**Table S1:** Basic information on the participating cohort studies for the pooled analysis on androgens and EOC: the Ovarian Cancer Cohort Consortium (OC3)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cohort (cases) Population Recruitment  period** |  **Fasting  status** | **Storage**  |  | **Matching criteria** |
|  |  |  |  | **Controls per case** | **Age at blood donation** | **Date of blood sample** | **Day of****cycle** | **Menopausal status** | **Other criteria** |
| Clue II (46) | Residents of Washington Country, USA | 1989 | Non-fasting | -70°C | 1:2 | ± 1 years | ± 14 days | ± 1 day | Menopausal status at blood collection | Current OC / HT use |
| EPIC (451) | Volunteers in Denmark, France, Germany, Greece, Italy, Netherlands, Spain, Sweden and UK | 1992-2000 | Matched | -196°C1 | 1:2 | ± 6 months | No (incidence density sampling) | 5 phases | Menopausal status at blood collection  | Recruitment center, Time of the day of blood collection,  |
| FMC (576) | Population based maternity cohort | 1986-2007 | Not available | -25°C | 1:3 | ± 6 months | ± 3 months | Not applicable | Not available | Parity (1,2,>2), parity at diagnosis (1,2,>2) |
| NHS and II2 (138) | Registered nurses in the USA | 1996-99 | Matched | -130°C | 1:3 | ± 2 years | ± 2 months | ± 1 day for luteal blood sample3 | Menopausal status at baseline and diagnosis | Time of day, use of postmenopausal hormones at blood collection  |
| WHS (63) | US female health professionals; RCT4 | 1992-95 | Matched | -170°C | 1:2 | ± 1 year | ± 3 months | 5 phases and day | Menopausal status at baseline / diagnosis | Postmenopausal hormones at baseline /diagnosis, time since randomization (± 6 months), |
| NYUWHS (63) | Women attending breast cancer screening center, NY USA | 1985-91 | Non-fasting | -80°C | 1:2 | ± 6 months | ± 3 months | day of menstrual cycle | Menopausal status at blood donation | Number of blood donations |

CLUE II = Washington County, MD Study ‘Give us a clue to cancer and heart disease’. EPIC= European Prospective Investigation into Cancer and Nutrition. FMC= Finnish Maternity Cohort. NHS= Nurses’ Health Study. NYU WHS = New York University Women’s Health Study. 1Most samples were stored in liquid nitrogen at -196°C, apart from Denmark and Sweden were samples were stored locally at -150°C and -70°C. 2NHS phase 1 (1999-2003 follow-up cycles) and phase 2 (2005-09 follow-up cycles). 3Patients were asked to provide follicular sample at 3-5 days and luteal sample at 7-9 days before anticipated start of the next cycle. 4 RCT = Randomized Controlled Trial.

**Table S2:** Laboratory assays and Intra- and Inter-batch CVs for the participating cohorts: the Ovarian Cancer Cohort Consortium (OC3)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Biomarker** | **Sample** | **Assay** | **Intra-Batch CV****(%)** | **Inter-Batch CV****(%)** |
| **Testosterone** |
|  CLUE II | heparin plasma | direct RIA | 12.9 | 22.2 |
|  EPIC phase 1 | serum | direct RIA1direct RIA1 | 6.6 | 11.0 |
|  EPIC phase 2 | 12.7 | 7.6 |
|  FMC | serum | HPLC tandem mass spec. | 9.7 | 8.1 |
|  NHS | heparin plasma | liquid chromatography/mass spec. | 13.3\* | - |
|  NHS II | - |
|  WHS | EDTA plasma | Liquid chromatography/mass spec. | - |
|  NYUWHS | serum | Direct RIA1 | 9.6 | 14 |
| **DHEAS** |
|  CLUE II | heparin plasma | direct RIA | < 3 | <10 |
|  EPIC phase 1 | serum | direct RIA1 | 3.4 | 11.6 |
|  EPIC phase 2 | direct RIA1 | 8.2 | 6.2 |
|  FMC | - | - | - | - |
|  NHS | heparin plasma heparin plasma | chemiluminescent immunoassay | 3.8\* | - |
|  NHS II | chemiluminescent immunoassay | - |
|  WHS | EDTA plasma | chemiluminescent immunoassay | - |
|  NYUWHS | serum | direct RIA1 | 4.6 | 11.5 |
| **Androstenedione** |
|  CLUE II | heparin plasma | double-antibody RIA | 8.6 | 10.0 |
|  EPIC phase 1 | serum | direct RIA3 | 3.0 | 8.4 |
|  EPIC phase 2 | direct RIA3 | 20.5 | 10.4 |
|  FMC | serum | HPLC tandem mass spectrometry | 8.3 | 7.7 |
|  NHS | heparin plasma | Liquid chromatography/mass spec. | 9.4\* | - |
|  NHS II | Liquid chromatography/mass spec. | - |
|  WHS | EDTA plasma | Liquid chromatography/mass spec. | - |
|  NYUWHS | serum | double-antibody RIA3 | 7.0 | 13.8 |
| **SHBG** |
|  CLUE II | heparin plasma | direct “sandwich” immunoradiometric | 1.4 | 22.2 |
|  EPIC phase 1 | serum | direct “sandwich” immunoradiometric5 direct “sandwich” immunoradiometric5 | 4.2 | 10.7 |
|  EPIC phase 2 | serum | 5.9 | 3.2 |
|  FMC | serum | chemiluminescence | 8.7 | 3.7 |
|  NHS  | - | - | - | - |
|  NHS II | - | - | - | - |
|  WHS | - | - | - | - |
|  NYUWHS | serum | direct ‘sandwich’immunoradiometric5 | 6.2 | 11.5 |

1Radio-Immuno-Assay (RIA) Immunotech, Marseille, France; 2Beckman Coulter, Brea, California; 3Diagnostic System Laboratories (DSL),
Webster, Texas, USA; 4Beckman and Coulter, Brea, California, USA; 5CIS-Bio, Gif-sur-Yvette, France; 6Enzyme-linked
immunosorbent assay (ELISA); DSL, Webster, Texas, USA; 7Immunodiagnostics Systems, Germany. \*average intra-batch coefficient from NHS / NHS II and WHS

Table S3. Geometric means of hormone concentrations (95% CI) by cohort and case-control status after log2 transformation and standardization: the Ovarian Cancer Cohort Consortium (OC3)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study** |  |  | **Testosterone (ng/ml)** | **Free Testosterone (nmol/l)** | **Androstenedione (ng/ml)** | **DHEAS (ug/dl)** | **SHBG (nmol/l)** |
| **Clue II** | **Cases** |  46 | 1.10 (0.96-1.27) | 1.11 (0.92-1.32) | 1.10 (0.95-1.28) | 1.01 (0.83-1.23) | 1.04 (0.90-1.22) |
|  | **Controls**  |  91 | 1.00 (0.90-1.11) | 1.00 (0.88-1.13) | 1.00 (0.90-1.11) | 1.00 (0.87-1.15) | 1.00 (0.90-1.11) |
| **EPIC** | **Cases** |  451 | 1.00 (0.96-1.05) | 1.04 (0.98-1.10) | 0.98 (0.94-1.03) | 1.03 (0.97-1.10) | 0.97 (0.92-1.02) |
|  | **Controls**  |  867 | 1.00 (0.97-1.03) | 1.00 (0.96-1.04) | 1.00 (0.97-1.04) | 1.00 (0.96-1.05) | 1.00 (0.97-1.03) |
| **FMC** | **Cases** |  576 | 1.07 (1.03-1.11) | 1.07 (1.00-1.14) | 1.07 (1.03-1.12) | ≠≠ | 1.04 (0.98-1.10) |
|  | **Controls**  | 1,433 | 1.00 (0.97-1.03) | 1.00 (0.96-1.04) | 1.00 (0.97-1.03) | ≠≠ | 1.00 (0.90-1.11) |
| **NHS** | **Cases** |  117 | 1.05 (0.96-1.15) | ≠≠ | 0.93 (0.84-1.02) | 0.87 (0.77-0.99) | ≠≠ |
|  | **Controls**  |  348 | 1.00 (0.95-1.05) | ≠≠ | 1.00 (0.94-1.06) | 1.00 (0.93-1.07) | ≠≠ |
| **NHS II** | **Cases** |  15 | 1.17 (0.91-1.50) | ≠≠ | 1.03 (0.79-1.36) | 0.94 (0.67-1.34) | ≠≠ |
|  | **Controls**  |  45 | 1.00 (0.86-1.16) | ≠≠ | 1.00 (0.85-1.18) | 1.00 (0.82-1.22) | ≠≠ |
| **NYU WHS** | **Cases** |  63 | 0.98 (0.87-1.11) | 0.92 (0.79-1.08) | 0.95 (0.84-1.09) | ≠≠ | 1.08 (0.95-1.23) |
|  | **Controls**  |  112 | 1.00 (0.91-1.10) | 1.00 (0.89-1.12) | 1.00 (0.91-1.10) | ≠≠ | 1.00 (0.91-1.10) |
| **WHS** | **Cases** |  63 | 0.90 (0.80-1.02) | ≠≠ | 1.01 (0.88-1.15) | 1.00 (0.85-1.19) | ≠≠ |
|  | **Controls**  |  122 | 1.00 (0.92-1.09) | ≠≠ | 1.00 (0.91-1.09) | 1.00 (0.89-1.13) | ≠≠ |

Table S4. Tumor characteristics in pooled analysis of prospective data on circulating androgens, SHBG and EOC risk: the Ovarian Cancer Cohort Consortium (OC3)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Clue II | EPIC | FMC | NHS | NHS II | NYUWHS | WHS | Total |
| References | ≠ | Ose et al. 2014 | Schock et al. 2014 | Tworoger et al. 2008 | Tworoger et al. 2008 | Lukanova et al. 2002 | Tworoger et al. 2008 |  |
| No | 46 | 451 | 576 | 117 | 15 | 63 | 63 | 1,331 |
| Age at dx, yrs 1 | 67.4 (13.0) | 62.5 (8.9) | 44.7 (8.1) | 65.0 (7.3) | 48.8 (3.8) | 59.8 (8.8) | 60.1 (8.0) | 54.8 (12.4) |
| Lag time, yrs 1 | 6.6 (3.0) | 6.6 (3.6) | 12.3 (6.8) | 7.3 (4.0) | 2.7 (1.9) | 7.2 (3.5) | 4.3 (2.6) | 9.0 (6.0) |
| Histology |  |  |  |  |  |  |  |  |
| Serous | 19 (41%) | 238 (53%) | 263 (46%) | 62 (53%) | 5 (33%) | 38 (60%) | 42 (67%) | 667 (50%) |
| Endometrioid | 5 (11%) | 45 (10%) | 92 (16%) | 11 (9%) | 4 (27%) | 4 (6%) | 5 (8%) | 166 (12%) |
| Mucinous | 2 (4%) | 30 (7%) | 143 (25%) | 9 (8%) | 1 (7%) | 6 (10%) | 2 (3%) | 193 (15%) |
| Clear cell | 2 (4%) | 25 (6%) | 23 (4%) | 4 (3%) | 2 (13%) | 5 (8%) | - | 61 (5%) |
| Others | 18 (39%) | 113 (25%) | 55 (10%) | 31 (27%) | 3 (20%) | 10 (16%) | 14 (22%) | 244 (18%) |
| Grade 2 |  |  |  |  |  |  |  |  |
| Low grade | 1 (4%) | 31 (12%) | - | 11 (12%) | 3 (25%) | 7 (14%) | 3 (7%) | 56 (12%) |
| High grade | 24 (96%) | 220 (88%) | - | 79 (88%) | 9 (75%) | 43 (86%) | 42 (93%) | 417 (88%) |
| Stage 2 |  |  |  |  |  |  |  |  |
| Low stage | 3 (9%) | 57 (14%) | 150 (31%) | 27 (23%) | 7 (47%) | 13 (24%) | - | 257 (23%) |
| High stage | 29 (91%) | 341 (86%) | 332 (69%) | 88 (77%) | 8 (53%) | 41 (76%) | - | 839 (77%) |
| Type 2 |  |  |  |  |  |  |  |  |
| Type I | 5 (24%) | 76 (32%) | 166 | 20 (24%) | 6 (55%) | 14 (30%) | 4 (11%) | 291 (48%) |
| Type II | 16 (76%) | 163 (68%) | - | 65 (76%) | 5 (45%) | 33 (70%) | 32 (89%) | 314 (52%) |

1presented as mean (SD)
2Among cases with data. grade missing for 64%, stage missing for 18%, Type I/II missing for 55%
≠ Data from Clue II have not been published.

|  |
| --- |
| Table S5. Odds ratios (95% CI) for invasive EOC overall and the serous subtype in quintiles of androgen and SHBG concentrations: OC31 |
|  | **Invasive EOC** |  | **Serous EOC** |
|  | **Sets** | **OR (95% CI)** | **ptrend2** |  | **Sets** | **OR (95% CI)** | **ptrend2** |
| **Testosterone** |
| Q1 | 254 | ref |  |  | 145 | ref |  |
| Q2 | 250 | 1.00 (0.81-1.23) |  |  | 128 | 0.88 (0.66-1.18) |  |
| Q3 | 251 | 1.10 (0.89-1.36) |  |  | 140 | 1.13 (0.84-1.50) |  |
| Q4 | 267 | 1.12 (0.90-1.38) |  |  | 121 | 0.88 (0.65-1.19) |  |
| Q5 | 279 | 1.22 (0.99-1.52) | 0.03 |  | 121 | 0.88 (0.65-1.20) | 0.56 |
| **Free Testosterone** |
| Q1 | 159 | ref |  |  | 84 | ref |  |
| Q2 | 179 | 1.08 (0.83-1.41) |  |  | 96 | 1.04 (0.72-1.49) |  |
| Q3 | 177 | 1.15 (0.87-1.50) |  |  | 90 | 1.10 (0.76-1.60) |  |
| Q4 | 149 | 0.97 (0.73-1.28) |  |  | 85 | 0.95 (0.65-1.38) |  |
| Q5 | 201 | 1.29 (0.99-1.68) | 0.04 |  | 80 | 0.88 (0.60-1.29) | 0.63 |
| **Androstenedione** |
| Q1 | 260 | ref |  |  | 138 | ref |  |
| Q2 | 265 | 1.07 (0.87-1.33) |  |  | 141 | 1.02 (0.76-1.37) |  |
| Q3 | 220 | 0.88 (0.70-1.10) |  |  | 114 | 0.77 (0.57-1.06) |  |
| Q4 | 276 | 1.10 (0.89-1.37) |  |  | 145 | 1.14 (0.74-1.54) |  |
| Q5 | 286 | 1.20 (0.95-1.51) | 0.13 |  | 118 | 0.90 (0.64-1.25) | 0.79 |
| **DHEAS** |
| Q1 | 135 | ref |  |  | 74 | ref |  |
| Q2 | 133 | 0.97 (0.73-1.31) |  |  | 74 | 1.01 (0.68-1.50) |  |
| Q3 | 158 | 1.18 (0.88-1.58) |  |  | 78 | 1.22 (0.81-1.82) |  |
| Q4 | 116 | 0.88 (0.64-1.20) |  |  | 66 | 0.93 (0.61-1.41) |  |
| Q5 | 149 | 1.13 (0.82-1.55) | 0.87 |  | 74 | 0.99 (0.63-1.54) | 0.45 |
| **SHBG** |
| Q1 | 186 | ref |  |  | 85 | ref |  |
| Q2 | 183 | 0.99 (0.77-1.28) |  |  | 96 | 1.18 (0.83-1.69) |  |
| Q3 | 137 | 0.77 (0.59-1.00) |  |  | 76 | 0.89 (0.62-1.28) |  |
| Q4 | 180 | 0.99 (0.76-1.27) |  |  | 92 | 1.05 (0.73-1.50) |  |
| Q5 | 200 | 1.14 (0.88-1.48) | 0.56 |  | 96 | 1.31 (0.90-1.89) | 0.39 |
| 1Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing); 2The p value for trend across quintiles is based on acontinuous probit score (generating a rank for each person in each cohort by hormone level). DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin |

**Figure S1.** Odds ratios (95% CI) for doubling of androgen concentrations and Type I EOC restricted to cases with data on tumor grade (phet comparing type I and type II: testosterone, 0.09; free testosterone, 0.38; androstenedione, <0.01; DHEAS, 0.03; SHBG, 0.14; type II ORs shown in Figure 1): the Ovarian Cancer Cohort Consortium (OC3)

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Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing). Pair-wise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (1) the same association between exposure and outcomes compared to (2) a model assuming different associations for each subtype. DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin