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Development of Serum Biomarker Panels for The Early Detection of Breast Cancer

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Abstract

Purpose: We aimed to develop noninvasive and early detection breast cancer biomarkers panel that may serve as assistant diagnostic method.

Methods: 61 biomarkers were detected in sea of 101 healthy controls, 46 benign breast diseases and 77 breast cancer patients in the training group. A metropolis algorithm with Monte Carlo simulation was used for choosing the model. 444 individuals were used for validation. Serum from 245 female cancer patients including 5 kinds of cancers were also collected to evaluate cancer selectivity.

Results: Panel consisting of Apolipoprotein A Ⅰ (ApoA Ⅰ), ApopB, C-reactive protein (CRP) and interleukin (IL)-8 had the highest value for discriminating between breast cancer and healthy control. The sensitivity (SN) was 98.70% for all-stage, 100.00% for early-stage and 97.92% for advanced-stage with 90% specificity (SP). In the validation group, the sensitivities were 96.43%, 100.00% and 94.21% at 90% SP. This panel identified 14.29% cervical cancer, 0% lung cancer, 20.29% pancreatic cancer, 25.00% gastric cancer, and 17.50% colorectal cancer as non-breast cancer. Panel consisting of Pepsinogen (PG) Ⅰ/II, CRP, Superoxide dismutase, Tumor necrosis factor α had the highest value for discriminating between breast cancer and benign breast diseases. The SN was 88.31% for all-stage, 72.41% for early-stage and 97.92% for advanced-stage with 90% SP. In the validation group, the sensitivities were 81.25%, 69.77% and 88.41% at 90% SP.

Conclusions: The biomarker panels showed an improved performance when compared to CA153. It may serve as assistant tools for breast cancer screening and early detection to improve the clinical outcome.

Keywords: Serum; Breast Cancer; Metropolis Algorithm, Monte Carlo Simulation; Early Detection

1. Introduction

Breast cancer is the most common type of cancer in worldwide. It is the second leading cause of cancer death among women in the United States with nearly 234580 new cases and 40030 deaths expected in the year 2013[1]. Five year survival of breast cancer women is highly correlated with the breast cancer stage. Overall 5 year survival is 98% when diagnosed at an early stage as opposed to 23% when the disease has already spread to distant organs[2], thus, early detection of breast cancer is critical to reduce breast cancer morbidity and mortality.

Unfortunately, now there are no effective diagnostic tools and biomarkers for the early detection of breast cancer in clinical practice. For example, mammography, which was known as the gold standard diagnostic tool for breast cancer diagnosis, may miss some small lesions which were not visible, particularly in young women with dense breast tissue[3]. In addition, mammograms have a high false positive rate, which will result in costly and invasive follow up tests, including biopsies. Its diagnostic value for early detection of breast cancer greatly limited its application in clinical practice. Magnetic resonance imaging may improve the sensitivity to 92%, however, its specificity was low, with a specificity of 52%[4]. Noninvasive detection, such as, serum biomarker test, would be less expensive and easier to perform on a large scale. The most commonly serum biomarker used for monitoring breast cancer, CA15-3, however, the sensitivity was low, and it was not useful for the early detection of breast cancer[5, 6]. It is increased in 10% of patients with stage I disease, 20% with stage II disease, 40% with stage III disease, and 75% with stage IV disease[7].

Lots of potential novel biomarkers for breast cancer detection and recurrence have been developed in the past years, such as, CA 27.29, carcinoembryonic antigen, estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, urokinase plasminogen activator, plasminogen activator inhibitor 1, prostate apoptosis response 4, splA/ryanodine receptor domain and SOCS box containing 1 [8-11], however, most of them were limited by their diagnostic value for early stage breast cancer, or lack of effective methods for large scale clinical diagnosis. Multi-biomarker detection methods were also developed[12, 13], but most of them only discriminated the healthy control and breast cancer, not evaluated the diagnostic value of discriminating the benign breast diseases and breast cancer. In addition, one strategy may be feasible by exploring the diagnostic value of the conventional biomarkers in clinical practice, because the biomarkers were usually not specific for one kind of cancer. Noninvasive, sensitive, accurate and early detection breast cancer biomarkers panel which may serve as assistant diagnostic method is crucial for clinical practice.

In this study, we used multi-biomarker analysis method to analyze 61 conventional serum biomarkers which have been approved by ISO 15189 to ensure stable and comparable results. We aimed to identify and validate biomarker panels for discriminating breast cancer and healthy control, benign breast diseases which may serve as assistant tools for early detection of breast cancer to improve the clinical outcome. The flowchart of our experimental design was shown in Figure 1.

2. Materials and Methods

2.1 Sample collection and patients

With patients consent, serum from 224 individuals including 77 breast cancer patients, 101 healthy control individuals, and 46 benign breast diseases patients (15 fibroadenoma, 8 intraductal papilloma, 6 cysts, 14 fibrocystic changes, 3 atypical ductal hyperplasia patients) were collected as training group from Chinese PLA General Hospital. The research was approved by the Ethics Committee of the Chinese PLA General Hospital. Independent serum from 444 individuals including 168 healthy control individuals, 112 breast cancer patients and 164 benign breast diseases (48 fibroadenoma, 13 intraductal papilloma, 31 cysts, 39 fibrocystic changes, 17 atypical ductal hyperplasia patients, and 16 others patients) were collected for cross-validation of the training group. Serum from 245 female cancer patients including 77 cervical cancer, 23 lung cancer, 69 pancreatic cancer, 36 gastric cancer and 40 colorectal cancer patients were also collected for evaluating cancer selectivity. Stage I and II were defined as early stage breast cancer. Stage III and IV were defined as advanced stage breast cancer [14-17]. 10 mL peripheral blood samples were collected, after centrifuging at 3400 rpm for 7 minutes, the serum was aliquoted and stored at -80° C until detection. No freeze thawing was allowed prior to detection. Serum samples were collected before any treatment, such as surgery, chemotherapy or radiation therapy. None of the patients enrolled had a family history of breast cancer. Healthy control individuals were detected based on based on their negative results including blood biomarker test, physical examination, and mammography. Clinical characteristics of the samples were shown in Table 1.

2.2 Biomarkers list

61 clinical conventional serum biomarkers including Carcinoembryonic antigen (CEA), Alpha-fetoprotein (AFP), Carbohydrate anigen 125 (CA125), CA19-9, CA153, CA72-4, Cytokeratin 19 fragment antigen (CY21-1), Ferritin (FERR), Neuron-specific enolase (NSE), Sguamous cell carcinoma associated antigen (SCC), Pepsinogen I (PG I), PGII, PG I /II, Sialic acid (SA), Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF), Interferon-γ (IFN-γ), Interleukin-10 (IL-10), IL-1β, IL-2, IL-4, IL-6, IL-8, Monocyte chemotactic protein-1(MCP-1), Tumor necrosis factor α (TNF-α), C reactive protein (CRP), High-density lipoprotein cholesterol (HDL), Low-density lipoprotein cholesterol (LDL), Apolipoprotein AI (ApoAI), Apolipoprotein B (ApopB), Total cholesterol (TC), Total triglyceride (TG), Lipoprotein a [Lp (a)], Calcium (Ca), Phosphorus (P), Magnesium (Mg), Potassium (K), Sodium (Na), Chlorine (Cl), Carbon dioxide (CO2), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total protein (TP), Albumin (ALB), Total bilirubin (TB), Direct bilirubin (DB), Total bile acid (TBA), Alkaline phosphatase (ALP), Glutamyl aminotransferase (GGT), Creatine kinase (CK), Lactate dehydrogenase (LDH), Creatine kinase isoenzyme (CK-MB), Ischemia modified albumin (IMA), Urea

(UR), Creatinine (CR), Uric acid (UA), Cystatin C (Cys C), Amylase (AMY), Lipase (LPS), Superoxide dismutase (SOD), Homocysteic acid (HCY), Glucose (GLU) were detected. All these serum biomarkers manufacturers and testing equipment suppliers were listed as our previous study[18].

Table 1 Clinical characteristic of the breast cancer patients

Characteristic	Training group (n=77)	Validation group (n=112)
Age, years		
Median	54	52
Range	44-69	39-73
Tumor size, n (%)		
T1	23 (29.87)	37 (33.04)
T2	28 (36.36)	36 (32.14)
T3	20 (25.97)	27 (24.11)
T4	6 (7.80)	12 (10.71)
Lymph node metastasis, n (%)		
N0	25	31
N1	21	38
N2	18	28
N3	13	15
Distant metastasis, n (%)		
Present	71 (92.21)	104 (92.86)
Absent	6 (7.79)	8 (7.14)
Staging, n (%)		
I	9 (11.69)	12 (10.71)
II	20 (25.97)	31 (27.69)
III	42 (54.55)	61 (54.46)
IV	6 (7.79)	8 (7.14)
Histological subtype, n (%)		
Ductal	54 (70.13)	77 (68.75)
Lobular	11 (14.29)	13 (11.61)
Ductal/Lobular	3 (3.90)	6 (5.36)
Others	9 (11.68)	16 (14.28)
ER, n (%)		
Positive	60 (77.92)	93 (83.04)
Negative	17 (22.08)	19 (16.96)
PR, n (%)		
Positive	56 (72.73)	85 (75.89)
Negative	21 (27.27)	27 (24.11)
HER2, n (%)		
Positive	12 (15.58)	18 (16.07)
Negative	65 (84.42)	94 (83.93)

2.3 Statistical Analysis

The different serum biomarkers between the groups were tested using one-way analysis of variance with Tukey's multiple comparison tests. A value of $P<0.05$ was considered to indicate a significant difference. The false discovery rate was controlled at 5% according to the method described by Benjamini and Hochberg. All development of statistical models for distinguishing between the

breast cancer versus healthy control group and breast cancer versus benign breast diseases group were restricted to the training set until one panel and one model of combining the candidate biomarkers in the panel were selected. A Metropolis algorithm with Monte Carlo simulation (MMC) was used for analysis of the data as described previously [19-21]. In MMC analysis, all possible panels consisting of two-, three-, and four-biomarkers were evaluated for sensitivity (SN) at 90% specificity (SP) in the preliminary training set. For each panel size, the panels with the best SN at 90% SP on the full data set were re-estimated with cross validation. For cross validation, 20% of samples were randomly excluded from the data set, and the rest were used as at training set to build the optimal Scoring Function. The resultant model was applied to the excluded participants, and this process was repeated 400 times to obtain a smooth average receiver operator characteristic (ROC) curve. For each comparison (breast cancer vs healthy control, breast cancer vs benign breast diseases), a multi-biomarker panel which had the best cross-validated SN at 90% SP was evaluated in the validation group. The diagnostic SN at 90 % SP for early stage and advanced stage of breast cancer in the training and validation groups were also evaluated. The diagnostic value of multi-biomarker panels which were chosen for discriminating breast cancer from healthy control or benign breast diseases were compared with diagnostic biomarker CA15-3. The best-performing multibiomarker panel for discriminating breast cancer and healthy control group was further evaluated for cancer selectivity in patients diagnosed with cervical, lung, pancreatic, gastric and colorectal cancer.

3. Results

3.1 Differential biomarkers in breast cancer group when compared with healthy controls and benign breast diseases group

The serum levels of the 61 biomarkers in the breast cancer group were compared with the the healthy control and benign breast diseases group. The differential biomarkers were shown in Table 2. Of the 61 biomarkers, 22 biomarkers in the breast cancer group showed significant difference when compared with the healthy control group. Of the 22 differential biomarkers, 11 biomarkers showed significantly increased in the breast cancer group when compared with the healthy control group, the other 11 biomarkers showed significantly decreased. Of the 61 biomarkers, 17 biomarkers in the breast cancer group showed significant difference when compared to the benign breast diseases group. Of the 17 differential biomarkers, 14 biomarkers showed significantly increased when compared to the benign breast diseases group, the other 3 biomarkers showed significantly decreased.

3.2 Multi-biomarker panels for discriminating between the breast cancer versus healthy control group and breast cancer versus benign breast diseases group using the MMC algorithm

The differential biomarkers in the breast cancer group compared with the healthy control and benign breast diseases group were further analyzed using the MMC algorithm. All possible two, three, and four biomarker panels were evaluated for SN at 90% SP in the training group. The 20 best performance two- and three-biomarker panels for discriminating between the breast cancer versus healthy control group and breast cancer versus benign breast diseases group were shown in Supplementary Table 1. The four-biomarker panel consisting of ApoAI, ApopB, CRP and IL-8 showed the best performance of discriminating the breast cancer group and the healthy control group when compared to the other four-biomarker panels shown in Table 3. The diagnostic value of the panel consisting of ApoAI, ApopB, CRP and IL-8 and that of CA153 were shown in Figure 2A. The AUC were 0.991 (95% CI: 0.982, 1.000) and 0.714 (95% CI: 0.637, 0.792), and the SN values were 98.70% and 31.17% at 90% SP. The diagnostic value of the panel consiting ApoAI, ApopB, CRP and IL-8 and that of CA153 for discriminating between early-stage or advanced-stage breast cancer and healthy control are shown in Supplementary Figure 1A and Figure 1B. The AUC values of the panel consisting of ApoAI, ApopB, CRP and IL-8 for discriminating between early-stage or advanced-stage breast cancer and healthy control were 1.000 (0.998, 1.000) and 0.986 (0.972, 1.000). The SN values were 100.00 and 97.92% at 90% SP. The AUC values of CA153 for discriminating between early-stage or advanced-stage breast cancer and healthy control were 0.697

(0.590, 0.805) and 0.715 (0.627, 0.804). The SN values were 24.14% and 35.42% at 90% SP. The diagnostic value of the panel consisting of ApoAI, ApopB, CRP and IL-8 for discriminating between the breast cancer group and the healthy control group was better than that of CA153 alone.

The four-biomarker panel consisting of PGI /II, CRP, SOD and TNF-α showed the best performance of discriminating the breast cancer group and the benign breast diseases group when compared to the other four-biomarker panels also shown in Table 3. The diagnostic value of the panel consisting of PGI /II, CRP, SOD and TNF-α and that of CA153 were shown in Figure 2B. The AUC were 0.951 (95% CI: 0.914, 0.989) and 0.612 (95% CI: 0.512, 0.712), and the SN were 88.31% and 22.08% at 90% SP. The diagnostic value of the panel consisting PGI/II, CRP, SOD and TNF-α and CA153 for discriminating between early-stage or advanced-stage breast cancer and the benign breast diseases are shown in Supplementary Figure 1C and Figure 1D. The AUC values of the panel consisting of PGI/II, CRP, SOD and TNF-αfor discriminating between early-stage or advanced-stage breast cancer and the benign breast diseases were separately 72.41% and 97.92%. The AUC values of CA153 for discriminating between early-stage or advanced-stage breast cancer and the benign breast diseases were 0.607 (0.473, 0.740) and 0.615 (0.502, 0.729). The SN values were separately 20.69% and 22.92%. The diagnostic value of the PGI /II, CRP, SOD and TNF-α panel showed improved performance when compared to the conventional biomarker CA153 for discriminating the breast cancer group and benign breast diseases group.

3.3 Independent validation of the optimal multi-biomarkers panels for discriminating between breast cancer group versus healthy control group and breast cancer group versus benign breast diseases group

After the two panels with the best performance in discriminating between the breast cancer group versus healthy control group and breast cancer group versus benign breast diseases group were calculated by MMC algorithm analysis, they were validated by a blinded validation group consisting 168 healthy control individuals, 164 benign breast diseases and 112 breast cancer patients.

In the validation group, the AUC of the panel consisting of ApoAI, ApopB, CRP and IL-8 for discriminating between the breast cancer group and healthy control individuals was 0.982 (95% CI: 0.966, 0.998) and the SN was 96.43% at 90% SP. The AUC of CA153 was 0.722 (95% CI: 0.661, 0.784), and the SN was 25.89 % at 90% SP, as shown in Figue 2C. The diagnostic value of the panel consisting of ApoAI, ApopB, CRP and IL-8 and that of CA153 for discriminating between early-stage or advanced stage breast cancer and healthy control individuals are shown in Supplementary Figure 2A and Figure 2B. The AUC values of the panel consisting of ApoAI, ApopB, CRP and IL-8 for discriminating between early-stage or advanced stage breast cancer and healthy control individuals were 0.994 (0.988, 1.000) and 0.975 (0.949, 1.000). The SN values were 100.00% and 94.21% at 90% SP. The AUC values of CA153 for discriminating between early-stage or advanced stage breast cancer and healthy control individuals were 0.601 (0.503, 0.699) and 0.798 (0.738, 0.857). The SN values were 18.61% and 30.44% at 90% SP. The AUC of the panel consisting of PGI /II, CRP, SOD and TNF-α and that of CA153 for discriminating between the breast cancer group and the benign breast diseases group were 0.936 (95% CI: 0.907, 0.964) and 0.513 (95% CI: 0.438, 0.589), the SN values were 81.25% and 18.75 % at 90% SP, as shown in Figure 2D. For discriminating between early-stage or advanced stage breast cancer and the benign breast diseases group are shown in Supplementary Figure 2C and Figure 2D, the AUC values of the panel for discriminating between early-stage or advanced stage breast cancer and the benign breast diseases group were 0.916 (0.877, 0.956) and 0.967 (0.939, 0.994). The SN values were 69.77% and 88.41% at 90% SP. The AUC values of CA153 for discriminating between early-stage or advanced stage breast cancer and healthy control individuals were 0.563 (0.467, 0.658) and 0.636 (0.537, 0.734). The SN values were 20.93% and 17.39%.

Table 2 Levels of significant differential biomarkers in breast cancer, benign breast disease, and healthy controls group

Indicator	Units	Cancer	Benign	Ctrl	Cancer VS Control	Cancer VS Benign
CA153	U/mL	10.90 (6.90, 16.29)	8.45 (6.01, 12.64)	7.17 (4.98, 10.17))	< 0.001	0.006
CY21-1	ng/mL	1.93 (1.26, 3.22)	1.32 (0.87, 1.83)	1.57 (1.13, 2.17)	0.013	< 0.001
NSE	ng/mL	8.82 (7.37, 10.99)	7.38 (4.85, 8.98)	8.70 (7.44, 10.38)	NS	0.001
PGI/II	1	5.00 (4.00, 6.00)	7.08 (3.79, 16.97)	5.13 (3.38, 6.46)	NS	< 0.001
PGII	ng/mL	9.00 (6.00, 14.00)	7.58 (4.35, 10.25)	12.60 (6.70, 21.65)	0.012	0.047
PGI	ng/mL	46.00 (33.50, 63.00)	23.15 (6.68, 56.60)	60.50 (40.30, 88.45)	0.009	0.009
ALT	U/L	12.00 (9.30, 15.00)	19.05 (9.65, 46.25)	14.60 (11.60, 19.10)	0.015	0.001
TP	g/L	68.70 (65.45, 72.00)	66.80 (64.18, 70.85)	76.00 (73.65, 79.25)	0.001	NS
ALB	g/L	40.40 (38.80, 42.70)	39.40 (37.18, 41.90)	45.40 (43.85, 46.90)	< 0.001	NS
TBA	μmol/L	3.40 (1.70, 4.75)	2.60 (1.65, 3.60)	3.50 (2.40, 5.90)	NS	0.043
Mg	mmol/L	0.88 (0.81, 0.96)	0.87 (0.76, 0.94)	0.95 (0.91, 1.01)	< 0.001	NS
K	mmol/L	3.97 (3.69, 4.18)	4.06 (3.77, 4.28)	4.26 (4.07, 4.51)	0.002	NS
Cl	mmol/L	106.00 (104.40, 107.90)	106.35 (104.28, 110.53)	107.70 (106.05, 109.30)	0.026	NS
HDL	mmol/L	1.27 (1.03, 1.47)	1.17 (0.99, 1.37)	1.44 (1.24, 1.66)	0.005	NS
ApoA1	g/L	1.30 (1.13, 1.50)	1.22 (1.10, 1.33)	1.50 (1.33, 1.62)	< 0.001	NS
ApoB	g/L	0.74 (0.63, 0.89)	0.69(0.53, 0.87)	0.83 (0.71, 0.93)	0.004	NS
CRP	g/L	0.60 (0.30, 2.50)	2.00 (0.20, 10.88)	0.06 (0.04, 0.11)	0.015	< 0.001
SOD	U/mL	147.30 (126.60, 169.10)	139.20 (118.65, 158.30)	130.00 (118.80, 143.60)	0.002	0.042
GM-CSF	pg/mL	37.00 (0.82, 62.00)	1.65 (0.76, 5.18)	0.66 (0.21, 1.25)	0.008	0.005
IFN Y	pg/mL	10.00 (0.55, 14.50)	0.57 (0.28, 1.00)	0.11 (0.00, 0.42)	0.011	0.013
IL-10	pg/mL	38.00 (2.82, 65.75)	4.15 (2.32, 5.63)	1.49 (0.91, 2.21)	0.005	0.005
IL-2	pg/mL	10.00 (0.64, 35.00)	0.63 (0.15, 3.57)	0.05 (0.00, 0.27)	< 0.001	< 0.001
IL-8	pg/mL	3451.00 (73.29, 7076.50)	125.22 (59.16, 198.24)	9.61 (5.17, 18.24)	< 0.001	< 0.001
MCP-1	pg/mL	7075.00 (343.94, 8957.00)	427.89 (267.32, 724.07)	300.58 (225.68, 356.99)	< 0.001	< 0.001
TNF a	pg/mL	141.50 (6.89, 243.13)	5.91 (3.82, 8.53)	5.15 (3.91, 7.07)	< 0.001	< 0.001

Table 3 The sensitivity of top 20 performing 4 biomarker panels for discriminating the breast cancer and healthy control, benign breast diseases identified by MMC algorithm applied to the training set at 90% specificity

Breast cancer *Vs* Control					**Breast cancer *Vs* Benign breast diseases**				
Four Biomarkers				Sensitivity	Four Biomarkers				Sensitivity
ApoAI	ApopB	CRP	IL-8	98.70%	PGI /II	CRP	SOD	TNF-α	88.31%
CYFRA21-1	ApopB	CRP	IL-8	97.41%	PGI /II	CRP	SOD	IFN-γ	87.01%
Mg	ApopB	CRP	IL-8	97.41%	PGI /II	CRP	SOD	IL-10	87.01%
Mg	ApopB	CRP	TNF-α	97.41%	ALT	CRP	SOD	TNF-α	87.01%
K	ApopB	CRP	IL-8	97.41%	CA153	ALT	IL-8	TNF-α	87.01%
Cl	ApopB	CRP	IL-8	97.41%	CYFRA21-1	PGI/II	CRP	SOD	87.01%
ApoAI	ApopB	CRP	TNF-α	97.41%	CYFRA21-1	ALT	CRP	SOD	87.01%
CA153	ALB	Cl	IL-8	97.41%	PGI/II	ALT	CRP	SOD	87.01%
ALT	ApopB	CRP	TNF-α	97.41%	PGI/II	CRP	SOD	IL-8	85.71%
TP	Mg	Cl	IL-8	97.41%	CA153	ALT	CRP	MCP-1	84.42%
Mg	ApopB	CRP	SOD	97.41%	PGI/II	CRP	SOD	MCP-1	84.42%
ApopB	CRP	IL-10	IL-8	97.41%	ALT	CRP	SOD	IFN-γ	84.42%
CA153	ALB	ApoAI	IL-8	96.10%	CA153	PGI/II	CRP	SOD	83.12%
ALT	ALB	Cl	IL-8	96.10%	CA153	ALT	TBA	TNF-α	83.12%
ALB	Cl	CRP	IL-8	96.10%	NES	PGI/II	CRP	SOD	83.12%
Mg	ApoAI	CRP	IL-2	96.10%	PGI/II	TBA	CRP	SOD	83.12%
Mg	ApopB	CRP	ALT	96.10%	PGI/II	CRP	SOD	GM-CSF	83.12%
Mg	ApopB	CRP	IL-10	96.10%	PGI/II	CRP	SOD	IL-2	83.12%
K	ApopB	CRP	TNF-α	96.10%	CA153	CYFRA21-1	ALT	TNF-α	81.82%
ApoAI	ApopB	CRP	ALT	96.10%	CA153	CYFRA21-1	TBA	TNF-α	81.82%

Abbreviation: MMC, Metropolis algorithm with Monte Carlo simulation

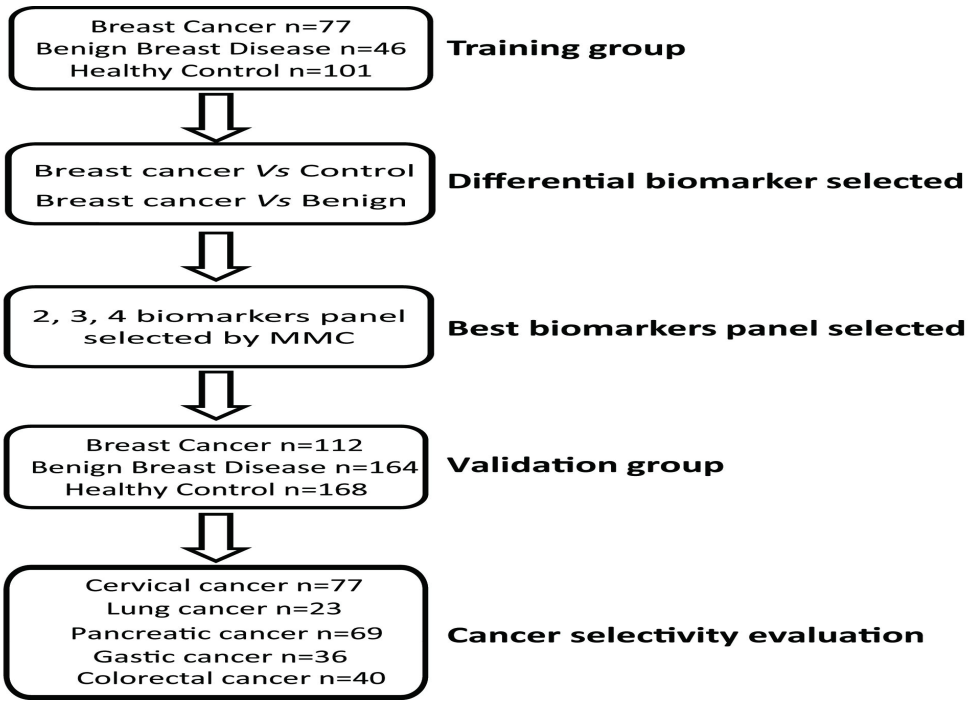

Figure 1 Flowchart of our experiment design.

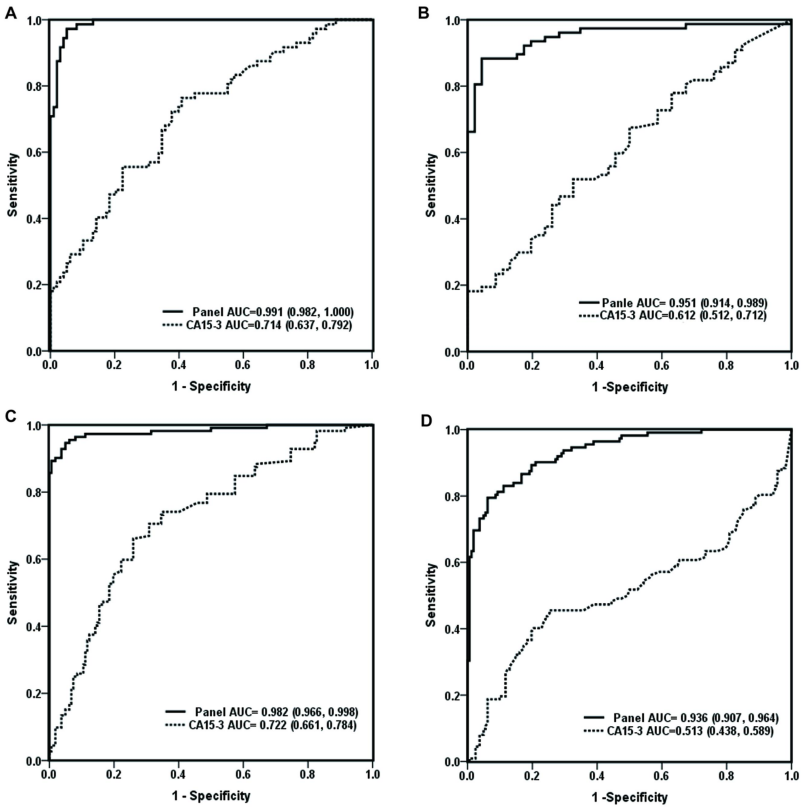

Figure 2 ROC curves of ApoAI, ApopB, CRP and IL-8 panel (solid line) and CA15-3 (dotted line) for discriminating between breast cancer and healthy control in the training group (A) and validation group (C). ROC curves of the panel consisting of PGI /II, CRP, SOD and TNF-α (solid line) and CA15-3 (dotted line) for discriminating between breast cancer and benign breast diseases in the training group (B) and validation group (D).

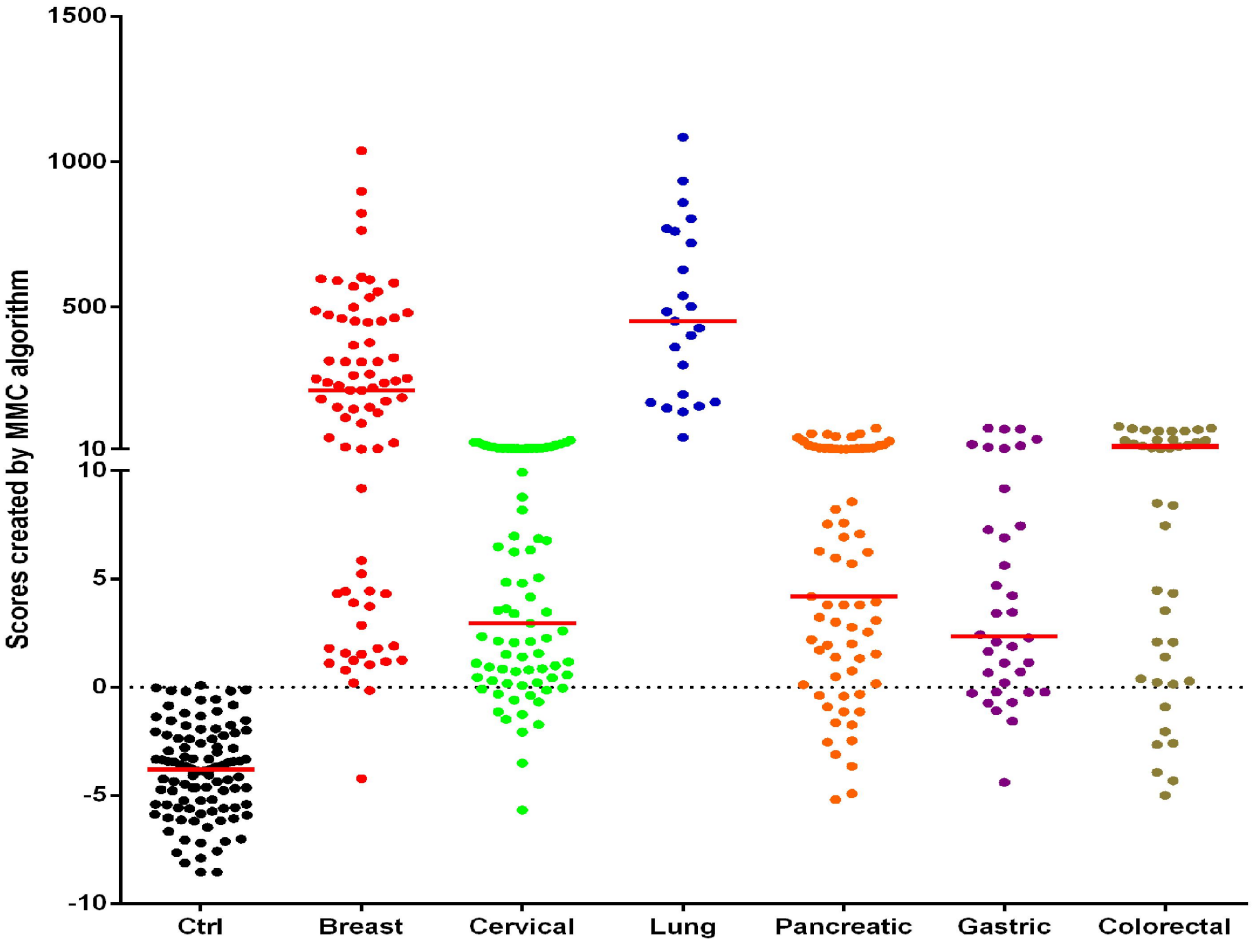

Figure 3 Distributions of the scores calculated by the MMC algorithm.

3.4 Cancer selectivity of the multi-biomarker panel for breast cancer versus other cancers

The panel consisting of ApoAI, ApopB, CRP and IL-8, which was identified by our MMC algorithm for discriminating between the breast cancer and healthy control, was used to classify a blinded mixed set of 245 female cancer patients including 77 cervical cancer, 23 lung cancer, 69 pancreatic cancer, 36 gastric cancer and 40 colorectal cancer patients. The distributions of the scores calculated by our MMC algorithm for each cancer type are shown in Figure 3. Our multi-parameter panel identified 14.29% of cervical cancer [AUC=0.814, (0.746–0.883)], 0% of lung cancer [AUC=0.733, (0.625–0.842)], 20.29% of pancreatic cancer [AUC=0.786, (0.711–0.862)], 25.00% of gastric cancer [AUC=0.821, (0.746–0.895)], and 17.50% of colorectal cancer [AUC=0.761, (0.676–0.847)] as non-breast cancer.

4. Discussion

Although lots of breast cancer biomarkers had been developed in recent years, however, up to now, little effective biomarker for early stage breast cancer diagnosis was applied in the clinical practice. There were several reasons. First, the carcinogenesis was a very complex process with diverse mechanism still to be elucidated. It was difficult for single biomarker which can meet the clinical practice. Second, lots of novel biomarkers had been explored as mentioned previous, however, their validity were far from clinical application mainly because their reliability, validity, sensitivity, specificity, ascertainment bias, confounding were not evaluated in clinical practice which greatly limited their clinical diagnosis[22, 23]. Third, several multi-biomarker panels were developed for the breast cancer detection [12, 24, 25], however, their

clinical validity were also limited as same to the novel single biomarker. Our strategy may be more feasible and closer to clinical practice for early stage breast cancer diagnosis by exploring the diagnostic value of the conventional biomarkers in clinical practice[26]. One reason is that the conventional biomarker, such as, CA125, CEA, CA19-9, lipids, cytokines were not specific for one kind of tumor. They also showed elevated levels in other cancer types which may have potential diagnostic value for the early stage breast cancer diagnosis. Another reason is that the multi-parameter analyze method can improve the diagnostic sensitivity and specificity compared to single biomarker[27-30]. Combination of conventional biomarker and multi-parameter analyze method may be closer to the clinical application compared to the other methods. In our study, we aimed to utilize 61 conventional biomarkers in clinical practice, and to interpret the results with MMC algorithm and cross validation to provide an assistant diagnostic method for early stage breast cancer diagnosis in clinical practice.

In our study, the panel consisting of ApoAI, ApopB, CRP and IL-8 showed improved performance for discriminating between the breast cancer group and healthy control group, and the panel consisting of PGI /II, CRP, SOD and TNF-αshowed improved performance for discriminating between the breast cancer group and the benign breast diseases group. Study had demonstrated that serum lipoproteins were related to the cancer development and progression[31]. In our study, compared with the healthy control, ApoAI in the breast cancer showed significantly decreased. ApoAI is a major lipoprotein component of HDL[32, 33]. It involved the development and progression of breast cancer[34]. Serum HDL showed significantly lower levels in the breast cancer when compared with the healthy control[35]. The serum levels of ApoAI also showed significantly lower levels in breast cancer when compared to the healthy control[12, 36]. Our study validated the previous results, and the ApoAI and HDL may have potential diagnostic value for breast cancer. ApopB is the major protein component of LDL. In our study, the LDL showed no significant difference between the breast cancer and healthy control, however, ApopB showed significantly lower level in the breast cancer. It may be related to the lipid metabolic disorder in the breast cancer[37, 38]. CRP is an acute-phase protein secreted by hepatocytes during the inflammatory response, and it is regulated by pro-inflammatory cytokines, such as, TNF-α and IFN-γ. Many studies have demonstrated that serum CRP levels showed significantly increased when compared to the healthy control[39, 40]. Elevated serum CRP levels are positively associated with early breast cancer, predominantly among overweight and postmenopausal women[41, 42]. In our study, CRP showed significantly increased in breast cancer when compared to the healthy control. It may have potential diagnostic value for breast cancer diagnosis. IL-8 is one member of the CXC chemokine family. It is known for its function in recruitment and activation of immune and inflammatory cells during inflammation. Clinical studies have also shown that the levels of IL-8 are higher in breast tumor tissue than in normal breast tissue, and an increased serum concentration of IL-8 has been suggested to be associated with advanced stages of breast cancer[43, 44]. Our result of IL-8 was consistent with the previous studies. SOD is a family of antioxidant enzymes that convert harmful superoxide radicals into H2O2, which in turn is metabolized to harmless water and oxygen by catalase and glutathione peroxidase. In the previous studies, some showed significantly increased in breast cancer compared to the healthy control[45, 46], but others showed significantly decreased[47, 48]. In our study, SOD levels showed significantly increased in breast cancer compared to the healthy control group. The reason may be oxidative environment in breast cancer may provoke oxidants to activate the corresponding antioxidant responsive elements[49]. PG II, also known as progastricsin or Pepsinogen C, is the precursor of pepsin C, an aspartic proteinase that is synthesized primarily in the gastric mucosa and secreted into the gastric lumen[50]. Studies have reported that the PG II was not only related to gastric cancer, but also breast cancer[51]. Its expression in breast carcinomas was associated with pathological and biochemical features of less aggressive disease and with favorable prognostic outcome[52, 53]. Its serum levels in breast cancer were little researched by now. In our study, PG II showed significantly decreased in

the breast cancer compared to the healthy control, and had potential diagnostic value for breast cancer diagnosis. It may be a protective factor for the breast cancer.

In conclusions, we described two panels for discriminating between the breast cancer and control, breast cancer and benign breast diseases. But there were also several limitations in our study. First, more samples and multi-centers needed in the future study to evaluate the clinical utility of the two panels. Second, our multi-parameter panel consisting of ApoAI, ApopB, CRP and IL-8 identified most of the other kinds of cancers as breast cancer. So in our study, our biomarker panels may serve as potential biomarker panel for cancer detection, in addition, it can also serve as an assistant tool for the detection of breast cancer, such as, mammography and magnetic resonance imaging. Third, because of the sample size was little, so the diagnostic values of breast cancer subtypes (such as, HER2 positive or negative) were not analyzed, in our future study, we will enlarge the sample size and analyze the diagnostic value of breast cancer subtypes. In conclusion, in our study, ApoAI, ApopB, CRP and IL-8 panel and the PGI/II, CRP, SOD and TNF-α panel showed improved diagnostic values for breast cancer when compared to CA15-3. The biomarker panels may serve as an assistant tool for breast cancer screening and early detection to improve the clinical outcome.

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Competing interests

All authors declare that they have no competing interests.

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