Nuclear alterations of cells and atypical metaplastic cells in cervical smears are predictive criteria of high-grade cervical intraepithelial neoplasia

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Summary

Diagnoses based on the screening of cervical smears show low interobserver reproducibility and are frequently discordant with the final histological diagnosis. The aim of this study was to identify which of the cytomorphologic criteria used in the screening of cervical smears were most predictive of the histopathological grades of cervical intraepithelial neoplasia. The abnormal cervical smears of 206 women were reviewed blindly according to 22 pre-established cytomorphological criteria. Colposcopic evaluation was carried out in all cases. The marked presence of several nuclear criteria frequently found together in the same smear was associated with high grade intraepithelial neoplasia regardless of the presence of any other criteria. On the other hand, when the nuclear criteria were less evident, the cluster of criteria related to metaplastic cells was predictive of a diagnosis of high-grade intraepithelial neoplasia. Focusing on selected cytological criteria can aggregate predictive value to cervical smear diagnoses.

Key words: Cervical smear; Metaplastic cells; Predictive value; Screening; Cervical cancer.

Introduction

A woman with an abnormal Pap smear should undergo colposcopy and this clinical decision is based on the probability that the cytological diagnosis could represent true cervical intraepithelial neoplasia (CIN). Many studies have been carried out in an attempt to identify markers capable of predicting the histological diagnosis or the progression of squamous intraepithelial lesions [1]. Nevertheless cytology is still the main tool for screening for cervical cancer and the Bethesda System was recently reviewed in an attempt to improve the correlation between cytological and histological diagnoses [2].

Diagnoses based on cervical smear analysis show low interobserver reproducibility and are frequently discordant with the final histological diagnosis. This could be partially attributable to low uniformity in the interpretation of cytological criteria [3, 4-7].

The aim of this study was to identify the cytomorphologic criteria more capable of distinguishing grades of intraepithelial neoplasia. We therefore reviewed abnormal smears obtained during cervical cancer screening to analyze the association of cytomorphologic criteria with the diagnosis reached during the subsequent histological evaluation. Our objective was to improve cervical smear analysis and to achieve a more accurate prediction of the final diagnosis.

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Materials and Methods

Between October 2001 and March 2002, 206 women with a single abnormal Pap smear who had been referred from primary healthcare for colposcopy were selected for this study. Women with more than one abnormal Pap smear and pregnant women were not included. A total of 47 cases of ASCUS (atypical squamous cells of undetermined significance), 30 cases of LSIL (low-grade squamous intraepithelial lesion), 124 cases of HSIL (high-grade squamous intraepithelial lesion), three cases of AGUS (atypical glandular cells of undetermined significance) and two cases of invasive squamous carcinoma were admitted to the trial. Primary clinical healthcare recommendations establish that all women with HSIL should have colposcopy carried out immediately. Women with ASCUS or LSIL may have a second Pap smear performed six months after the first test, but some clinicians preferred to refer these women for immediate colposcopy and they were therefore included in this study.

Every woman underwent colposcopy and had a second cervical smear carried out during the first study visit. Biopsies were performed whenever a suspicious image was found and cervical conization was carried out when the cervical biopsy was judged to be insufficient for establishing diagnosis. Of the 206 women admitted to the study, 180 were submitted to histological examination of the cervix and the resulting diagnosis was considered final. When a woman underwent more than one histological examination, the most severe diagnosis was considered the final diagnosis. The remaining 26 women in the study had a satisfactory negative colposcopic evaluation and a negative second cervical smear. They were therefore considered free of neoplasia and were analyzed together with another 41 cases that received a negative histological diagnosis for neoplasia.

Every cervical smear was completely and carefully reviewed by two cytopathologists according to 22 pre-established cytomorphological criteria that considered both cytoplasmic and

nuclear cellular alterations, types of atypical cells and general features of smears. Table 1 lists all cytological criteria selected for this study. The number of atypical cells, clear perinuclear space, increased nuclear cytoplasmic ratio, anisokaryosis and hyperchromasia criteria were classified as absent, slight, moderate or high. The other criteria were classified simply as absent or present.

The cytopathologists who reviewed the cervical smears were unaware of the histological diagnoses and the results of the other examinations. Every histological diagnosis required the consensus of two pathologists who were blinded to the cytological diagnosis.

The cases were grouped according to the final diagnosis: absence of neoplasia, CIN 1 or CIN 2/CIN 3. CIN 2 and CIN 3 were analyzed together because they both correspond to a cytological diagnosis of high-grade squamous intraepithelial lesion. The association of cytomorphological criteria with the final diagnosis was analyzed between cases of CIN 1 and absence of neoplasia, and between cases of CIN 1 and CIN 2/CIN 3. Odds ratio (OR) and 95% confidence interval (CI 95%) were used to estimate the strength of the association of each cytomorphological criterion with the final diagnosis. The logistic regression model was used to assess this association and the results were expressed as OR and CI 95% [9]. These analyses were also adjusted for the woman's age and for the time interval between cervical cytological screening and colposcopic evaluation. Chi-square Automatic Interaction Detection (CHAID) was used to select a set of cytomorphological criteria and their interactions that would optimally predict the final diagnosis. The model developed was a classification tree that shows how major cytomorphological criteria were able to differentiate and predict the final diagnosis [8].

Results

The mean age of women in this study was 33.2 years (range 13 - 73 years). The time interval between cytological screening and cervical biopsy ranged from 1 to 24 months (mean interval 5.4 months). Of the 180 women who were submitted to histological examination of the cervix, 76 received a histological diagnosis of CIN 3, 22 women were diagnosed CIN 2, 41 were CIN 1 and 41 cases were negative for malignancy. The remaining 26 women received a negative diagnosis from their second Pap smear and a negative colposcopy. These women did not undergo biopsy and were also considered to be free of malignancy (data not shown). The predominant feature of the cervical smears of the women diagnosed as negative for neoplasia was either the absence or slight presence of the following criteria: number of atypical cells, increased nuclear/cytoplasmic ratio, anisokaryosis, and hyperchromasia. In the case of women who were diagnosed with CIN 1, these criteria were classified predominantly as slight or moderate, and in cases of CIN 2/CIN 3, these criteria were rated moderate or high (Table 1). A high number of atypical cells, increased nuclear cytoplasmic ratio, anisokaryosis and hyperchromasia were criteria present in almost half the cervical smears that received a final diagnosis of CIN 2/CIN 3. The predictive value of these criteria for this diagnosis proved to be around 90% (Table 1).

Atypical mature metaplastic cells and metaplastic cells with nuclear volume more than 1.5 times the normal nuclear volume of intermediate cells were present in approximately 80% of the smears that received a final diagnosis of CIN 2/CIN 3. The predictive value of these criteria for this diagnosis proved to be 70%. Atypical parabasal cells showed a similar predictive value but were present in only 19% of cases of CIN 2/CIN 3. A dense cytoplasmic border was a criterion twice as frequent in cervical smears with a final diagnosis of CIN 1 as in cervical smears without neoplasia or in those that received a diagnosis of CIN 2/CIN 3 (Table 1).

Taking the negative neoplastic cases as a reference, the criteria associated with CIN 1 were dense cytoplasmic border, moderate number of atypical cells, a moderate

Table 1. — *Frequency (%) and predictive value (%) of each morphologic criterion in cervical smear screening according to the final diagnosis.*

Morphologic criteria		Absence of neoplasia		CIN 1		CIN2/CIN3	
		Freq.	PV	Freq.	PV	Freq.	PV
No. of atypical cells	Absent	13	69	10	31	0	0
	Slight	48	55	27	19	15	26
	Moderate	31	26	59	30	35	44
	High	8	9	5	4	49	87
Atypical parabasal cells		3	8	10	16	19	76
Atypical intermediate cells		69	29	73	19	84	52
Atypical superficial cells		58	29	68	20	71	50
Atypical mature metaplastic cells		30	18	32	12	80	70
Atypical immature metaplastic cells		11	16	15	14	31	70
Atypical endocervical cells		6	44	5	22	3	33
Clear perinuclear space	Absent	43	40	32	18	31	42
	Slight	52	30	56	20	60	50
	Moderate	5	18	12	29	9	53
	High	–	–	–	–	–	–
Dyskeratosis		34	23	46	19	60	58
Dense cytoplasmic border		21	23	46	31	29	46
Binucleation		75	28	85	20	93	52
Multinucleation		21	23	15	10	41	67
Increased N/C ratio	Absent	25	71	12	21	2	8
	Slight	51	49	44	26	18	26
	Moderate	19	21	39	26	34	53
	High	5	6	5	4	46	90
Anisokaryosis	Absent	25	61	17	23	5	16
	Slight	51	47	44	27	18	27
	Moderate	19	22	34	24	32	53
	High	5	6	5	4	45	90
Hyperchromasia	Absent	16	73	10	27	0	0
	Slight	51	49	39	23	20	29
	Moderate	30	27	49	27	35	46
	High	3	4	2	2	45	94
Irregular nuclear membrane		37	21	39	14	79	65
Thick nuclear membrane		16	14	17	9	61	77
Irregularly distributed chromatin		28	17	44	16	77	67
Nucleoli		5	60	2	20	1	20
NV of metaplastic cells > 1.5		36	19	42	14	85	67
NV of mature cells > 2.5-3.0		33	56	15	15	11	28
NV of mature cells > 3.0		36	25	54	23	51	52
Total cases		67		41		98	

Freq: Frequency; PV: predictive value; N/C: nuclear/cytoplasmic; NV: nuclear volume.

increase in the nuclear cytoplasmic ratio and moderate hyperchromasia (Table 2). The OR values and respective confidence intervals showed no statistically significant variation when they were adjusted for age and/or the time interval between cervical cytological screening and colposcopic evaluation (data not shown).

Taking the CIN 1 cases as reference and according to OR values, the criteria that expressed the strongest association with a diagnosis of CIN 2 or CIN 3 were high hyperchromasia, high increase of nuclear/cytoplasmic ratio, high number of atypical cells and high anisokaryosis. Atypical mature metaplastic cells, atypical immature metaplastic cells and metaplastic cells with nuclear volume greater than 1.5 times the nuclear volume of intermediate cells were criteria also associated with CIN 2/CIN 3 (Table 2). The OR values and respective confidence intervals did not vary significantly when they were adjusted for age and/or the time interval between cervical cytologic screening and colposcopic evaluation (data not shown).

Table 2. — *Cytomorphological criteria in cervical smear screening associated with histological diagnosis of CIN 1, taking the cases negative for neoplasia as a reference.*

Cytomorphologic criteria	OR	(CI 95%)
Dense cytoplasmic border	3.3	(1.4-7.7)
Moderate number of atypical cells	3.1	(1.4-7.2)
Moderate increase NC ratio	2.7	(1.1-6.6)
Moderate hyperchromasia	2.3	(1.0-5.1)
NV of mature cells > 2.5-3.0	0.4	(0.1-1.0)

N/C: nuclear/cytoplasmic; NV: nuclear volume; OR: odds ratio; CI: confidence interval. Absence of neoplasia: refers to negative histological diagnoses of neoplasia or cases with negative colposcopy and negative second cervical smears.

According to the logistic regression analysis, the criteria associated with a diagnosis of CIN 2/CIN 3 were high increases in the nuclear/cytoplasmic ratio and in the number of atypical mature metaplastic cells. When the number of atypical cells was excluded as one of the criteria in this analysis, high hyperchromasia also showed an association. A dense cytoplasmic border was the only factor that was independently associated with the diagnosis of CIN 1 (Table 3).

Table 3. — *Cytomorphological criteria in cervical smear screening associated with CIN2/CIN3, taking final diagnosis of CIN 1 as reference.*

Cytomorphologic criteria	OR	(CI 95%)
High hyperchromasia	44.0	(5.5-351.0)
High increase N/C ratio	25.9	(5.6-120.5)
High number of atypical cells	24.0	(4.9-117.1)
High anisokaryosis	23.9	(5.2-110.0)
Atypical mature metaplastic cells	8.4	(3.7 - 19.0)
NV of metaplastic cells > 1.5	7.8	(3.4 - 17.9)
Thick nuclear membrane	7.7	(3.1 - 19.0)
Irregular nuclear membrane	5.7	(2.6 - 12.6)
Irregularly distributed chromatin	4.2	(1.9 -9.0)
Multinucleation	4.0	(1.6 - 10.5)
Atypical immature metaplastic cells	2.6	(1.0 - 6.8)
Moderate anisokaryosis	2.4	(1.0-5.6)
Dense cytoplasmic border	0.5	(0.2 - 1.0)

N/C: nuclear/cytoplasmic; NV: nuclear volume; OR: odds ratio; CI: confidence interval.

CHAID identified an increased nuclear/cytoplasmic ratio as the single factor most predictive of the final diagnosis. Ninety percent of cervical smears with cells showing a high increase in the nuclear/cytoplasmic ratio received a diagnosis of CIN 2/CIN 3, but when the nuclear/cytoplasmic ratio was normal or only slightly increased, 54% of cervical smears revealed no neoplastic lesion. When high anisokaryosis was associated with a high increase in the nuclear cytoplasmic ratio, 95.2% of these cervical smears resulted in a diagnosis of CIN 2/CIN 3. Sixty-six percent of the cervical smears that contained cells showing a moderate increase in the nuclear/cytoplasmic ratio as well as metaplastic cells with nuclear volume greater than 1.5 received a final diagnosis of CIN 2/CIN 3, but when the latter factor was absent, only 13% were diagnosed as CIN2/CIN 3 (Figure 1).

Discussion

Our results show that an increased nucleocytoplasmic ratio is the single cytomorphologic criterion most capable of distinguishing cervical smears with no neoplasia from those that will result in a diagnosis of CIN 1 or CIN 2/CIN 3. When the increase in the nuclear/cytoplasmic ratio is only moderate, its ability to predict diagnosis is weak, but when it is associated with an increase in the nuclear volume of metaplastic cells, the most probable diagnosis will be CIN 2/CIN 3; otherwise the most likely diagnosis will be CIN 1 or absence of neoplasia.

Logistic regression showed that a high number of atypical cells and atypical mature metaplastic cells were criteria independently associated with CIN 2/CIN 3. When the high number of atypical cells was excluded from the analysis, high hyperchromasia together with atypical mature metaplastic cells became the criteria most strongly associated with this diagnosis. In the bivariate analysis, high hyperchromasia showed the highest OR, followed by an increased nucleocytoplasmic ratio, high number of atypical cells and high anisokaryosis.

We can assume that the cluster of criteria related to cellular nuclei is frequently found together in the same smear and that when their combined presence is marked and independent from other criteria, these criteria are associated with high-grade SIL. When the nuclear criteria are less evident, the second cluster of criteria that should be considered are those related to metaplastic cells, consisting of a nuclear volume of metaplastic cells greater than 1.5 times the intermediate cells, atypical mature metaplastic cells and atypical immature metaplastic cells.

The association of atypical metaplastic cells with high-grade cervical intraepithelial neoplasia has already been described. Sheils & Wilbus [9] analyzed the follow-up data on ASCUS and showed that in cases of mature metaplastic and immature metaplastic ASCUS, high-grade SIL accounted, respectively, for 42% and 60% of those subsequently diagnosed with a squamous intraepithelial

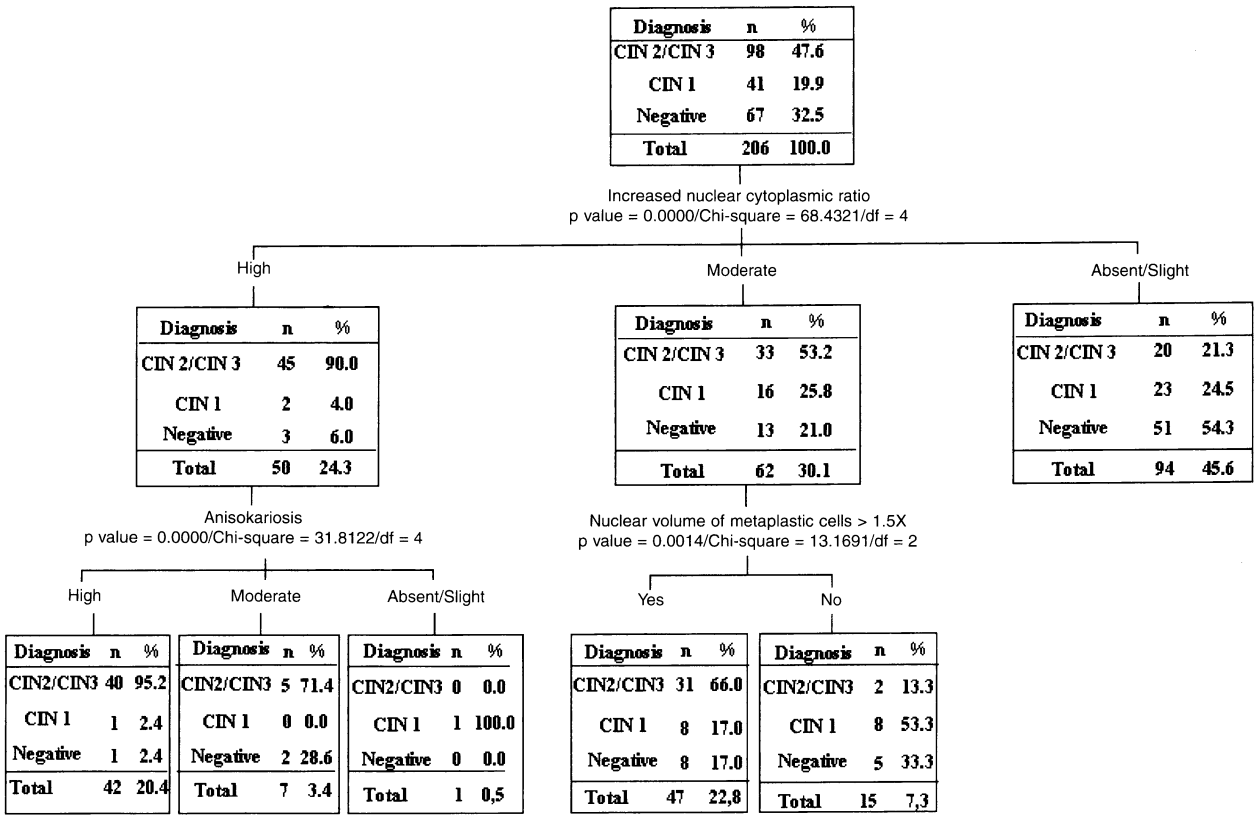

Figure 1. — Classification tree according to Chi-Square Automatic Interaction Detection (CHAID): cytomorphological criteria present in the screening of cervical smears and the interactions of these criteria that optimally predict the final diagnosis.

lesion, as opposed to 30% in cases of mature ASCUS [9]. They concluded that when features of mature metaplasia and immature metaplasia are found in the cells of ASCUS, patients were observed to be at an increasingly greater risk of SIL and these cases were proportionately more likely to be high grade.

Nasser *et al.* (2003) described cervical cytological specimens showing LSIL that occasionally contained a few cells that were suggestive of HSIL, but did not constitute a definitive diagnosis [10]. Reviewing these Pap smears, they found that the types of suspicious cells most indicative of a high-grade lesion were atypical squamous metaplastic cells (62%), followed by atypical keratinized cells (20%), and dysplastic cells with a borderline nuclear/cytoplasmic ratio (18%).

Diagnosis of CIN 1 is weakly associated with some criteria that have low interobserver agreement [11]. In this study, the classical criteria of atypical superficial or intermediate cells were not associated with a histological diagnosis of CIN 1 and this could explain the low correlation between cytological and histological diagnoses of low-grade SIL [12]. A dense cytoplasmic border showed a fairly independent association with CIN 1 but it was insufficient to give strength to this diagnosis.

Although this study was performed with conventional smears, its results with respect to increased nuclear/cytoplasmic ratio, hyperchromasia and atypical metaplastic cells are also applicable to liquid-based cytology. It is also difficult in either of these techniques to distinguish squamous metaplastic cells from reserve cells with reactive, reparative, or degenerative nuclei and from atypical glandular cells [4, 13, 14].

Smears were grouped according to the final diagnosis using the Bethesda System that considers CIN 2 and CIN 3 as HSIL [6, 15-18]. Therefore, the criteria related to CIN 2/CIN 3 should represent a cytological diagnosis of HSIL. We must emphasize that the aim of this study was not to make a direct cytological-histological correlation since the cervical smears and the cervical biopsies were not performed at the same time. In fact, we reproduced the system of cervical cancer screening to evaluate how cervical smear analysis could be predictive of the subsequent diagnosis established using colposcopy and biopsy.

Currently many markers such as HPV typing and $p16^{INK4A}$ have been assessed in the diagnosis of high-grade lesions, but the clinical use of these techniques still requires further investigation. In fact, these alternatives will most probably increase the cost of cervical cancer screening. Cytological detection of high-grade squamous intraepithelial lesions is critical in the prevention of cervical cancer. Therefore, focusing more attention on selected cytological criteria that could aggregate predictive value to cervical smear screening could reduce the subjectiveness of microscopic screening without increasing the cost.

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