

Distribution and incidence of atypical glandular lesions in cervical cytology focusing on the association with high-risk human papillomavirus subtypes

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Abstract. Adenocarcinoma *in situ* (AIS) is considered a precursor of adenocarcinoma. Cervical adenocarcinoma has been associated with human papillomavirus (HPV), while other subtypes of AIS and endocervical adenocarcinoma have no precursor lesions and are not associated with HPV. Cervical cytology and HPV genotyping are important in the detection of these different subtypes. Notably, endometrial lesions and infiltration with secondary adenocarcinoma may lead to misdiagnosis of endocervical lesions. The aim of the present study was to avoid misdiagnosis of squamous cell changes and endometrial lesions as endocervical lesions in cervical screening. A total of 210,510 female cytological samples were analyzed between the beginning of January 2020 and the beginning of January 2021. The samples were processed for conventional cytological techniques, and for molecular detection and subtyping of high-risk HPV (HPV-HR) according to the advice and measurements of BD Biosciences (117,765 samples) and the PapilloCheck® HPV test (5,579 samples). The present study was carried out in Germany using the Munich classification III. II-g (Bethesda classification: atypical glandular cells not otherwise specified) was detected in 0.12% of cases under the age of 35 years. Another peak was noticed within the 41-60-years age group (0.11%). In the 41-50-years age group, a peak for II-e (Bethesda classification: Endometrial cells) (1.5%) was identified. An association was revealed between HPV16, HPV18 and HPV45 with cervical intraepithelial neoplasia III, AIS, endocervical adenocarcinoma and squamous cell carcinoma, in addition to other HPV-HR subtypes, such as HPV33/58, as well as 52, 56/59/66 in the different age groups. In patients aged <35 years, 0.03% of cases were vaccinated cases

against HPV. In the 35-40-years age group, there was only one vaccinated case (0.0045%); in the 41-50-years age group, there were 11 vaccinated cases (0.031%); and in the 51-60-years age group, there was one vaccinated case (0.002%). No patients aged >60 years were vaccinated against HPV in the analyzed cohort. In conclusion, most cases of HPV-associated glandular dysplastic changes and neoplasia occurred in sexually active women aged between 35 and 60 years. In addition, endocervical adenocarcinoma may occur at any age with or without an HPV infection.

Introduction

Cervical cancer is the fourth most common type of cancer in women according to the World Health Organization and a worldwide analysis from 2018 (1). Adenocarcinoma *in situ* (AIS) is considered a precursor of adenocarcinoma (2). The current global incidence rate of AIS is 6.6 per 100,000 individuals, with a mean age of 35-37 years at diagnosis (3). The proportion of cytological smears that display dysplasia and/or abnormalities varies considerably between countries, from 0.98 to 15.5% (4,5). Notably, histological diagnosis of AIS can develop from either glandular or squamous abnormalities (6).

In Germany, there are guiding rules regarding the performance of cervical screening for women and cytological diagnosis is graded according to the Munich classification III (7) (Table I). This classification system is quite similar to the Bethesda classification (8) but with some details in subgroup II-e, which is defined as when abnormal endometrial cells are detected in the cytology from women >40 years old in the second phase of the menstrual cycle. In addition, in the Muenchener classification III, there is a separation of high-grade squamous lesions into IIID2 and IVa-p, whereas in the Bethesda system, both moderate and high dysplasia are classified under one grade, high-grade squamous intraepithelial lesion (Table I) (8).

In Germany (9), under the age of 30 years, cervical screening depends mainly on conventional cytological methods, including either cervical smear or liquid-based techniques. Between the ages of 30 and 34 years, cervical screening is performed according to conventional methods, with the possibility of

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performing human papillomavirus (HPV) testing 6 months after conventional cytology in cases with atypical morphology (II-p, II-g and IIID1). From the age of 35 years, cervical screening consists of both conventional cytological methods and HPV testing. Other countries have investigated the differences between cervical screening methods, and countries such as Australia and the Netherlands perform cervical screening only with detection of high-risk HPV (HPV-HR) (10). Currently, different approaches are used as some researchers depend completely on the detection of HPV subtypes, focusing on HPV16 and HPV18, as the major causes for the development of squamous and glandular cervical lesions.

While assessing the literature, no study was identified that had examined the association between the presence of II-e or III-e cytology with histological findings in cervical biopsies. Furthermore, to the best of our knowledge, no previous study assessed the distribution of lesions, such as those with II-g and III-g cytology, in different age groups and their association with HPV subtypes. The present study assessed glandular lesions (II-g, II-e, III-g, III-e, IVa-g, IVb-g, V-g and V-e) in different age groups, and determined their association with HPV subtypes and the histopathological diagnosis of cervical biopsies. The aim of the present study was to avoid the misdiagnosis of squamous cell changes and endometrial glandular lesions as endocervical glandular lesions in the presence of metaplasia or atypia in cervical screening; therefore, a colposcopy and histopathological diagnosis of biopsies should follow cytological investigation in highly suspicious cases. The present study highlighted the importance of combining cytological screening and HPV genotyping as a predictive method in cases at risk of developing dysplastic, premalignant and malignant glandular lesions. The present study also aimed to ensure that screening by performing cytological examination and HPV genotyping could detect HPV-negative adenocarcinoma, HPV-negative gastric-type AIS and non-cervical adenocarcinoma (metastasis/infiltration). Vaccination against HPV infection should be further increased to protect women against dangerous subtypes.

Materials and methods

Patient samples, age and methods of investigation. At the Institute for Pathology and Cytology (Schüttorf, Germany), 210,510 female cytological samples were analyzed between the beginning of January 2020 and the beginning of January 2021. A total of 63,710 samples were from women <35 years old and 146,791 samples were from women ≥35 years old. The samples were processed for both conventional cytological techniques, and for molecular detection and subtyping of HPV-HR according to the advice and measurements of BD Biosciences (117,765 samples) using Viper-Test and the PapilloCheck® HPV test (5,579 samples). The difference of ~87,000 samples resulted either from women <35 years old without medical reasons to detect the HPV subtypes because they exhibited normal cytology (grade I, according to Munich classification) or they were not tested at our institute or due to samples with few cells.

Study approach. The present study included the cases (n=123,344) for which both conventional cytology and HPV

molecular analysis are available. According to BD Biosciences, certain HPV-HR subtypes were studied, namely HPV16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 51, 39, 68, 73, 82, 53, 66, 70, 6, 40, 42, 43, 44/45, 33/58, 56/59/66 and 35/39/68. The present study focused on the HPV vaccination history of the patients and divided the dataset into the following age groups: <35, 35-40, 41-50, 51-60 and >60 years. In these groups, the present study focused on the presence of atypical glandular lesions, their association with HR-HPV subtypes and the results of histopathology after colposcopy. The present study also investigated the presence of atypical, precancerous and cancerous endometrial glands in cervical cytology to detect the incidence of occurrence in different age groups and to compare with the presence of endocervical gland lesions. The grading of cytological diagnoses was performed according to the Muenchener classification III (Table I).

Ethics approval. The present study was approved by the Ethics Committee of the Medical Association, Hannover, Germany (approval no. Bo/14/2021). Informed consent for inclusion in the present study was waived as patient records were anonymized and retrospectively analyzed. The samples were anonymized to ensure data protection.

Results

Distribution of glandular lesions in relation to different age groups. In individuals <35 years old, 0.12% cases had II-g cytology. Another peak was noticed in the age groups 41-50 and 51-60 years (0.11%). There was a peak in the 41-50-years age group for II-e (1.5%). There were few cases of III-g cytology in all age groups, with the maximum detected in the 41-50-years age group (0.023%). There were more cases of III-e in the 51-60 and >60-years age groups (0.12%). A peak for IVa-g was observed in the 41-50-years age group (0.028%). V-g and V-e cytology were highest in the >60-years age group (0.012 and 0.016%, respectively) (Table II; Fig. 1).

Vaccine status, HPV status and histopathological diagnosis in the different age groups. Among individuals <35 years old, 0.03% were vaccinated (Table III). There were four cases with IVa-g (two cases), IVb-g (one case) and V-g (one case), and no endometrial carcinoma (V-e) was detected in this age group. Among individuals aged 35-40 years old, there was prominence of II-g (0.126%) (Table IV). Notably, there was only one vaccinated case in this group (35-40 years old). In addition, in this age group, the following HPV-HR subtypes were detected: 16, 18, 45, 56/59/66, 35/39/68 and 52. There was no IVb-g, V-g or V-e cytology detected in this age group. There were two cases negative for HPV-HR subtypes, and with histopathological diagnoses of cervical intraepithelial neoplasia (CIN) II and CIN III. Among individuals aged 41-50 years old, there was prominence of II-e (1.54%) (Table V). There was only one vaccinated case (0.0028%) in this group. In addition, in this age group, there was one case negative for HPV-HR but that was histopathologically diagnosed with AIS. Among individuals aged 51-60 years old, there was prominence of II-e (0.73%) and there was one vaccinated case in this group (Table VI). In addition, in this age group, the following HPV-HR subtypes were detected: 16, 18, 45, 51, 52, 31, 33/58,

Table I. Association of Muenchener classification III with Bethesda classification.

Group	Definition	Bethesda classification
0	Not enough material	Unsatisfactory for evaluation
I	No abnormality	NILM
II-a	No abnormality with a history of abnormal cytological diagnosis	NILM
II-p	Squamous cells with morphological changes but not CIN I	ASC-US
II-g	Endocervical glands with morphological changes e.g., irritation	AGC endocervical NOS
II-e	Endometrial glands in women >40 years old in second phase of menstrual cycle	Normal endometrial cells
III-p	Atypical squamous cells: CIN II/III or squamous cell carcinoma are to be considered	ASC-H
III-g	Atypical glandular cells: AIS/Adenocarcinoma are to be considered	AGC endocervical favor neoplastic
III-e	Abnormal endometrial glands especially postmenopausal	AGC endometrial
III-x	Anormal glands not endocervical or endometrial	AGC favor neoplastic
IIID1	Squamous cells with mild dysplasia (CIN I)	LSIL
IIID2	Squamous cells with moderate dysplasia (CIN II)	HSIL
IVa-p	Squamous cells with high dysplasia (CIN III)	HSIL
IVa-g	Endocervical cells with high dysplasia (AIS)	AIS
IVb-p	Squamous cells with high dysplasia that may be squamous cell carcinoma	HSIL with features of invasion
IVb-g	Endocervical cells with high dysplasia (AIS) and may be invasion	AIS with features of invasion
V-p	Squamous cell carcinoma	Squamous cell carcinoma
V-g	Endocervical adenocarcinoma	Endocervical adenocarcinoma
V-e	Endometrial adenocarcinoma	Endometrial adenocarcinoma
V-x	Other type of malignancy	Other malignant neoplasms

NILM, negative for intraepithelial lesion or neoplasm; ASC-US, atypical squamous cells of undetermined significance; AGC endocervical NOS, atypical glandular cells not otherwise specified; ASC-H, atypical squamous cells of undetermined significance, cannot exclude HSIL; AGC endocervical favor neoplastic, atypical glandular endocervical cells favor neoplastic; AGC endometrial, atypical glandular endometrial cells; AGC favor neoplastic, atypical glandular cells favor neoplastic; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AIS, adenocarcinoma *in situ*; CIN, cervical intraepithelial neoplasia.

Table II. Distribution of glandular lesions in relation to different age groups.

Age group (number of patients)	II-g	II-e	III-g	III-e	IVa-g	IVb-g	V-g	V-e
<35 years old (63,710)	81	0	7	1	2	1	1	0
35-40 years old (22,136)	28	0	3	1	9	0	0	0
41-50 years old (34,667)	40	534	8	23	10	0	1	0
51-60 years old (41,276)	38	302	7	59	8	3	0	4
>60 years old (48,721)	11	78	1	61	2	0	6	8

35/39/68 and 56/59/66. A total of 10 cases of endometrial carcinoma and 19 cases with molecular pathological detection of HPV-HR (HPV18 and 52) and without histopathological dysplasia were detected. Among individuals aged >60 years old, there were more cases of III-e (0.125%) and no vaccinated cases (Table VII). In addition, in this age group, the following HPV-HR subtypes were detected: 16, 35/39/68, 33/58, 18, 52 and 56/59/66. Endometrial carcinoma was detected in 21 cases and endocervical adenocarcinoma in five cases, and there were single cases of CIN I, CIN III and squamous cell carcinoma detected.

Association between glandular lesions of endocervical glands and the different age groups. The prevalence of III-g cytology (15 cases) was high in the 41-60-years age group (0.02%), whereas the prevalence of IVa-g cytology was high in all age groups (0.04% in the 35-40-years age group; 0.028% in the 41-60-years age group). Furthermore, V-g cytology was detected in the <35-years (one case), 41-50-years (one case) and >60 years (six cases) age groups, which may indicate that there is no direct relationship between precursor non-invasive lesions of endocervical glands and invasive endocervical glandular lesions in almost all age groups (Table VIII; Fig. 2).

Table III. Vaccination status, HPV status and histopathological diagnosis in women <35 years old.

Variable	II-g	II-e	III-g	III-e	IVa-g	IVb-g	V-g	V-e
Total patients (n=63,710)	81 ^a	0	7	1	2 ^b	1	1	0
Vaccinated, n	20	0	1	0	1	0	0	0
Not vaccinated, n	60	0	6	1	0	0	0	0
HPV status, n (HPV type)	70 (n=1)	0	0	0	0	0	0	0
Histopathology	0	0	AIS (n=1), WD (n=6)	0	WD (n=2)	Sq.c.ca. (n=1)	endo.aden. (n=1)	0

^aA total of 20 cases were vaccinated cases, 60 were not vaccinated, and one case was unknown regarding vaccination status; ^ba total of two cases, one case was vaccinated and one case was unknown regarding vaccination status. HPV, human papillomavirus; AIS, adenocarcinoma *in situ*; WD, without dysplasia; Sq.c.ca, squamous cell carcinoma; endo.adeno., endocervical adenocarcinoma.

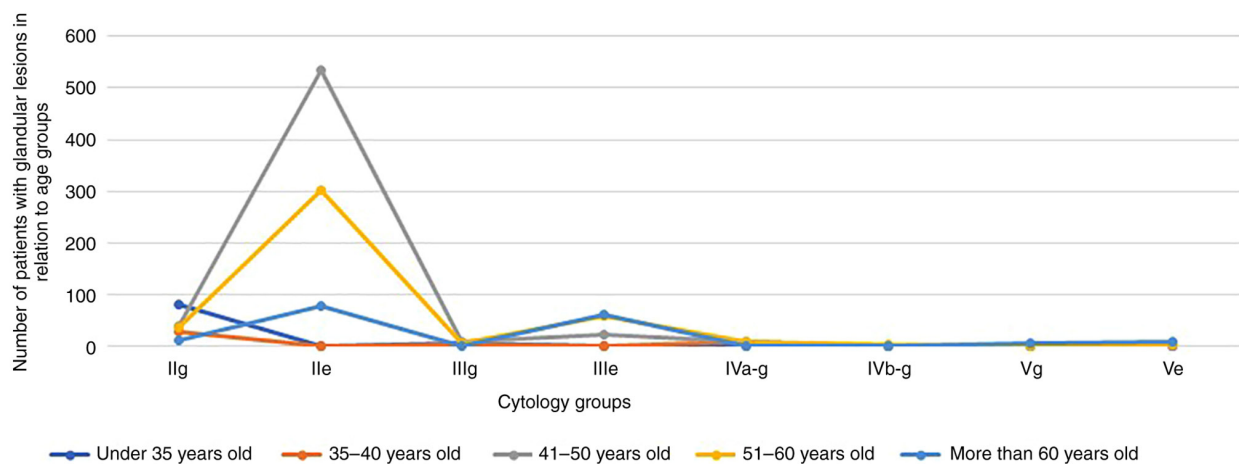


Figure 1. Prevalence of glandular lesions in all investigated age groups.

Furthermore, different HPV-HR subtypes were detected in all investigated age groups which may be due to sexual activity, as HPV is mainly transmitted sexually. In individuals >60 years old, only the following HPV subtypes were detected: 16, 33/58, 18, 52 and 56/59/66, which may indicate the persistence of these subtypes throughout life and the hazardousness of these subtypes, due to the higher incidence of invasive cervical carcinoma in older age groups (Table IX).

There was an association identified between HPV16, HPV18 and HPV45 with CIN III, AIS, endocervical adenocarcinoma and squamous cell carcinoma. In addition, other HPV-HR subtypes, such as HPV 33/58 (>40 years old), as well as 52 (>35 years old), 56/59/66 (>35 years old) were associated with the occurrence of CIN II and CIN III in the different age groups (Tables IV-VI).

Discussion

The current retrospective study based on 210,510 cases included not only dysplastic, premalignant and malignant endocervical glandular lesions, but also reactive changes of endocervical glands. In addition, endometrial reactive changes were included, as well as premalignant and malignant changes of the endometrium. This was not only to monitor the endometrial

changes in the cases assessed, but also as a control group for endocervical gland changes, to avoid misinterpretation and overinterpretation, which have been noticed in previous works as reported by Schnatz *et al* (11) and Risse *et al* (12) that have described the dysplastic changes in the endocervical epithelium and atypical glandular lesions. The present study also included the vaccination status of individuals against HPV, as until now, to the best of our knowledge, the importance of vaccination has not been well understood. Because of the importance of vaccination to protect women from HPV infection, the study also included the status of HPV-HR infection. The present study aimed to provide an overview of the most common HPV subtypes, which were found in the examined age groups. Finally, histological diagnosis was included in the present study as a gold standard to compare with and also to control the cytological findings.

Schnatz *et al* (11), DeSimone *et al* (13) and Marques *et al* (14) previously reported an II-g incidence (Atypical glandular cells not otherwise specified in Bethesda classification) of 0.1-2.1% in investigated women. In the present study, the II-g prevalence ranged from 0.09% (51-60-years age group) to 0.12% (>35-years age group). A cohort study from Sweden reported that women with II-g in cervical screening had a higher risk of cervical carcinoma incidence, particularly adenocarcinoma,

Table IV. Vaccination status, HPV status and histopathological diagnosis in women 35-40 years old.

Variable	II-g	II-e	III-g	III-e	IVa-g	IVb-g	V-g	V-e
Total patients (n=22,136)	28	0	3	1 ^a	9	0	0	0
Vaccinated, n	0	0	0	0	1	0	0	0
Not vaccinated, n	28	0	3	0	8	0	0	0
HPV status	18 (n=1), 45 (n=2), 56/59/66 (n=1), 35/39/68 (n=1)	0	68 (n=1), 56/59/ 66 (n=1), 45 (n=1)	0	52 (n=1), 18 (n=1), 18 and 31 (n=1), 16 (n=1)	0	0	0
Histopathology	CIN II with HPV18 (n=1)	0	CIN II with HPV45 (n=1)	WD (n=1)	AIS with HPV18 (n=1), CIN III with HPV16 (n=1)	0	0	0
Histopathology negative for HPV-HR	0	0	0	0	CIN III (n=1), CIN II (n=1), WD (n=2)	0	0	0

AIS, adenocarcinoma *in situ*; HPV-HR, high-risk human papillomavirus; CIN, cervical intraepithelial neoplasia; WD, without dysplasia.

^aA case with III-e had unknown vaccination status.

compared with women with high-grade squamous lesions (15). In the present study, cytologically, the highest incidence of II-g was in women <35 years old (0.12%), whereas the highest incidence of V-g (invasive adenocarcinoma of endocervical epithelium) was in women >60 years old (0.012%). The highest incidence of cytological dysplastic changes (III-g) was 0.016% in the 51-60-years age group, whereas the lowest incidence of III-g was 0.002% in women >60 years old, which may indicate that the dysplastic changes have reduced with age, whereas the incidence of invasive cervical adenocarcinoma has increased with age, which may explain the finding of the previous study (15). The incidence of premalignant changes (IVa-g), which is equivalent to AIS, was lowest in women <35 years old (0.003%) but was higher in women in the 35-40-years (0.04%) and 41-50-years (0.02%) age groups. Furthermore, IVa-g was again lower in women in the 51-60-years (0.019%) and >60-years (0.004%) age groups, which may suggest that there are numerous factors that lead to invasive cervical lesions in the older age groups.

The present study also assessed the vaccination status of individuals against HPV and HPV-infection status. The vaccination status against HPV infection was low in all age groups: <35 years, 0.034%; 35-40 years, 0.004%; 41-50 years, 0.002%; 51-60 years, 0.002%; and >60 years, 0%. The HPV infection status was lowest in women <35 years old (0.001%; one case out of 63,710 cases), whereas it was higher in the 35-40 and 41-50-years age groups (0.06 and 0.09% respectively; common subtypes: HPV16, 18, 31, 45, 51, 52, 68, 56/59/66, 35/39/68 and 33/58), and relatively lower in the 51-60-years age group (0.05%) than younger age groups (<50 years old) and even lower in the >60-years age group (0.016%), with common HPV-subtypes including HPV 16, 18, 31, 45, 51, 52, 33/58, 35/39/68 and 56/59/66, which may point to the highest incidence of HPV in ages (<50 years old) associated with higher sexual activity. The similarity of HPV subtypes between the younger and older age groups may indicate persistence of some subtypes of HPV and may indicate the relative risk of developing cervical cancer in older ages; this partially agrees with the key findings

of Graue *et al* (6). This previous study stated that there is a high risk of cervical adenocarcinoma following atypical glandular changes. The present study may add to this key message that atypical glandular changes could be associated with HPV subtypes. Pirog *et al* (16) and Holl *et al* (17) reported that most cases of cervical adenocarcinoma are related to persistent infection with oncogenic HPV-HR subtypes. The present study identified other HPV subtypes than Ronnett *et al* (18) and Pirog *et al* (16), who documented only HPV16, 18 and 45, and reported that these glandular lesions with HPV association were common in younger patients <40 years old.

The present study included histopathological diagnosis as the gold standard and to compare with and control the cytological findings. In women <35 years, there were seven cases with III-g, one of which was confirmed as having AIS by biopsy. In the same age group, there was one case of V-g which was not associated with HPV infection. In the 35-40-years age group, there were 28 cases with II-g, one of which was diagnosed with CIN II with HPV18; three cases with III-g, one of which was diagnosed with CIN II with HPV45; nine cases with IVa-g, one of which was diagnosed with AIS with HPV18, another one was diagnosed with CIN III with HPV16, and two cases were diagnosed as CIN II and CIN III without HPV infection. These findings indicated that HPV infection may be an associated infection in some cases, but that it may not have initiated aetiology in several other cases, which was consistent with Schiffmann M and de Sanjose (19). In the 41-50-years age group, there were eight cases with III-g, one of which was diagnosed with CIN II with HPV52, one with CIN III with HPV31 and 52, one with endocervical adenocarcinoma with HPV18 and one with AIS without HPV infection; 10 cases with IVa-g, one of which was diagnosed with CIN III with HPV18, one with AIS with HPV18, one with AIS with HPV16 and 31, one with CIN III with HPV52 and 33/58, one with CIN III with HPV35/39/68, one with endocervical adenocarcinoma with HPV16 and one with squamous cell carcinoma with HPV16; and one case with V-g, which was diagnosed with invasive carcinoma without HPV infection.

Table V. Vaccination status, HPV status and histopathological diagnosis in women 41-50 years old.

Variable	II-g	II-e	III-g	III-e	IVa-g	V-g
Total patients (n=34,667)	40	534	8	23	10	1
Vaccinated, n	0	0	0	0	1	0
Not vaccinated, n	40	534	8	23	9	1
HPV status	56/59/66 (n=1)	16 (n=3), 18 (n=2), 31 (n=5), 52, 56/59/66 (n=1), 51 (n=1), 33/58 (n=1), 56/59/66 (n=2), 35/39/68 (n=3)	18 (n=2), 52 (n=1), 31 and 52 (n=1)	16 (n=1)	16 (n=2), 18 (n=3), 52 and 33/58 (n=2), 16 and 31 (n=1), 35/39/68 (n=1)	0
Histopathology	WH (n=1)	WH (n=17)	endo.ca with HPV18 (n=1), WD with HPV18 (n=1), CIN II with HPV52 (n=1), CIN III with HPV31 and 52 (n=1), AIS with HPV-HR (n=1)	Sq.c.ca with HPV16 (n=1), WD (n=1), WD with HPV-HR (n=22)	sq.c.ca with HPV16 (n=1), endo.ca. with HPV16 (n=1), CIN III with HPV18 (n=1), AIS with HPV18 (n=1), AIS with HPV16 and 31 (n=1), CIN III with HPV52 and 33/58 (n=1), CIN III with HPV35/39/68 (n=1)	endo.ca.

WH, without histopathological biopsy; WD, without dysplasia; HPV-HR⁺, negative for HPV-HR; HPV-HR⁻, high-risk human papillomavirus; CIN, cervical intraepithelial neoplasia; Sq.c.ca, squamous cell carcinoma; AIS, adenocarcinoma *in situ*; endo.ca, endocervical adenocarcinoma.

Table VI. Vaccination status, HPV status and histopathological diagnosis in women 51-60 years old.

Variable	II-g	II-e	III-g	III-e	IVa-g	IVb-g	V-e
Total patients (n=41,276)	38	302	7	59	8	3	4
Vaccinated, n	0	1	0	0	0	0	0
Not vaccinated, n	38	301	7	59	8	3	4
HPV status	16 (n=1), 52 (n=1), 35/39/68 (n=1), 56/59/66 (n=1), 16 and 56/59/66 (n=1)	35/39/68 (n=2), 56/59/66 (n=2), 45 (n=1), 18 (n=3), 51 (n=1), 31 (n=1)	45, 33/58 (n=2)	0	18 (n=4), 16 (n=1), 52 (n=1)	18 (n=2), 16 (n=1)	16 (n=1)
Histopathology	WH with HPV 16, 52, 35/39/68, 56/59/66 and 16 and 56/59/66 (n=5)	WH with HPV18 (n=3), 31 (n=1), 45 (n=1), 51 (n=1), 35/39/68 (n=2) and 56/59/66 (n=2). CIN II with HPV-HR ⁻ (n=1)	Sq.c.ca with HPV45 (n=1), WH with HPV33/58 (n=1), CIN I with HPV-HR ⁻ (n=2), WD with HPV-HR ⁻ (n=1) CIN II with HPV-HR ⁻ (n=1)	WD (n=27), end.ca. (n=1), endomet.ca. (n=7)	AIS with HPV18 (n=1), CIN II with HPV18 (n=1), WD with HPV18 (n=1), AIS with HPV16 (n=1), WD with HPV52 (n=1)	endo.ca with HPV18 (n=1), endomet.ca with HPV16 (n=1), WD with HPV16 (n=1)	sq.c.ca with HPV16 (n=1), endomet.ca. with HPV-HR ⁻ (n=2) WD (n=1)

WH, without histopathological biopsy; WD, without dysplasia; HPV-HR⁻, negative for HR-HPV HPV-HR, high-risk human papillomavirus; CIN, cervical intraepithelial neoplasia; Sq.c.ca, squamous cell carcinoma; AIS, adenocarcinoma *in situ*; endo.ca, endocervical adenocarcinoma; endomet.ca, endometrial carcinoma.

Table VII. Vaccination status, HPV status and histopathological diagnosis in women >60 years old.

Variable	II-g	II-e	III-g	III-e	IVa-g	V-g	V-e
Total patients (n=48,721)	11	78	1	61	2	6	8
Vaccinated, n	0	0	0	0	0	0	0
Not vaccinated, n	11	78	1	61	2	6	8
HPV status	16 (n=1)	16 (n=1), 35/39/68 (n=)	0	16 (n=1)	33/58 (n=1)	18 (n=1), 52,56/59/66 (n=1)	35/39/68 (n=1)
Histopathology	WH with HPV16 (n=1)	endomet.ca with HPV 16 and 35//39/68 (n=2).	WH with HPV-V-HR ⁺ (n=1)	endomet.ca. with HPV-V-HR ⁺ (n=15), WH with HPV16 (n=1), endo.ca. with HPV-V-HR ⁺ (n=3)	CIN III with HPV33/58 (n=1), CIN I with HPV-V-HR ⁺ (n=1)	endomet.ca. with HPV 52 und 56/59/66 (n=1), endomet.ca. with HPV-V-HR ⁺ (n=2), Sq.c.ca with HPV18 (n=1)	endomet.ca. with HPV-V-HR ⁺ (n=2), endo. Ca. with HPV35/39/68 (n=1) WD with HPV-V-HR ⁺ (n=2), WH with HPV-V-HR ⁺ (n=3)

WD, without dysplasia; AIS, adenocarcinoma *in situ*; Sq.c.ca, squamous cell carcinoma; endo.ca, endocervical adenocarcinoma; endomet.ca, endometrial carcinoma; WH, without histopathological biopsy; HPV, human papillomavirus. HPV-V-HR⁺, negative for HPV-V-HR

Table VIII. Association between glandular lesions of endocervical glands and age.

Age group	II-g	III-g	IVa-g	V-g
<35 years old	81	7	2	1
35-40 years old	28	3	9	0
41-50 years old	40	8	10	1
51-60 years old	38	7	8	0
>60 years old	11	1	2	6

These findings provided further evidence of the association of HPV infection with the lesions, but not necessarily the initial aetiology. Notably, different HPV subtypes were associated with the same histological lesions and the same HPV subtype were associated with different invasive subtypes of carcinoma. In the 51-60-years age group, there were seven cases with III-g, one case was diagnosed was squamous cell carcinoma with HPV45, and two cases with CIN I and CIN II without HPV infection; eight cases with IVa-g, one case was diagnosed with CIN II with HPV18, one with AIS with HPV18 and one with AIS with HPV16; and three cases with IVb-g (equivalent to *in situ* lesion suspicious of already present invasive carcinoma in Bethesda classification), one of which was diagnosed with endocervical adenocarcinoma with HPV18. In the >60-years age group, there were two cases with IVa-g, which were diagnosed with CIN I with HPV33/58 and CIN III without HPV infection; six cases with V-g, two of which were diagnosed with endocervical adenocarcinoma with HPV18, 52 and 56/59/66, and one with squamous cell carcinoma without HPV infection, and another two cases were diagnosed with endometrial carcinoma without HPV infection. These findings differ from those of Clifford and Franceschi (20); this previous study stated that endocervical neoplasia was commonly associated (50-75%) with HPV18 and the remainder of lesions were associated with HPV16 and 45, whereas these findings agree with the previous study by Abbas *et al* (21).

To detect the prevalence of endometrial lesions in cervical screening and to determine the actual role of HPV infection in developing cervical lesions, the present study also included the other cytological groups, II-e, III-e and V-e. In individuals <35 years old, there were no cases of II-e or V-e; and there was one case (0.0015%) of III-e, without histopathological diagnosis or HPV infection. In the 35-40-years age group, there were no cases of II-e or V-e; and there was one case (0.004%) of III-e, without HPV infection and diagnosed without dysplasia. In the 41-50-years age group, there were 534 (1.54%) cases of II-e without histopathological diagnosis, with HPV infection in 17 cases (16, 18, 31, 52, 56/59/66, 51, 33/58 and 35/39/68); 23 cases (0.066%) of III-e, one of which was diagnosed with squamous cell carcinoma and HPV16, and 22 without dysplasia; and no cases of V-e. In the 51-60-years age group, there were 302 (0.73%) cases of II-e, 10 cases of which had HPV infection (35/39/68, 56/59/66, 31, 45, 18 and 51) without histopathological biopsy; 59 cases (0.14%) of III-e, all of them without HPV infection, one of which was diagnosed as endocervical adenocarcinoma and seven

Table IX. Association between glandular lesions and HPV status in the different age groups.

Cytology	Number of patients (HPV type)				
	<35 years old	35-40 years old	41-50 years old	51-60 years old	>60 years old
II-g	1 (70)	1 (18), 2 (45), 1 (56/59/66), 1 (35/39/68)	1 (56/59/66)	1 (16), 1 (52), 1 (35/39/68), 1 (56/59/66), 1 (16,56/59/66)	1 (16)
III-g	0	1 (68), 1 (56/59/66), 1 (45)	2 (18), 1 (52), 1 (31 and 52)	1 (45), 1 (33/58)	0
IVa-g	0	1 (52), 2 (18), 1 (16)	3 (18), 1 (16), 2 (33/58,52), 1 (16,31), 1 (35)	4 (18), 1 (16), 1 (52)	1 (33/58)
V-g	0	0	0	0	1 (18), 1 (52,56/59/66)

HPV, human papillomavirus.

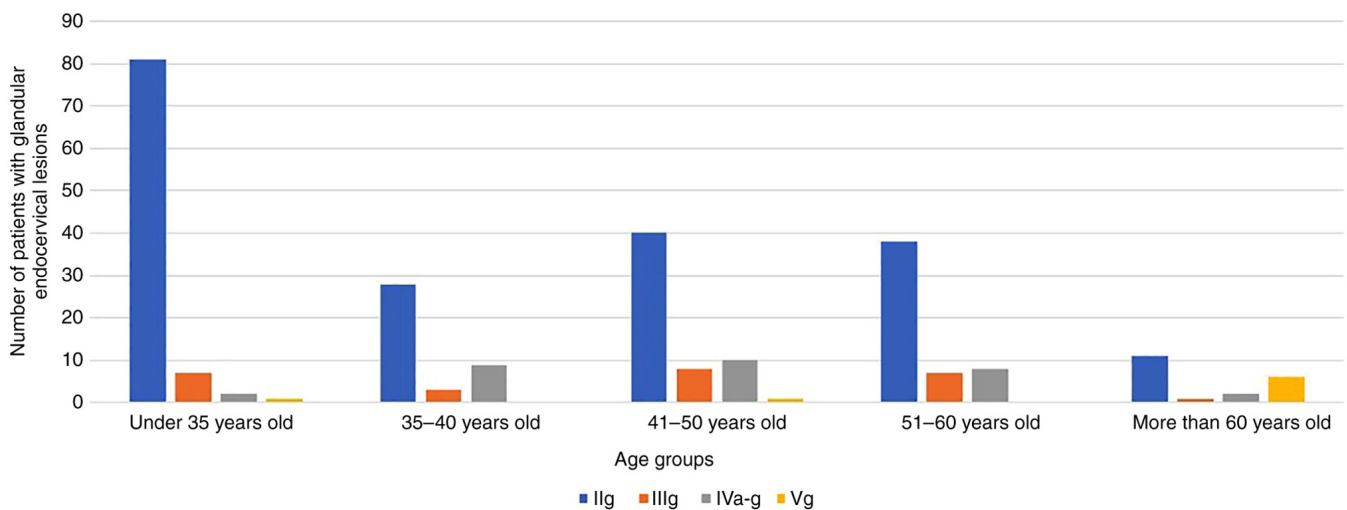


Figure 2. Patients with glandular lesions of endocervical glands separated by age.

with endometrial carcinoma; and four cases (0.009%) of V-e, only one of which had HPV16 and was diagnosed with squamous cell carcinoma, two of which were diagnosed with endometrial carcinoma and one without dysplasia. In the >60-years age group, there were 78 cases (0.16%) of II-e, two of which were diagnosed with endometrial carcinoma and two with HPV16 and 35/39/68, but without histopathological biopsy; 61 cases (0.125%) of III-e, one of which had HPV16 and was without dysplasia, 15 were diagnosed with endometrial carcinoma and without HPV-infection, and three were diagnosed with endocervical adenocarcinoma without HPV infection; and eight cases (0.0164%) of V-e, one of which had HPV35/39/68 and without dysplasia, and two were diagnosed with endometrial carcinoma. These findings indicated that there may be no relationship between HPV infection and endometrial lesions, but that endocervical lesions may be misdiagnosed under endometrial lesions as previously reported by Schnatz *et al* (11). Notably, endocervical carcinoma can occur without HPV infection and HPV

infection does not mean that there should necessarily be cervical epithelial changes.

In conclusion, endocervical glandular dysplastic changes usually occur in younger individuals aged between 35 and 60 years old, and may or may not be associated with HPV infection. Furthermore, endocervical adenocarcinoma can occur at any age in individuals with or without HPV infection. Squamous cell changes and endometrial lesions can be misdiagnosed as endocervical lesions in cervical screening; therefore, a histopathological investigation should follow in highly suspicious cases. The association of cytological screening and HPV genotyping is a predictive method in cases of risk for developing dysplastic, premalignant and malignant glandular lesions. The combination of both cytological examination and HPV genotyping is also important to detect HPV-negative adenocarcinoma, AIS from gastric type with negativity for HPV and non-cervical adenocarcinoma (metastasis/infiltration). Vaccination against HPV infection should be performed to protect women against dangerous subtypes. One of the limitations of the present study is the

lack of follow-up data for these cases; however, we aim to rectify this in future.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MA developed the idea of the work and study design, interpreted the results, drafted the manuscript and provided final approval. OB collected data and interpreted the results. JDJ interpreted the results and was involved in analysis of figures. MA and OB confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The approval was granted by the Ethics Committee of the Medical Association (Hannover, Germany). Informed consent for inclusion in the study was waived, as patient records were anonymized and retrospectively analysed. The samples were anonymized to ensure data protection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J and Bray F: Estimates of incidence and mortality of cervical cancer in 2018: A worldwide analysis. *Lancet Glob Health* 8: e191-e203, 2020.
- Sopracordevole F, Clemente N, Alessandrini L, Di Giuseppe J, Cigolot F, Buttignol M, Ciavattini A and Canzonieri V: Detection of occult endocervical glandular dysplasia in cervical conization specimens for squamous lesions. *Pathol Res Pract* 213: 210-216, 2017.
- Teoh D, Musa F, Salani R, Huh W and Jimenez E: Diagnosis and management of adenocarcinoma in situ: A society of gynecologic oncology evidence-based review and recommendations. *Obstet Gynecol* 135: 869-878, 2020.
- Engineer AD and Misra JS: The role of routine outpatient cytological screening for early detection of carcinoma of the cervix in India. *Diagn Cytopathol* 3: 30-34, 1987.
- Ranabhat SK, Shrestha R and Tiwari M: Analysis of abnormal epithelial lesions in cervical Pap smears in Mid-Western Nepal. *J Pathol Nepal* 1: 30-33, 2011.
- Graue R, Lönnberg S, Skare GB, Sæther SMM and Bjørge T: Atypical glandular lesions of the cervix and risk of cervical cancer. *Acta Obstet Gynecol Scand* 99: 582-590, 2020.
- Marquardt K, Schenk U and Griesser H: Muenchen classification for gynaecological cytological diagnoses. *Verband deutscher cytologisch tätiger Assistenten e.V., Langen*, 2014 (In German).
- Griesser H, Marquardt K, Jordan B, Kühn W, Neis K, Neumann HH, Bollmann R, Pöschel B, Steiner PM and Schenck U: Münchner nomenklatur III. *Frauenarzt* 11: 2-7, 2013.
- Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF (Hrsg): S3-Leitlinie Prävention des Zervixkarzinoms. Langversion 1.0-Dezember 2017. AWMF-Registernummer 015/027OL. https://www.awmf.org/uploads/tx_szleitlinien/015-027OL_Praevention_Zervixkarzinom_2018-01.pdf (Last accessed on 9 January 2019).
- Hillemanns P: Krebsfrüherkennung: Zervixkarzinom-doppelter paradigmwechsel. *Dtsch Arztebl* 113: A282-A285, 2016.
- Schnatz PF, Guile M, O'Sullivan DM and Sorosky JI: Clinical significance of atypical glandular cells on cervical cytology. *Obstet Gynecol* 107: 701-708, 2006.
- Risse EK, Ouwerkerk-Noordam E and Boon ME: Endometrial cells in liquid-based cervical cytology: A diagnostic pitfall solved by preparing cytohistology from the residual thin layer sample. *Acta Cytol* 55: 327-333, 2011.
- DeSimone CP, Day ME, Tovar MM, Dietrich CS III, Eastham ML and Modesitt SC: Rate of pathology from atypical glandular cell Pap tests classified by the Bethesda 2001 nomenclature. *Obstet Gynecol* 107: 1285-1291, 2006.
- Marques JP, Costa LB, Pinto AP, Lima AF, Duarte ME, Barbosa AP and Medeiros PL: Atypical glandular cells and cervical cancer: Systematic review. *Rev Assoc Med Bras* (1992) 57: 234-238, 2011.
- Wang J, Andrae B, Sundström K, Ström P, Ploner A, Elfström KM, Arnheim-Dahlström L, Dillner J and Sparén P: Risk of invasive cervical cancer after atypical glandular cells in cervical screening: Nationwide cohort study. *BMJ* 352: i276, 2016.
- Pirog EC, Lloveras B, Molijn A, Tous S, Guimerà N, Alejo M, Clavero O, Klaustermeier J, Jenkins D, Quint WG, *et al*: HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. *Mod Pathol* 27: 1559-1567, 2014.
- Holl K, Nowakowski AM, Powell N, McCluggage WG, Pirog EC, Collas De Souza S, Tjalma WA, Rosenlund M, Fiander A, Castro Sánchez M, *et al*: Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: Results from a European multinational epidemiological study. *Int J Cancer* 137: 2858-2868, 2015.
- Ronnett BM, Manos MM, Ransley JE, Fetterman BJ, Kinney WK, Hurley LB, Ngai JS, Kurman RJ and Sherman ME: Atypical glandular cells of undetermined significance (AGUS): Cytopathologic features, histopathologic results, and human papillomavirus DNA detection. *Hum Pathol* 30: 816-825, 1999.
- Schiffmann M and de Sanjose S: False positive cervical HPV screening test results. *Papillomavirus Res* 7: 184-187, 2019.
- Clifford G and Franceschi S: Members of the human papillomavirus type 18 family (alpha-7 species) share a common association with adenocarcinoma of the cervix. *Int J Cancer* 122: 1684-1685, 2008.
- Abbas M, de Jonge J and Bettendorf O: HPV-genotyping versus conventional cervical cytology as a screening method to detect dysplastic cervical epithelial changes. *Sci Rep* 12: 17828, 2022.



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