

# Auto-antibodies against P53—a useful complementary biomarker for ovarian cancer screening?

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More than 60% of ovarian cancers are diagnosed in late stage, when 5-year survival is less than 30%. Cases diagnosed at early stage have markedly better survival outcomes (1), and this survival difference has prompted extensive research on methods for early detection and ovarian cancer screening. Current strategies for earlierstage detection of ovarian cancer use a combination of blood-based biomarkers and trans-vaginal ultrasound (TVUS) imaging. To date, the best available biomarker for ovarian cancer is ovarian tumor-associated antigen CA125; however, CA125 demonstrates limited sensitivity for early stage disease. While the vast majority (~80%) of ovarian tumors express CA125, only 50-60% of women with early stage cancers have elevated circulating CA125 levels (concentrations >35 U/mL) at presentation, possibly because small tumors may not shed sufficient amounts of CA125 into the circulation (2). In prospective studies using prediagnosis samples, we (3) and others (4,5) have found that CA125 provides good diagnostic discrimination for ovarian cancer only when blood samples had been taken relatively shortly ( $\leq 6$  months) before diagnosis, and that it mostly detected tumors that were clinically advanced at the time of diagnosis. Similar observations were made for human epididymis protein 4 (HE4), the second best available ovarian cancer marker to date (3-5). In screening trials, the combination of CA125 with TVUS provided either no reduction in ovarian cancer mortality [Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO),

USA] (6), or only a modest and marginally significant reduction when using an algorithm ("Risk of Ovarian Cancer", ROCA) based on serial CA125 measurements [United Kingdom Collaborative Trial on Ovarian Cancer Screening (UKCTOCS)] (7). Although the prospective ROCA algorithm clearly improves sensitivity of screening by CA125, a substantial proportion of ovarian cancer cases remain undetected by this method, underscoring the need for complementary and more sensitive biomarkers for the detection of earlier-stage ovarian cancer.

A promising class of markers for early cancer diagnosis is tumor-associated autoantibodies (AAbs). These AAbs are produced as an immune response to proteins exhibiting locally aberrant expression, that are mutated or post-translationally modified, or other auto-antigens associated with tumors (8-10). AAbs are attractive as candidate early detection markers as they may amplify a signal from low-concentration antigens in early stages of tumorigenesis (10,11), and they may circulate at much higher concentrations than their corresponding antigen. Furthermore, antibodies are more stable than their corresponding antigens in blood samples, and can be measured in small serum (or plasma) volumes using laboratory methods that can be easily translated to clinical applications.

In recent years, the discovery of AAbs has been accelerated by the emergence of novel high-throughput technologies such as serological expression cloning (SEREX) and affinity array proteomics (8), with the identification of an increasing number of AAbs showing elevated serum levels among cancer patients, relative to cancerfree controls, for a variety of cancers (8,9,12-14). These studies show that distributions of AAbs are often highly skewed with elevated right-tail values for cancer patients, suggesting high diagnostic specificity at elevated antibody titers. However, data to date also indicate substantial heterogeneity in autoantibody response to antigens across cancer patients, translating into limited diagnostic sensitivity for individual AAb. Nonetheless, for a variety of cancer sites [e.g., lung, colorectum, breast, reviewed in (8,9)], it has been shown that combined panels of multiple AAb biomarkers can reach substantial levels of diagnostic sensitivity with only moderate reductions in specificity [e.g., 7-AAb EarlyCDT lung cancer panel, 37-41% sensitivity at 91% specificity (15,16)].

Although the search for diagnostically useful AAb markers for ovarian cancer is less advanced in comparison to some other cancers, in a systematic review we recently identified 29 studies examining 85 individual AAbs with regard to their capacity to discriminate between ovarian cancer cases and controls; of these, 32 demonstrated at least 15% sensitivity at minimally 95% specificity in at least one study (17). P53-AAbs were the most frequently investigated AAb, evaluated in 10 separate studies. These AAbs discriminated significantly between cases and controls in the majority of these studies generally with greater than 20% sensitivity at about 95% or higher specificity, in part depending on specific ovarian cancer sub-types (P53-AAbs may provide stronger discrimination for serous tumors than tumors of other histologic sub-type). Of note, with one exception, these investigations were conducted in prevalent cases.

An overriding methodological limitation of discovery studies for diagnostic cancer biomarkers is that, in most cases, the markers have been first identified and tested exclusively in patients already diagnosed with (often advanced) cancer, and have been compared to either hospital controls or, more rarely, cancer-free control subjects from the general population. To examine whether markers can detect cancer prior to the occurrence of symptoms and diagnosis under usual care, the consensus view is that they should be further validated in the context of populationbased prospective cohort studies, using samples collected pre-diagnosis and following the recommended PRoBE principles [Prospective-specimen-collection, Retrospective-Blinded-Evaluation (18)]. Prospective studies allow an evaluation of diagnostic marker discrimination at variable time intervals between blood collection and cancer diagnosis under usual care, providing insight into the average lead-time by which the markers may allow earlier cancer detection. Furthermore, the prospective study design avoids potential biases that may be caused, for example, by differences in general conditions under which blood samples from cancer cases and disease-free controls are collected or processed (18).

In a recent publication in *Clinical Cancer Research*, Yang and colleagues (19) reported findings from a first prospective evaluation of auto-antibodies against TP53 (P53-AAbs), alone and in combination with CA125, as an early detection marker for invasive epithelial ovarian/ tubal/peritoneal cancer (OC). The study was performed in context of the multi-modal screening arm (n=50,640) of the UKCTOCS-a population-based, multi-center randomized controlled trial of ovarian cancer screening among more than 200,000 post-menopausal women in the United Kingdom (7)-and in two smaller studies in the USA [MD Anderson Cancer Center-Normal Risk Ovarian Screening Study trial (MDACC-NROSS)] and Australia [Australian Ovarian Cancer Study (AOCS)]. The UKCTOCS study sample included 1,053 serial serum samples collected up to 5 years prior to OC diagnosis for a total of 220 women who went on to develop ovarian cancer; 3,069 longitudinal sera were collected from 619 age-matched control women who did not develop ovarian cancer. Of the 220 OC cases, 164 (74.5%) were detected with rising CA125 using the ROCA algorithm and TVUS (screen positives) whereas 56 cases (25.5%) were screen negative; the latter population was oversampled by design for this study. Each OC patient had 1-8 serial sera drawn at least annually during the trial, whereas each control had 4-5 serial sera drawn annually. The higher maximum number of serial sera drawn from the cases (i.e., up to 8), as compared to controls, stems from the fact that the UKCTOCS protocol mandates the drawing of a further serum sample after 3 months for repeat CA125 measurement and ROCA scoring for women showing an intermediate risk in on the ROCA score (20). Across the three studies, almost all OC cases in the selected study samples from MDACC-NROSS and AOCS, and about 67% of cases in the UKCTOCS study sample, had tumors with serous, or mixed-serous histology.

Yang and colleagues developed a highly sensitive immunoassay to detect autoantibodies against wild-type P53 protein, determined the fractions of OC patients in each study with elevated P53-AAb titers, and used the preclinical sera and data from the UKCTOCS study sample to determine whether TP53-AAbs can provide lead time over diagnostic ROCA risk scoring or single-time CA125 measurements at the usual clinical cut-point of 35 U/mL, and detect cases that did not have elevated CA125 (i.e., that were screen-negative in the trial). Applying a P53-AAb cut-point (78 U/mL) that yielded 97.4% overall specificity across the three studies, they found a positive antibody signal in 30% of pre-treatment sera (15 of 50) from invasive EOC cases in MDAC/NROSS, in 21.3% of pre-treatment sera (23 of 108) in AOCS, and in 19.5% of sera (43 of 220) from invasive OC cases in the UKCTOCS preclinical samples—values broadly in line with those from clinical case-control comparisons [reviewed in (17)].

In the UKCTOCS study, evaluating pre-diagnosis samples, P53-AAb levels above the 78 U/mL cut-point were observed in 34 of the 164 screen-positive cases (20.7%), on average preceding detection by ROCA or elevated CA125 by 9.2 and 8.1 months, respectively. Notably, P53-AAbs also appeared to provide a lead-time benefit among the 9 cases undetected by ROCA but with P53-AAb positivity. Among these cases, TP53-AAb levels were elevated an average of 22.9 months prior to diagnosis. On this basis, the authors concluded that P53-AAbs may be a promising biomarker with clinically significant lead time over either ROCA based risk assessment calculated from serial CA125 measurements or single-time elevation of CA125 (>35 U/mL), and for identification of cases undetected by ROCA or CA125. However, while Yang and colleagues presented the percentage of false-positives identified by P53-AAbs among the control participants (2.7%), the overall false-positive rate associated with a diagnostic algorithm based on the combination of P53-AAb and ROCA (or single-time elevation of CA125) remains unclear. The overall specificity of the markers combined would be lower than that for either marker alone [i.e., false positive rate (FPR; 1-specificity) for the combination of two independent markers is approximately their sum when the FPR for each marker separately is low] and it is possible that the improvement in OC detection observed for the combination of CA125 and TP53-AAbs could have been achieved on the basis of ROCA (or even single-time elevation of CA125) alone with an equivalent relaxation of specificity (i.e., lower marker/ROCA diagnostic cut-points). As a hypothetical example, if the combined specificity of CA125 and TP53-AAbs is 95%, it is plausible that lowering the specificity threshold for CA125 from ~98% to 95% also would have allowed identification of an additional 16% of cases, irrespective of P53-AAbs.

To explore further whether P53-AAb levels are complementary to CA125 for the detection ovarian cancer, Yang and his team performed area under the receiver operating curve (AUC) analyses within the UKCTOCS samples, to examine diagnostic discrimination potential of joint (P53-AAb plus CA125) vs. single-marker (CA125 only) approaches for early detection. Statistical analyses were based on serum measurements first reaching either a P53-AAb threshold of 78 U/mL (97.3% specificity in UKCTOCS) or a CA125 threshold of 35 U/mL (corresponds to 98.1% specificity in UKCTOCS); this simulated a virtual screening approach. Using these marker cut-points, adding P53-AAb values to CA125 moderately but significantly increased the AUC, from 0.838 (CA125 alone) to 0.867 (CA125 + P53-AAb) (P=0.001). By contrast a much larger increase in AUC was observed (from 0.751 to 0.861) when analyses were restricted to serum measurements preceding ovarian cancer diagnosis by at least 3 months. This much larger difference in AUC, however, was due to a reduction in the AUC for CA125 in the time-restricted analyses (>3 months restriction: 0.751; all cases: 0.838). A general issue with the post-hoc evaluation of singletime CA125 measurements (35 U/mL cut-point) as an early-diagnostic marker in the UKCTOCS is that the OC diagnoses in the multi-modal screening arm were driven by ROCA analyses of longitudinal change in the same biomarker, which may have largely eclipsed the performance for single-time assessments. It thus seems possible that the substantial reduction in the AUC for CA125 in the timerestricted analyses was an effect of the selective exclusion of women diagnosed with OC after an intermediate elevated risk estimate by ROCA followed by 3-month confirmatory CA125 measurement and ROCA scoring.

Further, the overall increase in AUC was related to improved sensitivity levels exclusively upwards of an FPR of about 8–10%, though this is not described in detail. At higher levels of specificity (e.g., 98%), as required for practical implementation in screening settings, no improvement in diagnostic sensitivity was apparent when using a combined score of P53-AAbs and CA125, as compared to either marker alone (pAUC at 98% specificity: CA125 alone: 0.006; TP53-AAb alone: 0.003; TP53-AAb and CA125: 0.007; P=0.097.

The TP53 gene is mutated in close to 100% of highgrade serous ovarian tumors, and it has been hypothesized that AAbs are formed against modified protein products of mutated genes as neo-antigens. Thus, as a further study component Yang and his team examined whether autoantibodies more specifically related to modified P53 protein epitopes corresponding to patient-specific TP53 gene mutations would further improve diagnostic sensitivity for OC compared to assays for auto-antibodies against P53 wild-type protein. Diagnostic discrimination was similar in both analyses, in line with findings from other recent studies on the proteomic mapping of P53 immunogenicity by independent research groups (12). A general review of AAbs identified in relation to ovarian cancer (17) and other cancer types (8,9) suggests that, indeed, the vast majority of AAbs may be formed as a polyclonal reaction against wild-type proteins or wild-type protein epitopes, and that AAb response may be mostly driven by locally aberrant expression in tumor tissue, possibly in interaction with local inflammation and immune cell infiltration.

Overall, the results from this first prospective evaluation of auto-antibodies against P53 protein as complementary markers for early detection for OC seem to hold promise, in that a substantial proportion of patients were found to have elevated AAb titers many months ahead of screening diagnosis by ROCA and TVUS, as well as among screennegative cases. The results presented from this first prospective study will need to be investigated and further explored in detail in future prospective studies to evaluate whether, or not, adding P53-AAbs as complementary marker to either a longitudinal ROCA analysis or singletime CA125 measurements will indeed improve overall diagnostic sensitivity at equivalent specificity.

While the discovery and validation of AAbs as early detection markers for ovarian cancer is still an emerging field, more extensive studies on other cancers have led to the establishment of combined AAb panels showing substantial diagnostic potential. For example, for lung cancer the "EarlyCDT-Lung" AAb panel (16) is currently being tested in a multi-modal randomized lung cancer screening trial in Scotland (21), using antibody testing as a first screen to identify participants for low-dose computer tomography imaging as a second screen. Recent studies using highthroughput immuno-proteomics technologies have resulted in a rapidly increasing number of AAbs with diagnostic discrimination potential for OC (12,13), and analyses in clinical case-control sets suggest that a selected panel of 11 AAbs may provide up to 45% sensitivity at 98% specificity, discriminating between serous OC cases vs. healthy controls (22). To date, however, these novel antibody panels have not yet been independently crossvalidated in case-control sets, or prospectively evaluated in

cohort studies. For ovarian cancer, additional prospective investigations, similar to that by Yang and colleagues, are a critical future step to advance AAbs as potentially clinically useful early detection markers for OC.

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