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HUMAN PAPILLOMAVIRUS-POSITIVE ATYPICAL GLANDULAR CELLS IN CERVICAL SCREENING: A HIGH-RISK FINDING.

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HUMAN PAPILLOMAVIRUS-POSITIVE ATYPICAL GLANDULAR CELLS IN CERVICAL SCREENING: A HIGH-RISK FINDING.

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Keywords: Adenocarcinoma, atypical glandular cells, cervical cancer screening, human papilloma-virus, liquid based cytology.

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Abbreviations: ADCA: adenocarcinoma; AGC: atypical glandular cells; AIS: adenocarcinoma in situ; CI: confidence interval; HPV: human papillomavirus; HSIL: high-grade squamous intraepithelial lesion; LBC: liquid based cytology; LSIL: low-grade squamous intraepithelial lesion; OR: odds ratio; PPV: positive predictive values; SCC: squamous cell cancer.

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WHAT THIS ARTICLE ADDS TO THE LITTERATURE:

What is already known on this topic:

- AGC found at cervical screening is associated with a high and persistent risk of cervical cancer for up to 15 years, particularly for ADCA and among women aged 30-39.
- A large proportion of AGC is negative for HPV.
- AGC may represent cancer precursor lesions, but may also signal benign conditions.

What this study adds:

- HPV positivity among AGC greatly increases (OR>40) the predictive ability for presence of high-grade cervical lesions.
- As >95% of the cervical high-grade lesions were found in HPV-positive AGC, the switch to HPV-based screening appears to be safe.
- An HPV-positive AGC has a very high PPV for a high-grade cervical lesion (about 60%), indicating that clinical management algorithms may need to be revised to minimize the risk that women will be lost to follow-up.

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ABSTRACT

Objectives: To determine how human papillomavirus (HPV) positivity of atypical glandular cells (AGC) affects the predictive values for presence of high-grade cervical lesions.

Design: Population-based cohort study.

Setting: Stockholm-Gotland region, Sweden.

Participants: Between 2014-02-17 and 2016-06-30, there were 562 women with AGC in a cervical smear in the region. Registry linkages up to 2016-06-30 identified that 392 women had also had an associated HPV test and a histopathological follow-up.

Main outcome measure: Presence of high-grade cervical lesions in the cervical biopsies taken after the AGC smear, in relation to the HPV status of the AGC-containing index smear.

Results: The proportion of HPV-positive AGC was 56% (n=222). In this group, there were 6 cases of invasive cervical adenocarcinoma, 33 cases of cervical adenocarcinoma in situ and 93 cases of high grade squamous intraepithelial lesion (HSIL), giving a positive predictive value (PPV) for a cervical lesion to treat of 60% (132/222). Among the 170 women with HPV-negative AGC, there was 1 invasive cervical squamous cell cancer and 4 HSIL, giving a PPV for a cervical lesion to treat of 2.9% (5/170). This group also contained 5 endometrial cancers and 1 breast cancer.

Conclusions: HPV triaging of AGC will greatly increase the predictive ability for cervical lesions to treat [Odds Ratio: 48.4 (95% Confidence Interval: 19.1-122.6)]

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and the high sensitivity (96%; 132/137 women) implies safety of primary HPV screening strategies. The measurable risk for endometrial cancer among women with HPV-negative AGC (2.9%) suggests that research on screening for endometrial cancer is needed.

Keywords: Adenocarcinoma, atypical glandular cells, cervical cancer screening, human papilloma-virus, liquid based cytology.

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Strengths of study:

- A large and population-based study, nested within an organized screening program that mandated identical clinical follow-up for HPV-positive and HPV-negative AGC.
- The entire cohort was followed using comprehensive registries.

Limitations of study:

- Not all women with AGC had had an HPV test performed and not everyone had a histopathology follow-up.
- Cytological diagnosis of AGC may be variable between settings.

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BACKGROUND

Organized cervical screening programs have resulted in a marked decline in the incidence of cervical squamous cell carcinomas (SCC) (1). However, the effect on invasive cervical adenocarcinoma (ADCA) (1) has been much less. Several countries now report that 20% or more of all remaining invasive cervical carcinoma are ADCA (2) and some studies have even reported an increasing incidence of cervical ADCA (3, 4). Whereas the precursor lesions of SCC and their management strategies are well recognized, the precursors of ADCA and their optimal management strategies are less clear. Cytological criteria for premalignant columnar cell lesions were recognized as late as in the mid 1990-ties (5). This increased the possibility to identify and treat such lesions. Women with a history of AGC have a greatly increased risk for later development of cervical cancer, probably because of persisting uncertainty regarding how to identify and manage these high-risk women (6).

Most countries have switched to the use of liquid-based cytology (LBC) and this sample allows for easier identification of AGC diagnosis (7, 8). In Sweden, atypical glandular cells (AGC) are reported in less than 0.3% of the cervical samples (9). AGC are not only signaling an ADCA precursor lesion, they may also be caused by benign conditions such as cervical polyps, hyperplasia and tubar metaplasia (10). AGC is a high-risk marker for high-grade squamous intraepithelial lesion (HSIL), SCC (11), adenocarcinoma in situ (AIS) or ADCA (12). AGC found at cervical screening is associated with a high and persistent risk of cervical cancer for up to 15 years, particularly for ADCA and among women aged 30-39 (6). The current management of AGC thus seems to not be optimally effective in preventing cervical cancer (6). Cervical cytology is primarily a screening test for squamous intraepithelial

lesions and SCC. Sensitivity for glandular lesions is more variable, because of both sampling and interpretation issues and because glandular lesions are less common, less well defined and may include also reactive conditions (13).

Human papilloma (HPV) reflex testing on ASCUS and low-grade squamous intraepithelial lesions (LSIL) is routinely used to increase the HSIL predictive value of referral to colposcopy (14). A systematic review found 12 studies of HPV testing in glandular lesions and found that about 40% of AGC were high-risk positive and that predictive values were increased if AGC was triaged with HPV testing (15). However, HPV-negative AGC above 50 years of age may contain a substantial number of non-cervical cancers (15) and the use of HPV triaging in the management of AGC is therefore not clear.

While cervical screening does not aim to detect endometrial carcinoma, occasionally the cervical sample will detect abnormal endometrial cells and may contribute to the earlier diagnosis of endometrial carcinoma (16). There are no guidelines or cost-effectiveness evaluations that consider a possible benefit of cervical screening on early diagnosis of endometrial carcinoma. Nevertheless, most guidelines recommend clinical follow-up of abnormal endometrial cells, should they be found (16).

As endometrial cancer and abnormal endometrial cells are negative for HPV (11) and as primary cervical screening with HPV is now a globally recommended practice (17), there is a concern that the switch to HPV-based screening may risk losing a possible benefit on early diagnosis of endometrial cancer. The organized cervical screening program of the Stockholm-Gotland region in Sweden decided on 2014-02-17 to introduce HPV triaging for all women with AGC, while retaining the same management guidelines for both HPV-positive and HPV-negative women with AGC.

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The present study included all cases of AGC in the region during 2014-02-17 to 2016-06-30. We determined how HPV triaging of AGC affected the predictive values for subsequent diagnosis of high-grade cervical or endometrial lesions.

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MATERIAL AND METHODS

Study population, data collection and analysis. To minimize risk for selection biases in inclusion or follow-up, we included all women who lived in the Stockholm-Gotland region of Sweden and had a primary cytology screening from 17. February 2014 to 30. June 2016. A comprehensive cervical screening registry was then used to identify all of these women who had an AGC diagnosis on their Pap test, if there had been an HPV test done and if there had been a subsequent histopathological diagnosis. HPV tests performed within 40 days after or before the AGC index sample [most HPV tests 378/392 (96%) were performed within 5 days of the AGC diagnosis] were considered to likely reflect the HPV status of the index sample.

The cervical LBC samples were transferred to cytology glass slides using a ThinPrep® 5000 processor (ThinPrep®, Hologic, Marlborough, MA, USA) and the remaining cell suspension was analysed for HPV DNA by the Cobas 4800 HPV test, with robotic decapping of ThinPrep vials (p480, Roche Molecular Diagnostic, Pleasanton, CA, USA). Qualitative detection of HPV DNA was obtained by amplification of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (with HPV 16 and HPV 18 in separate channels, the remaining HR-types reported as a group).

Cytological and histopathological classification used the SNOMED system (Standardised Nomenclature of Medical diagnoses). In case of several histopathological diagnoses, the most severe histological diagnosis was taken as outcome. Follow-up ended on 30 June 2016. Odds Ratios and confidence intervals were calculated using conditional logistic regression using EpiInfo (www.cdc.gov). Individual level data will be shared on request, to be sent to JD.

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Patient involvement

No patient was involved in setting the research question or the outcome measures. The participants were not involved in developing plans for recruitment, design, or implementation of the study. No patients were asked to advice on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community. Women in the intervention group were informed about their HPV and cytology results. Participants in the control group were only informed about their cytology results (regular screening).

Ethics. The study was approved by the regional ethical board of Stockholm, decision number 2016/1103-31.

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RESULTS

During 2014-02-17 to 2016-06-30, altogether 564 primary cervical cytology samples (ThinPrep®, Hologic) were diagnosed as AGC. For 76 (13%) samples, there was no associated HPV test. For 96 (17%) cases there was no histological follow-up. There were 392 (70%) women who had had an AGC that both had an associated HPV test and a subsequent histopathological follow-up. Mean age of these women: 38 years, range 23-86 (figure 1).

The risk of being HR-HPV positive decreased with increasing age: Women <40 years, 40-50 years and ≥50 years had 62%, 60% and 25% HPV-positive tests, respectively (table 1).

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Table 1: Histological follow-up after HPV-positive or HPV-negative AGC, by age.

	<40 year		40-50 year		>50 year		Total	
	HPV+	**HPV-**	**HPV+**	**HPV-**	**HPV+**	**HPV-**	**HPV+**	**HPV-**
WNL	38	50	12	55	6	22	56	127
LSIL	25	12	6	13	3	7	34	32
HSIL	71	1	20	2	2	1	93	4
SCC	0	0	0	0	0	1	0	1
AIS	26	0	6	0	1	0	33	0
ADCA cx	5	0	1	0	0	0	6	0
Tot	**165**	**63**	**45**	**70**	**12**	**31**	**222**	**164**
PPV	62%	1.6%	60%	2.8%	25%	6%	60%	3%

Abbreviations: WNL indicates with normal limits; LSIL=low grade intraepithelial lesion; HSIL=high-grade intraepithelial lesion, SCC=squamous cervical cancer; AIS=endocervical adenocarcinoma in situ; ADCA cx=adenocarcinoma cervix.

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Among the HPV-positive AGC, the subsequent histologies identified 6 (3%) cases of cervical ADCA, 33 (15%) cases of AIS and 93 (42%) cases of HSIL. The PPV for a lesion to treat was 132/222 (60 %). The corresponding figures for the HPV negative AGC group was 5 endometrial cancers, one cervical SCC and 4 HSIL, giving a PPV of 5/170 (3%) for a cervical high-grade lesion and 3% also for an endometrial cancer. HR-HPV was thus found in 132/137 cases (sensitivity 96%) with cervical high-grade lesions to treat [OR: 48.4 (95% CI: 19.1-122.6)] but in none of the 5 endometrial cancers (figure 2).

In the HPV positive group of 165 women younger than 40 year, 71 cases (41%) had HSIL, 26 cases (16%) had AIS and 5 cases (3%) were cervical ADCA. Among the 45 HPV-positive women between 40 and 50 years of ages, 20 cases (44%) were HSIL, 6 cases (13%) were AIS and 1 case (2%) was cervical ADCA. Among the 12 women older than 50 years, there were 2 (16%) HSIL and 1 (8%) AIS case.

All 5 endometrial ADCA were found among the 36 HPV-negative women >50 years of age (PPV: 14%) (table 2).

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Table 2: Histological follow-up after AGC in cytology

	Age, year				
	<40	**40-50**	**>50**	**Total**	**%**
WNL	88	67	28	183	(46.3)
LSIL	37	19	10	66	(16.8)
HSIL	72	22	3	97	(25.4)
SCC	0	0	1	0	(0.3)
AIS	26	6	1	33	(8.1)
ADCA, Cx	5	0	1	6	(1.5)
ADCA, endom	0	0	5	5	(1.3)
ADCA met	0	0	1	1	(0.3)
Total	**228**	**114**	**50**	**392**	
PPV	45%	25%	12%		

Abbreviations: WNL indicates with normal limits; LSIL=low grade intraepithelial lesion; HSIL=high-grade intraepithelial lesion, SCC=squamous cervical cancer; AIS=endocervical adenocarcinoma in situ; ADCA cx=adenocarcinoma cervix; ADCA endom=adenocarcinoma endometrial; ADCA met=adenocarcinoma metastas.

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DISCUSSION

Statement of main findings: The high predictive values for cervical lesions to treat of an HPV-positive AGC, indicates that ambitious clinical management algorithms with minimal risks for loss to follow-up would need to be followed.

The sensitivity of HPV-positivity for cervical high grade lesions, suggest that HPV-based screening strategies are safe with regards to catching AGC-associated cervical cancer precursor lesions.

The fact that all endometrial cancers were HPV negative suggest that further research is warranted on the possible benefit of cervical cytological screening on early diagnosis of endometrial cancer.

Strengths of study: The present study is large and population-based, nested within an organized screening program that mandated identical clinical follow-up for HPV-positive and HPV-negative AGC. The entire cohort was followed using comprehensive registries and the risk for ascertainment bias in follow-up is thus minimal. The setting also implies high generalizability.

Limitations of study: Not all women with AGC had had an HPV test performed, only 87%. About 70% of cervical samples in the region are taken as a result of an organized invitation with an appointment – the remainder being taken in other settings for example during clinical follow-up of cytological abnormalities referred from the screening program. Whereas it is straightforward to ensure adherence to policies for organized samples, it is more complicated for other settings and we consider an 87% compliance with the HPV-triaging policy as a high compliance.

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A substantial proportion of women (17%) had no histological follow-up. A nationwide audit found that lack of histological follow-up was present in about 1/3d of women with AGC (6). As this lack of follow-up was associated with very high risks of cancer (6), there is today a greatly increased awareness of the need for histological follow-up after AGC. For women with an index AGC close to the end of the study, lack of follow-up may simply reflect insufficient follow-up time in our study. However, late histopathologies not recorded in the study are not likely to be biased in relation to the outcomes of the study.

AGC is not an easily recognized entity and there may be differences in diagnostic practices between different laboratories. As the organized screening program of region Stockholm-Gotland uses a single laboratory (Karolinska University Laboratory), the laboratory of the present study can be characterized as a high-volume, highly specialized laboratory. It is thus not for certain that the predictive values found can be generalized to other settings.

Comparisons with others: The systematic review of Verdoodt et al (15) that identified 12 studies on HPV and AGC, reported that on average 40% of AGC were HPV-positive. We find a somewhat higher figure [56 % (n=222) of AGC were HPV-positive], which may be related to the fact that the program uses a single, specialized laboratory.

Castle et al (11) reported that all endometrial cancers detected were HPV-negative, which is in accordance with our results.

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CONCLUSION

The very high predictive values of an HPV-positive AGC indicates that current clinical management algorithms may need to be revised to minimize the risk that existing lesions may escape detection and in particular to minimize the risks that women will be lost to follow-up.

The high sensitivity of HPV-positivity for cervical high grade lesions among AGC suggests that the switch to HPV-based screening is safe with regards to catching AGC-associated cervical cancer precursor lesions.

Although the sensitivity is not 100%, the greatly increased PPV for the HPV-positive women with AGC that are referred implies that only HPV-positive AGC needs to be referred. If more stringent management algorithms are used for these women, this may increase safety although fewer women are referred. An example of this phenomenon has been reported from our randomized implementation of HPV triaging of ASCUS/LSIL samples, where the arm referring only HPV-positive ASCUS/LSIL found more high-grade lesions in spite of referring fewer women (18).

The HPV-negativity of endometrial cancers is in accordance with other studies (11) and indicates that with the ongoing switch to HPV-based screening, there will be no benefit of early diagnosis of endometrial cancers. It is not entirely clear if such occasionally found early diagnoses of endometrial cancer ever did involve a measureable health benefit. Further research to establish whether this was indeed the case seems warranted.

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IMPLICATIONS

With the switch to HPV-based cervical screening, there will no longer be any early detection of endometrial cancers and further studies to elucidate whether this should be remedied are warranted.

The new screening modality also will involve detecting only HPV-positive AGC. This involves a greatly increased PPV compared to AGC with unknown HPV status and with HPV-based screening the management guidelines for AGC would need to be substantially changed to reflect this.

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CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

CONTRIBUTORSHIP STATEMENT

IN: Retrieved and evaluated data, is guarantor of the study and coordinated the study. AH: Provided supervision. JD: Provided supervision and resources.

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All authors revired the manuscript for important intellectual content and have approved the final version for submission. IN affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

FUNDING STATEMENT

The study was funded by Stockholm County Clinical Research.

DATA SHARING STATEMENT

Individual level data is available from the corresponding author.

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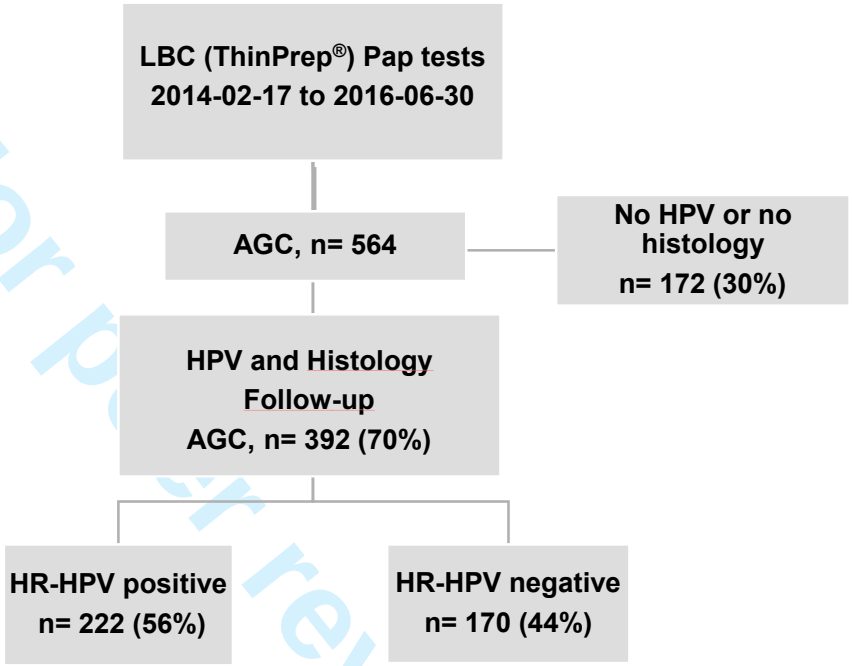
FIGURE LEGENDS:

Figure 1: The study flow chart



Abbreviations: LBC = Liquid-based cytology; Pap = Papanicolaou; AGC = atypical glandular cells; HR-HPV = high-risk human papillomavirus

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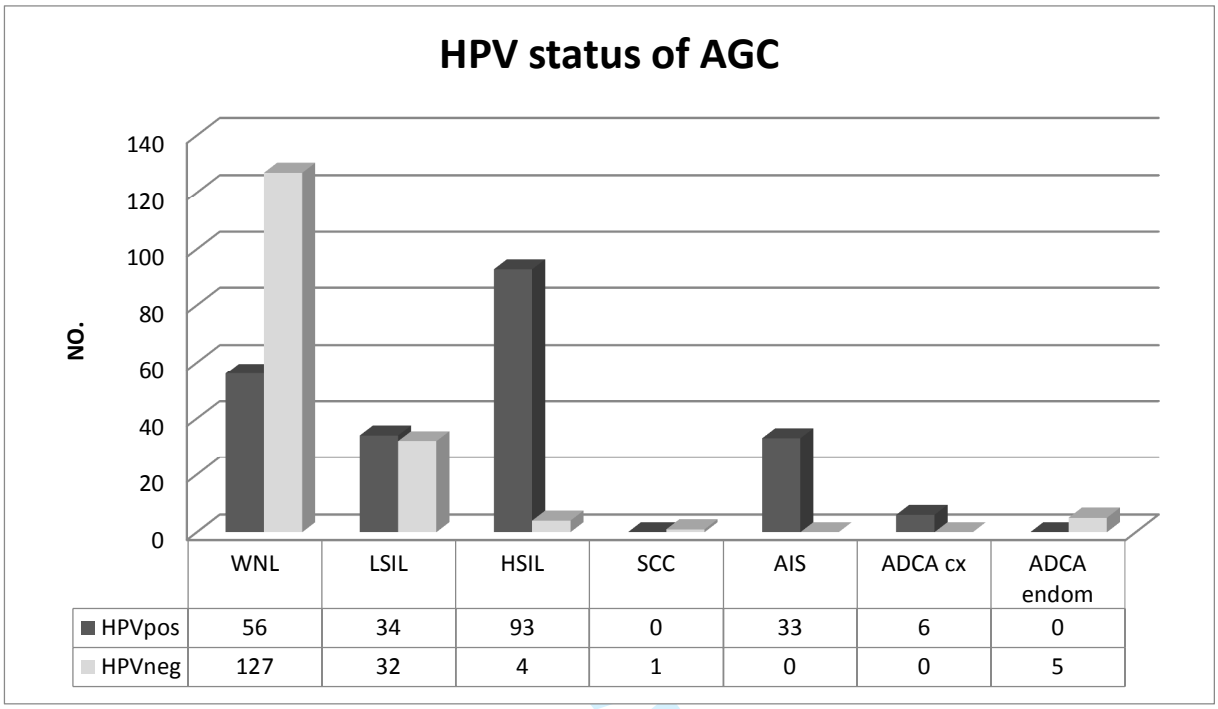
Figure 2: Histological findings in HPV positive and HPV negative AGC cases. Advanced cervical lesions were found in 60% of HPV positive AGC cases while only in 3% of the HPV negative ones, corresponding to a sensitivity of 96% (132/137).



	WNL	LSIL	HSIL	SCC	AIS	ADCA cx	ADCA endom
HPVpos	56	34	93	0	33	6	0
HPVneg	127	32	4	1	0	0	5

Abbreviations: WNL indicates with normal limits; LSIL=low grade intraepithelial lesion; HSIL=high-grade intraepithelial lesion, SCC=squamous cervical cancer; AIS=endocervical adenocarcinoma in situ; ADCA cx=adenocarcinoma cervix; ADCA endom=adenocarcinoma endometrial.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			**7**
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	N/A
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	9-10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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RISK OF HIGH-GRADE LESIONS AFTER ATYPICAL GLANDULAR CELLS IN CERVICAL SCREENING: A POPULATION-BASED COHORT STUDY

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Keywords: Adenocarcinoma, atypical glandular cells, cervical cancer screening, human papilloma-virus, liquid based cytology.

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Word count Text: 2830

Abbreviations: ADCA: adenocarcinoma; AGC: atypical glandular cells; AIS: adenocarcinoma in situ; CI: confidence interval; HPV: human papillomavirus; HSIL: high-grade squamous intraepithelial lesion; LBC: liquid based cytology; LSIL: low-grade squamous intraepithelial lesion; OR: odds ratio; PPV: positive predictive values; SCC: squamous cell cancer.

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ABSTRACT

Objectives: To determine how human papillomavirus (HPV) positivity of atypical glandular cells (AGC) affects the predictive values for presence of high-grade cervical lesions.

Design: Population-based cohort study.

Setting: Stockholm-Gotland region, Sweden.

Participants: Between 2014-02-17 and 2016-06-30, there were 562 women with AGC detected in a cervical smear. Registry linkages up to 2016-06-30 identified 392 women with an associated HPV test and a histopathological follow-up.

Main outcome measure: Presence of a high-grade cervical lesion in the cervical biopsy taken after the AGC smear, in relation to the HPV status of the AGC-containing index smear.

Results: The proportion of HPV-positive AGC was 56% (n=222). In this group, there were 6 cases of invasive cervical adenocarcinoma, 33 cases of cervical adenocarcinoma in situ and 93 cases of high-grade squamous intraepithelial lesion (HSIL), giving a positive predictive value (PPV) for a cervical high-grade lesion of 60% (132/222). Among the 170 women with HPV-negative AGC, there was 1 invasive cervical squamous cell cancer and 4 HSIL, giving a PPV for a cervical high-grade lesion of 2.9% (5/170). This group also contained 5 endometrial cancers and 1 breast cancer.

Conclusions: HPV triaging of AGC will greatly increase the predictive ability for identifying cervical high-grade lesions[Odds Ratio: 48.4 (95% Confidence Interval: 19.1-122.6)] and the high sensitivity (96%; 132/137 women) implies safety of primary

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HPV screening strategies. The measurable risk for endometrial cancer among women with HPV-negative AGC (2.9%) suggests that research on screening for endometrial cancer is needed.

Keywords: Adenocarcinoma, atypical glandular cells, cervical cancer screening, human papilloma-virus, liquid based cytology.

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Strengths of study:

- A large and population-based study, nested within an organized screening program that mandated identical clinical follow-up for HPV-positive and HPV-negative AGC.
- The entire cohort was followed using comprehensive registries.

Limitations of study:

- Not all women with AGC had had an HPV test performed and not everyone had a histopathology follow-up.
- Cytological diagnosis of AGC may be variable between settings.

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BACKGROUND

Organized cervical screening programs have resulted in a marked decline in the incidence of cervical squamous cell carcinomas (SCC) (1). However, the effect on invasive cervical adenocarcinoma (ADCA) (1) has been much less. Several countries now report that 20% or more of all remaining invasive cervical carcinoma are ADCA (2) and some studies have even reported an increasing incidence of cervical ADCA (3, 4). Whereas the precursor lesions of SCC and their management strategies are well recognized, the precursors of ADCA and their optimal management strategies are less clear. Cytological criteria for premalignant columnar cell lesions were recognized as late as in the mid 1990-ties (5). This increased the possibility to identify and treat such lesions. Women with a history of AGC have a greatly increased risk for later development of cervical cancer, probably because of persisting uncertainty regarding how to identify and manage these high-risk women (6).

Most countries have switched from conventional to liquid-based cytology (LBC), which has made it easier to identify AGC (7, 8). In Sweden, atypical glandular cells (AGC) are reported in less than 0.3% of cervical samples (9). AGC not only signal an ADCA precursor lesion, they may also be caused by benign conditions such as cervical polyps, hyperplasia, and tubar metaplasia (10). AGC is a high-risk marker for high-grade squamous intraepithelial lesion (HSIL), SCC (11), adenocarcinoma in situ (AIS), or ADCA (12). AGC found at cervical screening is associated with a high and persistent risk of cervical cancer for up to 15 years, particularly for ADCA and among women aged 30-39 (6). The current management of AGC thus does not seem to be optimally effective in preventing cervical cancer (6). Cervical cytology is primarily a screening test for squamous intraepithelial lesions and SCC. Sensitivity

for glandular lesions is more variable due to sampling and interpretation issues, and because glandular lesions are less common, less well-defined, and may also include reactive conditions (13).

Human papilloma (HPV) reflex testing on ASCUS and low-grade squamous intraepithelial lesions (LSIL) is routinely used to increase predictive value HSIL and referral to colposcopy (14). A systematic review found 12 studies of HPV testing in glandular lesions and found that about 40% of AGC were high-risk positive and that predictive values were increased if AGC was triaged with HPV testing (15). However, HPV-negative AGC above 50 years of age may contain a substantial number of non-cervical cancers (15) and the value of HPV triage in the management of AGC is therefore not clear.

While cervical screening does not aim to detect endometrial carcinoma, occasionally abnormal endometrial cells are detected in cervical samples and may lead to an earlier diagnosis of endometrial carcinoma (16). There are no guidelines or cost-effectiveness evaluations that consider a possible benefit of cervical screening on early diagnosis of endometrial carcinoma. Nevertheless, most guidelines recommend clinical follow-up of abnormal endometrial cells, should they be found (16).

As endometrial cancer and abnormal endometrial cells are negative for HPV (11) and as primary cervical screening with HPV is now a globally recommended practice (17), there is a concern that switching to HPV-based screening may result in losing a possible benefit of early diagnosis of endometrial cancer. The organized cervical screening program of the Stockholm-Gotland region in Sweden decided on 2014-02-17 to introduce HPV triaging for all women with AGC, while retaining the same management guidelines for both HPV-positive and HPV-negative women with AGC.

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The present study included all cases of AGC in the region during the period 2014-02-17 to 2016-06-30. We determined how HPV triaging of AGC affected the predictive values for subsequent diagnosis of high-grade cervical lesions and cancer or endometrial cancers.

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MATERIAL AND METHODS

Study population, data collection and analysis. The Swedish cervical screening program invites all women for screening every third year (23-49 years of age) or fifth year (50-64 years of age) (18). Annually, nearly 800,000 cervical samples are reported in Sweden and about 100,000 samples were collected in the greater Stockholm County and Gotland region. About 75-80,000 samples per year are taken as a result of an invitation within the organized program and about 20-25,000 samples are taken during follow-up or opportunistically.. To minimize risk for selection biases in inclusion or follow-up, this population-based cohort study included all women who lived in the Stockholm-Gotland region of Sweden and had a primary cervical screening result from February 17th, 2014 to June 30th, 2016 (about 200,000 women). A comprehensive screening registry where all samples in the region are registered was used to identify all of women who had an AGC diagnosis on their Pap test (n=564), if there was a corresponding HPV test, and if there was a subsequent histopathological diagnosis. HPV tests performed within 40 days before or after the AGC index sample [most HPV tests 378/392 (96%) were performed within 5 days of the AGC diagnosis] were considered to likely reflect the HPV status of the index sample. In the organized program, LBC samples were collected by midwives using plastic Ayre-like spatula and an endocervical brush (Medscand, Cooper Surgical company, Berlin, Germany). The cervical cells were obtained from the ectocervix and endocervix of the uterus and suspended in PreservCyt, a methanol-based fixative medium, as recommended by the manufacturer (ThinPrep®, Hologic, Marlborough, MA, USA). The cervical LBC samples were transferred to cytology glass slides using a ThinPrep® 5000 processor (Hologic) and the remaining cell suspension was analysed for HPV DNA using the Cobas 4800 HPV test, with robotic

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decapping of ThinPrep vials (p480, Roche Molecular Diagnostic, Pleasanton, CA, USA). Qualitative detection of high-risk HPV DNA was obtained by amplification of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (with HPV 16 and HPV 18 in separate channels, the remaining HR-types were reported as a group).

LBC samples were prepared and evaluated at the Department of Clinical Pathology and Cytology, Karolinska University Hospital, Sweden. A modification of the Bethesda system was used for cytological diagnostics including the diagnostic system for AGC but without further subgrouping within the AGC diagnosis (19). The Systematized Nomenclature of Medicine (SNOMED) system was used for cytological and histological classification coding (20). When several histopathological diagnoses were given, the most severe histological diagnosis was taken as outcome. All 564 women were followed up for histopathologies, using registry linkages, until June 30th, 2016. Odds ratios and confidence intervals were calculated using conditional logistic regression using EpiInfo (www.cdc.gov). Individual level data will be shared on request, to be sent to JD.

Patient involvement

No patient was involved in setting the research question or the outcome measures. The participants were not involved in developing plans for recruitment, design, or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community. Women were informed about their HPV and cytology results.

Ethics. The study was approved by the regional ethical board of Stockholm, decision number 2016/1103-31.

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In Sweden, the Ethical Review Boards (ERB) are appointed by government, chaired by a senior judge and have the authority to decide on the requirements for consent. For this study, the ERB decided that consent was not required and all women resident in the population of the Stockholm/Gotland region could be included in the study.

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RESULTS

During 2014-02-17 to 2016-06-30, altogether 564 primary cervical cytology samples (ThinPrep®, Hologic) were diagnosed as AGC. A total of 172 samples were excluded in the study. Of these, 76 (13%) samples had no associated HPV test and 96 (17%) samples had no histological follow-up. In total, there were 392 (70%) women who had had an associated HPV test and a subsequent histopathological follow-up. The mean age of these women was 38 years (range 23-86) (figure 1).

The risk of being HR-HPV positive decreased with increasing age: 62%, 60%, and 25% of samples were HPV-positive among women <40 years, 40-50 years, and ≥50 years had, respectively (table 1).

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Table 1: Histological follow-up after HPV-positive or HPV-negative AGC, by age.

	<40 years old		40-50 years old		>50 years old		Total	
	HPV+	HPV-	HPV+	HPV-	HPV+	HPV-	HPV+	HPV-
WNL	38	50	12	55	6	22	56	127
LSIL	25	12	6	13	3	7	34	32
HSIL	71	1	20	2	2	1	93	4
SCC	0	0	0	0	0	1	0	1
AIS	26	0	6	0	1	0	33	0
ADCA cx	5	0	1	0	0	0	6	0
Tot	**165**	**63**	**45**	**70**	**12**	**31**	**222**	**164**
PPV	62%	1.6%	60%	2.8%	25%	6%	60%	3%

Abbreviations: WNL indicates with normal limits; LSIL=low grade intraepithelial lesion; HSIL=high-grade intraepithelial lesion, SCC=squamous cervical cancer; AIS=endocervical adenocarcinoma in situ; ADCA cx=adenocarcinoma cervix.

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Among the HPV-positive AGC, the subsequent histologies identified 6 (3%) cases of cervical ADCA, 33 (15%) cases of AIS and 93 (42%) cases of HSIL. The PPV for a high-grade lesion was 132/222 (60 %). The corresponding figures for the HPV negative AGC group was 5 endometrial cancers, one cervical SCC and 4 HSIL, giving a PPV of 5/170 (3%) for cervical high-grade lesions and 3% for endometrial cancer. HR-HPV was thus found in 132/137 cases (sensitivity 96%) with cervical high-grade lesions to treat [OR: 48.4 (95% CI: 19.1-122.6)] but in none of the 5 endometrial cancers (figure 2).

In the HPV positive group of 165 women younger than age 40, 71 cases (41%) had HSIL, 26 cases (16%) had AIS, and 5 cases (3%) were cervical ADCA. Among the 45 HPV-positive women between the ages of 40 and 50, 20 cases (44%) were HSIL, 6 cases (13%) were AIS, and 1 case (2%) was cervical ADCA. Among the 12 women older than 50 years of age, there were 2 (16%) HSIL cases and 1 (8%) AIS case.

All 5 endometrial ADCA were found among the 36 HPV-negative women >50 years of age (PPV: 14%) (table 2).

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Table 2: Histological follow-up after AGC in cytology

	Age, year				
	<40	**40-50**	**>50**	**Total**	**%**
WNL	88	67	28	183	(46.3)
LSIL	37	19	10	66	(16.8)
HSIL	72	22	3	97	(25.4)
SCC	0	0	1	0	(0.3)
AIS	26	6	1	33	(8.1)
ADCA, Cx	5	0	1	6	(1.5)
ADCA, endom	0	0	5	5	(1.3)
ADCA met	0	0	1	1	(0.3)
Total	**228**	**114**	**50**	**392**	
PPV	45%	25%	12%		

Abbreviations: WNL indicates with normal limits; LSIL=low grade intraepithelial lesion; HSIL=high-grade intraepithelial lesion, SCC=squamous cervical cancer; AIS=endocervical adenocarcinoma in situ; ADCA cx=adenocarcinoma cervix; ADCA endom=adenocarcinoma endometrial; ADCA met=adenocarcinoma metastasis?

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DISCUSSION

Statement of main findings: The high predictive values for cervical lesions to treat of an HPV-positive AGC indicates that ambitious clinical management algorithms that minimize risk the for loss to follow-up would need to be followed.

The sensitivity of HPV-positivity for cervical high grade lesions suggest that HPV-based screening strategies are safe with regards to finding AGC-associated cervical cancer precursor lesions.

The fact that all endometrial cancers were HPV negative suggest that further research is warranted on the possible benefit of cervical cytological screening on early diagnosis of endometrial cancer.

Strengths of study: The present study is large and population-based, nested within an organized screening program that mandated identical clinical follow-up for HPV-positive and HPV-negative AGC. The entire cohort was followed using comprehensive registries and the risk for ascertainment bias in follow-up is thus minimal. The setting also implies high generalizability.

Limitations of study: Not all women with AGC had had an HPV test performed (only 87%). About 70% of cervical samples in the region are taken following an invitation with an appointment in the organized program – the remainder are taken in other settings, for example, during clinical follow-up of cytological abnormalities detected in the screening program. Whereas it is straightforward to ensure adherence to policies for samples taken in the organized program, it is more complicated for samples taken in other settings and we consider an 87% compliance with the HPV-triaging policy as high compliance.

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A substantial proportion of women (17%) had no histological follow-up. A nationwide audit found that one third of women with AGC lacked histological follow-up (6). As this lack of follow-up was associated with very high risks for cancer (6), there is now a greatly increased awareness of the need for histological follow-up after AGC. For women with an index AGC close to the end of the study, lack of follow-up may simply reflect insufficient follow-up time in our study. However, late histopathologies not recorded in the study are not likely to be biased in relation to the outcomes of the study.

AGC is not an easily recognized entity and there may be differences in diagnostic practices between different laboratories. As the organized screening program of region Stockholm-Gotland uses a single laboratory (Karolinska University Laboratory), the laboratory of the present study can be characterized as a high-volume, highly specialized laboratory. Thus, it is not certain that the predictive values found in this study can be generalized to other settings.

Strengths and weaknesses in relation to other studies, discussing important differences in results : The systematic review of Verdoodt et al (15) identified that there were only 12 studies on HPV in AGC and reported that, on average, 40% of AGC were HPV-positive. We find a somewhat higher figure [56 % (n=222) of AGC were HPV-positive], which may be related to the fact that the screening program uses a single, specialized laboratory. As far as we have could determine, ours is the largest population-based cohort study that has included subsequent histopathological diagnoses after AGC in relation to the HPV status of the index cytology.

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The major previous study that reported on the HPV status of endometrial cancers detected after AGC found that all of them were HPV-negative (11), which is in accordance with our results.

The meaning of the study and implications for clinicians and policy-makers: The very high predictive values of an HPV-positive AGC indicate that current clinical management algorithms may need to be revised to minimize the risk that existing lesions escape detection and most importantly, to minimize the risk that women will be lost to follow-up.

The high sensitivity of HPV-positivity for cervical high grade lesions among AGC suggests that the switch to HPV-based screening is safe with regards to catching AGC-associated cervical cancer precursor lesions: Only 3% of the HSIL lesions detected after AGC would be missed by not referring HPV-negative AGC. Some programs are contemplating the use of double testing with both HPV and cytology at least once per lifetime and this would enable detection of this small subset of HSIL that occurs after HPV-negative AGC.

Although the sensitivity is not 100%, the greatly increased PPV for the HPV-positive women with AGC that are referred implies that only HPV-positive AGC need to be referred. If more stringent management algorithms are used for these women, this may increase safety although fewer women will be referred. This phenomenon has been demonstrated in our randomized implementation of HPV triaging of ASCUS/LSIL samples, where the arm referring only HPV-positive ASCUS/LSIL found more high-grade lesions despite referring fewer women (21).

:Unanswered questions and future research:

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The HPV-negativity of endometrial cancers is in accordance with other studies (11) and indicates that with the ongoing switch to HPV-based screening, there will be no benefit of early diagnosis of endometrial cancers. It is not entirely clear if endometrial cancer detected early through cervical screening ever resulted in a measurable health benefit(22). Further research to establish whether this was indeed the case seems warranted.

With the switch to HPV-based cervical screening, there will no longer be any early detection of endometrial cancers and further studies to elucidate whether this should be remedied are warranted.

The new screening modality will detect only HPV-positive AGC. This will result in a greatly increased PPV compared to AGC with unknown HPV status, and with HPV-based screening the management guidelines for AGC would need to be substantially changed to reflect this. Recent guidelines in France recommend HPV triaging of AGC (22), a strategy supported by our results.

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CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

CONTRIBUTORSHIP STATEMENT

IN: Retrieved and evaluated data, is guarantor of the study and coordinated the study. AH: Provided supervision. JD: Provided supervision and resources.

All authors revised the manuscript for important intellectual content and have approved the final version for submission. IN affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

FUNDING STATEMENT

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DATA SHARING STATEMENT

Individual level data is available from the corresponding author.

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FIGURE LEGENDS:

Figure 1: The study flow chart.
(LBC = Liquid-based cytology; Pap = Papanicolaou; AGC = atypical glandular cells; HR-HPV = high-risk human papillomavirus).

Figure 2: Histological findings in HPV positive and HPV negative AGC cases. Advanced cervical lesions were found in 60% of HPV positive AGC cases while only in 3% of the HPV negative ones, corresponding to a sensitivity of 96% (132/137).
(WNL = within normal limits; LSIL= low grade intraepithelial lesion; HSIL= high grade intraepithelial lesion; SCC = squamous cell carcinoma (cervical); AIS = adenocarcinoma in situ (cervical); ADCA cx = cervical adenocarcinoma; ADCA endom = endometrial adenocarcinoma).

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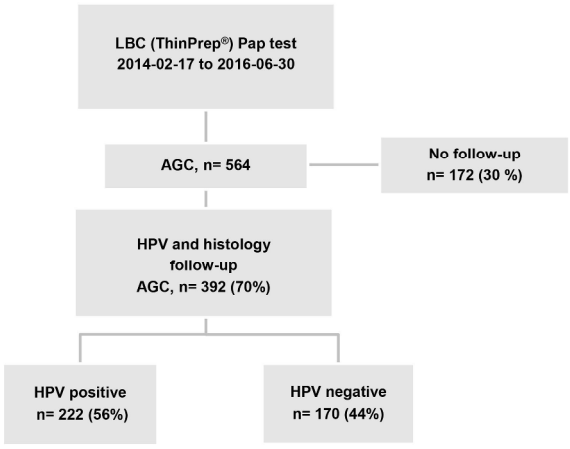

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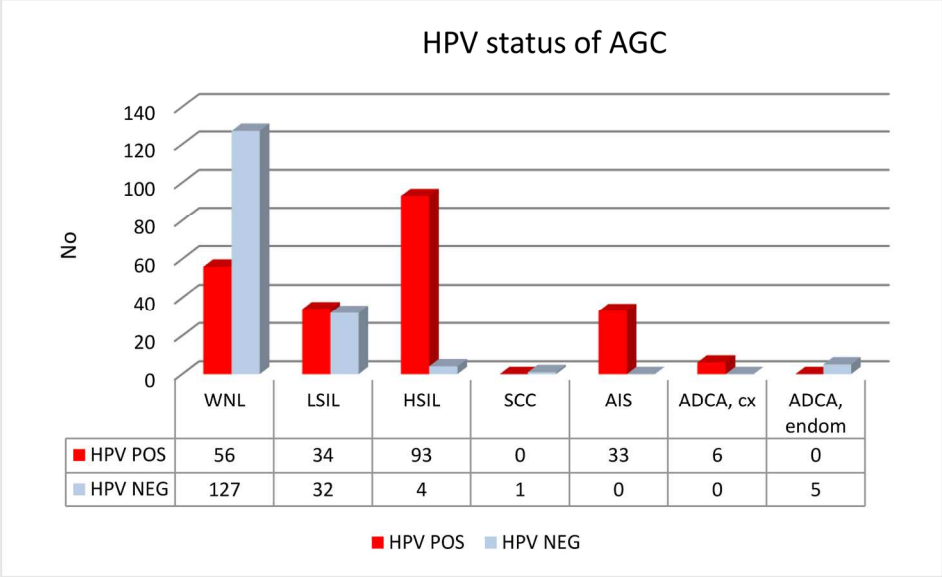

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			**7**
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	N/A
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	9-10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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RISK OF HIGH-GRADE LESIONS AFTER ATYPICAL GLANDULAR CELLS IN CERVICAL SCREENING: A POPULATION-BASED COHORT STUDY

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Keywords:	CYTOPATHOLOGY, EPIDEMIOLOGY, GYNAECOLOGY

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RISK OF HIGH-GRADE LESIONS AFTER ATYPICAL GLANDULAR CELLS IN CERVICAL SCREENING: A POPULATION-BASED COHORT STUDY

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Keywords: Adenocarcinoma, atypical glandular cells, cervical cancer screening, human papilloma-virus, liquid based cytology.

Word count Abstract: 249

Word count Text: 2830

Abbreviations: ADCA: adenocarcinoma; AGC: atypical glandular cells; AIS: adenocarcinoma in situ; CI: confidence interval; HPV: human papillomavirus; HSIL: high-grade squamous intraepithelial lesion; LBC: liquid based cytology; LSIL: low-grade squamous intraepithelial lesion; OR: odds ratio; PPV: positive predictive values; SCC: squamous cell cancer.

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ABSTRACT

Objectives: To determine how human papillomavirus (HPV) positivity of atypical glandular cells (AGC) affects the predictive values for presence of high-grade cervical lesions.

Design: Population-based cohort study.

Setting: Stockholm-Gotland region, Sweden.

Participants: Between 2014-02-17 and 2016-06-30, there were 562 women with AGC detected in a cervical sample. Registry linkages up to 2016-06-30 identified 392 women with an associated HPV test and a histopathological follow-up.

Main outcome measure: Presence of a high-grade cervical lesion in the cervical biopsy taken after the AGC smear, in relation to the HPV status of the AGC-containing index smear.

Results: The proportion of HPV-positive AGC was 56% (n=222). In this group, there were six cases of invasive cervical adenocarcinoma, 33 cases of cervical adenocarcinoma in situ and 93 cases of high-grade squamous intraepithelial lesion (HSIL), giving a positive predictive value (PPV) for a cervical high-grade lesion of 60% (132/222). Among the 170 women with HPV-negative AGC, there was one invasive cervical squamous cell cancer and four HSIL, giving a PPV for a cervical high-grade lesion of 2.9% (5/170). This group also contained five endometrial cancers and one breast cancer.

Conclusions: HPV triaging of AGC will greatly increase the predictive ability for identifying cervical high-grade lesions [Odds Ratio: 48.4 (95% Confidence Interval: 19.1-122.6)] and the high sensitivity (96%; 132/137 women) implies safety of primary

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HPV screening strategies, with regard to this subset of patients. The measurable risk for endometrial cancer among women with HPV-negative AGC (2.9%) suggests that research on screening for endometrial cancer is needed.

Keywords: Adenocarcinoma, atypical glandular cells, cervical cancer screening, human papilloma-virus, liquid based cytology.

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Strengths of study:

- A large and population-based study, nested within an organized screening program that mandated identical clinical follow-up for HPV-positive and HPV-negative AGC.
- The entire cohort was followed using comprehensive registries.

Limitations of study:

- Not all women with AGC had had an HPV test performed and not everyone had a histopathology follow-up.
- Cytological diagnosis of AGC may be variable between settings.

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BACKGROUND

Organized cervical screening programs have resulted in a marked decline in the incidence of cervical squamous cell carcinomas (SCC) (1). However, the effect on invasive cervical adenocarcinoma (ADCA) (1) has been much less. Several countries now report that 20% or more of all remaining invasive cervical carcinoma are ADCA (2) and some studies have even reported an increasing incidence of cervical ADCA (3, 4). Whereas the precursor lesions of SCC and their management strategies are well recognized, the precursors of ADCA and their optimal management strategies are less clear. Cytological criteria for premalignant columnar cell lesions were recognized as late as in the mid 1990-ties (5). This increased the possibility to identify and treat such lesions. Women with a history of AGC have a greatly increased risk for later development of cervical cancer, probably because of persisting uncertainty regarding how to identify and manage these high-risk women (6).

Most countries have switched from conventional to liquid-based cytology (LBC), which has made it easier to identify AGC (7, 8). In Sweden, atypical glandular cells (AGC) are reported in less than 0.3% of cervical samples (9). AGC not only signal an ADCA precursor lesion, they may also be caused by benign conditions such as cervical polyps, hyperplasia, and tubar metaplasia (10). AGC is a high-risk marker for high-grade squamous intraepithelial lesion (HSIL), SCC (11), adenocarcinoma in situ (AIS), or ADCA (12). AGC found at cervical screening is associated with a high and persistent risk of cervical cancer for up to 15 years, particularly for ADCA and among women aged 30-39 (6). The current management of AGC thus does not seem to be optimally effective in preventing cervical cancer (6). Cervical cytology is primarily a screening test for squamous intraepithelial lesions and SCC. Sensitivity

for glandular lesions is more variable due to sampling and interpretation issues, and because glandular lesions are less common, less well-defined, and may also include reactive conditions (13).

Human papilloma (HPV) reflex testing on ASCUS and low-grade squamous intraepithelial lesions (LSIL) is routinely used to increase predictive value HSIL and referral to colposcopy (14). A systematic review found 12 studies of HPV testing in glandular lesions and found that about 40% of AGC were high-risk positive and that predictive values were increased if AGC was triaged with HPV testing (15). However, HPV-negative AGC above 50 years of age may contain a substantial number of non-cervical cancers (15) and the value of HPV triage in the management of AGC is therefore not clear.

While cervical screening does not aim to detect endometrial carcinoma, occasionally abnormal endometrial cells are detected in cervical samples and may lead to an earlier diagnosis of endometrial carcinoma (16). There are no guidelines or cost-effectiveness evaluations that consider a possible benefit of cervical screening on early diagnosis of endometrial carcinoma. Nevertheless, most guidelines recommend clinical follow-up of abnormal endometrial cells, should they be found (16).

As endometrial cancer and abnormal endometrial cells are negative for HPV (11) and as primary cervical screening with HPV is now a globally recommended practice (17), there is a concern that switching to HPV-based screening may result in losing a possible benefit of early diagnosis of endometrial cancer. The organized cervical screening program of the Stockholm-Gotland region in Sweden decided on 2014-02-17 to introduce HPV triaging for all women with AGC, while retaining the same management guidelines for both HPV-positive and HPV-negative women with AGC.

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The present study included all cases of AGC in the region during the period 2014-02-17 to 2016-06-30. We determined how HPV triaging of AGC affected the predictive values for subsequent diagnosis of high-grade cervical lesions and cancer or endometrial cancers.

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MATERIAL AND METHODS

Study population, data collection and analysis. The Swedish cervical screening program invites all women for screening every third year (23-49 years of age) or fifth year (50-64 years of age) (18). Annually, nearly 800,000 cervical samples are reported in Sweden and about 100,000 samples were collected in the greater Stockholm County and Gotland region. About 75-80,000 samples per year are taken as a result of an invitation within the organized program and about 20-25,000 samples are taken during follow-up or opportunistically. To minimize risk for selection biases in inclusion or follow-up, this population-based cohort study included all women who lived in the Stockholm-Gotland region of Sweden and had a primary cervical screening result from February 17th, 2014 to June 30th, 2016 (about 200,000 women). A comprehensive screening registry where all samples in the region are registered was used to identify all of women who had an AGC diagnosis on their Pap test (n=564), if there was a corresponding HPV test, and if there was a subsequent histopathological diagnosis. HPV tests performed within 40 days before or after the AGC index sample [most HPV tests 378/392 (96%) were performed within five days of the AGC diagnosis] were considered to likely reflect the HPV status of the index sample. In the organized program, LBC samples were collected by midwives using plastic Ayre-like spatula and an endocervical brush (Medscand, Cooper Surgical company, Berlin, Germany). The cervical cells were obtained from the ectocervix and endocervix of the uterus and suspended in PreservCyt, a methanol-based fixative medium, as recommended by the manufacturer (ThinPrep®, Hologic, Marlborough, MA, USA). The cervical LBC samples were transferred to cytology glass slides using a ThinPrep® 5000 processor (Hologic) and the remaining cell suspension was analysed for HPV DNA using the Cobas 4800 HPV test, with robotic

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decapping of ThinPrep vials (p480, Roche Molecular Diagnostic, Pleasanton, CA, USA). Qualitative detection of high-risk HPV DNA was obtained by amplification of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (with HPV 16 and HPV 18 in separate channels, the remaining HR-types were reported as a group).

LBC samples were prepared and evaluated at the Department of Clinical Pathology and Cytology, Karolinska University Hospital, Sweden. A modification of the Bethesda system was used for cytological diagnostics including the diagnostic system for AGC but without further subgrouping within the AGC diagnosis (19). The Systematized Nomenclature of Medicine (SNOMED) system was used for cytological and histological classification coding (20). When several histopathological diagnoses were given, the most severe histological diagnosis was taken as outcome. All 564 women were followed up for histopathologies, using registry linkages, until June 30th, 2016. Odds ratios and confidence intervals were calculated using conditional logistic regression using EpiInfo (www.cdc.gov). Individual level data will be shared on request, to be sent to JD.

Patient involvement

No patient was involved in setting the research question or the outcome measures. The participants were not involved in developing plans for recruitment, design, or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community. Women were informed about their HPV and cytology results.

Ethics. The study was approved by the regional ethical board of Stockholm, decision number 2016/1103-31.

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In Sweden, the Ethical Review Boards (ERB) are appointed by government, chaired by a senior judge and have the authority to decide on the requirements for consent. For this study, the ERB decided that consent was not required and all women resident in the population of the Stockholm/Gotland region could be included in the study.

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RESULTS

During 2014-02-17 to 2016-06-30, altogether 564 primary cervical cytology samples (ThinPrep®, Hologic) were diagnosed as AGC. A total of 172 samples were excluded in the study. Of these, 76 (13%) samples had no associated HPV test and 96 (17%) samples had no histological follow-up. In total, there were 392 (70%) women who had had an associated HPV test and a subsequent histopathological follow-up. The mean age of these women was 38 years (range 23-86) (figure 1).

The risk of being HR-HPV positive decreased with increasing age: 62%, 60%, and 25% of samples were HPV-positive among women <40 years, 40-50 years, and ≥50 years had, respectively (table 1).

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Table 1: Histological follow-up after HPV-positive or HPV-negative AGC, by age.

	<40 years old		40-50 years old		>50 years old		Total	
	HPV+	HPV-	HPV+	HPV-	HPV+	HPV-	HPV+	HPV-
WNL	38	50	12	55	6	22	56	127
LSIL	25	12	6	13	3	7	34	32
HSIL	71	1	20	2	2	1	93	4
SCC	0	0	0	0	0	1	0	1
AIS	26	0	6	0	1	0	33	0
ADCA cx	5	0	1	0	0	0	6	0
Tot	**165**	**63**	**45**	**70**	**12**	**31**	**222**	**164**
PPV	62%	1.6%	60%	2.8%	25%	6%	60%	3%

Abbreviations: WNL indicates with normal limits; LSIL=low grade intraepithelial lesion; HSIL=high-grade intraepithelial lesion, SCC=squamous cervical cancer; AIS=endocervical adenocarcinoma in situ; ADCA cx=adenocarcinoma cervix.

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Among the HPV-positive AGC, the subsequent histologies identified six (3%) cases of cervical ADCA, 33 (15%) cases of AIS and 93 (42%) cases of HSIL. The PPV for a high-grade lesion was 132/222 (60 %). The corresponding figures for the HPV negative AGC group was five endometrial cancers, one cervical SCC and 4 HSIL, giving a PPV of 5/170 (3%) for cervical high-grade lesions and 3% for endometrial cancer. HR-HPV was thus found in 132/137 cases (sensitivity 96%) with cervical high-grade lesions to treat [OR: 48.4 (95% CI: 19.1-122.6)] but in none of the five endometrial cancers (figure 2).

In the HPV positive group of 165 women younger than age 40, 71 cases (41%) had HSIL, 26 cases (16%) had AIS, and five cases (3%) were cervical ADCA. Among the 45 HPV-positive women between the ages of 40 and 50, 20 cases (44%) were HSIL, six cases (13%) were AIS, and one case (2%) was cervical ADCA. Among the 12 women older than 50 years of age, there were two (16%) HSIL cases and one (8%) AIS case.

All five endometrial ADCA were found among the 36 HPV-negative women >50 years of age (PPV: 14%) (table 2).

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Table 2: Histological follow-up after AGC in cytology

	Age, year				
	<40	**40-50**	**>50**	**Total**	**%**
WNL	88	67	28	183	(46.3)
LSIL	37	19	10	66	(16.8)
HSIL	72	22	3	97	(25.4)
SCC	0	0	1	0	(0.3)
AIS	26	6	1	33	(8.1)
ADCA, Cx	5	0	1	6	(1.5)
ADCA, endom	0	0	5	5	(1.3)
ADCA met	0	0	1	1	(0.3)
Total	**228**	**114**	**50**	**392**	
PPV	45%	25%	12%		

Abbreviations: WNL indicates with normal limits; LSIL=low grade intraepithelial lesion; HSIL=high-grade intraepithelial lesion, SCC=squamous cervical cancer; AIS=endocervical adenocarcinoma in situ; ADCA cx=adenocarcinoma cervix; ADCA endom=adenocarcinoma endometrial; ADCA met=adenocarcinoma metastasis?

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DISCUSSION

Statement of main findings: The high predictive values for cervical lesions to treat of an HPV-positive AGC indicates that ambitious clinical management algorithms that minimize risk the for loss to follow-up would need to be followed.

The sensitivity of HPV-positivity for cervical high-grade lesions suggest that HPV-based screening strategies are safe with regards to finding AGC-associated cervical cancer precursor lesions.

The fact that all endometrial cancers were HPV negative suggest that further research is warranted on the possible benefit of cervical cytological screening on early diagnosis of endometrial cancer.

Strengths of study: The present study is large and population-based, nested within an organized screening program that mandated identical clinical follow-up for HPV-positive and HPV-negative AGC. The entire cohort was followed using comprehensive registries and the risk for ascertainment bias in follow-up is thus minimal. The setting also implies high generalizability.

Limitations of study: Not all women with AGC had had an HPV test performed (only 87%). About 70% of cervical samples in the region are taken following an invitation with an appointment in the organized program – the remainder are taken in other settings, for example, during clinical follow-up of cytological abnormalities detected in the screening program. Whereas it is straightforward to ensure adherence to policies for samples taken in the organized program, it is more complicated for samples taken in other settings and we consider an 87% compliance with the HPV-triaging policy as high compliance.

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A substantial proportion of women (17%) had no histological follow-up. A nationwide audit found that one third of women with AGC lacked histological follow-up (6). As this lack of follow-up was associated with very high risks for cancer (6), there is now a greatly increased awareness of the need for histological follow-up after AGC. For women with an index AGC close to the end of the study, lack of follow-up may simply reflect insufficient follow-up time in our study. However, late histopathologies not recorded in the study are not likely to be biased in relation to the outcomes of the study.

AGC is not an easily recognized entity and there may be differences in diagnostic practices between different laboratories. As the organized screening program of region Stockholm-Gotland uses a single laboratory (Karolinska University Laboratory), the laboratory of the present study can be characterized as a high-volume, highly specialized laboratory. Thus, it is not certain that the predictive values found in this study can be generalized to other settings.

Strengths and weaknesses in relation to other studies, discussing important differences in results: The systematic review of Verdoodt et al (15) identified that there were only 12 studies on HPV in AGC and reported that, on average, 40% of AGC were HPV-positive. We find a somewhat higher figure [56 % (n=222) of AGC were HPV-positive], which may be related to the fact that the screening program uses a single, specialized laboratory. As far as we have could determine, ours is the largest population-based cohort study that has included subsequent histopathological diagnoses after AGC in relation to the HPV status of the index cytology.

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The major previous study that reported on the HPV status of endometrial cancers detected after AGC found that all of them were HPV-negative (11), which is in accordance with our results.

The meaning of the study and implications for clinicians and policy-makers:

The very high predictive values of an HPV-positive AGC indicate that current clinical management algorithms may need to be revised to minimize the risk that existing lesions escape detection and most importantly, to minimize the risk that women will be lost to follow-up.

The high sensitivity of HPV-positivity for cervical high-grade lesions among AGC suggests that the switch to HPV-based screening is safe with regards to catching AGC-associated cervical cancer precursor lesions: Only 3% of the HSIL lesions detected after AGC would be missed by not referring HPV-negative AGC, although this percentage may not reflect HPV-negative lesions in screening tests as a whole. Some programs are contemplating the use of double testing with both HPV and cytology at least once per lifetime and this would enable detection of this small subset of HSIL that occurs after HPV-negative AGC along with others without AGC.

Although the sensitivity is not 100%, the greatly increased PPV for the HPV-positive women with AGC that are referred implies that only HPV-positive AGC need to be referred. If more stringent management algorithms are used for these women, this may increase safety although fewer women will be referred. This phenomenon has been demonstrated in our randomized implementation of HPV triaging of ASCUS/LSIL samples, where the arm referring only HPV-positive ASCUS/LSIL found more high-grade lesions despite referring fewer women (21).

Unanswered questions and future research:

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The HPV-negativity of endometrial cancers is in accordance with other studies (11) and indicates that with the ongoing switch to HPV-based screening, there will be no benefit of early diagnosis of endometrial cancers. It is not entirely clear if endometrial cancer detected early through cervical screening ever resulted in a measurable health benefit (22). Further research to establish whether this was indeed the case seems warranted.

With the switch to HPV-based cervical screening, there will no longer be any early detection of endometrial cancers and further studies to elucidate whether this should be remedied are warranted.

The new screening modality will detect only HPV-positive AGC. This will result in a greatly increased PPV compared to AGC with unknown HPV status, and with HPV-based screening the management guidelines for AGC would need to be substantially changed to reflect this. Recent guidelines in France recommend HPV triaging of AGC (22), a strategy supported by our results.

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CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

CONTRIBUTORSHIP STATEMENT

IN: Retrieved and evaluated data, is guarantor of the study and coordinated the study. AH: Provided supervision. JD: Provided supervision and resources.

All authors revised the manuscript for important intellectual content and have approved the final version for submission. IN affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

FUNDING STATEMENT

The study was funded by Stockholm County Clinical Research.

DATA SHARING STATEMENT

Individual level data is available from the corresponding author.

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FIGURE LEGENDS:

Figure 1: The study flow chart.
(LBC = Liquid-based cytology; Pap = Papanicolaou; AGC = atypical glandular cells; HR-HPV = high-risk human papillomavirus).

Figure 2: Histological findings in HPV positive and HPV negative AGC cases. Advanced cervical lesions were found in 60% of HPV positive AGC cases while only in 3% of the HPV negative ones, corresponding to a sensitivity of 96% (132/137).
(WNL = within normal limits; LSIL= low grade intraepithelial lesion; HSIL= high grade intraepithelial lesion; SCC = squamous cell carcinoma (cervical); AIS = adenocarcinoma in situ (cervical); ADCA cx = cervical adenocarcinoma; ADCA endom = endometrial adenocarcinoma).

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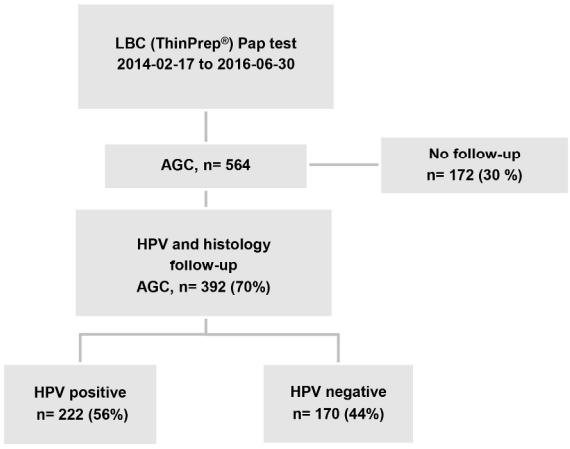

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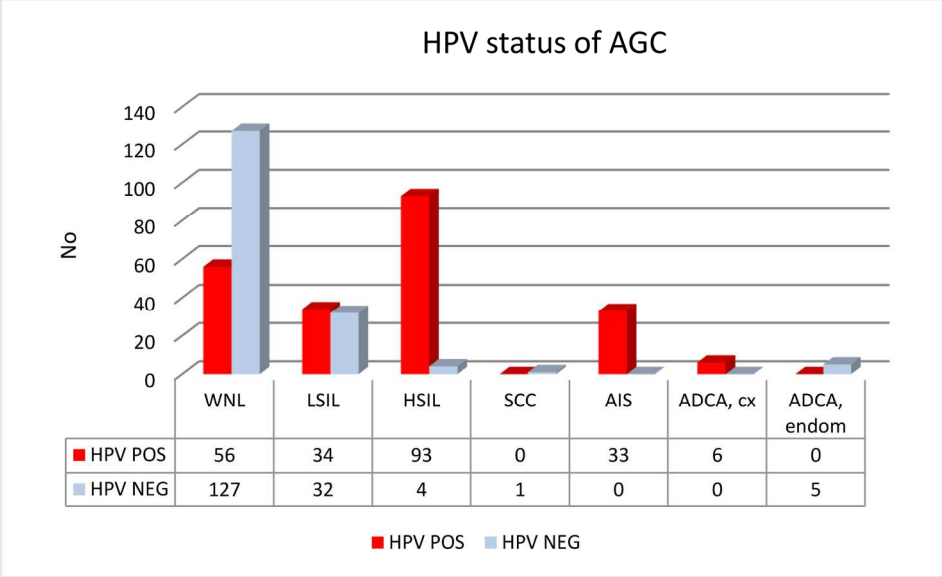

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			**7**
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	N/A
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	9-10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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