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Review Article

Systematic review: Tumor-associated antigen autoantibodies and ovarian cancer early detection

Renée Turzanski Fortner, Antje Damms-Machado, Rudolf Kaaks *

Division of Cancer Epidemiology, German Cancer Research Center (DFKZ), Heidelberg, Germany

HIGHLIGHTS

- AAbs for ovarian cancer earlier detection is an emerging area.
- Reviewed diagnostic discrimination of 85 AAbs for ovarian cancer early detection.
- AAb panels may eventually reach diagnostic discrimination for earlier detection.

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ABSTRACT

Objectives. Tumor-associated autoantibodies (AAbs), produced as an immune response to tumor-associated antigens (TAAs), are a novel pathway of early detection markers.

Methods. We conducted a systematic review on AAbs and ovarian cancer to summarize the diagnostic performance of individual AAbs and AAb panels. A total of 29 studies including 85 AAbs were included; 27 of the studies were conducted in prevalent cases and cancer-free controls and 2 investigations included pre-diagnosis samples. The majority of studies were hypothesis-driven, evaluating AAbs to target TAAs; 10 studies used screening approaches such as serological expression cloning (SEREX) and nucleic acid-programmable protein arrays (NAPPA).

Results. The highest sensitivities for individual AAbs were reported for RhoGDI-AAbs (89.5%) and TUBA1C-AAbs (89%); however, specificity levels were relatively low (80% and 75%, respectively). High sensitivities at high specificities were reported for HOXA7-AAbs for detection of moderately differentiated ovarian tumors (66.7% sensitivity at 100% specificity) and IL8-AAbs in stage I–II ovarian cancer (65.5% sensitivity at 98% specificity). A panel of 11 AAbs (ICAM3, CTAG2, p53, STYXL1, PVR, POMC, NUDT11, TRIM39, UHMK1, KSR1, and NXF3) provided 45% sensitivity at 98% specificity for serous ovarian cancer, when at least 2 AAbs were above a threshold of 95% specificity. Twelve of the AAbs identified in this review were investigated in more than one study. Data on diagnostic discrimination by tumor histology and stage at diagnosis are sparse. Limited data suggest select AAb markers improve diagnostic discrimination when combined with markers such as CA125 and HE4.

Conclusions. AAbs for ovarian cancer early detection is an emerging area, and large-scale, prospective investigations considering histology and stage are required for discovery and validation. However, data to date suggests panels of AAbs may eventually reach sufficient diagnostic discrimination to allow earlier detection of disease as a complement to existing markers and transvaginal ultrasound.

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* Corresponding author at: German Cancer Research Center (DKFZ), Division of Cancer Epidemiology, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany.
E-mail address: r.kaaks@dkfz.de (R. Kaaks).

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1. Introduction

Ovarian cancer is a lethal gynecologic malignancy, with relatively poor long-term survival (e.g., 5-year survival of 46.5% in the United States [1] and 37.6% in Europe [2]) given most cases are diagnosed at late stage after the cancer has spread [3]. Survival rates are substantially better for cases diagnosed at early stage (e.g., 5-year survival 89% for stage I disease vs. 17% for stage IV disease [3]); however, there are no effective strategies for screening or early detection, and early disease symptoms, if any, are non-specific. Ovarian tumor-associated antigens CA125 (MUC16) and human epididymis protein 4 (HE4) are the most sensitive and specific ovarian cancer early detection markers identified to date. However, diagnostic discrimination remains suboptimal, with limited predictive utility for screening given low sensitivity for early stage disease [4,5]. Studies evaluating serial CA125 measures using the Risk of Ovarian Cancer Algorithm (ROCA) show that this algorithm may improve sensitivity for earlier stage disease [6,7]. However, additional markers for screening, and to be used together with transvaginal ultrasound (TVUS), are needed.

Tumor-associated autoantibodies (AAbs) are produced as an immune response to aberrantly expressed, mutated or post-translationally modified proteins or other auto-antigens associated with tumors and may be enhanced by tumor-associated inflammation [8]. These markers represent one novel pathway of early detection markers for cancer. AAbs are particularly attractive as diagnostic biomarkers for cancer given they may circulate at higher concentrations than their corresponding antigen, demonstrate higher stability over time [9,10], and may be detectable at earlier disease stage [11].

Searches for AAbs have been accelerated by the advent of proteomics technologies, and led to increasing numbers of AAbs for which elevated serum levels were found in cancer patients, for a variety of cancer types [12]. For ovarian cancer, >80 circulating AAbs have been investigated, with select antibodies demonstrating high tumor specificity (>95–98%) at elevated serum levels, though at limited sensitivity (generally <20%). We present a systematic review on circulating AAbs examined as potential biomarkers for early detection of ovarian cancer.

2. Methods

2.1. Literature search

A systematic search in PubMed was conducted independently by two investigators (RK and ADM). We searched for articles written in English with citations indexed up to November 8, 2016 and not limited to publication year using the following search string: [(antibodies OR autoantibodies OR immunoglobulin) AND (tumor-associated antigen OR tumor-specific antigen) AND (diagnosis OR diagnostic OR detection OR biomarker) AND (cancer OR carcinoma OR neoplasm OR tumor) AND (ovar OR ovary OR ovarian) AND (blood OR serum OR plasma) NOT (therapy AND survival)]. Additionally, we performed a hand search of the reference lists of studies identified through PubMed. The search was re-run on June 28, 2017 to ensure inclusion of recent relevant studies.

2.2. Eligibility criteria

This systematic review focused on AAbs against tumor-associated or tumor-specific antigens, as potential diagnostic blood-based biomarkers for ovarian cancer. Therefore, we selected only articles reporting on circulating AAbs and immunoglobulins against these proteins, or immune complexes, with comparisons between ovarian cancer patients and cancer-free control subjects. For articles to be eligible for inclusion, we required the study to include information about sample size for case and control groups, as well as about diagnostic performance (sensitivities, specificities, area under the receiver operator characteristic [ROC] curve [AUC]). Articles providing basic information on frequencies of positive test results or false positive/false negative rates within the cases or control groups, which allowed for calculation of sensitivities and specificities, were also considered. Review articles were excluded, but used for identification of further original articles.

2.3. Data extraction

From the articles that met the inclusion criteria, the following data were retrieved: target antigen of the detected AAbs, first author, publication year, information on ovarian cancer case characteristics (histologic subtype and tumor stage at diagnosis, if available), numbers of cases and controls (differentiating between healthy controls and control subjects with benign disease), and AAb detection method(s). As indicators for diagnostic performance of the AAbs as biomarkers for ovarian cancer detection, we extracted data on overall and/or stage specific sensitivities and specificities, areas under the ROC curve (if reported), and p-values for case-control differences. Any ambiguity about extracted data was discussed and resolved among the co-authors.

3. Results

The primary search in PubMed resulted in 803 records, of which 63 non-English articles were excluded in a first step. The remaining 740 reports were screened for thematic relevance by reading titles and abstracts. We selected 62 studies, including 14 studies found through cross-referencing, for full text review. After full-text review, a total of 29 studies were included in the systematic review. Main reasons for study exclusion were: lack of information on diagnostic performance of the respective AAbs as a detection biomarker (n = 18); direct measurement of antigens instead of their corresponding AAbs (n = 7); AAbs analyzed in tumor tissue instead of blood circulation (n = 4); lack of suitable control groups (n = 3); or, lack of information about the antigens the AAbs were targeted against (n = 1). The flowchart of the study selection process is shown in Fig. 1. The majority of studies were restricted to epithelial ovarian cancer cases (n = 18; 62%) or the majority of cases were defined as epithelial disease (n = 2; 7%); histology was not provided in 9 studies (31%) [13–21] (Table 1; see "tumor subtypes").

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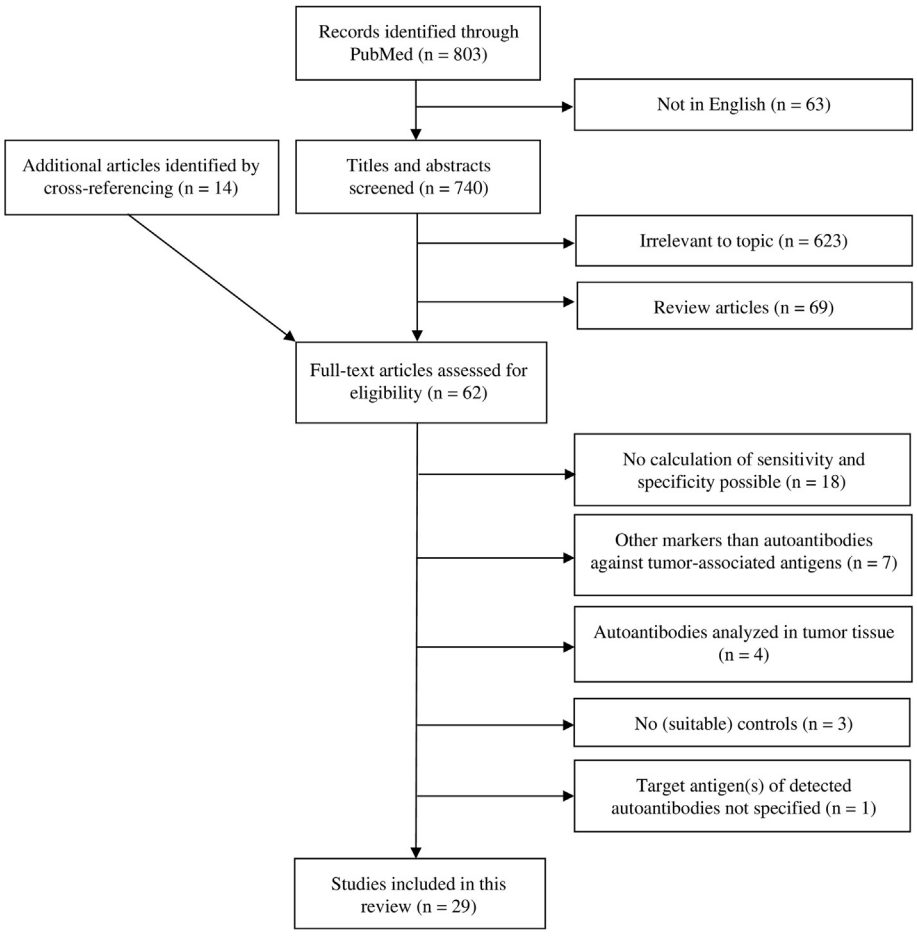

Fig. 1. Flow diagram of the literature selection process.

3.1. Investigated autoantibody markers

In total, diagnostic performance data for 85 AAbs were retrieved (Table 1). Eighteen studies analyzed the potential of one specific AAb as a detection marker, while 11 studies reported results for multiple AAbs (median number of AAbs reported per study: 8 (range 2–15)).

In most studies, the selection of investigated AAbs was hypothesis-driven, focusing on AAbs against proteins known to be frequently mutated and/or over-expressed in ovarian tumors (i.e., TAAs) (Table 1). The most common method for detection of AAbs was enzyme-linked immunosorbent assay (ELISA); 16 studies used this method for quantification of specific AAbs in relation to pre-identified candidate TAAs (Supplemental Table 1).

Ten studies performed a more generalized top-down screening approach to identify candidate AAbs discriminating cases from controls. Six studies started with pre-screening approaches for identification of immunogenic TAAs, e.g., from cancer cell lysates, using serological expression cloning (SEREX) [16,18,22,23] or immunoblotting methods [20,24]; antibodies corresponding to the selected TAAs were then quantified in blood serum of ovarian cancer patients and controls using ELISA or immunofluorescence assays. Finally, 4 recent studies used microarrays of thousands of human proteins as candidate antigen binders to directly capture and quantify AAbs in sera from ovarian cancer patients and controls [25–28]. The two most recent screening studies used nucleic acid-programmable protein arrays (NAPPA), which comprised panels of 5177 [27] and 10,247 [28] antigens, directly synthesized on-slide starting from human protein DNA sequences. Two other studies also assayed sera for antibody reactivity, using an array of >8000 proteins translated from genes randomly selected throughout the human genome and fluorescence detection [25] or a combination of purified protein microarrays and iTRAQ (isobaric tag for relative and absolute quantitation) multiplex quantitative proteomics using ovarian cancer cell lysates [26].

Twelve AAbs were described by more than one article (c-myc [15,21], cyclin B1 [15,21], HE4 [29,30], IMP1 [15,17,21], p62/IMP2 [15,17,21], koc/IMP3 [15,21], mesothelin [21,31], MUC1 [32,33], NY-ESO-1 [14,34], p16 [15,21], p53 [15,19,21,25,27,28,35–38], and survivin [15,21]).

3.2. Characteristics of the study populations

The characteristics of the study populations are provided in Table 1 and Supplementary Table 1. The majority of the included studies (n = 21) were conducted in North America, with further studies in Europe (n = 6) [29,32,36,38,39,40] and Asia (n = 2) [24,27]. Some articles derived from the same investigators or overlapping author groups, which resulted in some overlap of cases and/or control groups originating from the same hospital [13,27,28,33,35,37,41], medical university ([39,40] and [22,23]), serum bank [13,17,20,21] or trial [32,38]. All but 2 of the included studies used a case-control study design in which blood samples were obtained from women clinically diagnosed with ovarian cancer and cancer-free controls. Two studies analyzed the diagnostic potential of the biomarkers for early detection prospectively, using blood samples collected prior to cancer diagnosis; these studies were completed in the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) [32,38]. The number of ovarian cancer patients enrolled into the case groups ranged from 17 to 220 (median 51), and the number of healthy control subjects ranged from

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Table 1
Diagnostic performance of single AAb markers (alphabetical order).

Target antigen of detected AAbs		Description	First author. Year [ref]	Tumor subtypes (%)	Tumor stage (%)	No. of OC cases (a)	No. of Controls (b: benign/c: healthy)	p-Value (a vs. c)	Sensitivity (%)	Specificity (%) (c)	AUC
14–3-3 Zeta	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta	Involved in signal transduction	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	31.82	96.0	0.809
ACSBG1	Acyl-CoA Synthetase Bubblegum Family Member 1	Frequently overexpressed in ovarian cancer tissue	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.2287	13.3	95.0	0.539
AFP	Alpha Fetoprotein	Overexpression prevalent in ovarian cancer; tumor marker for diagnosis and management of ovarian germ cell tumors	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.1971	15.0	95.0	0.544
BRCA1	Breast Cancer 1, Early Onset	Acts as a tumor suppressor; mutations are responsible for >80% of inherited breast and ovarian cancers	Zhu, 2015 [13]	n/a	n/a	34	0/135	<0.001	50.0	99.3	–
BRCA2	Breast Cancer 2, Early Onset	Inherited mutations in BRCA1 and BRCA2 confer increased risk of developing breast or ovarian cancer	Zhu, 2015 [13]	n/a	n/a	34	0/135	<0.05	5.9	99.3	–
Carbonic anhydrase		Amplified gene product, overexpressed in a variety of tumor types	Stockert, 1998 [14]	n/a	n/a [metastatic disease]	32	0/70	–	0.0	100.0	–
C16orf45	Chromosome 16 Open Reading Frame 45	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	14.0	96.0	–
C20orf67	PDX1 C-Terminal Inhibiting Factor 1, Chromosome 20 Open Reading Frame 67	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	10.0	100.0	–
CA125	Cancer antigen 125, Mucin 16 (MUC16), cell surface associated	Overexpressed in ovarian cancer tissue, associated with ovarian cancer and endometriosis	Bandiera, 2013 [29]	Serous (32), endometrioid (15), clear cell (13), mucinous (5), undifferentiated (10), unknown (2)	n/a	60	120 (b1: 60 endometriosis, b2: 60 benign cyst)/60	–	b1: 13.0 b1: 8.0 b1: 8.0 b2: 12.0 b2: 8.0 b2: 7.0 c: 15.0 c: 13.0 c: 13.0	90.0 96.0 100.0 90.0 96.0 100.0 90.0 96.0 100.0	
CCDC44	Coiled-Coil Domain-Containing Protein 44	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	16.0	96.0	–
CDK2	Cyclin-dependent kinase 2	Gene amplification present in 15–20% of ovarian tumors	Li, 2008 [15]	n/a	n/a	32	0/82	<0.05	9.4	100.0	–
CFL1	Cofilin 1	–	Karabudak, 2013 [26]	Epithelial OC	I (50), II–IV (50)	40	20/20	0.096 (a vs. b + c)	55.0	89.0 (b + c)	0.794
c-myc		Oncogene; mutated in many cancers, including ovarian cancer	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	65.91	92.0	0.867
			Li, 2008 [15]	n/a	n/a	32	0/82	n.s.	3.1	100.0	–
CSNK1A1L	Casein kinase 1 alpha 1 like	–	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.2819	10.0	95.0	0.529
CTAG2	Cancer/testis antigen 2	Expressed in a wide array of cancers, mutational cancer driver in ovarian serous cystadenocarcinoma	Katchman, 2017 [28]	Serous OC (100)	I–II (5), III–IV (95)	34	0/32	0.016	23.5	96.8	–

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Cyclin A		Cyclin gene amplification and overexpression occurs in breast and ovarian cancers	Li, 2008 [15]	n/a	n/a	32	0/82	<0.01	18.8	100.0	–
Cyclin B1			Sun, 2017 [21]	n/a	n/a	44	0/50	<0.001	38.64	92.0	0.733
			Li, 2008 [15]	n/a	n/a	32	0/82	<0.01	25.0	97.6	
Cyclin D1			Li, 2008 [15]	n/a	n/a	32	0/82	<0.01	25.0	98.8	–
Cyclin E			Li, 2008 [15]	n/a	n/a	32	0/82	<0.01	21.9	100.0	–
DHFR	Dihydrofolate reductase	Gene amplification in ovarian cancer	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.3722	13.3	95.0	0.520
			Anderson, 2015 [27]	Nonserous OC: endometrioid (33), clear cell (33), mucinous (33)	n/a	30	0/60	0.1311	16.7	93.3	0.587
DRAP1	DR1 associated protein 1	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	14.0	100.0	–
EpCAM	Epithelial cell adhesion molecule	Oncogenic potential; appears to play a role in tumorigenesis and metastasis of carcinomas	Kim, 2003 [41]	Serous (50), mucinous (19), endometrioid (19), clear cell/others (12)	I (31), II (12), III (50), IV (8)	52	26/26	<0.05	42.3 73.1 73.1	100.0 (c) 80.8 (c) 76.9 (b)	0.851
EZR	Ezrin	Implicated in various human cancers; prognostic marker in ovarian and other cancers	Karabudak, 2013 [26]	Epithelial OC	I (50), II-IV (50)	40	20/20	0.087 (a vs. b + c)	50.0	89.0 (b + c)	0.700
FER		Overexpression in ovarian cancer cells; involved in the metastatic process of ovarian cancer cells	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	12.0	100.0	–
FGFR1	Fibroblast growth factor receptor 1	Gene amplification observed in lung, prostate, breast and ovarian cancer	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	12.0	96.0	–
HCC1.4	RNA binding motif protein 39	Interacts with estrogen receptors	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	20.45	98.0	0.753
HE4	Human Epididymis Protein 4	Frequently overexpressed in ovarian carcinoma: used as a serum biomarker for ovarian cancer detection	Bandiera, 2013 [29]	Serous (32), endometrioid (15), clear cell (13), mucinous (5), undifferentiated (10), unknown (2)	I + II (28), II + IV (72)	60	120 (b1: 60 endometriosis, b2: 60 benign cyst)/60	–	b1: 7.0 b1: 3.0 b1: 0.0 b2: 7.0 b2: 3.0 b2: 0.0 c: 10.0 c: 2.0 c: 0.0	90.0 96.0 100.0 90.0 96.0 100.0 90.0 96.0 100.0	–
			Hellstrom, 2013 [30]	Epithelial OC	I-II (18), III-IV (82)	92	0/71	<0.01	14.0	97.0	–
HOXA7	Homebox A7	HOX genes are strongly expressed in ovarian cancer; play a role in the oncogenesis of ovarian cancer	Naora, 2001a [22]	Serous (94), endometrioid (6)	II (2), III (74), IV (24)	51	19/30	Serous OC $p < 0.0001$; poorly differentiated $p = 0.4$, moderately differentiated $p < 0.0001$	Poorly differentiated: 4.2 Moderately differentiated: 66.7	32.0 (b) 100.0 (c)	–
HOXB7	Homebox B7		Naora, 2001b [23]	Epithelial OC	II (3), III (77), IV (20)	39	0/29	$p < 0.0001$	33.3	96.6	–
Hsp27	Heat shock protein 27	Hsps are excessively expressed in numerous malignant neoplasms in humans, including genital cancers	Olejek, 2009 [39]	Serous (55), mucinous (30), endometrioid (15)	I (17), II (20), III (42), IV (22)	158	0/80	<0.05	44.0	87.5	–
Hsp60	Heat shock protein 60		Bodzek, 2014 [40]	Serous (55), mucinous (30), endometrioid (15)	I (17), II (21), III (40), IV	149	0/80	0.024	21.8	90.0	–

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Table 1 (*continued*)

Target antigen of detected AAbs	Description		First author. Year [ref]	Tumor subtypes (%)	Tumor stage (%)	No. of OC cases (a)	No. of Controls (b: benign/c: healthy)	p-Value (a vs. c)	Sensitivity (%)	Specificity (%) (c)	AUC
					(22)						
Hsp65	Heat shock protein 65		Bodzek, 2014 [40]	Serous (55), mucinous (30), endometrioid (15)	I (17), II (21), III (40), IV (22)	149	0/80	0.039	20.6	90.0	–
Hsp70	Heat shock protein 70		Liu, 2017 [20]	n/a	n/a	120	0/85	<0.01	21.7	97.6	
Hsp90	Heat shock protein 90		Luo, 2002 [16]	n/a	I-II (69), III-IV (31)	32	20/22	–	I/II:10.0 III/IV: 32.0	95.0 (b) 100.0 (c)	–
ICAM3	Intercellular adhesion molecule 3	May induce cancer cell proliferation and contribute to cancer progression	Katchman, 2017 [28]	Serous OC (100)	I-II (5), III-IV (95)	34	0/32	–	17.6	96.8	–
IMP1	Insulin-like growth factor 2 (IGF2) mRNA binding protein 2	Oncofetal factor in various neoplasias including ovarian carcinoma	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	36.36	92.0	0.748
			Liu, 2014 [17]	n/a	n/a	34	0/89	<0.01	26.5	98.9	–
			Li, 2008 [15]	n/a	n/a	32	0/82	ns	9.4	97.6	–
P62/IMP2	IGF2 mRNA binding protein 2	Frequently overexpressed in various cancers; oncofetal antigen	Sun, 2017 [21]	n/a	n/a	44	0/50	0.555	0.0	96.0	0.536
P62			Liu, 2014 [17]	n/a	n/a	34	0/89	<0.01	29.4	98.9	–
P62/IMP2			Li, 2008 [15]	n/a	n/a	32	0/82	n.s.	9.4	98.8	–
Koc/IMP3	IGF2 binding protein 3	Onco-fetal gene; K homology domain containing protein overexpressed in cancer	Sun, 2017 [21]	n/a	n/a	44	0/50	0.058	2.27	100	0.614
			Li, 2008 [15]	n/a	n/a	32	0/82	<0.01	18.8	98.8	–
IL8	Interleukin 8	Elevated in ovarian cyst fluid, ascites, serum, and tumor tissue from ovarian cancer patients; increased expression correlates with poor prognosis and survival	Lokshin 2006 [18]	n/a	I-II (47), III-IV (53)	94	37/80	I–II: <0.01, III–IV: <0.05	I-II:65.5	98.0	0.867
KLK6	Kallikrein related peptidase 6	Expression elevated in multiple human cancers, including ovarian cancer; potential serum biomarker of ovarian cancer	Bandiera, 2013 [29]	Epithelial OC: Serous (32), endometrioid (15), clear cell (13), mucinous (5), undifferentiated (10), unkown (2)	I + II (28), II + IV (72)	60	120 (b1: 60 endometriosis, b2: 60 benign cyst)/60	–	b1: 7.0 b1: 0.0 b1: 0.0 b2: 7.0 b2: 2.0 b2: 2.0 c: 17.0 c: 7.0 c: 3.0	90.0 96.0 100.0 90.0 96.0 100.0 90.0 96.0 100.0	–
KSR1	Kinase suppressor of Ras 1	–	Katchman, 2017 [28]	Serous (100)	I–II (5), III–IV (95)	34	0/32		11.8	96.8	–
MAGE1	Melanoma associated antigen 1	MAGE family = cancer-germline genes, aberrantly expressed in a wide variety of cancer types	Stockert, 1998 [14]	n/a	n/a [metastatic disease]	32	0/70	–	3.1	100.0	–
MAGE3	Melanoma associated antigen 3		Stockert, 1998 [14]	n/a	n/a [metastatic disease]	32	0/70	–	0.0	100.0	–
MBNL1	Muscleblind Like Splicing Regulator 1	–	Anderson, 2015 [27]	Serous (100)	n/a	60	0/60	0.5076	0.0	95.0	0.500
MDM2	MDM2 proto-oncogene	Detected in a variety of cancers; degrades tumor suppressor proteins	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	38.64	98.0	0.823
Mesothelin	MSLN	Overexpressed in ovarian tumors, shed into the circulation with high specificity for ovarian cancer	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.004	13.64	98.0	0.675
			Luborsky,	Serous (57), endometrioid	I–II (25),	28	47 (b1: 23	–	36.0	81.0 (c)	–

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			2011 [31]	(32), mucinous (4), clear cell (4), mixed (4)	III–IV (75)		endometriosis, b2: 24 benign)/152		14.0	65.0 (b1) 79.0 (b2) 89.0 (c) 74.0 (b1) 79.0 (b2)	
MelanA	Melanoma-associated antigen	Differentiation antigen	Stockert, 1998 [14]	n/a	n/a [metastatic disease]	32	0/70	–	0.0	100.0	–
MUC1	Mucin 1; Cancer antigen 15.3 (CA15.3)	Overexpressed in most epithelial ovarian cancers	Cramer, 2005 [33]	n/a (epithelial)	n/a	48	0/705	–	25.0 45.8	87.7 66.2	–
MUC1core3			Burford, 2013 [32]	Serous (64), endometrioid (15), clear cell (9), adenocarcinoma NOS (3), mucinous (3), other OC (5)	I (23), II (13), III (58), IV (6)	86	0/247	ns	3.4	94.1	–
MUC1STn			Burford, 2013 [32]	Serous (64), endometrioid (15), clear cell (9), adenocarcinoma NOS (3), mucinous (3), other OC (5)	I (23), II (13), III (58), IV (6)	86	0/247	ns	0.0	97.7	–
MYST2	MYST histone acetyltranferase 2	Overexpressed in primary cancers (breast, testis, ovarian)	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	14.0	100	–
NPM1	Nucleophosmin/Nucleoplasmin 1	–	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	36.36	98.0	0.836
NPM3	Nucleophosmin/Nucleoplasmin 3	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	16.0	96.0	–
NUDT11	Nudix Hydrolase 11	Overexpression obeserved in prostate cancer	Katchman, 2017 [28]	Serous OC (100)	I-II (5), III-IV (95)	34	0/32	0.023 (a vs. c)	32.4	100.0 (c)	
NXF3	Nuclear RNA export factor 3	–	Katchman, 2017 [28]	Serous OC (100)	I-II (5), III-IV (95)	34	0/32	–	8.8	96.8	
NY-ESO-1	New York Esophageal Squamous Cell Carcinoma 1; Cancer testes antigen 1A, 1B (CTAG1A, CTAG 1B)	Cancer-germline gene, high immunogenicity in various cancers; overexpression in ovarian cancer	Milne, 2008 [34]	Serous OC (100)	I (11), II (9), III (54), IV (11), unknown (15)	35	0/60	–	25.7	95.0	–
			Stockert, 1998 [14]	n/a	n/a [metastatic disease]	32	0/70	–	12.5	100.0	–
OPN	Osteopontin	Usually overexpressed in ovarian cancer, serum OPN levels generally elevated in ovarian neoplasm patients	Bandiera, 2013 [29]	Serous (32), endometrioid (15), clear cell (13), mucinous (5), undifferentiated (10), unkown (2)	I + II (28), II + IV (72)	60	120 (b1: 60 endometriosis, b2: 60 benign cyst)/60	–	b1: 10.0 b1: 2.0 b1: 0.0 b2: 12.0 b2: 0.0 b2: 0.0 c: 22.0 c: 0.0 c: 0.0	90.0 96.0 100.0 90.0 96.0 100.0 90.0 96.0 100.0	–
P16	Tumor protein 16	Frequently overexpressed in ovarian carcinoma	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	31.82	96.0	0.814
			Li, 2008 [15]	n/a	n/a	32	0/82	<0.01	25.0	98.8	–
P53	Tumor protein 53	Tumor suppressor, overexpression and mutation have been described in cancer, including ovarian cancer	Yang, 2017 [38]	Serous (96), serous & endometrioid (4)	I (4), II (2), III (74), IV (20)	50	0/216	<0.05	30.0	97.7	–
			Yang, 2017 [38]	Serous (96), mixed (1), endometrioid (1), poorly	I (7), II (4), III (75), IV	108	109/464	<0.05 (b) <0.05 (c)	21.3	97.2	–

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Table 1 (*continued*)

Target antigen of detected AAbs		Description	First author. Year [ref]	Tumor subtypes (%)	Tumor stage (%)	No. of OC cases (a)	No. of Controls (b: benign/c: healthy)	p-Value (a vs. c)	Sensitivity (%)	Specificity (%) (c)	AUC
				differentiated (2)	(8), not known (6)						
			Yang, 2017 [38]	Serous (67), endometrioid (11), clear cell (6), mucinous (1), poorly differentiated (13), mixed (2)	I (26), II (11), III (54), IV (9)	220	0/619	<0.05	17.7	98.0	0.698
			Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	34.09	98.0	0.810
			Katchman, 2017 [28]	Serous OC (100)	I–II (5), III–IV (95)	34	30/32	0.002 (a vs. c)	44.1	96.8 (c)	–
			Katchman, 2016 [35]	Serous OC (100)	II (74), III (26)	17	0/19	0.0032	58.8	94.0	–
			Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.0024	21.7	95.0	0.648
			Anderson, 2015 [27]	Serous OC (100)	n/a	30	30/0	–	53.3	93.3 (b)	0.86
			Anderson, 2015 [27]	Nonserous OC: endometrioid (33), clear cell (33), mucinous (33)	n/a	30	0/30	0.1931	20.0	93.3	0.574
			Anderson, 2010 [37]	Serous (67), nonserous (33) (endometrioid, clear cell, mucinous)	n/a	90	30/120	<0.001	Serous: 41.7 41.7 33.3 Nonserous: 13.3	 91.7 (c) 90.0 (b) 96.7 (c) 91.7 (c) 90.0 (b)	0.69
			Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	10.0	100.0	–
			Li, 2008 [15]	n/a	n/a	32	0/82	<0.01	25.0	97.6	
			Høgdall, 2002 [36]	Papillary (7), serous (57), other (36)	I (26), II (7), III (58), IV (10)	193	0/86		I: 8.0 II: 0.0 III 15.3 IV:15.8	98.8	–
			Angelopoulou, 1994 [19]	n/a	n/a	86	0/150	–	15.0	100.0	–
P90		–	Li, 2008 [15]	n/a	n/a	32	0/82	<0.05	9.4	100.0	
PARP1	Poly(ADP ribose) polymerase 1	Overexpressed in endometrial cancer and BRCA-mutated ovarian cancer	Zhu, 2015 [13]	n/a	n/a	34	0/135	<0.001	29.4	99.3	–
PDZD11	PDZ Domain containing 11	–	Karabudak, 2013 [26]	Epithelial OC	I (50), II–IV (50)	40	20/20	<0.001 (a vs. b + c)	40.0	89.0	0.66
PLAT	Plasminogen activator, tissue type		Sun, 2017 [21]	n/a	n/a	44	0/50	<0.0001	40.91	98.0	0.791
POMC	Proopiomelanocortin	–	Katchman, 2017 [28]	Serous OC (100)	I–II (5), III–IV (95)	34	0/32	–	11.8	93.7	–
PRL	Prolactin	Serum marker for ovarian cancer	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.2122	10.0	95.0	0.539
PSMC1	Proteasome 26S Subunit, ATPase 1	–	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.3743	10.0	95.0	0.516
			Anderson, 2015 [27]	Nonserous OC: endometrioid (33), clear	n/a	30	0/30	0.6612	6.7	93.3	0.461

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				cell (33), mucinous (33)							
PTGFR	Prostaglandin F receptor	Overexpression prevalent in endometrial adenocarcinomas	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.0019	21.7	95.0	0.652
			Anderson, 2015 [27]	Serous OC (100)	n/a	30	30/0	–	16.7	93.3 (b)	0.570
			Anderson, 2015 [27]	Nonserous OC: endometrioid (33), clear cell (33), mucinous (33)	n/a	30	0/30	0.4127	10.0	93.3	0.514
PTPRA	Protein Tyrosine Phosphatase, Receptor Type A	Associated with Estrogen-Receptor Negative Breast Cancer, overexpressed in gastric cancer	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.0019	31.7	95.0	0.652
			Anderson, 2015 [27]	Serous OC (100)	n/a	30	30/0	–	13.3	93.3	0.610
			Anderson, 2015 [27]	Nonserous OC: endometrioid (33), clear cell (33), mucinous (33)		30	0/30	0.4631	20.0	93.3	0.510
PVR	Poliovirus receptor	–	Katchman, 2017 [28]	Serous OC (100)	III (5), III–IV (95)	34	30/32	0.013 (a vs. c)	17.6	96.8 (c)	–
RAB7L1	Member RAS oncogene family-like 1	–	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.2554	11.7	95.0	0.539
			Anderson, 2015 [27]	Nonserous OC: endometrioid (33), clear cell (33), mucinous (10)	n/a	30	0/30	0.7204	10.0	93.3	0.460
RalA	RAS like proto-oncogene A		Sun, 2017 [21]	n/a	n/a	44	0/50	<0.001	9.09	98.0	0.721
RhoGDI	RHO protein GDP dissociation inhibitor	Upregulation of gene expression on oral squamous cell carcinoma	Yoneyama, 2015 [24]	Serous (33), mucinous (29), clear cell (29), endometrioid (19)	n/a	49	43/54	0.0065	89.5	80.0 (c) 71.0 (b)	–
SCYL3	SCY1 like pseudokinase 3	–	Anderson, 2015 [27]	Serous OC (100)	n/a	60	0/60	0.2735	8.3	95.0	0.534
			Anderson, 2015 [27]	Nonserous OC: endometrioid (33), clear cell (33), mucinous (33)	n/a	30	0/30	0.5234	6.7	93.3	0.502
SMRP	Soluble mesothelin-related peptide	Serum/plasma biomarker for ovarian cancer diagnosis and prognosis; overexpressed in epithelial mesotheliomas, ovarian cancers and in specific squamous cell carcinomas	Bandiera, 2013 [29]	Serous (32), endometrioid (15), clear cell (13), mucinous (5), undifferentiated (10), unkown (2)	I + II (28), II + IV (72)	60	120 (b1: 60 endometriosis, b2: 60 benign cyst)/60	–	b1: 13.0 b1: 5.0 b1: 0.0 b2: 15.0 b2: 2.0 b2: 0.0 c: 5.0 c: 3.0 c. 0.0	90.0 96.0 100.0 90.0 96.0 100.0 90.0 96.0 100.0	–
SSX2	Synovial sarcoma X 2	Cancer-testis antigen	Stockert, 1998 [14]	n/a	n/a [metastatic disease]	32	0/70	–	0.0	100.0	–
STYLX1		–	Katchman, 2017 [28]	Serous OC (100)	I–II (5), III–IV (95)	34	0/32	–	8.8	96.8	–
Survivin		Highly expressed in most cancers	Sun, 2017 [21]	n/a	n/a	44	0/50	<0.001	20.45	98.0	0.730
			Li, 2008 [15]	n/a	n/a	32	0/82	<0.01	21.9	97.6	–
TGIF2	TGFB induced factor homebox 2	Amplified and overexpressed in some ovarian cancers	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	10.0	96.0	–
TGIF2LX	TGFB induced factor homebox 2, x-linked	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	10.0	96.0	–
TUBA1C	Tubulin alpha 1c	–	Karabudak, 2013 [26]	Epithelial OC	I (50), II–IV (50)	40	20/20	0.001 (a vs. b + c)	89.0	75.0 (b + c)	0.771

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Table 1 (*continued*)

Target antigen of detected AAbs		Description	First author. Year [ref]	Tumor subtypes (%)	Tumor stage (%)	No. of OC cases (a)	No. of Controls (b: benign/c: healthy)	p-Value (a vs. c)	Sensitivity (%)	Specificity (%) (c)	AUC
TRIM39	Tripartite Motif Containing 39	–	Katchman, 2017 [28]	Serous OC (100)	I–II (5), III–IV (95)	34	0/32	0.039 (a vs. c)	14.7	96.8 (c)	–
TSC22D4	TSC22 domain family member 4	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	12.0	98.0	
Tyrosinase		Differentiation antigen	Stockert, 1998 [14]	n/a	n/a [metastatic disease]	32	0/70	–	0.0	100.0	–
UBL4A	Ubiquitin like 4A	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	8.0	98.0	
UBTD2	Ubiquitin domain containing 2	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4), mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	24.0	94.0	
UHMK1	U2AF Homology Motif Kinase 1	–	Katchman, 2017 [28]	Serous OC (100)	I–II (5), III–IV (95)	34	0/32	–	11.8	96.8	–
ZNF434	Zinc finger protein 434	–	Gnjatic, 2010 [25]	Serous (82), mixed (8), clear cell (4),mucinous (2), endometrioid (2), unknown (2)	III (92), IV (8)	51	0/53	–	14.0	100.0	

Abbreviations: n/a not available, ns not significant, a: OC cases, b: controls with benign disease, c: healthy controls.

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19 to 705 (median 60). Eleven studies incorporated a second arm of controls consisting of women with benign ovarian conditions (e.g., endometriosis, benign ovarian cysts); median group size: 30, range: 19–120) [16,18,22,24,26–29,31,37,41].

Cases and controls were explicitly group-matched for age in 15 studies. Detailed information about the age distribution in the case and control groups was given in 10 studies, while 8 other studies only stated that the groups were age-matched. In 3 studies, the median age of the control group was ≥10 years younger than that of the cases [24,29,31]. Eleven articles [13–17,19–23,41] did not report the age structure in the study population.

3.3. Diagnostic performance characteristics of autoantibody markers

For each of the single AAb markers examined, Table 1 shows combined sensitivity and specificity estimates for the detection of ovarian cancer. Reported sensitivities ranged from 0 to 89.5% (median: 13%) at specificities between 32% and 100% (median: 96%). The highest sensitivities were reported for RhoGDI-AAbs [24] (89.5%) and TUBA1C-AAbs [26] (89%), but at low specificity levels of only 80% and 75%, respectively. At high specificity levels (>98%), best sensitivities were reported for HOXA7-AAbs for detection of moderately differentiated ovarian tumors (66.7% sensitivity at 100% specificity) [22], IL8-AAbs in stage I–II ovarian cancer (65.5% sensitivity at 98% specificity) [18], and BRCA1-AAbs (50% sensitivity at 99.3% specificity) [13]. For 31 different AAbs the AUC was calculated, with estimates ranging from 0.460 for RAB7L1-AAbs [27] to a maximum of 0.867 for both IL8-AAbs for detection of stage I–II ovarian cancer [4] and c-myc antibodies [21] (median: 0.656). One study reported the partial AUC (pAUC) at fixed 95% specificity [27] observing pAUCs of 5.56, 8.00, and 7.11 for p53-AAbs, PTGFR-AAbs, and PTPRA-AAbs, respectively ($p < 0.01$). A second study reported pAUC of 0.003 at 98% specificity for p53-AAbs [38].

P53-AAbs were the most frequently investigated AAb, evaluated in 10 separate studies [15,19,21,25,27,28,35–38]; these AAbs discriminated significantly between cases and cancer-free controls in the majority of these studies [15,21,27,28,35,37,38] with a range of 21.7% sensitivity at 95% specificity [27] to 58.8% sensitivity at 94% specificity [35] in studies comparing prevalent cases to controls. The single prospective study on p53-AAbs reported 17.3% sensitivity at 98% specificity comparing controls and cases diagnosed >3 months to 5 years after blood collection [38]. AAbs to c-myc [15,21], cyclin B1 [15,21], HE4 [29,30], koc/IMP3 [15,21], mesothelin [21,31], MUC1 [32,33], NY-ESO-1 [14,34], p16 [15,21] and survivin [15,21] were each investigated in 2 studies, and IMP1 [15,17,21] and p62/IMP2 [15,17,21] in 3 studies with relatively low between-study agreement. For example, for HE4-AAbs one study observed 2% sensitivity at 96% specificity [29], whereas the second observed 14% sensitivity at 97% specificity [30]. Similarly, for IMP1-AAbs, one study observed 36.36% sensitivity at 92% specificity [21], a second study observed 26% sensitivity at 98.9% specificity [17], and a third study observed 9.4% sensitivity at 97.6% specificity [15].

Nine studies investigated the diagnostic discrimination offered by combined panels of multiple AAbs, or by single AAbs in combination with other ovarian cancer detection markers such as CA125 and HE4 (Table 2) [13,15,18,21,27,28,37,38,41]. Five of these studies evaluated the diagnostic discrimination of multiple AAbs in panels including 3 [13,27], 11 [28], and 13 [15,21] markers. The diagnostic classification criterion used for AAb panels varied by study. For example, 2 studies evaluated sensitivity and specificity of the panel when at least 2 AAbs were above a pre-specified threshold of 95% specificity defined individually for each AAb [27,28]. In the first study, the combination of p53, PTGFR, PTPRA provided 23.3% sensitivity at 98.3% specificity for serous ovarian cancer when at least 2 of 3 AAbs were above the threshold [27]. Likewise, in the second study, the panel provided 47% sensitivity at 93.7% specificity for serous ovarian cancer when more than 2 of 11 AAbs (ICAM3, CTAG2, p53, STYXL1, PVR, POMC, NUDT11, TRIM39, UHMK1, KSR1, and NXF3) were above the threshold [28]. Li et al. [15] evaluated the sensitivity and specificity of a 13 AAb panel, observing 62.5% sensitivity at 86.4% specificity for the full panel (survivin, p53, p16, cyclin B1, cyclin D1, cyclin A, cyclin E, Koc, IMP1, p62, CDK2, p90, c-myc) when cases were positive for one or more AAbs, each passing a threshold level corresponding to 3 standard deviations below or above the mean in the controls. Sun et al. [21] observed 72.7% sensitivity at 88% specificity when any of 13 AAbs were above a threshold of 3 standard deviations above the mean in the controls (panel included: MDM2, PLAT, NPM1, 14-3-3 Zeta, p53, RalA, c-myc, mesothelin, HCC1.4, survivin, cyclin B1, p16, and IMP1). In a decision tree analysis, only c-myc-AAbs were retained after "pruning" to correct for overfitting [21]. An additional investigation considered diagnostic performance when concentrations of each of 3 included AAbs (PARP, BRCA1, BRCA2) exceeded a threshold of 3 standard deviations above the mean in the controls [13]; with this stringent criterion, however, the panel had zero diagnostic sensitivity, in spite of relatively high sensitivity estimates of each of the three markers independently. Case histology was not provided in the latter 3 studies.

Further studies evaluated whether diagnostic discrimination was improved by evaluating individual AAb markers together with CA125 [38,41], CA125 and HE4 [37], or CA125 and IL8 [18]. In the most recent study, TP53-AAbs improved the AUC beyond CA125 alone (AUCs, TP53-AAbs: 0.698; CA125: 0.838; TP53-AAbs + CA125: 0.867), even in samples collected 1 to 5 years before diagnosis (AUCs, p53-AAbs: 0.636; CA125: 0.751; p53-AAbs + CA125: 0.861); however, pAUCs at 98% specificity were similar in both models [38]. In another evaluation, the combination of p53-AAb with CA125 and HE4 did not improve diagnostic discrimination relative to CA125 and HE4 alone [37]. In another study, by contrast, the combination of IL8-AAbs, IL-8 and CA125 resulted in better classification (sensitivity 87.5% at 98% specificity) compared to any of the individual markers alone (e.g., CA125, sensitivity 76.8% at 98% specificity) [18]. Likewise, a third study reported that the diagnostic discrimination of EpCAM-AAb combined with CA125 (90.4% sensitivity at 92.3% specificity) was higher than that of CA125 alone (86.5% sensitivity at 88.5% specificity) [41]. Notably, 5 of these 9 studies combining multiple markers considered model overfitting; these studies evaluated test and validation sets to avoid model over-optimism [18,27,28,38], or utilized "pruning" in a decision tree analysis [21].

3.4. Tumor type-specific diagnostic characteristics of autoantibody markers

Information about the histopathological subtypes of ovarian carcinoma was available in 15 studies. However, only 5 studies compared the diagnostic values of the AAb markers by tumor histology [27,36–39] and one by tumor grade [22]. Anderson et al. [27] found that the 2 most promising AAb biomarkers identified in their study for serous ovarian cancer, PTGRF and PTPRA, were not detected in the serum of women with non-serous ovarian cancers. Moreover, in the same study, the AUCs for AAbs against p53 were higher in serous vs. non-serous tumor types (0.648 vs. 0.574), as would be expected given somatic TP53 gene mutations are more frequent in high grade serous tumors [42]. These findings of better diagnostic performance for p53-AAbs in serous vs. non-serous disease were also observed in an earlier study by Anderson et al. (sensitivities: 41.7% in serous ovarian cancer and 13.3% in non-serous tumors; both at 90% specificity) [37] and are in line with findings in the recent study by Yang et al. [38]. However, in contrast, Høgdall et al. [36] did not observe significant associations between p53-AAb and ovarian cancer, regardless of histological subtype. In the single investigation on Hsp27-AAbs, the mean concentration of antibodies in ovarian cancer patients did not differ by tumor histology [39].

Naora et al. [22] provided the only study with analyses by tumor grade. This study on HOXA7-AAbs reported significantly higher sensitivity among women with poorly as compared to moderately differentiated tumors (4.2% vs. 66.7%; both at 100% specificity; $p < 0.001$).

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Table 2
Diagnostic performance of AAb marker combinations.

First author. Year [ref]	No. of OC cases (a)	No. of controls (b:benign/c:healthy)	Single AAb markers and combinations	Sensitivity (%)	Specificity (%) (c)	AUC	Comment
Anderson, 2015 [27]	60	0/60	P53	21.7	95.0	0.6475	P53, PTPRA, and PTGFR are potential biomarkers for the detection of ovarian cancer.
			PTGFR	21.7	95.0	0.6522	
			PTPRA	31.7	95.0	0.6525	
			P53 + PTGFR + PTPRA				
			(1) at least 1 positive	(1) 45.0	(1) 86.7	n/a	
			(2) at least 2 positive + specificity >95%	(2) 23.3	(2) 98.3	n/a	
Anderson, 2010 [37]	90	30/120	P53-AAb	n/a	n/a	0.64	P53-AAb did not improve the detection of cases or the discrimination of benign vs. malignant disease compared with CA125 or HE4.
			HE4	n/a	n/a	0.97	
			CA125	n/a	n/a	0.94	
			P53-AAb + CA125 + HE4	n/a	n/a	0.98	
Katchman, 2017 [28]	34	30/32	Top 11 AAbs combined: CTAG2 + ICAM3 + KSR1, NUDT11 + NXF3 + POMC + PVR + STYLX1 + p53 + TRIM39 + UHMK1	37.9 47.1 45.0	93.0 (b) 93.7 (c) >99 (c)	n/a n/a 0.72	Distinguished serous ovarian cancer from healthy controls with a combined 45% sensitivity at 98.0% specificity.
Kim, 2003 [41]	52	0/26	EpCAM	73.1	80.8	0.851	Combination of EpCAM AAb and CA125 increased specificity as compared with CA125 alone without lowering sensitivity.
			CA125	86.5	88.5	0.965	
			EpCAM + CA125	90.4	92.3	n/a	
Lokshin, 2006 [18]	94	37/80	αIL8AAb	65.5	98.0	n/a	Combining IL-8 and anti-IL8 IgG with Ca125 resulted in increased classification power as compared to individual markers analyzed separately.
			IL8	62.6	98.0	n/a	
			CA125	76.8	98.0	n/a	
			αIL8AAb + IL8 + CA125	87.5	98.0	n/a	
Li, 2008 [15]	32	0/82	Individual AAbs	3.1–25.0	97.6–100.0	n/a	Stepwise increase in sensitivity of up to 62.5% and in specificity of 90.2 with the successive addition of AAbs to a total of 7 antigens.
			Cumulative 13 AAb panel: Survivin + p53 + p16 + cyclin B1 + cyclin D1 + cyclin A + cyclin E + Koc + IMP1 + p62 + CDK2 + p90 + c-myc	62.5	86.4	n/a	
Sun, 2017 [21]	44	0/50	Individual AAbs	9.09–65.91	92.0–98.0		Stepwise increase in sensitivity to 72.3% at specificity of 96.0 with the addition of AAbs to a total of 6 AAbs; no further gain in sensitivity and lower specificity with addition of further AAbs. Decision tree analysis identified only c-myc from the set of 13 AAbs.
			13 AAb panel: MDM2 + PLAT + NPM1 + 14-3-3 Zeta + p53 + RalA + c-myc + mesothelin + HCC1.4 + survivin + cyclin B1 + p16 + IMP1	72.73	88.0		
Yang, 2017 [38]	220	0/619	P53	17.7	98.0	0.698	P value <0.01 comparing AUCs for Ca125 vs. CA125 + TP53-Abbs.
			CA125	47.3	98.0	0.838	
			P53 + CA125	50.3	98.0	0.867	
Zhu, 2015 [13]	34	0/125	PARP1	29.4	99.3		Autoantibody responses slightly decreased and the AAb reactions varied from 0% to 50.0%.
			BRCA1	50.0	99.3		
			BRCA2	5.9	99.3		
			PARP1 + BRCA1 + BRCA2	0	100.0		

Abbreviations: n/a not available, ns not significant, a: OC cases, b: controls with benign disease, c: healthy controls.

3.5. Tumor stage-specific diagnostic characteristics of autoantibody markers

Eighteen studies characterized the study population by ovarian cancer stage at diagnosis, but only 6 studies performed analyses evaluating diagnostic discrimination by stage (Table 3) [16,18,30,36,38,39]. Hsp27-AAbs showed better discrimination between cases diagnosed at relatively early vs. late stage [39]. The sensitivity of Hsp27-AAbs was 62% for stage I disease but 31% for stage IV disease, both at 87.5% specificity. Further, EpCAM-AAb detection in sera of stage I or stage II ovarian cancer cases showed significantly higher levels compared with stage IV tumors; however, no stage-specific sensitivities were reported in this study [41]. In contrast, diagnostic performance of Hsp90-AAbs [16] was lower in early-stage compared to late-stage tumors (e.g., Hsp90-AAb sensitivity: stages I–II: 10%, stages III/IV: 32%; both at 100% specificity). Hellstrom et al. [30] did not detect a statistically significant difference in HE4-AAb positivity by disease stage at diagnosis. IL8-AAbs had 65.5% sensitivity at 98% specificity for stage I–II disease; however, diagnostic discrimination for stages III-IV disease was not provided [18]. On balance, p53-AAbs have been shown to have somewhat higher discrimination in cases diagnosed at later stage [36,38].

4. Discussion

More than 60 years ago, Graham et al. first reported that 25% of patients with various gynecologic tumors presented with antibodies to antigens specific to their excised tumor [43]. Since that first publication, numerous studies have documented the presence of increased AAb levels against a wide variety of auto-antigens expressed in different tumors, including tumors of the lung [44], colorectum [45], breast [46] and other organ sites. For some cancer types, e.g. lung cancer, the systematic search and evaluation of AAbs as potential markers for early cancer detection have resulted in combined AAb marker panels that have considerable diagnostic discrimination capacity [44] and are already being tested as adjunct early detection tool in population screening studies [47].

AAbs exhibit several properties that make them attractive as early detection biomarkers. First, circulating antibodies are relatively stable, exhibiting greater stability than their corresponding antigen over time, given antigens are subject to proteolysis, whereas antibodies are not [48]. Second, the immune response to tumor-associated antigens results in an amplified signal, such that AAbs may be detectable earlier than the antigens themselves [49], potentially allowing detection of earlier stage disease. Finally, AAb ELISA assays are readily translatable to clinical chemistry platforms. Therefore, AAb panels providing validated diagnostic discrimination could readily be added to the best available screening markers, CA125 and HE4, for clinical implementation. For ovarian cancer, although systematic searches for diagnostically useful AAb markers are less advanced than, for example, for lung cancer, we identified 29 studies examining 85 individual AAbs with regard to their capacity to discriminate between ovarian

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Table 3
Tumor-stage specific diagnostic performance of AAb markers.

Target antigen of detected AAbs	First author. Year [ref]	Tumor stage (no. of cases (a))	Sensitivity (%)	Specificity (%) (c)	*p*-value	Comment
HE4	Hellstrom, 2013 [30]	I–II (17)	20.0	97.0	I–II vs. control: <0.05	No statistically significant difference between patients who had stage I/II as compared to stage III/IV disease.
		III–IV (75)	12.0		III–IV vs. control: <0.05	
Hsp27	Olejek, 2009 [39]	I (26) II (32) III (66) IV (34)	62.0 55.0 35.0 36.0	87.5	I–II vs. III–IV: <0.05	Less advanced cancers associated with higher anti-Hsp27 antibody concentrations.
Hsp90	Luo, 2002 [16]	I–II (22) III–IV (10)	10.0 32.0	95.0 (b) 100.0 (c)	n/a	Correlation of Hsp90 AAbs and late stage cancer implies that it may have utility as a prognostic biomarker.
IL-8	Lokshin, 2005 [18]	I–II (47)	65.5	98.0	I–II vs. control: <0.01	Logistic regression analysis of circulating concentrations of anti-IL8-IgG in patients with stages I–II ovarian cancer vs. healthy controls allowed for prediction of early ovarian cancer.
		III–IV (53)	n/a		III–IV vs. control: <0.05	
P53	Høgdall, 2002 [36]	I (50) II (13) III (111) IV (19)	8.0 0.0 15.3 15.8	98.8	0.13 across stage categories	No significant association between p53 AAb and clinical stage.
	Yang, 2017 [38]	I (2) II (1) III (37) IV (10)	0.0 0.0 29.7 40.0	97.7	n/a	MD Anderson Normal Risk Ovarian Screening Study population.
	Yang, 2017 [38]	I (8) II (4) III (81) IV (9)	25.0 0.0 22.2 11.1	97.2	n/a	Australian Ovarian Cancer Study population.
	Yang, 2017 [38]	I (25) II (11) III (54) IV (9)	7.1 28.0 22.7 25.0	97.4	n/a	UKCTOCS population. Elevated TP53-AAbs based on overall study specificity of 97.4%.

Abbreviations: n/a not available, ns not significant, a: OC cases, b: controls with benign disease, c: healthy controls.

cancer cases and controls. An independent recent review also described TAAs and some selected AAbs in relation to ovarian cancer [50]; however, this earlier review focused largely on other (non-AAb) biomarkers for ovarian cancer and included only 7 publications on AAbs overlapping with the current review [18,26,29,30,36,37,41].

The production of AAbs is thought to be elicited by proteins presenting neoepitopes, for example resulting from somatic missense mutations in gene coding domains or from post-translational modifications (e.g., glycosylation, phosphorylation, polyadenylation, adenosine diphosphate-ribosylation, alteration of glycation side-chains [8]). Alternatively, it has been proposed that AAb production may also be elicited by locally aberrant expression levels of proteins [8]. Each of these aberrations can result in detection by immune surveillance systems, activation of B- and T-lymphocytes, and the release of chemokines and cytokines, which, in turn, further stimulate the immune response [8,10]. B-lymphocytes provide a targeted antibody response to TAAs, resulting in antigen-specific AAbs [8,10]. Local inflammation and immune cell infiltration in the tumor micro-environment are a likely enhancer, if not a pre-requisite, for immune reaction and antibody formation.

Few proteins identified as potential TAAs in ovarian cancer are encoded by genes that are known to undergo somatic mutation. Proteins that fall into this category include p53, BRCA1 and BRCA2. p53 has been studied intensively as a potential TAA in ovarian cancer, as the TP53 gene is known to be somatically mutated in almost all high-grade serous ovarian tumors, the predominant ovarian cancer subtype, and not consistently observed in low-grade serous disease and ovarian tumors of other histologies [51]. Interestingly, antibodies against p53, while antigen-specific, have been shown to be of polyclonal origin and directed against epitopes mostly outside the mutated protein regions [35], suggesting that AAbs may result from antibody formation against both normal and mutated proteins. This observation was confirmed in a recent study reporting similar sensitivity in analyses evaluating AAbs to wild-type and mutant TP53 [38].

The vast majority of TAAs investigated in the studies reported here, as well as those reported in association with other cancer types, are non-mutated antigens [8] and for most of these no specific neo-epitopes caused by post-translational mechanisms have been identified or reported. These and other observations suggest that aberrant expression levels, possibly combined with inflammation and immune cell infiltration in the tumor micro-environment, may be the most frequent driver of AAb production. Aberrantly expressed proteins include cancer testis antigens (e.g., CTAG1 (NY-ESO-1), CTAG2, MAGE1, and MAGE3) which normally are expressed only at immuno-privileged locations within the body. Further aberrant expressions are observed for onco-fetal antigens such as IMP1, which are normally expressed only during prenatal development but can be re-expressed in cells undergoing malignant transformation, and onco-proteins such as c-myc, cyclins and CDK2, which are highly over-expressed in malignant cells. Many of these aberrantly expressed proteins and their antibodies have been observed in more than one cancer type (e.g., c-myc and p53 in lung, colon, and breast cancers; reviewed in [44–46]). Some of the auto-antigens associated with ovarian cancer, including CA125, HE4, MUC1 (CA15.3), and mesothelin are (glyco)proteins that are produced in large amounts by ovarian tumors and are themselves used blood-based biomarkers for ovarian tumor burden or diagnosis [4–6,52,53].

Of the 85 AAbs included in this review, 32 demonstrated at least 15% sensitivity at minimally 95% specificity in at least one study. However, only 12 AAbs included in this review have been investigated in two or more studies: c-myc-AAbs, cyclin B1-AAbs, HE4-AAbs, IMP1-AAbs,

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p62/IMP2-AAbs, koc/IMP3-AAbs, mesothelin-AAbs, MUC1-AAbs, NY-ESO-1-AAbs, p16-AAb, p53-AAbs and survivin-AAbs. We observed between-study heterogeneity in diagnostic performance for these markers, which in part may have been due to differences in the cut-points used for specificity for reported sensitivity, and/or methodology used for AAb quantification. Standardized cross-validation studies are needed to validate and more definitively quantify the diagnostic potential of these markers, and preferably such cross-validation studies should be conducted following the recommended PRoBE principles (Prospective-specimen-collection, Retrospective-Blinded-Evaluation [54]) for marker discovery and validation. For a wide variety of AAbs, accumulating data suggest that generally their distributions are highly skewed, with elevated right-tail values for relatively small proportions of individuals that show strong immune response; these AAbs may have high tumor specificity, but with limited corresponding diagnostic sensitivity. However, as already demonstrated for other cancer sites (e.g., lung [44], colorectum [45], breast [46]), combinations of AAb biomarkers can reach higher levels of diagnostic sensitivity, while only moderately compromising on specificity.

A further factor that may contribute to heterogeneity in diagnostic marker performance is distribution of ovarian cancer histologic subtypes, grade, and stage at diagnosis. In terms of disease subtypes, epithelial ovarian cancer is increasingly recognized as a heterogeneous disease, with four major histologic subgroups (serous, mucinous, endometrioid, and clear cell) [55] and two hypothesized developmental pathways (type I and type II) [51]. These tumor subtypes are hypothesized to have different tissues of origin and present with distinct morphological and molecular characteristics, and plausibly elicit both different degrees of immune response, and subtype-specific AAb. AAbs to p53, PTGRF, PTPRA, and hsp27 were studied by tumor histologic subgroups, with five studies evaluating diagnostic discrimination in serous and/or non-serous ovarian cancers [27,35–37,39]. P53-AAbs, PTGRF-AAbs, PTPRA-AAbs provided better diagnostic discrimination for serous than for non-serous disease, whereas diagnostic discrimination for hsp27-AAbs did not differ by histologic subgroup. In their investigations of p53-AAbs, Katchman et al. [35] observed sensitivity of 58.8% at 94% specificity among serous ovarian cancers, whereas Anderson et al. [27] observed 20% sensitivity at 93% specificity in their study of non-serous ovarian cancers. Future investigations should consider diagnostic performance by ovarian cancer subtype.

Advances in technology have moved the field from investigations of individual candidate AAbs (e.g., antibodies against individual antigens selected based on antigen associations) - the majority of studies in this review – to high-throughput, larger-scale discovery efforts using methods such as SEREX and protein microarrays for the identification of novel TAAs and their corresponding AAbs. The emergence of these proteomics approaches has facilitated identification of promising AAbs. However, given the number of AAbs included in these discovery efforts, relatively large study sample size is required for discovery and validation to minimize false positive findings. From statistical power simulations as described by Pepe et al. [54,56], we estimated that >500 cases (plus cancer-free controls) would be necessary for identification and subsequent validation of markers with 12% sensitivity at 98% specificity with >80% power and <0.01% false-positive markers, when using these methods. Studies using this approach have been smaller and therefore statistically powered to identify AAb with relatively high sensitivities [27,28]. Thus, we expect that the AAbs identified thus far may represent only a fraction of the potentially diagnostically useful AAbs for ovarian cancer.

Further, to date, AAb studies for ovarian cancer have focused on discovery and validation in "Phase II" studies [54], comparing prevalent cases to cancer-free controls. Ovarian cancer is generally diagnosed at late stage; therefore, these AAb discovery efforts may have resulted in the identification of markers that are predictive for late, but not early, stage disease. Only 6 studies evaluated diagnostic performance by stage at diagnosis in this review, three of which reported sensitivities ≥20% at specificities ≥87.5% for earlier-stage disease (i.e., stages I/II; Hsp27-AAbs [39]; IL8-AAbs [18]; HE4 [30]). The remaining studies reported at least suggestively better performance in later-stage disease (i.e., stages III/IV; p53-AAbs [36,38]; Hsp90-AAbs [16]). A related issue is that the performance of AAb for early detection (i.e., in samples collected pre-diagnosis) is not well described. To our knowledge, there are only two prospective studies ("Phase III") to date on the early detection potential of AAbs: one investigating MUC1-AAbs [32] and a second investigating p53-AAbs [38]. Both investigations were conducted in the UKCTOCS. The first evaluated AAbs against MUC1 (also known as CA15.3) among subsequent cases and cancer-free controls in samples collected median 1.5 years prior to diagnosis [32]; MUC1-AAbs did not discriminate cases from non-cases. In contrast, a subsequent study on p53-AAbs reported significant discrimination between cases and controls in prediagnosis samples; samples were collected up to 5 years prior to diagnosis [38]. Additional prospective validation of other promising AAbs identified and evaluated in prevalent cases is needed to determine whether these AAbs discriminate in blood samples drawn prior to diagnosis, and if so, over what "lag-time" between blood collection and subsequent diagnosis. While this field is emerging for ovarian cancer, data from studies on lung cancer show the early detection potential of AAbs, with markers identified for pre-cancerous lung lesions [57] and in samples collected up to 5 years prior to lung cancer diagnosis [58–60]. Further, the earlyCDT test, a panel of AAb for lung cancer [61,62], is being evaluated in a large-scale screening trial in Scotland (clinicaltrials.gov, NCT01925625); this panel identifies both early and late-stage disease [62,63].

To date, AAb panels, or AAb in the context of CA125 and HE4, have been minimally explored with respect to diagnostic discrimination in ovarian cancer. Katchman et al. observed 45% sensitivity at 98% specificity, discriminating between serous ovarian cancer cases vs. healthy controls, with a AAb panel of 11 markers [28]. To date, this panel has not been tested against or together with CA125. However, other studies suggest AAbs plus CA125 results in better diagnostic discrimination relative to CA125 alone [18,41]. In the UKCTOCS, the combination of CA125 and TP53-AAbs resulted in a significantly higher AUC than either marker alone, though the pAUC at 98% specificity did not differ significantly between the two models [38]. Interestingly, 16% of cases not identified using CA125 were identified by high TP53-AAbs. It should be noted that CA125 and HE4 together or as individual markers have only limited predictive capacity, and only discriminate between cases and controls in the relative near-term prior to diagnosis (<6 months to 1 year) and for tumors diagnosed at more advanced stage [4,5]. AAbs that discriminate minimally 6–12 months prior to diagnosis may be useful when integrated into screening algorithms together with established protein markers. Pepe et al. [56] propose a framework for calculating the estimated sensitivity and specificity required for a clinically meaningful net benefit to result from a new screening test (e.g., blood-based AAbs) used in conjunction with an independent screening test (e.g., TVUS). TVUS has estimated sensitivity of ~75% at ~98% specificity, based on data from screening trials [52,64], and TVUS-detectable ovarian cancer has an estimated prevalence of 0.04–0.07% with estimates of 0.02–0.04% for stages I/II disease [52,64,65]. TVUS alone has a positive predictive value of ~5% for ovarian cancer overall (i.e., 5.3% of women screened positive have disease) and ~3% for invasive disease. Using Pepe's framework and published diagnostic performance estimates for TVUS, marker panels with a true positive rate 7.4 times higher than the false positive rate, and implemented together with TVUS, would yield PPV of ~10% - approximately double that of TVUS alone. A PPV of 10% has been suggested as a prediction threshold for ovarian early detection cancer markers [66], at which the potential benefit of earlier detection is generally judged to outweigh the harms of more invasive diagnostic procedures among the false positives. Marker panels may include a panel of TAAbs alone, or together with CA125 and HE4. This is translatable to a range of sensitivities at a range of specificities (e.g., sensitivity of 74% at specificity 90%; sensitivity of 37% at specificity 95%). Additional

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details on these analyses and further examples are provided in the Appendix A.

While AAbs for ovarian cancer have been recognized since the 1950s, AAbs for early detection remains an emerging field due in large part to the relative rarity of this lethal malignancy. Substantial progress has been made in identifying promising AAbs for ovarian cancer; however, we observed substantial heterogeneity across studies, due to differences in study setting and design, case characteristics (e.g., stage at diagnosis, tumor histology), and the statistical methods, reported results (sensitivity and specificity vs. AUC) and AAb cut-points used. The field now requires large-scale prospective investigations, including detailed data on case characteristics, comprehensive reporting of results using standardized cut-points both within study and across studies, and ultimately focusing on the development of diagnostic detection algorithms based on multi-marker panels including AAbs as well as existing markers such as CA125 and HE4. With this, the promising findings from clinical case-control studies will progress AAbs toward clinical utility for ovarian cancer early detection.

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Conflict of interest statement

The authors declare no conflicts of interest.

Appendix

We used the framework described by Pepe et al. [56] to estimate target sensitivities and specificities for a clinically useful intervention:

$$\frac{TPR}{FPR} \geq \frac{(1-\rho)^*}{\rho} r$$

where TPR is the true positive rate, FPR is the false positive rate, ρ is the prevalence of disease in the population to be screened, and r is the cost/benefit ratio or risk threshold. In analyses including two independent screening tests, the TPR (or FPR) of the screening tests combined is the product of the TPR (or FPR) from each of the tests independently; this allows the estimation of target specificities and sensitivities for marker panels accounting for the performance of TVUS. The formula we evaluated to calculate target sensitivities and specificities for a marker panel combined with TVUS was:

$$\frac{TPR_{marker\ panel}{}^* TPR_{TVUS}}{FPR_{marker\ panel}{}^* FPR_{TVUS}} \geq \frac{(1-\rho)^*}{\rho} r$$

or

$$\frac{TPR_{marker\ panel}}{FPR_{marker\ panel}} \geq \frac{(1-\rho)^*}{\rho} r^* \frac{FPR_{TVUS}}{TPR_{TVUS}}$$

In our calculations, we used the following parameters: population prevalence of TVUS detectable stage I or II ovarian cancer of 0.04%; risk threshold of 1 true case for every 10 tested ($r = 1/9$ or 0.111); TVUS sensitivity of 75% and specificity of 98%. These analyses indicated that marker panels with a TPR that is 7.4 times higher than the FPR, when implemented together with TVUS, would result in a PPV of 10% indicating a potential clinical benefit. The $\frac{TPR}{FPR} \geq 7.4$ threshold corresponds to sensitivities of 15%, 37%, 44%, 59% and 74% at specificities of 98%, 95%, 94%, 92% and 90% respectively.

In the UKCTOCS trial [64], sensitivity of 100% for TVUS was observed for invasive epithelial ovarian cancer diagnosed at stage I or II (i.e., 12 of 12 cases in the TVUS group with disease were identified); we used 75% sensitivity as a more conservative estimate in our primary analysis. We repeated the analysis above with 100% sensitivity for TVUS. In this analysis, marker panels with a TPR that is 5.5 times higher than the FPR may have clinical benefit for earlier detection of ovarian cancer. This corresponds to sensitivities of 11%, 27%, 33% 44% and 55% at specificities of 98%, 95%, 94%, 92% and 90%, respectively.

References

[1] N. Howlader, A.M. Noone, M. Krapcho, D. Miller, K. Bishop, C.L. Kosary, et al., SEER Cancer Statistics Review, 1975–2014, National Cancer Institute, Bethesda, MD, April 2017 (Based on November 2016 SEER data submission, posted to the SEER web site).
[2] R. De Angelis, M. Sant, M.P. Coleman, S. Francisci, P. Baili, D. Pierannunzio, et al., Cancer survival in Europe 1999–2007 by country and age: results of EUROCARE—5 - a population-based study, Lancet Oncol. 15 (2014) 23–34.
[3] L.A. Baldwin, B. Huang, R.W. Miller, T. Tucker, S.T. Goodrich, I. Podzielinski, et al., Ten-year relative survival for epithelial ovarian cancer, Obstet. Gynecol. 120 (2012) 612–618.
[4] D.W. Cramer, R.C. Bast Jr., C.D. Berg, E.P. Diamandis, A.K. Godwin, P. Hartge, et al., Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens, Cancer Prev. Res. 4 (2011) 365–374.
[5] K.L. Terry, H. Schock, R.T. Fortner, A. Husing, R.N. Fichorova, H.S. Yamamoto, et al., A prospective evaluation of early detection biomarkers for ovarian cancer in the European EPIC cohort, Clin. Cancer Res. 22 (2016) 4664–4675.
[6] I.J. Jacobs, U. Menon, A. Ryan, A. Gentry-Maharaj, M. Burnell, J.K. Kalsi, et al., Ovarian cancer screening and mortality in the UK collaborative trial of ovarian cancer screening (UKCTOCS): a randomised controlled trial, Lancet 387 (2016) 945–956.
[7] K.H. Lu, S. Skates, M.A. Hernandez, D. Bedi, T. Bevers, L. Leeds, et al., A 2-stage ovarian cancer screening strategy using the risk of ovarian cancer algorithm (ROCA) identifies early-stage incident cancers and demonstrates high positive predictive value, Cancer 119 (2013) 3454–3461.
[8] P. Zaenker, E.S. Gray, M.R. Ziman, Autoantibody production in cancer—the humoral immune response toward autologous antigens in cancer patients, Autoimmun. Rev. 15 (2016) 477–483.
[9] H. Lu, V. Goodell, M.L. Disis, Humoral immunity directed against tumor-associated antigens as potential biomarkers for the early diagnosis of cancer, J. Proteome Res. 7 (2008) 1388–1394.
[10] K.S. Anderson, J. LaBaer, The sentinel within: exploiting the immune system for cancer biomarkers, J. Proteome Res. 4 (2005) 1123–1133.
[11] P. Zayakin, G. Ancans, K. Silina, I. Meistere, Z. Kalnina, D. Andrejeva, et al., Tumor-associated autoantibody signature for the early detection of gastric cancer, Int. J. Cancer 132 (2013) 137–147.
[12] P. Zaenker, M.R. Ziman, Serologic autoantibodies as diagnostic cancer biomarkers - a review, Cancer Epidemiol. Biomark. Prev. 22 (2013) 2161–2181.
[13] Q. Zhu, S.X. Han, C.Y. Zhou, M.J. Cai, L.P. Dai, J.Y. Zhang, Autoimmune response to PARP and BRCA1/BRCA2 in cancer, Oncotarget 6 (2015) 11575–11584.
[14] E. Stockert, E. Jager, Y.T. Chen, M.J. Scanlan, I. Gout, J. Karbach, et al., A survey of the humoral immune response of cancer patients to a panel of human tumor antigens, J. Exp. Med. 187 (1998) 1349–1354.
[15] L. Li, K. Wang, L. Dai, P. Wang, X.X. Peng, J.Y. Zhang, Detection of autoantibodies to multiple tumor-associated antigens in the immunodiagnosis of ovarian cancer, Mol. Med. Rep. 1 (2008) 589–594.
[16] L.Y. Luo, I. Herrera, A. Soosaipillai, E.P. Diamandis, Identification of heat shock protein 90 and other proteins as tumour antigens by serological screening of an ovarian carcinoma expression library, Br. J. Cancer 87 (2002) 339–343.
[17] X. Liu, H. Ye, L. Li, W. Li, Y. Zhang, J.Y. Zhang, Humoral autoimmune responses to insulin-like growth factor II mRNA-binding proteins IMP1 and p62/IMP2 in ovarian cancer, J Immunol Res 2014 (2014) 326593.
[18] A.E. Lokshin, M. Winans, D. Landsittel, A.M. Marrangoni, L. Velikokhatnaya, F. Modugno, et al., Circulating IL-8 and anti-IL-8 autoantibody in patients with ovarian cancer, Gynecol. Oncol. 102 (2006) 244–251.
[19] K. Angelopoulou, E.P. Diamandis, D.J. Sutherland, J.A. Kellen, P.S. Bunting, Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers, Int. J. Cancer 58 (1994) 480–487.
[20] X.X. Liu, H. Ye, P. Wang, L.X. Li, Y. Zhang, J.Y. Zhang, Proteomic-based identification of HSP70 as a tumor-associated antigen in ovarian cancer, Oncol. Rep. 37 (2017) 2771–2778.
[21] H. Sun, J.X. Shi, H.F. Zhang, M.T. Xing, P. Li, L.P. Dai, et al., Serum autoantibodies against a panel of 15 tumor-associated antigens in the detection of ovarian cancer, Tumour Biol. 39 (2017) 1010428317699132. (the journal of the International Society for Oncodevelopmental Biology and Medicine).
[22] H. Naora, F.J. Montz, C.Y. Chai, R.B. Roden, Aberrant expression of homeobox gene HOXA7 is associated with mullerian-like differentiation of epithelial ovarian tumors and the generation of a specific autologous antibody response, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 15209–15214.
[23] H. Naora, Y.Q. Yang, F.J. Montz, J.D. Seidman, R.J. Kurman, R.B. Roden, A serologically identified tumor antigen encoded by a homeobox gene promotes growth of ovarian epithelial cells, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4060–4065.
[24] K. Yoneyama, S. Kojima, Y. Kodani, N. Yamaguchi, A. Igarashi, K. Kurose, et al., Proteomic identification of autoantibodies in sera from patients with ovarian cancer as possible diagnostic biomarkers, Anticancer Res. 35 (2015) 881–889.
[25] S. Gnjatic, E. Ritter, M.W. Buchler, N.A. Giese, B. Brors, C. Frei, et al., Seromic profiling of ovarian and pancreatic cancer, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 5088–5093.
[26] A.A. Karabudak, J. Hafner, V. Shetty, S. Chen, A.A. Secord, M.A. Morse, et al., Autoantibody biomarkers identified by proteomics methods distinguish ovarian cancer

ARTICLE IN PRESS

from non-ovarian cancer with various CA-125 levels, J. Cancer Res. Clin. Oncol. 139 (2013) 1757–1770.
[27] K.S. Anderson, D.W. Cramer, S. Sibani, G. Wallstrom, J. Wong, J. Park, et al., Autoantibody signature for the serologic detection of ovarian cancer, J. Proteome Res. 14 (2015) 578–586.
[28] B.A. Katchman, D. Chowell, G. Wallstrom, A.F. Vitonis, J. LaBaer, D.W. Cramer, et al., Autoantibody biomarkers for the detection of serous ovarian cancer, Gynecol. Oncol. (2017).
[29] E. Bandiera, L. Zanotti, A.S. Fabricio, E. Bucca, E. Squarcina, C. Romani, et al., Cancer antigen 125, human epididymis 4, kallikrein 6, osteopontin and soluble mesothelin-related peptide immunocomplexed with immunoglobulin M in epithelial ovarian cancer diagnosis, Clin. Chem. Lab. Med. 51 (2013) 1815–1824.
[30] I. Hellstrom, E. Swisher, K.E. Hellstrom, Y.Y. Yip, K. Agnew, J.L. Luborsky, Anti-HE4 antibodies in infertile women and women with ovarian cancer, Gynecol. Oncol. 130 (2013) 629–633.
[31] J.L. Luborsky, Y. Yu, S.L. Edassery, J. Jaffar, Y.Y. Yip, P. Liu, et al., Autoantibodies to mesothelin in infertility, Cancer Epidemiol. Biomark. Prev. 20 (2011) 1970–1978.
[32] B. Burford, A. Gentry-Maharaj, R. Graham, D. Allen, J.W. Pedersen, A.S. Nudelman, 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 108 (2013) 2045–2055.
[33] D.W. Cramer, L. Titus-Ernstoff, J.R. McKolanis, W.R. Welch, A.F. Vitonis, R.S. Berkowitz, et al., Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer, Cancer Epidemiol. Biomark. Prev. 14 (2005) 1125–1131.
[34] K. Milne, R.O. Barnes, A. Girardin, M.A. Mawer, N.J. Nesslinger, A. Ng, et al., Tumor-infiltrating T cells correlate with NY-ESO-1-specific autoantibodies in ovarian cancer, PLoS One 3 (2008), e3409. .
[35] B.A. Katchman, R. Barderas, R. Alam, D. Chowell, M.S. Field, L.J. Esserman, et al., Proteomic mapping of p53 immunogenicity in pancreatic, ovarian, and breast cancers, Proteomics Clin. Appl. 10 (2016) 720–731.
[36] E.V. Hogdall, C.K. Hogdall, J. Blaakaer, N.H. Heegaard, E. Glud, L. Christensen, et al., P53 autoantibodies in sera from Danish ovarian cancer patients and their correlation with clinical data and prognosis, APMIS 110 (2002) 545–553.
[37] K.S. Anderson, J. Wong, A. Vitonis, C.P. Crum, P.M. Sluss, J. Labaer, et al., p53 autoantibodies as potential detection and prognostic biomarkers in serous ovarian cancer, Cancer Epidemiol. Biomark. Prev. 19 (2010) 859–868.
[38] W.L. Yang, A. Gentry-Maharaj, A.R. Simmons, A. Ryan, E.O. Fourkala, Z. Lu, et al., Elevation of TP53 autoantibody prior to CA125 in preclinical invasive epithelial ovarian cancer, Clin. Cancer Res. (2017).
[39] A. Olejek, A. Damasiewicz-Bodzek, P. Bodzek, T. Wielkoszynski, J. Zamlynski, P. Stoltny, et al., Concentrations of antibodies against heat shock protein 27 in the sera of women with ovarian carcinoma, Int. J. Gynecol. Cancer 19 (2009) 1516–1520.
[40] P. Bodzek, R. Partyka, A. Damasiewicz-Bodzek, Antibodies against Hsp60 and Hsp65 in the sera of women with ovarian cancer, J. Ovarian Res. 7 (2014) 30.
[41] J.H. Kim, D. Herlyn, K.K. Wong, D.C. Park, J.O. Schorge, K.H. Lu, et al., Identification of epithelial cell adhesion molecule autoantibody in patients with ovarian cancer, Clin. Cancer Res. 9 (2003) 4782–4791.
[42] I.-M. Shih, R.J. Kurman, Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis, Am. J. Pathol. 164 (2004) 1511–1518.
[43] J.B. Graham, R.M. Graham, Antibodies elicited by cancer in patients, Cancer 8 (1955) 409–416.
[44] I. Broodman, J. Lindemans, J. van Sten, R. Bischoff, T.M. Luider, Serum protein markers for the early detection of lung cancer: a focus on autoantibodies, J. Proteome Res. (2016).
[45] H. Chen, S. Werner, S. Tao, I. Zornig, H. Brenner, Blood autoantibodies against tumor-associated antigens as biomarkers in early detection of colorectal cancer, Cancer Lett. 346 (2014) 178–187.
[46] J. Xia, J. Shi, P. Wang, C. Song, K. Wang, J. Zhang, et al., Tumour-associated autoantibodies as diagnostic biomarkers for breast cancer: a systematic review and meta-analysis, Scand. J. Immunol. 83 (2016) 393–408.
[47] F.M. Sullivan, E. Farmer, F.S. Mair, S. Treweek, D. Kendrick, C. Jackson, et al., Detection in blood of autoantibodies to tumour antigens as a case-finding method in lung cancer using the EarlyCDT(R)-lung test (ECLS): study protocol for a randomized controlled trial, BMC Cancer 17 (2017) 187.
[48] J.W. Pedersen, H.H. Wandall, Autoantibodies as biomarkers in cancer, Labmedicine 42 (2011) 623–628.
[49] S. Hanash, Harnessing immunity for cancer marker discovery, Nat. Biotechnol. 21 (2003) 37–38.
[50] J.X. Shi, J.J. Qin, H. Ye, P. Wang, K.J. Wang, J.Y. Zhang, Tumor associated antigens or anti-TAA autoantibodies as biomarkers in the diagnosis of ovarian cancer: a systematic review with meta-analysis, Expert. Rev. Mol. Diagn. 15 (2015) 829–852.
[51] R.J. Kurman, Ie M. Shih, The dualistic model of ovarian carcinogenesis: revisited, revised, and expanded, Am. J. Pathol. 186 (2016) 733–747.
[52] H. Kobayashi, Y. Yamada, T. Sado, M. Sakata, S. Yoshida, R. Kawaguchi, et al., A randomized study of screening for ovarian cancer: a multicenter study in Japan, Int. J. Gynecol. Cancer 18 (2008) 414–420.
[53] J. Huang, W. Hu, A.K. Sood, Prognostic biomarkers in ovarian cancer, Cancer Biomark. 8 (2010) 231–251.
[54] M.S. Pepe, Z. Feng, H. Janes, P.M. Bossuyt, J.D. Potter, Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design, J. Natl. Cancer Inst. 100 (2008) 1432–1438.
[55] R.A. Soslow, Histologic subtypes of ovarian carcinoma: an overview, Int. J. Gynecol. Pathol. 27 (2008) 161–174 (official journal of the International Society of Gynecological Pathologists).
[56] M.S. Pepe, C.I. Li, Z. Feng, Improving the quality of biomarker discovery research: the right samples and enough of them, Cancer Epidemiol. Biomark. Prev. 24 (2015) 944–950.
[57] F.J. Lowe, W. Shen, J. Zu, J. Li, H. Wang, X. Zhang, et al., A novel autoantibody test for the detection of pre-neoplastic lung lesions, Mol. Cancer 13 (2014) 78.
[58] J. Qiu, G. Choi, L. Li, H. Wang, S.J. Pitteri, S.R. Pereira-Faca, et al., Occurrence of autoantibodies to annexin I, 14-3-3 theta and LAMR1 in prediagnostic lung cancer sera, J. Clin. Oncol. 26 (2008) 5060–5066 (official journal of the American Society of Clinical Oncology).
[59] S.R. Pereira-Faca, R. Kuick, E. Puravs, Q. Zhang, A.L. Krasnoselsky, D. Phanstiel, et al., Identification of 14-3-3 theta as an antigen that induces a humoral response in lung cancer, Cancer Res. 67 (2007) 12000–12006.
[60] L. Zhong, S.P. Coe, A.J. Stromberg, N.H. Khattar, J.R. Jett, E.A. Hirschowitz, Profiling tumor-associated antibodies for early detection of non-small cell lung cancer, J. Thorac. Oncol. 1 (2006) 513–519 (official publication of the International Association for the Study of Lung Cancer).
[61] C.J. Chapman, G.F. Healey, A. Murray, P. Boyle, C. Robertson, L.J. Peek, et al., EarlyCDT(R)-lung test: improved clinical utility through additional autoantibody assays, Tumour Biol. 33 (2012) 1319–1326.
[62] J.R. Jett, L.J. Peek, L. Fredericks, W. Jewell, W.W. Pingleton, J.F. Robertson, Audit of the autoantibody test, EarlyCDT(R)-lung, in 1600 patients: an evaluation of its performance in routine clinical practice, Lung Cancer 83 (2014) 51–55.
[63] S. Lam, P. Boyle, G.F. Healey, P. Maddison, L. Peek, A. Murray, et al., EarlyCDT-lung: an immunobiomarker test as an aid to early detection of lung cancer, Cancer Prev. Res. (Phila.) 4 (2011) 1126–1134.
[64] U. Menon, A. Gentry-Maharaj, R. Hallett, A. Ryan, M. Burnell, A. Sharma, et al., Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK collaborative trial of ovarian cancer screening (UKCTOCS), Lancet Oncol. 10 (2009) 327–340.
[65] S.S. Buys, E. Partridge, M.H. Greene, P.C. Prorok, D. Reding, T.L. Riley, et al., Ovarian cancer screening in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial: findings from the initial screen of a randomized trial, Am. J. Obstet. Gynecol. 193 (2005) 1630–1639.
[66] I. Jacobs, R.C. Bast Jr., The CA 125 tumour-associated antigen: a review of the literature, Hum. Reprod. 4 (1989) 1–12.

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