Risk of gynaecological cancer after atypical glandular cells found on cervical cytology: a population-based cohort study

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ABSTRACT

Background

Atypical glandular cells (AGC) are rare abnormalities found on cervical cytology associated with a range of lesions of the female reproductive system. We compared the risk of cervical and other gynaecological cancers following AGC on cervical cytology with the risk following squamous cell abnormalities of comparable severity.

Methods

We used data from the Dutch Pathology Archive (PALGA) from 2000-2015 to categorise cervical cytology tests into groups based on most severe cytological abnormality and correlated follow-up advice (normal cytology and 'no follow-up' advice, squamous-cell based-, AGC-based, and combined AGC/squamous-cell based each with either repeat testing or referral advice). Cancer data were linked from the Netherlands Cancer Registry. Cox proportional hazard models were calculated stratified by age (younger (<50 years) and older (50+ years)), adjusted for number of previous primary cytology tests.

Results

8,537,385 cytology smears and 9,061 cancers were included. When repeat cytology testing was advised, hazard ratios (HR) of cervical cancer (younger women – HR: 6.91, 95% CI: 5.48 – 8.71; older– HR: 3.98, 95% CI: 2.38 - 6.66) or other gynaecological cancer diagnosis in younger women (HR: 2.82, 95% CI: 1.39 - 5.74) were significantly higher after an AGC-based abnormality compared with squamous-based abnormalities. Hazards were also significantly higher for 'referral' advice cytology, except for cervical cancer amongst older women (HR: 0.88, 95% CI: 0.63 – 1.21).

Conclusions

AGC indicates an increased risk of gynaecological cancer compared to squamous-based abnormalities of comparable severity.

Impact

Gynaecologists should be alert for cervical and endometrial cancers when examining women referred following AGC.

INTRODUCTION

Atypical glandular cells (AGC) are rare cytological abnormalities, detected in less than 1% of cervical smears.[1] Interpretation of AGC can be challenging for cytotechnicians and pathologists,[2] partly because associated conditions can range widely, from benign lesions to cancers.[3] Cancers include cervical adenocarcinoma, which organised cervical screening has had little impact on the incidence of,[4 5] as well as other gynaecological cancers such as endometrial and ovarian cancers.[6-9] The type of lesion that is indicated by AGC has been shown to differ by age, with squamous cell abnormalities (cervical intraepithelial neoplasia (CIN) grades 2 or 3) most commonly found in younger women and endometrial cancer most commonly found in women aged 50 years and older.[6]

Cervical cancer screening programmes are aimed at detecting pre-malignant and malignant cervical lesions, with the goal of preventing incidence of, and mortality from, cervical cancer. This has been largely achieved in the Netherlands, with incidence and mortality reduction in the period following the introduction of organised screening.[10] Although detection of other gynaecological cancers is beyond the scope of organised cervical cancer screening programmes, some women may have benefited from the incidental detection of non-cervical, AGC-related lesions following cervical cytology.

Large, population-based datasets are needed for investigating the risk of gynaecological cancer following AGC because AGC is a uncommon diagnosis. However, these type of studies are scarce, with most AGC research focusing on only limited numbers of clinical samples. One large population-based study[11] found that AGC was associated with a persistent, long-term risk of cervical cancer in particular AGC cervical adenocarcinoma. However, other AGC-related cancers were not considered as endpoints in this study. Robust, population-based estimates of the risk of non-cervical gynaecological cancers after AGC are needed to inform and refine management strategies for these women. This is particularly pertinent in the Netherlands because not all women with an AGC on cervical cytology were referred directly to the gynaecologist prior to 2017. To that end, we aimed to investigate what is the risk of particular gynaecological cancers for women after a smear where AGC are detected as compared to this risk after squamous abnormalities with comparable severity.

MATERIALS AND METHODS

Study design

We conducted a population-based retrospective cohort study including cervical cytology tests taken in the Netherlands from the Dutch cervical cancer screening programme between 1 January 2000 and 31 December 2015. Women aged 29 to 63 years were

included. In the case that a woman has participated more than once in the screening programme, each screen was included (when the screen met the criteria outlined in section *"Data analysis and variables"*). In our study, the risk of cancer is the immediate risk following a cytology smear, as we identified which screen was most proximal in time to the cancer diagnosis.

Setting

Between 1996 and 2016, primary cervical cytology screening was offered as part of the nationally-coordinated organised screening programme in the Netherlands. In 2017, the national screening programme transitioned to primary high-risk human papillomavirus (hrHPV) screening. Women between 30 to 60 years were invited for screening every five years. Quality of the programme is high, with low rates of cancers after normal cytology[12] and a participation rate of around 65%.[13]

During the period of this study, the primary screening test was either conventional cytology or liquid-based cytology, with an increase in the use of liquid-based cytology over the period 2000 to 2012.[14] Cervical cytology is graded using the CISOE-A system, the Dutch nationwide, pro-forma classification system for cervical cytology[15] which is easily convertible to other classification systems, such as Pap classification and The Bethesda System for Reporting Cervical Cytology (Table 1).[16] CISOE-A is tri-axial classification with specific information on squamous cells, glandular cells and 'other' cells. The CISOE-A classification is used to determine the follow-up advice that a woman receives following primary screening. The screening programme algorithm can be found in Figure S1.

Based on the results of primary cytology screening, women receive an advice to either return to regular screening (negative cytology; NILM), receive a repeat cytology test after 6 months (low-grade cytological abnormalities; ASC-US/LSIL) or were referred directly to a gynaecologist (high-grade cytology abnormalities; HSIL). Women with lower-grade AGC lesions (AGC of endocervical origin only) were advised to attend repeat cytology testing after 6 months. Women with AGC of endometrial or ovarian origin or women with cytology indicating adenocarcinoma in situ were referred directly to a gynaecologist.

During most of the period of this study, national guidelines were in place for the diagnosis and treatment of cervical abnormalities; one set of guidelines covered the period 2004 – 2014[17] and one set covering 2015 to present.[18] The CISOE-A score is used by gynaecologists to determine the correct diagnostic strategy. Women with squamous cervical abnormalities on their cytology receive colposcopy and dependant on the colposcopic image and the CISOE-A score and other factors (such as age, depth and visibility of lesion, transformation zone type), gynaecologists determine whether biopsies or a large excision of the cervix were required for diagnosis and/or treatment. Random biopsies of the cervix are not standard practice in the Netherlands. In the case of AGC abnormalities, the CISOE-A score is used to determine if ultrasound or colposcopy is

Table 1: *Concordance between CISOE-A, Pap and Bethesda grading systems. Adapted from Oncoline*[30]

CISOE-A	Papanicolaou (Pap)	Bethesda 2001
C0	Pap 0	Inadequate
S1, O1-2*, E1-2#	Pap 1	Negative for intraepithelial lesion or malignancy (NILM) # *E2: no endocervical cells* * *O2: atrophy*
S2-3, O3	Pap 2	Atypical squamous cells of undetermined significance (ASC-US)
E3	Pap 2	Atypical glandular cells (AGC), of endocervical origin only
E4-5	Pap 3a1	AGC, endocervical origin only *(E4 low grade, E5 intermediate grade)*
S4	Pap 3a1	Low-grade squamous intraepithelial lesion (LSIL)
S5	Pap 3a2	High-grade squamous intraepithelial lesion (HSIL)
O4-5	Pap 3a2	AGC, endometrial origin or ovarian (but not endocervical origin)
E6, O6	Pap 3b	AGC, E6 high grade neoplasia or AIS. *(If not endocervical, then O6 in case of endometrial-, ovarian- or other cells)*
S6	Pap 3b	HSIL (including atypical squamous cells, includes ASC-H in NL)
E7	Pap 4	Adenocarcinoma in situ (AIS). *(Used interchangeable with E6)*
S7	Pap 4	Carcinoma in situ *(Used interchangeable with S6)*
S9, O7-9, E9	Pap 5	Invasive carcinoma
S1, E1-5, O1-3 in combination with EX 15	Pap 3a2	ASC-H

required for diagnosis. When endometrial cancer is suspected, the most recent national guidelines state that imaging can be conducted and pre-operative histological samples can be taken within an outpatient clinic to help gynaecologists reach a diagnosis.[19]

Data sources

We used data from the nationwide network of cyto- and histopathology in the Netherlands (PALGA) and from the Netherlands Cancer Registry (NCR). PALGA has complete coverage of all pathology labs within the Netherlands.[20] The NCR is the national oncological registry in the Netherlands with data on all cancer patients and has data from 1989 onwards.[21] For this study, we collaborated with PALGA to create a dataset comprising of an extract of cervical cytology, hrHPV test results and histology records for cervical, uterine and ovarian cancers. Data from PALGA contains an individual, pseudonimised identifier (PALGA ID) based on the first eight letter of the woman's surname (maiden surnames are used for married women) and date of birth which can be used to follow screening histories.

Data analysis and variables

We used CISOE-A to group cytology smears into seven categories based on the type of cytological abnormality [either normal, squamous cell abnormality, AGC abnormality, combined squamous/AGC abnormality] and the follow-up advice that is given on the basis of the severity of the abnormality [return to regular screening, repeat cytology testing, referral to gynaecologist]. The combination of these categories resulted in seven groups:

- Normal cytology [NILM equivalent];
- Repeat, Squamous [ASC-US/LSIL equivalent];
- Repeat, AGC [Endocervical origin only];
- Repeat, AGC/Squamous;
- Referral, Squamous [HSIL/Carcinoma-in-situ equivalent];
- Referral AGC [Endometrial/ovarian origin/adenocarcinoma-in-situ]; and,
- Referral AGC/Squamous.

AGC or squamous cell abnormalities were classified based on the highest CISOE-A score; that is, if both AGC and squamous abnormalities were reported, the most severe abnormality was used for grouping. Cytology results were only classified as combined AGC/squamous when the squamous and other cell abnormalities were of equal severity. Coding of these categories were reviewed by a pathologist and cytology data expert. Any cytology tests that could not be classified in one of the seven groups (e.g. due to invalid or incomplete CISOE-A coding) were excluded from the analysis.

We also received data from the NCR for gynaecological cancers (cervical, uterine [including endometrial], ovarian and other gynaecological cancers: ICD-10 codes C53, C54, C56, C57) including information about topography and morphology. Vulva (C51) and vaginal (C52) cancer were excluded as they are not associated with AGC. We included only invasive cancers in our analysis. We created six diagnosis groups: cervical cancer –squamous, cervical cancer – adenocarcinoma, cervical cancer – other, endometrial cancers, ovarian cancers and other cancers. Morphology groupings of different cervical types are based on the International Classification of Disease for Oncology, 3rd edition.[22] Detailed information about coding of these groups can be found in Table S1. For survival analysis, we collapsed these categories into cervical cancers and other cancers.

There is also information about cancer diagnoses available in PALGA. We used the PALGA diagnosis date to calculate follow-up time, as these records had a more exact date of diagnosis than available in our NCR extract. We included cancers where there was both a NCR record, a histological record of cancer, adenocarcinoma-in-situ or CIN 3 recorded in PALGA and the difference between the dates of these records was no more than 90 days (to compensate for potential differences in dates of registration in the two datasets). In cases where one woman had more than one cancer diagnosis, we only considered the first diagnosis.

Figure S2 shows the process of data management and linkage. Linkage keys were created deterministically, based on matching of variables including PALGA ID, postcode, date of birth, date of diagnosis and lab number. Depending of the number of variables that matched, a rating was given from 1 (most trustworthy link) to 12 (least trustworthy link). We included cases with linkage rating 1 to 9.

Statistical methods

Data analysis and management was conducted using SAS Base 9.4 and RStudio (R v3.6.1, packages epitools, survminer and survival). For the purposes of statistical analysis, we split the sample into women aged 49 years and younger and women aged 50 years and older, because differences in risk have been shown by age in previous research.[23] Life-years at risk will be counted until whichever of these events occurs first: a diagnosis of one of the cancers listed above; the next primary cytology screening (either within the screening programme or by indication/opportunistic screening); 31st December 2015; a hysterectomy recorded in PALGA; or 8.5 years of follow-up, as this contains one complete screening round and beyond, covering women overdue for screening. We excluded all cytology tests of a woman that occurred after either a cancer diagnosis or a hysterectomy.

We calculated relative risks within advice groups. We calculated cumulative incidence of cervical squamous cell cancers, cervical adenocarcinomas and endometrial cancers using Kaplan-Meier estimates. Cox proportional hazards models were calculated both unadjusted and adjusted for the number of previous primary cytology tests. We used the number of previous cytology tests as a proxy for screening history, in order to adjust for differences in risk of invasive cervical cancer in women who have never been screened. Any groups in which there are 5 or less cancer diagnoses are not presented. *P* values less than or equal to 0.05 were considered significant.

Patient involvement

No patients or patient groups were involved in the design or conduct of this study.

Ethical approval

This study used a retrospective, anonymised dataset from PALGA. The board of PALGA approved the use of data for this study. The request for NCR data was approved by the NCR.

Funding

This study was conducted as part of the Evaluation of the Dutch cervical cancer screening programme, which is funded by the Dutch National Institute from Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu).

RESULTS

Table 2 shows the number of cytology tests included in this study by smear-result group and number of cancers by type. A total of 8,537,385 primary screening programme cytology smears could be classified in one of the seven categories. The majority of cytology smears were classified as normal (96.5%), followed by low-grade ('Repeat, squamous': 2.6%) and high-grade ('Referral, squamous': 0.7%) squamous cell abnormalities. AGC was diagnosed rarely; 'Repeat, AGC' results accounted for 0.16% of all smear results and 'Referral, AGC' results accounted for 0.04% of all smear results. The mean age per smear-result group differed: 'Referral, squamous' women were the youngest (38.2 years) and 'Referral, AGC' women were the oldest (47.2 years).The mean and median time to diagnosis following screening was shortest for cancers diagnosed following 'Referral' screens and longest following normal cytology.

Figures 1a and 1b show the proportion of cancer diagnoses for each smear-result group by age for the three cancers with the most diagnoses in our dataset (cervical squamous-cell, cervical adenocarcinoma, endometrial). In both younger and older woman, a higher proportion of cancers were diagnosed following 'Referral, AGC' smears, however, the type of cancers diagnosed following 'Referral, AGC' smears differed by age. Amongst younger women the proportion of cervical adenocarcinoma is highest whereas in older women the highest proportion of cancers diagnosed were endometrial cancers.

Table 3 shows the crude relative risk of a cancer diagnosis compared with squamous smear-result groups for five cancer types (results for 'other gynaecological cancers' are not shown due to small numbers). The crude absolute risk of cancer was higher for the 'Referral, AGC' smear-result group compared to 'Referral, Squamous' as indicated by the higher relative risk for almost all cancer types. The relative risk of an adenocarcinoma diagnosis was higher than the relative risk of a squamous cervical cancer diagnosis for 'Repeat, AGC' (squamous – RR: 1.94; adenocarcinoma – RR: 46.97), 'Referral, AGC' (squamous – RR: 0.50; adenocarcinoma – RR: 26.43), and 'Referral, AGC/Squamous' (squamous – RR: 1.69; adenocarcinoma – RR: 13.38) smear-result groups compared to the respective squamous smear-result groups.

Figures S3 to S8 show cumulative event curves for the three cancers with the most diagnoses in our dataset (cervical squamous-cell, cervical adenocarcinoma, endometrial). Cumulative event curves were higher following 'AGC' cytology than 'squamous' cytology for cervical adenocarcinomas and for endometrial cancers following a 'referral'-advice cytology test. For endometrial cancers diagnosed following 'repeat'-advice cytology tests, the cumulative event curves were higher for following 'AGC' cytology than 'squamous' cytology, however, the confidence interval overlapped at some points on the curve.

Table 2: *Descriptive statistics by result of primary cytology screening and cancer type, 1 January 2000 to 31 December 2015*

CYTOLOGY SCREENS							
	Return to regular screening	***Repeat cytology within six months***			***Referral to gynaecologist***		
	Normal cytology	AGC	Squamous	AGC/ Squamous	AGC	Squamous	AGC/ Squamous
N (% of total)	8,241,096 (96.53%)	13,237 (0.16%)	218,458 (2.56%)	2,126 (0.02%)	3,164 (0.04%)	57,780 (0.68%)	1,524 (0.02%)
Mean age (SD)	44.3 (9.4)	42.5 (8.4)	41.4 (8.8)	42.5 (8.5)	47.2 (9.4)	38.2 (8.1)	39.2 (8.4)
CANCERS (N, % cases following all screens with smear-result group)							
Mean time to diagnosis in months (SD)	35.1 (25.6)	15.6 (19.2)	24.7 (21.2)	22.2 (25.9)	4.5 (8.0)	4.9 (9.7)	4.7 (11.5)
Median time to diagnosis in months	31	9	16	11	2	2	2
Cervical cancer –squamous	365 (0.00%)	30 (0.23%)	255 (0.12%)	6 (0.28%)	34 (1.07%)	1,235 (2.14%)	55 (3.61%)
Cervical cancer - adenocarcinoma	141 (0.00%)	74 (0.56%)	26 (0.01%)	6 (0.28%)	123 (3.89%)	85 (0.15%)	31 (2.03%)
Cervical cancer - other	113 (0.00%)	19 (0.14%)	18 (0.01%)	–	43 (1.36%)	77 (0.13%)	14 (0.92%)
Endometrial cancers	3,634 (0.04%)	19 (0.14%)	118 (0.05%)	–	276 (8.72%)	24 (0.04%)	
Ovarian cancers	1,897 (0.02%)	–	48 (0.02%)	–	18 (0.57%)	11 (0.02%)	–
Other gynaecological cancers	238 (0.00%)	–	8 (0.00%)	–	7 (0.00%)	–	–

AGC: Atypical glandular cells, SD: Standard deviation

– Cells suppressed as contains cells less than 5.

Table 3. *Relative risk of cancer compared to squamous cytology, by cancer type, cytology type and advice class*

	Cervical cancer – Squamous	Cervical cancer - Adenocarcinoma	Cervical cancer - other	Endometrial cancer	Ovarian cancer
	RR (95% CI)	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
Repeat cytology within six months					
Squamous (ref)	1.0	1.0	1.0	1.0	1.0
AGC	1.94* (1.33 – 2.83)	46.97* (30.06 – 73.41)	17.42* (9.15 – 33.18)	2.66* (1.64 – 4.31)	–
AGC/Squamous	2.42* (1.08 – 5.43)	23.71* (9.78 – 57.55)	–	–	–
Referral to gynaecologist					
Squamous (ref)	1.0	1.0	1.0	1.0	1.0
AGC	0.50* (0.36 – 0.75)	26.43* (20.10 – 34.76)	10.20* (7.03 – 14.78)	210.01* (138.60 – 318.21)	29.88* (14.12 – 63.20)
AGC/Squamous	1.69* (1.30 – 2.20)	13.83* (9.19 – 20.80)	6.89* (3.91 – 12.15)	–	–

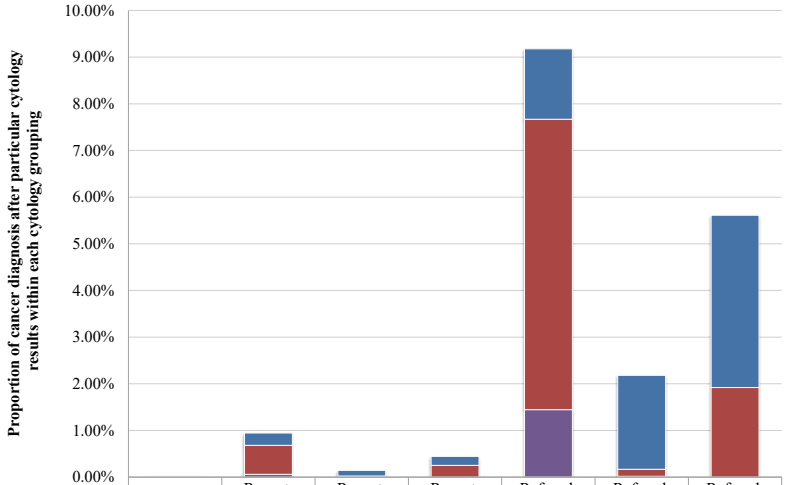
* *P* value < 0.05.
– Estimates not presented as less than 5 cancers were in these cells.
AGC: Atypical glandular cells, RR: Relative risk, CI: Confidence interval

Table 4 shows unadjusted and adjusted hazards of a cancer diagnosis after AGC and AGC/squamous cytology result compared with squamous cytology results by age and referral type. Amongst younger women, the hazards of both a cervical cancer diagnosis or other gynaecological cancer diagnosis was significantly higher for 'Repeat, AGC' and 'Referral, AGC' smear-result groups. The same was found for women aged 50 years and older, except for 'Repeat, AGC' smear-result group for other cancers diagnoses and 'Referral, AGC' smear-result group for cervical cancer diagnoses, both of which had no significant difference in hazards compared to squamous smear-result groups.

DISCUSSION

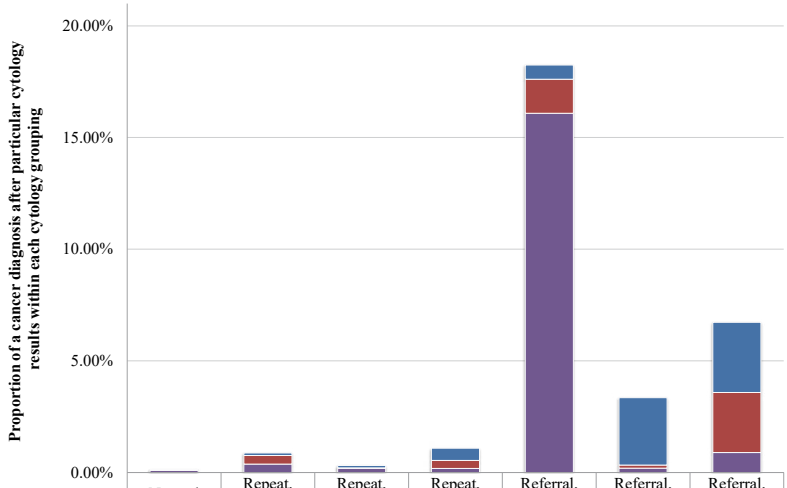
Results of our study confirm that AGC found on cervical cytology indicates an increased risk of a cancer diagnosis compared with normal cytology and squamous cell abnormalities of equal severity. The risk of a cancer diagnosis after a normal cytology test is very low in comparison to the other cytology groups, which is expected given the low rate of cervical cancer after normal cytology in the Netherlands.[12 14] Therefore, the comparisons between AGC and Squamous cytology results are of more interest for the management of patients, as cytology smears of comparable severity were given similar advice for follow-up. For all but two smear-result groups , AGC smears had a higher risk of cancer than squamous cell abnormalities of equal severity. The increased risk of a

FIGURE 1a: YOUNGER WOMEN



	Normal	Repeat, AGC	Repeat, Squamous	Repeat, AGC/Sq	Referral, AGC	Referral, Sq	Referral, AGC/Sq
Cervical - squamous	0.00%	0.26%	0.12%	0.19%	1.51%	2.01%	3.69%
Cervical - adeno	0.00%	0.62%	0.01%	0.25%	6.22%	0.15%	1.92%
Endometrial	0.01%	0.06%	0.01%	0.00%	1.45%	0.02%	0.00%

FIGURE 1b: OLDER WOMEN



	Normal	Repeat, AGC	Repeat, Squamous	Repeat, AGC/Sq	Referral, AGC	Referral, Sq	Referral, AGC/Sq
Cervical - squamous	0.00%	0.12%	0.11%	0.54%	0.64%	3.01%	3.14%
Cervical - adeno	0.00%	0.38%	0.00%	0.36%	1.53%	0.14%	2.69%
Endometrial	0.11%	0.38%	0.21%	0.18%	16.08%	0.20%	0.90%

Figure 1: *Proportion of cervical squamous-cell cancers, cervical adenocarcinomas and endometrial cancers diagnosed after various cytology results (numerator: total cancers detected after specific cytology result; denominator: all smears within each cytology result group), by cancer diagnosis*

a. Women aged 49 years and younger

b. Women aged 50 years and older

Table 4: *Results of Cox Proportional Hazards models comparing AGC and AGC/Squamous cytology results with Squamous cytology results within repeat/referral groups, by age and cancer type*

	Cervical cancers		Other cancers	
	Unadjusted HR (95% CI)	**Adjusted HR* (95% CI)**	**Unadjusted HR (95% CI)**	**Adjusted HR* (95% CI)**
Women aged 29 to 49 years				
Repeat cytology within six months				
Squamous (ref)	1.0	1.0	1.0	1.0
AGC	7.05 (5.59 – 8.87)**	6.91 (5.48 – 8.71)**	2.79 (1.37 – 5.66)**	2.82 (1.39 – 5.74)**
AGC/Squamous	3.39 (1.68 – 6.86)**	3.32 (1.64 – 6.71)**	–	–
Referral to gynaecologist				
Squamous (ref)	1.0	1.0	1.0	1.0
AGC	4.56 (3.86 – 5.39)**	4.57 (3.86 – 5.40)**	47.37 (27.01 – 83.06)**	44.43 (25.28 – 78.10)**
AGC/Squamous	2.98 (2.39 - 3.71)**	2.98 (2.39 - 3.71)**	–	–
Women aged 50 years and older				
Repeat cytology at six months				
Squamous (ref)	1.0	1.0	1.0	1.0
AGC	4.38 (2.64 – 7.29)**	3.98 (2.38 – 6.66)**	1.48 (0.86 – 2.52)	1.41 (0.82 – 2.42)
AGC/Squamous	6.68 (2.68 – 16.65)**	6.06 (2.42 – 15.16)**		
Referral to gynaecologist				
Squamous (ref)	1.0	1.0	1.0	1.0
AGC	0.89 (0.65 – 1.23)	0.88 (0.63 – 1.21)	79.31 (48.58 – 129.50)**	79.75 (48.85 – 130.20)**
AGC/Squamous	1.92 (1.12 - 3.30)**	1.86 (1.09 – 3.19)**	–	–

* Adjusted for number of previous primary cytology tests

** *P* value < 0.05

– Estimates not presented as less than 5 cancers were in these cells.

AGC: Atypical glandular cells, HR: hazard ratio

cervical cancer diagnosis is related to the significantly higher risk of adenocarcinoma after AGC. These results are similar to those reported by Wang and colleagues.[11]

Age is also a key factor associated with the risk of cancer after AGC. Cheng and colleagues found that women aged 60 years and older were more likely to have a diagnosis of cervical, uterine or ovarian cancer following AGC than women aged 35 years and younger.[24] Our study supports this finding, with a higher proportion of cancer diagnosis following screening amongst women aged 50 years and older. However, only comparing between age groups misses an important finding – that in both younger and older women, AGC cytology has a higher risk of a cancer diagnosis compared with squamous cell abnormalities. We also found that the type of cancer AGC indicates varied by age; in younger women, the largest proportion of the risk was due to cervical adenocarcinoma and, in older women, endometrial cancers. Other studies have also shown the association between age and type of cancer diagnosed after AGC.[23] Age is associated with the

likelihood of an hrHPV infection, which is highest in younger women and declines with age,[25] which is, in turn, related to whether the cancer diagnosis after AGC is hrHPV related (i.e. cervical cancers) or not.

The impact of hrHPV screening on AGC cytology and cancer detection

The impact of the introduction of primary hrHPV screening on the detection of malignancies following AGC is of interest, as many countries are transitioning from cytology-based to hrHPV-based screening. In 2017, primary hrHPV screening replaced cytology-based screening in the organised screening programme in the Netherlands. We did not include information about hrHPV status in our analysis, as the use of hrHPV testing was limited during the study period and only used for triage of low-grade repeat cytology (see Figure A1). In the new hrHPV-based Dutch screening programme, all women who are hrHPV positive and have a cytological abnormality of any kind are now directly referred. Several studies have reported on the impact of hrHPV positivity on AGC cytology[8 26 27] and results indicate that primary hrHPV screening is capable of detecting relevant cervical lesions, regardless of whether the origin is cervical squamous or cervical glandular epithelium.

However, a previous review found that women aged over 50 years with hrHPV-negative AGC cytology had an increased risk of a non-cervical cancer diagnosis.[23] We found that in both younger and older women, there was increased risk of a non-cervical cancer diagnosis after AGC cytology. Looking from the perspective of those women already diagnosed with cancer, one study of women with endometrial cancer found the majority of women with a high-grade cancer diagnosis had abnormal cervical cytology.[28] These two findings indicate that cervical cytology may have been of some use in the detection of other gynaecological cancers. As primary hrHPV screening is implemented, we expect to see a reduction in the number of cytology tests with AGC results in women over 50 years, as AGC in this age group is more likely to indicate a non-hrHPV related cancer. While the detection of these cancers is not the goal of cervical screening, some women may have benefited from incidental detection. Within the new hrHPV screening programme, women who test hrHPV negative at 40 or 50 years are given a longer screening interval of 10 years. It should be communicated explicitly to these women that if they experience clinical symptoms, they should see their GP for assessment, irrespective of their prior hrHPV test results.

Management strategies for women with AGC cytology

Our results show that the risk of a cancer diagnosis is high immediately following an AGC cytology result and suggest that management strategies for women with AGC must ensure that women receive adequate diagnostics and surveillance immediately following a AGC cytology result. As referral and treatment advice within cervical cancer

screening programmes is based on the risk of pre-cancerous lesions, our results support referring all women with AGC, given the increased risk of cancer. Given the small number of AGC abnormalities found, referring all women with AGC would be unlikely to impact on colposcopy capacity and may mean clinically significant lesions are detected sooner.

Glandular cell abnormalities may be more difficult to detect at colposcopy, as most lesions are located higher in the cervical canal. Because of this, Dutch guidelines recommend conisation, which allows for better assessment of the endocervical canal.[18] Furthermore, these guidelines suggest discussing hysterectomy with women when adenocarcinoma in situ is suspected, in cases where the possibility of invasive cancer has been excluded as far as possible. Hysterectomy may be appropriate for women over 50 years given the high risk of endometrial cancer found in our study, and the fact these women may have reached menopause. However, for women who are younger than 50 or women who do not want a hysterectomy, offering both cervical and endometrial biopsy in order to determine the origin of the AGC and inform further treatment strategies may be a practical alternative to more invasive treatment.

Strengths and limitations

Our study has several strengths. To our knowledge, this is the largest study of cancer risk following AGC cytology, with over 8 million screening tests included. We used a large and comprehensive national dataset to select cytological smears taken within our study period. We then supplemented this data with detailed information from the NCR to provide accurate diagnostic data. As AGC is an uncommon cytological abnormality, the size of our dataset has allowed us to explore trends in the incidence of multiple types of gynaecological cancers after AGC with robust estimates.

Our study also has some limitations. We did not have access to data on deaths and emigration to censor life-years at risk. In the Netherlands, linkage of demographic and medical record data on the individual level is difficult for practical and privacy reasons. However, emigration rates amongst women aged 40 years and older are low.[29] Therefore, we do not believe this impacted significantly on our results. We used data from PALGA on hysterectomy to censor screens from women following a hysterectomy. There is a small proportion of women (estimated between 1.5% – 2%, based on a small check by PALGA) who had a partial excision of the uterus who are classified as having a hysterectomy. Therefore, some women who were still at risk of a cancer may have been censored too early. Finally, there may be some degree of verification bias in types of cancers diagnosed after an abnormal cytology, as the CISOE-A score is used by gynaecologists to inform diagnostic strategies.

CONCLUSION

The presence of AGC on cervical cytology indicates an increased risk of cervical adenocarcinoma and other gynaecological cancers compared to both normal cytology and squamous cell abnormalities of comparable severity. Our results indicate that women who present with AGC on cervical cytology warrant direct referral and should be provided with diagnostic assessment of both endocervical and endometrial tissue given the risk of both cervical and other cancers in this group. Managing these women effectively and ensuring a complete diagnostic workup is important to ensure that cancers are detected and treated as early as possible.

Acknowledgements

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Conflict of interest disclosure

The funder had no involvement in the design, conduct or reporting of the study; the writing of the manuscript; or the decision to publish the manuscript. All authors declare that they have no financial disclosures or conflicts of interest.

Contributions to authorship

I.M.C.M.de Kok, F.J. van Kemenade and A.G. Siebers were responsible for the developing the concept for the study. I.M.C.M.de Kok, F.J. van Kemenade, A.G. Siebers, C.A. Aitken and E.E.L. Jansen were involved in designing the project and outlining the outcomes to be measured. A.G. Siebers created the PALGA dataset with linkage to the national cancer registry data, with input from C.A. Aitken and E.E.L. Jansen. C.A. Aitken performed the data analysis, with assistance from E.E.L. Jansen and I.M.C.M. de Kok in checking the methods and from A.G. Siebers and F.J. van Kemenade in validating the groupings of cytology results and cancer diagnoses. A.L.D. van Haaften-de Jong provided clinical knowledge and advice. C.A. Aitken wrote the manuscript with contribution from all other authors.

Ethical approval

This study used a retrospective, anonymised dataset from PALGA. The board of PALGA approved the use of data for this study. The request for NCR data was approved by the NCR.

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SUPPLEMENTARY MATERIALS

APPENDIX A: Methodological information

List of tables and figures

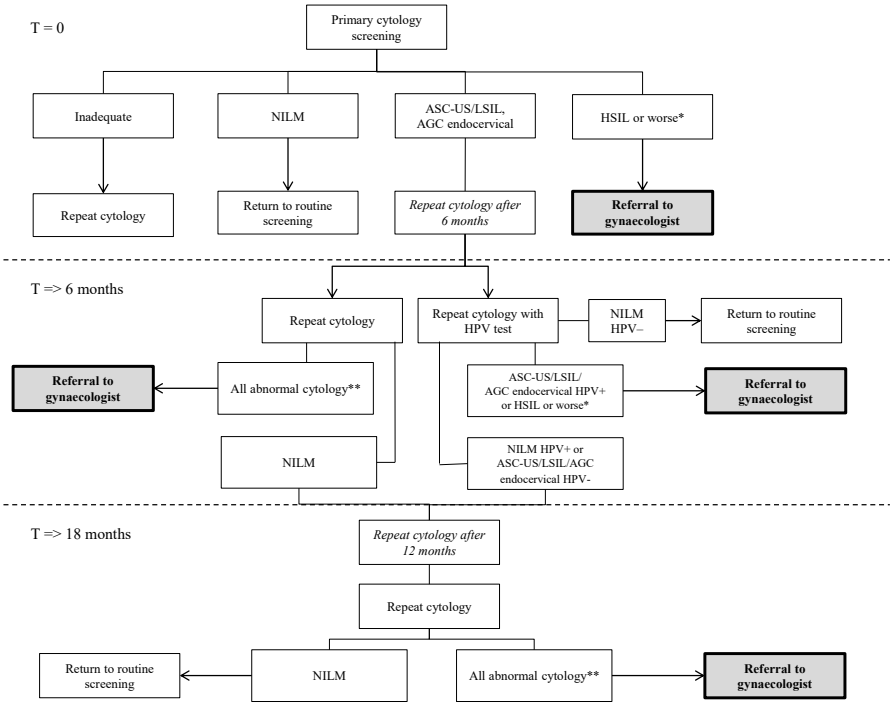

Figure S1: *Flowchart of referral pathways within Dutch cervical cancer screening programme (adapted from Bekkers et al.[1] and Aitken et al.[2])*

NB. At T => 6 months, either cytology only or HPV co-testing was performed, depending on the laboratory that processed the test.

* Includes HSIL, AGC endometrial, AGC favouring neoplasia, adenocarcinoma in situ, ASC-H and cancer irrespective of hrHPV status.

** Includes ASC-US, LSIL, AGC endometrial and HSIL or worse* cytology results.

T: time, NILM: Negative for intraepithelial lesion or malignancy, ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, AGC: atypical glandular cells, HPV: human papillomavirus

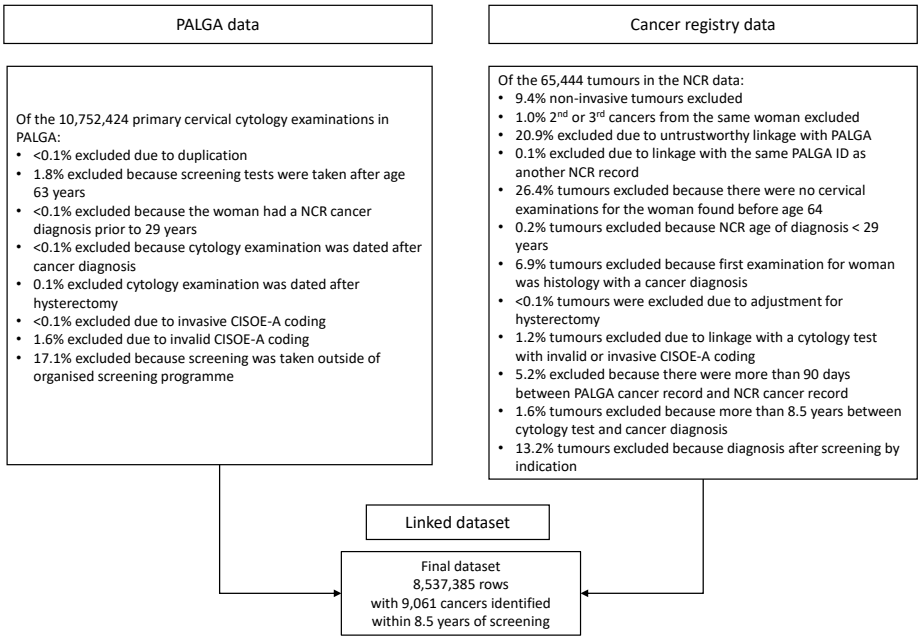

Figure S2: *Data linkage and management flowchart*

Table S1: *Cancer groups by topography and morphology codes*

Cancer type	Cancer group	Topography	Morphology*
Cervical	Cervical - squamous	C53.0, C53.1, C53.8, C53.9	M-805 – M-808, M-812, M-813
	Cervical - adenocarcinoma	C53.0, C53.1, C53.8, C53.9	M-814, M-816, M-819 – M-822, M-826 – M-833, M-835 – M-855, M-857, M-894
	Cervical - other	C53.0, C53.1, C53.8, C53.9	All other morphology codes not specified in other cervical groups
Other	Endometrial	C54.1	
	Ovarian	C56.9	
	Other	C54.0, C54.2, C54.3, C54.8, C54.9, C55.9, C57.0, C57.1	

* Grouping of morphology codes for differentiating different cervical types is based on Table 25 from the International Classification of Disease for Oncology, 3rd edition.[3]

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APPENDIX B: Further analysis and results

List of tables and figures

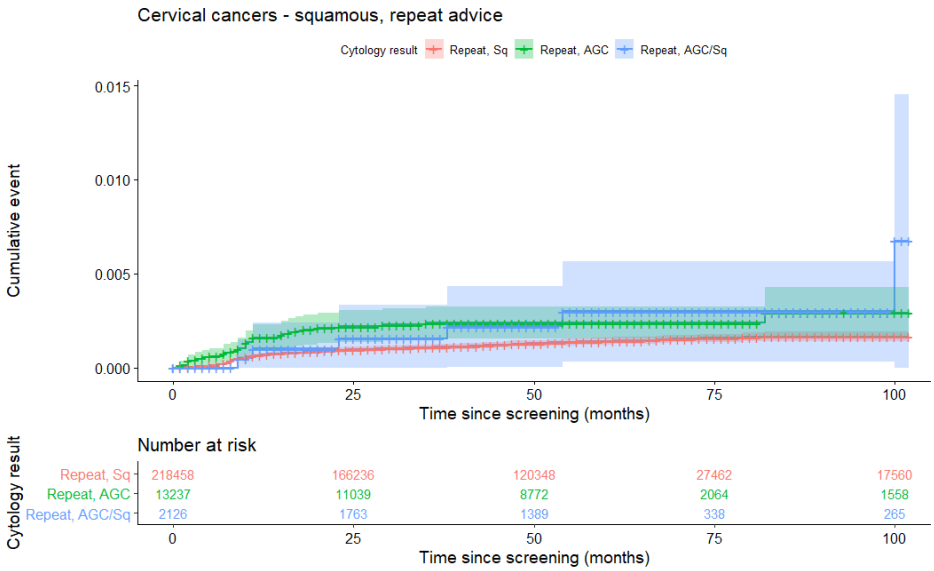

Figure S3: *Cumulative event curves for squamous cervical cancers by cytology type, repeat advice*

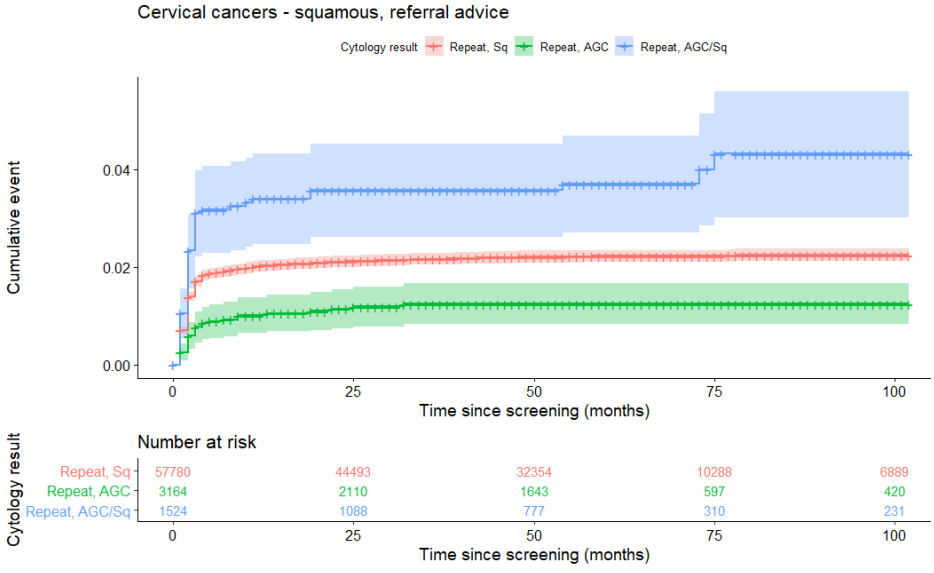

Figure S4: *Cumulative event curves for squamous cervical cancers by cytology type, referral advice*

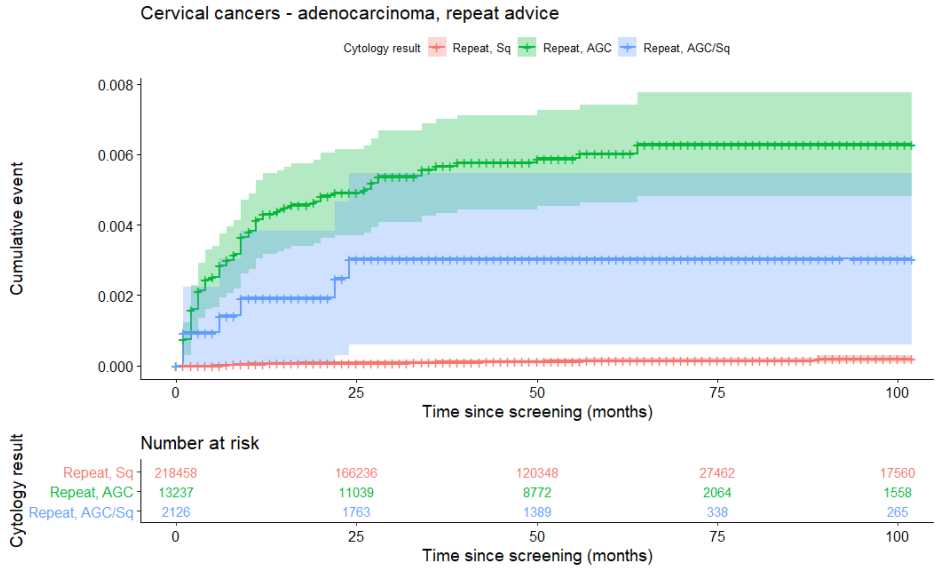

Figure S5. *Cumulative event curves for cervical adenocarcinomas by cytology type, repeat advice*

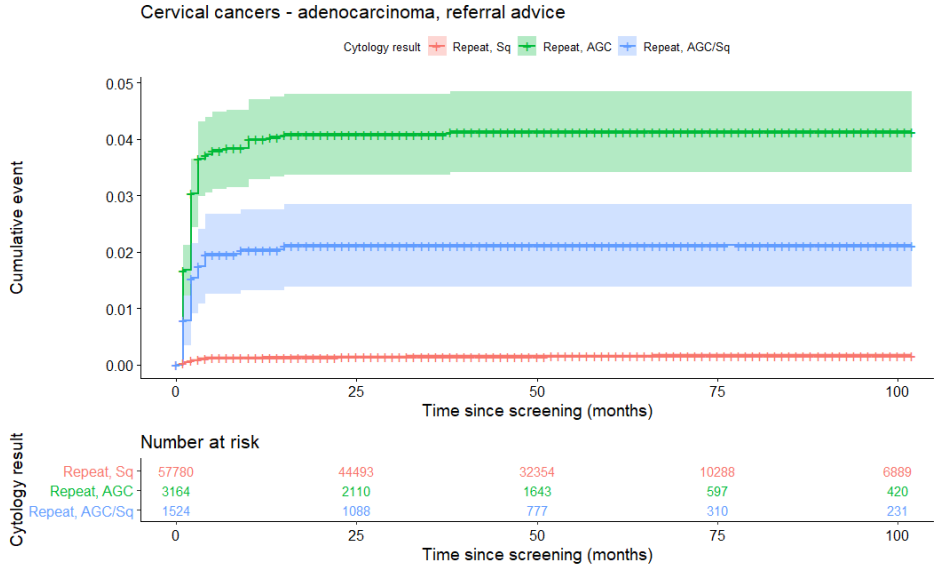

Figure S6. *Cumulative event curves for cervical adenocarcinomas by cytology type, referral advice*

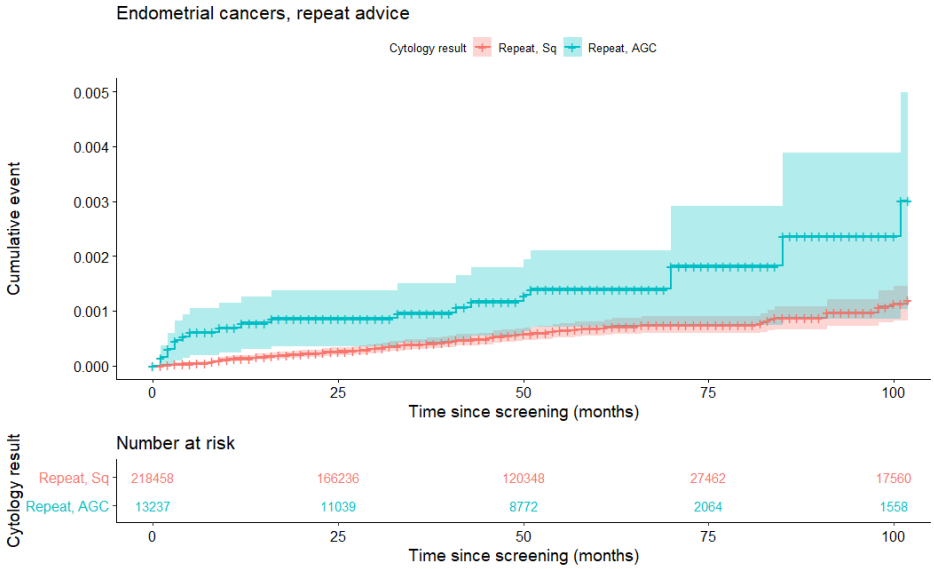

Figure S7: *Cumulative event curves for endometrial cancers by cytology type, repeat advice*

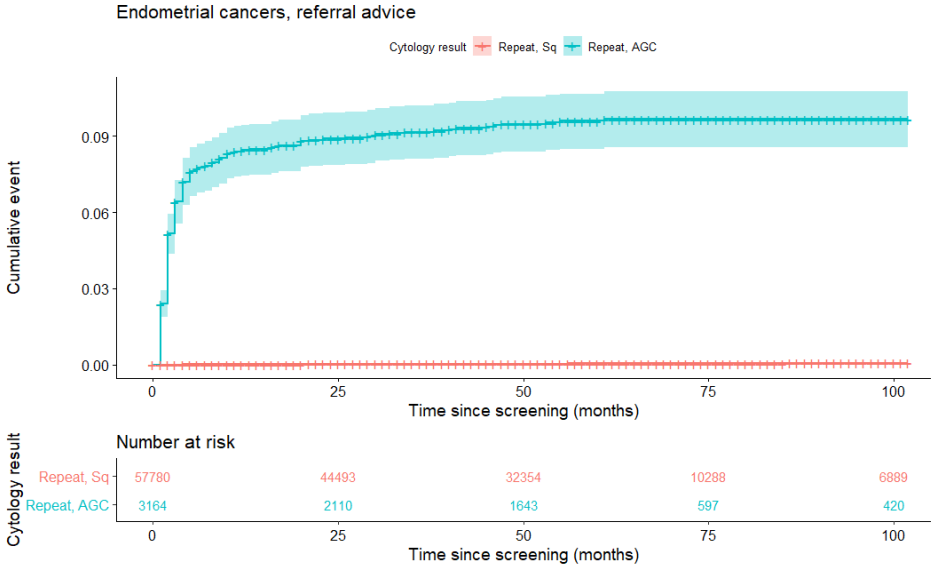

Figure S8: *Cumulative event curves for endometrial cancers by cytology type, referral advice*