Selenium-Binding Protein 1 (SBP1) autoantibodies in ovarian disorders and ovarian cancer

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Abstract

Infertility is a risk factor for ovarian cancer (OvCa). The goal was to determine if antibodies to selenium-binding protein 1 (SBP1), an autoantibody we identified in patients with premature ovarian failure (POF), occurs in both infertility and OvCa patients, and thus could be associated with preneoplasia. Anti-SBP1 was measured by immunoassay against recombinant SBP1, in sera from OvCa ($n = 41$), infertility ($n = 92$) and control ($n = 87$) patients. Infertility causes were POF, unexplained, irregular ovulation or endometriosis. The percent of anti-SBP1-positive sera was higher in POF ($P = 0.02$), irregular ovulation ($P = 0.001$), unexplained causes ($P = 0.02$), late (III–IV)-stage OvCa ($P = 0.02$) but was not significant in endometriosis, benign ovarian tumors/cysts, early stage (I–II) OvCa or uterine cancer compared to healthy controls. Anti-SBP1 was significantly higher in women with serous ($P = 0.04$) but not non-serous ($P = 0.33$) OvCa compared to controls. Also, we determined if anti-SBP1 was associated with CA125 or anti-TP53, markers often studied in OvCa. Anti-TP53 and CA125 were measured by established immunoassays. The ability of anti-SBP1 alone to discriminate infertility or OvCa from controls or when combined with anti-TP53 and CA125, to identify OvCa was evaluated by comparing the area under the curve (AUC) in ROC analysis. Anti-SBP1 alone discriminated infertility (AUC = 0.7; $P = 0.001$) or OvCa (AUC = 0.67; $P = 0.03$) from controls. The sensitivity and specificity of OvCa identification was increased by combining CA125, anti-TP53 and anti-SBP1 (AUC = 0.96). Therefore, anti-SBP1 occurs in infertile women with POF, ovulatory disturbances or unexplained infertility and in serous OvCa. This suggests an autoimmune process is associated with the development of serous OvCa.
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Introduction

Longitudinal studies show that ovarian cancer is significantly increased in women who experienced certain subtypes of infertility compared to the population (Brinton *et al.* 2004, 2005, Jensen *et al.* 2008). Although there are recent reports of potential genetic links between some types of infertility, such as endometriosis and ovarian cancer (Prowse *et al.* 2006, Munksgaard & Blaakaer 2012, Lee *et al.* 2016), little is known of the biologic basis for the link between other causes of infertility, such as anovulation and ovarian cancer.

Autoimmunity has been implicated in cancer risk (de Visser *et al.* 2006, Tan & Coussens 2007, Franks & Slansky 2012, Hemminki *et al.* 2012, Vendramini-Costa & Carvalho 2012). Many patients with tumors, including ovarian cancer, produce autoantibodies to tumor antigens (Stockert *et al.* 1998, Barua *et al.* 2007, Tan & Zhang 2008, Bei *et al.* 2009, Gnjatic *et al.* 2009, 2010, Chatterjee & Tainsky 2010). Autoantibodies have been shown to precede many tumors (Casiano *et al.* 2006, Qiu & Hanash 2009, Zaenker & Ziman 2013). For example, autoantibodies associated with liver cirrhosis precede liver cancer and colitis-associated autoantibodies predict colon cancer (Zhang & Tan 2010, Dai *et al.* 2013, Zaenker & Ziman 2013). This is consistent with the concept that inflammation may be a part of an early mechanism in cancer development (Lundin *et al.* 2009, Trabert *et al.* 2014).

Autoantibodies could potentially be used to identify women at increased risk for ovarian cancer. We identified ovarian autoantibodies in premature

Table 1   Summary of patient characteristics.

Patient category	n	Age mean ± s.d. (range) (years)	FSH mean ± s.d. (range), (IU/L) (range) (IU/mL)
Infertility	92		
Endometriosis	18	41.3 ± 15.0 (28–70)	7.1 ± 2.6 (4.9–12.1)
Ovulatory dysfunction	12	31.7 ± 4.7 (24–40)	16.9 ± 24.9 (1.2–72)
Unexplained infertility	37	33.2 ± 4.7 (21–49)	7.4 ± 3.1 (1.9–16.5)
Premature ovarian failure	25	30.1 ± 6.6 (19–42)	60.4 ± 37.8 (19.1–123)
Ovarian cancer	41		
Early stage	14	58.3 ± 13.6 (37–93)	nd
Late stage	27	56.5 ± 15.7 (26–85)	nd
Benign tumor or cyst	23	56.5 ± 15.7 (26–85)	nd
Uterine cancer	18	66.3 ± 11.5 (47–83)	nd
Controls			
Normal (random population)	30	38.7 ± 15.9 (18–65)	nd
Assay (selected)	16	56.5 ± 15.7 (26–85)	nd
Total	220		

nd, not determined.

ovarian failure (POF), suggesting that there is an autoimmune process against the ovary (Luborsky *et al.* 1990, 1999, Meyer *et al.* 1990, Luborsky 2002). POF is also known as ovarian insufficiency, a term which includes the continuum of reduced ovarian function (e.g., disturbances in ovulation) and failure of ovarian function (Nelson 2009, Jin *et al.* 2012). Ovarian insufficiency is associated with ovarian autoantibodies (Luborsky 2002, Forges *et al.* 2006, Sundblad *et al.* 2006, Nelson 2009, Pires & Khole 2009). Specific autoantigens such as HSP90 (Pires & Khole 2009, Pires *et al.* 2011, Choudhury & Khole 2015) and enolase (Sundblad *et al.* 2006) were reported in POF and infertility, although autoantibodies to these antigens also occur as part of the background antibody repertoire in healthy individuals (Pashov *et al.* 2002). Using immune-proteomics, we found several unique autoantigens including selenium-binding protein 1 (SBP1) associated with infertility and POF (Edassery *et al.* 2010). SBP1 is thought to have a tumor suppressor function, and it inhibits tumor growth in nude mice (Pohl *et al.* 2009, Fang *et al.* 2010). SBP1 is involved in selenium metabolism, and its expression is reduced in many cancers including ovarian cancer (Huang *et al.* 2006, 2012, Li *et al.* 2008, Silvers *et al.* 2010, Zhang *et al.* 2011, Yang & Diamond 2013).

The primary objective was to determine if SBP1 autoantibodies occur in infertile women and women with ovarian cancer. If true, this would support the concept that there is a link between ovarian autoimmunity and ovarian cancer. Secondarily we examined the relationship of anti-SBP1 with CA125 levels and anti-TP53 to determine if these identified similar or different patients. CA125, although lacking strong specificity, remains the best marker for ovarian cancer (Cramer *et al.* 2011). Anti-TP53 is frequent in ovarian cancer (Erkanli *et al.* 2006, Anderson *et al.* 2010) as well as other malignancies (Soussi 2000, Li *et al.* 2005).

Materials and methods

Patients and sera

Patients with cancer, infertility and healthy women ($n = 220$) (Table 1) contributed blood samples after informed consent. All procedures followed protocols approved by the review boards for protection of human subjects in research at each institution. Blood was collected into a red top tube and aliquots of serum were stored at −80°C. Blood collection from women with regular menstrual cycles was collected on cycle day 3 at the time a clinical sample was taken for hormone evaluation. For women with irregular or no menstrual cycles (i.e., cancer patients, premature ovarian failure and ovulatory disturbances), blood was taken at the time of a clinic visit. For normal healthy female experimental controls, the timing of the blood sample was unavailable. Women were not on hormone replacement or hormone contraceptives.

Infertility

Infertility sera ($n = 92$) were collected from infertility clinics at Rush University Medical Center, the Center for Human Reproduction (courtesy of Dr Carolyn Coulam) and from the University of Ulm, Germany (courtesy of Dr Cosima Brucker). Sera included endometriosis ($n = 18$), ovulatory dysfunction ($n = 12$), unexplained infertility ($n = 37$) and premature ovarian failure (POF) ($n = 25$). Female infertility includes diagnostic sub-categories of non-ovarian etiologies such as endometriosis (endometrial cells outside the uterus) and ovarian causes such as ovulatory dysfunction (irregular or absent ovulation), diminished ovarian reserve (reduced or absent oocyte content, which includes premature ovarian failure (POF) and idiopathic infertility (Molinaro *et al.* 2009). Unexplained infertility is a diagnostic category used when standard clinical and laboratory data are normal. POF is spontaneous menopause before age 40 years (Luborsky 2002, Luborsky *et al.* 2003, Goswami & Conway 2005) and includes elevated FSH (usually over 40 IU/L (Jin *et al.* 2012)). POF is also known as ovarian insufficiency, a term that includes reduced ovarian functions (e.g., irregular menstrual cycles and ovulation, and reduced steroid hormone production and POF) before age 40

(Nelson 2009, Jin *et al.* 2012). For the purpose of this study, impaired ovulation and POF are evaluated as separate groups, but in the discussion, the combined results are referred to as ovarian insufficiency for simplicity.

Ovarian cancer

Sera from patients with early- ($n = 14$) and late-stage ($n = 27$) ovarian cancer or a benign ovarian tumor or cyst ($n = 23$) were obtained from women entering the clinic for the evaluation of abdominal discomfort or pelvic mass at Rush University Medical Center or the University of Washington. The diagnosis of ovarian cancer was confirmed by surgery and pathology evaluation of tissue. Stages were defined by a board-certified pathologist, guided by the FIGO system, as early stage with no spread beyond the pelvis and late stage involving metastasis beyond the pelvic area.

Controls

Assay reference controls ($n = 16$) were selected from self-defined healthy women without diagnosed autoimmune disease or cancer and within a similar age range as those with infertility. The assay reference control sera were used to monitor inter-assay performance and to determine a cutoff value for a positive antibody result. Experimental normal healthy female control sera from healthy woman (without detailed histories) were obtained from a commercial source (ProMedDx, $n = 30$). Patients with uterine cancer ($n = 18$) or with benign ovarian tumors or cysts ($n = 23$) were used as comparison/control groups to assess the specificity of autoantibodies to ovarian cancer.

Proteins and assays

Recombinant selenium-binding protein 1

His-tagged recombinant SBP1 (rSBP1) was produced in E Coli using a PET28 expression vector. The rSBP1 was purified initially using a Nickel column (Ni-NTA, Qiagen) to at least 95% purity. In initial studies, some control sera had anti-SBP1 in immunoassay and one-dimensional Western blot. Using proteomics, the immunoreaction of control sera was identified as a contaminant of a similar molecular size as rSBP1. Therefore, the rSBP1 was subjected to additional purification using size exclusion (Superdex 200 16/60 column, GE Healthcare) and ion exchange columns (Hi-Trap Q column, GE Healthcare), and this highly purified protein was used in anti-SBP1 immunoassays. The immunogenicity of the rSBP1 was tested in Western blots using several anti-SBP1 antibodies; the rSBP1 was reactive against two anti-SBP1 monoclonal antibodies and a polyclonal antibody to rSBP1 raised in chicken that were developed in our laboratory and a commercial monoclonal antibody (clone 4D4 MBL, Nagoya, Japan).

Anti-SBP1 immunoassay

Plates (Medisorp, Nunc, Thermo Scientific) were coated (overnight, 4°C) with rSBP1 (200 ng/well) in bicarbonate buffer (50 mM, pH 9.7). For each serum, wells without antigen (bicarbonate buffer only) were incubated as control for nonspecific binding of serum to plates. Nonspecific binding sites were blocked with 5% BSA in wash buffer (PBS with 0.05% Triton X-100; 2 h, 22°C). Diluted patient sera (1:100, 0.1 mL/well) in wash buffer containing 1% BSA were added (90 min; 22°C). Autoantibodies to SBP1 were detected with goat anti-human IgG-HRP (1:50,000/0.1 mL/well, 1 h, 22°C) (Jackson Immunoresearch). After washing, TMB peroxidase substrate (1-STEP Ultra TMB–ELISA, Thermo Scientific) was added (0.1 mL, 22°C in dark), and the reaction was stopped after 15 min by adding stop solution (1 M sulfuric acid, 0.05 mL, VWR, West Chester, PA, USA). The anti-SBP1 level in the serum was measured as the optical density (OD) at 450 nm with 580 nm as reference using a SPECTRA Max Plus. For each serum, OD values in wells without antigen were subtracted from the OD values in wells with SBP1. The cutoff value for a positive result was calculated as the mean OD value for assay reference controls ($n = 16$) plus two standard deviations (95% confidence level).

CA125 assay

CA125 level was measured by an established singleplex Luminex bead-based assay (Scholler *et al.* 2006). The mean fluorescence intensity (MFI) data were normalized to the pooled control sera. On each plate, several replicates of a pooled control sample of sera from nine healthy post-menopausal women (Scholler *et al.* 2006) were included. To normalize the results, the MFI readings from each unknown were divided by the average reading from the pooled controls within each plate. The resulting normalized values are multiples of the average reading from the control pool.

Anti-TP53 antibody immunoassay

Serum anti-TP53 was measured using an anti-TP53 commercial kit (MESACUP; MBL International Corporation, Woburn, MA, USA). Sera were diluted to 1:100 in the Assay Diluent provided and anti-TP53 determined according to the manufacturer's instructions. The absorbance was read at 450 nm with reference at 620 nm. Data were obtained as the OD value, and the level of anti-TP53 was calculated from the standard curve as supplied by the manufacturer with 'arbitrary' units of U/mL. A cutoff value for anti-TP53 positivity was determined from 15 normal female sera, which were randomly selected from normal controls as the value above the mean OD plus two standard deviations (95% confidence level).

Statistical analysis

The significance of differences in mean OD values for anti-SBP1, CA125 and anti-TP53 in cases and controls were assessed by ANOVA (Dunnett's multiple comparison test). Significant differences in the proportion of anti-SBP1 and anti-TP53 positive sera were determined using the Fishers exact test. Correlations between CA125 and other biomarkers were determined, and significant correlations were identified using Spearman's rank correlation. For all tests, $P < 0.05$ was considered significant.

The ability of markers to discriminate cancer from control specimens was evaluated by receiver-operating characteristic

(ROC) curve; the area under curve (AUC) and the probability (*P*) that the AUC differed significantly from random (AUC = 0.5) was calculated using SAS or GraphPad Prism. Logistic regression was used to combine multiple biomarker levels into a linear relationship to analyze the discrimination ability of combined biomarkers using ROC curve analysis.

Results

Anti-SBP antibody levels

Overall, the mean OD (0.72; *P* = 0.04) and the proportion of sera positive (30%; *P* = 0.03) for anti-SBP1 was significantly higher in infertility compared to normal controls (Table 2). Among women with infertility, the mean OD value for anti-SBP1 was significantly higher in women with ovulatory dysfunction (*P* < 0.0001), unexplained infertility (*P* = 0.01) and POF (*P* = 0.03) but not endometriosis (*P* = 0.07) compared with healthy controls (Fig. 1 and Table 2). The proportion of anti-SBP1 positive sera was significantly higher in women with ovulatory dysfunction (50.0%; *P* = 0.007), unexplained infertility (24.3%; *P* = 0.02) and POF (28.0%; *P* = 0.02), but not endometriosis (5.6%; *P* = 1.00), compared to control (Table 2).

In ovarian cancer, anti-SBP1 was higher than that in healthy controls, and the difference approached significance whether using mean OD values (0.69 ± 0.37 vs 0.53 ± 0.21 respectively; *P* = 0.07) or the percent of positive sera (18.1% vs 3.3% respectively; *P* = 0.07) (Table 2). Anti-SBP1 was low and did not differ among control sera (e.g., benign ovarian tumors or cysts, uterine cancer and healthy controls).

As serous ovarian tumors are more often associated with anti-tumor antibody responses (Gnjatic *et al.* 2010),

Table 2 SBP1 antibody in infertility, ovarian cancer and controls compared to healthy women.

|	**OD value** mean ± s.d. (range)	**% POS** (95% confidence) % (*n*/total)
Infertility		
All	0.72 ± 0.26 (0.28–1.40)**	30 (22/74)*
Endometriosis	0.67 ± 0.19 (0.27–1.02)	6 (1/18)
Ovulatory dysfunction	0.87 ± 0.24 (0.55–1.30)***	50 (6/12)**
Unexplained infertility	0.68 ± 0.25 (0.38–1.31)*	24 (9/37)*
Premature ovarian failure	0.68 ± 0.28 (0.28–1.35)*	28 (7/25)*
Ovarian cancer		
All	0.69 ± 0.37 (0.22–1.93)	18 (7/33)
Early	0.54 ± 0.28 (0.29–0.99)	17 (1/6)
Late	0.72 ± 0.38 (0.22–1.93)*	22 (6/27)*
Serous	0.76 ± 0.43 (0.22–1.93)*	26 (5/19)*
Non-serous	0.59 ± 0.25 (0.26–1.14)	14 (2/14)
Controls		
Normal	0.53 ± 0.21 (0.16–0.99)	3 (1/30)
Uterine cancer	0.58 ± 0.17 (0.27–0.97)	6 (1/18)
Benign ovary tumor/ cyst	0.57 ± 0.16 (0.31–0.88)	0 (0/23)

Significance is indicated as *P = 0.05–0.01; **P = 0.01–0.001; ***P < 0.001 compared to control.

we compared anti-SBP1 by serous and non-serous histology. Compared to controls (mean OD = 0.53 ± 0.21), anti-SBP1 was significantly higher in serous (mean OD = 0.76 ± 0.43; *P* = 0.03) but not non-serous (mean OD = 0.59 ± 0.25; *P* = 0.9) ovarian cancer (Table 2).

Early stage (I–II) with late stage (III–IV) ovarian cancer was also compared. The mean OD value for anti-SBP1 was higher in late stage (0.72 ± 0.38; *P* = 0.02) but not in early stage (0.56 ± 0.25; *P* = 0.73) ovarian cancer than that in controls (0.53 ± 0.21). However, this may reflect the presence of significantly more serous histology tumors in late stage (70%; 19 of 27) than early stage in the study groups (21%; 3 of 14) (early vs late, *P* = 0.007).

CA125 level

As expected, CA125 was significantly higher in patients with ovarian cancer (mean MFI = 44.33 ± 55.25; *P* = 0.001) compared to healthy controls (mean MFI = 1.31 ± 1.79). CA125 was not elevated in infertility
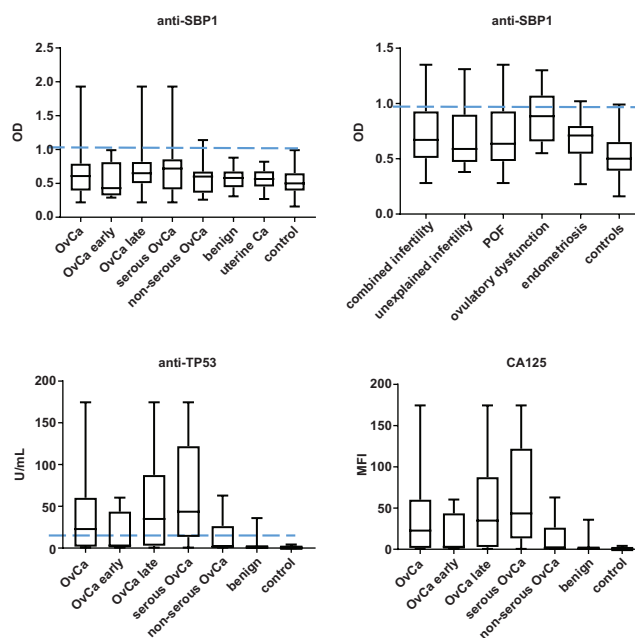
Figure 1 Box and whiskers plot of anti-SBP1, anti-TP53 and CA125 by study group. Anti-SBP1 in infertility (upper right), ovarian cancer (upper left) and controls is shown as the optical density (OD) value. For anti-SBP1 in ovarian cancer (OvCa), the subgroups serous vs non-serous histology tumors, as well as early- (stage I–II) vs late-stage (III–IV), are shown in comparison to women with benign tumors (benign) or uterine cancer (uterine Ca) and healthy controls. Anti-SBP1 in infertility (experimental groups of infertility combined) and the subgroups premature ovarian failure (POF), unexplained infertility, ovulatory dysfunction (irregular or absent ovulation) and endometriosis are shown in comparison to healthy controls. Anti-TP53 (lower left) is shown as U/mL. CA125 (lower right) is displayed as mean fluorescence intensity units (MFI). Sera were tested at 1:100. The cutoff value for a positive result in the antibody graphs is shown as a dotted horizontal line (anti-SBP1 = 0.93; anti-TP53 = 2.6).

(mean MFI = 1.82 ± 1.75; *P* = 0.89) compared to healthy controls.

Anti-TP53 antibody level

The mean OD value for anti-TP53 was significantly higher in women with ovarian cancer (12.33 ± 21.20 U/mL; *P* = 0.001), but not in women with infertility (1.0 ± 3.48; *P* = 0.91) or a benign ovarian tumor or cyst (4.62 ± 8.19; *P* = 0.86) compared to healthy controls (0.65 ± 1.05 U/mL). The number of anti-TP53 positive sera was significantly higher in women with ovarian cancer (40.7% positive; *P* = 0.03) and was significantly higher in patients with late-stage serous (52.6% positive; *P* = 0.01) but not non-serous (12.5% positive; *P* > 0.9) histology ovarian tumors compared to control.

Sensitivity and specificity of anti-SBP1 alone or with CA125 and anti-TP53

Anti-SBP1 was not correlated with CA125 in ovarian cancer (correlation coefficient = 0.10; *P* = 0.61) or in infertility (correlation coefficient = 0.07; *P* = 0.59). Likewise, anti-SBP1 was not correlated with anti-TP53 in ovarian cancer (correlation coefficient = 0.03; *P* = 0.88) or in infertility (correlation coefficient = −0.25; *P* = 0.07).

Anti-SBP1 discriminated infertility (POF, unexplained infertility, ovulatory dysfunction and endometriosis) from healthy controls with an AUC of 0.7 (*P* = 0.001) (Table 3). As anti-TP53 and CA125 were not elevated in infertility, analysis of combined markers was not performed.

Consistent with prior studies in ovarian cancer (Cramer *et al.* 2011, Zhu *et al.* 2011), CA125 was the best single marker by itself, with the highest AUC (AUC = 0.85; *P* < 0.001) compared to anti-SBP1 (AUC = 0.67; *P* = 0.03), anti-TP53 (AUC = 0.75; *P* = 0.008). Combination of CA125, anti-TP53 and anti-SBP1 increased the discrimination of ovarian cancer from control (AUC = 0.96; *P* < 0.001) (Table 3).

Discussion

The results show that anti-SBP1 occurs primarily in women with infertility related to ovarian insufficiency and serous ovarian cancer. This study extends our original identification of SBP1 as an autoantigen target of anti-ovarian antibodies in premature ovarian failure (i.e., premature menopause) (Edassery *et al.* 2010). It suggests the possibility that very early changes, which may show up as infertility or suboptimal reproductive function, may occur in the ovary long before a tumor is detectable. The role of organ-specific autoantibodies in infertility is not known. Antibodies could be pathogenic, due to tissue damage or could indicate an etiology such as autoimmunity (Naparstek & Plotz 1993, Rowley & Whittingham 2015).

Table 3 Area under the curve (AUC) for ovarian cancer and infertility.

Comparison groups	Marker	AUC	*P* value
Infertility vs healthy	Anti-SBP1	0.7	0.001
OVCA vs healthy	Anti-SBP1	0.67	0.03
Serous OvCa vs healthy	Anti-SBP1	0.65	0.05
OVCA vs healthy	Anti-TP53	0.75	0.008
OVCA vs healthy	CA-125	0.85	<0.001
OVCA vs healthy	Anti-SBP/anti-TP53/CA125	0.96	<0.001

Ovarian cancer is significantly increased in women with a history of certain subtypes of infertility compared to the population (Brinton *et al.* 2004, 2005, Jensen *et al.* 2008, Pearce *et al.* 2015). For example, a longitudinal study that followed women with infertility for over 25 years, showed that various causes of infertility such as endometriosis, had increased risk for ovarian cancer compared to the general population (Brinton *et al.* 2004).

Recent studies show that endometriosis related to ovarian cancer is associated with genetic mutations (Lee *et al.* 2016). Endometriosis is more often associated with the development of endometrioid or clear-cell ovarian cancer (Pearce *et al.* 2012). Endometrioid or clear-cell histotypes may be less immunogenic as there were significantly less serum autoantibodies than those in serous ovarian cancer (Gnjatic *et al.* 2010). As we did not find anti-SBP1 antibodies in infertility associated with endometriosis or in non-serous ovarian cancer, it is possible that this is related to different etiologies for specific histological types of ovarian cancer. This would be consistent with the differences in biochemistry and gene mutation profiles among different histological types of ovarian tumors (Marquez *et al.* 2005, Zorn *et al.* 2005, Kobel *et al.* 2008). Thus, the shared SBP1 autoantibodies in ovarian insufficiency and serous ovarian cancer may reflect the involvement of an autoimmune process in the etiology of serous ovarian cancer. We speculate that this could occur in multiple ways. For example, autoimmunity could occur as a response to ovarian cells shed during ovulation, a response to fallopian tube cells shed onto the ovarian surface and/or as a response to mutations.

The results are congruent with other reports of shared autoantibody repertoires in autoimmunity and cancer (Tan & Coussens 2007, Bei *et al.* 2009, Franks & Slansky 2012) and with a relationship of autoantibodies in cirrhosis to the development of liver cancer (Tan & Zhang 2008), melanoma (Ramirez-Montagut *et al.* 2003) and other cancers (Franks & Slansky 2012). The finding in this study is similar to our previous study of anti-mesothelin (Luborsky *et al.* 2011). Overall, the results suggest that anti-SBP1 in women with infertility related to ovarian insufficiency may be a measurable indicator of risk for ovarian cancer. Although further investigation is needed, it is possible that tests for these antibodies could form part of an assessment for identifying young women at risk for serous ovarian cancer.

A recent multi-center study evaluated existing ovarian cancer biomarkers using pre-diagnostic serial sera samples from the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) screening trial (Cramer *et al.* 2011) found that several biomarkers, including mesothelin and HE4, when combined with CA125, improved the detection of ovarian cancer over CA125 alone. CA125 alone had limited sensitivity in detecting ovarian cancer, especially early-stage ovarian cancer (Cramer *et al.* 2011). Our findings agree with others that anti-TP53 may have utility in ovarian cancer detection (Erkanli *et al.* 2006, Cramer *et al.* 2011) when combined with other markers. Based on the ROC curve analysis, combining anti-SBP1 and anti-TP53 with CA125 increased the area under the ROC curve over CA125 alone to a level that suggests the combination could be investigated for the detection of ovarian cancer.

Interestingly, the association of specific antibodies with both prematurely reduced ovarian function and ovarian cancer is consistent with our results in the hen, a spontaneous animal model of human ovarian cancer. The histology, biochemistry, genetic mutations and tumor immunology in this model have striking similarities with human ovarian tumors (Rodriguez-Burford *et al.* 2001, Barua *et al.* 2009*a*,*b*, Hakim *et al.* 2009, Gonzalez Bosquet *et al.* 2011, Bradaric *et al.* 2013). Both anti-SBP1 (Stammer *et al.* 2008) and anti-mesothelin (Yu *et al.* 2011) are associated with reduced ovarian function and with ovarian tumors in the hen.

In summary, this is the first study to demonstrate SBP1 autoantibodies in ovarian insufficiency and cancer and is an extension of our previous reports of anti-mesothelin (Luborsky *et al.* 2011) and anti-HE4 (Hellstrom *et al.* 2013) in ovarian cancer. Thus, there appears to be a link between autoantibodies in infertility and serous ovarian cancer. Our data suggest that autoantibodies may predict risk and could be useful in identifying women for more intense study and evaluation.

Declaration of interest

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