], while others prefer to keep HSIL patients under close observation until early signs of malignant transformation are detected for, only then, treat patients properly[30]. However, for both lines of conduct, it is important to precisely recognize histopathological lesions of HSIL or greater gravity. Taking this into account, the two pathologists who had the highest number of individual readings tended to agree more with the consensus reading, although the kappa obtained was only moderate. Greater agreement was not reached because, again, the reading scores of pathologist 2 (44.7% or 21/47) and pathologist 3 (71.4% or 5 / 7) subclassified the lesions, which could exert an decisive influence in the clinical management of the lesions if the consensus reading had not been carried out.

The authors conclude that, for the pathologists who participated in this study, the average interobserver agreement was only fair, although replication of diagnosis for lesions equal to or more severe than HSIL have been moderate. There was the distinct impression that the early diagnosis of anal cancer in centers that lack pathologists with extensive experience in the area (as commonly observed in most Pathology Services due to the low incidence of anal cancer in the general population) should be based in consensus diagnosis, preferably with three or more observers, in order to facilitate the resolution of any differences of interpretation that could occur between two pathologists. The use of surrogate markers of dysplastic lesions of high grade anal could help increase the replication diagnosis of lesions with greater potential for malignant transformation and decrease the learning curve of not especially skilled pathologists in the diagnosis of anal intraepithelial lesions.

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R E S U M O

Objetivo: *Analisar a variabilidade interobservadores no diagnóstico de lesões precursoras do câncer anal no cenário mais comum de um serviço constituído por patologistas sem experiência prévia no diagnóstico destas lesões.* ***Métodos:*** *Quinhentas e duas lâminas histopatológicas com espécimes anais retirados de 372 pacientes HIV-positivos e HIV-negativos foram analisadas no Departamento de Patologia da Fundação de Medicina Tropical do Amazonas por três patologistas com ampla experiência no diagnóstico de doenças tropicais e infecciosas, mas sem experiência prévia importante no diagnóstico de lesões precursoras do câncer anal. As leituras individuais de cada patologista foram comparadas com a que se seguiu a diagnóstico de consenso em microscópio de ótica compartilhada. Os diagnósticos individuais foram confrontados com os de consenso mediante análise da estatística* kappa. ***Resultados:*** *A concordância absoluta entre cada diagnóstico individual e o de consenso correspondente foi ruim (*kappa*=-0,002). Considerando os resultados apenas positivos ou negativos para lesões intraepiteliais escamosas anais, obteve-se concordância regular entre os observadores (*kappa*=0,35), enquanto que a concordância foi moderada quando os resultados histopatológicos foram considerados positivos ou negativos para lesão intraepitelial de alto grau ou câncer (*kappa*=0,52).* ***Conclusão:*** *A variabilidade interobservadores no diagnóstico histopatológico do câncer anal e de suas lesões precursoras entre patologistas sem grande experiência na área, apesar de* experts *em outras, é tal que os diagnósticos neste campo e neste cenário comum devem sempre ser de consenso.*

Descritores: *Canal anal. Neoplasias do ânus. Variações dependentes do observador. Patologia.*

REFERENCES

1. Sobhani I, Walker F, Roudot-Thoraval F, Abramowitz L, Johanet H, Hénin D, et al. Anal carcinoma: incidence and effect of cumulative infections. AIDS 2004;18(11):1561-9.
2. Palefsky JM. Anal cancer prevention in HIV-positive men and women. Curr Opin Oncol 2009;21(5):433-8.
3. Longacre TA, Kong CS, Welton ML. Diagnostic problems in anal pathology. Adv Anat Pathol 2008;15(5):263-78.
4. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J Acquir Immune Defic Syndr 2008;48(4):491-9.
5. Johnson LG, Madeleine MM, Newcomer LM, Schwartz SM, Daling JR. Anal cancer incidence and survival: the surveillance, epidemiology, and end results experience, 1973-2000. Cancer 2004;101(2):281-8.
6. Goedert JJ, Coté TR, Virgo P, Scoppa SM, Kingma DW, Gail MH, et al. Spectrum of AIDS-associated malignant disorders. Lancet 1998;351(9119):1833-9.
7. Bower M, Powles T, Newsom-Davis T, Thirlwell C, Stebbing J, Mandalia S, et al. HIV-associated anal cancer: has highly active antiretroviral therapy reduced the incidence or improved the outcome ? J Acquir Immune Defic Syndr 2004;37(5):1563-5.
8. Nadal SR, Calore EE, Manzione CR, Assakawa MA, Felix LM, Horta SHC. Incidência de neoplasias intraepiteliais anais em doentes HIV-positivos portadores de condilomas acuminados, comparando período anterior e posterior aos inibidores da protease. Rev bras Coloproctol 2005;25(3):217-22.
9. Melbye M, Sprogel P. Aetiological parallel between anal cancer and cervical cancer. Lancet 1991;338(8768):657-9.
10. Hernandez BY, McDuffie K, Zhu X, Wilkens LR, Killeen J, Kessel B, et al. Anal human papillomavirus infection in women and its relationship with cervical infection. Cancer Epidemiol Biomarkers Prev 2005;14(11 Pt 1):2550-6.
11. Fenger C, Nielsen VT. Intraepithelial neoplasia in the anal canal. The appearance and relation to genital neoplasia. Acta Pathol Microbiol Immunol Scand A 1986;94(5):343-9.
12. Cai B, Ronnett BM, Stoler M, Ferenczy A, Kurman RJ, Sadow D, et al. Longitudinal evaluation of interobserver and intraobserver agreement of cervical intraepithelial neoplasia diagnosis among an experienced panel of gynecologic pathologists. Am J Surg Pathol 2007;31(12):1854-60.
13. Ceballos KM, Chapman W, Daya D, Julian JA, Lytwyn A, McLachlin CM, et al. Reproducibility of the histological diagnosis of cervical dysplasia among pathologists from 4 continents. Int J Gynecol Pathol 2008;27(1):101-7.
14. Lacey HB, Wilson GE, Tilston P, Wilkins EG, Bailey AS, Corbitt G, et al. A study of anal intraepithelial neoplasia in HIV positive homosexual men. Sex Transm Infect 1999; 75(3):172-7.
15. Colquhoun P, Nogueras JJ, Dipasquale B, Petras R, Wexner SD, Woodhouse S. Interobserver and intraobserver bias exists in the interpretation of anal dysplasia. Dis Colon Rectum 2003;46(10):1332-6; discussion 1336-8.
16. Bethesda 2001 Workshop. 2001 Terminology [database on the Internet]. NCI Bethesda System 2001. 2001 [citado 2008 sep 18]; Disponível em: http://bethesda2001.cancer.gov/terminology.html
17. 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(7): 1447-56.
18. Fenger C. The anal transition zone. Acta Pathol Microbiol Immunol Scand Suppl 1987; 289:1-42.
19. Fenger C, Frisch M, Jass JJ, Williams GT, Hilden J. Anal cancer subtype reproducibility study. Virchows Arch 2000;436(3):229-33.
20. Tramujas da Costa e Silva I, de Lima Ferreira LC, Santos Gimenez F, Gonçalves Guimaraes RA, Botinelly Fujimoto L, Barbosa Cabral CR, et al. High-resolution anoscopy in the diagnosis of anal cancer precursor lesions in renal graft recipients. Ann Surg Oncol 2008;15(5):1470-5.
21. Abramowitz L, Benabderrahmane D, Ravaud P, Walker F, Rioux C, Jestin C, et al. Anal squamous intraepithelial lesions and condyloma in HIV-infected heterosexual men, homosexual men and women: prevalence and associated factors. AIDS 2007;21(11):1457-65.
22. BioEstat. Aplicações estatísticas nas áreas das ciências bio-médicas. [computer program]. Version 5.0. Belém (PA): Instituto de Desenvolvimento Sustentável Mamirauá; 2007.
23. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33(1):159-74.
24. Malpica A, Matisic JP, Niekirk DV, Crum CP, Staerkel GA, Yamal JM, et al. Kappa statistics to measure interrater and intrarater agreement for 1790 cervical biopsy specimens among twelve pathologists: qualitative histopathologic analysis and methodologic issues. Gynecol Oncol 2005;99(3 Suppl 1):S38-52.
25. Ryan DP, Compton CC, Mayer RJ. Carcinoma of the anal canal. N Engl J Med 2000; 342(11):792-800.
26. Kreuter A, Jesse M, Potthoff A, Brockmeyer NH, Gambichler T, Stücker M, et al. Expression of proliferative biomarkers in anal intraepithelial neoplasia of HIV-positive men. J Am Acad Dermatol 2010;63(3):490-8.

27. Panther LA, Wagner K, Proper J, Fugelso DK, Chatis PA, Weeden W, et al. High resolution anoscopy findings for men who have sex with men: inaccuracy of anal cytology as a predictor of histologic high-grade anal intraepithelial neoplasia and the impact of HIV serostatus. Clin Infect Dis 2004;38(10):1490-2.
28. Carter PS, Sheffield JP, Shepherd N, Melcher DH, Jenkins D, Ewings P, et al. Interobserver variation in the reporting of the histopathological grading of anal intraepithelial neoplasia. J Clin Pathol 1994;47(11):1032-4.
29. Martin F, Bower M. Anal intraepithelial neoplasia in HIV positive people. Sex Transm Infect 2001;77(5):327-31.
30. Devaraj B, Cosman BC. Expectant management of anal squamous dysplasia in patients with HIV. Dis Colon Rectum 2006;49(1):36-40.

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