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Screening effectiveness of less common histological types of invasive cervical cancer: a population-based nested case-control study

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What is this article adds to the literature:

What is already known on this topic:

- Evidence on screening effectiveness for invasive cervical cancer, which are neither squamous cell carcinoma nor adenocarcinoma, is limited.
- Cervical screening is related to lower risk of adenosquamous cell carcinoma (ASC), however, literature on screening effectiveness of the rare types of invasive cervical cancer (RICC) is absent.
- Human papillomavirus (HPV) distribution varies between different histological types of invasive cervical cancer (ICC), and the HPV distribution for less common types of ICC 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, and the magnitude of risk reduction in relation to cervical screening was less for RICC compared to ASC.
- Most of the less common types of ICC are high-risk human papillomavirus positive in tumors.

Abstract

Objectives: To examine the association of cervical cytology screening with the risk of less common invasive cervical cancer (ICC) using comprehensive registry data, and to assess tumor human papillomavirus (HPV) status of less common ICC.

Design: Nationwide, population-based nested case-control study.

Setting: Sweden.

Participants: We included all cases of cervical cancer in Sweden during 2002-2011 (4254 confirmed cases after clinical and histo-pathological review). We identified 338 cases of less common ICC, which were neither squamous cell carcinoma nor adenocarcinoma, including 164 cases of adenosquamous cell carcinoma (ASC) and 174 rare invasive cervical cancer cases (RICC) (glassy cell carcinoma, clear cell carcinoma, small cell carcinoma, neuroendocrine cell carcinoma, large cell carcinoma, and undifferentiated carcinoma). Thirty birth-year-matched controls from the general Swedish population were matched to each case applying incidence density sampling.

Main outcome measures: Conditional logistic regression was used to calculate odds ratios, interpreted as incidence rate ratios (IRRs), on risk of less common ICC in relation to screening status and screening history adjusting for education. HPV distribution of less common ICC was based on available archival tumor tissues from the majority of Swedish pathology biobanks.

Results: Women with two screening tests in the last two recommended screening intervals had a lower risk of ASC (IRR 0.22, 95% CI 0.14 to 0.34) and RICC (IRR 0.34, 95% CI 0.21 to 0.55), compared to women without any test. High-risk human papillomavirus (hrHPV) was

detected in 148/211 (70.1%) cases of less common ICC with valid HPV results from tumor tissues. The risk reduction among women with hrHPV-positive (IRR 0.28, 95% CI 0.18 to 0.46) and women with hrHPV-negative (IRR 0.27, 95% CI 0.13 to 0.59) tumours was similar, compared to women not attending any test.

Conclusions: Cervical screening is associated with reduced risk of ASC and RICC, and majority of less common ICC are hrHPV positive. Our evidence gives a benchmark for evaluating future cervical screening strategies.

Introduction

Cervical screening with cytology, which aims to detect precancerous lesions and invasive cervical cancer (ICC) before the onset of symptoms, has been implemented in most high-income countries.[1] The introduction of organized screening from the early 1960s and onwards in Nordic countries has been estimated to have prevented almost half of the expected ICC cases.[2] Effectiveness of screening for squamous cell carcinoma (SCC) and adenocarcinoma (AC) has been evaluated, and to some extent also adenosquamous cell carcinoma (ASC).[3-5] However, no studies on screening effectiveness for other rare types of invasive cervical cancer (RICC) have been performed, although RICC include histological types reported as highly aggressive, with a worse prognosis and distinct characteristics compared to SCC and AC.[6]

Among less common histological types of ICC besides SCC and AC, ASC is a histological type that is composed of a mixture of malignant glandular and squamous components.[7] RICC includes a group of histological types with overlapping morphology, and the histopathological classification of these types is relatively difficult.[7] In previous studies on the effectiveness of screening, RICC have been classified as “non-squamous cell carcinoma”, “other types” besides SCC and AC, or simply excluded from the analysis.[3 5] Furthermore, there is limited evidence available on the human papillomavirus (HPV) status of these histological types.

In Sweden, women ages 23 - 60 were invited to cervical cytology screening every three years until age 50 and every five years thereafter, according to national guidelines before 2015.[8] The aim of our study was to examine the association of cervical cytology screening with the risk of less common ICC using comprehensive registry data, and to assess the tumor HPV status of less common ICC.

Material and Methods

Study population

We conducted a population-based nested case-control study, which represented a cohort of all Swedish women that were born during 1909-1986 in Sweden. A total of 4533 ICC cases were identified during 2002-2011, through cross-linkage to the Swedish Cancer Register[9] (Supplementary Figure 1). Subsequently, 279 cases were excluded for the following reasons a) not invasive cancer, b) not cervical origin, c) not primary tumor, and d) relapse of a previous cancer according to medical charts review by a single expert gynecologist (BA), leaving 4254 confirmed primary ICC. A senior pathologist (WR) performed histo-pathological review for 91% of all sample slides collected from pathological laboratories in Sweden. Among the confirmed cases, there were 338 cases classified as less common ICC, which were neither SCC nor AC, including ASC and RICC (glassy cell carcinoma (GCC), clear cell carcinoma (CCC), and other rare types of ICC such as small cell carcinoma, neuroendocrine carcinoma, large cell carcinoma, and undifferentiated carcinoma). Date of diagnosis was used as the index date for these cases.

For each case of less common ICC, thirty controls were randomly selected from the Total Population Register,[10] using incidence density sampling and individual matching by year of birth. All controls were alive, with no history of cervical cancer, and living in Sweden on the date of diagnosis of their matched case. Date of cancer diagnosis of the corresponding case was used as the index date for the controls. Controls that had a history of hysterectomy were subsequently excluded based on information from the Swedish Patient Register,[11] because they were no longer at risk of cervical cancer after hysterectomy, leaving a total of 9691 controls eligible for analysis.

HPV genotyping

Archived formalin-fixed paraffin-embedded (FFPE) blocks were retrieved from the archives of the diagnosing pathology laboratory, and 2909 of the confirmed cases were HPV genotyped (68.4%). FFPE blocks were extracted and tested in parallel with β-globin real-time polymerase chain reaction (PCR) and HPV genotyping using general primers (MGP)-PCR targeting the L1 region,[12] 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.[13] We used a blank-block containing only paraffin as a control for contamination which was sectioned and analyzed in between each case block. In total, 2850 confirmed cases had valid HPV genotyping, including 211 cases of less common ICC which accounted for 62.4% of all less common ICC cases (n=338).

Exposure

The exposure was cervical screening measured as screening status and history of the last two recommended screening intervals (calculated according to women's age at the index date) more than six months before the index date (Supplementary Table 1). Pap smear tests within six months before the diagnosis of cervical cancer were assumed to be part of the diagnostic work-up and not considered to be screening tests. Cases and controls were linked to the Swedish National Cervical Screening Registry[14] (NKCx) to retrieve information on screening attendance and the eventual results of the screening tests.

Screening status was categorized as "no test", "one test", and "two tests" based on screening attendance in the defined two screening intervals (Supplementary Table 2). Screening history was defined by the cytology (Pap smear) result of the two screening intervals according to Systematized Nomenclature of Medicine (SNOMED) codes (Supplementary Table 3) defined

by the Swedish Association of Clinical Cytology. An abnormal smear included a diagnosis of atypical squamous cell of unknown significance (ASCUS) or worse. Screening history was categorized 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 "≥ 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 4). Mode of detection was categorized as screen-detected or symptomatic cancer according to the medical charts.

Statistical analysis

We described the distribution of less common ICC according to age, cancer stage, and mode of detection. Age at diagnosis was categorized into three groups (22-29, 30-60, and >60). Cancer stage was classified according to the International Federation of Gynecology and Obstetrics (FIGO) guidelines, and categorized into IA (microinvasive), IB (localized), and II or higher (advanced).[15] We included women age 30 or above for the analysis of the associations of screening status and history with the risk of less common ICC, because they had two full screening intervals before the index date.

Conditional logistic regression was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) of less common ICC, 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 Labor Market Studies database.[16] The matching variable birth year 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 ORs as incidence rate ratios (IRRs)[17 18] which served as estimates for the association between cervical screening and risk of less common ICC. We also

stratified the analysis by age at index date and FIGO stage, to examine the heterogeneity of the studied association by age and FIGO stage.

Among cases with available HPV genotype data, we tabulated tumor HPV genotypes distribution by histological type, classified as HPV16, HPV18, other high-risk (hr)HPV-positive, and hrHPV-negative. We further examined the association with screening by tumor HPV status. We also evaluated screening attendance and risk of RICC, analyzing by GCC, CCC and other rare types of ICC respectively in a sensitivity analysis. All statistical tests were 2-sided and SAS 9.4 was used for data management and statistical analysis.

This study was approved by the Regional Ethical Review Board in Stockholm, which determined that informed consent from the study participants was not required (Dnr 02-556; Dnr 2011/921-32; Dnr 2011/1026-31/4; Dnr 2012/1028/32; Dnr 2013/1836-32).

Patient 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. There are no plans to disseminate the results of the research to study participants or the relevant patient community.

Results

Cases of less common ICC

Among 338 cases of less common ICC, 164 (48.5%) were ASC, 43 (12.7%) were GCC, 31 (9.2%) were CCC, and 100 (29.6%) were other rare types (Table 1). Women with less common ICC were mostly (52.7%) diagnosed between age 30 and 60, and only 9.1% of the cases were diagnosed before age 30. Five percent of cases were stage IA (microinvasive), 50.9% stage IB (localized), and 44.1% stage II+ (advanced). The majority (87.9%) of cases were symptomatic cancers, and this proportion was even higher for CCC (96.8%) and other rare types of cancers (97.0%).

Screening status and risk of less common ICC

Compared to having no test in either of the last two screening intervals, having had two tests was associated with a substantially lower risk of less common ICC (IRR 0.22, 95% CI 0.14 to 0.34 for ASC and IRR 0.34, 95% CI 0.21 to 0.55 for RICC) (Table 2). The risk reduction was more pronounced for women with two tests compared to women with only one test. Overall, the risk reduction was greatest among women at age 30-60 (IRR 0.21, 95% CI 0.14 to 0.31). Stratifying by FIGO stage, screening was associated with significantly reduced risk of stage IB+ less common ICC through both one test (IRR 0.54, 95% CI 0.35 to 0.82 for stage IB; IRR 0.38, 95% CI 0.24 to 0.60 for stage II+) and two tests (IRR 0.27, 95% CI 0.17 to 0.42 for stage IB; IRR 0.24, 95% CI 0.15 to 0.39 for stage II+) (Table 2).

Screening history and risk of less common ICC

Women with two normal results in the last two screening intervals had significantly lower risk of less common ICC (IRR 0.21, 95% CI 0.15 to 0.30), and the risk reduction was also noted

for women with only one test with normal result (IRR 0.46, 95% CI 0.34 to 0.63) (Table 3). However, women with at least one abnormal test in any of the two intervals had an elevated, although not statistically significant, risk for less common ICC (IRR 1.35, 95% CI 0.78 to 2.37 for ASC, and IRR 1.83, 95% CI 0.92 to 3.66 for RICC). The risk reduction in relation to two normal test results was larger for ASC compared to RICC and did not differ by age or FIGO stage in general.

Tumor HPV status, screening status and risk of less common ICC

hrHPV was detected in 148 cases of the total 211 cases of less common ICC (70.1%), among which HPV18 (37.4%) was the dominant type, followed by HPV16 (22.3%), and other hrHPV types (10.4%). We observed a similar risk reduction associated with screening for hrHPV-positive (one test: IRR 0.42, 95% CI 0.26 to 0.67; two tests: IRR 0.28, 95% CI 0.18 to 0.46) and hrHPV-negative (one test: IRR 0.46, 95% CI 0.23 to 0.92; two tests: IRR 0.27, 95% CI 0.13 to 0.59) less common ICC, comparing women that attended one or both screening tests to women not attending any.

Sensitivity analysis

Stratified by each histological types of RICC, we showed that having two tests was also related to reduced risk of GCC (IRR 0.24, 95% CI 0.09 to 0.68), CCC (IRR 0.17, 95% CI 0.05 to 0.57) and other rare types of ICC (IRR 0.44, 95% CI 0.24 to 0.83) (Supplementary Table 5).

Discussion

Women who routinely attended screening according to recommended intervals had a significantly reduced risk of both ASC and RICC. The risk reduction was more pronounced for women with two tests compared to women with only one test. The magnitude of risk reduction in relation to cervical screening was less for RICC compared to ASC. Moreover, attending screening was associated with a significantly decreased risk of advanced stage cancers, and substantial down-staging for all less common ICC.

RICC tended to be diagnosed at a more advanced stage compared to the rest of the histological types,[3] which might subsequently result in a worse prognosis as previously reported.[6] RICC may indeed be a group of ICC with distinctive histological types compared to ASC given that RICC have a rapid progression[7] and we found that the risk reduction in relation to one test in the last screening intervals was not significant. Of note, as we defined it, RICC consists of several specific histological types, which are rare variants of AC. 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 to squamous-cell lesions.

Our finding on greater risk reduction in relation to having two tests compared to one test in the last two screening intervals confirmed the general principle that the sensitivity of cytology is improved through repeated tests. Attending all recommended screening tests can increase the probability of detecting existing precursors. The smaller magnitude of risk reduction could explain why less common types of ICC, especially RICC, tend to be diagnosed at a more advanced stage and to be symptomatic cancer compared to SCC and AC.[3] This is true even among the present study population with good screening coverage.[19] When stratifying the analyses by age at cancer diagnosis, attending screening was related to decreased risk of less

common ICC among women age below 60 compared to women with no test, and we had limited power to estimate screening for women age above 60 in this study. However, a previous study showed that the risk of ICC after age 60 was highly related to screening at the age of 51-60.[20]

In terms of screening history, our results highlight that having normal tests in the last two screening intervals was associated with a risk reduction of ASC and RICC, especially for women at age 30-60. In contrast, having at least one abnormal test was associated with increased risk of both ASC and RICC compared to women without any test. We considered that clinical management of abnormal smears could potentially alter the risk of less common ICC after abnormal smear, and timely assessment through biopsies or treatment (if needed) could be essential for histological types that progress rapidly.[21]

The proportions of hrHPV-positivity in ASC and RICC were somehow lower compared to SCC and AC.[22] Recently, a meta-analysis showed that AC had a lower prevalence of hrHPV compared to adenocarcinoma *in situ*.[23] Some variants of less common ICC were reported as having low prevalence of HPV[24] or even not HPV-related.[25] However, the lower proportion of hrHPV-positivity noted in our study does not have to signify that hrHPV is not the main cause of less common ICC. On the contrary, it might indicates that HPV might become undetectable in advanced cancers.[26] In another sample tested with the same methodology, we found 97% of women with cervical intraepithelial neoplasia 3+ (CIN3+) were hrHPV-positive.[27]

Our findings on risk reduction of ASC are similar to the British studies.[4 5] However, no previous studies have addressed the association between screening and risk of RICC due to the rarity of these diagnoses and limited information on cervical screening. Compared to SCC and AC, we saw similar trend of risk reduction associated with cervical screening in less common ICC as well as a down-staging effect.[3 4 28] The risk reduction for ASC associated

with cervical screening is similar to SCC,[3] while the risk reduction attenuate for RICC. It suggests that ASC might evolve from squamous component and acquire glandular involvement later on,[5] or might be a glandular cancer exhibiting squamous differentiation as shown in previous studies.[24]

Since the great majority of hrHPV-positive less common ICC were HPV16/HPV18 positive, current HPV vaccination with bivalent or quadrivalent HPV vaccines will be a significant strategy for prevention. With the 9-valent HPV vaccine, which covers five additional HPV types, additional prevention of less common ICC could be expected in future. Besides, HPV as a primary testing method for women at age above 29 has been recommended for organized, population-based screening program according to European guidelines from 2015.[29] A continuation of monitoring HPV testing and less common ICC is necessary in the future.

Although this is to date the largest study of less common ICC and we included all cases over a 10-year period, the number of cases were limited which resulted in reduced precision in some of the sub-group analyses. Smoking status and sexually transmitted infections were not adjusted for in the analysis, however, we controlled for educational level which to some extent accounts for these factors.[30] Misclassification of histological types might have occurred due to the overlap of morphology, but we employed strict clinical and histo-pathological review to ensure classification.

To the best of our knowledge, this is the first population-based study to examine cervical screening and risk of less common ICC, and to present the HPV prevalence in tumors. All cases were strictly reviewed according to a cervical cancer case audit protocol. We used complete screening data extracted from the NKCx 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 regarding hysterectomy, death, and migration limited bias due to loss of follow-up. We used highly sensitive methods for HPV genotyping in FFPE material[13] with strict quality control to avoid HPV contamination and ensure the accuracy of the detected HPV genotypes. Our results should be generalizable to countries or regions with similar screening programs and settings.

Conclusion

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 less common ICC were positive for hrHPV, the switch to primary HPV screening and prevention by HPV vaccination are also expected to decrease the risk of less common ICC, but this will need to be monitored.

Statements

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Contributors

JL did the literature search and review. BA and PS conceived the research questions and hypotheses, and designed the study. PS and SNK collected diagnostic samples. BA reviewed medical charts and supported histo-pathological classification of all cases. CL and CE did the laboratory analysis on HPV genotyping and validation. JL and AP conducted statistical analysis, and JL, BA, AP, JD, FF, KME and PS interpreted the data. JL wrote the original draft of this manuscript. All authors are involved in the critical revision of article, and have read and approved submission of the manuscript.

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

Data sharing: Data sharing is not available.

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 have been explained.

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Table 1 Distribution of less common histological types of invasive cervical cancer.

	Adenosquamous cell carcinoma n (%)	Glassy cell carcinoma n (%)	Clear cell carcinoma n (%)	Other rare types[a] n (%)	Total n (%)
Number of cases	164 (100)	43 (100)	31 (100)	100(100)	338 (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 (IQR)	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 (5.5)	15 (34.9)	0 (0.0)	7 (7.0)	31 (9.1)
30-60	105 (64.0)	19 (44.2)	13 (41.9)	41 (41.0)	178 (52.7)
>60	50 (30.5)	9 (20.9)	18 (58.1)	52 (52.0)	129 (38.2)
FIGO stage[b]					
IA	12 (7.3)	1 (2.3)	1 (3.2)	3 (3.0)	17 (5.0)
IB	98 (59.8)	30 (69.8)	18 (58.1)	26 (26.0)	172 (50.9)
II+	54 (32.9)	12 (27.9)	12 (38.7)	71 (71.0)	149 (44.1)
Mode of detection					
Screen-detected	31 (18.9)	6 (14.0)	1 (3.2)	3 (3.0)	41 (12.1)
Symptomatic cancer	133 (81.1)	37 (86.0)	30 (96.8)	97 (97.0)	297 (87.9)

[a] Other rare types include small cell carcinoma, large cell carcinoma, neuroendocrine carcinoma and undifferentiated cell carcinoma.
[b] FIGO (International Federation of Gynecology and Obstetrics) stage: IA=microinvasive; IB=localized cancer; II or higher=advanced cancer

Table 2 Incidence rate ratio (IRR) of less common histological types of invasive cervical cancer by screening status in last two screening intervals.

	Screening status	All less common ICC (n=307)		ASC (n=155)		RICC[c] (n=152)	
		Cases (%)	IRR[b] (95% CI)	Cases (%)	IRR[b] (95% CI)	Cases (%)	IRR[b] (95% CI)
Cases age≥30	No test	135 (44.0)	Ref	65 (41.9)	Ref	70 (46.1)	Ref
	One test	91 (29.6)	0.51 (0.38 to 0.69)	44 (28.4)	0.39 (0.26 to 0.59)	47 (30.9)	0.69 (0.45 to 1.06)
	Two tests	81 (26.4)	0.27 (0.19 to 0.37)	46 (29.7)	0.22 (0.14 to 0.34)	35 (23.0)	0.34 (0.21 to 0.55)
Age at diagnosis							
30to60	No test	58 (32.6)	Ref	35 (33.3)	Ref	23 (31.5)	Ref
	One test	61 (34.3)	0.44 (0.30 to 0.64)	33 (31.4)	0.38 (0.23 to 0.62)	28 (38.4)	0.52 (0.29 to 0.94)
	Two tests	59 (33.1)	0.21 (0.14 to 0.31)	37 (35.2)	0.21 (0.13 to 0.34)	22 (30.1)	0.22 (0.12 to 0.40)
>60	No test	77 (59.7)	Ref	30 (60.0)	Ref	47 (59.5)	Ref
	One test	30 (23.3)	0.62 (0.38 to 1.01)	11 (22.0)	0.42 (0.19 to 0.90)	19 (24.1)	0.82 (0.44 to 1.54)
	Two tests	22 (17.1)	0.44 (0.25 to 0.78)	9 (18.0)	0.28 (0.11 to 0.67)	13 (16.5)	0.61 (0.29 to 1.27)
FIGO stage[a]							
IA	No test	3 (20.0)	Ref	2 (20.0)	Ref	1 (20.0)	Ref
	One test	9 (60.0)	3.42 (0.72 to 16.26)	5 (50.0)	3.00 (0.37 to 24.31)	4 (80.0)	3.46 (0.35 to 33.94)
	Two tests	3 (20.0)	0.76 (0.11 to 5.19)	3 (30.0)	1.10 (0.10 to 11.81)	0 (0.0)	- (to)
IB	No test	53 (35.6)	Ref	34 (37.0)	Ref	19 (33.3)	Ref
	One test	50 (33.6)	0.54 (0.35 to 0.82)	27 (29.3)	0.38 (0.22 to 0.66)	23 (40.4)	0.92 (0.46 to 1.86)
	Two tests	46 (30.9)	0.27 (0.17 to 0.42)	31 (33.7)	0.23 (0.13 to 0.39)	15 (26.3)	0.34 (0.16 to 0.75)
II+	No test	79 (55.2)	Ref	29 (54.7)	Ref	50 (55.6)	Ref
	One test	32 (22.4)	0.38 (0.24 to 0.60)	12 (22.6)	0.29 (0.14 to 0.60)	20 (22.2)	0.44 (0.24 to 0.81)
	Two tests	32 (22.4)	0.24 (0.15 to 0.39)	12 (22.6)	0.15 (0.07 to 0.33)	20 (22.2)	0.32 (0.17 to 0.61)

ICC: invasive cervical cancer; ASC: adenosquamous cell carcinoma; RICC: rare types invasive cervical cancer.
[a] FIGO (International Federation of Gynecology and Obstetrics) stage: IA=microinvasive; IB=localized cancer; II or higher=advanced cancer.
[b] Incidence rate ratio adjusted by education level.
[c] RICC includes glassy cell carcinoma, clear cell carcinoma and other rare types.
Note: women age 30 and above were included.

Table 3 Incidence rate ratio (IRR) of less common histological types of invasive cervical cancer by screening history in last two screening intervals.

	Screening history	All less common ICC (n=307)		ASC (n=155)		RICC[c] (n=152)	
		Cases (%)	IRR[b] (95% CI)	Cases (%)	IRR[b] (95% CI)	Cases (%)	IRR[b] (95% CI)
Cases age≥30	No test	135 (44.0)	Ref	65 (41.9)	Ref	70 (46.1)	Ref
	Double normal	59 (19.2)	0.21 (0.15 to 0.30)	31 (20.0)	0.16 (0.10 to 0.26)	28 (18.4)	0.29 (0.17 to 0.48)
	One normal only	80 (26.1)	0.46 (0.34 to 0.63)	38 (24.5)	0.34 (0.22 to 0.53)	42 (27.6)	0.63 (0.41 to 0.99)
	≥ One abnormal	33 (10.7)	1.57 (1.02 to 2.43)	21 (13.5)	1.35 (0.78 to 2.37)	12 (7.9)	1.83 (0.92 to 3.66)
Age at diagnosis							
30to60	No test	58 (32.6)	Ref	35 (33.3)	Ref	23 (31.5)	Ref
	Double normal	39 (21.9)	0.15 (0.10 to 0.23)	23 (21.9)	0.14 (0.08 to 0.24)	16 (21.9)	0.17 (0.08 to 0.32)
	One normal only	51 (28.7)	0.38 (0.25 to 0.56)	27 (25.7)	0.32 (0.19 to 0.54)	24 (32.9)	0.47 (0.26 to 0.85)
	≥ One abnormal	30 (16.9)	1.64 (1.01 to 2.65)	20 (19.0)	1.58 (0.86 to 2.90)	10 (13.7)	1.70 (0.76 to 3.81)
>60	No test	77 (59.7)	Ref	30 (60.0)	Ref	47 (59.5)	Ref
	Double normal	20 (15.5)	0.43 (0.24 to 0.77)	8 (16.0)	0.27 (0.11 to 0.67)	12 (15.2)	0.59 (0.28 to 1.26)
	One normal only	29 (22.5)	0.62 (0.38 to 1.00)	11 (22.0)	0.42 (0.20 to 0.91)	18 (22.8)	0.80 (0.42 to 1.50)
	≥ One abnormal	3 (2.3)	0.71 (0.21 to 2.42)	1 (2.0)	0.34 (0.04 to 2.81)	2 (2.5)	1.29 (0.29 to 5.72)
FIGO[a] stage							
IA	No test	3 (20.0)	Ref	2 (20.0)	Ref	1 (20.0)	Ref
	Double normal	2 (13.3)	0.59 (0.07 to 4.96)	2 (20.0)	0.76 (0.06 to 9.27)	0 (0.0)	- (to)
	One normal only	7 (46.7)	2.88 (0.55 to 15.11)	5 (50.0)	2.99 (0.36 to 24.82)	2 (40.0)	2.35 (0.18 to 31.23)
	≥ One abnormal	3 (20.0)	22.63 (2.53 to 202.17)	1 (10.0)	6.96 (0.32 to 150.07)	2 (40.0)	- (to)
IB	No test	53 (35.6)	Ref	34 (37.0)	Ref	19 (33.3)	Ref
	Double normal	29 (19.5)	0.19 (0.12 to 0.31)	19 (20.7)	0.16 (0.09 to 0.29)	10 (17.5)	0.24 (0.10 to 0.57)
	One normal only	42 (28.2)	0.47 (0.30 to 0.73)	22 (23.9)	0.33 (0.18 to 0.58)	20 (35.1)	0.84 (0.41 to 1.73)
	≥ One abnormal	25 (16.8)	2.12 (1.22 to 3.66)	17 (18.5)	1.77 (0.90 to 3.48)	8 (14.0)	2.90 (1.11 to 7.53)

II+	No test	79 (55.2)	Ref	29 (54.7)	Ref	50 (55.6)	Ref
	Double normal	28 (19.6)	0.22 (0.13 to 0.37)	10 (18.9)	0.14 (0.06 to 0.31)	18 (20.0)	0.30 (0.16 to 0.59)
	One normal only	31 (21.7)	0.37 (0.23 to 0.60)	11 (20.8)	0.27 (0.12 to 0.57)	20 (22.2)	0.45 (0.25 to 0.83)
	≥ One abnormal	5 (3.5)	0.53 (0.20 to 1.38)	3 (5.7)	0.52 (0.15 to 1.81)	2 (2.2)	0.49 (0.11 to 2.16)

ICC: invasive cervical cancer; ASC: adenosquamous cell carcinoma; RICC: rare types invasive cervical cancer.
[a] FIGO (International Federation of Gynecology and Obstetrics) stage: IA=microinvasive; IB=localized cancer; II or higher=advanced cancer
[b] Incidence rate ratio adjusted by education level.
[c] RICC includes glassy cell carcinoma, clear cell carcinoma and other rare types.
Note: women age 30 and above were included.

Table 4 Tumor human papillomavirus (HPV) status of less common histological types of invasive cervical cancer.

Less common ICC	Cases	Tumor HPV status			
		HPV 16 n (%)	HPV 18 n (%)	Other hrHPV + n (%)	hrHPV - n (%)
Total	211	47 (22.3)	79 (37.4)	22 (10.4)	63 (29.9)
Adenosquamous cell carcinoma	119	25 (21.0)	49 (41.2)	14 (11.8)	31 (26.1)
Glassy cell carcinoma	24	5 (20.8)	12 (50.0)	3 (12.5)	4 (16.7)
Clear cell carcinoma	14	1 (7.1)	5 (35.7)	1 (7.1)	7 (50.0)
Other rare types[a]	54	16 (29.6)	13 (24.1)	4 (7.4)	21 (38.9)

ICC: invasive cervical cancer; hrHPV, high-risk human papillomavirus.

[a] Other rare types include small cell carcinoma, large cell carcinoma, neuroendocrine carcinoma and undifferentiated cell carcinoma.

Note: women had valid tumor HPV genotypes were included (n=211).

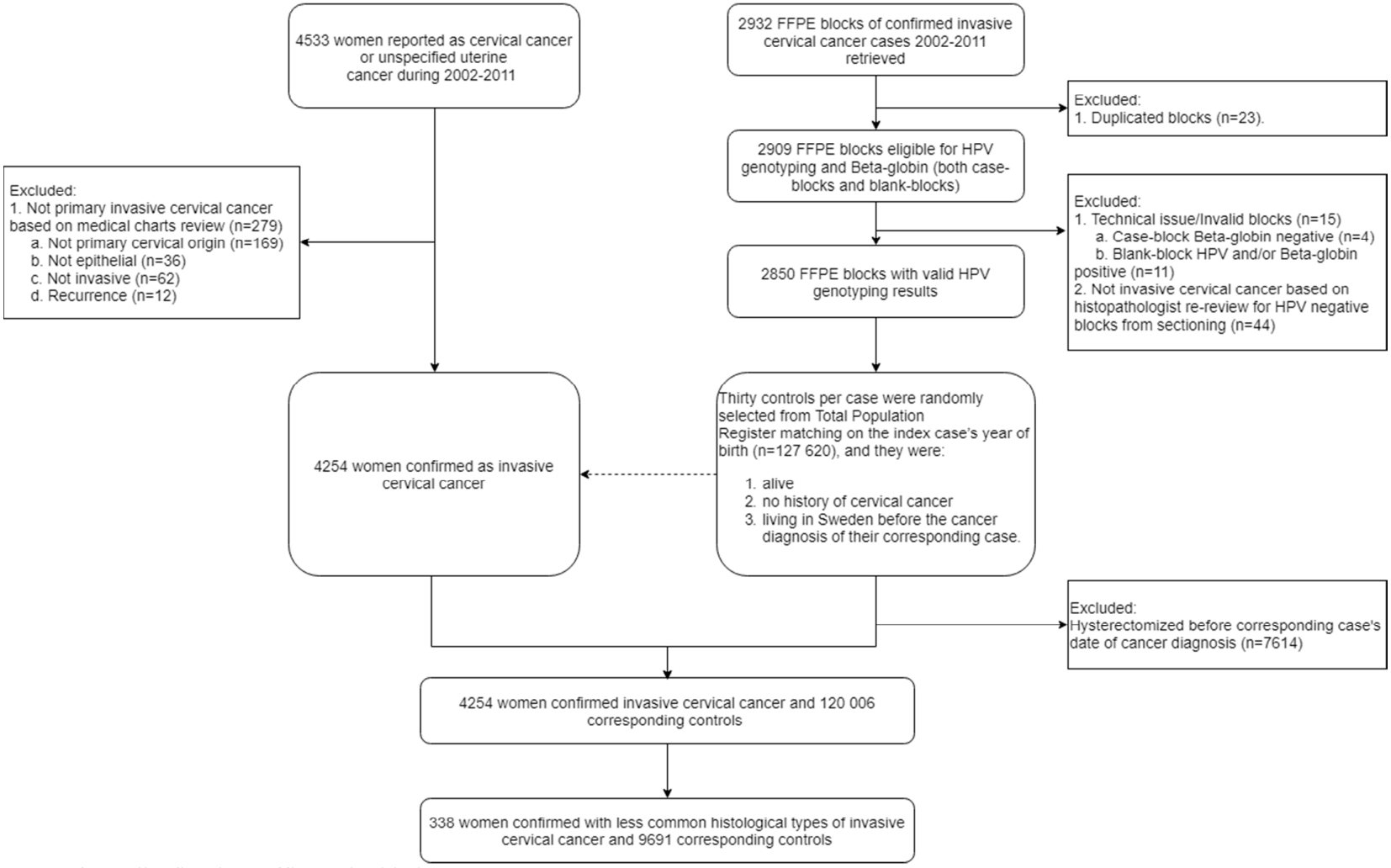
Table 5 Incidence rate ratio (IRR) of less common histological types of invasive cervical cancer by screening status in last two screening intervals, and tumor human papillomavirus (HPV) status.

	Screening status	Cases n (%)	IRR[a] (95% CI)
Cases age≥30	No test	86 (44.6)	Ref
	One test	52 (26.9)	0.43 (0.29 to 0.63)
	Two tests	55 (28.5)	0.28 (0.19 to 0.42)
Tumor HPV status			
hrHPV +	No test	53 (40.5)	Ref
	One test	37 (28.2)	0.42 (0.26 to 0.67)
	Two tests	41 (31.3)	0.28 (0.18 to 0.46)
hrHPV -	No test	33 (53.2)	Ref
	One test	15 (24.2)	0.46 (0.23 to 0.92)
	Two tests	14 (22.6)	0.27 (0.13 to 0.59)

hrHPV, high-risk human papillomavirus.
[a] Incidence rate ratio adjusted by education level.
Note: women age 30 and above and had valid tumor HPV genotypes were included (n=193).

Supplementary

Figure 1 Flow chart of study population.



FFPE, formalin-fixed paraffin-embedded.

Table 1 Evaluated screening intervals depend on age at cancer diagnosis.

Age at cancer diagnosis	1st screening interval (years before cancer diagnosis)	2nd screening interval (years before cancer diagnosis)
23-53	0.5-3.5	3.5-6.5
54-58	0.5-5.5	5.5-8.5
59-65	0.5-5.5	5.5-10.5
66+	age 61-65	age 56-60

Table 2 Classification of screening status.

Screening status	1st screening interval	2nd screening interval
No test	Unscreened	Unscreened
One test	Unscreened	Screened
	Screened	Unscreened
Two tests	Screened	Screened

Table 3 Systematized Nomenclature of Medicine (SNOMED) codes for cytological diagnosis.

	SNOMED code	Cytological diagnoses
Abnormal cytological diagnosis	M69710	Atypical squamous cell of undetermined significance (ASCUS)
	M69719	Suspected high-grade dysplasia (ASC-H)
	M74006	Mild dysplasia/Cervical intraepithelial neoplasia (CIN) 1
	M74007	Moderate dysplasia/Cervical intraepithelial neoplasia (CIN) 2
	M80702	Severe dysplasia/Cervical intraepithelial neoplasia (CIN) 3/Cancer in situ (CIS)
	M80703	Squamous cell carcinoma
	M69720	Atypical glandular cells (AGC)
	M81403	Adenocarcinoma/Adenocarcinoma in situ (AIS)
	M69700	Atypia in cells of uncertain origin
Normal cytological diagnosis	M00110	Benign sample

Table 4 Classification of screening history.

Screening history	1st screening interval	2nd screening interval
No test	Unscreened	Unscreened
Double normal	Normal smear	Normal smear
One normal only	Unscreened	Normal smear
	Normal smear	Unscreened
≥ One abnormal	Unscreened	Abnormal smear
	Normal smear	Abnormal smear
	Abnormal smear	Normal smear
	Abnormal smear	Unscreened
	Abnormal smear	Abnormal smear

Table 5 Incidence rate ratio (IRR) of glassy cell carcinoma, clear cell carcinoma and other rare types of invasive cervical cancer (ICC) by screening status.

Histological types	Screening status	Cases (%)	IRR[b] (95% CI)
Glassy cell carcinoma	No test	12 (42.9)	Ref
	One test	8 (28.6)	0.36 (0.13 to 0.98)
	Two tests	8 (28.6)	0.24 (0.09 to 0.68)
Clear cell carcinoma	No test	14 (45.2)	Ref
	One test	13 (41.9)	0.97 (0.40 to 2.36)
	Two tests	4 (12.9)	0.17 (0.05 to 0.57)
Other rare types[a]	No test	44 (47.3)	Ref
	One test	26 (28.0)	0.72 (0.40 to 1.28)
	Two tests	23 (24.7)	0.44 (0.24 to 0.83)

[a] Other rare types include small cell carcinoma, large cell carcinoma, neuroendocrine carcinoma and undifferentiated cell carcinoma.
[b] Incidence rate ratio adjusted by education level.
Note: women age 30 and above were included.