Cervical screening and risk of adenosquamous and rare histological types of invasive cervical carcinoma: population based nested case-control study

Jiayao Lei,¹ Bengt Andrae,^{1,2} Alexander Ploner,¹ Camilla Lagheden,³ Carina Eklund,³ Sara Nordqvist Kleppe,^{1,3} Jiangrong Wang,^{1,3} Fang Fang,¹ Joakim Dillner,^{3,4} K Miriam Elfström,^{3,5} Pär Sparén¹

¹Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, 171 77 Stockholm, Sweden

²Center for Research and Development, Uppsala University/Region of Gävleborg, 801 88 Gävle, Sweden

³Department of Laboratory Medicine, Karolinska Institutet, 141 83 Stockholm, Sweden

⁴Karolinska University Laboratory, Karolinska University Hospital, 171 76 Stockholm, Sweden

⁵Regional Cancer Center Stockholm-Gotland, 118 27 Stockholm, Sweden

Correspondence to: J Lei
jiayao.lei@ki.se
(or @JiayaoL on Twitter
ORCID 0000-0002-4718-1414)
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ABSTRACT

OBJECTIVES

To examine the association of cervical cytology screening with the risk of adenosquamous cell carcinoma (ASC) and rare histological types of invasive cervical carcinoma (RICC), using comprehensive registry data, and to assess tumour human papillomavirus status of ASC and RICC.

DESIGN

Nationwide, population based, nested case-control study.

SETTING

Sweden.

PARTICIPANTS

All cases of invasive cervical carcinoma in Sweden during 2002-11 (4254 confirmed cases after clinical and histopathological review). 338 cases were neither squamous cell carcinoma nor adenocarcinoma, including 164 cases of ASC and 174 cases of RICC (glassy cell carcinoma, clear cell carcinoma, small cell carcinoma, neuroendocrine cell carcinoma, large cell carcinoma, and undifferentiated carcinoma). 30 birth year matched controls from the general Swedish population were matched to each case by applying incidence density sampling.

MAIN OUTCOME MEASURES

Conditional logistic regression was used to calculate odds ratios, interpreted as incidence rate ratios, for risk of ASC and RICC in relation to screening status and screening history, adjusted for education. Human papillomavirus distribution of ASC and RICC was

based on available archival tumour tissues from most Swedish pathology biobanks.

RESULTS

Women with two screening tests in the previous two recommended screening intervals had a lower risk of ASC (incidence rate ratio 0.22, 95% confidence interval 0.14 to 0.34) and RICC (0.34, 0.21 to 0.55), compared with women without any test. High risk human papillomavirus was detected in 148/211 (70%) cases with valid human papillomavirus results from tumour tissues. The risk reduction among women with tumours that were positive (incidence rate ratio 0.28, 0.18 to 0.46) and negative (0.27, 0.13 to 0.59) for high risk human papillomavirus was similar, compared with women who did not attend any test.

CONCLUSIONS

Cervical screening is associated with reduced risk of ASC and RICC, and most ASC and RICC are positive for high risk human papillomavirus. This evidence provides a benchmark for evaluating future cervical screening strategies.

Introduction

The primary goal of cervical screening is to prevent invasive cervical carcinoma by detecting and removing precancerous lesions (cervical intraepithelial neoplasia 3 or adenocarcinoma in situ), which if left untreated could lead to invasive cervical carcinoma. Hence, cervical screening can effectively reduce the incidence of and mortality from cervical carcinoma and concurrently detect asymptomatic invasive malignancies early, which affects the prognosis of invasive disease.^{1 2} Previous studies have shown that screening is associated with reduced risk of squamous cell carcinoma and adenocarcinoma, and to some extent of adenosquamous cell carcinoma (ASC).²⁻⁴ However, no studies have looked at screening and risk of rare types of invasive cervical carcinoma (RICC).

ASC is a histological type of invasive cervical carcinoma that is composed of a mixture of malignant glandular and squamous components.⁵ RICC include a group of histological types that are of glandular origin, with overlapping morphology, and the histopathological classification of these types is relatively difficult.⁵ RICC have also been reported as highly aggressive, with a worse prognosis than squamous cell carcinoma and adenocarcinoma.¹ In previous studies on screening and risk of invasive cervical carcinoma, RICC have been classified as “non-squamous cell carcinoma” or “other types” besides

WHAT IS ALREADY KNOWN ON THIS TOPIC

Evidence on cervical screening and risk of invasive cervical carcinoma that is neither squamous cell carcinoma nor adenocarcinoma is limited

Cervical screening is related to lower risk of adenosquamous cell carcinoma (ASC), but literature on the rare histological types of invasive cervical carcinoma (RICC) is absent

The distribution of human papillomavirus varies between different histological types of invasive cervical carcinoma, and its distribution for ASC and RICC is not well known

WHAT THIS STUDY ADDS

Women who attended screening according to routinely recommended intervals had a significantly reduced risk of both ASC and RICC

The magnitude of risk reduction in relation to cervical screening was less for RICC than for ASC

Most of the ASC and RICC tumours were positive for high risk human papillomavirus

squamous cell carcinoma and adenocarcinoma or have simply been excluded from the analysis.²⁻⁴ Limited evidence is available on the human papillomavirus status of these histological types.

In Sweden, women aged 23-60 years were invited to cervical cytology screening every three years until age 50 and every five years thereafter, according to national guidelines before 2015.⁶ The aim of our study was to examine the association of cervical cytology screening with the risk of ASC and RICC, by using comprehensive registry data, and to assess the tumour human papillomavirus status of ASC and RICC.

Methods

Study population

We did a population based, nested case-control study in a cohort of all women born during 1909-86 in Sweden. We identified 4533 cases of cervical cancer and unspecified uterine cancer during 2002-11, through cross linkage to the Swedish Cancer Register⁷ (supplementary figure A). We did a thorough clinical and histopathological review of all cases to ascertain the final diagnosis. Subsequently, we excluded 279 cases for the following reasons: not primary cervical origin, not epithelial, not invasive, and recurrence of a previous cancer according to review of medical charts by a single expert gynaecologist (BA), leaving 4254 confirmed cases of primary invasive cervical carcinoma. A senior pathologist (WR) did a histopathological review of 91% of all sample slides collected from pathological laboratories in Sweden. Among the confirmed cases, 338 were classified as neither squamous cell carcinoma nor adenocarcinoma, including ASC and RICC (glassy cell carcinoma, clear cell carcinoma, and other rare types of invasive cervical carcinoma such as small cell carcinoma, neuroendocrine carcinoma, large cell carcinoma, and undifferentiated carcinoma). We used date of diagnosis as the index date for these cases.

For each case, we randomly selected 30 controls from the Total Population Register,⁸ using incidence density sampling and individual matching by year of birth, which corresponds to age at time of diagnosis of the case. All controls were alive, with no history of cervical cancer, and living in Sweden up to the date of diagnosis of their matched case. We used date of diagnosis of the corresponding case as the index date for the controls. We subsequently excluded controls (n=449) who had a history of total hysterectomy, because they were no longer at risk of cervical cancer after total hysterectomy, leaving a total of 9691 controls eligible for analysis. Information on total hysterectomy came from the Swedish Patient Register,⁹ established in 1964 and with complete national coverage of information on inpatient care since 1987.

Human papillomavirus genotyping

We retrieved archived formalin fixed, paraffin embedded blocks from the archives of the diagnosing pathology laboratory, and 2909 of the confirmed cases were genotyped for human papillomavirus (68.4%).

The blocks were extracted and tested in parallel with -globin real time polymerase chain reaction and human papillomavirus genotyping by using general primers polymerase chain reaction targeting the L1 region,¹⁰ followed by typing with Luminex for 13 high risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and non-high risk types (6, 11, 26, 30, 40, 42, 43, 53, 54, 61, 66, 67, 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, 91) as previously described.¹¹ We used a blank block containing only paraffin as a control for contamination, which was sectioned and analysed in between each case block. In total, 2850 confirmed cases had valid human papillomavirus genotyping, including 211 cases of ASC and RICC, which accounted for 62% of all cases (n=338) included in this study.

Exposure

The exposure was cervical screening measured as screening status and history of the previous two recommended screening intervals (calculated according to women's age at the index date) more than six months before the index date (supplementary table A). We assumed Pap smear tests within six months before the diagnosis of cervical cancer to be part of the diagnostic investigation and did not consider them to be screening tests. We linked cases and controls to the Swedish National Cervical Screening Registry to retrieve information on attendance for screening and the eventual results of the screening tests.¹²

We categorised screening status as "no test," "one test," and "two tests" on the basis of attendance for screening in the defined two screening intervals (supplementary table B). Screening history was defined by the cytology (Pap smear) result of the two screening intervals according to Systematized Nomenclature of Medicine codes (supplementary table C) defined by the Swedish Association of Clinical Cytology. An abnormal smear included a diagnosis of atypical squamous cell of unknown significance or worse. We categorised screening history as "no test," "double normal results," "one normal result only" (including women with a normal test in one of the two screening intervals but without a test in the other interval), and "at least one abnormal result" (including women with at least one abnormal test during the two screening intervals, regardless of whether they participated or had a normal test in the other interval) (supplementary table D). We categorised mode of detection as screen detected or symptomatic cancer according to the medical charts.

Statistical analysis

We described the distribution of ASC and RICC according to age, cancer stage, and mode of detection. We categorised age at diagnosis into three groups (22-29, 30-60, and >60 years). We classified cancer stage according to the International Federation of Gynecology and Obstetrics guidelines and categorised it into IA (microinvasive), IB (localised), and II or higher (advanced).¹³ We included women aged 30 or above for the analysis of the associations of screening status and history with the risk of ASC and RICC,

because they had two full screening intervals before the index date.

We used conditional logistic regression to calculate odds ratios with 95% confidence intervals of ASC and RICC, after adjusting for the highest completed education by the year of cancer diagnosis (classified as low, middle, and high). We retrieved this information from the Longitudinal Integration Database for Health Insurance and Labour Market Studies database, which includes everyone aged 16 and above registered in Sweden at the end of each year since 1990.¹⁴ The educational information in the database is derived from the National Swedish Education Register, established in 1985. The matching variable birth year, which corresponds to age at the time of diagnosis of the case, was automatically adjusted for in the model. Given that we tracked incident cases in a dynamic population, matched on time at event, we interpreted the odds ratios as incidence rate ratios,^{15 16} which served as estimates for the association between cervical screening and risk of ASC and RICC. We also stratified the analysis by age at index date and cancer stage, to examine the heterogeneity of the studied association by age and cancer stage.

Among cases with available genotype data for human papillomavirus, we tabulated tumour human papillomavirus genotypes distribution by histological type, classified as positive or negative for HPV16, HPV18, and other high risk human papillomavirus. We further examined the association with screening by tumour human papillomavirus status. We also evaluated attendance for screening and risk of RICC, analysing by glassy cell carcinoma, clear cell carcinoma, and other rare types in a sensitivity analysis. All data were linked at the individual level on the basis of a sequential study number corresponding to the Swedish personal identity number, which allows for virtually 100% coverage.¹⁷ All statistical tests were two sided, and we used SAS 9.4 for data management and statistical analysis.

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were

they involved in developing plans for design or implementation of the study. No patients were asked to advise on interpretation or writing up of results. Our findings will be disseminated through updates of the National Swedish Guidelines for Cervical Cancer Prevention, Diagnosis, and Treatment.¹⁸ These guidelines are written by expert groups and are updated regularly to reflect advances in the scientific evidence. Patients and professional organisations are involved in reviewing and commenting on these guidelines before they are adopted into practice.

Results

Characteristics of ASC and RICC

Among the 338 cases, 164 (49%) were ASC, 43 (13%) were glassy cell carcinoma, 31 (9%) were clear cell carcinoma, and 100 (30%) were other rare types (table 1). ASC and RICC were mostly (178/338; 53%) diagnosed in women aged between 30 and 60, and only 9% (31/338) of the cases were diagnosed before age 30. Five per cent (17/338) of all cases were stage IA (microinvasive), 51% (172/338) were stage IB (localised), and 44% (149/338) were stage II+ (advanced). Most (297/338; 88%) cases were symptomatic cancers, and this proportion was even higher for CCC (30/31; 97%) and other rare types of carcinoma (97/100; 97%).

Screening status and risk of ASC and RICC

Compared with having no test in either of the previous two screening intervals, having two tests was associated with a substantially lower risk of ASC (incidence rate ratio 0.22, 95% confidence interval 0.14 to 0.34) and RICC (0.34, 0.21 to 0.55) (table 2). The risk reduction was more pronounced for women with two tests than for women with only one test. Overall, the risk reduction was greatest among women at age 30-60 (ASC: incidence rate ratio 0.21 0.13 to 0.34; RICC: 0.22, 0.12 to 0.40). Stratified by cancer stage, screening was associated with a significantly reduced risk of stage IB+ ASC through both one test (stage IB: incidence rate ratio 0.38, 0.22 to 0.66; stage II+: 0.29, 0.14 to 0.60) and two tests (stage IB: 0.23,

Table 1 | Distribution of adenosquamous cell carcinoma and rare types of invasive cervical carcinoma. Values are numbers (percentages) unless stated otherwise

	Adenosquamous cell carcinoma (n=164)	Rare types of invasive cervical carcinoma			Total (n=338)
		Glassy cell carcinoma (n=43)	Clear cell carcinoma (n=31)	Other rare types* (n=100)	
Age at diagnosis					
Mean (SD)	51.5 (16.5)	43.2 (20.0)	60.6 (17.5)	61.4 (18.9)	54.2 (18.8)
Median (interquartile range)	48.0 (38.0-64.5)	38.0 (28.0-51.0)	64.0 (42.0-75.0)	63.0 (46.0-77.0)	52.0 (39.0-71.0)
22-29	9 (5)	15 (35)	0 (0)	7 (7)	31 (9)
30-60	105 (64)	19 (44)	13 (42)	41 (41)	178 (53)
>60	50 (30)	9 (21)	18 (58)	52 (52)	129 (38)
FIGO stage†					
IA	12 (7)	1 (2)	1 (3)	3 (3)	17 (5)
IB	98 (60)	30 (70)	18 (58)	26 (26)	172 (51)
II+	54 (33)	12 (28)	12 (39)	71 (71)	149 (44)
Mode of detection					
Screen detected	31 (19)	6 (14)	1 (3)	3 (3)	41 (12)
Symptomatic cancer	133 (81)	37 (86)	30 (97)	97 (97)	297 (88)

*Includes small cell carcinoma, large cell carcinoma, neuroendocrine carcinoma, and undifferentiated cell carcinoma.

†International Federation of Gynecology and Obstetrics stage: IA=microinvasive; IB=localised cancer; II or higher=advanced cancer.

Table 2 | Incidence rate ratio (IRR) of adenosquamous cell carcinoma and rare types of invasive cervical carcinoma by screening status in previous two screening intervals in women aged over 30

Screening status	Adenosquamous cell carcinoma (n=155)				Rare types of invasive cervical carcinoma* (n=152)			
	Cases—No (%)	Controls—No (%)	Crude IRR (95% CI)	Adjusted† IRR (95% CI)	Cases—No (%)	Controls—No (%)	Crude IRR (95% CI)	Adjusted† IRR (95% CI)
Cases aged ≥30 years								
No test	65 (42)	954 (21.5)	Reference	Reference	70 (46)	1461 (33.8)	Reference	Reference
One test	44 (28)	1290 (29.0)	0.40 (0.26 to 0.60)	0.39 (0.26 to 0.59)	47 (31)	1169 (27.1)	0.64 (0.42 to 0.99)	0.69 (0.45 to 1.06)
Two tests	46 (30)	2197 (49.5)	0.22 (0.15 to 0.34)	0.22 (0.14 to 0.34)	35 (23)	1691 (39.1)	0.31 (0.19 to 0.49)	0.34 (0.21 to 0.55)
Age at diagnosis								
30–60 years:								
No test	35 (33)	367 (12.0)	Reference	Reference	23 (32)	281 (13.3)	Reference	Reference
One test	33 (31)	904 (29.5)	0.38 (0.23 to 0.63)	0.38 (0.23 to 0.62)	28 (39)	633 (29.9)	0.54 (0.30 to 0.95)	0.52 (0.29 to 0.94)
Two tests	37 (35)	1789 (58.5)	0.21 (0.13 to 0.34)	0.21 (0.13 to 0.34)	22 (30)	1200 (56.8)	0.22 (0.12 to 0.40)	0.22 (0.12 to 0.40)
>60 years:								
No test	30 (60)	587 (42.5)	Reference	Reference	47 (59)	1180 (53.5)	Reference	Reference
One test	11 (22)	386 (28.0)	0.43 (0.20 to 0.92)	0.42 (0.19 to 0.90)	19 (24)	536 (24.3)	0.76 (0.41 to 1.42)	0.82 (0.44 to 1.54)
Two tests	9 (18)	408 (29.5)	0.29 (0.12 to 0.69)	0.28 (0.11 to 0.67)	13 (16)	491 (22.2)	0.53 (0.26 to 1.11)	0.61 (0.29 to 1.27)
FIGO stage‡								
IA:								
No test	2 (20)	87 (30)	Reference	Reference	1 (20)	48 (34)	Reference	Reference
One test	5 (50)	82 (28)	3.49 (0.44 to 27.52)	3.00 (0.37 to 24.31)	4 (80)	47 (33)	3.75 (0.39 to 36.30)	3.46 (0.35 to 33.94)
Two tests	3 (30)	122 (42)	1.42 (0.14 to 14.25)	1.10 (0.10 to 11.81)	0 (0)	48 (34)	-	-
IB:								
No test	34 (37.0)	453 (17.0)	Reference	Reference	19 (33)	415 (25.4)	Reference	Reference
One test	27 (29.3)	797 (30.0)	0.40 (0.23 to 0.68)	0.38 (0.22 to 0.66)	23 (40)	457 (28.0)	0.98 (0.49 to 1.95)	0.92 (0.46 to 1.86)
Two tests	31 (33.7)	1411 (53.0)	0.24 (0.14 to 0.41)	0.23 (0.13 to 0.39)	15 (26)	762 (46.6)	0.36 (0.17 to 0.77)	0.34 (0.16 to 0.75)
II+:								
No test	29 (55)	414 (27.8)	Reference	Reference	50 (56)	998 (39.2)	Reference	Reference
One test	12 (23)	411 (27.6)	0.29 (0.14 to 0.60)	0.29 (0.14 to 0.60)	20 (22)	665 (26.1)	0.40 (0.22 to 0.74)	0.44 (0.24 to 0.81)
Two tests	12 (23)	664 (44.6)	0.15 (0.07 to 0.33)	0.15 (0.07 to 0.33)	20 (22)	881 (34.6)	0.28 (0.15 to 0.52)	0.32 (0.17 to 0.61)

*Includes glassy cell carcinoma, clear cell carcinoma, and other rare types.

†Adjusted for educational level and age.

‡International Federation of Gynecology and Obstetrics stage: IA=microinvasive; IB=localised cancer; II or higher=advanced cancer.

0.13 to 0.39; stage II+: 0.15, 0.07 to 0.33). For RICC, screening was related to a significantly reduced risk of stage II+ RICC through both one test (incidence rate ratio 0.44, 0.24 to 0.81) and two tests (0.32, 0.17 to 0.61) but a significantly reduced risk of stage IB RICC only through two tests (0.34, 0.16 to 0.75).

Screening history and risk of ASC and RICC

Women with two normal results in the previous two screening intervals had significantly lower risk of ASC (incidence rate ratio 0.16, 0.10 to 0.26) and RICC (0.29, 0.17 to 0.48). The risk reduction was also noted for women with only one test with normal result (incidence rate ratio 0.34, 0.22 to 0.53, for ASC; 0.63, 0.41 to 0.99, for RICC) (table 3). However, women with at least one abnormal test in any of the two intervals had an elevated, although not statistically significantly, risk of ASC incidence rate ratio 1.35, 0.78 to 2.37) and RICC (1.83, 0.92 to 3.66). The risk reduction in relation to two normal test results was larger for ASC than RICC and did not differ by age or cancer stage in general.

Tumour human papillomavirus status, screening status, and risk of ASC and RICC

High risk human papillomavirus was detected in 148/211 (70%) cases, among which HPV18 (79/211; 37%) was the dominant type, followed by HPV16 (47/211; 22%) and other high risk human

papillomavirus types (22/211; 10%) (table 4). We observed a similar risk reduction associated with screening for cases positive (one test: incidence rate ratio 0.42, 0.26 to 0.67; two tests: 0.28, 0.18 to 0.46) and negative (one test: 0.46, 0.23 to 0.92; two tests: 0.27, 0.13 to 0.59) for high risk human papillomavirus, comparing women who attended one or both screening tests with women who did not attend any (table 5).

Sensitivity analysis

When we stratified by each histological type of RICC, we found that having two tests was also related to reduced risks of glassy cell carcinoma (incidence rate ratio 0.24, 0.09 to 0.68), clear cell carcinoma (0.17, 0.05 to 0.57), and other rare types (0.44, 0.24 to 0.83) (supplementary table E).

Discussion

Women who routinely attended for cervical screening according to recommended intervals had a significantly reduced risk of both ASC and RICC compared with those who did not attend for screening. The risk reduction was more pronounced for women with two tests than for women with only one test. The magnitude of risk reduction in relation to cervical screening was less for RICC than for ASC. Moreover, attending screening was associated with a significantly decreased risk of advanced stage cancers and substantial down-staging for ASC and RICC.

Table 3 | Incidence rate ratio (IRR) of adenosquamous cell carcinoma and rare types of invasive cervical carcinoma by screening history in previous two screening intervals in women aged over 30

Screening history	Adenosquamous cell carcinoma (n=155)				Rare types of invasive cervical carcinoma* (n=152)			
	Cases— No (%)	Controls— No (%)	Crude IRR (95% CI)	Adjusted† IRR (95% CI)	Cases— No (%)	Controls— No (%)	Crude IRR (95% CI)	Adjusted† IRR (95% CI)
Cases aged ≥30 years								
No test	65 (42)	954 (21.5)	Reference	Reference	70 (46)	1461 (33.8)	Reference	Reference
Double normal	31 (20)	2059 (46.4)	0.17 (0.11 to 0.27)	0.16 (0.10 to 0.26)	28 (18)	1608 (37.2)	0.26 (0.16 to 0.43)	0.29 (0.17 to 0.48)
One normal only	38 (25)	1269 (28.6)	0.35 (0.23 to 0.54)	0.34 (0.22 to 0.53)	42 (28)	1145 (26.5)	0.59 (0.38 to 0.92)	0.63 (0.41 to 0.99)
At least one abnormal	21 (14)	159 (3.6)	1.41 (0.81 to 2.45)	1.35 (0.78 to 2.37)	12 (8)	107 (2.5)	1.70 (0.86 to 3.37)	1.83 (0.92 to 3.66)
Age at diagnosis								
30-60 years:								
No test	35 (33)	367 (12.0)	Reference	Reference	23 (32)	281 (13.3)	Reference	Reference
Double normal	23 (22)	1681 (54.9)	0.14 (0.08 to 0.25)	0.14 (0.08 to 0.24)	16 (22)	1142 (54.0)	0.17 (0.09 to 0.32)	0.17 (0.08 to 0.32)
One normal only	27 (26)	889 (29.1)	0.32 (0.19 to 0.54)	0.32 (0.19 to 0.54)	24 (33)	619 (29.3)	0.48 (0.26 to 0.87)	0.47 (0.26 to 0.85)
At least one abnormal	20 (19)	123 (4.0)	1.62 (0.89 to 2.94)	1.58 (0.86 to 2.90)	10 (14)	72 (3.4)	1.69 (0.76 to 3.75)	1.70 (0.76 to 3.81)
>60 years:								
No test	30 (60)	587 (42.5)	Reference	Reference	47 (59)	1180 (53.5)	Reference	Reference
Double normal	8 (16)	378 (27.4)	0.28 (0.11 to 0.68)	0.27 (0.11 to 0.67)	12 (15)	466 (21.1)	0.52 (0.25 to 1.11)	0.59 (0.28 to 1.26)
One normal only	11 (22)	380 (27.5)	0.43 (0.20 to 0.93)	0.42 (0.20 to 0.91)	18 (23)	526 (23.8)	0.74 (0.39 to 1.38)	0.80 (0.42 to 1.50)
At least one abnormal	1 (2)	36 (2.6)	0.35 (0.04 to 2.83)	0.34 (0.04 to 2.81)	2 (3)	35 (1.6)	1.16 (0.26 to 5.16)	1.29 (0.29 to 5.72)
FIGO stage‡								
IA:								
No test	2 (20)	87 (30)	Reference	Reference	1 (20)	48 (34)	Reference	Reference
Double normal	2 (20)	115 (40)	0.99 (0.09 to 11.32)	0.76 (0.06 to 9.27)	0 (0)	46 (32)	-	-
One normal only	5 (50)	81 (28)	3.50 (0.44 to 27.83)	2.99 (0.36 to 24.82)	2 (40)	47 (33)	2.40 (0.20 to 29.06)	2.35 (0.18 to 31.23)
At least one abnormal	1 (10)	8 (3)	7.16 (0.39 to 131.29)	6.96 (0.32 to 150.07)	2 (40)	2 (1)	48.40 (2.08 to 1129.05)	-
IB:								
No test	34 (37)	453 (17.0)	Reference	Reference	19 (33)	415 (25.4)	Reference	Reference
Double normal	19 (22)	1323 (49.7)	0.17 (0.09 to 0.31)	0.16 (0.09 to 0.29)	10 (18)	721 (44.1)	0.25 (0.11 to 0.59)	0.24 (0.10 to 0.57)
One normal only	22 (24)	783 (29.4)	0.34 (0.19 to 0.60)	0.33 (0.18 to 0.58)	20 (35)	446 (27.3)	0.89 (0.44 to 1.81)	0.84 (0.41 to 1.73)
At least one abnormal	17 (18)	102 (3.8)	1.87 (0.96 to 3.64)	1.77 (0.90 to 3.48)	8 (14)	52 (3.2)	2.97 (1.16 to 7.58)	2.90 (1.11 to 7.53)
II+:								
No test	29 (557)	414 (27.8)	Reference	Reference	50 (56)	998 (39.2)	Reference	Reference
Double normal	10 (19)	621 (41.7)	0.14 (0.06 to 0.31)	0.14 (0.06 to 0.31)	18 (20)	841 (33.1)	0.26 (0.14 to 0.49)	0.30 (0.16 to 0.59)
One normal only	11 (21)	405 (27.2)	0.27 (0.13 to 0.57)	0.27 (0.12 to 0.57)	20 (22)	652 (25.6)	0.41 (0.23 to 0.75)	0.45 (0.25 to 0.83)
At least one abnormal	3 (6)	49 (3.3)	0.52 (0.15 to 1.83)	0.52 (0.15 to 1.81)	2 (2)	53 (2.1)	0.46 (0.11 to 2.03)	0.49 (0.11 to 2.16)

*Includes glassy cell carcinoma, clear cell carcinoma, and other rare types.

†Adjusted for educational level and age.

‡International Federation of Gynecology and Obstetrics stage: IA=microinvasive; IB=localised cancer; II or higher=advanced cancer.

Interpretation of findings

RICC tended to be diagnosed at a more advanced stage than the other histological types,² which might subsequently result in a worse prognosis as previously reported.¹ RICC may be a group of invasive cervical carcinomas with distinctive histological types compared with ASC, given that RICC have a rapid progression,⁵ and we found that the risk reduction in relation to one test in the previous screening intervals was not significant. Of note, as we defined it, RICC consist of several specific histological types, which

are rare variants of adenocarcinoma. As a result, they might be more likely to have glandular precancerous lesions, which are located in the endocervical canal and typically more difficult to detect through cytology and manage through colposcopy assessment compared with squamous cell lesions.

Our finding of a greater risk reduction in women who had two tests compared with one test in the previous two screening intervals confirmed the general principle that the sensitivity of cytology is improved through repeated tests. Attending all recommended screening

Table 4 | Tumour human papillomavirus (HPV) status of adenosquamous cell carcinoma and rare types of invasive cervical carcinoma. Values are numbers (percentages)

Characteristics	Total	Tumour HPV status			
		HPV16	HPV18	Other hrHPV positive	hrHPV negative
Adenosquamous cell carcinoma	119 (100)	25 (21)	49 (41)	14 (12)	31 (26)
Rare types of invasive cervical carcinoma:					
Glassy cell carcinoma	24 (100)	5 (21)	12 (50)	3 (13)	4 (17)
Clear cell carcinoma	14 (100)	1 (7)	5 (36)	1 (7)	7 (50)
Other rare types*	54 (100)	16 (30)	13 (24)	4 (7)	21 (39)
Total	211 (100)	47 (22)	79 (37)	22 (10)	63 (30)

Women with valid tumour HPV genotypes were included (n=211).

hrHPV=high risk human papillomavirus.

*Includes small cell carcinoma, large cell carcinoma, neuroendocrine carcinoma, and undifferentiated cell carcinoma.

Table 5 | Incidence rate ratio (IRR) of adenosquamous cell carcinoma and rare types of invasive cervical carcinoma by screening status in previous two screening intervals and tumour human papillomavirus (HPV) status

Screening status	Cases—No (%)	Controls—No (%)	Crude IRR (95% CI)	Adjusted* IRR (95% CI)
Cases aged ≥30 years				
No test	86 (45)	1456 (26.3)	Reference	Reference
One test	52 (27)	1613 (29.2)	0.40 (0.28 to 0.59)	0.43 (0.29 to 0.63)
Two tests	55 (28)	2459 (44.5)	0.26 (0.18 to 0.38)	0.28 (0.19 to 0.42)
Tumour HPV status				
hrHPV positive:				
No test	53 (40)	831 (21.9)	Reference	Reference
One test	37 (28)	1143 (30.1)	0.40 (0.25 to 0.63)	0.42 (0.26 to 0.67)
Two tests	41 (31)	1829 (48.1)	0.26 (0.17 to 0.41)	0.28 (0.18 to 0.46)
hrHPV negative:				
No test	33 (53)	625 (36.2)	Reference	Reference
One test	15 (24)	470 (27.2)	0.42 (0.21 to 0.84)	0.46 (0.23 to 0.92)
Two tests	14 (23)	630 (36.5)	0.25 (0.12 to 0.54)	0.27 (0.13 to 0.59)

Women aged 30 and above with valid tumour HPV genotypes were included (n=193).

hrHPV=high risk human papillomavirus.

*Adjusted for education level and age.

tests can increase the probability of existing precursors being detected. When we stratified the analyses by age at diagnosis of cancer, attending screening was related to decreased risk of ASC and RICC among women aged below 60 compared with women with no test, and we had limited power to estimate screening for women aged above 60 in this study. However, a previous study showed that the risk of invasive cervical cancer after age 60 was highly related to screening at age 51–60.¹⁹ Most (13/17; 76%) of the stage IA cases in our study were detected through screening, so it was not unexpected that women with screening tests showed an increased risk of cancer at stage IA compared with women without any tests, whose cancers are usually diagnosed at more advanced stages.

In terms of screening history, our results highlight that having normal tests in the previous two screening intervals was associated with a reduction in the risk of ASC and RICC, especially for women at age 30–60. In contrast, having at least one abnormal test was associated with increased risks of both ASC and RICC compared with women without any test. This could be due to the inadequate execution of the management practice after an abnormality.² In addition, clinical management of abnormal smears could alter the risk of ASC and RICC after an abnormal smear, and timely assessment through biopsies or treatment (if needed) could be essential for histological types that progress rapidly. Also, the recommended management strategy might not be perfectly effective for certain scenarios of risk elevation.^{20 21}

The proportions of positivity for high risk human papillomavirus in ASC and RICC were somewhat lower than for squamous cell carcinoma and adenocarcinoma.²² Recently, a meta-analysis showed that adenocarcinoma had a lower prevalence of high risk human papillomavirus compared with adenocarcinoma in situ.²³ Some variants of RICC were reported as having a low prevalence of human papillomavirus or even as not being related to human papillomavirus.^{24 25} However, the lower proportion of positivity for high risk human papillomavirus noted in our study does not have to signify that this is not

the main cause of ASC and RICC. On the contrary, it indicates that human papillomavirus might become undetectable in advanced cancers.^{26 27} Therefore, the fact that negativity for high risk human papillomavirus is preferentially found in advanced and symptomatic cases might have resulted in a greater proportion of such cases among RICC. In another sample tested with the same method, we found that 97% of women with cervical intraepithelial neoplasia 3+ were positive for high risk human papillomavirus.²⁸

Comparisons with other studies

Our findings on risk reduction of ASC are similar to those of UK studies.^{3 4} However, no previous studies have looked at the association between screening and risk of RICC owing to the rarity of these diagnoses and limited information on cervical screening. Compared with squamous cell carcinoma and adenocarcinoma, we saw a similar trend of risk reduction associated with cervical screening in ASC and RICC as well as a downstaging effect.^{23 29} The risk reduction for ASC associated with cervical screening was similar to that for squamous cell carcinoma,² whereas the risk reductions for RICC and adenocarcinoma were attenuated (supplementary table F). The smaller magnitude of risk reduction for RICC might explain why RICC tend to be diagnosed at a more advanced stage and to be symptomatic compared with the more common types.² Similar risk reductions for ASC and squamous cell carcinoma also suggest that ASC might evolve from a squamous component and acquire glandular involvement later on,⁴ or it might be a glandular cancer exhibiting squamous differentiation as shown in previous studies.²⁴

Strengths and limitations of study

Although this is the largest study to date of ASC and RICC, and we included all cases over a 10 year period, further assessment of clinical management after abnormal tests was still not feasible owing to the limited number of cases. The small number of cases resulted in reduced precision in some of the subgroup analyses. We did not have information on lifestyle factors (such as smoking status) and sexually

transmitted infections. However, we had virtually complete information on educational level, which is a good proxy indicator for both socioeconomic status and lifestyle factors.³⁰ We thus controlled for educational level in our analysis, and the association between screening and risk of ASC and RICC remained robust. Nevertheless, a healthy volunteer effect for women participating in cervical screening cannot be ruled out. If existing, it should be fairly small and not likely to account for the risk differences observed in this study. Finally, misclassification of histological types might have occurred owing to the overlap of morphology, but we used strict clinical and histopathological review to ensure accurate classification.

To the best of our knowledge, this is the first population based study to examine cervical screening and risk of ASC and RICC and to present the prevalence of human papillomavirus in tumours. All cases were strictly reviewed according to a cervical cancer case audit protocol. We used complete screening data extracted from the Swedish National Cervical Screening Registry with limited selection and information bias. Moreover, all controls were selected randomly from the Swedish Total Population Register and individually matched to the cases on year of birth, further eliminating selection bias and confounding by age. High quality information on hysterectomy, death, and migration limited bias due to loss of follow-up. We used a xylene-free extraction method that has been shown to be robust and result in improved detectability of human papillomavirus compared with standard xylene methods.³¹ This method is highly sensitive for human papillomavirus genotyping in formalin fixed, paraffin embedded material,¹¹ and it was used with strict quality control to avoid contamination with human papillomavirus and ensure the accuracy of the detected genotypes. Our results should be generalisable to countries or regions with similar screening programmes and settings.

Future research

As the great majority of ASC and RICC positive for high risk human papillomavirus were HPV16/HPV18 positive, vaccination against human papillomavirus with bivalent or quadrivalent vaccines will be a significant strategy for prevention. With the nine-valent human papillomavirus vaccine, which covers five additional virus types, additional prevention of ASC and RICC could be expected in future. European guidelines from 2015 recommend human papillomavirus as a primary testing method for organised, population based screening in women at age 30 and above.³² Continued monitoring of ASC and RICC is necessary in the post-human papillomavirus vaccination era and after the switch to primary human papillomavirus testing.

Conclusions

Cervical screening is associated with a reduced risk of both ASC and RICC, especially for advanced stage cancers, which gives a benchmark for evaluating future

cervical screening strategies. As most of ASC and RICC were positive for high risk human papillomavirus, the switch to primary human papillomavirus screening and prevention by vaccination are also expected to decrease the risk of ASC and RICC, but this will need to be monitored.

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Contributors: JL did the literature search and review. BA, JD, and PS conceived the research questions and hypotheses and designed the study. PS, JD, and SNK collected diagnostic samples. BA reviewed medical charts and supported histopathological classification of all cases. CL and CE did the laboratory analysis on human papillomavirus genotyping and validation. JL and AP did statistical analysis, and JL, BA, AP, JD, FF, KME, and PS interpreted the data. JL wrote the original draft of this manuscript. All authors were involved in the critical revision of article and have read and approved submission of the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. PS is the guarantor.

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Ethical approval: This study was approved by the Regional Ethical Review Board in Stockholm, which determined that, owing to the population based nature of the study, informed consent from the study participants was not needed (Dnr 2011/1026-31/4; Dnr 02-556; Dnr 2012/1028/32; Dnr 2011/921-32).

Data sharing: Data from the study are available on request from corresponding author.

Transparency declaration: The lead author (PS) 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.

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- Andrae B, Andersson TM, Lambert PC, et al. Screening and cervical cancer cure: population based cohort study. *BMJ* 2012;344:e900. doi:10.1136/bmj.e900
- Andrae B, Kemetli L, Sparén P, et al. Screening-preventable cervical cancer risks: evidence from a nationwide audit in Sweden. *J Natl Cancer Inst* 2008;100:622-9. doi:10.1093/jnci/djn099
- Castanon A, Landy R, Sasieni PD. Is cervical screening preventing adenocarcinoma and adenosquamous carcinoma of the cervix? *Int J Cancer* 2016;139:1040-5. doi:10.1002/ijc.30152
- Sasieni P, Castanon A, Cuzick J. Screening and adenocarcinoma of the cervix. *Int J Cancer* 2009;125:525-9. doi:10.1002/ijc.24410
- Kurman RJ, Carcangiu ML, Herrington CS, Young RH. *WHO Classification of Tumours of Female Reproductive Organs*. 4th ed. International Agency for Research on Cancer (IARC), 2014.
- Dillner J. Cervical cancer screening in Sweden. *Eur J Cancer* 2000;36:2255-9. doi:10.1016/S0959-8049(00)00320-8
- National Board of Health and Welfare. Swedish Cancer Registry: Socialstyrelsen. <http://www.socialstyrelsen.se/register/halsodataregister/cancerregistret/inenglish>.

- 8 Ludvigsson JF, Almqvist C, Bonamy AK, et al. Registers of the Swedish total population and their use in medical research. *Eur J Epidemiol* 2016;31:125-36. doi:10.1007/s10654-016-0117-y
- 9 Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011;11:450. doi:10.1186/1471-2458-11-450
- 10 Söderlund-Strand A, Carlson J, Dillner J. Modified general primer PCR system for sensitive detection of multiple types of oncogenic human papillomavirus. *J Clin Microbiol* 2009;47:541-6. doi:10.1128/JCM.02007-08
- 11 Lagheden C, Eklund C, Lamin H, et al. Nationwide comprehensive human papillomavirus (HPV) genotyping of invasive cervical cancer. *Br J Cancer* 2018;118:1377-81. doi:10.1038/s41416-018-0053-6
- 12 NKCC. Swedish National Cervical Screening Registry_Analysis. http://www.nkcx.se/index_e.htm.
- 13 Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet* 2009;105:103-4. doi:10.1016/j.ijgo.2009.02.012
- 14 Statistics Sweden. Longitudinal integration database for health insurance and labour market studies (LISA). <https://www.scb.se/lisa-en>.
- 15 Knol MJ, Vandenbroucke JP, Scott P, Egger M. What do case-control studies estimate? Survey of methods and assumptions in published case-control research. *Am J Epidemiol* 2008;168:1073-81. doi:10.1093/aje/kwn217
- 16 Pearce N. What does the odds ratio estimate in a case-control study? *Int J Epidemiol* 1993;22:1189-92. doi:10.1093/ije/22.6.1189
- 17 Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. *Eur J Epidemiol* 2009;24:659-67. doi:10.1007/s10654-009-9350-y
- 18 Regional Cancer Centers in Sweden in Cooperation. National Swedish Guidelines for Cervical Cancer Prevention, Diagnosis, and Treatment. 2018. <https://www.cancercentrum.se/globalassets/vara-uppdrag/prevention-tidig-upptackt/gynekologisk-cellprovskontroll/wardprogram/nationellt-wardprogram-cervixcancerprevention.pdf>.
- 19 Wang J, Andrae B, Sundström K, et al. Effectiveness of cervical screening after age 60 years according to screening history: Nationwide cohort study in Sweden. *PLoS Med* 2017;14:e1002414. doi:10.1371/journal.pmed.1002414
- 20 Wang J, Andrae B, Sundström K, et al. Risk of invasive cervical cancer after atypical glandular cells in cervical screening: nationwide cohort study. *BMJ* 2016;352:i276. doi:10.1136/bmj.i276
- 21 Strander B, Hällgren J, Sparén P. Effect of ageing on cervical or vaginal cancer in Swedish women previously treated for cervical intraepithelial neoplasia grade 3: population based cohort study of long term incidence and mortality. *BMJ* 2014;348:f7361. doi:10.1136/bmj.f7361
- 22 de Sanjose S, Quint WG, Alemany L, et al. Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol* 2010;11:1048-56. doi:10.1016/S1470-2045(10)70230-8
- 23 Guan P, Clifford GM, Franceschi S. Human papillomavirus types in glandular lesions of the cervix: a meta-analysis of published studies. *Int J Cancer* 2013;132:248-50. doi:10.1002/ijc.27663
- 24 Holl K, Nowakowski AM, Powell N, et al. Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: Results from a European multinational epidemiological study. *Int J Cancer* 2015;137:2858-68. doi:10.1002/ijc.29651
- 25 Hasegawa K, Nagao S, Yasuda M, et al. Gynecologic Cancer InterGroup (GIG) consensus review for clear cell carcinoma of the uterine corpus and cervix. *Int J Gynecol Cancer* 2014;24(Suppl 3):S90-5. doi:10.1097/IGC.0000000000000297
- 26 Lei J, Ploner A, Lagheden C, et al. High-risk human papillomavirus status and prognosis in invasive cervical cancer: A nationwide cohort study. *PLoS Med* 2018;15:e1002666. doi:10.1371/journal.pmed.1002666
- 27 Schiffman M, Kinney WK, Cheung LC, et al. Relative Performance of HPV and Cytology Components of Cotesting in Cervical Screening. *J Natl Cancer Inst* 2018;110:501-8. doi:10.1093/jnci/djx225
- 28 Hortlund M, Sundström K, Lamin H, Hjerpe A, Dillner J. Laboratory audit as part of the quality assessment of a primary HPV-screening program. *J Clin Virol* 2016;75:33-6. doi:10.1016/j.jcv.2015.12.007
- 29 Sasieni P, Castanon A, Cuzick J. Effectiveness of cervical screening with age: population based case-control study of prospectively recorded data. *BMJ* 2009;339:b2968. doi:10.1136/bmj.b2968
- 30 Cavelaars AE, Kunst AE, Geurts JJ, et al. Educational differences in smoking: international comparison. *BMJ* 2000;320:1102-7. doi:10.1136/bmj.320.7242.1102
- 31 Lagheden C, Eklund C, Kleppe SN, Unger ER, Dillner J, Sundström K. Validation of a standardized extraction method for formalin-fixed paraffin-embedded tissue samples. *J Clin Virol* 2016;80:36-9. doi:10.1016/j.jcv.2016.04.016
- 32 von Karsa L, Arbyn M, De Vuyst H, et al. European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination. *Papillomavirus Res* 2015;1:22-31. doi:10.1016/j.pvr.2015.06.006

Supplementary materials