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Smoking and risk of ovarian cancer by histological subtypes: an analysis among 300 000 Norwegian women

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Background: We prospectively investigated the association between different measures of smoking exposure and the risk of serous, mucinous, and endometrioid ovarian cancers (OC) in a cohort of more than 300 000 Norwegian women.

Methods: We followed 300 398 women aged 19–67 years at enrolment until 31 December 2013 for OC incidence through linkage to national registries. We used Cox proportional hazards models with attained age as the underlying time scale to estimate multivariable-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) adjusted for relevant confounders.

Results: During more than 5.9 million person-years and a median follow-up time of 19 years, 2336 primary invasive (1647, 71%) and borderline (689, 29%) OC were identified (53% serous, 19% mucinous). Compared with never smokers, current smokers who had smoked for $\geqslant$10 years had a higher risk of mucinous OC ($HR_{10–19\ years\ vs\ never} = 1.73$, 95% CI 1.24–2.42; $HR_{\geqslant 20\ vs\ never} = 2.26$, 95% CI 1.77–2.89, $P_{trend} < 0.001$). When stratified by invasiveness, current smokers had a higher risk of invasive mucinous OC (HR = 1.78, 95% CI 1.20–2.64) and borderline mucinous OC (HR = 2.26 95% CI, 1.71–2.97) ($P_{heterogeneity} = 0.34$) than never smokers. Smoking was not associated with serous or endometrioid OC.

Conclusions: Using a very large cohort of women, the current analysis provides an important replication for a similar risk of invasive and borderline mucinous OC related to smoking.

Cigarette smoking is an established risk factor for mucinous ovarian cancer (OC) with an estimated population attributable fraction of 14% (Agudo *et al*, 2012; International Agency for Research on Cancer, 2012). Serous ($\approx$60%), mucinous ($\approx$10%), endometrioid, and clear cell (both $\approx$17%) are the most common histological subtypes of OC (Kurman *et al*, 2014). The relationship between smoking and endometrioid and clear cell OC remains a controversial topic (Beral *et al*, 2012; Faber *et al*, 2013), as results are not consistent among studies (Terry *et al*, 2003). A recent pooled analysis included 51 epidemiological studies and found a 79% increased risk of mucinous OC when comparing participants who were current and never smokers at study enrolment. This increased risk was driven by borderline malignant mucinous OC rather than invasive mucinous tumours (Beral *et al*, 2012). The authors found reduced risks of both endometrioid and clear cell OC, and no association for serous OC, in current smokers. The pooled analysis did not study the association between OC other measures of smoking exposure, such as number of cigarettes smoked per day, smoking duration, pack-years smoked or age at smoking initiation, and histological subtypes of OC.

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Moreover, few prospective studies have investigated the association between these measures of smoking exposure and histological subtypes of OC (Gram *et al*, 2008; Tworoger *et al*, 2008; Gram *et al*, 2012; Licaj *et al*, 2016; Wentzensen *et al*, 2016), and the results of the prospective studies are inconsistent. None of them found an association between mucinous OC and smoking duration, and only one (Gram *et al*, 2012) reported that mucinous OC was associated with the number of cigarettes smoked per day. In a recent study (Wentzensen *et al*, 2016), number of pack-years, which was the only measure of smoking exposure studied, was associated with a higher risk of invasive mucinous OC among ever smokers. Two reports from the same study population found a positive association between number of cigarettes smoked per day, smoking duration in years, and the risk of borderline OC (Gram *et al*, 2008; Licaj *et al*, 2016). One reason for the variation in these results may be the different methods employed to assess and analyse these measures of smoking exposure.

Thus, there is a need for large prospective studies on the relationship between smoking and the risk of OC. On the basis of a cohort of a more than 300 000 Norwegian women, the purpose of this study was to investigate the role of smoking, including different measures of smoking exposure, in the risk of different histological subtypes of OC.

MATERIALS AND METHODS

Study population. The study population has been described previously (Næss *et al*, 2008; Bjerkaas *et al*, 2013). Briefly, it comprises Norwegian women recruited into different prospective cohort studies conducted by the Norwegian Institute of Public Health: the Norwegian Counties Study (1974–1988), the 40 Years Study (1985–1999), and the Cohort of Norway (CONOR) Study (1994–2003). All these cohorts are part of the Norwegian Health Screening Surveys. The response rate in the three studies ranged from 56 to 88% (Stocks *et al*, 2010). Overall, 330 342 women were eligible and 300 398 remained in the analytical cohort after exclusions due to missing vital status ($n=95$), prevalent cancer excluding non-melanoma skin cancer ($n=7180$), emigration or death prior to study enrolment, and with inconsistencies in immigration and/or emigration dates ($n=3264$). We further excluded women with missing information on smoking status ($n=2811$) and other main covariates included in the analyses ($n=16\,594$). The present study was approved by the Regional Committee for Medical Research Ethics South-East, Norway.

Data collection. Smoking status was defined at cohort entry as never, former, current, or ever smoker. The smoking questions were similar, across all three cohorts. The questionnaires collected information on current and former daily smoking habits, smoking duration, and average number of cigarettes smoked per day. Only the CONOR Study asked about age at smoking initiation. Thus, in the other surveys, we calculated this variable for both current (age at enrolment minus smoking duration in years) and former (age at enrolment minus years since quitting and smoking duration in years) smokers.

Information on number of children, age at first childbirth, and leisure time physical activity was also obtained from the questionnaires. Number of children was categorised into four groups (0, 1, 2, and $\geqslant 3$), as was age at first childbirth (<20, 20–24, 25–29, and $\geqslant 30$ years). Physical activity was categorised into three groups: low (reading, watching television, and sedentary activity), moderate (walking, bicycling, or similar activities $\geqslant 4$ h per week), and heavy (light sports or heavy gardening $\geqslant 4$ h per week, heavy exercise or daily competitive sports). The most recent information regarding education was obtained from Statistics Norway and used to assign participants to one of the three categories: low (<10 years), moderate (10–12 years), and high (>12 years). Height and weight were measured at enrolment by a trained nurse and used to calculate body mass index (BMI), which was categorised as (<25, 25–29, and $\geqslant 30\,\mathrm{kg\,m^{-2}}$).

Information on menopausal status was available from questionnaires. For women with missing age at menopause, 50 years of age was used as a proxy measure of menopausal status.

Participants were followed for cancer incidence through record linkage with the Cancer Registry of Norway, and for death and emigration through linkage to the Norwegian Central Population Register. These national registries contain correct, detailed, virtually complete information on cancer incidence and mortality (Larsen *et al*, 2009). The International Classification of Diseases (ICD-7 code 175 or corresponding ICD-9 or ICD-10 codes) and the World Health Organization's International Histological Classification of Tumours were used to identify four histological subtypes of OC (serous, mucinous, endometrioid, and clear cell) and to determine invasiveness (borderline and invasive). However, due to a small number of cases, we chose not to show results for clear cell OC.

Statistical analysis. Cox proportional hazards models with attained age as the underlying time scale were used to estimate multivariable-adjusted hazard ratios (HRs) with 95% confidence intervals (CI) for the associations between smoking status and OC overall, for the three histological subtypes we investigated (serous, mucinous, endometrioid), and for invasiveness of OCs. We then carried out the same analyses after reclassifying ever smokers according to different measures of exposure: duration (<10, 10–19, $\geqslant 20$ years, and missing $n=1795$), number of pack-years (<6, 6–15, $\geqslant 16$, and missing $n=2707$), number of cigarettes smoked per day (<6, 6–15, $\geqslant 16$, and missing $n=1587$), and age at smoking initiation (<20, 20–24, $\geqslant 25$ years, and missing $n=34\,397$). For the these analyses, we included missing indicators after checking that the parameters associated with these indicators were not statistically significantly associated with risk of OC overall or by histological subtype. Never smokers were used as the reference group. The start of the follow-up was defined as age at enrolment, and exit time as age at any incident cancer diagnosis (including OC) (except basal cell carcinoma), emigration, death, or the end of follow-up (31 December 2013), whichever occurred first.

Similar models were used to estimate multivariable-adjusted HRs with 95% CIs for the association between different measures of smoking exposure and serous and mucinous OC stratified by invasiveness. We were not able to do this for endometrioid and clear cell OC due to small numbers.

Wald χ^2 statistics were used to test for heterogeneity between histological subtypes. Models were stratified by cohort study and birth cohort ($\leqslant 1950$ and >1950). If a linear trend was observed for a specific measure of smoking exposure, that exposure was also tested as a continuous variable.

Analysis were adjusted for available established risk factors for OC (World Cancer Research Fund & American Institute for Cancer Research, 2014). In order to be retained in the final model, the removal of the covariate had to lead to a change in the regression coefficients of at least 10% in any of the smoking status groups. The final model included the covariates: number of children, age at first childbirth, physical activity level, and BMI.

We did not include use of hormone therapy (information available for $n=89\,637$) or oral contraceptives (information available for $n=80\,736$) in the main analyses as this information was only available in the 40 Years Study and the CONOR Study. Instead, we performed sensitivity analyses in these cohorts that included these two covariates. Tests for trend across categories of measures of smoking exposure were based on the median value in each category and included never smokers. We created separate models that included and excluded never smokers.

We tested if the proportional hazard assumption was met using Schoenfeld residuals. The modifying effect of number of children, education, physical activity, BMI, and menopausal status on the relationship between smoking status and OC was also assessed. Models with main effects and interaction terms were fitted and compared with models with only main effects. The difference in log-likelihood (likelihood ratio test statistics) was compared with a χ^2 distribution with degrees of freedom equal to the number of interaction terms.

All analyses were performed in STATA version 14.0 (StataCorp, College Station, TX, USA) and in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Median age at cohort entrance was 41 years. During more than 5.9 million person-years and a median follow-up of 19 years, 2336 cases of primary OC were identified. There were 1242 (53%) serous OC, 440 (19%) mucinous OC, 190 (8%) endometrioid OC, and 82 (4%) clear cell OC. Altogether 1647 (71%) were diagnosed with invasive and 689 (29%) with borderline tumours. In this study 38% of women were current, 21% former, and 41% never smokers at enrolment. Current smokers had an earlier age at first childbirth, more often reported low physical activity, had less years of education when compared with never smokers (Table 1).

When compared with women with serous OC, those with mucinous OC had an older age at first childbirth, more often reported low physical activity and were more often overweight (BMI $\geqslant$ 25 kg/m^2). Borderline OC was more often seen in obese (BMI $\geqslant$ 30 kg/m^2) and nulliparous women when compared with women with invasive OC (Supplementary Table 1).

Current smokers had a higher risk of all borderline OC but smoking was not associated with all invasive, serous, or endometrioid OC (Table 2). No increased risk of mucinous OC was observed for former smokers, whereas current smokers had a more than two-fold higher risk (HR = 2.09, 95% CI 1.67–2.62) when compared with never smokers. The elevated risk of mucinous OC was somewhat lower in ever smokers (HR = 1.70, 95% CI 1.37–2.10). Compared with never smokers, current smokers who had smoked for 10–19 years had a higher risk of mucinous OC (HR = 1.73, 95% CI 1.24–2.42) as did those who smoked for more than 20 years (HR = 2.26, 95% CI 1.77–2.89; $P_{\text{linear trend}} < 0.001$). We observed a higher risk of mucinous OC among current smokers who smoked 6–15 pack-years (HR = 2.44, 95% CI 1.90–3.14), those who smoked more than 16 pack-years (HR = 1.95, 95% CI 1.41–2.70), those who smoked 6–15 cigarettes per day (HR = 2.18, 95% CI 1.71–2.77), and those who smoked more than 16 cigarettes per day (HR = 2.46, 95% CI 1.73–3.50). The risk of mucinous OC was similar among current smokers who started smoking before 19 years of age (HR = 2.12, 95% CI 1.65–2.72) and those who started after 20 years of age (HR = 1.73, 95% CI 1.24–2.42). For current smokers, we observed a positive trend between smoking duration, number of pack-years, number of cigarettes smoked per day, and age at smoking initiation and the risk of mucinous OC and all borderline OCs (Table 2). We observed similar trends with and without never smokers. We found that each additional year of cigarette smoking increased the risk of mucinous OCs by 2%. The same elevated risk was found for each additional cigarette smoked per day.

Table 1. Baseline characteristics of the analytical sample according smoking status, Norwegian women, *N* = 300 398 (1974–2013)

	Never *N* = 122 594 41%	Former *N* = 63 434 21%	Current *N* = 114 370 38%	Ever *N* = 177 804 59%
Mean age at enrolment (95% CI)	43.6 (43.6–43.7)	43.0 (43.0–43.1)	41.7 (41.6–41.7)	42.2 (42.1–42.2)
Number of OC	926	454	956	1410
Number of invasive OC	704	314	629	943
Number of borderline OC	222	140	327	467
Number of serous OC	497	261	484	745
Number of mucinous OC	130	69	241	310
Number of endometrioid OC	90	36	64	100
Number of clear cell OC	40	22	20	42
Number of other OC	169	66	147	213
Arithmetic mean age at diagnosis for OC (95% CI)	58.6 (57.9–59.2)	56.5(55.6–57.4)	55.5 (55.1–56.1)	55.9 (55.4–56.3)
Mean age at diagnosis for invasive (95% CI)	59.5 (58.7–60.2)	57.5 (56.5–58.5)	56.1 (55.4–56.8)	56.6 (56.0–57.1)
Mean age at diagnosis for borderline (95% CI)	55.7 (54.4–57.0)	54.2 (52.6–55.8)	54.5 (53.6–55.4)	54.4 (53.6–55.2)
Mean age at diagnosis for serous (95% CI)	59.1 (58.2–59.9)	56.4 (55.3–57.5)	55.8 (55.1–56.5)	56.0 (55.4–56.6)
Mean age at diagnosis for mucinous (95% CI)	56.9 (55.1–58.8)	55.4 (53.0–57.8)	54.6 (53.5–55.7)	54.8 (53.8–55.8)
Mean age at diagnosis for endometrioid (95% CI)	56.7 (54.6–58.8)	55.0 (52.6–57.4)	54.4 (52.4–56.5)	54.6 (53.1–56.2)
Mean age at diagnosis for clear cells (95% CI)	57.3 (54.0–60.6)	56.2 (52.4–60.1)	56.6 (53.2–60.1)	56.4 (53.9–58.9)
Mean age at diagnosis for other OC (95% CI)	59.8 (58.0–61.5)	58.9 (56.2–61.6)	56.5 (54.9–58.1)	57.3 (55.9–58.7)
Follow-up time in years (age exit and age at enrolment)	20.1 (20.0–20.1)	18.9 (18.9–19.0)	19.9 (19.8–19.9)	19.5 (19.5–19.6)
Null parity, %	13.2	10.2	11.8	11.2
Mean number of children[a]	2.6 (2.6–2.6)	2.4 (2.4–2.4)	2.4 (2.4–2.4)	2.4 (2.4–2.4)
Mean age at first childbirth	25.0 (25.0–25.0)	24.5 (24.4–24.5)	22.9 (22.9–22.9)	23.5 (23.5–23.5)
Physical activity, %				
Low	20.3	19.5	25.2	23.1
Middle	58.1	55.8	56.7	56.4
High	21.6	24.7	18.1	20.5
Education in years				
Low (< 10)	20.0	20.51	32.0	27.9
Moderate(10–12)	51.9	57.34	57.3	57.3
High(⩾13)	28.1	22.15	10.7	14.8
BMI (kg m^{-2})	25.0 (24.9–25.0)	25.0 (25.0–25.1)	24.1 (24.0–24.1)	24.4 (24.4–24.4)
Age at smoking initiation[b]	NA	18.3 (18.2–18.3)	20.5 (20.5–20.6)	20.1 (20.0–20.2)

Abbreviations: BMI = body mass index; CI = confidence interval; NA = not applicable; OC = ovarian cancer.
[a]Parous women.
[b]For current and former smokers.

Table 2. Multivariable[a] HRs (95% CI) of invasiveness status and by histological subtypes according of OCs according to various measures of smoking status compared with never smokers 300 398 Norwegian women

	All borderline[b]		All invasive[b]		Serous[c]		Mucinous[c]		Endometrioid[c]	
	n_{cases}	HR (95% CI)	n_{cases}	HR (95% CI)	n_{cases}	HR (95% CI)	n_{cases}	HR (95% CI)	n_{cases}	HR (95% CI)
Smoking status										
Never	222	1.00	704	1.00	497	1.00	130	1.00	90	1.00
Former	140	1.23 (0.99–1.52)	314	0.95 (0.83–1.08)	261	1.03 (0.88–1.20)	69	1.09 (0.81–1.47)	36	0.83 (0.56–1.23)
Current	327	1.55 (1.29–1.85)	629	0.97 (0.86–1.08)	484	0.99 (0.87–1.13)	241	2.09 (1.67–2.62)	64	0.74 (0.53–1.03)
Ever	467	1.42 (1.20–1.68)	943	0.96 (0.87–1.06)	745	1.00 (0.89–1.13)	310	1.70 (1.37–2.10)	100	0.77 (0.57–1.04)
Smoking duration in years										
Former										
⩽9	56	1.23 (0.91–1.65)	98	0.95 (0.78–1.15)	98	0.99 (0.80–1.23)	26	1.05 (0.68–1.60)	15	0.87 (0.50–1.50)
10–19	56	1.18 (0.87–1.58)	108	0.96 (0.80–1.16)	108	1.04 (0.84–1.28)	28	1.07 (0.71–1.62)	19	1.06 (0.64–1.74)
20+	25	1.25 (0.82–1.91)	51	0.89 (0.68–1.18)	51	1.13 (0.84–1.52)	13	1.12 (0.63–2.00)	2	0.28 (0.07–1.14)
P_{trend}[d]		0.07		0.53		0.47		0.04		0.18
Current										
⩽9	18	1.25 (0.74–2.09)	28	0.91 (0.65–1.28)	28	0.95 (0.64–1.42)	13	1.64 (0.90–2.98)	6	1.01 (0.42–2.45)
10–19	64	1.27 (0.95–1.68)	96	0.87 (0.72–1.05)	96	0.85 (0.68–1.07)	51	1.73 (1.24–2.42)	11	0.54 (0.28–1.01)
20+	243	1.68 (1.38–2.04)	359	1.02 (0.90–1.16)	359	1.04 (0.90–1.21)	175	2.26 (1.77–2.89)	47	0.84 (0.57–1.23)
P_{trend}[d]		<0.001		0.75		0.24		<0.001		0.11
Pack-years										
Former										
⩽5	76	1.15 (0.88–1.49)	153	0.98 (0.83–1.15)	153	1.05 (0.87–1.26)	36	0.98 (0.67–1.42)	20	0.79 (0.48–1.28)
6–15	44	1.22 (0.88–1.69)	68	0.84 (0.67–1.05)	68	0.86 (0.67–1.11)	24	1.21 (0.78–1.88)	15	1.11 (0.64–1.93)
16+	15	1.60 (0.94–2.72)	33	1.11 (0.76–1.62)	33	1.64 (1.14–2.34)	5	0.97 (0.39–2.39)	1	0.31 (0.04–2.24)
P_{trend}[d]		0.025		0.61		0.89		0.13		0.28
Current										
⩽5	48	1.17 (0.85–1.62)	86	0.86 (0.70–1.05)	86	0.96 (0.76–1.22)	32	1.37 (0.92–2.04)	9	0.52 (0.26–1.06)
6–15	165	1.51 (1.22–1.86)	237	1.03 (0.90–1.18)	237	0.92 (0.78–1.08)	147	2.44 (1.90–3.14)	36	0.83 (0.55–1.24)
16+	111	1.90 (1.49–2.43)	159	0.96 (0.80–1.15)	159	1.15 (0.95–1.39)	60	1.95 (1.41–2.70)	19	0.88 (0.52–1.48)
P_{trend}[d]		<0.001		0.50		0.84		<0.001		0.29
Number of cigarettes per day										
Former										
⩽5	41	1.31 (0.94–1.83)	74	0.97 (0.78–1.20)	74	1.06 (0.83–1.36)	17	0.95 (0.57–1.59)	13	1.1 (0.61–1.98)
6–15	73	1.10 (0.84–1.43)	136	0.92 (0.78–1.09)	136	0.93 (0.77–1.13)	43	1.19 (0.84–1.69)	17	0.67 (0.4–1.14)
16+	23	1.53 (0.99–2.36)	45	1.03 (0.75–1.42)	45	1.43 (1.05–1.95)	8	1.02 (0.50–2.10)	6	1.11 (0.48–2.55)
P_{trend}[d]		0.08		0.68		0.29		0.98		0.25
Current										
⩽5	31	1.21 (0.83–1.77)	52	0.80 (0.62–1.03)	52	0.89 (0.67–1.19)	21	1.44 (0.91–2.29)	4	0.38 (0.14–1.05)
6–15	221	1.49 (1.22–1.81)	342	1.02 (0.90–1.16)	342	0.99 (0.85–1.14)	175	2.18 (1.71–2.77)	48	0.82 (0.57–1.19)
16+	73	2.02 (1.53–2.67)	88	0.94 (0.75–1.17)	88	1.05 (0.83–1.33)	46	2.46 (1.73–3.50)	12	0.89 (0.48–1.65)
P_{trend}[d]		<0.001		0.66		0.81		<0.001		0.28
Age at smoking initiation										
Former										
20+	14	1.03 (0.58–1.83)	34	1.16 (0.80–1.68)	34	1.42 (0.97–2.08)	5	0.61 (0.24–1.54)	3	1.10 (0.32–3.80)
⩽19	37	1.11 (0.75–1.66)	67	1.19 (0.89–1.59)	67	1.18 (0.87–1.59)	20	1.30 (0.75–2.25)	8	1.08 (0.46–2.54)
P_{trend}[d]		0.04		0.12		0.69		0.51		0.28
Current										
20+	163	1.48 (1.20–1.82)	237	0.95 (0.84–1.09)	237	0.90 (0.77–1.06)	136	2.12 (1.65–2.72)	34	0.72 (0.48–1.09)
⩽19	163	1.63 (1.30–2.03)	246	1.01 (0.86–1.17)	246	1.11 (0.94–1.32)	105	2.03 (1.53–2.69)	30	0.84 (0.54–1.33)
P_{trend}[d]		<0.001		0.51		0.57		<0.001		0.23

Abbreviations: BMI = body mass index; CI = confidence interval; HR = hazard ratios; OC = ovarian cancer.
[a]Adjusted for number of children (0, 1, 2, and ⩾3), age at first childbirth (<20, 20–24, 25–29, and ⩾30 years), BMI (<25, 25–29, and ⩾30 kg m^{-2}), and level of physical activity (low, moderate, and heavy).
[b]All histological subtypes.
[c]Include invasive and borderline tumours.
[d]Trend tests include never smokers.

When we stratified serous and mucinous OC by invasiveness (Table 3), current compared with never smokers were at higher risk of invasive mucinous OC (HR = 1.78, 95% CI 1.20–2.64) and of borderline mucinous OC (HR = 2.26, 95% CI 1.71–2.97). The test for heterogeneity showed that there was no difference between invasive and borderline mucinous OC ($P_{heterogeneity} = 0.34$). We observed a positive trend between smoking duration and risk of invasive and borderline mucinous OC, but not for borderline serous or invasive serous OC (Table 3). The risk for all histological subtypes of OC was similar in former and never smokers.

We combined three smoking characteristics age at smoking initiation, duration, and cigarettes smoked per day (Table 4). In women who smoked for more than 10 years and more than 10 cigarettes per day, the age at smoking initiation (<18 years or older) had little impact on the risk of mucinous OC when compared with never smokers. They were HR = 1.77 (95% CI 1.26–2.49) and HR = 2.42 (95% CI 1.88–3.10), respectively (Table 4).

We found no statistically significant interactions between smoking status and number of children, physical activity, education, or BMI in relation to mucinous or other histological

Table 3. Multivariable[a] HR (95% CI) of serous and mucinous epithelial OC overall by invasive status according to various measures of smoking status at enrolment compared with never smokers in 300 398 Norwegian women

OCs	N_{cases}	Invasive mucinous $N=138$	N_{cases}	Borderline mucinous $N=302$	N_{cases}	Invasive serous $N=888$	N_{cases}	Borderline serous $N=354$
Smoking status								
Never	45	1.00	85	1.00	371	1.00	126	1.00
Former	21	1.01 (0.60–1.70)	48	1.14 (0.80–1.63)	175	0.94 (0.79–1.13)	86	1.27 (0.96–1.67)
Current	72	1.78 (1.20–2.64)	169	2.26 (1.71–2.97)	342	0.94 (0.81–1.10)	142	1.11 (0.86–1.43)
Ever	93	1.49 (1.03–2.16)	217	1.81 (1.40–2.35)	517	0.94 (0.82–1.08)	228	1.17 (0.93–1.47)
Smoking duration in years								
Ever smokers								
⩽9	7	0.65 (0.29–1.44)	32	1.42 (0.94–2.15)	88	0.95 (0.75–1.20)	38	1.10 (0.76–1.60)
10–19	27	1.42 (0.88–2.31)	52	1.42 (1.00–2.01)	143	0.90 (0.74–1.09)	61	1.07 (0.79–1.47)
20+	58	1.89 (1.25–2.86)	130	2.25 (1.68–3.01)	283	0.97 (0.83–1.15)	127	1.25 (0.97–1.63)
P_{trend}		0.001		<0.001		0.46		0.12
Pack-years								
Ever smokers								
⩽5	19	0.94 (0.55–1.61)	49	1.22 (0.85–1.74)	169	0.98 (0.81–1.18)	70	1.14 (0.85–1.53)
6–15	55	1.96 (1.30–2.96)	116	2.20 (1.65–2.95)	225	0.90 (0.76–1.07)	80	0.92 (0.69–1.22)
16+	17	1.37 (0.77–2.46)	48	2.05 (1.41–2.96)	118	1.00 (0.80–1.24)	74	1.76 (1.30–2.38)
P_{trend}		0.07		0.01		0.35		0.01
Number of cigarettes per day								
Ever smokers								
⩽5	11	1.02 (0.52–1.98)	26	1.21 (0.78–1.88)	84	0.89 (0.70–1.12)	42	1.28 (0.90–1.83)
6–15	67	1.68 (1.13–2.49)	151	1.93 (1.46–2.54)	349	0.96 (0.82–1.12)	129	1.00 (0.77–1.29)
16+	15	1.68 (0.92–3.08)	39	2.18 (1.48–3.23)	80	0.95 (0.74–1.22)	53	1.66 (1.19–2.31)
P_{trend}		0.008		<0.001		0.60		0.02
Age at smoking initiation								
Ever smokers								
25+	14	1.36 (0.74–2.48)	28	1.61 (1.05–2.48)	78	0.94 (0.74–1.21)	24	0.95 (0.61–1.47)
20–24	32	2.02 (1.27–3.23)	67	2.33 (1.68–3.23)	120	0.88 (0.71–1.08)	49	1.07 (0.76–1.50)
<20	32	1.61 (0.98–2.64)	93	2.12 (1.53–2.92)	213	1.06 (0.88–1.28)	100	1.19 (0.89–1.59)
P_{trend}		0.97		0.04		0.27		0.03

Abbreviations: BMI = body mass index; CI = confidence interval; HR = hazard ratios; OC = ovarian cancer.
[a]Adjusted for number of children (0, 1, 2, and ⩾3), age at first childbirth (<20, 20–24, 25–29, and ⩾30 years), BMI (<25, 25–29, and ⩾30 kg/m2), and level of physical activity (sedentary, moderate, and heavy).

Table 4. Multivariable[a] hazard ratios (95% confidence interval) of three combined smoking characteristics[b] (duration in years, number of cigarettes per day and age at start) and serous mucinous and endometrioid tumours in 300 398 Norwegian women

	All invasive[c]		All borderline[c]		Serous[d]		Mucinous[d]		Endometrioid[d]	
Never	**704**	**1.00**	**222**	**1.00**	**497**	**1.00**	**130**	**1.00**	**90**	**1.00**
Category 1	25	0.73 (0.49–1.09)	18	1.48 (0.91-2.4)	22	0.87 (0.57-1.34)	10	1.47 (0.77-2.82)	4	-
Category 2	10	0.99 (0.53–1.88)	4	–	8	–	1	–	2	–
Category 3	20	0.99 (0.63–1.54)	5	–	13	0.85 (0.49–1.47)	4	–	2	–
Category 4	13	1.38 (0.78–2.44)	6	–	12	1.30 (0.71–2.37)	4	–	1	–
Category 5	132	0.94 (0.78–1.13)	46	1.10 (0.80–1.51)	91	0.93 (0.74–1.17)	34	1.36 (0.93–2.00)	9	–
Category 6	34	0.95 (0.67–1.35)	17	1.13 (0.68–1.87)	28	0.90 (0.61–1.33)	10	1.35 (0.70–2.61)	7	–
Category 7	330	1.00 (0.87–1.14)	178	1.69 (1.38–2.08)	239	0.98 (0.83–1.15)	143	2.42 (1.88–3.10)	35	0.83 (0.55–1.24)
Category 8	166	1.03 (0.85–1.23)	99	1.55 (1.20–2.00)	165	1.20 (0.99–1.45)	56	1.77 (1.26–2.49)	15	0.71 (0.40–1.28)

Category 1: smoked <10 years, <10 years cigarettes per day, age at start ⩾18 years. Category 2: smoked <10 years, <10 years cigarettes per day, age at start <18 years. Category 3: smoked <10 years, ⩾10 years cigarettes per day, age at start ⩾18 years. Category 4: smoked ⩾10 years, <10 years cigarettes per day, age at start ⩾18 years. Category 5: smoked <10 years, ⩾10 years cigarettes per day, age at start <18 years. Category 6: smoked ⩾10 years, <10 years cigarettes per day, age at start <18 years. Category 7: smoked ⩾10 years, ⩾10 years cigarettes per day, age at start ⩾18 years. Category 8: smoked ⩾10 years, ⩾10 years cigarettes per day, age at start <18 years.
[a]Adjusted for number of children (0, 1, 2, ⩾3), age at first childbirth (<20, 20–24, 25–29, ⩾30 years), BMI (<25, 25–29, ⩾30 kg m^{-2}) and level of physical activity (sedentary, moderate, heavy).
[b]Only HRs and 95% CI with more than 10 cases are shown.
[c]All histological subtypes.
[d]Include invasive and borderline tumours.

subtypes of OC. We observed similar higher risks of mucinous OC when current smokers were compared with never smokers in analyses stratified by BMI (<25 kg m^{-2}: HR = 2.36, 95% CI 1.74–3.19; 25–29 kg m^{-2}: HR = 1.62, 95% CI 1.07–2.44; ⩾30 kg m^{-2}: HR = 2.20, 95% CI 1.17–4.13), but also when stratified by physical activity and number of children (data not shown).

We observed no differences in sensitivity analyses that were additionally adjusted for hormone therapy and oral contraceptive use (data not shown).

DISCUSSION

In this large, prospective study, cigarette smoking was associated with invasive and borderline mucinous OC, but not with other histological subtypes of OC. For invasive and borderline mucinous OC, we observed a significant, dose–response association with smoking duration, pack-years, and number of cigarettes smoked per day. When restricting the analyses to smokers, we found that each additional year of cigarette smoking and each increase of one cigarette per day increased the risk of mucinous OC by 2%.

Our study complements the results from two recent pooled analyses of smoking and OC (Beral *et al*, 2012; Faber *et al*, 2013) that included 14 724 and 28 114 cases of OC, respectively. One was from a collaborative group on epidemiological studies of OC including both prospective cohorts and case–control studies and reported that borderline mucinous (HR = 2.25, 95% CI 1.91–2.65) and invasive mucinous (HR = 1.49, 95% CI 1.28–1.73; *P* for heterogeneity 0.01) OC incidence were increased when current smokers were compared with never smokers. This collaborative study did not include dose–response associations for different measures of cigarette smoking or for subtypes of OC. The second pooled analysis studied dose–response associations with different measures of cigarette smoking, but only included case–control studies (Faber *et al*, 2013). Both studies reported lower smoking-related risks of invasive mucinous OC among current smokers than we observed in our study. In contrast to the first pooled analysis of 51 epidemiological studies, our study demonstrated that smoking increased the risk of both invasive and borderline mucinous OC to a similar extent. In contrast to the pooled analysis of 21 case–controls studies (Faber *et al*, 2013), we did not find an association between cigarette smoking and risk of borderline serous OC.

The few prospective cohort studies that have investigated the association between measure of smoking exposure and the risk of subtypes of OC (Tworoger *et al*, 2008; Gram *et al*, 2012; Licaj *et al*, 2016) found no association between smoking duration and mucinous OCs. Each 20 additional pack-years in ever compared with never smokers was associated with a significant 20% higher risk of invasive mucinous OC (Wentzensen *et al*, 2016).

In our study, neither BMI nor education was revealed as an effect modifier for the association between smoking and histological subtypes of OC, as there was no evidence of statistically significant interaction.

Mucinous OC shares its histological appearance and its association with smoking with colorectal cancer (Newcomb *et al*, 1995; Parajuli *et al*, 2014). As a potential biological mechanism, somatic mutations in the *KRAS* gene are common in borderline tumours but not in invasive epithelial OC, and were found to be more frequent in borderline mucinous than in borderline serous OC (Mayr *et al*, 2006). Cigarette smoking was found to induce KRAS mutations in patients with lung, pancreatic, and colon cancers (Diergaarde *et al*, 2003; Jiao *et al*, 2007; Baykara *et al*, 2013). A similar mechanism of oncogenesis might be applicable to mucinous OC. Adducts of benzo(a)pyrene, a potent local carcinogen, have been found in ovarian follicular cells of women exposed to cigarette smoke (Zenzes *et al*, 1998). The DNA-damaging effect of adducts might partially explain a biological plausibility between smoking and mucinous ovarian cancer.

Strengths and limitations. The main strength of this study is the large number of enrolled women, representing all counties in Norway, the virtually complete long-term follow-up, the large number of OC overall, and of histological subtypes of OC. In our analysis, we stratified the analyses by two birth cohorts ($\leqslant$1950 and $>$1950), as reproductive and lifestyle factors changed during follow-up.

The limitations include lack of complete information for established risk factors such as age at menopause and menarche, use of oral contraceptives and hormone therapy. The sensitivity analyses controlling for hormone therapy and oral contraceptive use cannot be representative of our entire sample and should be interpreted with caution. The use of HT became more widespread after 1990 (Bakken *et al*, 2004), and may be a more important limitation in younger birth cohorts than it is in our study. In the recent consortia study by Wentzensen *et al* (2016), ever use of oral contraceptive was associated with a lower risk of all invasive OC, whereas ever use of hormone therapy was associated with a higher risk; but none of these two factors was associated with invasive mucinous OC. When we additionally adjusted for hormone therapy and oral contraceptive in our sensitivity analyses, we observed similar results.

Another limitation of our study is the possible misclassification of histological subtypes of OC and invasiveness. We relied on pathology information recorded by local pathologists. We believe that a differential misclassification of OC subtypes between current/former and never smokers is unlikely. In addition, we observe significant differences between subtype and invasiveness of OC, which is not supporting significant misclassification in our data.

The prevalence of smoking in Norway continuously decreased during the follow-up period of this study (1974–2013), but we had no updated information on smoking status for all participants. Comparison of OC risk between ever and never smokers gave similar results as the comparison between current and never smokers. As only never smokers could change their smoking status during follow-up (women who quit smoking will still be ever smokers), misclassification in smoking status in the follow-up period can be assumed to be low and there is little chance of misclassification bias, which would tend to attenuate the current findings. However, the long study period without follow-up information limits our ability to infer causality. Possible misclassification of histological types of OC and invasiveness may also be present, but we believe that differential misclassification of OC subtypes between current and never smokers is unlikely.

CONCLUSION

We found a dose–response association between smoking duration, pack-years, and number of cigarettes smoked per day, and both invasive and borderline mucinous OC. This large confirmation study add further evidence that smoking increases the risk of invasive and borderline mucinous OC to a similar extent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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