Author Manuscript Published OnlineFirst on April 16, 2018; DOI: 10.1158/1055-9965.EPI-17-0744
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Anti-CA15.3 and anti-CA125 antibodies and ovarian cancer risk: Results from the EPIC cohort

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Conflict of Interest: The authors declare no potential conflicts of interest.

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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

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Word count, text: 4076

Word count, abstract: 248

Tables: 6 tables; 1 supplemental table

Figures: 0

Key words: Mucin, Anti-Mucin Antibodies, Ovarian Cancer

Funding

Research reported in this publication was supported by The National Institutes of Health, R01 CA 158119 (DW Cramer [PI] and RF Fichorova [Co-Investigator]); NIH R35 CA197605 (DW Cramer [PI] and RF Fichorova [Co-Investigator]); Cancer Research UK C8221/A19170 R Travis; and The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food, Nutrition and Health (Norway); Health Research Fund (FIS), PI13/00061 to Granada; PI13/01162 to EPIC-Murcia), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk, MR/M012190/1 to EPIC-Oxford) (United Kingdom). For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php

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Abstract

Background:

Neoplastic and non-neoplastic events may raise levels of mucins, CA15.3 and CA125, and generate antibodies against them; but their impact on epithelial ovarian cancer (EOC) risk has not been fully defined.

Methods:

CA15.3, CA125, and IgG1 antibodies against them were measured in 806 women who developed EOC and 1,927 matched controls from the European Prospective Investigation of Nutrition and Cancer. Associations between epidemiologic factors and anti-mucin antibodies were evaluated using generalized linear models; EOC risks associated with anti-mucin antibodies, by themselves or in combination with respective antigens, were evaluated using conditional logistic regression.

Results:

In controls, lower antibodies against both mucins were associated with current smoking; and, in postmenopausal women, higher levels with longer oral contraceptive use and later-age-at and shorter-interval-since last birth. Lower anti-CA15.3 antibodies were associated with higher body mass and, in premenopausal women, more ovulatory cycles. Higher anti-CA15.3 and anti-CA125 antibodies were associated with higher risk for mucinous EOC occurring ≥ 3 years from enrollment. Long-term risk for serous EOC was reduced in women with low CA125 and high anti-CA125 antibodies relative to women with low concentrations of both.

Conclusions:

We found general support for the hypothesis that anti-mucin antibody levels correlate with risk factors for EOC. Antibodies alone or in combinations with their antigen may predict longer term risk of specific EOC types.

Impact:

Anti-CA125 and anti- CA15.3 antibodies alone or in perspective of antigens may be informative in the pathogenesis of EOC subtypes, but less useful for informing risk for all EOC.

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Introduction

In the 1980s, two heavily-glycosylated proteins were discovered as potential markers for breast and epithelial ovarian cancer(EOC)(1, 2). Monoclonal antibodies were raised against selected epitopes and assays developed whose names became synonymous with the markers CA15.3 for the breast biomarker and CA125 for the EOC biomarker. Genes for these proteins were eventually cloned and proved to be members of the human mucin family(3, 4) - CA15.3 as human mucin 1 (MUC1) and CA125 as human mucin 16 (MUC16). Both proteins were found to be expressed on mucosal barriers lining the genital, digestive, and respiratory tracts and breast ducts and over expressed in many of the neoplasms originating from these tissues (5, 6). Specifically, for invasive EOC and depending upon the antibody used for histochemical analysis, a majority of all EOC subtypes express CA15.3(7). Tissue expression of CA125 varies by subtype from 12% (mucinous) to 85% (serous)(8). Higher serum levels of CA15.3 in patients with breast and colorectal cancers were associated with poorer prognosis(9, 10) and thought to reflect an immunosuppressive effect on T cell proliferation(11). Similarly, in EOC, high serum levels of CA125 predict advanced disease and poorer survival(12). Like CA15.3, this may have an immune basis since CA125 can bind with natural killer(NK) cells and blunt NK response to ovarian tumors cells(13).

Antibodies against CA15.3 and CA125 have also been described. Anti-CA15.3 antibodies were found in some in patients with CA15.3-expressing cancers and associated with better prognosis (14). Both CA15.3 and anti-CA15.3 antibodies can also be found in healthy women, including those who are pregnant or breastfeeding(15). This led to the theory that anti-CA15.3 antibodies evoked by these events could explain why they protect against breast cancer(16). CA125 is

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elevated in pregnancy, but whether anti-CA125 antibodies also form during pregnancy has not been studied. However, events which reduce EOC risk like pelvic surgery, mumps, or puerperal mastitis, may lead to elevated (and presumably protective) levels of both anti-CA125 and anti-CA15.3 antibodies(17-20). Conversely, chronic events that increase EOC risk like repeated damage and repair to the ovary from "incessant ovulation" (ovulations not interrupted by pregnancy or oral contraceptive use) were associated with lower levels of anti-CA15.3 antibodies, suggesting immune tolerance that allows emergence of a CA15.3-expressing cancer(20, 21). These observations form the basis for a general paradigm that risk factors for mucin-expressing cancers may operate because of humoral (and, likely, cellular) immune reactions to mucin-expression invoked by the events themselves. Chronicity of the exposure may determine whether the event raises or lowers risk for the cancer.

In this study, we measured anti-CA15.3 and anti-CA125 antibodies and their corresponding antigens in sera from female participants in the European Prospective Investigation of Nutrition and Cancer(EPIC) including 806 who subsequently developed invasive EOC and 1,927 matched controls. We sought to identify factors associated with levels of anti-CA15.3 and anti-CA125 antibodies by menopausal status and determine whether these antibodies were associated with early detection (elevated levels within three years of diagnosis) or risk (elevated levels three or more years before diagnosis) of EOC overall and by histologic subtypes.

Material and Methods

The EPIC cohort – background and collection of blood samples

EPIC is an ongoing multicenter prospective cohort study designed to investigate relationships between diet and cancer(22). Briefly, 519,978 participants (366,521 women) were enrolled from 1992 to 2000 in 23 centers in 10 European countries: Denmark, France, Germany, Greece, Italy,

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Norway, the Netherlands, Spain, Sweden, and the United Kingdom. A total of 385,747 participants (226,673 women) provided a baseline blood sample, kept frozen in long-term storage at ≤-150^{o}C, with the exception of Sweden, where samples are stored at -70^{o}C.

Ascertainment of incident cancer cases

Cancer occurrence was documented through record linkage with cancer registries or active follow-up. Self-report was verified by clinical record review. Vital status was determined by linkage with mortality registries. When the present study was initiated, follow-up was complete through 2005 (France) to 2008 (Germany). Among women who had provided a baseline blood sample, a total of 806 incident cases of invasive EOC were identified including ovary (International Classification of Diseases for Oncology (ICD) O–3code: C569), fallopian tube (C570) or peritoneal cancer (C480, C481, C482, C488). Low malignant potential (borderline) tumors were not included. For the 806 EOC cases, complete information on tumor grade was available for 471 patients (58%) and information on tumor stage was available for 709 patients (88%).

Design of nested case-control study

For each case, up to four controls were randomly selected from female cohort members who were alive and cancer-free at diagnosis of the index case using a sampling protocol described previously(23). Case and control participants were matched on study center, age at blood donation, time of the day of blood collection, fasting status, menopausal status, menstrual cycle phase for premenopausal women, current use of oral contraceptives(OC) or hormonal replacement therapy (HRT).

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Exposure Data

Baseline data were self-reported through questionnaires or interview except for body mass index (BMI) where height and weight were directly measured. Categorized, the variables included: age at blood draw (<41, 41-50, 51-60, 61-70, >70 years), BMI (<18.5, 18.5-24.99, 25-29.99, ≥30 kg/m^2), smoking (never, former, current), pack years (≤11, 12-19, 20-31, >31), age at menarche (<12, 12, 13, 14, >14), OC use (never/ever), OC duration (≤2, >2-5, >5-10, >10 years), number (0, 1, 2, 3, 4+) and timing of pregnancies, age at first birth (<20, 20-24, 25-29, ≥30), age at last birth (<25, 25-29, 30-34, ≥35), years since last birth (<21, 21-27, 28-32, >32 years), hysterectomy, oophorectomy, family history of breast cancer, age at menopause (<46, 47-49, 50-51, 52-53, >53), and HRT use (postmenopausal never used HRT, ≤2.5 years, >2.5 years). The number of ovulatory cycles was estimated by calculating years between menarche and current age or age at menopause (if postmenopausal) and subtracting time using oral contraceptives, pregnant, or breastfeeding. Those missing an exposure of interest were excluded from analyses for that exposure.

Laboratory Methods

Assays were performed at the Brigham and Women's Hospital Laboratory of Genital Tract Biology using a multiplex platform (Meso Scale Discovery, MSD). Laboratory personnel were blind to case-control and quality-control sample status. Methods related to the measurement of CA125 and CA15.3 were previously described(23). Similar batching rules used for the mucin antigens were employed for the mucin antibodies; i.e. keep matching pairs together and as many from the same center as possible with at least 6 QC samples. The following reagents were provided by Fujirebio Diagnostics, Inc. (Malvern, PA): anti-CA125 antibody, anti-CA15.3 antibody. To measure antibodies against CA15.3 and CA125, antigen-grade CA15.3 and CA125

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purified from human breast and ovarian cancer cell lines (Meridian Life Sciences Inc., Memphis, TN) were coated on multi-spot plates. The assays included blocking with a blocking buffer for 1h followed by wash; 2h incubation with samples at multiple dilutions followed by PBS/0.05% Tween-20 wash; detection of human IgG1 bound to the specific protein spots with MSD sulfo-Tag-labeled antibodies (1µg/ml) for 2h; washing and adding read buffer followed by detection of electro-chemiluminescence (ECL) using an MSD Imager 2400. Split aliquots of this pool were tested at the same dilutions as the test samples on each assay plate and served to assess inter-batch variation. Coefficients of variation were calculated using measurements from blinded aliquots. Average intra-plate CVs were 14% for anti-CA125, 11% for anti-CA15.3, and the inter-plate CVs were 35% and 31%, respectively.

Statistical analyses

To account for batch-to-batch variation, we recalibrated antibodies to have a comparable distribution to an average batch according to methods described by Rosner and colleagues(24). Other than batch number, no other variables were used in the recalibration models. Anti-CA15.3 and anti-CA125 antibody levels were log-transformed (as were CA15.3 and CA125). Correlation between antigens and antibodies was assessed using Spearman partial correlations adjusted for the study matching factors. We used generalized linear models to estimate mean antibody values separately in controls and cases overall and by menopausal status since risk factors, especially reproductive variables, may differ by menopausal status(25). Results were exponentiated to obtain geometric mean values in the original scale. Linear regression models were adjusted for the study matching factors, BMI, and smoking status. Indicators were used to account for missing data for covariates. Trend tests of continuous variables were based on the medians of each category. We included interaction terms in the linear regression models to test

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for heterogeneity in antibody levels by menopausal status. To examine the associations between antibodies and ovarian cancer, we classified antibodies into quartiles based on the control distribution and calculated odds ratios and 95% confidence intervals (CI) using conditional logistic regression. In the conditional logistic regression analyses, we individually added each potential confounder to the model and examined the change in the OR estimate from the crude OR. The covariates examined were age at menarche, OC use, parity, hysterectomy, oophorectomy, duration of HRT use, BMI, and smoking status (using categories described above). The percent changes in the adjusted ORs from the crude ORs were minimal, ranging from 0-3%, therefore we present crude conditional logistic regression results. We evaluated risk associations separately for cases diagnosed within three years (to reflect early detection) and greater than three years (to reflect risk); these lag-time cutpoints were based on prior studies in the EPIC cohort(23) and by others(20, 26). Finally, using median values as cutpoints, we cross classified participants as having high or low antibodies and antigens and examined the association between these biomarker combinations and ovarian cancer. We used the contrast test method to test whether the association between increasing antibodies and risk, and between antigen-antibody combination and risk, varied by histologic type(27). Analyses were conducted using SAS version 9.4 (Cary, NC). Statistical tests were two-tailed and significant at $p<0.05$.

Informed consent and data protection

All participants gave consent for future analyses of their blood samples in their written consent. The principles expressed in the Helsinki Declaration of 1996, conventions of the Council of Europe on Human Rights and Biomedicine, and the UNESCO Declaration on Human Genome have been respected. The present study was approved by the ethics committees at the International Agency for Research on Cancer(IARC) and the University of Heidelberg

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(Germany). Since the identity of subjects providing specimens was anonymous to Brigham and Women's Hospital (BWH) investigators, the research was declared exempt at BWH.

Results

Table 1 compares the characteristics of EOC cases and controls in this nested case-control study. Cases did not differ from controls in age at enrollment or menopausal status, but were more likely to be heavier, nulliparous, and to have never used oral contraceptives (and used oral contraceptives for a shorter duration). Concentrations of anti-CA15.3 antibodies by epidemiologic variables in all, premenopausal, and postmenopausal controls are shown in **Table 2**. Among postmenopausal women, we observed higher anti-CA15.3 antibodies with older age at baseline (P_{trend}=0.04). Overall, anti-CA15.3 antibodies were lower among women with higher BMI (P_{trend}=0.03) and current smoking (P<0.0001). Among current smokers, more pack-years were associated with lower anti-CA15.3 antibodies (P_{trend}=0.002). Among women premenopausal at baseline, ever versus never use of OCs was associated with lower anti-CA15.3 antibodies (P=0.05); and higher number of ovulatory cycles was associated with a trend for lower anti-CA15.3 antibodies (P_{trend}=0.02). Among postmenopausal women, higher anti-CA15.3 antibodies were associated with later age at last birth (P_{trend}=0.02), shorter interval since last birth (P_{trend}=0.04), and longer duration of OC use (P_{trend}=0.01). The difference in the trend in anti-CA15.3 antibody levels by duration and OC use between pre- and postmenopausal women was significant (P for heterogeneity = 0.03).

Similar to associations seen for anti-CA15.3 antibodies, current smoking was associated with lower anti-CA125 antibodies (P<0.0001), and among smokers, anti-CA125 levels declined with increasing pack-years (P_{trend}=0.004) (**Table 3**). Among postmenopausal women, later age at last

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birth (P_{trend}=0.002) and shorter interval since last birth (P_{trend}=0.005) were associated with higher anti-CA125 antibodies. Among premenopausal women, duration of OC use was associated with a lower level of anti-CA125 antibodies when non-users were included in the referent non-exposed category.

Among cases, we observed no associations between tumor characteristics and antibody levels (Supplemental Table 1). When we evaluated all cross-sectional associations adjusting for the corresponding antigen concentration, results were unchanged. Other variables that were examined and found not to affect anti-mucin antibodies in either cases or controls included: miscarriage, hysterectomy or unilateral oophorectomy, family history of breast cancer, IUD use, and duration of HRT use. Information on tubal sterilization was not collected at all EPIC sites and data was too limited to evaluate this exposure.

We then evaluated quartiles of anti-CA15.3 and anti-CA125 antibodies and EOC risk in all subjects and subjects stratified by menopausal status at blood collection, histologic type of EOC, and time between blood collection and diagnosis (<3 years and ≥3 years) (**Table 4**). For mucinous EOC arising three or more years after baseline, trends for increased risk were found with both increasing anti-CA15.3 antibodies (P_{trend}=0.02) and increasing anti-CA125 antibodies (P_{trend}=0.05). Of borderline significance, there was a trend for higher levels of anti-CA15.3 antibodies to be associated with lower risk for serous EOC (P_{trend}=0.06) that developed within 3 years of their blood draw. Levels of anti-CA15.3 in the fourth quartile were associated with an OR (and 95% CI) of 0.51 (0.27, 0.96) compared to women with levels in the first quartile.

Next, we evaluated risk for EOC by anti-CA15.3 and anti-CA125 antibody levels jointly classified by the corresponding antigen level with both dichotomized as high or low based on

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median cutpoints in controls (**Table 5**). Although combining CA15.3 antigen levels with anti-CA15.3 antibody levels was not informative, combining CA125 levels with anti-CA125 antibody yielded several interesting findings. Relative to women with low concentrations of both markers, EOC risk overall was elevated for women with high CA125 levels regardless of anti-CA125 antibody levels, but only for disease arising within 3 years of blood draw. These effects were most apparent in postmenopausal women and those with serous or endometrioid EOC. Conversely, a reduction in risk for all EOC, both in short and long-term risk was associated with low CA125 antigen and high anti-CA125 antibody. For women who developed serous EOC, the combination of low CA125 antigen and high anti-CA125 antibody was associated with an OR (and 95% CI) of 0.60 (0.40, 0.89) for disease that developed more than 3 years after blood draw.

Anti-CA15.3 and anti-CA125 antibodies were strongly correlated in cases (r=0.75, $P<0.0001$) and controls (r=0.75, $P<0.0001$. In all premenopausal EOC cases, there was a weak but significant inverse correlation between CA15.3 and anti-CA15.3 antibodies of -0.15 ($P=0.04$) that was strongest for premenopausal serous cases r=-0.34 ($P=0.001$). In all EOC cases, there was a weak but significant positive correlation between CA125 and anti-CA125 antibodies of 0.10 ($P=0.004$) that was strongest for mucinous tumors r=0.40 ($P=0.01$). Overall, no significant correlations were noted between anti-mucin antibodies and their corresponding antigen in controls (**Table 6**).

Discussion

We examined levels of IgG1 anti-CA15.3 and IgG1 anti-CA125 antibodies in baseline specimens from women who went on to develop EOC and matched controls from the EPIC

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cohort. We sought to identify those epidemiologic factors in control subjects that were associated with anti-mucin antibody levels. Our general premise was that events known to increase (or decrease) EOC risk will be those that decrease (or increase) anti-CA15.3 or anti-CA125 antibody levels. Using both case and control participants, we then sought to determine whether anti-CA15.3 or anti-CA125 antibodies, either by themselves or considered in the context of the corresponding antigen, were associated with EOC risk.

Because of their importance as correlates of EOC risk, the effect of reproductive variables on antibody levels were of principal interest. Decreased EOC risk with increasing parity and duration of OC use are two consistent epidemiologic findings. No clear effect of higher parity on either anti-CA15.3 or anti-CA125 antibody levels was seen, although longer duration of OC use was associated with higher anti-CA15.3 antibody levels (P_{trend}=0.01) in postmenopausal women. There is clear evidence that a later age at last birth (and a shorter interval since last birth) lowers EOC risk (25). Among postmenopausal women, but not premenopausal women, we observed higher levels of both anti-CA15.3 and anti-CA125 antibodies to be associated with a later age at and shorter interval since last birth. These late reproductive events are clearly set for postmenopausal women but may not be for premenopausal controls in EPIC. Both observations suggest that anti-CA125 antibodies may be generated during pregnancy, as has been reported for anti-CA15.3(15) antibodies, and wane with time since pregnancy.

We observed that lower anti-CA15.3 antibodies were associated with a higher number of ovulatory cycles in control subjects who were premenopausal at blood donation. This observation confirms an inverse correlation between anti-CA15.3 antibodies and ovulatory cycles seen in two other independent studies(20, 21) and is again consistent with the proposition that low antibodies predict greater risk since an increasing number of estimated ovulatory cycles

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is associated with increasing EOC risk(28-30). On the other hand, La Vecchia etal(31) expressed doubts about disentangling independent effects of the components of the algorithm used to construct the estimate (i.e. age at menarche, number of pregnancies, duration of OC use or breastfeeding, and current age or age at menopause). Particularly for premenopausal women, current age is an obvious confounder since older women will have a greater number of potential ovulatory years than younger women. However, the association we observed between ovulatory cycles and anti-CA15.3 antibodies seen in the linear regression models included continuous age as an adjustment variable. That the association was confined to premenopausal women is compatible with the observation that ovulatory cycles are a stronger predictor of EOC risk in premenopausal women than in postmenopausal women(30). Indeed, Purdie et al. proposed that more ovulations during ages 20-29 were the strongest predictor of greater EOC risk(29).

The lack of association between ovulatory cycles and anti-CA15.3 antibodies in postmenopausal women in our study and weak correlation of ovulatory cycles with EOC risk in postmenopausal women in published studies could reflect poorer recall of early reproductive events in postmenopausal women. Alternatively, the transition to menopause may take several years during which anovulatory cycles are common; therefore, the assumption that ovulation occurs regularly until menopause would introduce error in calculating ovulatory cycles for postmenopausal women.

Age, BMI, and smoking were non-reproductive variables that influenced antibody levels. Anti-CA15.3 antibodies increased with older age and declined with higher BMI. The latter observation is compatible with the fact that high BMI may increase risk for EOC, as well as other CA15.3-expressing cancers including endometrial cancer and postmenopausal breast cancer(32). Current smoking was associated with both lower anti-CA15.3 and anti-CA125

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antibodies and levels decreased further with increasing pack-years. Smoking does not appear to affect risk for EOC overall but is associated with increased risk of mucinous EOC(33, 34). The observation that smoking lowers anti-CA15.3 antibodies and increases risk for mucinous tumors fits with our hypothesis regarding how risk factors may operate; but not our hypotheses that higher levels of antibodies lowers the risk for EOC in general. This may relate to the uniqueness of mucinous tumors compared to the other subtypes.

Despite finding general support for our premise that anti-CA15.3 and anti-CA125 antibodies may correlate with risk factors for EOC, we found no evidence that, by themselves, they predicted risk for EOC overall; but they may predict risk for specific histologic types of EOC. Thus, we found higher levels of both anti-CA15.3 and anti-CA125 antibodies were associated with higher risk for mucinous tumors developing three or more years after baseline, though our numbers for this rarer subtype were limited, especially cases developing mucinous tumor premenopausally. As mentioned above this finding is contrary to our original hypothesis that anti-mucin antibodies may decrease risk for EOC, but may be explained by the similarities of mucinous ovarian tumors to colorectal cancers (CRC) which have been described(35). This is of interest in that a study looking at the ability of MUC1 auto-antibodies to predict risk for CRC in prospective specimens from United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) found that elevated levels predicted greater risk for CRC with a lead time of at least 2 years(36).

In our study, we found a modest trend (P=0.06) for a greater level of anti-CA15.3 antibodies to be associated with lower risk for serous ovarian cancer developing within 3 years of baseline; but no effect on long-term risk. The ability of anti-CA15.3 antibodies to predict risk for EOC was previously addressed in the Nurses' Health Studies(20). This study excluded cases diagnosed

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within three years to focus on longer term risk. In the NHS, higher levels of anti-CA15.3 antibodies decreased EOC risk in women who were <64 at blood draw but increased risk for women who were ≥ 64 at blood draw. We examined age 64 as a cutpoint for long-term risk in the current study, but did not confirm the NHS finding. In the NHS, antibodies against an unglycosylated tandem repeat were measured while antibodies against intact CA15.3 were measured in this study. The ability of anti-MUC1 antibodies to aid in the early detection of EOC overall was also addressed in the UKCTOCS study. In this study, antibodies were measured against various glycopeptide "pieces" of MUC1 and recombinant (unglycosylated) MUC1 containing the 16 tandem repeats. Antibody reactivity to MUC1 tandem repeat peptides or glycoforms did not differ between controls and EOC cases, nor cases with other MUC1-expressing cancers, including breast, lung, and pancreas(37).

Neither the NHS nor UKCTOCS examined the combined effect of antigen and antibody levels as done in this study. Several new findings emerged in the current study with respect to both short and long-term risk for EOC, primarily related to CA125 antigen and its antibody. High CA125 levels, regardless anti-CA125 antibody level, predicted increased short-term risk for all EOC, EOC arising postmenopausally, and serous EOC. This is not surprising since both EPIC(23) and other prospective studies(38,39) reported elevated CA125 is the strongest predictor of EOC development within a year of the blood draw, but diagnostic discrimination wanes rapidly with lag-times between blood collection and diagnosis of more than 1 to 2 years. Perhaps more important is our novel observation that the combination of low CA125 and high anti-CA125 antibodies lowers risk for all EOC in both the long and short-term and for serous EOC in the long-term. The contribution of anti-CA125 antibody levels to CA125 in early detection is explored more fully in a separate publication(40).

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Limitations of our study are that standards for anti-CA15.3 or anti-CA125 antibody assays have not been established both in terms of antigen epitope to be used and which phenotype, IgG or IgM, to measure. In particular, epitopes used may be based on unique peptide sequences or the whole protein in either glycosylated or unglycosylated forms. It has been postulated that mucin antigens shed by inflammatory or hormonal events may be less glycosylated than unshed mucins and more similar to mucins shed by a tumor, but precise differences are unclear as well as whether anti-mucin antibodies formed in response to tumor mucins differ from those formed from non-neoplastic events. It is also possible that complexes involving the mucin antigens and antibodies can form which could hide the mucin antigen from its detection assay and be relevant to EOC cases presenting with low CA125(41). Finally, results of these analyses should be considered in the context of the number of tests performed and possibility of chance findings, particularly for subgroup analyses in which numbers are limited.

In conclusion, we found some support for our premise that events known to increase (or decrease) EOC risk generally correlate with those that decrease (or increase) anti-CA15.3 or anti-CA125 antibody levels. Although by themselves, anti-mucin antibodies were not strongly associated with overall EOC, higher levels of both anti-CA15.3 and anti-CA125 were associated with greater longer term risk for mucinous EOC. This might reflect the similarities of mucinous tumors to CRC where higher anti-MUC1 antibodies predict greater, not lower, risk. Not surprisingly, high CA125 regardless of anti-CA125 antibodies predicted higher risk for EOC in the short-term especially for women who developed serous EOC. Notably, the combination of low CA125 and high anti-CA125 antibodies is associated with lower long-term risk for serous EOC; i.e. disease developing 3 or more years after baseline. The latter observation may be more useful in defining the role of CA125 in the pathogenesis of serous EOC and less so for

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improving early detection. Advancing this research will require a greater understanding of the biology and immunology of CA125 and CA15.3, the nature of the antibodies that develop against them, and how they interact with traditional risk factors for EOC.

Acknowledgements

We would like to thank Fujirebio Diagnostics for supplying the reagents for the anti-mucin antibody assays.

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1. Burchell J, Gendler S, Taylor-Papadimitriou J, Girling A, Lewis A, Millis R, *et al.* Development and characterization of breast cancer reactive monoclonal antibodies directed to the core protein of the human milk mucin. Cancer research. 1987;47:5476-82.

2. Bast RC, Jr., Klug TL, St John E, Jenison E, Niloff JM, Lazarus H, *et al.* A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med. 1983;309:883-7.

3. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J, *et al.* Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J Biol Chem. 1990;265:15286-93.

4. Yin BW, Lloyd KO. Molecular cloning of the CA125 ovarian cancer antigen: identification as a new mucin, MUC16. J Biol Chem. 2001;276:27371-5.

5. Ho SB, Niehans GA, Lyftogt C, Yan PS, Cherwitz DL, Gum ET, *et al.* Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer research. 1993;53:641-51.

6. Kufe DW. Mucins in cancer: function, prognosis and therapy. Nature reviews Cancer. 2009;9:874-85.

7. Van Elssen CH, Frings PW, Bot FJ, Van de Vijver KK, Huls MB, Meek B, *et al.* Expression of aberrantly glycosylated Mucin-1 in ovarian cancer. Histopathology. 2010;57:597-606.

8. Hogdall EV, Christensen L, Kjaer SK, Blaakaer J, Kjaerbye-Thygesen A, Gayther S, *et al.* CA125 expression pattern, prognosis and correlation with serum CA125 in ovarian tumor patients. From The Danish "MALOVA" Ovarian Cancer Study. Gynecologic oncology. 2007;104:508-15.

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Author Manuscript Published OnlineFirst on April 16, 2018; DOI: 10.1158/1055-9965.EPI-17-0744
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

9. Berruti A, Tampellini M, Torta M, Buniva T, Gorzegno G, Dogliotti L. Prognostic value in predicting overall survival of two mucinous markers: CA 15-3 and CA 125 in breast cancer patients at first relapse of disease. Eur J Cancer. 1994;30A:2082-4.

10. Nakamori S, Ota DM, Cleary KR, Shirotani K, Irimura T. MUC1 mucin expression as a marker of progression and metastasis of human colorectal carcinoma. Gastroenterology. 1994;106:353-61.

11. Agrawal B, Krantz MJ, Reddish MA, Longenecker BM. Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. Nat Med. 1998;4:43-9.

12. Cooper BC, Sood AK, Davis CS, Ritchie JM, Sorosky JI, Anderson B, *et al.* Preoperative CA 125 levels: an independent prognostic factor for epithelial ovarian cancer. Obstetrics and gynecology. 2002;100:59-64.

13. Gubbels JA, Felder M, Horibata S, Belisle JA, Kapur A, Holden H, *et al.* MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells. Mol Cancer. 2010;9:11.

14. von Mensdorff-Pouilly S, Verstraeten AA, Kenemans P, Snijdewint FG, Kok A, Van Kamp GJ, *et al.* Survival in early breast cancer patients is favorably influenced by a natural humoral immune response to polymorphic epithelial mucin. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2000;18:574-83.

15. Richards ER, Devine PL, Quin RJ, Fontenot JD, Ward BG, McGuckin MA. Antibodies reactive with the protein core of MUC1 mucin are present in ovarian cancer patients and healthy women. Cancer Immunol Immunother. 1998;46:245-52.

16. Agrawal B, Reddish MA, Krantz MJ, Longenecker BM. Does pregnancy immunize against breast cancer? Cancer research. 1995;55:2257-61.

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Author Manuscript Published OnlineFirst on April 16, 2018; DOI: 10.1158/1055-9965.EPI-17-0744
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

17. Cramer DW, Titus-Ernstoff L, McKolanis JR, Welch WR, Vitonis AF, Berkowitz RS, *et al.* Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2005;14:1125-31.

18. Cramer DW, Vitonis AF, Pinheiro SP, McKolanis JR, Fichorova RN, Brown KE, *et al.* Mumps and ovarian cancer: modern interpretation of an historic association. Cancer Causes Control. 2010;21:1193-201.

19. Cramer DW, Williams K, Vitonis AF, Yamamoto HS, Stuebe A, Welch WR, *et al.* Puerperal mastitis: a reproductive event of importance affecting anti-mucin antibody levels and ovarian cancer risk. Cancer Causes Control. 2013;24:1911-23.

20. Pinheiro SP, Hankinson SE, Tworoger SS, Rosner BA, McKolanis JR, Finn OJ, *et al.* Anti-MUC1 antibodies and ovarian cancer risk: prospective data from the Nurses' Health Studies. Cancer Epidemiol Biomarkers Prev. 2010;19:1595-601.

21. Terry KL, Titus-Ernstoff L, McKolanis JR, Welch WR, Finn OJ, Cramer DW. Incessant ovulation, mucin 1 immunity, and risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2007;16:30-5.

22. Riboli E. Nutrition and cancer: background and rationale of the European Prospective Investigation into Cancer and Nutrition (EPIC). Annals of oncology : official journal of the European Society for Medical Oncology. 1992;3:783-91.

23. Terry KL, Schock H, Fortner RT, Husing A, Fichorova RN, Yamamoto HS, *et al.* A Prospective Evaluation of Early Detection Biomarkers for Ovarian Cancer in the European EPIC Cohort. Clinical cancer research : an official journal of the American Association for Cancer Research. 2016;22:4664-75.

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24. Rosner B, Cook N, Portman R, Daniels S, Falkner B. Determination of blood pressure percentiles in normal-weight children: some methodological issues. Am J Epidemiol. 2008;167:653-66.

25. Moorman PG, Calingaert B, Palmieri RT, Iversen ES, Bentley RC, Halabi S, *et al.* Hormonal risk factors for ovarian cancer in premenopausal and postmenopausal women. Am J Epidemiol. 2008;167:1059-69.

26. Anderson GL, McIntosh M, Wu L, Barnett M, Goodman G, Thorpe JD, *et al.* Assessing lead time of selected ovarian cancer biomarkers: a nested case-control study. Journal of the National Cancer Institute. 2010;102:26-38.

27. Wang M, Spiegelman D, Kuchiba A, Lochhead P, Kim S, Chan AT, *et al.* Statistical methods for studying disease subtype heterogeneity. Stat Med. 2016;35:782-800.

28. Casagrande JT, Louie EW, Pike MC, Roy S, Ross RK, Henderson BE. "Incessant ovulation" and ovarian cancer. Lancet (London, England). 1979;2:170-3.

29. Purdie DM, Bain CJ, Siskind V, Webb PM, Green AC. Ovulation and risk of epithelial ovarian cancer. Int J Cancer. 2003;104:228-32.

30. Tung KH, Wilkens LR, Wu AH, McDuffie K, Nomura AM, Kolonel LN, *et al.* Effect of anovulation factors on pre- and postmenopausal ovarian cancer risk: revisiting the incessant ovulation hypothesis. Am J Epidemiol. 2005;161:321-9.

31. La Vecchia C, Franceschi S, Gallus G, Decarli A, Liberati A, Tognoni G. Incessant ovulation and ovarian cancer: a critical approach. Int J Epidemiol. 1983;12:161-4.

32. Reeves GK, Pirie K, Beral V, Green J, Spencer E, Bull D, *et al.* Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. BMJ. 2007;335:1134.

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Author Manuscript Published OnlineFirst on April 16, 2018; DOI: 10.1158/1055-9965.EPI-17-0744
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

33. Beral V, Gaitskell K, Hermon C, Moser K, Reeves G, Peto R. Ovarian cancer and smoking: individual participant meta-analysis including 28,114 women with ovarian cancer from 51 epidemiological studies. The Lancet Oncology. 2012;13:946-56.

34. Gram IT, Lukanova A, Brill I, Braaten T, Lund E, Lundin E, *et al.* Cigarette smoking and risk of histological subtypes of epithelial ovarian cancer in the EPIC cohort study. Int J Cancer. 2012;130:2204-10.

35. Marquez RT, Baggerly KA, Patterson AP, Liu J, Broaddus R, Frumovitz M, *et al.* Patterns of gene expression in different histotypes of epithelial ovarian cancer correlate with those in normal fallopian tube, endometrium, and colon. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005;11:6116-26.

36. Pedersen JW, Gentry-Maharaj A, Nostdal A, Fourkala EO, Dawnay A, Burnell M, *et al.* Cancer-associated autoantibodies to MUC1 and MUC4--a blinded case-control study of colorectal cancer in UK collaborative trial of ovarian cancer screening. Int J Cancer. 2014;134:2180-88.

37. Burford B, Gentry-Maharaj A, Graham R, Allen D, Pedersen JW, Nudelman AS, *et al.* Autoantibodies to MUC1 glycopeptides cannot be used as a screening assay for early detection of breast, ovarian, lung or pancreatic cancer. Br J Cancer. 2013;108:2045-55.

38. Cramer DW, Bast RC, Jr., Berg CD, Diamandis EP, Godwin AK, Hartge P, *et al.* Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prev Res (Phila). 2011;4:365-74.

39. Jacobs IJ, Menon U, Ryan A, Gentry-Maharaj A, Burnell M, Kalsi JK, *et al.* Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet (London, England). 2016;387:945-56.

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Author Manuscript Published OnlineFirst on April 16, 2018; DOI: 10.1158/1055-9965.EPI-17-0744
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

40. Fortner RT, Schock H, LeCornet C, Hüsing A, Vitonis AF, Johnson T, *et al.* Ovarian cancer early detection by CA125, in the context of anti-CA125 antibody concentrations: Results from the EPIC cohort. International Journal of Cancer (IJC) 2017 (in press).

41. Cramer DW, O'Rourke DJ, Vitonis AF, Matulonis UA, Dijohnson DA, Sluss PM, *et al.* CA125 immune complexes in ovarian cancer patients with low CA125 concentrations. Clin Chem. 2010;56:1889-92.

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Table 1. Characteristics of cases and controls in the EPIC ovarian nested case-control study.

	Controls N=1927	Cases N=806
Age at blood draw (years)		
Mean (SD)	56.2 (8.3)	56.1 (8.1)
Menopausal status, N (%)		
Premenopausal	485 (25.5%)	201 (25.4%)
Postmenopausal	1416 (74.5%)	589 (74.6%)
BMI		
Mean (SD)	25.8 (4.5)	26.1 (4.8)
Smoking status, N (%)		
Never	1096 (57.9%)	429 (54.3%)
Former	431 (22.8%)	185 (23.4%)
Current	367 (19.4%)	176 (22.3%)
Oral contraceptive use, N (%)		
Never	924 (49.8%)	431 (55.7%)
Ever	933 (50.2%)	343 (44.3%)
Duration of oral contraceptive use		
Mean (SD)	8.5 (8.1)	7.0 (7.0)
Parity, N (%)		
Nulliparous	202 (11.3%)	129 (17.3%)
Parous	1579 (88.7%)	616 (82.7%)
Number of births among parous women		
Mean (SD)	2.4 (1.1)	2.3 (1.1)
Ovulatory cycles		
Mean (SD)	406.1 (64.4)	412.2 (62.2)

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Table 2. Associations between epidemiologic characteristics and anti-CA15.3 antibodies in healthy controls, stratified by menopausal status.

	All			Premenopausal			Postmenopausal			p-het
	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	
Age at blood draw (years)										
< 41	75 (4)	8253 (5569, 12232)	0.97	75 (15)	7802 (5467, 11134)	0.78	0 (0)	--		
41-50	327 (17)	8188 (6747, 9936)	Ref.	269 (55)	7372 (6183, 8789)	Ref.	47 (3)	9571 (6066, 15100)	Ref.	
51-60	853 (44)	7464 (6714, 8298)	0.43	141 (29)	6751 (5138, 8870)	0.62	697 (49)	7664 (6801, 8638)	0.35	
61-70	585 (30)	9257 (8118, 10555)	0.34	0 (0)	--	--	585 (41)	9543 (8384, 10862)	0.99	
> 70	87 (5)	10135 (7181, 14302)	0.30	0 (0)	--	--	87 (6)	10758 (7476, 15481)	0.70	
p_{trend}			0.07			0.55			0.04	
BMI (kg/m^2)										
<18.5	30 (2)	11362 (6597, 19571)	0.35	9 (2)	7718 (3064, 19441)	0.93	21 (2)	12090 (6193, 23605)	0.39	0.77
18.5-24.99	872 (48)	8715 (7865, 9656)	Ref.	267 (59)	8063 (6822, 9529)	Ref.	589 (44)	8946 (7867, 10174)	Ref.	Ref.
25 - 29.99	658 (36)	8092 (7204, 9091)	0.36	131 (29)	6513 (5125, 8277)	0.16	522 (39)	8658 (7570, 9902)	0.73	0.18
>=30	271 (15)	7065 (5858, 8521)	0.06	46 (10)	6165 (4101, 9267)	0.24	222 (16)	7425 (5983, 9214)	0.16	0.60
p_{trend}			0.03			0.15			0.12	0.41
Smoking										
Never	1096 (58)	9012 (8232, 9865)	Ref.	273 (57)	7830 (6632, 9245)	Ref.	816 (59)	9439 (8470, 10520)	Ref.	Ref.
Former	431 (23)	8855 (7664, 10230)	0.84	114 (24)	7872 (6051, 10242)	0.97	310 (22)	9181 (7701, 10945)	0.8	0.88
Current	367 (19)	5782 (4942, 6764)	<0.0001	94 (20)	5168 (3869, 6902)	0.02	266 (19)	6123 (5058, 7413)	0.0002	0.98
Packyears (among current smokers)										
<=11 packyears	89 (25)	9069 (6836, 12031)	Ref.	34 (37)	7737 (5203, 11505)	Ref.	53 (21)	10459 (7140, 15321)	Ref.	Ref.
12-19	91 (25)	5443 (4114, 7202)	0.01	21 (23)	4183 (2502, 6991)	0.08	67 (26)	5695 (4008, 8091)	0.03	0.88
20-31	95 (27)	4216 (3231, 5502)	0.0002	26 (28)	4604 (2914, 7275)	0.11	68 (26)	4370 (3146, 6070)	0.0009	0.63
>31	82 (23)	4716 (3505, 6345)	0.002	12 (13)	3070 (1582, 5956)	0.03	69 (27)	4998 (3569, 6998)	0.005	0.82
p_{trend}			0.002			0.02			0.008	0.87
Age at menarche (years)										
< 12	266 (14)	7531 (6243, 9084)	0.77	93 (20)	5632 (4196, 7561)	0.8	171 (13)	8528 (6708, 10842)	0.56	0.76
12	352 (19)	7260 (6191, 8513)	Ref.	94 (20)	5940 (4440, 7948)	Ref.	257 (19)	7789 (6432, 9434)	Ref.	Ref.
13	422 (23)	8395 (7263, 9703)	0.19	118 (26)	9347 (7227, 12089)	0.02	301 (22)	8214 (6886, 9797)	0.69	0.15
14	433 (24)	8550 (7405, 9873)	0.14	94 (20)	7934 (5918, 10638)	0.18	336 (25)	8836 (7474, 10445)	0.33	0.58
> 14	365 (20)	8883 (7584, 10405)	0.08	60 (13)	6940 (4820, 9994)	0.52	299 (22)	9426 (7879, 11277)	0.16	0.63
p_{trend}			0.07			0.13			0.23	0.82

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Table 2. Associations between epidemiologic characteristics and anti-CA15.3 antibodies in healthy controls, stratified by menopausal status. (continued)

	All			Premenopausal			Postmenopausal			p-het
	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	
Oral contraceptive use										
Never	924 (50)	7947 (7156, 8826)	Ref.	145 (31)	8831 (6880, 11334)	Ref.	775 (56)	8040 (7159, 9029)	Ref.	Ref.
Ever	933 (50)	8408 (7575, 9332)	0.48	320 (69)	6434 (5487, 7546)	0.05	604 (44)	9371 (8199, 10711)	0.11	0.08
<= 2 years	257 (14)	8002 (6630, 9658)	Ref.	101 (22)	7675 (5743, 10256)	Ref.	153 (12)	8034 (6270, 10294)	Ref.	Ref.
> 2 to 5 years	180 (10)	6530 (5221, 8165)	0.15	68 (15)	5093 (3603, 7200)	0.11	111 (8)	6950 (5189, 9309)	0.39	0.37
> 5 to 10 years	207 (12)	9107 (7370, 11252)	0.43	77 (17)	6278 (4527, 8705)	0.26	127 (10)	10867 (8241, 14329)	0.13	0.09
> 10 years	225 (13)	9451 (7668, 11650)	0.32	62 (14)	5971 (3993, 8929)	0.46	161 (12)	11152 (8683, 14324)	0.14	0.04
p_{trend} †			0.09			0.24			0.01	0.06
p_{trend} §			0.11			0.78			0.06	0.03
Parity										
Nulliparous	202 (11)	7725 (6268, 9521)	Ref.	48 (11)	5577 (3689, 8432)	Ref.	152 (12)	8511 (6642, 10905)	Ref.	Ref.
Parous	1579 (89)	8339 (7742, 8982)	0.5	406 (89)	7366 (6425, 8445)	0.22	1162 (88)	8781 (8032, 9598)	0.82	0.36
p_{diff}										
1 child	277 (16)	8308 (6938, 9948)	Ref.	74 (17)	6452 (4630, 8993)	Ref.	200 (15)	9197 (7396, 11438)	Ref.	Ref.
2 children	730 (42)	7960 (7128, 8891)	0.69	215 (50)	8085 (6666, 9805)	0.33	512 (40)	7959 (6948, 9118)	0.29	0.13
3 children	348 (20)	9728 (8297, 11406)	0.19	75 (17)	7237 (5212, 10049)	0.77	270 (21)	10523 (8741, 12669)	0.34	0.98
4+ children	180 (10)	8346 (6636, 10496)	0.92	22 (5)	9049 (4872, 16805)	0.36	157 (12)	8508 (6612, 10949)	0.66	0.27
p_{trend} †			0.22			0.13			0.52	0.65
p_{trend} §			0.31			0.55			0.52	0.31
Age at first birth (years)										
<20	120 (8)	8234 (6247, 10852)	Ref.	28 (7)	7934 (4573, 13765)	Ref.	90 (8)	8148 (5870, 11311)	Ref.	Ref.
20-24	683 (43)	7931 (7075, 8890)	0.81	179 (44)	7221 (5842, 8925)	0.76	500 (43)	8281 (7222, 9496)	0.93	0.67
25-29	543 (34)	8784 (7726, 9987)	0.68	140 (34)	8352 (6559, 10636)	0.87	399 (34)	8958 (7679, 10450)	0.61	0.85
≥30	228 (14)	8460 (6934, 10321)	0.88	59 (15)	5274 (3605, 7717)	0.23	168 (15)	10080 (7951, 12779)	0.31	0.11
p_{trend}			0.48			0.3			0.15	0.11
Age at last birth (years)										
<25	217 (14)	7313 (5962, 8971)	Ref.	64 (17)	7442 (5181, 10688)	Ref.	150 (13)	7319 (5693, 9409)	Ref.	Ref.
25-29	532 (35)	8166 (7173, 9297)	0.37	137 (35)	8639 (6762, 11038)	0.51	394 (35)	7906 (6770, 9233)	0.61	0.63
30-34	508 (33)	8552 (7492, 9763)	0.21	133 (34)	5933 (4646, 7577)	0.31	369 (33)	9820 (8372, 11518)	0.05	0.07
≥35	273 (18)	9682 (8058, 11633)	0.05	52 (13)	9787 (6576, 14566)	0.32	221 (19)	9844 (7982, 12140)	0.08	0.87
p_{trend}			0.05			0.86			0.02	0.13

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Table 2. Associations between epidemiologic characteristics and anti-CA15.3 antibodies in healthy controls, stratified by menopausal status. (continued)

	All			Premenopausal			Postmenopausal			p-het
	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	
Years since last birth										
<21	404 (26)	8859 (7284, 10776)	Ref.	266 (69)	7034 (5863, 8439)	Ref.	133 (12)	10008 (7451, 13440)	Ref.	Ref.
21-27	395 (26)	9545 (8194, 11118)	0.54	95 (25)	8395 (6131, 11496)	0.37	297 (26)	10571 (8766, 12747)	0.74	0.62
28-32	349 (23)	8407 (7130, 9912)	0.71	22 (6)	12239 (6458, 23194)	0.11	325 (29)	8632 (7286, 10225)	0.39	0.11
>32	382 (25)	7004 (5759, 8518)	0.16	3 (1)	2878 (523, 15829)	0.31	379 (33)	7318 (6074, 8817)	0.11	0.26
p_{trend}			0.15			0.27			0.04	0.14
Ovulatory cycles¶										
<= 368	380 (25)	8477 (7254, 9905)	Ref.	143 (34)	9521 (6598, 13739)	Ref.	236 (22)	8860 (7260, 10811)	Ref.	Ref.
369-414	385 (25)	8147 (7006, 9474)	0.72	120 (29)	9585 (7328, 12538)	0.98	262 (24)	7540 (6251, 9095)	0.25	0.04
415-450	363 (24)	8097 (6929, 9460)	0.69	80 (19)	4569 (3161, 6605)	0.02	279 (25)	9236 (7701, 11077)	0.76	0.28
> 450	395 (26)	7839 (6740, 9117)	0.49	73 (18)	4820 (3018, 7696)	0.07	318 (29)	8468 (7138, 10046)	0.74	0.94
p_{trend}			0.50			0.02			0.85	0.72
Age at menopause (years)										
≤ 46	--	--	--	--	--	--	303 (27)	7982 (6676, 9542)	Ref.	
47 - 49	--	--	--	--	--	--	236 (21)	9052 (7417, 11048)	0.36	
50 - 51	--	--	--	--	--	--	235 (21)	9521 (7794, 11631)	0.2	
52 - 53	--	--	--	--	--	--	182 (16)	7948 (6334, 9972)	0.98	
> 53	--	--	--	--	--	--	170 (15)	9450 (7441, 12000)	0.27	
p_{trend}									0.37	
Type of HRT										
Never used HRT	--	--	--	--	--	--	778 (75)	7931 (6792, 9260)	Ref.	
Estrogen alone	--	--	--	--	--	--	81 (8)	13562 (8121, 22648)	0.18	
Estrogen + Progestin	--	--	--	--	--	--	177 (17)	9304 (6256, 13836)	0.82	

*Geometric means adjusted for matching factors, including study center (grouped by country), age at blood draw, fasting status, date and time of blood draw, menopausal status at blood draw, menstrual cycle phase for premenopausal women at blood draw, OC/HRT use at blood draw, length of follow up. HRT use adjusted for hysterectomy. Age at menarche, OC use, parity, ovulatory cycles, hysterectomy, oophorectomy, menopausal status, age at menopause, and HRT use additionally adjusted for BMI and smoking status.

† Trend including nulliparous women and those who never used oral contraceptives.

§ Trend among parous women and OC users.

¶Time between menarche and menopause with time subtracted for oral contraceptive use, pregnancy and breastfeeding; categories based on quartile cutpoints

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Table 3. Associations between epidemiologic characteristics and anti-CA125 antibodies in healthy controls, stratified by menopausal status.

	All			Premenopausal			Postmenopausal			p-het
	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	
Age at blood draw (years)										
< 41	75 (4)	5428 (4032, 7307)	0.04	75 (15)	5494 (4136, 7298)	0.03	0 (0)	--	--	
41-50	327 (17)	3971 (3431, 4596)	Ref.	269 (55)	3827 (3326, 4404)	Ref.	47 (3)	4260 (3032, 5985)	Ref.	
51-60	853 (44)	4039 (3728, 4376)	0.85	141 (29)	3852 (3098, 4790)	0.96	697 (49)	4091 (3742, 4472)	0.82	
61-70	585 (30)	4382 (3968, 4839)	0.31	0 (0)	--	--	585 (41)	4436 (4028, 4885)	0.82	
> 70	87 (5)	3812 (2939, 4946)	0.79	0 (0)	--	--	87 (6)	3843 (2929, 5041)	0.64	
p_{trend}			0.95			0.09			0.68	
BMI (kg/m^2)										
<18.5	30 (2)	4292 (2848, 6469)	0.92	9 (2)	4366 (2088, 9129)	0.87	21 (2)	3989 (2423, 6568)	0.79	0.56
18.5-24.99	872 (48)	4199 (3887, 4537)	Ref.	267 (59)	4104 (3592, 4690)	Ref.	589 (44)	4273 (3883, 4703)	Ref.	Ref.
25 - 29.99	658 (36)	4131 (3784, 4510)	0.79	131 (29)	4115 (3398, 4983)	0.98	522 (39)	4158 (3762, 4596)	0.70	0.82
>=30	271 (15)	4087 (3548, 4708)	0.75	46 (10)	3438 (2483, 4761)	0.33	222 (16)	4246 (3615, 4987)	0.95	0.32
p_{trend}			0.71			0.4			0.95	0.27
Smoking										
Never	1096 (58)	4456 (4161, 4773)	Ref.	273 (57)	4301 (3764, 4914)	Ref.	816 (59)	4512 (4160, 4892)	Ref.	Ref.
Former	431 (23)	4188 (3754, 4673)	0.35	114 (24)	4184 (3387, 5169)	0.83	310 (22)	4191 (3675, 4779)	0.36	0.64
Current	367 (19)	3348 (2973, 3772)	<0.0001	94 (20)	3271 (2593, 4127)	0.05	266 (19)	3433 (2976, 3961)	0.001	0.99
Packyears (among current smokers)										
<=11 packyears	89 (25)	4703 (3778, 5854)	Ref.	34 (37)	3775 (2653, 5373)	Ref.	53 (21)	5060 (3777, 6778)	Ref.	Ref.
12-19	91 (25)	3150 (2536, 3913)	0.01	21 (23)	3228 (2044, 5097)	0.62	67 (26)	3194 (2441, 4180)	0.03	0.68
20-31	95 (27)	2635 (2144, 3239)	0.0002	26 (28)	2778 (1849, 4172)	0.29	68 (26)	2834 (2203, 3644)	0.004	0.93
>31	82 (23)	2948 (2342, 3710)	0.005	12 (13)	3104 (1722, 5596)	0.59	69 (27)	2856 (2207, 3695)	0.004	0.40
p_{trend}			0.004			0.40			0.008	0.92
Age at menarche (years)										
< 12	266 (14)	4075 (3534, 4700)	0.81	93 (20)	3889 (3071, 4924)	0.74	171 (13)	4129 (3450, 4941)	0.98	0.80
12	352 (19)	3982 (3527, 4494)	Ref.	94 (20)	3677 (2911, 4644)	Ref.	257 (19)	4115 (3565, 4749)	Ref.	Ref.
13	422 (23)	4234 (3792, 4727)	0.46	118 (26)	4737 (3854, 5823)	0.12	301 (22)	4082 (3577, 4657)	0.94	0.16
14	433 (24)	4042 (3623, 4509)	0.86	94 (20)	4198 (3319, 5311)	0.44	336 (25)	4027 (3553, 4564)	0.82	0.43
> 14	365 (20)	4362 (3868, 4920)	0.30	60 (13)	3107 (2319, 4162)	0.38	299 (22)	4696 (4107, 5370)	0.19	0.15
p_{trend}			0.47			0.64			0.28	0.35

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Table 3. Associations between epidemiologic characteristics and anti-CA125 antibodies in healthy controls, stratified by menopausal status. (continued)

	All			Premenopausal			Postmenopausal			p-het
	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	
Oral contraceptive use										
Never	924 (50)	4110 (3796, 4450)	Ref.	145 (31)	4657 (3814, 5686)	Ref.	775 (56)	4054 (3717, 4422)	Ref.	Ref.
Ever	933 (50)	4167 (3850, 4510)	0.82	320 (69)	3688 (3247, 4189)	0.07	604 (44)	4404 (3985, 4867)	0.25	0.11
>= 2 years	257 (14)	4402 (3813, 5082)	Ref.	101 (22)	4565 (3624, 5750)	Ref.	153 (12)	4358 (3614, 5255)	Ref.	Ref.
> 2 to 5 years	180 (10)	3534 (2979, 4193)	0.03	68 (15)	3324 (2524, 4379)	0.10	111 (8)	3580 (2871, 4463)	0.16	0.61
> 5 to 10 years	207 (12)	4059 (3453, 4771)	0.38	77 (17)	3276 (2525, 4249)	0.07	127 (10)	4487 (3642, 5529)	0.82	0.12
> 10 years	225 (13)	4360 (3716, 5115)	0.72	62 (14)	3073 (2231, 4233)	0.09	161 (12)	4903 (4059, 5923)	0.58	0.04
p_{trend} †			0.57			0.04			0.07	0.13
p_{trend} §			0.65			0.19			0.24	0.01
Parity										
Nulliparous	202 (11)	4376 (3740, 5120)	Ref.	48 (11)	3731 (2688, 5181)	Ref.	152 (12)	4582 (3815, 5503)	Ref.	Ref.
Parous	1579 (89)	4124 (3900, 4361)	0.49	406 (89)	3985 (3575, 4441)	0.71	1162 (88)	4184 (3918, 4469)	0.36	0.50
p_{diff}										
1 child	277 (16)	3996 (3487, 4578)	Ref.	74 (17)	3535 (2710, 4612)	Ref.	200 (15)	4238 (3606, 4982)	Ref.	Ref.
2 children	730 (42)	3994 (3674, 4341)	0.98	215 (50)	4351 (3728, 5079)	0.24	512 (40)	3852 (3483, 4261)	0.36	0.11
3 children	348 (20)	4565 (4048, 5148)	0.14	75 (17)	4033 (3101, 5245)	0.59	270 (21)	4718 (4111, 5414)	0.29	0.90
4+ children	180 (10)	4306 (3621, 5120)	0.49	22 (5)	3646 (2221, 5986)	0.94	157 (12)	4421 (3667, 5331)	0.74	0.90
p_{trend} †			0.53			0.54			0.72	0.73
p_{trend} §			0.15			0.84			0.20	0.82
Age at first birth (years)										
<20	120 (8)	3681 (2993, 4528)	Ref.	28 (7)	3624 (2334, 5628)	Ref.	90 (8)	3710 (2912, 4727)	Ref.	Ref.
20-24	683 (43)	3978 (3651, 4335)	0.63	179 (44)	3886 (3281, 4603)	0.77	500 (43)	4082 (3689, 4516)	0.47	0.70
25-29	543 (34)	4277 (3884, 4711)	0.35	140 (34)	4280 (3529, 5191)	0.50	399 (34)	4184 (3734, 4688)	0.38	0.99
≥30	228 (14)	4446 (3831, 5160)	0.23	59 (15)	3674 (2711, 4979)	0.96	168 (15)	4755 (3990, 5666)	0.11	0.38
p_{trend}			0.14			0.90			0.09	0.51
Age at last birth (years)										
<25	217 (14)	3567 (3056, 4164)	Ref.	64 (17)	4131 (3082, 5536)	Ref.	150 (13)	3419 (2838, 4118)	Ref.	Ref.
25-29	532 (35)	4022 (3647, 4437)	0.21	137 (35)	4330 (3552, 5279)	0.80	394 (35)	3919 (3494, 4397)	0.22	0.79
30-34	508 (33)	4245 (3841, 4692)	0.09	133 (34)	3571 (2931, 4352)	0.42	369 (33)	4465 (3967, 5025)	0.02	0.06
≥35	273 (18)	4772 (4152, 5483)	0.01	52 (13)	4561 (3306, 6290)	0.66	221 (19)	4833 (4137, 5645)	0.006	0.29
p_{trend}			0.01			0.78			0.002	

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Table 3. Associations between epidemiologic characteristics and anti-CA125 antibodies in healthy controls, stratified by menopausal status. (continued)

	All			Premenopausal			Postmenopausal			p-het
	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	N (%)	Mean (95% CI)*	p	
Years since last birth										
<21	404 (26)	4669 (4026, 5415)	Ref.	266 (69)	3896 (3364, 4513)	Ref.	133 (12)	5120 (4114, 6371)	Ref.	Ref.
21-27	395 (26)	4455 (3969, 5001)	0.71	95 (25)	4299 (3336, 5541)	0.53	297 (26)	4710 (4100, 5411)	0.50	0.41
28-32	349 (23)	4129 (3645, 4677)	0.31	22 (6)	5220 (3116, 8746)	0.30	325 (29)	4214 (3717, 4778)	0.13	0.12
>32	382 (25)	3422 (2951, 3969)	0.02	3 (1)	2797 (706, 11074)	0.64	379 (33)	3529 (3074, 4052)	0.01	0.58
p_{trend}			0.03			0.42			0.005	0.10
Ovulatory cycles¶										
<= 368	380 (25)	4139 (3675, 4660)	Ref.	143 (34)	3661 (2722, 4924)	Ref.	236 (22)	4185 (3606, 4856)	Ref.	Ref.
369-414	385 (25)	3994 (3560, 4481)	0.67	120 (29)	4284 (3448, 5322)	0.42	262 (24)	3844 (3342, 4423)	0.42	0.34
415-450	363 (24)	4083 (3626, 4598)	0.88	80 (19)	3522 (2615, 4744)	0.88	279 (25)	4356 (3803, 4990)	0.70	0.28
> 450	395 (26)	4374 (3898, 4908)	0.52	73 (18)	5010 (3432, 7313)	0.30	318 (29)	4395 (3868, 4994)	0.63	0.62
p_{trend}			0.48			0.44			0.36	0.91
Age at menopause (years)										
≤ 46	--	--	--	--	--	--	303 (27)	3993 (3495, 4562)	Ref.	
47 - 49	--	--	--	--	--	--	236 (21)	3792 (3268, 4400)	0.61	
50 - 51	--	--	--	--	--	--	235 (21)	4576 (3941, 5313)	0.19	
52 - 53	--	--	--	--	--	--	182 (16)	4060 (3428, 4808)	0.88	
> 53	--	--	--	--	--	--	170 (15)	5009 (4192, 5986)	0.05	
p_{trend}									0.08	
Type of HRT										
Never used HRT	--	--	--	--	--	--	778 (75)	4065 (3620, 4564)	Ref.	
Estrogen alone	--	--	--	--	--	--	81 (8)	4992 (3402, 7325)	0.53	
Estrogen + Progestin	--	--	--	--	--	--	177 (17)	4192 (3115, 5641)	0.88	

†Geometric means adjusted for matching factors, including study center (grouped by country), age at blood draw, fasting status, date and time of blood draw, menopausal status at blood, menstrual cycle phase for premenopausal women at blood, OC/HRT use at blood, length of follow up. HRT use adjusted for hysterectomy. Age at menarche, OC use, parity, ovulatory cycles, hysterectomy, oophorectomy, menopausal status, age at menopause, and HRT use additionally adjusted for BMI and smoking status.

† Trend including nulliparous women and those who never used oral contraceptives.

§ Trend among parous women and OC users.

¶Time between menarche and menopause with time subtracted for oral contraceptive use, pregnancy and breastfeeding; categories based on quartile cutpoints

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Table 4. Associations between anti-CA15.3 and anti-CA125 antibodies and risk of ovarian cancer.

	All				<3 years between blood and diagnosis				≥3 years between blood and diagnosis			
	Controls N (%)	Cases N (%)	OR (95% CI)	p	Controls N (%)	Cases N (%)	OR (95% CI)	p	Controls N (%)	Cases N (%)	OR (95% CI)	p
Anti-CA15.3 antibodies												
All EOC												
Q1	481 (24.9)	218 (27.0)	1.00 (referent)		170 (23.8)	56 (28.9)	1.00 (referent)		311 (25.6)	162 (26.5)	1.00 (referent)	
Q2	482 (25.0)	200 (24.8)	0.90 (0.71, 1.14)	0.39	172 (24.1)	40 (20.6)	0.72 (0.45, 1.14)	0.16	310 (25.6)	160 (26.1)	0.98 (0.74, 1.29)	0.88
Q3	484 (25.1)	175 (21.7)	0.80 (0.63, 1.01)	0.06	168 (23.5)	45 (23.2)	0.83 (0.53, 1.30)	0.42	316 (26.1)	130 (21.2)	0.78 (0.59, 1.04)	0.09
Q4	481 (24.9)	213 (26.4)	1.01 (0.80, 1.27)	0.93	205 (28.7)	53 (27.3)	0.81 (0.52, 1.26)	0.34	276 (22.8)	160 (26.1)	1.11 (0.84, 1.45)	0.47
p-trend				0.62				0.65				0.20
All EOC, premenopausal												
Q1	129 (26.9)	51 (25.4)	1.00 (referent)		50 (29.2)	12 (26.1)	1.00 (referent)		79 (25.6)	39 (25.2)	1.00 (referent)	
Q2	119 (24.8)	54 (26.9)	1.12 (0.71, 1.78)	0.63	40 (23.4)	9 (19.6)	0.97 (0.38, 2.48)	0.95	79 (25.6)	45 (29.0)	1.16 (0.68, 1.99)	0.58
Q3	118 (24.6)	47 (23.4)	1.00 (0.62, 1.59)	0.98	40 (23.4)	9 (19.6)	1.04 (0.40, 2.68)	0.93	78 (25.2)	38 (24.5)	0.98 (0.57, 1.69)	0.93
Q4	114 (23.8)	49 (24.4)	1.12 (0.70, 1.81)	0.63	41 (24.0)	16 (34.8)	1.92 (0.79, 4.66)	0.15	73 (23.6)	33 (21.3)	0.91 (0.52, 1.59)	0.74
p-trend				0.88				0.15				0.55
All EOC, postmenopausal												
Q1	347 (24.6)	163 (27.7)	1.00 (referent)		117 (22.0)	42 (29.0)	1.00 (referent)		230 (26.2)	121 (27.3)	1.00 (referent)	
Q2	351 (24.9)	144 (24.4)	0.87 (0.66, 1.15)	0.32	129 (24.3)	31 (21.4)	0.67 (0.39, 1.16)	0.15	222 (25.3)	113 (25.5)	0.95 (0.69, 1.31)	0.76
Q3	355 (25.2)	122 (20.7)	0.74 (0.56, 0.98)	0.03	127 (23.9)	35 (24.1)	0.76 (0.45, 1.28)	0.31	228 (26.0)	87 (19.6)	0.72 (0.52, 1.00)	0.05
Q4	356 (25.3)	160 (27.2)	0.99 (0.76, 1.30)	0.96	158 (29.8)	37 (25.5)	0.64 (0.38, 1.09)	0.10	198 (22.6)	123 (27.7)	1.18 (0.86, 1.61)	0.31
p-trend				0.60				0.28				0.09
Serous cases												
Q1	264 (24.8)	123 (27.8)	1.00 (referent)		97 (24.3)	35 (32.4)	1.00 (referent)		167 (25.2)	88 (26.3)	1.00 (referent)	
Q2	244 (23.0)	109 (24.6)	0.96 (0.69, 1.32)	0.78	91 (22.8)	21 (19.4)	0.65 (0.34, 1.22)	0.18	153 (23.0)	88 (26.3)	1.09 (0.75, 1.59)	0.64
Q3	273 (25.7)	103 (23.3)	0.81 (0.59, 1.10)	0.18	89 (22.3)	29 (26.9)	0.92 (0.52, 1.62)	0.77	184 (27.7)	74 (22.1)	0.77 (0.53, 1.12)	0.17
Q4	282 (26.5)	108 (24.4)	0.85 (0.62, 1.16)	0.30	122 (30.6)	23 (21.3)	0.51 (0.27, 0.96)	0.04	160 (24.1)	85 (25.4)	1.02 (0.71, 1.48)	0.92
p-trend*				0.28				0.06				0.89
Mucinous cases												
Q1	41 (27.5)	15 (25.9)	1.00 (referent)		18 (25.7)	7 (38.9)	1.00 (referent)		23 (29.1)	8 (20.0)	1.00 (referent)	
Q2	43 (28.9)	17 (29.3)	1.03 (0.46, 2.32)	0.94	18 (25.7)	5 (27.8)	0.65 (0.18, 2.39)	0.52	25 (31.6)	12 (30.0)	1.45 (0.52, 4.07)	0.48
Q3	43 (28.9)	11 (19.0)	0.67 (0.26, 1.70)	0.39	20 (28.6)	2 (11.1)	0.21 (0.04, 1.26)	0.09	23 (29.1)	9 (22.5)	1.27 (0.39, 4.10)	0.69
Q4	22 (14.8)	15 (25.9)	2.01 (0.78, 5.20)	0.15	14 (20.0)	4 (22.2)	0.76 (0.18, 3.26)	0.71	8 (10.1)	11 (27.5)	4.44 (1.16, 17.0)	0.03
p-trend*				0.10				0.93				0.02
Endometrioid cases												
Q1	51 (22.3)	21 (21.9)	1.00 (referent)		15 (16.9)	6 (24.0)	1.00 (referent)		36 (25.7)	15 (21.1)	1.00 (referent)	
Q2	68 (29.7)	24 (25.0)	0.85 (0.41, 1.76)	0.67	29 (32.6)	4 (16.0)	0.36 (0.09, 1.47)	0.15	39 (27.9)	20 (28.2)	1.23 (0.52, 2.90)	0.64
Q3	52 (22.7)	20 (20.8)	1.00 (0.47, 2.11)	0.99	20 (22.5)	7 (28.0)	0.91 (0.26, 3.17)	0.88	32 (22.9)	13 (18.3)	0.98 (0.39, 2.43)	0.96
Q4	58 (25.3)	31 (32.3)	1.37 (0.69, 2.71)	0.37	25 (28.1)	8 (32.0)	0.88 (0.26, 2.96)	0.83	33 (23.6)	23 (32.4)	1.68 (0.73, 3.86)	0.22
p-trend*				0.21				0.66				0.19

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Table 4. Associations between anti-CA15.3 and anti-CA125 antibodies and risk of ovarian cancer. (continued)

	All				<3 years between blood and diagnosis				≥3 years between blood and diagnosis			
	Controls N (%)	Cases N (%)	OR (95% CI)	p	Controls N (%)	Cases N (%)	OR (95% CI)	p	Controls N (%)	Cases N (%)	OR (95% CI)	p
Anti-CA125 antibodies												
All EOC												
Q1	480 (24.9)	213 (26.4)	1.00 (referent)		167 (23.4)	46 (23.7)	1.00 (referent)		313 (25.8)	167 (27.3)	1.00 (referent)	
Q2	483 (25.1)	224 (27.8)	1.04 (0.82, 1.31)	0.75	173 (24.2)	56 (28.9)	1.10 (0.70, 1.72)	0.68	310 (25.6)	168 (27.5)	1.02 (0.77, 1.33)	0.91
Q3	481 (24.9)	169 (21.0)	0.80 (0.63, 1.02)	0.07	176 (24.6)	36 (18.6)	0.76 (0.46, 1.24)	0.27	305 (25.1)	133 (21.7)	0.81 (0.61, 1.07)	0.14
Q4	484 (25.1)	200 (24.8)	0.96 (0.76, 1.21)	0.70	199 (27.8)	56 (28.9)	1.01 (0.65, 1.57)	0.96	285 (23.5)	144 (23.5)	0.93 (0.71, 1.23)	0.63
p-trend				0.52				0.97				0.72
All EOC, premenopausal												
Q1	124 (25.8)	50 (24.9)	1.00 (referent)		47 (27.5)	11 (23.9)	1.00 (referent)		77 (24.9)	39 (25.2)	1.00 (referent)	
Q2	121 (25.2)	59 (29.4)	1.19 (0.74, 1.91)	0.47	42 (24.6)	13 (28.3)	1.15 (0.46, 2.88)	0.76	79 (25.6)	46 (29.7)	1.19 (0.68, 2.08)	0.53
Q3	113 (23.5)	40 (19.9)	0.86 (0.53, 1.40)	0.54	38 (22.2)	5 (10.9)	0.59 (0.19, 1.83)	0.36	75 (24.3)	35 (22.6)	0.92 (0.53, 1.59)	0.77
Q4	122 (25.4)	52 (25.9)	1.08 (0.68, 1.71)	0.75	44 (25.7)	17 (37.0)	1.69 (0.73, 3.91)	0.22	78 (25.2)	35 (22.6)	0.87 (0.50, 1.52)	0.63
p-trend				0.97				0.21				0.48
All EOC, postmenopausal												
Q1	345 (24.5)	160 (27.2)	1.00 (referent)		117 (22.0)	35 (24.1)	1.00 (referent)		228 (26.0)	125 (28.2)	1.00 (referent)	
Q2	356 (25.3)	161 (27.3)	0.97 (0.74, 1.27)	0.83	130 (24.5)	41 (28.3)	0.99 (0.58, 1.67)	0.96	226 (25.7)	120 (27.0)	0.96 (0.70, 1.32)	0.81
Q3	358 (25.4)	128 (21.7)	0.78 (0.59, 1.04)	0.09	136 (25.6)	31 (21.4)	0.77 (0.45, 1.33)	0.35	222 (25.3)	97 (21.8)	0.78 (0.56, 1.09)	0.15
Q4	350 (24.8)	140 (23.8)	0.88 (0.67, 1.16)	0.36	148 (27.9)	38 (26.2)	0.82 (0.48, 1.39)	0.46	202 (23.0)	102 (23.0)	0.90 (0.65, 1.25)	0.55
p-trend				0.34				0.52				0.72
Serous cases												
Q1	268 (25.2)	117 (26.4)	1.00 (referent)		99 (24.8)	25 (23.1)	1.00 (referent)		169 (25.5)	92 (27.5)	1.00 (referent)	
Q2	258 (24.3)	128 (28.9)	1.13 (0.83, 1.55)	0.44	98 (24.6)	30 (27.8)	1.13 (0.61, 2.09)	0.69	160 (24.1)	98 (29.3)	1.14 (0.79, 1.64)	0.49
Q3	246 (23.1)	89 (20.1)	0.84 (0.61, 1.17)	0.31	89 (22.3)	21 (19.4)	0.96 (0.50, 1.86)	0.91	157 (23.6)	68 (20.3)	0.81 (0.55, 1.18)	0.27
Q4	291 (27.4)	109 (24.6)	0.87 (0.63, 1.19)	0.37	113 (28.3)	32 (29.6)	1.10 (0.61, 1.99)	0.75	178 (26.8)	77 (23.0)	0.79 (0.54, 1.14)	0.21
p-trend†				0.17				0.85				0.11
Mucinous cases												
Q1	45 (30.2)	12 (20.7)	1.00 (referent)		20 (28.6)	4 (22.2)	1.00 (referent)		25 (31.6)	8 (20.0)	1.00 (referent)	
Q2	34 (22.8)	15 (25.9)	1.68 (0.67, 4.21)	0.27	14 (20.0)	5 (27.8)	1.61 (0.38, 6.73)	0.52	20 (25.3)	10 (25.0)	1.74 (0.53, 5.71)	0.36
Q3	46 (30.9)	17 (29.3)	1.44 (0.59, 3.57)	0.42	21 (30.0)	5 (27.8)	1.15 (0.29, 4.58)	0.84	25 (31.6)	12 (30.0)	1.74 (0.53, 5.79)	0.36
Q4	24 (16.1)	14 (24.1)	2.52 (0.95, 6.67)	0.06	15 (21.4)	4 (22.2)	1.37 (0.29, 6.45)	0.69	9 (11.4)	10 (25.0)	3.84 (1.06, 13.9)	0.04
p-trend†				0.15				0.93				0.05
Endometrioid cases												
Q1	52 (22.7)	27 (28.1)	1.00 (referent)		18 (20.2)	7 (28.0)	1.00 (referent)		34 (24.3)	20 (28.2)	1.00 (referent)	
Q2	68 (29.7)	23 (24.0)	0.60 (0.30, 1.21)	0.16	24 (27.0)	5 (20.0)	0.49 (0.13, 1.77)	0.27	44 (31.4)	18 (25.4)	0.67 (0.29, 1.53)	0.34
Q3	55 (24.0)	19 (19.8)	0.67 (0.33, 1.37)	0.27	20 (22.5)	5 (20.0)	0.62 (0.15, 2.49)	0.50	35 (25.0)	14 (19.7)	0.69 (0.30, 1.60)	0.39
Q4	54 (23.6)	27 (28.1)	1.07 (0.54, 2.11)	0.84	27 (30.3)	8 (32.0)	0.83 (0.26, 2.65)	0.75	27 (19.3)	19 (26.8)	1.24 (0.54, 2.86)	0.62
p-trend†				0.54				0.96				0.31

*p-heterogeneity comparing trend across serous, mucinous and endometrioid histologic types: all, p=0.06; <3 years, p=0.42, ≥3 years, p=0.07.

†p-heterogeneity comparing trend across serous, mucinous and endometrioid histologic types: all, p=0.08; <3 years, p=0.99, ≥3 years, p=0.03.

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Table 5. Association between anti-CA15.3 and anti-CA125 antibodies in perspective of CA15.3 and CA125 level.

Antigen/ antibody combinations	All				<3 years between blood and diagnosis				≥3 years between blood and diagnosis			
	Controls N (%)	Cases N (%)	OR (95% CI)	p	Controls N (%)	Cases N (%)	OR (95% CI)	p	Controls N (%)	Cases N (%)	OR (95% CI)	p
CA15.3/anti-CA15.3 antibodies												
All EOC												
Low/low	457 (23.8)	207 (25.7)	1.00 (referent)		168 (23.7)	44 (22.8)	1.00 (referent)		289 (23.9)	163 (26.7)	1.00 (referent)	
High/low	502 (26.2)	210 (26.1)	0.93 (0.74, 1.18)	0.57	172 (24.3)	51 (26.4)	1.20 (0.75, 1.90)	0.45	330 (27.3)	159 (26.0)	0.86 (0.65, 1.13)	0.26
Low/high	503 (26.2)	184 (22.9)	0.83 (0.66, 1.05)	0.13	200 (28.2)	45 (23.3)	0.90 (0.57, 1.44)	0.66	303 (25.0)	139 (22.7)	0.82 (0.62, 1.07)	0.14
High/high	457 (23.8)	203 (25.2)	1.00 (0.79, 1.27)	0.99	169 (23.8)	53 (27.5)	1.23 (0.77, 1.97)	0.38	288 (23.8)	150 (24.5)	0.93 (0.71, 1.22)	0.61
All EOC, premenopausal												
Low/low	133 (27.8)	56 (27.9)	1.00 (referent)		48 (28.2)	7 (15.2)	1.00 (referent)		85 (27.5)	49 (31.6)	1.00 (referent)	
High/low	114 (23.8)	49 (24.4)	1.02 (0.64, 1.62)	0.93	41 (24.1)	14 (30.4)	2.01 (0.74, 5.47)	0.17	73 (23.6)	35 (22.6)	0.84 (0.49, 1.43)	0.51
Low/high	132 (27.6)	55 (27.4)	1.01 (0.65, 1.58)	0.95	43 (25.3)	16 (34.8)	2.69 (1.01, 7.14)	0.05	89 (28.8)	39 (25.2)	0.76 (0.45, 1.26)	0.28
High/high	100 (20.9)	41 (20.4)	1.00 (0.62, 1.63)	0.99	38 (22.4)	9 (19.6)	1.63 (0.56, 4.72)	0.37	62 (20.1)	32 (20.6)	0.90 (0.51, 1.59)	0.72
All EOC, postmenopausal												
Low/low	318 (22.7)	149 (25.4)	1.00 (referent)		118 (22.4)	37 (25.7)	1.00 (referent)		200 (22.9)	112 (25.3)	1.00 (referent)	
High/low	377 (26.9)	157 (26.7)	0.90 (0.68, 1.19)	0.45	127 (24.1)	35 (24.3)	0.97 (0.57, 1.66)	0.92	250 (28.6)	122 (27.5)	0.87 (0.63, 1.21)	0.41
Low/high	357 (25.5)	124 (21.1)	0.77 (0.58, 1.02)	0.06	152 (28.9)	28 (19.4)	0.61 (0.35, 1.06)	0.08	205 (23.4)	96 (21.7)	0.84 (0.61, 1.17)	0.3
High/high	349 (24.9)	157 (26.7)	0.98 (0.75, 1.29)	0.88	129 (24.5)	44 (30.6)	1.13 (0.66, 1.91)	0.66	220 (25.1)	113 (25.5)	0.93 (0.67, 1.27)	0.63
Serous cases												
Low/low	249 (23.6)	124 (28.0)	1.00 (referent)		100 (25.3)	23 (21.3)	1.00 (referent)		149 (22.5)	101 (30.1)	1.00 (referent)	
High/low	257 (24.3)	108 (24.4)	0.83 (0.60, 1.15)	0.27	88 (22.2)	33 (30.6)	1.66 (0.90, 3.07)	0.11	169 (25.6)	75 (22.4)	0.64 (0.43, 0.93)	0.02
Low/high	288 (27.2)	113 (25.5)	0.80 (0.59, 1.09)	0.16	108 (27.3)	32 (29.6)	1.30 (0.71, 2.39)	0.39	180 (27.2)	81 (24.2)	0.67 (0.47, 0.96)	0.03
High/high*	263 (24.9)	98 (22.1)	0.75 (0.54, 1.03)	0.08	100 (25.3)	20 (18.5)	0.85 (0.43, 1.68)	0.64	163 (24.7)	78 (23.3)	0.71 (0.49, 1.03)	0.07
Mucinous cases												
Low/low	38 (25.5)	14 (24.1)	1.00 (referent)		11 (15.7)	5 (27.8)	1.00 (referent)		27 (34.2)	9 (22.5)	1.00 (referent)	
High/low	46 (30.9)	18 (31.0)	1.25 (0.54, 2.90)	0.61	25 (35.7)	7 (38.9)	0.53 (0.12, 2.32)	0.40	21 (26.6)	11 (27.5)	1.53 (0.54, 4.37)	0.42
Low/high	34 (22.8)	9 (15.5)	0.78 (0.30, 2.03)	0.61	16 (22.9)	2 (11.1)	0.26 (0.04, 1.66)	0.15	18 (22.8)	7 (17.5)	1.12 (0.35, 3.63)	0.84
High/high*	31 (20.8)	17 (29.3)	1.64 (0.69, 3.92)	0.26	18 (25.7)	4 (22.2)	0.43 (0.09, 2.06)	0.29	13 (16.5)	13 (32.5)	3.00 (0.99, 9.08)	0.05
Endometrioid cases												
Low/low	62 (27.2)	18 (18.9)	1.00 (referent)		20 (22.7)	6 (25.0)	1.00 (referent)		42 (30.0)	12 (16.9)	1.00 (referent)	
High/low	56 (24.6)	26 (27.4)	1.66 (0.82, 3.37)	0.16	23 (26.1)	3 (12.5)	0.49 (0.10, 2.28)	0.36	33 (23.6)	23 (32.4)	2.42 (1.04, 5.62)	0.04
Low/high	64 (28.1)	24 (25.3)	1.41 (0.69, 2.86)	0.35	27 (30.7)	4 (16.7)	0.51 (0.12, 2.20)	0.37	37 (26.4)	20 (28.2)	1.91 (0.83, 4.41)	0.13
High/high*	46 (20.2)	27 (28.4)	2.22 (1.06, 4.68)	0.04	18 (20.5)	11 (45.8)	1.96 (0.59, 6.58)	0.27	28 (20.0)	16 (22.5)	2.03 (0.80, 5.17)	0.14

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Table 5. Association between anti-CA15.3 and anti-CA125 antibodies in perspective of CA15.3 and CA125 level. (continued)

Antigen/ antibody combinations	All				<3 years between blood and diagnosis				≥3 years between blood and diagnosis			
	Controls N (%)	Cases N (%)	OR (95% CI)	p	Controls N (%)	Cases N (%)	OR (95% CI)	p	Controls N (%)	Cases N (%)	OR (95% CI)	p
CA125 / anti-CA125 antibodies												
All EOC												
Low/low	481 (25.0)	196 (24.3)	1.00 (referent)		174 (24.4)	31 (16.0)	1.00 (referent)		307 (25.3)	165 (27.0)	1.00 (referent)	
High/low	479 (24.9)	241 (29.9)	1.30 (1.03, 1.64)	0.03	164 (23.0)	71 (36.6)	2.67 (1.63, 4.35)	<0.0001	315 (26.0)	170 (27.8)	1.02 (0.78, 1.34)	0.88
Low/high	483 (25.1)	138 (17.1)	0.71 (0.55, 0.91)	0.01	192 (26.9)	18 (9.3)	0.54 (0.29, 1.01)	0.05	291 (24.0)	120 (19.6)	0.75 (0.56, 1.00)	0.05
High/high	483 (25.1)	231 (28.7)	1.26 (0.99, 1.60)	0.06	183 (25.7)	74 (38.1)	2.48 (1.53, 4.00)	0.0002	300 (24.7)	157 (25.7)	0.98 (0.74, 1.30)	0.91
All EOC, premenopausal												
Low/low	80 (16.7)	34 (16.9)	1.00 (referent)		30 (17.5)	9 (19.6)	1.00 (referent)		50 (16.2)	25 (16.1)	1.00 (referent)	
High/low	165 (34.4)	75 (37.3)	1.07 (0.65, 1.78)	0.79	59 (34.5)	15 (32.6)	0.91 (0.35, 2.36)	0.84	106 (34.3)	60 (38.7)	1.16 (0.64, 2.10)	0.64
Low/high	73 (15.2)	21 (10.4)	0.65 (0.34, 1.25)	0.2	25 (14.6)	2 (4.3)	0.26 (0.05, 1.37)	0.11	48 (15.5)	19 (12.3)	0.78 (0.38, 1.63)	0.51
High/high	162 (33.8)	71 (35.3)	1.07 (0.64, 1.77)	0.81	57 (33.3)	20 (43.5)	1.33 (0.53, 3.33)	0.54	105 (34.0)	51 (32.9)	0.98 (0.53, 1.80)	0.95
All EOC, postmenopausal												
Low/low	393 (27.9)	158 (26.8)	1.00 (referent)		143 (27.0)	22 (15.2)	1.00 (referent)		250 (28.5)	136 (30.6)	1.00 (referent)	
High/low	305 (21.7)	163 (27.7)	1.38 (1.06, 1.81)	0.02	102 (19.3)	54 (37.2)	3.69 (2.06, 6.60)	<0.0001	203 (23.1)	109 (24.5)	1.00 (0.73, 1.36)	0.99
Low/high	405 (28.8)	115 (19.5)	0.71 (0.54, 0.95)	0.02	166 (31.4)	16 (11.0)	0.66 (0.34, 1.31)	0.24	239 (27.2)	99 (22.3)	0.75 (0.54, 1.02)	0.07
High/high	304 (21.6)	153 (26.0)	1.32 (1.00, 1.74)	0.05	118 (22.3)	53 (36.6)	2.95 (1.67, 5.20)	0.0002	186 (21.2)	100 (22.5)	0.99 (0.71, 1.37)	0.95
Serous cases												
Low/low	247 (23.3)	108 (24.4)	1.00 (referent)		104 (26.2)	14 (13.0)	1.00 (referent)		143 (21.5)	94 (28.1)	1.00 (referent)	
High/low	276 (26.0)	137 (30.9)	1.16 (0.85, 1.58)	0.35	91 (22.9)	41 (38.0)	3.91 (1.92, 7.96)	0.0002	185 (27.9)	96 (28.7)	0.80 (0.55, 1.15)	0.22
Low/high	260 (24.5)	73 (16.5)	0.64 (0.45, 0.91)	0.01	106 (26.7)	12 (11.1)	0.84 (0.37, 1.89)	0.68	154 (23.2)	61 (18.2)	0.60 (0.40, 0.89)	0.01
High/high[†]	278 (26.2)	125 (28.2)	1.07 (0.77, 1.46)	0.70	96 (24.2)	41 (38.0)	3.51 (1.76, 7.02)	0.0004	182 (27.4)	84 (25.1)	0.71 (0.49, 1.03)	0.07
Mucinous cases												
Low/low	36 (24.2)	11 (19.0)	1.00 (referent)		12 (17.1)	6 (33.3)	1.00 (referent)		24 (30.4)	5 (12.5)	1.00 (referent)	
High/low	43 (28.9)	16 (27.6)	1.37 (0.51, 3.68)	0.53	22 (31.4)	3 (16.7)	0.22 (0.04, 1.20)	0.08	21 (26.6)	13 (32.5)	3.80 (1.00, 14.46)	0.05
Low/high	33 (22.1)	9 (15.5)	0.97 (0.35, 2.70)	0.95	15 (21.4)	1 (5.6)	0.12 (0.01, 1.17)	0.07	18 (22.8)	8 (20.0)	2.57 (0.68, 9.66)	0.16
High/high[†]	37 (24.8)	22 (37.9)	2.34 (0.91, 6.03)	0.08	21 (30.0)	8 (44.4)	0.65 (0.16, 2.56)	0.54	16 (20.3)	14 (35.0)	5.45 (1.42, 20.98)	0.01
Endometrioid cases												
Low/low	65 (28.4)	21 (21.9)	1.00 (referent)		24 (27.0)	3 (12.0)	1.00 (referent)		41 (29.3)	18 (25.4)	1.00 (referent)	
High/low	55 (24.0)	29 (30.2)	1.78 (0.89, 3.54)	0.1	18 (20.2)	9 (36.0)	3.67 (0.91, 14.82)	0.07	37 (26.4)	20 (28.2)	1.29 (0.58, 2.91)	0.53
Low/high	59 (25.8)	20 (20.8)	1.10 (0.53, 2.30)	0.8	28 (31.5)	4 (16.0)	1.04 (0.21, 5.25)	0.96	31 (22.1)	16 (22.5)	1.21 (0.52, 2.82)	0.66
High/high[†]	50 (21.8)	26 (27.1)	1.87 (0.90, 3.87)	0.09	19 (21.3)	9 (36.0)	4.06 (0.92, 17.86)	0.06	31 (22.1)	17 (23.9)	1.33 (0.57, 3.13)	0.51

*p-heterogeneity for high/high vs. low/low across serous, mucinous and endometrioid histologic types: all, p=0.01; <3 years, p=0.29; ≥3 years, p=0.01.

[†]p-heterogeneity for high/high vs. low/low across serous, mucinous and endometrioid histologic types: all, p=0.15; <3 years, p=0.08; ≥3 years, p=0.01.

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Author Manuscript Published OnlineFirst on April 16, 2018; DOI: 10.1158/1055-9965.EPI-17-0744
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Table 6. Spearman partial correlations between antigens and antibodies, stratified by menopausal status in EPIC cases and controls.

		All			Premenopausal			Postmenopausal		
		N	r*	p	N	r*	p	N	r*	p
CA15.3 and anti-CA15.3 antibodies										
Cases	**All**	804	-0.04	0.30	201	-0.15	0.04	587	-0.02	0.68
	Serous	443	-0.05	0.32	109	-0.34	0.001	326	0.03	0.64
	Mucinous	58	0.11	0.49	20	0.24	0.70	37	0.11	0.61
	Endometrioid	95	-0.07	0.52	29	-0.04	0.88	62	-0.05	0.72
	<1 year	61	-0.05	0.73	19	-0.35	0.56	42	0.06	0.74
	>1-3 years	132	0.09	0.36	27	0.28	0.40	102	0.06	0.59
	>3-6 years	199	-0.17	0.02	40	-0.05	0.83	156	-0.18	0.04
	>6-9 years	194	0.01	0.87	65	-0.05	0.72	124	0.06	0.55
	>9 years	218	-0.01	0.94	50	-0.13	0.48	163	0.04	0.67
Controls	**n/a**	1918	-0.04	0.06	484	-0.06	0.22	1408	-0.03	0.20
CA125 and anti-CA125 antibodies										
Cases	**All**	806	0.10	0.004	201	0.14	0.05	589	0.08	0.05
	Serous	443	0.10	0.04	109	0.22	0.03	326	0.08	0.14
	Mucinous	58	0.40	0.01	20	0.47	0.43	37	0.54	0.005
	Endometrioid	96	0.18	0.12	29	-0.19	0.52	63	0.07	0.62
	<1 year	61	-0.21	0.17	19	0.49	0.41	42	-0.34	0.08
	>1-3 years	133	0.22	0.02	27	-0.23	0.50	103	0.19	0.07
	>3-6 years	199	0.04	0.58	40	0.10	0.64	156	0.02	0.83
	>6-9 years	195	0.11	0.13	65	0.18	0.22	125	0.16	0.09
	>9 years	218	0.05	0.50	50	-0.25	0.16	163	0.03	0.73
Controls	**n/a**	1925	0.01	0.81	485	<0.01	0.99	1414	0.01	0.73

*Adjusted for matching factors, including age at blood draw, fasting status, date and time of blood draw, menopausal status at blood, menstrual cycle phase for premenopausal women at blood, OC/HRT use at blood, length of follow up.

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Cancer Epidemiology, Biomarkers & Prevention

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Daniel W. Cramer, Raina N Fichorova, Kathryn L. Terry, et al.

Cancer Epidemiol Biomarkers Prev Published OnlineFirst April 16, 2018.

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