Response to comments on Manuscript ID BMJ-2018-047292 entitled "Screening effectiveness of less common histological types of invasive cervical cancer: a population-based nested case-control study" > by Lei et al

Report from The BMJ's manuscript committee meeting

First, please revise your paper to respond to all of the comments by the reviewers. Their reports are available at the end of this letter, below.

Please also respond to these additional comments by the committee:

1. *It needs explanations and clarifications on the association between "screening and the risk of" other cancers. If screening doesn't or can't detect these cancers then why does screening appear to reduce the risk of developing the cancers? Do the author here mean the risk of having invasive cancer? The screening does not decrease the risk of having cancer (unless all rare cancers related to HPV and treatment of HPV eliminates the risk of cancer -but the study showed that not all RICC is associated with HPV infection) but rather decreases the risk of having advanced or invasive cancer at the time of diagnosis.*

Reply: The risk of cancer in this manuscript refers to risk of having invasive cervical carcinoma. It is correct that, generally, the aim of screening is to detect cancer at early stage and improve prognosis by preventing mortality from the disease. However, the main aim of cervical screening is to prevent the development of invasive cervical carcinoma by detecting and removing precancerous lesions (i.e. cervical intraepithelial neoplasia 3 or adenocarcinoma in situ), which if left untreated, could develop into invasive carcinoma (1). Treatment is not indicated for HPV infection, but only precancerous lesions. Concurrently, cervical screening can also early detect asymptomatic invasive malignancies and improve prognosis, an effect that has been reported in the BMJ previously (2).

In our analysis, we have been able to quantify the benefit of cervical screening on preventing adenosquamous cell carcinoma (ASC) and other rare types of invasive cervical carcinoma

(RICC), and we also see indications of detection at early stages. Please see also our response to Reviewer 1, point 8 below.

Revised in manuscript:

- Introduction – paragraph 1

2. *Highly invasive and aggressive cancers do not meet the criteria for screening programmes. The relevant criteria here are that the natural history should allow time for detection at an early stage and that there should be an effective treatment available that alters prognosis at the stage of detection. Table 1 shows that 88% of these cancers were not detected at screening and that's mostly because they progress so quickly that they go from nothing to nasty in the interval between screens.*

Reply: We agree that highly aggressive cancers are not expected to have precursors, and would therefore not fulfil the screening criteria. Cervical screening is designed to detect and remove precancerous lesions in order to prevent invasive cervical carcinoma. Most of the cervical cancers in Sweden have actually been prevented through the nation-wide population-based organized screening program in operation since late 1960s. Therefore, the cervical cancer cases presented in this study are an accumulation of cases that were not prevented by cervical screening. They can be detected at early stage, usually detected by screening, or at a later stage, diagnosed with symptoms.

The scientific basis for this study is that the preventability by screening has never been studied previously for ASC or RICC.

In this study we show there is a risk reduction for both ASC and RICC through cervical screening. The risk reduction of ASC is similar to squamous cell carcinoma (SCC), and the magnitude of risk reduction is slightly less for RICC and adenocarcinoma (AC). This indicates that there is in fact a pre-cursor stage for most ASC and RICC so they can be prevented by cervical screening. Truly aggressive cancers are a very small group (30%) among symptomatic cases, and most of the symptomatic cancers (66%) could have been prevented if they had participated to screening (Table A below). See also Table B in reply No. 8 to Reviewer 1.

Table A Screening status of symptomatic cancer in women age 26 and above (n=328).

Characteristics	Adenosquamous cell carcinoma n (%)	Rare invasive cervical carcinoma n (%)
Screening status[a]		
No test	87 (65.9)	103 (66.0)
Normal result	39 (29.6)	46 (29.5)
Abnormal result	6 (4.5)	7 (4.5)
Total	132 (100)	156 (100)

[a] Correspond to the 1st recommended screening interval before cancer diagnosis.

3. *It's an observational study and we know that people who attend screening also do other things that are beneficial for their health, so the apparent protective effect of screening is probably down to the other healthy behaviours of women who attend for screening and nothing to do with the screening itself. Consequently, these results don't provide strong evidence that screening prevents rare "other types" of cervical cancer.*

Reply: We agree that a healthy volunteer bias might affect all observational studies assessing the effect of screening for a disease. However, we do not consider that such a bias would completely account for the effect estimates presented in this study. To check this assumption, we conducted a matched case-control analysis exactly in the same manner as the present one, but with stomach cancer and rectal cancer as two separate outcomes. The results show that women participating to cervical screening in the last screening round had an IRR of 0.91 (95% CI: 0.84 to 0.99) for stomach cancer, compared to women not participating, while the corresponding figure for rectal cancer was IRR of 0.87 (95% CI: 0.82 to 0.91). Since cervical screening as such should not have any bearing on the risk of acquiring stomach or rectal cancer, the slightly reduced risk we see for women participating to cervical screening could be interpreted as an effect of more health consciousness among these women. Overall, a very small proportion of Swedish women do not participate to cervical screening at all (less than 2%). In this context, a healthy volunteer effect could account for a general risk reduction of cancer of say 10%, and certainly not more than 15%. Since our risk estimates generally indicate a much larger risk reduction from cervical screening than this, we conclude that most of the effect of screening we show in our analysis cannot solely be due to healthy volunteer bias, and if such a bias is acting, it is fairly small. Therefore we argue that our results actually do present strong arguments that cervical screening prevents ASC and RICC. We have added a sentence discussing the possible healthy volunteer bias to the Discussion.

Revised in manuscript:

- Discussion, strengths and weaknesses – paragraph 1

4. *Our statistician noted the authors could consider modelling age as a continuum.*

Reply: Year of birth (corresponding to age at time of diagnosis of the case) was individually matched between cases and controls, and it was used as a continuous variable. The matching variable birth year was automatically adjusted for in the conditional logistic regression. The comparisons between cases and controls were made only within the same risk set/matched set in the regression model.

Age as a categorical variable was used to evaluate whether the association between screening and the risk of ASC or RICC varied across age groups (as shown in Table 2 and 3 in the manuscript).

Revised in manuscript:

- Material and methods, study population – paragraph 2
- Material and methods, statistical analysis – paragraph 2
- Footnote in Table 2; Table 3; Table 5.

5. *The title might need revision to reflect the study better.*

Reply: The new title is "Cervical screening and risk of adenosquamous and rare histological types of invasive cervical carcinoma: a population-based nested case-control study". We use the terminology carcinoma to specify that we only include epithelial cancers in our study.

6. *For some general readers, some terms might be confusing. For example, RICC or less-common ICC. Could author consider terming them in a clearer way and be consistent across the paper?*

Reply: Thanks for the suggestion. We now present ASC and RICC separately through the revised paper considering their difference of risk reduction from screening, and therefore, the term less common ICC is not applied anymore.

7. *Could authors consider disseminating the findings to the public, patients, doctors, and allied groups as they might benefit from the study? The author may also want to thank those who made their data possible.*

Reply: Our findings will be disseminated through updates of the National Swedish Guidelines for Cervical Cancer Prevention, Diagnosis, and Treatment (3). These guidelines are written by expert groups and are updated regularly to reflect advances in the scientific evidence. Patients and professional organizations are involved in reviewing and commenting on these guidelines before they are adapted into practice. Previous results on screening and cervical cancer from our group have already been instrumental in previous versions of these guidelines; therefore, we believe that this is a viable arena for informing relevant stakeholders. We have now acknowledged all participants who contribute data to this study in the manuscript.

Revised in manuscript:

- Patient and public involvement
- Acknowledgement

In your response please provide, point by point, your replies to the comments made by the reviewers and the editors, explaining how you have dealt with them in the paper.

Comments from Reviewers

Reviewer: 1

There is undoubtedly a great amount of effort that went into this manuscript, and the authors should be commended for that. However, there are several points which could make this manuscript more informative:

Reply: Thank you for the very constructive and helpful comments on the manuscript.

8. *This is one of the very few reports on RICC, and so I find it a missed opportunity not to include a thorough comparison with SCC and AC. This would provide the context for the results and help clarify whether and how RICC differ from the common CC types; the lack of these data in this paper is particularly regrettable as the authors do seem have access to the relevant records (see Supplementary Figure 1).*

Reply: The results on screening and risk of SCC and AC are presented in another manuscript, which is currently pending publication. By analyzing the data of SCC and AC in the same manner as in our manuscript, the results are presented below for women age 30 and above. The risk reduction of cervical screening in preventing ASC is similar to SCC, while the magnitude of risk reduction is less for RICC and AC (Table B). We have included Table B as a supplementary table in this manuscript.

Table B Incidence rate ratios (IRR) of cervical carcinoma by screening status and histological types.

Screening status	SCC (n=2902) IRR[a] (95% CI)	AC (n=766) IRR[a] (95% CI)	ASC (n=155) IRR[a] (95% CI)	RICC (n=152) IRR[a] (95% CI)
No test	Ref	Ref	Ref	Ref
One Test	0.43 (0.39 to 0.47)	0.88 (0.71 to 1.08)	0.39 (0.26 to 0.59)	0.69 (0.45 to 1.06)
Two tests	0.19 (0.17 to 0.21)	0.57 (0.46 to 0.71)	0.22 (0.14 to 0.34)	0.34 (0.21 to 0.55)

SCC: squamous cell carcinoma; AC: adenocarcinoma; ASC: adenosquamous cell carcinoma; RICC: rare types of invasive cervical carcinoma.
[a] Incidence rate ratio adjusted by education level and age.
Note: only women age 30 and above were included.

Revised in manuscript:

- Supplementary Table 6

9. *Today, official cancer registry data in Nordcan report 4430 cases of cervical cancer diagnosed in 2002-2011 in Sweden at ages 25-85+ (which, if I understood correctly, is approximately the age group that the authors used in the study [birth cohorts 1909-1986]). If we assume that Nordcan data are the golden standard, then Nordcan reports 176 cases more than what the authors considered to be confirmed primary ICC (N=4254). As a proportion of the total, the 176 cases represent a small minority. However, if the "missing" cases are not randomly selected and (potentially) represent RICC, then in the worst-case scenario they would represent about 50% of all the RICC studied in the paper. I expect that the explanation for the "missing" cases is more benign, but I would welcome a report/discussion on these discrepancies.*

Reply: Nordcan receives a copy of data from the National Swedish Cancer Registry. Cancer registration in the Swedish Cancer Registry is usually done after the first biopsy, which is not necessarily the same as the final diagnosis.

In this study, we have reviewed all cervical cancers and unspecified uterine cancer reported to the Swedish Cancer Registry (as well as Nordcan) (n=4533), and excluded cases (n=279) after the full clinic diagnostic procedure, which is always performed to find the true diagnosis of primary invasive carcinomas of cervical origin before treatment. It is unfortunate that cancer registration is usually done before such pre-treatment review, and the revised diagnosis is not routinely reported back to the Swedish Cancer Registry and corrected (and hence not in Nordcan).

However, excluding cases which are not primary, invasive, epithelial (ie. sarcoma, endometrial cancer), or reoccurrent cases is very important for our study. Mistakenly including those cases, we could 1) potentially dilute the association between screening and risk of cervical carcinoma found in our study, 2) violate the distribution of HPV types, which probably would underestimate the proportion of hrHPV-positve cases.

Related in manuscript:

- Material and methods, study population – paragraph 1

10. *The authors analysed screening participation during periods of exactly the recommended lengths of the screening intervals, 3 or 5 years. In cervical screening, it is known that women may participate in slightly longer intervals, and so many national monitoring reports evaluate participation in 0.5-1 year longer-than-recommended intervals (e.g. 3.5-4 years, and 5.5-6 years). How do the results change if this reasonable routine variability in screening intervals is considered?*

Reply: Following this suggestion, we analyzed the screening in 0.5-1 year longer-than-recommended intervals. The tables below summarize the overall adjusted estimates of screening status (Table C), screening history (Table D) in relation to risk of ASC and RICC among cases aged 30 and above, with corresponding 0.5y- and 1y- longer-than-recommended intervals, respectively. The results are robust and we therefore decided not to change the definition of the screening interval.

Table C Incidence rate ratios (IRR) of adenosquamous and rare types of invasive cervical carcinoma by screening status, corresponds to the recommended screening interval, 0.5y longer-than-recommended intervals, and 1y longer-than-recommended intervals.

Screening status	ASC (n=155)			RICC (n=152)		
	IRR[a] (95% CI)	IRR[b] (95% CI)	IRR[c] (95% CI)	IRR[a] (95% CI)	IRR[b] (95% CI)	IRR[c] (95% CI)
No test	Ref	Ref	Ref	Ref	Ref	Ref
One test	0.39 (0.26 to 0.59)	0.42 (0.27 to 0.66)	0.38 (0.23 to 0.60)	0.69 (0.45 to 1.06)	0.68 (0.43 to 1.07)	0.65 (0.41 to 1.03)
Two tests	0.22 (0.14 to 0.34)	0.24 (0.16 to 0.37)	0.25 (0.16 to 0.38)	0.34 (0.21 to 0.55)	0.36 (0.22 to 0.57)	0.36 (0.22 to 0.57)

ASC: adenosquamous cell carcinoma; RICC: rare types of invasive cervical carcinoma.
[a] Incidence rate ratio corresponds to recommended screening interval, adjusted by education level and age.
[b] Incidence rate ratio corresponds to 0.5y longer-than-recommended intervals, adjusted by education level and age.
[c] Incidence rate ratio corresponds to 1y longer-than-recommended intervals, adjusted by education level and age.
Note: only women age 30 and above were included.

Table D Incidence rate ratios (IRR) of adenosquamous and rare types of invasive cervical carcinoma by screening history, corresponds to the recommended screening interval, 0.5y longer-than-recommended intervals, and 1y longer-than-recommended intervals.

Screening history	ASC (n=155)			RICC (n=152)		
	IRR[a] (95% CI)	IRR[b] (95% CI)	IRR[c] (95% CI)	IRR[a] (95% CI)	IRR[b] (95% CI)	IRR[c] (95% CI)
No test	Ref	Ref	Ref	Ref	Ref	Ref
Double normal	0.16 (0.10 to 0.26)	0.18 (0.11 to 0.28)	0.18 (0.11 to 0.28)	0.29 (0.17 to 0.48)	0.31 (0.19 to 0.50)	0.29 (0.18 to 0.48)
One normal only	0.34 (0.22 to 0.53)	0.38 (0.24 to 0.61)	0.36 (0.22 to 0.58)	0.63 (0.41 to 0.99)	0.63 (0.40 to 1.00)	0.63 (0.39 to 1.01)
≥ One abnormal	1.35 (0.78 to 2.37)	1.33 (0.77 to 2.30)	1.19 (0.69 to 2.05)	1.83 (0.92 to 3.66)	1.60 (0.80 to 3.20)	1.49 (0.75 to 2.93)

ASC: adenosquamous cell carcinoma; RICC: rare types of invasive cervical carcinoma.
[a] Incidence rate ratio corresponds to recommended screening interval, adjusted by education level and age.
[b] Incidence rate ratio corresponds to 0.5y longer-than-recommended intervals, adjusted by education level and age.

[c] Incidence rate ratio corresponds to 1y longer-than-recommended intervals, adjusted by education level and age. Note: only women age 30 and above were included.

11. *Please explain the completeness and accuracy of the Swedish Patient Register and Longitudinal Integration Database […]. Please clarify which types of hysterectomies were excluded from the analysis. Is the proportion of women excluded because of a hysterectomy approximately the same as the prevalence of hysterectomy in the general population of the same age? What is the source data on education within the Longitudinal Integration Database […]? Were all these data linked on the individual level?*

Reply: We have now clarified the quality and linkage of registries, as well as information on total hysterectomy in the manuscript.

Revised in manuscript:

- Material and methods, study population – paragraph 2
- Material and methods, statistical analysis – paragraph 2 & 3

In general, the Swedish Patient Register has three sources of data: somatic and psychiatric inpatient care, day-surgery, and hospital-based outpatient care. Hospital inpatient care has national coverage since 1987, day-surgery is reported to the register since 1997, and hospital-based outpatient care is reported since 2001. Diagnoses of inpatient care was found to have a PPV of 85%-95% and more than 99% of all somatic, surgical and psychiatric hospital discharges are registered (4). The Longitudinal Integration Database (LISA) is a compilation of many existing population based registers in Sweden, covering the entire Swedish population from age 16 since 1990. The database integrates existing data from the labour market, educational and social sectors and is updated annually (5). Data on education in LISA comes from the national Swedish Education Register, available since 1985, and reports highest achieved education on more than 98% of the Swedish population ages 16-64. A thorough documentation of the LISA database (in Swedish) can be found at https://www.scb.se/contentassets/f0bc88c852364b6ea5c1654a0cc90234/dokumentation-av-lisa.pdf.

Data from the Patient Register and LISA were linked to our case-control data on individual level.

Women with a total hysterectomy (449/10,140, 4.4%) were excluded from the matched controls (since they were not at risk of acquiring cervical cancer). As the overall rate of hysterectomy has been quite stable in Sweden over the past decades, we estimated that the prevalence of total hysterectomy in the general population of the same age as approximately 5.9% based on the calculation: *prevalence=incidence * duration of disease* (duration of disease was estimated by life expectancy of female in Sweden minus the mean age of total hysterectomy)

Prevalence = 211.55/100,000 * (80-52) * 100 = 5.9%

- Incidence of total hysterectomy for women age 20+ during year 1998-2011, taken as average (National Board of Health and Welfare, Sweden)
- Life expectancy of female in Sweden 1998-2011, taken as average (World Bank)
- Mean age of hysterectomy due to benign indications in Sweden 1987-2003 (6).

There is a slightly lower proportion of women with a total hysterectomy among the matched controls, which might be due to the low precision from the relatively smaller total number of controls for our case series, compared to the number of general female population (around 4.5 million). We further performed a calculation of women with total hysterectomy among matched controls of all invasive cervical carcinoma cases (n=4254) in our database. This proportion is 6.0% (7614/127,620) which is very similar to the estimated prevalence of total hysterectomy in the general female population of the same age.

12. *The risk of RICC was increased in women with at least one abnormal screening test, compared to unscreened women. Please clarify whether this was a consequence of insufficient follow-up after an abnormality.*

Reply: We believe that inadequate execution of the management practice will lead to increased risk of ASC and RICC. We showed in our previous publication based on nationwide Audit in Sweden that among women who had an abnormal Pap smear, those who did not have a follow-up biopsy had a higher risk of cervical cancer than those who did (OR:1.89 , 95% CI 1.19 to 3.02) (7). Moreover, the currently recommended management strategy might not be perfectly effective for certain scenarios of risk elevation (ie. atypia glandular cells) (8, 9).

Revised in manuscript:

- Discussion, principle findings and interpretations – paragraph 4

13. *Tables: data on controls should be added throughout, as should be crude IRRs – this would increase the transparency of the reporting and would help the readers follow the results.*

Reply: We have added information on controls and unadjusted IRRs.

Revised in manuscript:

- Table 2, Table 3, and Table 5.

14. *Minor comment: page 6, line 43-46, please clarify whether controls were required to be alive until the date of diagnosis of the matched case or until a later date (this may be just a misunderstanding because of how the sentence is written).*

Reply: All controls were required to be alive until the date of cancer diagnosis of the matched case. We revised the text to clarify this.

Revised in manuscript:

- Material and methods, study population- paragraph 2

15. *Minor comment: page 12, lines 48-50, when the authors talk about "a smaller magnitude of risk reduction", what comparison did they have in mind?*

Reply: It refers to smaller magnitude of risk reduction for RICC compared to common types of cervical cancer.

Revised in manuscript:

- Discussion, comparisons to other studies – paragraph 1

16. *Minor comment: Tables 2 and 3, although not statistically significant and based on small numbers, the IRRs for FIGO stage IA cases are oddly increased – please discuss.*

Reply: The estimation has limited precision for FIGO stage IA due to small number of cases. Besides, most of cases (76.5%) diagnosed at stage IA were screen-detected. Therefore, we are not surprised to see the IRRs of stage IA ASC and RICC to be higher for women who had screening tests compared to women who did not. Women who attend screening will benefit from early detection of cancers, while women who do not participate tend to be diagnosed at more advanced stages. We have now discussed this in the manuscript.

Being diagnosed at a lower stage (ie. IA and IB) with a screening test should however be considered as a success, rather than a failure of the screening programme, since there is evidence that almost all stage IA (microinvasive) cervical cancer can be cured, i.e. reach the same level of mortality as the general population, and the majority of microinvasive cancers can be treated conservatively (2). The 5-year survival of stage IB cervical cancer is also favourable (around 85% compared to general female population).

Revised in manuscript.

- Discussion, principle findings and interpretations – paragraph 3

Reviewer: 2

I thank you for asking me to review this manuscript. No doubt this is a very interesting study. It has highlighted the importance the rare types of invasive cervical cancer especially as the incidences of the more common types are decreasing in the western countries.

17. *HrHPV positivity was however very low in RICC compared with the common ICC and the relative distribution of the serotypes seem to be a reversal in the more common types of ICC, could this be attributed to the use of FFPE blocks?*

Reply: In our genotyping of 2850 cases we have extensively investigated the reasons for HPV-negativity and find that it is a biological phenomenon, preferentially found among women with symptomatic, late stage cervical carcinoma that have not attended screening (10). Also, direct comparisons have indicated no differences in HPV positivity between freshly frozen tissue and paraffin embedded tissue in general (11). Thus, we think that HPV-negativity attributed to the use of FFPE blocks seems to be unlikely.

The relative distribution of the genotypes has lower precision due to the low number of cases of RICC. However, since we have included all cases in a 10-year period in Sweden for comprehensive HPV genotyping for these RICC and it is also possible that the genotype distribution is indeed somewhat different.

Revised in manuscript:

- Discussion, principle findings and interpretations – paragraph 5

18. *Does the method of extraction lead to the reduced detection of hr HPV? It is important to clarify this with the increasing use of the HPV vaccine, does that mean that the vaccines will be less effective in the prevention of RICC whose relative incidence may increase because of the decreasing incidences of the more common types?*

Reply: The xylene-free extraction method used has been shown to be robust and result in improved detectability of HPV compared to the standard xylene method (12). Therefore, hrHPV negativity is unlikely to be due to the method of extraction.

The majority of hrHPV-positive ASC and RICC are indeed HPV16/18 positive in the tumor, based on our samples. Also, the fact that HPV-negativity is preferentially found in cases not detected by screening (10) might have resulted in a larger proportion of HPV-negative cases among RICC.

However, even if the proportion is lower compared to the common types of cervical carcinoma, it is key to note that hrHPV negativity in the tumor does not imply that hrHPV was not involved in the etiology of cancer development, since hrHPV-negative cases may have been infected with hrHPV at an earlier time point before cancer diagnosis. Using the same HPV detection method as in this study, we previously found that 97% of CIN3 or worse cases in the Swedish population were hrHPV positive (13). Additionally, we found a very similar risk reduction for women who had a screening test, both for cases who were hrHPV+ and for cases that were hrHPV-, compared to women without any tests (Table 5 in manuscript). This suggests that the HPV infections and subsequent cellular changes might be similar for both types of cases.

With a 9-valent HPV vaccine, and increased HPV vaccination coverage with subsequent herd effect, the incidence of all types of cervical cancer could be expected to further decrease. However, surveillance of the incidence of ASC and RICC is needed in the post-vaccination era.

Revised in manuscript:

- Discussion, strengths and weaknesses – paragraph 2

Reviewer: 3

This is a very good piece of research, the methods (laboratory, clinical confirmation, ascertainment of disease, statistical analysis) are well explained and applied, making the results very valid.

I only have two comments:

19. *Although there might not be enough cases of adenosquamous cell carcinoma (ASC) or the other rare types (RICC), the age categories of 30 to 60 and above 60 are not intuitive, can the authors produce a table with at least three age-groups, for instance: 30-39, 40-49 and 50 over?, or better discuss the lack of power to conduct such analysis?*

Reply: Following the reviewer's comments, we have generated two tables below with age groups as 30-39, 40-49 and 50 over (Table E & Table F). The lower risk of ASC and RICC for women who had a test in the recommended screening interval compared to women with no test remained across age groups. However, the estimations of RICC lose some precision.

Our rationale of classifying age as 30-60 and 60+ is to correspond with the screening guideline in Sweden under the relevant period that women age 23-60 were invited to cervical screening. Age classification in our study is also consistent with European screening guidelines (14). We therefore chose to keep the current age categories in the manuscript.

Table E Incidence rate ratio (IRR) of adenosquamous cell carcinoma and rare types of invasive cervical carcinoma by screening status in last two screening intervals.

	Screening status	All cases (n=307)			ASC (n=155)			RICC[b] (n=152)		
		Cases n (%)	Controls n (%)	IRR[a] (95% CI)	Cases n (%)	Controls n (%)	IRR[a] (95% CI)	Cases n (%)	Controls n (%)	IRR[a] (95% CI)
Age at diagnosis										
30-39	No test	14 (23.0)	258 (14.1)	Ref	9 (23.1)	153 (13.1)	Ref	5 (22.7)	105 (16.0)	Ref
	One test	27 (44.3)	606 (33.2)	0.71 (0.37 to 1.38)	17 (43.6)	395 (33.8)	0.61 (0.26 to 1.40)	10 (45.5)	211 (32.1)	0.77 (0.26 to 2.32)
	Two tests	20 (32.8)	963 (52.7)	0.33 (0.16 to 0.67)	13 (33.3)	621 (53.1)	0.29 (0.12 to 0.71)	7 (31.8)	342 (52.0)	0.32 (0.10 to 1.07)
40-49	No test	23 (37.7)	235 (13.1)	Ref	18 (45.0)	149 (12.8)	Ref	5 (23.8)	86 (13.9)	Ref
	One test	15 (24.6)	525 (29.4)	0.31 (0.16 to 0.61)	6 (15.0)	320 (27.4)	0.16 (0.06 to 0.42)	9 (42.9)	205 (33.1)	0.82 (0.26 to 2.62)
	Two tests	23 (37.7)	1028 (57.5)	0.24 (0.13 to 0.44)	16 (40.0)	699 (59.8)	0.20 (0.10 to 0.40)	7 (33.3)	329 (53.1)	0.38 (0.11 to 1.29)
>50	No test	98 (53.0)	1922 (37.3)	Ref	38 (50.0)	652 (31.0)	Ref	60 (55.0)	1270 (41.7)	Ref
	One test	49 (26.5)	1328 (25.8)	0.53 (0.36 to 0.79)	21 (27.6)	575 (27.3)	0.44 (0.24 to 0.80)	28 (25.7)	753 (24.7)	0.61 (0.36 to 1.04)
	Two tests	38 (20.5)	1897 (36.9)	0.25 (0.16 to 0.40)	17 (22.4)	877 (41.7)	0.19 (0.09 to 0.37)	21 (19.3)	1020 (33.5)	0.32 (0.18 to 0.58)

ASC: adenosquamous cell carcinoma; RICC: rare types of invasive cervical carcinoma.

[a] Incidence rate ratio adjusted by education level and age.

[b] RICC includes glassy cell carcinoma, clear cell carcinoma and other rare types.

Note: women age 30 and above were included.

Table F Incidence rate ratio (IRR) of adenosquamous cell carcinoma and rare types of invasive cervical carcinoma by screening history in last two screening intervals.

	Screening history	All cases (n=307)			ASC (n=155)			RICC[b] (n=152)		
		Cases n (%)	Controls n (%)	IRR[a] (95% CI)	Cases n (%)	Controls n (%)	IRR[a] (95% CI)	Cases n (%)	Controls n (%)	IRR[a] (95% CI)
Age at diagnosis										
30-39	No test	14 (23.0)	258 (14.1)	Ref	9 (23.1)	153 (13.1)	Ref	-	-	-
	Double normal	13 (21.3)	896 (49.0)	0.24 (0.11 to 0.51)	9 (23.1)	581 (49.7)	0.23 (0.09 to 0.59)	-	-	-
	One normal only	24 (39.3)	596 (32.6)	0.65 (0.33 to 1.28)	15 (38.5)	390 (33.4)	0.56 (0.24 to 1.31)	-	-	-
	≥ One abnormal	10 (16.4)	77 (4.2)	2.03 (0.85 to 4.86)	6 (15.4)	45 (3.8)	1.75 (0.57 to 5.41)	-	-	-
40-49	No test	23 (37.7)	235 (13.1)	Ref	18 (45.0)	149 (12.8)	Ref	5 (23.8)	86 (13.9)	Ref
	Double normal	15 (24.6)	981 (54.9)	0.17 (0.08 to 0.33)	10 (25.0)	664 (56.8)	0.13 (0.06 to 0.30)	5 (23.8)	317 (51.1)	0.30 (0.08 to 1.08)
	One normal only	12 (19.7)	517 (28.9)	0.26 (0.12 to 0.53)	5 (12.5)	315 (27.0)	0.14 (0.05 to 0.39)	7 (33.3)	202 (32.6)	0.72 (0.21 to 2.44)
	≥ One abnormal	11 (18.0)	55 (3.1)	2.12 (0.95 to 4.71)	7 (17.5)	40 (3.4)	1.48 (0.56 to 3.90)	4 (19.0)	15 (2.4)	5.39 (1.18 to 24.55)
>50	No test	98 (53.0)	1922 (37.3)	Ref	38 (50.0)	652 (31.0)	Ref	60 (55.0)	1270 (41.7)	Ref
	Double normal	31 (16.8)	1790 (34.8)	0.22 (0.14 to 0.36)	12 (15.8)	814 (38.7)	0.15 (0.07 to 0.32)	19 (17.4)	976 (32.1)	0.31 (0.16 to 0.57)
	One normal only	44 (23.8)	1301 (25.3)	0.49 (0.33 to 0.74)	18 (23.7)	564 (26.8)	0.38 (0.20 to 0.72)	26 (23.9)	737 (24.2)	0.59 (0.34 to 1.01)
	≥ One abnormal	12 (6.5)	134 (2.6)	1.08 (0.55 to 2.13)	8 (10.5)	74 (3.5)	1.07 (0.44 to 2.59)	4 (3.7)	60 (2.0)	0.97 (0.33 to 2.89)

ASC: adenosquamous cell carcinoma; RICC: rare types of invasive cervical carcinoma.

[a] Incidence rate ratio adjusted by education level and age.

[b] RICC includes glassy cell carcinoma, clear cell carcinoma and other rare types.

Note: women age 30 and above were included.

20. *Only 12% (41 of 338 cases including 31 less than 30y) were screen-detected (Table 1) while 56% of ASC and RICC (172 of 307 cases over 30y) attended screening (Table 2), however most of them were not detected by the screening process since these types of cancers are more aggressive and progress rapidly. The main results show that there is a risk reduction of developing ASC and RICC on women who attend screening, however, most cases are symptomatic and not screen-detected, can the authors discuss this further?*

Reply: Please see response to Editor's comment 2 above.

Apart from these comments, I think the manuscript is very valuable for the scientific audience, and should be published.

Reply: Thank you for the supportive comment.

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