Use of Blood-based mRNA profiling to Identify Biomarkers for Ovarian Cancer Screening

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Abstract

Purpose: To identify candidate genomic signatures for the early detection of ovarian cancer using whole-blood based gene expression profiles.

Experimental Design: We performed Affymetrix U133Plus 2.0 GeneChip microarray analyses on whole blood RNA samples obtained from 14 ovarian cancer patients and 15 age-matched, healthy women. Genes differentially expressed were identified using a parametric Welch t-test. Real-time qRT-PCR analyses were performed on RNA prepared from 96 ovarian cancer patients and 83 age-matched healthy women, using primer sets specific for 14 genes. A Mann Whitney U test assessed individual gene significance. CA125 levels were determined in the same set of samples. We used logistic regression analyses and cross validation to assess the ability of linear combinations of specific transcripts combined with CA125 to distinguish cancer from controls.

Results: Microarray analyses showed that 9583 probes were significantly different in blood gene expression profiles from healthy women as compared with those from ovarian cancer patients (p<0.05). Real-time RT-PCR analyses on the 96 cases and 83 controls validated 7 genes, which showed significantly different expression levels in cases and controls. Logistic regression analyses and cross validation identified an optimal panel of markers including CA125, BRCA1, and KIAA0562, that could improve the sensitivity of CA125 alone to over 90% at 98% specificity in the detection of early stage ovarian cancer.

Conclusion: Circulating blood gene expression profiles identified RNA markers that can improve the sensitivity of CA125 in the detection of early stage ovarian cancer. Further validation is warranted to confirm the clinical usefulness of these biomarkers.

Keywords: Blood; Biomarkers; Cancer

Introduction

Ovarian cancer is the fifth most common cancer in women, but is the leading cause of death of all gynecological cancers. This poor outcome is at least partly because ovarian cancer is hard to detect at an early stage. Currently, most ovarian cancers (75%) are found late in the course of disease, when prognosis is poor and five year survival rates are less than 20% [1]. However, when diagnosed early, the prognosis of ovarian cancer is usually excellent, with five-year survival exceeding 90% for well-differentiated disease [1].

The increase in survival when ovarian cancer is detected at early stages suggests the need for a screening test for the disease. The low incidence of ovarian cancer however 40-50 cases/year per 100,000 women over 50 years of age creates a significant hurdle to early detection. In addition, the morbidity associated with exploratory surgery for possible ovarian cancer has led to general agreement that an ovarian cancer screening program should refer to surgery at most ten women for every case of screen-detected ovarian cancer [2]. To achieve a PPV exceeding 10%, a screening program requires a highly sensitive test (>5%) and an extremely high overall screening specificity (>99.7%) [3].

Large prospective screening trials have investigated two testing modalities: blood tests for biomarkers such as CA125, HE4 and ultrasound. As compared with ultrasound, the blood test has the advantage of lower cost. But ultrasound outperforms blood testing with respect to sensitivity for early stage disease (although a direct randomized comparison has not yet been carried out) [4,5]. Trials of the antigen CA125 (MUC16) blood test, followed by ultrasound when CA125 values are elevated, have resulted in a PPV in excess of 20%, with a pre-clinical sensitivity of more than 70% [6,7]. Although in a large proportion of cases this strategy detected disease prior to the appearance of clinical symptoms, sensitivity for early stage disease using CA125>30U/ml was only about 40% [6]. The combined CA125 and HE4 has only minimal increase in sensitivity and there is only a marginally significant mortality reduction as shown in the most recent UKCTOCS study [8]. Thus a low cost and high sensitivity and specificity test for early-stage disease have not yet been developed. There is a critical need to continue searching for informative serum/plasma biomarkers for preclinical, early stage ovarian cancer.

Circulating blood is a dynamic, highly complex system that communicates with every tissue and organ in the body. Blood plays essential roles in homeostasis, in response to injury or infection, and...
in hormonal communication. It has been hypothesized that circulating blood may act as a "sentinel tissue" that can reflect states of health or disease within the body [9]. This concept is supported by recent studies showing that RNA profiles generated from circulating blood can be used as defined liquid biopsy to identify patients with cardiovascular disease [10,11], early osteoarthritis [12], schizophrenia, bipolar disorder [13-15], kidney diseases [16,17], Crohn’s disease [18], and liver cancer [19]. Thus, blood-derived RNA can be a novel alternative to proteins or peptides as a source of biomarkers.

In the present study, we describe mRNA expression analyses of circulating blood samples obtained from ovarian cancer patients and age-matched healthy control subjects, and we evaluate whether blood-derived RNA can be used for ovarian cancer detection.

Materials and Methods

Blood sample collection

Women of all ages and ethnic backgrounds were included in this study. Blood samples were collected in clinic from 96 patients with ovarian cancer (50 had serous tumors; 9, clear cell; 16, mucinous; 3, endometrioid; and 18 had mixed histological types of ovarian cancer) before surgery, and 83 age-matched (mean age=55) healthy women. These 179 samples included the 15 controls and 14 cancer patients whose samples were used in the initial microarray analyses. Among the 96 cases, 39 patients had early (stage I and II) ovarian cancer. Exclusion criteria include patients who received chemo or radiation therapy. The subjects were recruited equally from both Yongdong Severance Hospital, Korea, and Brigham and Women’s Hospital. All patients gave informed consent under protocols approved by the participating institutions. Blood samples from both cases and controls were collected in EDTA Vacutainer TM tubes (Becton Dickinson, Franklin Lakes, N.J.) and processed within 3 hours. The samples were centrifuged at 2000 rpm at 4°C for 5 minutes. The separated plasma was removed, aliquoted and frozen at -80°C for future analysis.

RNA isolation protocol

After plasma removal, a hypotonic buffer (1.6 mM EDTA, 10 mM KHCO3, 153 mM NH4Cl, pH 7.4) was added at a 3:1 volume ratio to lyse the red blood cells. The mixture was centrifuged to yield a white blood cell pellet, which was resuspended into 1.0 mL of TRizol® Reagent (Invitrogen Corp., Carlsbad, CA) and 0.2 mL of chloroform, according to the manufacturer’s instructions. RNA quality was assessed on an Agilent 2100 Bioanalyzer RNA 6000 Nano Chip, according to the manufacturer's instructions. RNA quantity was determined by absorbance at 260 nm in a Beckman-Coulter DU640 Spectrophotometer.

Microarray analysis

Five micrograms of white cell RNA from each patient were hybridized onto the Affymetrix U133Plus 2.0 Gene Chip oligonucleotide array (Affymetrix; Santa Clara, CA), according to the manufacturer’s instructions. Hybridization signals were scaled in the Affymetrix GCOS software (version 1.1.1), using a scaling factor determined by adjusting the global trimmed mean signal intensity value to 500 for each array, and imported into GeneSpring version 7.2 (Silicon Genetics; Redwood City, CA). Signal intensities were then centered to the 50th percentile for each chip, and for each individual gene, to the median intensity of each specific subset, first to minimize the possible technical bias, then for the whole sample set. Each blood sample yielded a "gene expression profile" representing each of the approximately 20,500 genes on the array.

Data from the microarray was loaded into dChip version 1.3 [20,21] for normalization and quantification. Normalization was performed using the default settings in the software. Expression values were quantified using the Perfect Match-only model [22]. The expression levels estimated by dChip were exported and loaded into Gene Spring version 7.2 for further analysis.

Differentially expressed genes were identified using a parametric test (Welch t-test). A p-value of 0.05 or less was applied and was considered significant, but due to the relatively large number of significant (p<0.05) genes, we used more stringent thresholds to identify genes with even greater significance. Thus, a list was generated of potential biomarker genes significantly up- or down-regulated in disease as compared with controls. Fourteen genes were chosen for qRT-PCR validation, based on a number of criteria, including, p-value, fold change, biological relevance and on support from the literature.

Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction validation Forward and reverse primers were designed using "Primer Quest" (http://biotools.idtdna.com/primerquest, Integrated DNA Technologies, Coralville, IA) and "Primer3" (http://frodo.wi.mit. edu/cgi-bin/primer3/ primer3_ www.cgi) for the following genes: ADAM9, ADIPOR1, KIAA0562, BCL11A, RAB11A, CEACAM1, TAGLN, MRIP, HIST1H2AC, TP53, BRCA1, HIST1H2B, HSPH1, and HSPA. The primer sets for the 14 genes are given in Table 1 (published online only). Serial dilution measurements for target gene and housekeeping gene, beta-actin (ACTB), were used to ensure that the values were within the linear range and the amplification efficiency was approximately equal for the target and ACTB. ACTB was selected as a housekeeping gene because no statistically significant differences were observed between control and disease group in this study. An automatically calculated melting point dissociation curve and agarose gels were used to examine and ensure the specific PCR amplification and the lack of primer-dimer formation in each well.

Amplification efficiency and specificity of the primer pairs were determined using serial dilution of reference cDNA generated from a normal blood RNA pool, with confirmation on agarose gel to ensure that the values were within the linear range and that the amplification efficiency was approximately equal for each of the target genes tested. For real-time RT-PCR assay, 1st strand cDNA was synthesized from 2 µg of total RNA using the ABI High Capacity cDNA Archive Kit (Applied Biosystems) on a Perkin–Elmer DNA Thermal Cycler according to the manufacturer's protocol. Further amplification and quantitation of specific cDNA were performed by Qiagen QuantiTec SYBR® Green PCR Kit using an ABI Prism 7500 real-time PCR system (Applied Biosystems, Foster City, CA). The confirmation of specific amplification and lack of primer dimer formation were determined by calculating melting dissociation curve. In each sample, the expression level of a target gene was quantified by its threshold cycle (Ct) value, which is the concentration-dependent PCR cycle number at which the amplicon was observed between control and disease group in this study. An automatically calculated melting point dissociation curve and agarose gels were used to examine and ensure the specific PCR amplification and the lack of primer-dimer formation in each well.

Amplification efficiency and specificity of the primer pairs were determined using serial dilution of reference cDNA generated from a normal blood RNA pool, with confirmation on agarose gel to ensure that the values were within the linear range and that the amplification efficiency was approximately equal for each of the target genes tested. For real-time RT-PCR assay, 1st strand cDNA was synthesized from 2 µg of total RNA using the ABI High Capacity cDNA Archive Kit (Applied Biosystems) on a Perkin–Elmer DNA Thermal Cycler according to the manufacturer's protocol. Further amplification and quantitation of specific cDNA were performed by Qiagen QuantiTec SYBR® Green PCR Kit using an ABI Prism 7500 real-time PCR system (Applied Biosystems, Foster City, CA). The confirmation of specific amplification and lack of primer dimer formation were determined by calculating melting dissociation curve. In each sample, the expression level of a target gene was quantified by its threshold cycle (Ct) value, which is the concentration-dependent PCR cycle number at which the amplicon becomes distinguishable over background. Relative fold change of each individual gene was calculated using the comparative Ct equation (User Bulletin #2, 2001, Applied Biosystems) as follows: 2ΔΔCt where ΔΔCt=ΔCt (of a sample)- ΔCt (control samples). ΔCt=Ct (target gene) - Ct (house-keeping gene) where Ct values of target genes were normalized to housekeeping gene (β-actin).

CA125 ELISA assay

An immunoradiometric assay was performed on CA 125, according to the manufacturer's instructions (Abbot Diagnostics). Results were expressed as the mean absorbance of triplicate wells after subtraction of background values.
Statistical Analysis

We employed logistic regression analyses using SAS (Statistical Analysis System Version 9.0, Cary, NC), with the branch and bound algorithm of Furnival and Wilson [23] to identify optimal panels of one to five markers with the highest (statistical) score. Following identification of the optimal panels, cross validation using “leave-one-out” prediction was performed to solve the overfitting bias problem. Sensitivities at 90%, 95%, and 98% specificity were estimated by ranking the predicted fit for each control subject, obtaining the cut-off points corresponding to these levels of specificity, and applying the cut-points to the ranked predictions for the ovarian cancer cases. Particular attention was paid to the results at 98% specificity, since a first line blood test at this level of specificity, followed by ultrasound, has been shown empirically to achieve an overall screening specificity of the required minimum of 99.7% [24]. Optimal panels were derived by, first, including CA125 in the selection set, and then, subsequently, by excluding CA125 from the selected marker set. This strategy was applied to cases of all stages of ovarian cancer cases (n=96) as compared with control subjects (n=83), and then to early stage cases (n=39) as compared with control subjects (n=83). Expression levels of single genes, as distinct from a panel, between cases and controls were compared by the non-parametric Mann Whitney U test.

Results

Expressed gene profile from blood

We profiled gene expression from blood samples obtained from 14 ovarian cancer patients and 15 controls. Parametric Welch t test analysis identified a set of 9,583 probes, with a P<0.05 that could differentiate controls from ovarian cancer samples. A hierarchical cluster analysis using a subset of 227 probes (P<0.001) of differentially expressed genes from these 29 blood samples is shown in Figure 1. The 14 ovarian cancer profiles clustered separately from the group of 15 healthy control profiles.

Real-time qRT-PCR validation

We first selected a list of 14 genes (ADAM9, ADIPOR1, KIAA0562, BCL11A, RAB11A, CEACAM1, TAGLN, MRIP, HIST1H2AC, TP53, BRCA1, HIST1H2B, HSPH1, and HSPA) for real time qRT-PCR.
Cross validation analysis

We used logistic regression to identify optimal combinations of the 14 candidate biomarkers with panels of one to five biomarkers and estimated the cross-validated sensitivities at three high levels of specificity. Panels with more than five biomarkers did not significantly improve the sensitivity, and in some cases, decreased it (data not shown). Joint complementarity was observed by combining different markers such as BRCA1 and BRIP (Figure 3). We first evaluated the RNA markers alone and subsequently included CA125, which is the most widely researched ovarian cancer marker, to determine whether the RNA biomarkers can be used to complement CA125 in the detection of early stage ovarian cancer. The optimal panel including CA125 had five markers (CA125, BRCA1, MRIP, ADAM9, BCL11A or RAB11A) and gave a sensitivity of 95.8% at 98% specificity, as compared with 88.5% sensitivity with CA125 alone. Additional markers did not improve sensitivity. When CA125 was omitted from our selection, the optimal panel contained three markers (HIST1H2B, MRIP, and ADIPOR1) which gave a sensitivity of 62.5% at a specificity of 98% (Table 2A and 2B). Statistical analysis identified the optimal panel for early stage disease (CA125, BRCA1, and KIAA0562), which provided a sensitivity of 92.3% at 98% specificity, as compared with a sensitivity of 84.6% with CA125 alone. Without CA125 in the selection set, the optimal panel (BRCA1, MRIP, HIST1H2B, ADAM9, and KIAA0562) had a sensitivity of 53.8% at 98% specificity (Table 3A and 3B).

Discussion

Because ovarian cancer is usually discovered only at a late, difficult to manage stage, there is a need for better methods for early detection. The most widely researched ovarian cancer marker is CA125, and in pilot screening studies the marker has shown some value in postmenopausal women; sensitivity of this marker for early stage, pre-clinical disease detection however remains unclear [6]. A number of tumor markers for ovarian cancer, alone or as a panel, have also been identified and evaluated recently [25-28], but a useful screening marker or panel of markers has not been clearly established. Most of these markers are proteins or peptides in serum or plasma. Other sources, such as RNA from whole blood, have not been thoroughly explored.

![Figure 2](Image)
the sensitivity at a fixed specificity. These data suggest that, although a single marker may not show a significant difference between cases and controls, it may still be useful in the development of a marker panel as demonstrated by the regression model. With the limited number of patients available in any single clinical study and the increasing number of genes and gene products measurable with recent technologies, it will be difficult to determine which candidates are true signals of cancer and which are spurious. Resolving this difference will require new biological insights, as well as empirical replication across multiple geographical sites and clinical cohorts of patients. Nonetheless, our panel of biomarkers complementing ovarian cancer biomarker CA125 is a promising step towards a blood test for ovarian cancer screening.

One component of our panel, BRCA1 RNA, is biologically related to ovarian carcinogenesis, and CA125 has empirically withstood the test of time through replication of positive results in many patient cohorts.

Our results demonstrate at a level of 98% specificity, the minimum required screening specificity for a first line test for ovarian cancer [3], our RNA marker panel including BRCA1, ADAM9, and MRIP increased the sensitivity of CA125 to more than 90% in detecting ovarian cancer in patients with early stage as well as all stages of the disease. These results suggest that these RNA markers together with CA125 may be used to develop a blood test for ovarian cancer screening.

Our data demonstrate the feasibility of using whole blood-based RNA biomarkers for ovarian cancer detection. The use of RNA biomarkers in screening is particularly attractive since it involves the use of multiplex quantitative RT-PCR analysis as a screening platform, which has a low coefficient of variation (CV) and does not require the generation of antibodies for protein biomarkers and the development of enzyme-linked immunosorbent assays (ELISA) for biomarker detection.

One limitation for this study is the relatively small size and they were collected from clinically identified patients. However, the pre-operative sensitivity estimates shown in this study are sufficiently high to encourage us to carry out future studies on a large collection of bio repository samples. These samples will be collected in prospective clinical screening studies, and include the panel of RNA markers alone or in combination with CA125 to obtain estimates of screening specificity and sensitivity. The crucial issue, which has yet to be addressed, is the sensitivity of a panel of biomarkers for detecting early stage ovarian cancer or screening subjects who would not have been.

The first indication that RNA profiles generated from circulating blood could serve as diagnostic markers emerged in studies of cardiovascular disease [10,11]. Blood-derived RNA has since been shown to have discriminatory power in other conditions, such as schizophrenia, bipolar disorder [13-15], early osteoarthritis [12], kidney diseases [16,17], Crohn’s disease [18], and liver cancer [19]. Using the RNA approach we have identified in circulating blood obtained from pre-operative ovarian cancer patients a gene expression signature that is distinct from the blood-based RNA signatures obtained from healthy women. Fourteen genes, which showed significant differences between cases and controls by parametric Welch t-test, were selected for further validation studies. Using the univariate non-parametric Mann-Whitney U test, expression levels of seven genes showed significant differences between cases and controls. Of interest, BRCA1, which did not show a significant difference between cases and controls by the non-parametric test, demonstrated complementarity with other markers using logistic regression analysis, in that it increased

Table 2A: Cross-validated sensitivity estimates of the best panels of 1-5 RNA markers including CA125 in early and late stage cases.

<table>
<thead>
<tr>
<th>Marker Panel</th>
<th>90% Specificity</th>
<th>95% Specificity</th>
<th>98% Specificity</th>
</tr>
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<tbody>
<tr>
<td>CA125</td>
<td>90.6</td>
<td>90.6</td>
<td>88.5</td>
</tr>
<tr>
<td>CA125 MRIP</td>
<td>95.8</td>
<td>91.7</td>
<td>91.7</td>
</tr>
<tr>
<td>CA125 MRIP BRCA1</td>
<td>95.8</td>
<td>95.8</td>
<td>94.8</td>
</tr>
<tr>
<td>CA125 MRIP BRCA1 ADAM9</td>
<td>95.8</td>
<td>95.8</td>
<td>94.8</td>
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<tr>
<td>CA125 MRIP BRCA1 ADAM9 MRIP BRCA1 ADIPOR1</td>
<td>95.8</td>
<td>96.8</td>
<td>94.8</td>
</tr>
<tr>
<td>CA125 MRIP BRCA1 ADAM9 MRIP BRCA1 ADIPOR1 HSPH1</td>
<td>95.8</td>
<td>96.8</td>
<td>94.8</td>
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<tr>
<td>CA125 MRIP BRCA1 KIAA0562 BCL11A</td>
<td>95.8</td>
<td>95.8</td>
<td>95.8</td>
</tr>
<tr>
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<td>95.8</td>
<td>95.8</td>
<td>95.8</td>
</tr>
</tbody>
</table>

Early Stage Cases (n=39) and Late Stage Cases (n=57) Versus Controls (n=83)

Table 2B: Cross-validated sensitivity estimates of the best panels of 1-5 RNA markers excluding CA125 from selection process in early and late stage cases.

<table>
<thead>
<tr>
<th>Marker Panel</th>
<th>90% Specificity</th>
<th>95% Specificity</th>
<th>98% Specificity</th>
</tr>
</thead>
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<tr>
<td>HSPA8</td>
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<td>41.7</td>
<td>39.6</td>
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<tr>
<td>MRIP</td>
<td>62.3</td>
<td>43.8</td>
<td>33.3</td>
</tr>
<tr>
<td>MRIP HIST1H2B</td>
<td>67.7</td>
<td>60.4</td>
<td>57.3</td>
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<tr>
<td>MRIP BRCA1 HSPH1</td>
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<td>MRIP BRCA1 HIST1H2B</td>
<td>71.9</td>
<td>65.6</td>
<td>50.0</td>
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<td>MRIP BRCA1 KIAA0562</td>
<td>69.8</td>
<td>67.7</td>
<td>54.2</td>
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<tr>
<td>MRIP HIST1H2B ADIPOR1</td>
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<td>62.5</td>
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<tr>
<td>MRIP BRCA1 KIAA0562 ADIPOR1</td>
<td>71.9</td>
<td>68.8</td>
<td>59.4</td>
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<tr>
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<tr>
<td>MRIP BRCA1 KIAA0562 HIST1H2B ADIPOR1</td>
<td>71.9</td>
<td>68.8</td>
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<tr>
<td>MRIP BRCA1 KIAA0562 ADAM9 BCL11A</td>
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Early Stage Cases (n=39) and Late Stage Cases (n=57) Versus Controls (n=83)
transcriptome profiling analysis on pre-operative circulating white cell RNA to identify a blood genomic signature (BGS) that is associated with ovarian cancer. Our results demonstrate that at a level of 98% specificity, our RNA marker panel increased the sensitivity of CA125 to more than 90% in detecting ovarian cancer in patients with early stage as well as all stages of the disease. These results suggest that these RNA markers together with CA125 may be used to develop a blood test for ovarian cancer screening.

Acknowledgements

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References


Table 3A: Cross-validated sensitivity estimates of the best panels of 1-5 RNA markers including CA125 in early stage cases.

<table>
<thead>
<tr>
<th>Marker Panel</th>
<th>Cross-validated Sensitivity (%)</th>
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<th>95% Specificity</th>
<th>98% Specificity</th>
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<tr>
<td>CA125</td>
<td>87.2</td>
<td>87.2</td>
<td>84.6</td>
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<tr>
<td>CA125 TP53</td>
<td>92.3</td>
<td>92.3</td>
<td>79.5</td>
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<tr>
<td>CA125 MRIP</td>
<td>92.3</td>
<td>87.2</td>
<td>84.6</td>
<td></td>
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<tr>
<td>CA125 BRCA1</td>
<td>89.7</td>
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<td>82.1</td>
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<tr>
<td>CA125 BRCA1 MRIP KIAA0562</td>
<td>94.9</td>
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<td>92.3</td>
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<tr>
<td>CA125 BRCA1 MRIP ADAM9 HSPH1</td>
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<td>94.9</td>
<td>92.3</td>
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<tr>
<td>CA125 BRCA1 MRIP ADAM9 BCL11A</td>
<td>94.9</td>
<td>94.9</td>
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Table 3B: Cross-validated sensitivity estimates of the best panels of 1-5 RNA markers excluding CA125 from selection process in early stage cases.

<table>
<thead>
<tr>
<th>Marker Panel</th>
<th>Cross-validated Sensitivity (%)</th>
<th>90% Specificity</th>
<th>95% Specificity</th>
<th>98% Specificity</th>
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<tr>
<td>HSPH1 or HSPA8</td>
<td>46.2</td>
<td>38.5</td>
<td>20.5</td>
<td>25.6</td>
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<td>BRCA1 MRIP</td>
<td>56.4</td>
<td>56.4</td>
<td>35.9</td>
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<td>BRCA1 MRIP CEACAM1</td>
<td>71.8</td>
<td>51.3</td>
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<tr>
<td>BRCA1 MRIP CEACAM1 ADAM9</td>
<td>76.9</td>
<td>51.3</td>
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<td>BRCA1 MRIP HIST12HB ADAM9</td>
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<td>KIAA0562</td>
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<td>62.9</td>
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<td>BRCA1 MRIP CEACAM1 ADAM9</td>
<td>74.4</td>
<td>59.0</td>
<td>43.6</td>
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In conclusion, this study has demonstrated the potential value of using microarray analysis to identify differentially expressed mRNA in circulating blood. The findings suggest evidence for an association between a circulating blood mRNA signature and ovarian cancer, pointing to a rationale for further research to assess potential clinical utility. The identification of specific mRNAs in circulating blood that can improve sensitivity and specificity for early ovarian cancer detection will allow us to develop an ovarian cancer screening test for women which can detect the disease at early stages and thus improve patient survival rates.

Translational Relevance

It has been hypothesized that circulating blood may act as a "sentinel tissue" that can reflect states of health or disease within the body. Thus, blood-derived RNA can be a novel alternative to proteins or peptides as a source of biomarkers. In the present study, for the first time we describe using transcriptome profiling analysis on pre-operative circulating white cell RNA to identify a blood genomic signature (BGS) that is associated with ovarian cancer. Our results demonstrate that at a level of 98% specificity, our RNA marker panel increased the sensitivity of CA125 to more than 90% in detecting ovarian cancer in patients with early stage as well as all stages of the disease. These results suggest


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