Epithelial ovarian cancer: testing the ‘androgens hypothesis’

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Abstract

In 1998, Risch proposed a hypothesis for the pathogenesis of ovarian cancer relating to the role of androgens in stimulating epithelial cell proliferation. Although this hypothesis has been widely discussed, direct evidence to support it is scant. To address this issue, we have conducted a detailed analysis of factors possibly associated with high circulating levels of androgens, including polycystic ovary syndrome (PCOS), hirsutism and acne (all clinically associated with hyperandrogenism) using the data collected in an Australia-wide, population-based case-control study. Cases aged 18–79 years with a new diagnosis of invasive epithelial ovarian cancer (n=1276) or borderline malignant tumour (n=315) were identified through a network of clinics and cancer registries throughout Australia. Controls (n=1508) were selected from the National Electoral Roll. Women self-reported a history of PCOS, acne, hirsutism and also use of testosterone supplements or the androgenic medication Danazol. We found no evidence that a history of PCOS, acne or hirsutism was associated with ovarian cancer overall, or with specific subtypes, with the exception of serous borderline tumours that were positively associated with a history of PCOS (OR 2.6; 95% CI 1.0–6.1). Women who had ever used testosterone supplements had an increased risk of ovarian cancer (OR 3.7; 95% CI 1.1–12.0); however, use of the androgenic medication Danazol did not increase risk (OR 1.0; 95% CI 0.4–2.9). Overall, our results do not support the hypothesis that androgen-related disorders increase the risk of ovarian cancer.

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Introduction

In 1998, Risch (1998) put forward a hypothesis for the pathogenesis of ovarian cancer relating to the role of androgens in stimulating epithelial cell proliferation. Although widely discussed in the aetiologic literature, there is a scant evidence to support this hypothesis. While a number of in vitro and animal experiments suggest a role for androgens in the development of ovarian cancer, epidemiological evidence is less convincing. Four prospective studies have examined the prediagnostic serum levels of androgens but, aside from the first very small study (n=31 cases; Helzlsouer et al. 1995), none has reported any significant associations with ovarian cancer risk (Helzlsouer et al. 1995, Lukanova et al. 2003, Rinaldi et al. 2007, Tworoger et al. 2007). These studies are, however, based on a single measure of androgens that may not accurately reflect long-term exposure. Others have assessed markers of high androgen levels such as polycystic ovary syndrome (PCOS), a disorder of functional androgen excess (Azziz 2003), and hirsutism and acne which are associated with high circulating levels of androgens (Lucky 1995), but these studies have also been limited by small numbers of exposed cases. One case-control study has reported an increased risk of ovarian cancer among women with PCOS (Schildkraut et al. 1996), although this finding was based on few exposed cases (n=7), and has not been confirmed in prospective studies (Coulam et al. 1983, Pierpoint et al. 1998); and only one previous study has investigated the association between acne or hirsutism and ovarian cancer, noting a positive association based on 13 and 3 exposed cases respectively (Wynder et al. 1969). These associations
have not been examined by tumour behaviour or histological subtype despite some known differences in risk factors (Risch et al. 1996, Titus-Ernstoff et al. 2001, Purdie et al. 2003).

To address this issue, we have conducted a detailed analysis of factors that have been clinically associated with high circulating levels of androgens, including PCOS, hirsutism and acne, in relation to risk of the major histological subtypes of ovarian cancer using the data collected from an Australia-wide, population-based case-control study. We also examined whether use of testosterone hormone therapy or the androgenic medication Danazol was related to ovarian cancer. Danazol (17-α-ethinyltestosterone; marketed as Danocrine in the US) is a synthetic androgen that binds to androgen receptors and sex hormone-binding globulin resulting in a threefold increase in free testosterone and is commonly used for the treatment of endometriosis (Olive & Pritts 2001). We assessed potential interaction between body mass index (BMI) and PCOS and ovarian cancer risk, since overweight or obese women with PCOS appear to suffer from a more severe form of hyperandrogenism than those of normal weight with PCOS (Gambineri et al. 2002).

Methods

Study participants

The Australian Ovarian Cancer Study was an Australia-wide population-based case-control study of epithelial ovarian cancer; full details of study design and participant recruitment have been reported previously (Merritt et al. 2008). Cases were women aged 18–79 years living in Australia with histologically confirmed epithelial ovarian, fallopian tube or primary peritoneal cancer newly diagnosed between January 2002 and June 2005. Cases were recruited by nurses who liaised with the treatment clinics, physicians and state cancer registries throughout Australia. Of the 3550 women identified with suspected ovarian cancer, 307 died before contact could be made, physicians refused to give consent to contact 133, usually because they were too sick or unable to give informed consent and 194 women could not be contacted. A further 171 (5%) were excluded on the basis of language difficulties (35) and illness (66). The remaining 2745 women with a clinically suspected diagnosis of ovarian cancer were invited to participate (prior to surgery, to facilitate fresh tissue collection) and, of these, 2319 (85%) agreed to take part. After surgery, pathology reports were obtained for all women and a further 608 women were excluded because their final diagnosis was a benign, non-epithelial or metastatic tumour and not primary epithelial ovarian cancer, 25 because their cancer was first diagnosed before the start of the study period and one women was excluded because she was not an Australian resident at the time of her initial diagnosis. Two researchers independently abstracted information on tumour site, histological subtype and tumour behaviour (invasive versus borderline) from the diagnostic histopathology reports. Discrepancies were resolved by consensus. To check the quality of the abstracted data, the pathology reports and the full set of diagnostic slides for a sample of 200 women were reviewed by a gynaecologic pathologist; agreement with the abstracted data was > 95% for tumour subtype and site, and 99% for tumour behaviour. Of the final 1685 eligible participants, 1591 (94%) returned a questionnaire.

Controls were randomly selected from the national electoral roll (enrolment is compulsory) and were frequency matched by age (in 5-year age bands) and state of residence to the case group. Selected women were mailed an invitation letter and information brochure explaining the study and then, where possible, followed up by telephone. Of the 3613 women contacted and invited to participate, 171 women were excluded due to illness (63), language difficulties (97) and death (11). Of the remaining 3442 women, 1613 agreed to participate and returned a questionnaire (47%). Six of them reported a history of ovarian cancer and 99 reported a previous bilateral oophorectomy and thus were excluded from the present study leaving 1508 population controls.

This study was approved by the Human Research Ethics Committees at the Peter MacCallum Cancer Centre, Queensland Institute of Medical Research, University of Melbourne, the Cancer Councils of New South Wales, South Australia and Victoria, the Cancer Foundation of Western Australia and all participating hospitals.

Data collection

After obtaining written informed consent, information was collected by a self-administered questionnaire that included questions about the demographic, medical, hormonal, reproductive, diet, family history and other potential risk factors for ovarian cancer. Women self-reported ever having a range of medical conditions including PCOS, severe acne as an adult, or excess body hair (face, chest or abdomen). The questionnaire included detailed questions about the use of hormone replacement therapy (tablets, implants, patches and gels/creams/pessaries) and other hormonal treatments
from which we were able to derive ever-use of testosterone or Danazol. Conditions or medication use after a reference date (defined as 1 year before the date of diagnosis for cases or date of first approach for controls) were excluded as they might have been influenced by the presence of preclinical disease.

Statistical analysis

Multivariable logistic models were used to adjust for potential confounders, including age at diagnosis/first approach, education, parity, hormonal contraceptive use and BMI. Other potential confounders that were considered for all analyses but not included in the final models since they did not substantially alter risk estimates were: state of residence, perineal talc use, history of hysterectomy or tubal sterilization, family history of breast or ovarian cancer in a first-degree relative, smoking, breastfeeding, menopausal status and level of recreational physical activity. For the analyses of PCOS, acne, hirsutism, testosterone supplements and Danazol use, the reference group was defined as women with no reported history of PCOS, hirsutism or acne. We also created a combined variable ‘any androgen-related disorder’ that included women who had a self-reported history of PCOS or acne or hirsutism.

Results

Based on the histopathology review, 1276 women had invasive cancer classified as follows: serous 847 (66%), endometrioid 142 (11%), clear cell 90 (7%), mucinous 42 (3%) and mixed or other histopathology 155 (12%). A further 315 women had borderline (low malignant potential) tumours classified as serous 152 (48%), mucinous 151 (48%) and other 12 (4%). Cases with mixed or other histopathology were excluded from the analyses by subtype. Descriptive statistics of the study population are presented in Table 1. Cases were significantly older than controls (mean age: cases, 57.9 years; controls, 56.4 years; \( P = 0.001 \)) and were less likely to have continued their education beyond high school. Cases were more likely to be nulliparous and to report a history of breast or ovarian cancer in a first-degree relative, and were less likely to have ever used oral contraceptives.

A history of PCOS was reported in 52 women; 130 reported a history of acne and 197 of hirsutism. Of the
women with PCOS, 12 of these also reported a history of hirsutism and 3 of acne. There was no overall association between PCOS, hirsutism or acne and all invasive cancers combined, but weak positive associations with PCOS and hirsutism were seen for borderline tumours (OR 1.8, 95% CI 0.8–3.9 and OR 1.5 95% CI 0.9–2.3 respectively; Table 2). The differences between the invasive and borderline tumours were statistically significant for PCOS ($P=0.049$) and borderline significant for hirsutism ($P=0.05$). When the histological subtypes were examined separately, a self-reported history of PCOS was associated with serous borderline tumours only (OR 2.5, 95% CI 1.0–6.1; Table 3). There was no significant association between self-reported acne or hirsutism and any of the subtypes of ovarian cancer although, as for borderline tumours overall, serous borderline tumours were non-significantly associated with hirsutism (OR 1.5, 95% CI 0.8–2.7). In our combined variable, history of any androgen-related disorder (PCOS or acne or hirsutism) was not associated with any of the subtypes of ovarian cancer (data not shown).

Table 4 considers the combined effects of PCOS and BMI (1 year prior to diagnosis) separately for all cancers, and by invasiveness and histology. There was no evidence of biological interaction between PCOS and BMI for invasive cancer. For borderline tumours, the OR was 0.9 (95%CI 0.2–4.4) for non-obese women with PCOS compared with non-obese women without PCOS, but rose to 3.0 (95% CI 1.2–7.5) for obese women with PCOS. This increased risk again appeared to be restricted to the single subtype of serous borderline tumours (OR 5.7; 95% CI 2.1–15.7 for obese women with PCOS). We did not observe any significant effect modification by OC use, menopausal status or parity.

Eleven cases (nine invasive and two borderline) and seven control women reported use of Danazol. After adjustment for age, education, parity, hormonal contraceptive use and self-reported endometriosis, the OR for the association between Danazol use and ovarian cancer (all cases) was 1.0 (95% CI 0.4–2.9). Eleven cases (all invasive) and four control women reported ever-use of testosterone (tablets, patches, troches and cream). The odds ratio for the association with invasive cancer (after adjustment for age, education, parity and hormonal contraceptive use) was 3.7 (95% CI 1.1–12.0).

### Discussion

In this large population-based case-control study, we found no evidence that self-reported histories of either PCOS, acne or hirsutism (all clinically associated with high circulating levels of androgens) were associated with increased ovarian cancer risk overall, although women with PCOS, who were also overweight, had a significantly increased risk of serous borderline tumours. Women who had ever used testosterone supplements had an increased risk of ovarian cancer while the use of the androgenic medication Danazol did not increase risk.


Other indirect evidence suggesting a possible aetiologic role for elevated androgens in the initiation and/or progression of ovarian cancer has come from

### Table 2 Multivariable adjusteda odds ratios (OR) and 95% confidence intervals (CI) of epithelial ovarian cancer for history of androgen-related disorders, by tumour invasiveness

<table>
<thead>
<tr>
<th>Androgen-related disorder</th>
<th>Controls</th>
<th>All cases</th>
<th>Invasive</th>
<th>Low malignant potential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>ORa (95% CI)</td>
<td>Cases</td>
<td>ORa (95% CI)</td>
</tr>
<tr>
<td>Polycystic ovary syndrome</td>
<td>24</td>
<td>28</td>
<td>1.1 (0.6–2.0)</td>
<td>16</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>96</td>
<td>101</td>
<td>1.0 (0.7–1.3)</td>
<td>72</td>
</tr>
<tr>
<td>Acne</td>
<td>68</td>
<td>62</td>
<td>0.9 (0.6–1.3)</td>
<td>51</td>
</tr>
</tbody>
</table>

aAdjusted for age, education, parity, hormonal contraceptive use, BMI 1 year ago.

bReference group was women with no self-reported history of PCOS, acne or hirsutism.
epidemiologic studies (Risch 1998). Firstly, it is well known that oral contraceptives, which suppress ovarian testosterone production (Gaspard et al. 1983, Murphy et al. 1990, Greer et al. 2005), protect against ovarian cancer. Secondly, there have been a number of case reports of ovarian cancer in female-to-male transsexuals who have undergone testosterone supplementation (Hage et al. 2000, Dizon et al. 2006), although the true incidence of the disease in this population is not yet known. Other potentially supportive evidence has come from a small number of population-based case-control studies. Schildkraut et al. (1996) observed a 2.5-fold increased risk of ovarian cancer among women with PCOS, although the analysis was based on a small number of women with PCOS (7 cases and 24 controls). Acne and hirsutism have also been associated with ovarian cancer (Wynder et al. 1969), while Cottreau et al. (2003) reported that after adjusting for age, gravidity, OC use and family history of ovarian cancer, women who used Danazol (n = 19) had over three times the risk for ovarian cancer compared with non-users.

Our data do not confirm these previous findings. We did not find any overall association with PCOS, acne or hirsutism, and our results for Danazol, based on a similar number of users (n = 18), did not confirm the findings of Cottreau et al. (2003). We did observe a relationship between PCOS and borderline serous tumours and can only speculate as to the reason for this. Current evidence derived from molecular and

Table 3 Multivariable adjusteda odds ratios (OR) and 95% confidence intervals (CI) of epithelial ovarian cancer for history of androgen-related disorders, by tumour invasiveness and histological subtype

<table>
<thead>
<tr>
<th>Androgen-related disorderb</th>
<th>Invasive cancer</th>
<th>Borderline tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serous (n = 847)</td>
<td>Endometrioid (n = 142)</td>
</tr>
<tr>
<td>Polycystic ovary syndrome (PCOS)</td>
<td>24</td>
<td>1.0 (0.5–2.0)</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>96</td>
<td>0.7 (0.5–1.1)</td>
</tr>
<tr>
<td>Acne</td>
<td>68</td>
<td>0.9 (0.6–1.5)</td>
</tr>
</tbody>
</table>

Estimates are not presented for the relationship between PCOS and endometrioid, clear cell and mucinous invasive cancers due to insufficient numbers of exposed cases.

aAdjusted for age, education, parity, hormonal contraceptive use and BMI 1 year ago.
bReference group was women with no self-reported history of PCOS, acne or hirsutism.

Table 4 Multivariable adjusteda odds ratios (OR) and 95% confidence intervals (CI) for the risk of epithelial ovarian cancer according to the combined effect of history of polycystic ovary syndrome (PCOS) and body mass index (BMI; reference group is BMI < 25, no PCOS)

<table>
<thead>
<tr>
<th>BMI 1 year ago</th>
<th>No PCOS</th>
<th>PCOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n)</td>
<td>Cases (n)</td>
</tr>
<tr>
<td>&lt; 25</td>
<td>684</td>
<td>600</td>
</tr>
<tr>
<td>≥ 25</td>
<td>777</td>
<td>786</td>
</tr>
<tr>
<td>Invasive cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>684</td>
<td>480</td>
</tr>
<tr>
<td>≥ 25</td>
<td>777</td>
<td>639</td>
</tr>
<tr>
<td>Borderline cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>684</td>
<td>120</td>
</tr>
<tr>
<td>≥ 25</td>
<td>777</td>
<td>147</td>
</tr>
<tr>
<td>Serous borderline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>684</td>
<td>48</td>
</tr>
<tr>
<td>≥ 25</td>
<td>777</td>
<td>79</td>
</tr>
<tr>
<td>Mucinous borderline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>684</td>
<td>68</td>
</tr>
<tr>
<td>≥ 25</td>
<td>777</td>
<td>63</td>
</tr>
</tbody>
</table>

aAdjusted for age, education, parity and hormonal contraceptive use.
genetic studies suggests that the borderline and invasive serous tumours develop through independent pathways (Singer et al. 2003, Shih le & Kurman 2004, Bell 2005), and thus the endocrine consequences of PCOS may have differential effects on the pathogenesis of these different tumour types. In addition, PCOS is a complex disorder associated with alterations in endogenous sex hormone levels, suppression of ovulation, infertility and a number of metabolic disorders including insulin resistance (Solomon 1999). This complex array of conditions make it difficult to assess the effect of increased androgen levels on ovarian cancer risk in isolation, and may also explain why we did not observe an association for some subtypes. We observed a threefold increased risk of ovarian cancer for women who had ever used testosterone supplements, and although our analysis was based on a very small number of women, this finding is potentially interesting and warrants further investigation.

Four prospective studies have examined the association between circulating androgens and the risk of ovarian cancer (Helzlsouer et al. 1995, Lukanova et al. 2003, Rinaldi et al. 2007, Tworoger et al. 2007). Although the first study, based on only 31 exposed cases, found an increasing risk of ovarian cancer with increasing levels of androstenedione and dehydroepiandrosterone (Helzlsouer et al. 1995), other larger and more recent studies have not observed an association between prediagnostic androgens and ovarian cancer risk (Lukanova et al. 2003, Tworoger et al. 2007). Rinaldi et al. (2007) observed an inverse association between free testosterone concentrations and ovarian cancer risk in postmenopausal women only (192 cases); however, other studies have not confirmed this finding, and our data do not suggest a differential effect by menopausal status. The results from prospective studies of circulating androgen levels are thus generally null, possibly because circulating androgens may not reflect androgen exposure at the tissue level. The ovarian epithelium is not vascular and thus paracrine hormonal influences may be more important than endocrine sources (Lukanova & Kaaks 2005).

Strengths of our study include the population-based design, large number of cases and detailed information on multiple exposures. A limitation was the relatively low participation rate among controls (47%), which could have resulted in selection bias; however, a comparison with the data from the Australian National Health Survey (NHS) conducted in 2004 (a representative survey of the Australian adult population; ABS 2006) revealed that the distributions of education level, parity and BMI among our control women were almost identical to those from the NHS (Jordan et al. 2007), and it is therefore unlikely that non-response could have resulted in appreciable bias. Another limitation was reliance upon self-reported medical history of PCOS, hirsutism and acne, and medication use. PCOS is a complex condition that may not be accurately reported by women. There may also be asymptomatic women with PCOS in our study population (Polson et al. 1988, Azziz et al. 2004); however, such under-reporting of PCOS would most likely have been random, and probably would have resulted in bias towards the null. It is possible that the cases were more likely to recall a history of PCOS and also use of medications/hormonal preparations than controls; however, this cannot explain the observed association between PCOS and the minority of borderline cases but not invasive cases. Women are unlikely to associate hirsutism and acne with ovarian cancer and therefore any misclassification of these conditions is expected to be non-differential.

In summary, we found no consistent evidence for a role of androgens in the aetiology of ovarian cancer, overall or by subtype, and thus our findings do not support the hypothesis that androgen-related disorders increase the risk of ovarian cancer. Although laboratory studies have suggested a role for androgens in the development of ovarian cancer, there is very little epidemiological evidence to support an association. Studies are also constrained by small sample sizes, and there is heterogeneity both in the types of exposure measures reported and the research findings. The results from prospective studies of circulating androgen levels are generally null. Large collaborative analyses are required to examine the associations between markers of high androgen levels and risk of ovarian cancer subtypes, and future research should target the relative roles of endocrine versus paracrine androgen sources.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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