ORIGINAL ARTICLE

Apolipoprotein A1 and Transferrin as Biomarkers in Ovarian Cancer Diagnostics

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Summary

Introduction. Advanced technologies such as matrix assisted laser desorption/ionization and surface enhanced laser desorption/ionization mass spectrometry has introduced promising insights into ovarian cancer detection. Several highly sensitive and specific protein peaks have been identified that discriminates ovarian cancer patients from patients with benign ovarian tumors and controls.

Aim of the Study. Objective of this study is to evaluate diagnostic accuracy each individual marker and combined serum biomarker assay consisting of apolipoprotein A1, transferrin and Ca125.

Materials and methods. A case-control study consisted of 99 women – 37 patients with ovarian cancer, 31 patients with benign ovarian diseases, and 31 age-matched healthy controls. Apolipoprotein A1 and transferrin was measured in sera using immunological turbidimetric assay. Tumor marker CA125 was analyzed by standard enzyme-labeled chemiluminescent immunometric assay. To compare the difference between variables in the study groups, ANOVA test was performed and for correlation Pearson's correlation analysis applied. Sensitivity and specificity of the diagnostic tests was calculated using statistical program Vassarstat.

Results. Serum apolipoprotein A1 and transferrin were down regulated, controversially to Ca125, which was up regulated among ovarian cancer patients. Negative correlation between transferrin and Ca 125 ($p < 0.00$), apolipoprotein A1 and Ca 125 ($p < 0.00$) and positive correlation between transferrin and apolipoprotein A1 ($p < 0.00$) was observed. For single biomarker test highest diagnostic sensitivity and specificity for Ca125 was observed. Addition of apolipoprotein A1 or transferrin to serum level of Ca125 with the condition, that both have to overlap the threshold (Ca125 > 21 U/ml and apolipoprotein A1 ≤ 139.1 mg/dl or transferrin ≤ 2.3 g/l), yielded test specificity of 96.7%. Addition of apolipoprotein A1 to Ca125 improved test sensitivity up to 94.5% maintaining high sensitivity at the same time 91.1%, respectively, when discriminating controls from ovarian cancer patients. Biomarker test consisting of apolipoprotein A1, transferrin and Ca125 had high specificity at unacceptable sensitivity.

Conclusions. Combined biomarker tests discovered using advanced technologies can aid more accurate ovarian cancer detection. Use of apolipoprotein A1 in combination with Ca125 at distinct thresholds can improve ovarian cancer detection.

Key words: apolipoprotein A1, transferrin, ovarian, cancer; screening.

INTRODUCTION

Ovarian cancer is the 5th leading cause of death between all cancers and most common between gynecological cancers worldwide. In year 2007–2008 mortality rates in Latvia were 194 and 200 cases each year with an incidence of 309 and 280 cases, respectively.

Cancer antigen 125 is the most studied and well characterized serologic tumor marker for advanced epithelial ovarian cancers. However, its use as a population-based screening tool for early detection and diagnosis of ovarian cancer is limited by its low sensitivity and specificity, therefore a variety of biomarkers have been investigated to improve ovarian cancer detection (9,12,21). Promising results have implemented advanced technologies such as matrix assisted laser desorption/ionization and surface enhanced laser desorption/ionization mass spectrometry. Several protein peaks have been identified that discriminates ovarian cancer patients from patients with benign ovarian tumors and controls. Particular technologies already have been used to detect other tumors such as prostate cancer, transitional cell carcinoma of the bladder, and cervical cancer (1,18,24,25).

A comprehensive review of these technologies and clinical applications are described in literature (4, 5, 8, 13, 17, 26).

Several authors have reported about different expression of transferrin and apolipoprotein A1 in serum among ovarian cancer patients and healthy controls improving sensitivity of ovarian cancer diagnostic tests to 74–98% at specificity of 92–98% (3,16,19,27).

AIM OF THE STUDY

Objective of this study is to evaluate diagnostic accuracy of each individual marker and combined serum biomarker assay consisting of apolipoprotein A1, transferrin and Ca125.

MATERIALS AND METHODS

Ethical approval was taken for this study from the Ethics Committee of Riga Stradins University. A case-control study consisted of 99 women – 37 patients with ovarian cancer in Group A, 31 patients with benign ovarian diseases in Group B, and 31 age-matched healthy controls in Group C. Patients were divided into the 2 study groups after surgery according to final histological

diagnosis. In Group B were patients thought to have had ovarian cancer before the operation. Patients with severe co-morbidities, previous or coexisting other malignancy were not included in the study. In the both study groups tumors arising only from epithelial origin were included (Table 1).

Table 1. Clinical characteristics and age distribution of 99 study samples

Diagnostic group	Age range	Mean age	Pathology		
			Serous	Endo-metroid	Mucinous
Group A	36–87	60.6	35	1	1
Group B	40–79	58.0	19	5	7
Group C	37–81	58.2	–	–	–

Serum samples from patients were collected into two Becton and Dickenson 6ml serum vacutainers in the early morning of the planned surgery day. Vacutainers according to protocol were shaken 6–8 times, left for clot formation between 60 and 120 minutes and centrifuged during the interval for 10 minutes at 1300 RPM. After that serum was aliquoted, divided and transferred into nine 0.5ml eppendorfs for storage at – 80 degrees C. For the control group women serum samples were taken after transvaginal ultrasonographic examination and ensuring of having no gynecological pathology.

In the control group were women chosen who attended gynecologist in out patient clinic.

Tumor marker CA125 was detected in patient's serum by standard enzyme-labeled chemiluminescent immunometric assay ADVIA Centaur CA125 II™, Multi-Diluent 1, Bayer, using Siemens analyzer Immulite-2000 (1,24).

Apolipoprotein A1 was measured in sera using immunological turbidimetric assay manufactured by Roche Diagnostics (2,16,20,22).

Transferrin was similarly measured in sera using immunological turbidimetric assay manufactured by Roche Diagnostics (7,11,23). Upper limit of 95% confidence interval from control group women was chosen as a cutoff level for apolipoprotein A1 and transferrin serum concentration.

For ovarian cancer patients a total abdominal hysterectomy with bilateral salpingoophorectomy and omentectomy was performed. Some ovarian cancer patients were subjected for pelvic lymphadenectomy as a staging or cytoreductive procedure. For patients with benign gynecological tumors a total abdominal hysterectomy with or without bilateral salpingoophorectomy was performed.

To evaluate mean biomarker concentrations in the groups, descriptive analysis were performed using statistical program SPSS 17.0. To reflect the standard deviations for the calculated values standard error of the mean was used. To compare the difference between variables in the study groups, ANOVA test was performed and for correlation Pearson's correlation analysis applied. Sensitivity and specificity of the diagnostic tests

was calculated using statistical program Vassarstat. To display diagnostic performance of each individual and combined biomarker test, receivers operating curve was drawn.

RESULTS

Both patient and control groups were similar according to the mean age ($p=0.59$). Level of Ca125 was analyzed as it is considered as a standard biomarker for ovarian cancer detection.

Serum apolipoprotein A1 and transferrin were down regulated in Group A, controversially to Ca125, which was up regulated among ovarian cancer patients (Figure 1)

Fig. 1. Distribution of mean serum Ca125 (a), Apolipoprotein A1 (b) and Transferrin (c) concentrations among study and control groups

Difference between mean serum concentrations in the study and control groups were statistically significant, except transferrin concentrations when compared between groups B and C (Tables 2, 3).

Table 2. Mean serum concentrations of serum Transferrin, Apolipoprotein A1 and Ca 125 in study and control groups

Diagnostic group	Transferrin, g/l (95% CI)	Apolipo A1, mg/dl (95% CI)	Ca 125, U/ml (95% CI)
Group A	2.21 ± 0.08 (2.0 – 2.3)	125.42 ± 6.75 (111.7 – 139.1)	627.80 ± 134.52 (353.7 – 901.8)
Group B	2.61 ± 0.08 (2.4 – 2.7)	142.36 ± 30.79 (131 – 153.6)	63.70 ± 17.29 (28.3 – 99.0)
Group C	2.52 ± 0.06 (2.4 – 2.6)	165.35 ± 26.74 (155.5 – 175.1)	7.37 ± 0.63 (6.0 – 8.6)

Table 3. Differences among study and control groups mean Transferrin, Apolipoprotein A1 and Ca 125 serum concentrations (p value)

Diagnostic group	Transferrin	Apolipo A1	Ca 125
Group A vs. B	0.00	0.06	0.00
Group B vs. C	0.41	0.00	0.00
Group C vs. A	0.00	0.00	0.00

Negative correlation between transferrin and Ca 125 ($p < 0.00$), apolipoprotein A1 and Ca 125 ($p < 0.00$) and positive correlation between transferrin and apolipoprotein A1 ($p < 0.00$) was observed.

For single biomarker test highest diagnostic sensitivity and specificity for Ca125 was observed. Addition of apolipoprotein A1 or transferrin to serum level of Ca125 with the condition, that both have to overlap the threshold (Ca125 > 21U/ml and apolipoprotein A1 ≤139.1 mg/dl or transferrin ≤2.3 g/l), improved specificity of the test up to 96.7%. But addition of apolipoprotein A1 or Transferrin to serum level of Ca125 with the condition, that one of them have to overlap the threshold, improved test sensitivity up to 94.5% with decrease in specificity to 59.6% – 62.9%. Further addition of third biomarker yielded prominent specificity with dramatic decrease in sensitivity (Table 4).

Table 4. Accuracy of individual and combined biomarker diagnostic tests evaluated between Groups A vs. B and C

Biomarkers (threshold)	Sensitivity	Specificity
Apolipoprotein A1 (≤139.1 mg/dl)	75.6%	70.9%
Transferrin (≤2.3 g/l)	72.9%	74.1%
Ca 125 (>21 U/ml)	91.9%	80.6%
Apolipoprotein A1 (≤139.1 mg/dl) and Ca 125 (>21 U/ml)	72.9%	88.7%
Apolipoprotein A1 (≤139.1 mg/dl) or Ca 125 (>21 U/ml)	94.5%	62.9%
Apolipoprotein A1 (≤139.1 mg/dl) and Transferrin (≤2.3 g/l)	64.8%	93.5%
Apolipoprotein A1 (≤139.1 mg/dl) or Transferrin (≤2.3 g/l)	83.7%	51.6%
Transferrin (≤2.3 g/l) and Ca 125 (>21 U/ml)	70.2%	96.7%
Transferrin (≤2.3 g/l) or Ca 125 (>21 U/ml)	94.5%	59.6%
Apolipo A1 (≤139.1 mg/dl) , Transferrin (≤2.3 g/l) and Ca 125 (>21 U/ml)	62.1%	100.0%

Similarly, the highest sensitivity and specificity was observed for Ca125 alone when applied to distinguish cancer patients from healthy controls in groups A and C. Only addition of apolipoprotein A1 improved test sensitivity up to 94.5% maintaining high sensitivity at the same time – 91.1% (Table 5). For all other biomarker combinations sensitivity or specificity was low.

Table 5. Accuracy of individual and combined biomarker diagnostic tests evaluated between Groups A and C

Biomarkers (threshold)	Sensitivity	Specificity
Apolipoprotein A1 (≤139.1 mg/dl)	75.6%	90.3%
Transferrin (≤2.3 g/l)	78.3%	70.9%
Ca 125 (>21 U/ml)	91.9%	100.0%
Apolipoprotein A1 (≤139.1 mg/dl) and Ca 125 (>21 U/ml)	72.9%	100.0%
Apolipoprotein A1 (≤139.1 mg/dl) or Ca 125 (>21 U/ml)	94.5%	91.1%
Apolipoprotein A1 (≤139.1 mg/dl) and Transferrin (≤2.3 g/l)	67.5%	100.0%
Apolipoprotein A1 (≤139.1 mg/dl) or Transferrin (≤2.3 g/l)	78.3%	61.2%
Transferrin (≤2.3 g/l) and Ca 125 (>21 U/ml)	78.3%	100.0%
Transferrin (≤2.3 g/l) or Ca 125 (>21 U/ml)	94.5%	70.9%

Ca125 alone was more accurate predictor of the disease when compared to combinations of Ca125+Apolipoprotein A1 or Ca125+Transferrin (Figure 2).

Fig. 2. Ability to separate ovarian cancer patients of single and combined biomarker tests

DISCUSSION

This study reflects facilities of advanced technologies which can separate differently expressed protein levels in ovarian cancer patients and healthy women. Information derived from innovative technologies can aid usage of conventional investigation methods more targeted.

Our sample set contained only 6 patients with early stage ovarian cancers; therefore we were not able to perform comparison of protein expression among early and late stage ovarian cancers.

Despite efforts to find new highly sensitive and specific biomarkers, CA125 still is a most accurate single biomarker for ovarian tumor malignancy prediction. Addition of new biomarkers can only improve diagnostic precision of Ca125.

Numerous studies have noted differently expressed protein peaks of apolipoprotein A1 and transferrin in ovarian cancer and control group patients (3,6,10,14,28). Most of studies had significant improvement in sensitivity and specificity of combined diagnostic tests. In literature there have been published articles about different prediction models, which can discriminate normal serum samples from serum samples derived from patients with low malignancy potential tumors with 91% sensitivity at specificity of 92%, and normal samples from early stage ovarian cancer with a sensitivity of 89% at specificity of 92% (3). Higher diagnostic accuracy of particular study can be explained by having another biomarker transthyretin included in malignancy prediction model. In our study merging of all biomarkers resulted in lower malignancy prediction

rates than use of algorithm when apolipoprotein A1 should be down regulated or Ca 125 elevated.

Consolidation of all biomarkers offered high specificity with essential decrease of sensitivity. Other authors have reported about high diagnostic sensitivity and specificity reaching 97% and 99% (AUC 99%), respectively, especially combining more than 2 newly discovered serum proteins with Ca125 (3).

CONCLUSIONS

Combined biomarker tests discovered using advanced technologies can aid more accurate ovarian cancer detection. Use of apolipoprotein A1 in combination with Ca125 at distinct thresholds can improve ovarian cancer detection. Other biomarker incorporation in diagnostic tests should be investigated and further prospective studies with larger sample size are needed to reach clear conclusions.

Conflict of interest: None

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