



# The role of Pap smear in the diagnostics of endocervical adenocarcinoma

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Pulkkinen J, Huhtala H, Kholová I. The role of Pap smear in the diagnostics of endocervical adenocarcinoma. *APMIS*. 2021; 129: 195–203.

In the high-income countries, the amount of cervical adenocarcinomas is on the rise. The pap smear sampling has a low sensitivity and a low specificity for endocervical malignancies, and there are only a few cytomorphological features, that are specifically associated with glandular atypia. In this study, 298 pap smears of 60 patients with endocervical adenocarcinoma or adenocarcinoma in situ (AIS) and 30 patients with high-grade intraepithelial lesion (HSIL) in histology were reviewed. The pap smear type (screening/clinical), the HPV status and the time from sampling to the histological confirmation of diagnosis were recorded for each case. Despite that no cytomorphological features could be associated with adenocarcinoma statistically, 70% of the pap smears were initially correctly diagnosed as an endocervical glandular lesion. Palisading cell borders, nuclear pleomorphism and the lack of single atypical cells present simultaneously were found to be associated with adenocarcinoma and AIS with the corresponding ORs of 5.89 (95% CI 1.96–17.70), 3.71 (95% CI 1.14–12.02) and 10.76 (95% CI 1.20–59.50). This combination of features was seen in smears taken up to 5 years before the histological diagnosis. Of all our screening samples, 10.9% were HPV-positive. There were no HPV-negative samples among patients with adenocarcinoma.

**Key words:** Endocervical adenocarcinoma; AIS; pap smear; cytological features; HPV; pathology.

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Worldwide cervical cancer is the fourth most frequent cancer in women (1). Since the cervical cancer screening programme in Finland started in the 1960s, the amount of cervical cancer deaths has decreased to one-fifth of its original number (2). In Finland, in addition to the national cervical cancer screening programme, there are also symptom-based and opportunistic (3) pap smears. While about 90% of the deaths caused by cervical cancer occur in low- and middle-income countries, in the high-income countries the total amount of cervical cancers has decreased (4). Yet, in the high-income countries, the relative and total amount of cervical adenocarcinomas seems to be rising, especially among the younger age groups (4).

While the diagnostic cytological and histological features of endocervical adenocarcinoma (EAC) and endocervical adenocarcinoma in situ (AIS) are

defined by The Bethesda System for Reporting Cervical Cytology (5) and the WHO Classification of the Tumours of Female Reproductive Organs (6), respectively, there are currently no accepted lower grade precursor lesions for adenocarcinoma like there are the intraepithelial neoplasias for cervical squamous cell carcinoma (5–8). In cytological cervical samples, a large amount of the lesions behind glandular diagnoses (atypical endocervical cells, NOS, atypical endocervical cells, favour neoplastic, endocervical adenocarcinoma in situ and endocervical adenocarcinoma) are non-neoplastic or of squamous or endometrial origin or other carcinomas leading to a low screening specificity for EAC and AIS (9–17).

Cytological diagnoses of atypical endocervical cells, NOS (AEC, NOS), atypical endocervical cells, favour neoplastic (AEC, FN) and adenocarcinoma in situ (AIS) were shown to have a progressively better association with neoplasia (10–11,17–18) and

Received 15 June 2020. Accepted 8 January 2021

**Table 1.** Cohort characteristics

	Number of patients	Age $\pm$ SD (range)	Number of pap smears	Average number of smears n (range)	Number of screening pap smears n (%)	Number of clinical pap smears n (%)	Abnormal <sup>1</sup>			Diagnostic <sup>2</sup>		
							Total n (%)	Screening pap smear n (%)	Clinical pap smear n (%)	Total n (%)	Screening pap smear n (%)	Clinical pap smear n (%)
AC total	60	43.0 $\pm$ 14.6 (22–83)	201	3.4 (1–10)	55 (27.4%)	146 (72.6%)	106 (52.7%)	36 (34.0%)	70 (66.0%)	60 (29.5%)	21 (35.0%)	39 (65.0%)
AC only	37	46.0 $\pm$ 16.0 (23–83)	109	2.9 (1–9)	27 (24.8%)	82 (75.2%)	57 (52.3%)	18 (31.6%)	39 (68.4%)	37 (33.3%)	2 (5.4%)	35 (94.6%)
AC + LSIL/HSIL	23	37.5 $\pm$ 9.9 (22–56)	92	4.0 (1–10)	28 (30.4%)	64 (69.6%)	49 (53.3%)	18 (36.7%)	31 (63.3%)	23 (25.0%)	6 (26.1%)	17 (73.9%)

<sup>1</sup>Abnormal pap smear sample was defined as sample other than Negative for Intraepithelial Lesion or Malignancy (NILM).

<sup>2</sup>Diagnostic pap smear sample was defined as sample after which histology confirmed the diagnosis of adenocarcinoma or adenocarcinoma in situ.

also specifically to cervical glandular malignancies (19). The reported sensitivity of a single pap smear for neoplasia in previous studies ranged from 15.3% up to 100% depending on the sample selections in the studies. Generally, it was lower in the studies that included also previous pap smears diagnosed as AEC, NOS, Atypical endometrial cells, NOS, atypical glandular cells, NOS, normal or insufficient on contrary to inclusion of only samples with possible or definite high-grade epithelial features (11–12,14–16,20–21).

In the studies, with pap smear samples diagnosed as AIS or EAC in cytology, in histology AIS was found in 13%–28.3% of the cases and EAC in 13% to 38.3% of the cases, although there was a clinically significant lesion in 98.3%–100% of the samples (11,16). When the pap smear samples of histologically confirmed EAC, AIS and AIS + HSIL were reanalysed a positive predictive value for a pap smear for AIS was reported to vary from 47.6% to 54.3% and for EAC from 45 to 76% (20,21).

There were only a few cytomorphological features associated specifically with AEC and AIS (9,10).

In previous studies, feathering was the cytological feature showing the strongest association specifically with endocervical glandular neoplasia, but also pseudostratified strips, rosettes, palisading borders and even papillary groups were reported (9,10).

The aim of the present study was to find out what type of cytological features and samples lead to the diagnosis of EAC or AIS and what are the reasons leading to sampling in these cases. In addition, we traced the first abnormal samples and the cytological progression of endocervical adenocarcinoma to find potential precursor cytological features.

## MATERIALS AND METHODS

A laboratory information system (LIS) search for histopathological diagnosis was made to find the patients operated for EAC or AIS at the Tampere University Hospital during the years 2008–2014. In total, 60 patients were found in the 7-year-study period, all of whom had pap smears taken prior to the diagnosis. The pap smear samples were conventional pap smears. All patients had histologically confirmed diagnosis either by conization or hysterectomy. The average age of the patients was 43.0 years (SD  $\pm$  14.6, range 22–83) (Table 1). The patients had altogether 201 pap smear samples taken, with the average of 3.4 samples per patient (Table 1).

This group of patients was further divided into the cases with EAC only or AIS only in the final histology, and to the cases with EAC or AIS together with a low or a high-grade squamous intraepithelial lesion (LSIL/HSIL) in the final histology. The first group had 37 patient-cases and 109 pap smear samples and the latter group 23 cases and 92 pap smears (Table 1). All the available information was retrieved from the clinical referral accompanying the pap smear samples.

A control group of patients with the diagnosis of high-grade intraepithelial lesion (HSIL) only in the final histology was retrieved from the LIS. Of the 83 samples signed out as HSIL during the year 2014, those cases lacking diagnostic pap smears or histological confirmation, either by conization or by biopsy, were excluded. Of the remaining cases, 30 were randomly selected for the study. Altogether, 97 pap smear samples were available in the control group. The patients in this group were between the ages of 21 and 54 with the average of 36.7 years (SD  $\pm$  10.0).

Altogether, the 90 patients included in the study, had 298 pap smears samples available for the revision. The number of pap smears per patient in this study varied from 1 to 10 with the average amount of samples being 3.3.

In some older samples, the diagnosis was given according to the Papanicolaou Classification System and those were converted to the corresponding diagnosis according to the Bethesda System for Reporting Cervical Cytology 2014 (5).

All pap smears were blindly reviewed by a senior cytopathologist in search for 38 different cytological features, consisting of background features (clean, inflammatory, necrotic, apoptotic debris, necrotic debris, bloody), cellular features (columnar cell shape, cuboidal cell shape, irregular cell borders, increased nuclear/cytoplasmic ratio, regeneration, degeneration, atrophy), nuclear features (enlarged nuclei, nuclear hyperchromasia, irregular nuclear membranes, oval nuclei, elongated nuclei, mitotic figures, nuclear pleomorphism, nucleoli, macronucleoli, finely granular chromatin, coarsely granular chromatin, chromatin clearing, nuclear vacuoles) and architectural features (high cellularity, scant cellularity, single atypical cells, nuclear crowding, loss of polarity, loss of honeycomb pattern, pseudostratified strips, palisading borders, papillary groups, rosettes, feathering). The time from cytological sampling to the histological confirmation of diagnosis was calculated for each pap smear. For statistical analyses, samples that had both a glandular and a squamous lesion in the final histopathology were excluded. Altogether, features of 256 pap smears representing 37 cases with EAC or AIS and 30 cases with HSIL in the final histopathology were analysed.

The statistical analysis was performed by an experienced statistician and the programme used was SPSS version 25 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) A further analysis of the cytomorphological features to define the combination of features associated with the occurrence of EAC and AIS was made with a forward stepwise multivariable logistic regression analysis using probability values of <0.05 for the entry of features.

The study was approved by the Ethical committee of Pirkanmaa Health Care District (R16022), and informed content of each individual was not requested. The study was conducted according to the Declaration of Helsinki.

## RESULTS

The oldest sample in this study was taken 19 years and 10 months before the cancer diagnosis and for 16 patients the first sample taken was already diagnostic for EAC or AIS.

Our material consisted of clinical and screening programme samples. Out of the 201 pap smear samples of the patients with EAC or AIS, 27.4% (55/201) were taken in the cervical screening programme and 72.6% (146/201) were clinical samples (Table 1). During the study period, in 2012, the city of Tampere started HPV-screening programme in the age group of 35–60 years (22,23). Of the screening samples, 10.9% (6/55) had a positive HPV-result representing genotypes HPV16 (2 samples), HPV18 (2 samples) and other high-risk HPV types (2 samples) (22). There were no negative HPV cases among patients with EAC or AIS in the final histology.

Of the screening samples, 65.5% (36/55) had an abnormal finding (Table 1) and of all diagnostic samples in our series 35.0% (21/60) were screening

samples. In 45.0% (27/60) of the cases, screening lead to biopsies or follow-ups, which eventually lead to the diagnosis of EAC or AIS.

Of the clinical pap smear samples, 12.3% (18/146) were taken because of an abnormal finding in a previous screening pap smear sample (Table 2).

Follow-ups of a previous abnormal clinical cytological or histological finding represented 19.2% (28/146) of the clinical samples. Testing in the association of general check-ups accounted for 13.7% (20/146) of the samples and 5.5% (8/146) were taken in association of birth control-related issues. In 4.8% (7/146) of the samples, there was a macroscopic tumour, either clinical or radiological. Symptoms reported by patients that led to sampling included abnormal bleeding, abdominal pain or symptoms related to infection like abnormal vaginal discharge, itching, burning sensation or a macroscopic condyloma.

In our material, issues related to infection were the most common symptoms, occurring in 62.5% (10/16) of the cases. They were also the most common non-specific cause leading to diagnosis as they were seen at a wide time range from one sample taken at the time of the final diagnosis to samples taken up to 106 months before the final histological diagnosis (average 53.8 months). The one case with serious infection symptoms at the time of the diagnosis also turned out to have a macroscopic tumour in the clinical check-up.

The second most common cause to symptom-based sampling was abnormal uterine bleeding, which was seen in 37.5% (6/16) of the cases. It was the most specific symptom leading straight to cancer diagnosis in 83% of the reported cases. In our material, only one patient presented with a coital bleeding. Abdominal pain was reported by 3 patients, and in one case, pain-following sampling lead to the cancer diagnosis.

Altogether, only 11.0% (16/146) of the clinical pap smears were taken because of a patient-reported symptom, two of those smears also presenting with a macroscopic tumour in the clinical check-up. Notably, 70.0% of the patients with adenocarcinoma or AIS did not report any symptoms during their history. Unfortunately, 34.9% (51/146) of the clinical samples had no clinical information given in their referral to the pathologist (Table 2).

In the cytomorphological analysis, coarsely granular nuclear chromatin and inflammatory debris were the only features associated specifically with HSIL with respective p-values of <0.001 and 0.024 (Table 3). Other studied features were seen also in EAC and AIS pap smears when all samples were taken into account. However, in HSIL pap smears certain features were recognized earlier. Cuboidal

**Table 2.** Indications of clinical pap smear sampling

	AC		AC only		AC + LSIL/HSIL	
	n	%	n	%	n	%
Number of clinical samples	146		82		64	
Number of indications	149		85		64	
Gynaecological check-up related	20	13.7	15	18.3	5	7.8
Follow-up <sup>1</sup>	28	19.2	17	20.7	11	17.2
Previous abnormal screening result	18	12.3	5	6.1	13	20.3
Birth control-related	8	5.5	4	4.9	4	6.3
Abnormal uterine bleeding	8 <sup>2</sup>	5.5	4	4.9	4 <sup>2</sup>	6.3
Macroscopic tumour	7	4.8	6	7.3	1	1.6
Abdominal pain	3	2.1	3	3.7	0	
Infection related symptoms	6	4.1	2	2.4	4	6.3
No data available	51	34.9	29	35.4	22	34.4

<sup>1</sup>Follow-up of a an abnormal finding in a previous clinical sample.

<sup>2</sup>Including 1 coital bleeding.

**Table 3.** Analysis of cytomorphological features

	Cytopathological feature	Time before histopathological diagnosis (years)	Total p-value
HSIL only <sup>1</sup>	Coarsely granular chromatin	1	0.001
	Inflammatory debris	1	0.024
HSIL earlier <sup>2</sup>	Cuboidal cell shape	3	0.002
	Single atypical cells	3	0.002
	High cellularity	1	0.007
	Papillary groups	1	<0.001
	Rosettes	1	0.006
AC/AIS earlier <sup>3</sup>	Palisading cell borders	2	<0.001

There were no cytopathological features associated with adenocarcinoma or AIS. The following features showed a similar association with squamous and endocervical glandular lesions: feathering, loss of polarity, loss of honeycomb pattern, nuclear crowding, irregular cell borders, columnar cell shape, elongated nuclei. The following features showed no association to squamous or endocervical glandular lesions: clean background, necrotic background, bloody background, apoptotic debris, necrotic debris, increased nuclear/cytoplasmic ratio, regeneration, degeneration, atrophy, enlarged nuclei, nuclear hyperchromasia, irregular nuclear membranes, oval nuclei, mitotic figures, nuclear pleomorphism, nucleoli, macronucleoli, finely granular chromatin, chromatin clearing, nuclear vacuoles, scant cellularity and pseudostratified strips.

<sup>1</sup>Features seen in pap smear samples in HSIL group only.

<sup>2</sup>Features seen in both groups, but presenting earlier in HSIL group compared to AC/AIS group.

<sup>3</sup>A feature seen in both groups, but presenting earlier in AC/AIS group compared to HSIL group.

cell shape and single atypical cells could be seen up to 36 months before histopathological diagnosis of HSIL ( $p = 0.002$  and  $0.002$ , respectively). High cellularity ( $p = 0.007$ ), inflammatory debris ( $p = 0.024$ ) and traditionally to glandular neoplasia-associated features as papillary groups ( $p < 0.001$ ) and rosettes ( $p = 0.006$ ) were seen up to 12 months before the diagnosis in the HSIL group. However, in the retrospective review of the conizates of the HSIL group, 26 out of 30 conizates showed HSIL extending to the endocervical glands, although in 4 cases the extension was very superficial.

In summary, feathering, palisading cell borders, nuclear crowding, loss of polarity, loss of honeycomb pattern, irregular cell borders, elongated nuclei and columnar cell shape were all seen in both squamous and glandular abnormalities in pap smear samples. Of those features, palisading

cell borders were the only feature seen earlier among the adenocarcinoma and AIS samples, as it presented in samples up to two years before histological diagnosis. Among HSIL samples, palisading cell borders were seen a year before the histological diagnosis. Nucleoli did not show an association with adenocarcinoma or AIS in the present study.

Rest of the studied features including clean background, necrotic background, bloody background, apoptotic debris, necrotic debris, increased nuclear/cytoplasmic ratio, regeneration, degeneration, atrophy, enlarged nuclei, nuclear hyperchromasia, irregular nuclear membranes, oval nuclei, mitotic figures, nuclear pleomorphism, nucleoli, macronucleoli, finely granular chromatin, chromatin clearing, nuclear vacuoles, scant cellularity and pseudostratified strips did not show any association with squamous or glandular lesions.

**Table 4.** The cytopathological diagnoses in the cohort<sup>1</sup>

		NILM	Glandular abnormality			
			AEC, NOS	AEC-FN	AIS	AC
AC total	N	95	27	29	2	4
	Time <sup>1</sup> ± SD (range)	84.9 ± 57.4 (0–229)	23.8 ± 29.4 (0–121)	4.2 ± 10.4 (0–41)	0 ± NA (NA)	0 ± NA (NA)
Ac only	n	53	17	16	1	4
	Time <sup>1</sup> ± SD (range)	93.4 ± 66.3 (0–229)	24.8 ± 32.5 (0–121)	3.7 ± 10.9 (0–41)	0 ± NA (NA)	0 ± NA (NA)
AC + LSIL/ HSIL	n	42	10	13	1	0
	Time <sup>1</sup> ± SD (range)	74.3 ± 42.2 (12–170)	22.1 ± 24.7 (0–69)	4.5 ± 10.1 (0–25)	0 ± NA (NA)	NA

  

		Squamous abnormality			
		ASC-US	LSIL	ASC-H	HSIL
AC total	n	27	6	10	13
	Time <sup>1</sup> ± SD (range)	41.2 ± 38.2 (0–173)	20 ± 17.2 (0–51)	8.7 ± 16.8 (0–48)	8.1 ± 15.6 (0–54)
Ac only	n	16	0	4	5
	Time <sup>1</sup> ± SD (range)	44.6 ± 44.5 (0–173)	NA	16 ± 24.0 (0–48)	13.8 ± 23.4 (0–54)
AC + LSIL/ HSIL	n	11	6	6	8
	Time <sup>1</sup> ± SD (range)	36.3 ± 25.4 (6–92)	20 ± 17.2 (0–51)	6.5 ± 12.6 (0–30)	4.5 ± 8.0 (0–22)

NA, not applicable.

<sup>1</sup>The average time of cytological diagnosis in months before the histological confirmation of adenocarcinoma or adenocarcinoma in situ.

Even though no single cytomorphological feature could be associated specifically with adenocarcinoma or AIS, the pap smears of patients with only EAC or AIS in histopathology were signed out as a glandular neoplasia more than twice as often as a squamous neoplasia (21 vs. 9), which means that 70% of the neoplastic diagnoses given in this group were glandular (Table 4). In the EAC/AIS + LSIL/HSIL – group, 41% of the neoplastic diagnosis given were glandular (14 vs. 20) while in the HSIL-only group, all the neoplastic diagnosis were squamous (0% glandular, data not shown).

The same although slightly in favour to glandular diagnoses ascending trend was seen in the pap smear samples taken within five years and during the last 12 months before the histological diagnosis. In the first mentioned group 71% of the neoplastic diagnoses given were glandular (22 vs. 9) in samples with AIS or EAC only in histology and 42% (11 vs. 15) in EAC/AIS + LSIL/HSIL –group (Table 5). During the last 12 months before the histological diagnosis, the corresponding figures were 76% (19 vs. 6) and 43% (10 vs. 13) (Table 5).

In the further analysis of the cytomorphological features, the combination of palisading cell borders, nuclear pleomorphism and the lack of single atypical cells were associated with EAC and AIS. An analysis was made with pap smear samples taken a

year before the histological diagnosis and another analysis with samples taken 5 years before the histological diagnosis. In the 1-year-analysis, the OR for palisading cell borders was 5.89 (95% CI 1.96–17.70), the OR for nuclear pleomorphism 3.71 (95% CI 1.14–12.02) and the OR for the lack of single atypical cells 10.76 (95% CI 1.20–59.50). The corresponding p-values for the features were 0.002, 0.034 and 0.005.

In the analysis of the samples taken within the preceding 5 years of the histological diagnosis, the OR for palisading cell borders was 4.98 (95% CI 1.78–13.88), the OR for nuclear pleomorphism 3.24 (95% CI 1.09–9.62) and the OR for the lack of single atypical cells 10.70 (95% CI 2.01–56.89). The corresponding p-values for the features were 0.02, 0.34 and 0.05.

The earliest single neoplastic diagnosis in AEC/AIS only group was HSIL, and it was given 54 months before the cancer diagnosis. The first diagnosis of AEC, NOS, that with current guidelines, would lead to colposcopy and histological sampling immediately was signed out as early as 121 months before the diagnosis. Also in the AEC/AIS + LSIL/HSIL– group, the earliest diagnosis that nowadays would lead to histological sampling, was AEC, NOS, and it was given 69 months before diagnosis. The first neoplastic diagnosis in this latter group was LSIL seen in a sample 51 months

**Table 5.** The cytopathological diagnoses in the cohort 12 months before the cancer diagnosis (A) and 5 years before the cancer diagnosis (B)<sup>1</sup>

		NILM	Glandular abnormality			
			AEC, NOS	AEC, FN	AIS	AC
(A)						
AC total	n	7	13	23	2	4
	Time <sup>1</sup> ± SD (range)	8.1 ± 4.3 (0–12)	2.8 ± 4.1 (0–12)	0.1 ± 0.4 (0–2)	0 ± 0 (0)	0 ± 0 (0)
AC only	n	4	9	14	1	4
	Time <sup>1</sup> ± SD (range)	5.5 ± 3.9 (0–9)	3.4 ± 4.5 (0–12)	0 ± 0 (0)	0 ± NA (NA)	0 ± 0 (0)
AC + LSIL/ HSIL	n	3	4	9	1	0
	Time <sup>1</sup> ± SD (range)	11.7 ± 0.6 (11–12)	1.5 ± 3.0 (0–6)	0.2 ± 0.7 (0–2)	0 ± NA (NA)	NA
(B)						
AC total	n	39	23	27	2	4
	Time <sup>1</sup> ± SD (range)	33.8 ± 18.0 (0–60)	11.8 ± 13.8 (0–44)	6.2 ± 14.8 (0–60)	0 ± 0 (0)	0 ± 0 (0)
AC only	n	19	15	17	1	4
	Time <sup>1</sup> ± SD (range)	30.2 ± 19.1 (0–60)	11.7 ± 14.6 (0–44)	7.0 ± 17.3 (0–60)	0 ± NA (NA)	0 ± 0 (0)
AC + LSIL/ HSIL	n	20	8	10	1	0
	Time <sup>1</sup> ± SD (range)	36.6 ± 16.8 (11–59)	11.9 ± 13.3 (0–38)	4.8 ± 10.1 (0–25)	0 ± NA (NA)	NA
		Squamous abnormality				
		ASC-US	LSIL	ASC-H	HSIL	
(A)						
AC total	n	6	1	8	10	
	Time <sup>1</sup> ± SD (range)	4.5 ± 5.3 (0–12)	0 ± NA (NA)	1.1 ± 3.2 (0–9)	1.4 ± 3.5 (0–11)	
AC only	n	4	0	3	3	
	Time <sup>1</sup> ± SD (range)	2.25 ± 4.5 (0–9)	NA	0 ± 0 (0)	0 ± 0 (0)	
AC + LSIL/ HSIL	n	2	1	5	7	
	Time <sup>1</sup> ± SD (range)	9 ± 4.2 (6–12)	0 ± NA (NA)	1.8 ± 4.0 (0–9)	2.3 ± 4.1 (0–11)	
(B)						
AC total	n	21	6	11	13	
	Time <sup>1</sup> ± SD (range)	27.8 ± 17.6 (0–60)	23.3 ± 17.6 (0–51)	7.9 ± 16.1 (0–48)	8.1 ± 15.6 (0–54)	
AC only	n	12	0	4	5	
	Time <sup>1</sup> ± SD (range)	25.2 ± 17.3 (0–54)	NA	12.0 ± 24.0 (0–48)	13.8 ± 23.4 (0–54)	
AC + LSIL/ HSIL	n	9	6	7	8	
	Time <sup>1</sup> ± SD (range)	30.7 ± 19.6 (6–60)	23.3 ± 20.1 (0–51)	5.6 ± 11.3 (0–30)	4.5 ± 8.0 (0–22)	

NA, not applicable.

<sup>1</sup>Average time of cytological diagnosis in months before the histological confirmation of adenocarcinoma or adenocarcinoma in situ.

before the histological diagnosis of endocervical neoplasia.

## DISCUSSION

In conclusion, although no single of the 38 cytomorphological features analysed could be associated specifically with EAC or AIS, the present study showed, that pathologists are, in fact, often able to differentiate neoplastic squamous lesions from neoplastic glandular lesions in cytology. There was not a single pap smear signed out with the diagnosis of atypical endocervical cells, favour neoplastic, AIS or adenocarcinoma among

histologically approved HSIL-only samples. Yet, in the series there were significant glandular extension of HSIL in 73% of the cases, which is known to be a common cause of false positive glandular diagnosis (24). Furthermore, among patients with EAC or AIS only in the final histology, the neoplastic diagnosis given was glandular in 70% of the cases.

In the further analysis of the cytomorphological features, a combination of palisading cell borders, nuclear pleomorphism and the lack of single atypical cells showed a positive association with EAC and AIS in pap smear samples taken up to five years before the histological diagnosis. A similar finding of a combination of cytological features predicting a positive finding in pap smears

diagnosed as AEC, NOS was also previously reported. In the study by Mariani et al. the combination of features included nuclear pleomorphism, as in the present study. In addition, nuclear enlargement, increased nuclear to cytoplasmic ratio and cells occurring in sheets and strips with cell crowding and nuclear overlap were described (25). In the study by Conrad et al., abundant tumour cellularity, nuclear size from 3 to 6 times normal, abundant 3-dimensional tumour cell groups, round cell shape and cytoplasmic neutrophils were reported in cases correctly diagnosed as adenocarcinoma in cytology (26).

In the present study, papillary groups and rosettes were seen in HSIL samples a year before the histological diagnosis. In a previous study by Rabelo-Santos et al., (9) papillary groups were reported to have a predictive value of 80 % for glandular neoplasia. In a study by Burja et al., (10) papillary groups were significantly associated with AIS in comparison to benign glandular lesions but did not differentiate AIS from squamous lesions. In the association of HSIL involving endocervical glands, round to oval clusters of abnormal cells with slightly irregular to smooth cell borders were described (27,28). Since there was a significant glandular extension of HSIL in the majority of our HSIL conizations, perhaps these were the cellular clusters interpreted as papillary. According to the Bethesda Criteria, papillary groups are not typical for HSIL (7). Rosettes associated with necrosis in HSIL extending into glands have been described (24), but in general, rosettes are not considered as a feature of HSIL involving glands (7,27–28).

In the studies with LBC samples cytologically diagnosed as glandular neoplasia, the PPV for any high-grade disease was reported to vary from 95.3% to 100% (29,30) and the PPV for endocervical neoplasia was reported to be 74.4% (29), which are in the same range as the best results seen with conventional pap smears.

In the study by Burnley et al., (29) LBC samples also showed higher PPVs in comparison to conventional smears both for high-grade lesions in general and also for endocervical malignancies, although the differences were not significant. The authors of the study described thin pseudostratified strips as a feature of glandular lesions often seen in LBC samples and also reported chromatin abnormalities to be more easily recognized in LBC samples.

Of the above studies by Conrad et al. and by Mariani et al., (26,25) studies were based on LBC samples. In the studies by Rabelo-Santos et al. and Burja et al. (9,10) on conventional pap smears, cytological features reported to be associated with

adenocarcinoma included pseudostratified strips, rosettes, palisading borders and papillary groups.

In summary, pseudostratified strips seem to be a feature recognized both in conventional and LBC samples. The 3-dimensional tumour cell groups can be interpreted as papillary groups and cells occurring in sheets and strips with cell crowding and nuclear overlap understood either as pseudostratified strips or fragments with palisading borders. In general, features recognized in conventional smears seem to be mainly architectural while in LBC samples also more cellular and nuclear features are seen.

In 25.5% of the cases in this study, there was a combined lesion of EAC or AIS and a squamous intraepithelial lesion, which is expected since most EACs and cervical squamous cell carcinomas share the same high-risk HPV-related aetiology (31–33).

During the study period, there was a change in the guidelines resulting in atypical glandular cells, NOS in cytology being sent straight to colposcopy (34). The colposcopy is known to have its limitations in the diagnostics of endocervical malignancies. Its sensitivity in detecting endocervical lesions has been reported to be even as low as 9.8% and the probability of significant lesion after a normal colposcopy as high as 87.5% (35,36).

Combining HPV testing to cytological sampling was shown to improve the diagnostic accuracy in cases with atypical endocervical cells in cytology and also to predict the outcome in conservatively treated *in situ* cases better than the pap smear only (36,37). Chen et al. reported a sensitivity of 91.0% and a specificity of 91.2% in diagnosing high-grade intraepithelial lesions and AIS or EAC among women with atypical endocervical cells in cytology and a positive high-risk HPV DNA result (37). Importantly, the combination of these two tests showed a high negative predictive value of 98.4% for the same lesions. In the study by Costa et al., (36) the combination of Pap smear and HPV test had a sensitivity of 90.0% and a negative predictive value of 88.9% at the first follow-up visit among patients treated for AIS by conization, and a sensitivity of 100% and a negative predictive value of 100% at the second follow-up.

In our study, the samples with high-grade cytological features were placed in the right diagnostic category according to the cell of origin in 100% of the cases in the HSIL group and in 70% of the cases in the EAC/AIS group. In the clinical practice, it can be argued that it does not matter whether the neoplastic cells are deemed of squamous or glandular origin as long as the patient is sent to a colposcopy. Recognizing and reporting atypical glandular cells, though, could guide the

colposcopist to specifically pay attention to the endocervical canal and lead to endocervical curettings and perhaps to an earlier diagnosis.

In our study, AEC, NOS was reported in 17 pap smears among patients with EAC/AIS only in the final histology and in 5 pap smears among patients with HSIL only in the final histology. The time range for those diagnoses varied from 121 months before the histological cancer diagnosis to the diagnostic '0 months' samples. Since before the change in the guidelines this diagnosis of AEC, NOS led only to a control pap smear sample instead of a colposcopy, it can only be speculated which of these prediagnostic pap smears already harboured a significant clinical lesion. Nevertheless, considering this very wide time range of presentation and also the above described challenges in this cytological diagnostic group, it would seem sensible with current guidelines, to accompany a pap smear with atypical glandular cells, NOS with a reflex HPV testing to avoid unnecessary procedures. On the other hand, among older women a large proportion of malignancies behind cytological glandular abnormalities are of endometrial origin, and in their diagnostics, HPV testing is not helpful (12–14).

As mentioned earlier, in Finland, there is a national screening programme for cervical cancer including women between ages of 30 and 60 and, in some municipalities, also women aged 25 and/or 65 years (2). Yet, of all the pap smear samples taken for screening purposes, only 40% were samples taken in the organized programme resulting in opportunistic screening accounting for 71% of the total screening costs annually (3). Since 55% of the EAC/AIS cases in the present study were diagnosed by clinical samples, of which only 11% were taken because of a patient-reported symptom, it is clear that screening is necessary in order to diagnose the endocervical glandular malignancies when they still are curable. Based on the previous studies from Finland, a national organized screening programme seems to be the most cost-effective way to do the screening (3,38).

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IK was supported by VTR grant.

## REFERENCES

1. <https://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/> (cited 05.03.2020).
2. <https://cancerregistry.fi/screening/cervical-cancer-screening/> (cited 05.03.2020).
3. Salo H, Nieminen P, Kilpi T, Auranen K, Leino T, Vänskä S, et al. Divergent coverage, frequency and costs of organised and opportunistic pap testing in Finland. *Int J Cancer*. 2014;135:204–13.
4. Shiliang L, Semenciw R, Mao Y. Cervical cancer: the increasing incidence of adenocarcinoma and adenosquamous carcinoma in younger women. *Can Med Assoc J*. 2001;164:1151–2.
5. Wilbur DC, Chhieng DC, Guidos B, Mody DR. Epithelial abnormalities: glandular. In: Nayar R, Wilbur DC, editors. *The Bethesda system for reporting cervical cytology*, 3rd ed. Cham: Springer, 2015.
6. Wilbur DC, Colgan TJ, Ferenczy AS, Hirschowitz L, Loening T, McCluggage WG, et al. Glandular tumors and precursors. In: Kurman RJ, Carcangiu ML, Herrington CS, Young RH, editors. *WHO Classification of Tumours of Female Reproductive Organs*, 4th edn, vol. 6. Lyon: International Agency for Research on Cancer, 2014;183–189.
7. Henry MR, Russel DK, Luff RD, Prey MU, Wright TC Jr, Nayar R. Epithelial abnormalities: squamous. In: Nayar R, Wilbur DC, editors. *The Bethesda System for Reporting Cervical Cytology*, 3rd ed. Cham: Springer, 2015.
8. Stoler M, Bergeron C, Colgan TJ, Ferenczy AS, Herrington CS, Kim KR, et al. Squamous cell tumors and precursors. In: Kurman RJ, Carcangiu ML, Herrington CS, Young RH, editors. *WHO Classification of Tumours of Female Reproductive Organs*, 4th ed., vol. 6. Lyon: International Agency for Research on Cancer, 2014;172–182.
9. Rabelo-Santos SH, Derchain SFM, Do Amaral Westin MC, Angelo-Andrade LAL, Sarian LOZ, Oliveira ERZM, et al. Endocervical glandular cell abnormalities in conventional cervical smears: evaluation of the performance of cytomorphological criteria and HPV testing in predicting neoplasia. *Cytopathology*. 2008;19:34–43.
10. Burja IT, Thompson SK, Sawyer WL Jr, Shurbaji MS. Atypical glandular cells of undetermined significance on cervical smears. *Acta Cytol*. 1999;43:351–6.
11. Zardo LMG, Thuler LCS, Zeferino LC, Horta NMSR, Fonseca RCSP. Performance of the cytologic examination for the diagnosis of endocervical adenocarcinoma in situ. *Acta Cytol*. 2009;53:558–64.
12. Kim M-K, Lee YK, Hong SR, Lim KT. Clinicopathological significance of atypical glandular cells on cervicovaginal pap smears. *Diagn Cytopathol*. 2017;45:867–72.
13. Pradhan D, Li Z, Ocque R, Patadji S, Zhao C. Clinical significance of atypical glandular cells in pap tests: an analysis of more than 3000 cases at a large academic women's center. *Cancer Cytopathology*. 2016;124:589–95.
14. Zhao C, Florea A, Onisko A, Austin RM. Histologic follow-up results in 662 patients with pap test findings of atypical glandular cells: results from a large academic womens hospital laboratory employing sensitive screening methods. *Gynecol Oncol*. 2009;114:383–9.
15. Kim HS, Underwood D. Adenocarcinoma in the cervicovaginal papanicolau smear: analysis of a 12-year experience. *Diagn Cytopathol*. 1991;7:119–24.
16. Geldenhuys L, Murray ML. Sensitivity and specificity of the pap smear for glandular lesions of the cervix and endometrium. *Acta Cytol*. 2007;51:47–50.



17. Lai C-R, Hsu C-Y, Tsay S-H, Li A. Clinical significance of atypical glandular cells by the 2001 Bethesda system in cytohistologic correlation. *Acta Cytol.* 2008;52:563–7.
18. Westin MC, Derchain SF, Rabelo-Santos SH, Angelo-Andrade LA, Sarian LO, Oliveira E, et al. Atypical glandular cells and adenocarcinoma in situ according to the Bethesda 2001 classification: cytohistological correlation and clinical implications. *Eur J Obstet Gynecol.* 2008;139:79–85.
19. Selvaggi SM. Glandular epithelial abnormalities on Thinprep® pap tests: clinical and cytohistologic correlation. *Diagn Cytopathol.* 2016;44:389–93.
20. Schoolland M, Segal A, Allpress S, Miranda A, Frost FA, Sterrett GF. Adenocarcinoma in situ of the cervix – sensitivity of detection by cervical smear. *Cancer Cytopathol.* 2002;96:330–7.
21. Krane JF, Granter SR, Trask CE, Hogan CL. Papanicolaou smear sensitivity for the detection of adenocarcinoma of the cervix: a study of 49 cases. *Cancer Cytopathol.* 2001;93:8–15.
22. Kares S, Veijalainen O, Kholová I, Tirkkonen M, Vuento R, Huhtala H, et al. High-risk HPV testing as the primary screening method in an organized regional screening program for cervical cancer: the value of HPV16 and HPV18 genotyping? *APMIS.* 2019;127:710–6.
23. Veijalainen O, Kares S, Kujala P, Tirkkonen M, Vuento R, Kholová I, et al. Human papillomavirus test with cytology triage in organized screening for cervical cancer. *Acta Obstet Gynecol Scand.* 2016;95:1220–7.
24. Kumar N, Bongiovanni M, Molliet M-J, Pelte M-F, Egger J-F, Pache J-C. Diverse glandular pathologies coexist with high-grade squamous intraepithelial lesion in cyto-histological review of atypical glandular cells on ThinPrep specimens. *Cytopathology.* 2009;20:351–8.
25. Mariani R, Grace C, Hughes K, Dietrich RM, Cabay RJ, David O. Can we improve the positive predictive value of atypical glandular cells not otherwise specified? *Diagn Cytopathol.* 2014;42:200–4.
26. Conrad RD, Liu AH, Wentzensen N, Zhang RR, Dunn ST, Wang SS, et al. Cytologic patterns of cervical adenocarcinomas with emphasis on factors associated with underdiagnosis. *Cancer Cytopathol.* 2018;121:950–8.
27. Selvaggi SM. Cytologic features of high-grade squamous intraepithelial lesions involving endocervical glands on ThinPrep® cytology. *Diagn Cytopathol.* 2002;26:181–5.
28. Selvaggi SM. Cytologic features of squamous cell carcinoma in situ involving endocervical glands in cervical cytobrush specimen. *Acta Cytol.* 1994;38:687–92.
29. Burnley C, Dudding N, Parker M, Parsons P, Whittaker CJ, Young W. Glandular neoplasia and borderline endocervical reporting rates before and after conversion to the Surepath™ liquid-based cytology (LBC) system. *Diagn Cytopathol.* 2011;39:869–74.
30. Thiryayi SA, Marshall J, Rana DN. An audit of liquid-based cervical cytology screening samples (ThinPrep and SurePath) reported as glandular neoplasia. *Cytopathology.* 2010;21:223–8.
31. Walboomers JMM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189:12–9.
32. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.* 2010;11:1048–56.
33. Pirog EC, Lloveras B, Molijn A, Tous S, Guimerà N, Alejo M, et al. HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. *Mod Pathol.* 2014;27:1559–67.
34. Current Care Working Group. Cytological changes in the cervix, vagina and vulva (online). Current Care Guideline. Working group set by the Finnish Medical Society Duodecim and the Finnish Colposcopy Association. Published 17.04.2019 [cited 05.03.2020]. Available at: [www.kaypahoito.fi](http://www.kaypahoito.fi)
35. Ullal A, Roberts M, Bulmer JN, Mathers ME, Wadehra V. The role of cervical cytology and colposcopy in detecting cervical glandular neoplasia. *Cytopathology.* 2009;20:359–66.
36. Costa S, Negri G, Sideri M, Santini D, Martinelli G, Venturoli S, et al. Human papillomavirus (HPV) test and PAP smear as predictors of outcome in conservatively treated adenocarcinoma in Situ (AIS) of the uterine cervix. *Gynecol Oncol.* 2007;106:170–6.
37. Chen L, Bin Y. Assessment of reflex human papillomavirus DNA testing in patients with atypical endocervical cells on cervical cytology. *Cancer Cytopathol.* 2008;114:236–41.
38. Lonnberg S, Anttila A, Luostarinen T, Nieminen P. Age-specific effectiveness of the Finnish cervical cancer screening programme. *Cancer Epidemiol Biomark Prev.* 2012;21:1354–61.