Insulin-like growth factor I and risk of epithelial invasive ovarian cancer by tumour characteristics: results from the EPIC cohort

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Insulin-like growth factor I (IGF-I)-related signalling pathways are implicated in the aetiology of epithelial cancer at various sites (e.g., prostate, colon and breast cancer), including ovarian cancer (reviewed in Bruchim and Werner, 2013; Singh et al, 2014). Insulin-like growth factor I drives cellular proliferation in several cell lines of epithelial neoplasms (reviewed in Pollak, 2008) and is additionally associated with invasion and angiogenesis in epithelial ovarian cancer cells (reviewed in Beauchamp et al, 2010). Recently, IGF-I was shown to be overexpressed in low-grade, but not high-grade, serous ovarian cancer cell lines, suggesting IGF-I may be differentially associated with risk across ovarian cancer subtypes (King et al, 2011). Further, low-grade ovarian cancer cells expressing IGF-I were more responsive to IGF-I stimulation and IGF-I receptor (IGF-IR) inhibition compared with high-grade serous ovarian cancer cells (King et al, 2011).

Prior prospective studies on the association between pre-diagnostic circulating IGF-I and epithelial invasive ovarian cancer (EOC) were inconclusive and evaluated EOC as a single disease entity, without addressing associations in EOC subgroups (i.e., histologic subtype, dualistic model of ovarian carcinogenesis) (Lukanova et al, 2002; Peeters et al, 2007; Tworoger et al, 2007). This is the largest prospective study to date (n = 565 cases; 1097 controls) investigating the role of IGF-I and EOC risk, and the first prospective investigation to assess IGF-I and EOC by tumour characteristics (histology, grade, stage and type I/II status).

A total of 565 eligible cases with biological samples and incident epithelial invasive ovarian, fallopian tube or primary peritoneal cancer were 1:2 matched to 1097 controls. An incidence density sampling protocol was used. We included 201 cases and 372 matched controls from a prior analysis in EPIC (phase 1; Peeters et al, 2007) and additional 364 cases (725 matched controls) subsequently diagnosed during follow-up (phase 2).

Information on tumour characteristics (histologic subtype (serous, endometrioid, clear cell, mucinous and not otherwise specified (NOS)), grade (well, moderately or poorly/undifferentiated) and stage (local, regional and metastatic)) was available from pathology reports and from cancer registries. Tumours were classified on the basis of histology and the proposed dualistic pathway of ovarian carcinogenesis (type I/II; Kurman and Shih, 2011). Clear cell carcinomas (n = 28) were excluded from type I/II analyses as they show unique clinical behaviour (Penson et al, 2013). All participants gave written informed consent. The Ethical Review Board of the International Agency for Research on Cancer and the Institutional Review Board of each EPIC centre approved these analyses.

Laboratory assays. Pre-diagnostic concentrations of IGF-I (nmol l$^{-1}$) were analysed with enzyme-linked immunosorbent assays at IARC (phase 1 (Peeters et al, 2007): DSL, Webster, TX, USA) and at the Division of Cancer Epidemiology at the German Cancer Research Center (phase 2: Immunodiagnostics Systems, Frankfurt, Germany). Cases and matched controls were analysed within the same analytical batch by laboratory technicians blinded to case–control status and identity of quality control samples. Intra- and inter-batch coefficients of variation from replicate quality control (QC) samples were 2.50% and 12.20% (phase 1: triplicate QCs), and 9.42% and 8.93% (phase 2: duplicate QCs).

Statistical analyses. Insulin-like growth factor I values were log2 transformed and centred to a mean value of zero in each phase. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression. Insulin-like growth factor I was examined continuously on the log2 scale and in tertiles with phase-specific cut-offs based on the distribution in controls.

The final model included full-term pregnancy (never/ever), as other covariates (BMI, height, smoking, physical activity, diabetes, alcohol, age at menarche, age at first birth, number of births, age at menopause, OC use and HRT use) did not change the OR by >10% (i.e., by a factor 1.10 or its reciprocal; Maldonado and Greenland, 1993).
Heterogeneity in the associations between IGF-I and EOC by tumour characteristics was assessed using likelihood ratio tests comparing logistic regression models with and without corresponding interaction terms (Rothman et al, 2008).

Sensitivity analyses included stratification by menopausal status at blood donation and age at diagnosis (<55 and ≥55 years); exclusion of women providing a blood sample <2 years before diagnosis to ensure any observed associations were not due to cancers influencing circulating concentrations of IGF-I, but not yet diagnosed) and women who had a prior hysterectomy.

All statistical tests were two-tailed and significant at the P<0.05 level. SAS 9.2 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analysis.

RESULTS

Cases and controls were similar with respect to most characteristics, except for established reproductive risk factors (e.g., cases were less likely to be parous, P<0.01 or to use OCs, P<0.01; Table 1). We observed no case–control differences in IGF-I concentrations overall or by study phase.

There was no association between EOC and IGF-I concentrations for doubling of hormone concentrations or comparing top to bottom tertiles in overall analyses (all histological subtypes combined (ORlog2 = 0.88; 95% CI 0.71–1.08)). A similar pattern was observed in subgroup analyses by tumour characteristics (e.g., serous tumours: ORlog2 = 0.98; 95% CI 0.74–1.30; Table 2). We did not observe heterogeneity between risk associations by tumour characteristics (e.g., P_het for histological subtypes = 0.12). Overall, risk estimates were similar when analyses were restricted to study phase 2 (data not shown).

Results were similar in sensitivity analyses by age at diagnosis (<55 vs ≥55) and menopausal status at blood donation (pre- vs postmenopausal at blood donation). Excluding women with unilateral oophorectomy/hysterectomy (n = 116) or women diagnosed within 2 years after blood donation (n = 84) led to results comparable to overall results (data not shown).

DISCUSSION

This is the largest prospective study on the relationship between IGF-I and EOC to date and the first to assess risk associations by tumour characteristics. We observed no association between IGF-I and EOC overall. The same pattern was observed in analyses stratified by tumour characteristics, age at diagnosis or menopausal status at blood donation.

Three prospective studies (range: 132 cases (Lukanova et al, 2002) to 222 cases (Tworoger et al, 2007)) have evaluated this association previously. Two of these studies observed a positive association between IGF-I and EOC in women <55 at diagnosis (Lukanova et al, 2002; Peeters et al, 2007); however, sample size in these subgroups was limited (n ≤66 younger than 55 at diagnosis) and confidence intervals were wide (i.e., ORlog2 = 5.10; 95% CI 1.50–18.20 (Peeters et al, 2007)). In a US-based study, no association was observed in women diagnosed before the age of 55, but there was an inverse association in women diagnosed after the age of 55 (ORlog2 = 0.52; 95% CI 0.28–0.95 (Tworoger et al, 2007)). Slightly different exclusion criteria might contribute to inconsistent results across studies (e.g., exclusion of cases diagnosed within 1 year after blood donation (Lukanova et al, 2002), fallopian tube cancers (Tworoger et al, 2007), unilateral oophorectomy/hysterectomy (EPIC phase 1; Peeters et al, 2007)). In the current study including 565 EOC cases, we observed no association between IGF-I and ovarian cancer risk regardless of the age at diagnosis.

Elevated IGF-I concentrations may lead to the development of a malignant cell rather than to apoptotic cell death in the early phases of carcinogenesis (reviewed in Pollak, 2008). Insulin-like growth factor I signalling is predominantly mediated by the IGF-IIR; higher IGF-IR expression is associated with development of epithelial neoplasms through anti-apoptotic and mitogenic

Table 1. Selected baseline characteristics of EOC cases and matched controls at enrolment in the EPIC study

|                     | Cases (n = 565) | Controls (n = 1097) | P-value |
|---------------------|----------------|---------------------|---------|
| Age at blood donation | 57.0 (33.6–80.7) | 56.9 (33.6–79.3) |         |
| Age at diagnosis     | 63.6 (37.4–86.5) |                     |         |
| Lag time between blood donation and diagnosis | 6.7 (0–16) |       |         |
| Menopausal status at blood donation |       |         |         |
| Pre                  | 112 (20%) | 219 (20%) | 878 (80%) |
| Post                 | 453 (80%) |                     |         |
| Age at menopause    | 50 (32–60) | 50 (30–59) | 0.03    |
| Ever full-term pregnancy | <0.01 |       |         |
| OC use               |       | <0.01   |         |
| Never                | 349 (62%) | 594 (54%) | 498 (46%) |
| Ever                 | 214 (38%) |                     |         |
| HRT use             |       | 0.57    |         |
| Never                | 452 (87%) | 867 (86%) | 145 (14%) |
| Ever                 | 69 (13%) |                     |         |

Histology

|                | Cases (%) | Controls (%) |
|----------------|-----------|--------------|
| Serous         | 302 (83%) |              |
| Mucinous       | 41 (12%)  |              |
| Endometrioid   | 66 (12%)  |              |
| Clear Cell     | 28 (5%)   |              |
| NOS            | 99 (18%)  |              |
| Other          | 29 (5%)   |              |

Grade f

|                | Cases (%) | Controls (%) |
|----------------|-----------|--------------|
| Low grade      | 35 (10%)  |              |
| High grade     | 308 (90%) |              |

Stage e,g

|                | Cases (%) | Controls (%) |
|----------------|-----------|--------------|
| Low stage      | 76 (15%)  |              |
| High stage     | 420 (85%) |              |

Type I/II h

|                | Cases (%) | Controls (%) |
|----------------|-----------|--------------|
| Type I         | 67 (22%)  |              |
| Type II        | 242 (78%) |              |

IGF-I (nmol l−1) h

|                | Cases (%) | Controls (%) |
|----------------|-----------|--------------|
| 13.98 (13.39–14.64) | 10.59 (13.63–14.5) | 0.26 |

Abbreviations: EOC = epithelial ovarian cancer; EPIC = European Prospective Investigation into Cancer and Nutrition; HRT = hormone replacement therapy; IGF-I = insulin-like growth factor I. Values are shown as median (range) or number (percentage).

*Cases and controls in both study phases were matched on: study recruitment centre, age at blood donation (±6 months), time of the day of blood collection (±1 h), fasting status (<3 h, 3–6 h, >6 h) and menopausal status at blood collection (premenopausal, perimenopausal and postmenopausal), as well as menstrual cycle phase for premenopausal women (‘early follicular’ (days 0–7 of the cycle), ‘late follicular’ (days 8–11), ‘peri-ovulatory’ (days 12–16), ‘mid-luteal’ (days 20–24) and ‘other luteal’ (days 17–19 or days 25–40). Cases missing data on the phase of menstrual cycle were matched to controls with missing information on menstrual cycle phase.

**Among postmenopausal women only.

†Matching factor.

* Differences between cases and matched controls based on conditional logistic regression.

** Percentages presented among women with data on tumour characteristics. Percentage of missing data on grade (39%), stage (12%) and type I/II status (45%).

Low-grade tumours: well differentiated tumours; high-grade tumours: moderately, poorly or undifferentiated tumours.

Low-stage tumours: localised tumours; high-stage tumours: regional metastatic or distant metastatic tumours.

a Differences in IGF-I concentrations between cases and matched controls based on geometric mean (95% confidence interval); values from each study phase are standardised to a mean of 0 for analyses.
activities and its role in oncogenic transformation (reviewed in Pollak, 2008). We hypothesised that circulating IGF-I would be differentially associated with ovarian cancer subtypes given the differential expression of IGF-I in low- and high-grade serous tumours. Insulin-like growth factor I has been shown to be overexpressed in low-grade serous ovarian cancer cell lines (i.e., type I), which were more responsive to IGF-I stimulation and IGF-IR inhibition compared with high-grade serous ovarian cancer cell lines (i.e., type II) (King et al., 2011). We did not observe the hypothesised associations; however, we had small sample size in some subgroups (i.e., low-grade serous tumours, n = 35).

Our study has important strengths and limitations. We investigated pre-diagnostic serum IGF-I and EOC risk in a large, well-characterised nested case–control study. However, circulating IGF-I may not be reflective of IGF-I exposure in the ovary. Despite evidence suggesting that IGF-I could be involved in EOC development (reviewed in Bruchim and Werner, 2013; Singh et al., 2014), our study shows no association between circulating IGF-I and EOC risk. Larger, pooled prospective studies are needed to confirm our results and to address the associations in small subgroups with more statistical power and assess risk associations with expression of IGF receptors.

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DISCLAIMER

The authors declare no conflict of interest.

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