Joint analysis of multiple biomarkers for identifying type 2 diabetes in middle-aged and older Chinese: a cross-sectional study

Hongyu Wu,[1] Zhijie Yu,[1] Qibin Qi,[2] Huaixing Li,[1] Qi Sun,[3] Xu Lin[1]

[1]Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Graduate School of the Chinese Academy of Sciences, Shanghai, China
[2]Department of Nutrtion, Harvard School of Public Health, Boston, Massachusetts, USA
[3]Department of Medicine, Channing Laboratory, Boston, Massachusetts, USA

Correspondence to
Dr Xu Lin; xlin@sibs.ac.cn

ABSTRACT

Objective: Identifying individuals with high risk of type 2 diabetes is important. To evaluate discriminatory ability of multiple biomarkers for type 2 diabetes in a Chinese population.

Methods: Plasma adiponectin, plasminogen activator inhibitor-1, retinol-binding protein 4, resistin, C-reactive protein, interleukin 6 (IL-6), tumour necrosis factor α receptor 2 and ferritin were measured in a population-based sample of 3189 Chinese (1419 men and 1770 women) aged 50–70 years. A weighted biomarkers risk score (BRS) was developed based on the strength of associations of these biomarkers with type 2 diabetes. The discriminatory ability was tested by the area under receiver operating characteristics curve (AUC).

Results: Adiponectin, plasminogen activator inhibitor-1, IL-6 and ferritin were independently associated with the prevalence of type 2 diabetes, and they were used to calculate the biomarkers risk score (BRS). After adjustment for the confounding factors, the ORs for type 2 diabetes and impaired fasting glucose with each point increment of BRS were 1.28 (95% CI 1.22 to 1.34) and 1.16 (1.12 to 1.20), respectively. Compared with those in the lowest quintile of the BRS, the participants in the highest quintile have an OR (95% CI) of 6.67 (4.21 to 10.55) for type 2 diabetes. The area under the curve for the BRS and conventional risk factors alone was 0.73 and 0.76, respectively, and substantially increased to 0.81 after combining both BRS and conventional risk factors (p<0.001).

Conclusions: These data suggest that combining multiple biomarkers and conventional risk factors might substantially enhance the ability to identify individuals with type 2 diabetes. More prospective data are warranted to confirm this observation.

INTRODUCTION

In parallel with rapid nutritional and lifestyle changes, such as an increased energy-dense diet and reduced physical activity, the prevalence of type 2 diabetes has reached epidemic level and currently affects 92.4 million (9.7%) Chinese adults.[1] About two-thirds of these patients had previously

ARTICLE SUMMARY

Article focus
- Development of type 2 diabetes could be delayed or even prevented by lifestyle modification. Identifying individuals with high risk of type 2 diabetes is important.
- This study evaluated the discriminatory ability of multiple biomarkers for type 2 diabetes in a Chinese population.

Key messages
- Plasma adiponectin, plasminogen activator inhibitor-1, retinol-binding protein 4, resistin, C reactive protein, interleukin 6, tumour necrosis factor-α receptor 2 and ferritin were measured in a population-based sample of 3189 Chinese. A weighted biomarkers risk score (BRS) was developed based on the strength of associations of these biomarkers with type 2 diabetes.
- After adjustment for the confounding factors, the OR for type 2 diabetes with each point increment of BRS was 1.28 (95% CI 1.22 to 1.34). The area under the curve for the BRS and conventional risk factors alone was 0.73 and 0.76, respectively, and substantially increased to 0.81 after combining both BRS and conventional risk factors (p<0.001).
- These finding suggested that combining multiple biomarkers and conventional risk factors might substantially enhance the ability to identify individuals with type 2 diabetes.

Strengths and limitations of this study
- This is the first study simultaneously assessing the discriminatory ability of multiple biomarkers together with conventional risk factors for type 2 diabetes in a large population-based sample from the north and south of China.
- Limitations including, the cross-sectional nature of the study, did not allow determination of the extent to which diabetes incidence would be predicted by the BRS; fasting glucose was measured only once. The oral glucose tolerance test, which was time-consuming and labour intensive, was not performed in such a large population.

undiagnosed diabetes.[2] Poor control of diabetes will lead to development of severe complications and substantially increase medical and socioeconomic burdens.[2] Compelling evidence has indicated that onset of type 2 diabetes could be delayed or even prevented by lifestyle modification.[3] Thus, identifying individuals at high risk at an early reversible stage of the disease is critical for controlling the type 2 diabetes epidemic trend.

To predict or classify type 2 diabetes, several scoring systems have been developed based on lifestyle factors,[4–7] anthropometric markers[4–6] or genetic factors.[8–10] Type 2 diabetes is a multifactorial disease, and its pathogenesis involves dysregulation of various biological pathways as well as an unfavourable profile of multiple cytokines such as adiponectin, plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP) and interleukin 6 (IL-6) in these pathways.[11] However, most studies have focused on the effect of a single cytokine on the risk of diabetes and few have investigated the collective effect of these biomarkers together with traditional risk factors in predicting the development of type 2 diabetes.[12] In a recent report based upon the finding from a subset population of the Inter99 Cohort, a lifestyle intervention trial, a diabetes risk score composed of adiponectin, CRP, ferritin, IL-2 receptor A, glucose and insulin was shown to provide a better predictive power for having type 2 diabetes than a single variable among overweight Caucasian people.[13] However, little is known about whether these circulating biomarkers might also be useful for predicting or screening high-risk individuals among free-living Chinese populations, who are considered to have a higher susceptibility to metabolic disorders even within 'low-risk' range of inflammatory cytokine such as CRP according to the criteria for Western populations.[14]

Therefore, this study aimed to evaluate whether the combination of eight plasma biomarkers, including adiponectin, PAI-1, retinol-binding protein 4 (RBP4), resistin, CRP, IL-6, tumour necrosis factor α receptor 2 (TNF-α-R2) and ferritin could improve the discriminatory ability for type 2 diabetes individuals in middle-aged and older Chinese men and women.

SUBJECTS AND METHODS
Study subjects
The study population included 3189 Shanghai and Beijing residents aged 50–70 years from the Nutrition and Health of Aging Population in China. This study was conducted simultaneously both in Beijing and Shanghai from March to June 2005. The participants were recruited by a multistage sampling method. In each city, two urban districts and one rural district were chosen to represent different socioeconomic statuses. We planned to select 400 participants from each urban district and 800 people from each rural district from the eligible candidates listed in the residential registration record. The eligibility of the candidates was defined as those who had been stable residents for at least 20 years in the

areas. Those with severe psychological disorders, physical disabilities, cancer, cardiovascular disease, Alzheimer's disease, or currently diagnosed with tuberculosis, AIDS, and other communicable diseases were excluded.

The study design and protocols have been described elsewhere.[14] In brief, information on demographic characteristics, health status, lifestyle practice and physical activity was collected using a standard questionnaire. Body weight and height were measured and body mass index (BMI) was calculated as weight (kg)/height (m^2). Overnight fasting plasma samples were collected and stored at −80°C until laboratory assays were performed. The study protocol was approved by the institutional review board of the Institute for Nutritional Sciences, and all participants provided written informed consents. A total of 3189 participants (1419 men and 1770 women) with none of the eight selected biomarkers missing were included in this study. Individuals were defined as having type 2 diabetes if they had a fasting glucose ≥7.0 mmol/l, or previously diagnosed type 2 diabetes, or used hypoglycaemic drugs including insulin. Impaired fasting glucose (IFG) and normal fasting glucose (NFG) were defined as a participant with a fasting glucose level ≥5.6 to <7.0 mmol/l and <5.6 mmol/l, respectively.

Laboratory measurement
Plasma fasting glucose was measured enzymatically on an automatic analyzer (Hitachi 7080, Japan) with reagents from Wako Pure Chemical Industries (Osaka, Japan).[14] HbA1c was quantified from erythrocyte with automated immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA).[14] Plasma adiponectin, PAI-1 and resistin concentrations were analysed by Luminex xMAP Technology on a Bio-Rad Multiplex Suspension Array System with an intra-assay coefficient of variation (CV) of 1.4–7.9% and an interassay CV<21%.[15 16] Circulating RBP4 was evaluated in duplicate by a sandwich ELISA kit developed in-house, with an intra-assay CV of 1.8–7.6% and an interassay CV of 3.7–8.8%.[17] Plasma high-sensitivity CRP was measured by a particle-enhanced immunoturbidimetric assay (Roche Diagnostics), and both intra-assay and interassay CV of CRP levels were <12%.[14] IL-6 was determined with a high-sensitivity ELISA (R&D Systems, Inc, Minneapolis, Minnesota, USA), with both intra-assay and interassay CVs <10%.[14] Soluble TNF-α-R2 was detected with a Human Death Receptor 3-Plex kit (Biosource International, Inc, Camarillo, California, USA) through the Multiplex Suspension Array System. the average intra-assay CV and interassay CVs for TNF-α-R2 were 7.9% and 10.1%, respectively.[18] Plasma ferritin was assayed using a commercially available particle-enhanced immunoturbidimetric kit (Shanghai Gensource Co, Ltd, Shanghai, China).[19] The intra-assay CV of ferritin measurements was <2.0%, whereas the interassay CV was <6.1%.

Statistical analyses
A multivariate logistic regression was applied to estimate the ORs for individual biomarkers. Results for the

association for each single biomarker have been reported elsewhere.[15] [16] [18] [19] The corresponding β coefficients (log ORs) in logistic regression models were employed to assign a score value for each biomarker; only the biomarkers with p values <0.05 after including all the eight biomarkers in the model with forward selection were then selected in the scoring system. A weighted biomarkers risk score (BRS) was developed based on the assumption that selected biomarkers have independent effects on type 2 diabetes and also contribute to diabetes risk in an additive manner.[20] Accordingly, adiponectin, PAI-1, IL-6 and ferritin were used to compute a BRS which was derived as the following equation: $BRS = \kappa \times (\beta_1 \times biomarker_1 + \beta_2 \times biomarker_2 + \ldots + \beta_\kappa \times biomarker_\kappa)/(\beta_1 + \beta_2 + \ldots + \beta_\kappa)$. Where κ represents the number of the biomarkers used (in this study $\kappa = 4$), β_i is the assigned sore value of each biomarker corresponding to the log (OR) in logistic regression, $biomarker_i$ assigned to 0, 1, 2 or 3 according to low to high quartile of the specific biomarker.[20] The quartiles of adiponectin were assigned as 3, 2, 1 or 0, because of its inverse association with diabetes risk. The confounding factors, which were adjusted in the logistic regression models included age,

sex, geographical region (Shanghai or Beijing), residence (urban or rural), smoking status (ex-smoker, current smoker and non-smoker), alcohol drinking (yes or no), physical activity (low, moderate or high), family history of diabetes (yes or no) and BMI. Area under the receiver operator characteristic curve (AUC) was calculated to evaluate the ability of BRS and conventional risk factors alone or collectively in discriminating the prevalence of type 2 diabetes. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) statistics were also applied to test the discriminative ability.[21] The categories <10%, 10−20%, 20−35% and >35% were used in the reclassification analyses. Analyses were performed using SAS V.9.2 (SAS Institute). All reported p values are two-sided and considered statistically significant for p<0.05.

RESULTS

The characteristics of the 3189 participants are presented in table 1, the prevalence of type 2 diabetes and IFG were 13.6% (n=434) and 26.9% (n=858), respectively. As expected, the participants with type 2 diabetes had lower plasma adiponectin, but higher concentrations of RBP4, PAI-1, CRP, IL-6 and ferritin than their IFG and NFG

Table 1 Characteristics of the study population*

Characteristics	NFG	IFG	Type 2 diabetes
n (%)	1897 (59.5)	858 (26.9)	434 (13.6)
Age (years)	58.4±6.0	58.6±6.0	59.7±5.7
Men, n (%)	789 (41.6)	414 (48.3)	216 (49.8)
Beijing residents, n (%)	727 (38.3)	564 (65.7)	282 (65.0)
Urban residents, n (%)	931 (49.1)	402 (46.9)	254 (58.5)
BMI (kg/m^2)	23.8±3.5	25.4±3.6	25.4±3.5
Family history of diabetes†, n (%)	181 (9.5)	110 (12.8)	142 (32.7)
Smoking status, n (%)			
Non-smoker	1230 (64.8)	484 (56.4)	263 (60.6)
Ex-smoker	152 (8.0)	111 (12.9)	61 (14.1)
Current smokers	515 (27.1)	263 (30.7)	110 (25.3)
Alcohol drinking, n (%)	470 (24.8)	302 (35.2)	133 (30.6)
Physical activity			
Low	136 (7.2)	74 (8.6)	25 (5.8)
Moderate	817 (43.1)	324 (37.8)	198 (45.6)
High	944 (49.8)	460 (53.6)	211 (48.6)
Fasting glucose (mmol/l)	5.06±0.36	6.01±0.34	8.94±3.02
Fasting insulin (pmol/l)	12.9 (9.4−17.5)	15.1 (11.1−20.4)	14.8 (10.3−22.4)
HbA1c (%)	5.64±0.42	5.85±0.45	7.81±2.01
Adiponectin (μg/ml)	14.9 (9.0−23.3)	12.4 (7.6−20.6)	10.1 (5.5−17.2)
RBP4 (μg/ml)	39.1±11.4	41.3±12.2	41.3±11.9
PAI-1 (ng/ml)	6.68 (2.25−15.4)	11.8 (4.45−23.8)	13.3 (5.5−25.6)
Resistin (μg/ml)	8.56 (5.67−13.9)	8.56 (5.92−14.4)	8.86 (6.03−13.7)
CRP (mg/l)	0.57 (0.30−1.27)	0.79 (0.36−1.76)	0.97 (0.46−2.37)
IL-6 (ng/l)	0.96 (0.62−1.48)	1.12 (0.72−1.65)	1.26 (0.81−1.89)
TNF-αR2 (μg/l)	1.62 (1.28−2.01)	1.61 (1.22−1.99)	1.64 (1.30−2.12)
Ferritin (g/l)	0.12 (0.08−0.17)	0.15 (0.10−0.20)	0.17 (0.12−0.24)
Biomarkers risk score	5.21 (3.40−7.18)	6.74 (4.77−8.60)	7.62 (5.86−9.26)

*Data are means±SD, medians (IQR) or n (%), unless otherwise indicated.
†First-degree relatives (parents or siblings) had a history of diabetes.
BMI, body mass index; CRP, C-reactive protein; IFG, impaired fasting glucose; IL, interleukin; NFG, normal fasting glucose; PAI-1, plasminogen activator inhibitor-1; RBP4, retinol-binding protein 4; TNF-αR2, tumour necrosis factor α receptor 2.
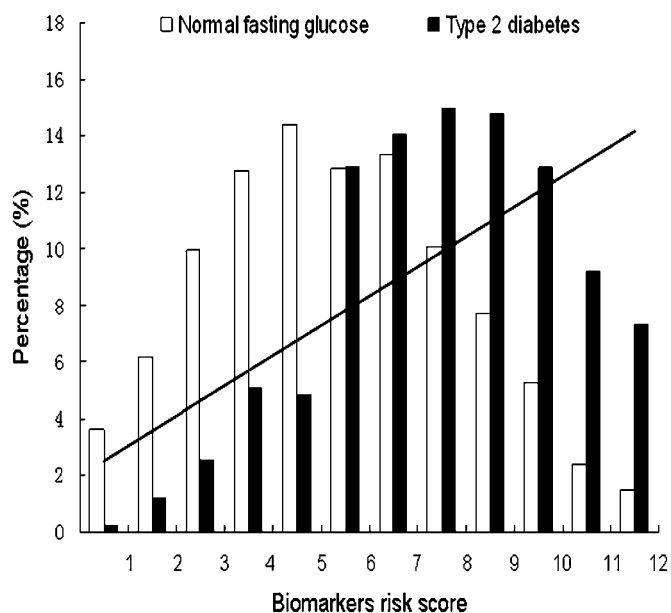
Figure 1 Percentage of participants in each biomarkers risk score category among those who had normal fasting glucose and type 2 diabetes. Risk of developing type 2 diabetes according to biomarkers risk score is shown as a fitted line from regression analysis.

counterparts. The risk for type 2 diabetes increased with lower adiponectin and higher PAI-1, CRP, IL-6 and ferritin (all p<0.001 for trend), but not with RBP4, resistin or TNF-α-R2 levels (supplemental table 1 and supplemental figure 1). Multiple logistic regression analysis with forward selection included all of these eight biomarkers in the model showed that adiponectin, PAI-1, IL-6 and ferritin were significantly and independently

associated with type 2 diabetes. Thus, these four biomarkers were used to develop a weighed BRS.

Compared with NFG participants, the participants with type 2 diabetes have significantly higher level of BRS (median (IQR): 7.62 (5.86−9.26) vs 5.21 (3.40−7.18), p<0.001, table 1). The distribution of BRS was shifted right in diabetic individuals compared with those with NFG (figure 1). After adjustment for age, sex, region, residence, smoking, alcohol drinking, physical activity, family history of diabetes and BMI, the ORs for type 2 diabetes and IFG with each point increment of BRS were 1.28 (95% CI 1.22 to 1.34; p<0.001) and 1.16 (95% CI 1.12 to 1.20; p<0.001), respectively (table 2). Similar ORs were observed among both sexes (p for interaction=0.48 and 0.49 for diabetes and IFG, respectively) and age groups defined by the cut-off point of 60 years (p for interaction=0.38 and 0.49 for diabetes and IFG, respectively). Among individuals with a score >10 (7.4%), the ORs were 9.95 (95% CI 5.46 to 18.13) for type 2 diabetes and 3.96 (95% CI 2.57 to 6.10) for IFG compared with those with BRS ≤3.0 (14.86%) (figure 2). A similar association was also found when the subjects were categorised into the BRS quintiles (table 2). Compared with the lowest quintile (BRS≤3.5), the ORs in the highest quintile (BRS>8.5) were 6.67 (95% CI 4.21 to 10.55) for type 2 diabetes and 3.09 (95% CI 2.29 to 4.19) for IFG after controlling for the confounding factors.

The interaction between BRS and BMI on the prevalence of type 2 diabetes was also examined (supplemental figure 2). In multivariate analysis adjusted for age, sex, region and residence, the odds of type 2 diabetes within each category of BMI increased significantly from the bottom to the top BRS quintile (p<0.0001 for

**Table 2** Association of biomarkers risk score (BRS) and risk of type 2 diabetes and impaired fasting glucose (IFG)

Variables	Quintiles of BRS					Continuous BRS	
	Q1	Q2	Q3	Q4	Q5	OR (95% CI)	p Value
N	637	642	629	648	633		
Median	2.3	4.4	6.0	7.5	9.7		
(range)	(0.0 to 3.5)	(3.6 to 5.2)	(5.2 to 6.7)	(6.8 to 8.5)	(8.5 to 12.0)		
Risk of type 2 diabetes							
Model 1*	1.0	1.45 (0.88 to 2.40)	3.29 (2.10 to 5.15)	4.19 (2.70 to 6.52)	6.45 (4.18 to 9.97)	1.27 (1.22 to 1.33)	<0.001
Model 2†	1.0	1.51 (0.91 to 2.50)	3.28 (2.08 to 5.18)	4.35 (2.77 to 6.81)	6.72 (4.32 to 10.45)	1.28 (1.22 to 1.34)	<0.001
Model 3‡	1.0	1.51 (0.91 to 2.50)	3.27 (2.06 to 5.18)	4.32 (2.73 to 6.83)	6.67 (4.21 to 10.55)	1.28 (1.22 to 1.34)	<0.001
Risk of IFG							
Model 1*	1.0	1.25 (0.94 to 1.66)	1.70 (1.28 to 2.25)	2.12 (1.60 to 2.80)	3.89 (2.92 to 5.19)	1.19 (1.15 to 1.24)	<0.001
Model 2†	1.0	1.25 (0.94 to 1.67)	1.69 (1.27 to 2.24)	2.13 (1.61 to 2.83)	3.94 (2.95 to 5.27)	1.20 (1.16 to 1.24)	<0.001
Model 3‡	1.0	1.16 (0.87 to 1.55)	1.50 (1.13 to 2.00)	1.76 (1.31 to 2.35)	3.09 (2.29 to 4.19)	1.16 (1.12 to 1.20)	<0.001

Data are OR (95% CI).
*Model 1 adjusted for age, gender, region and residence.
†Model 2 further adjusted for smoking, alcohol drinking, physical activity and family history of diabetes.
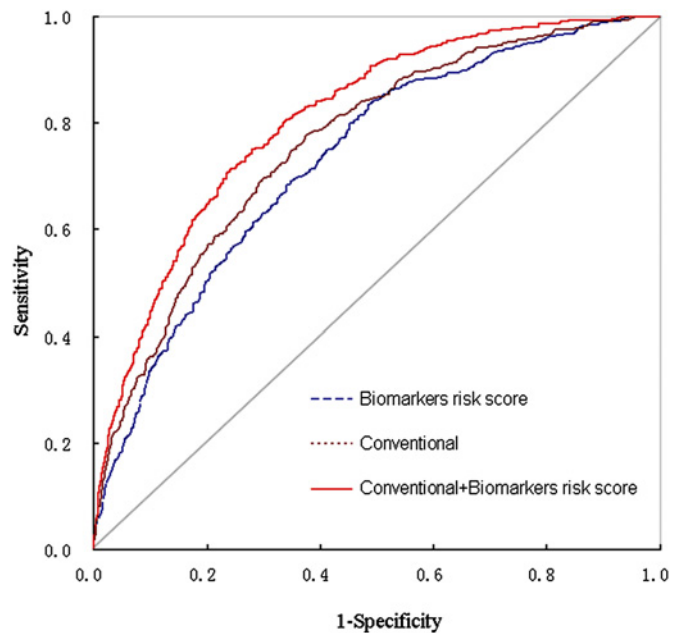‡Model 3 further adjusted for body mass index.
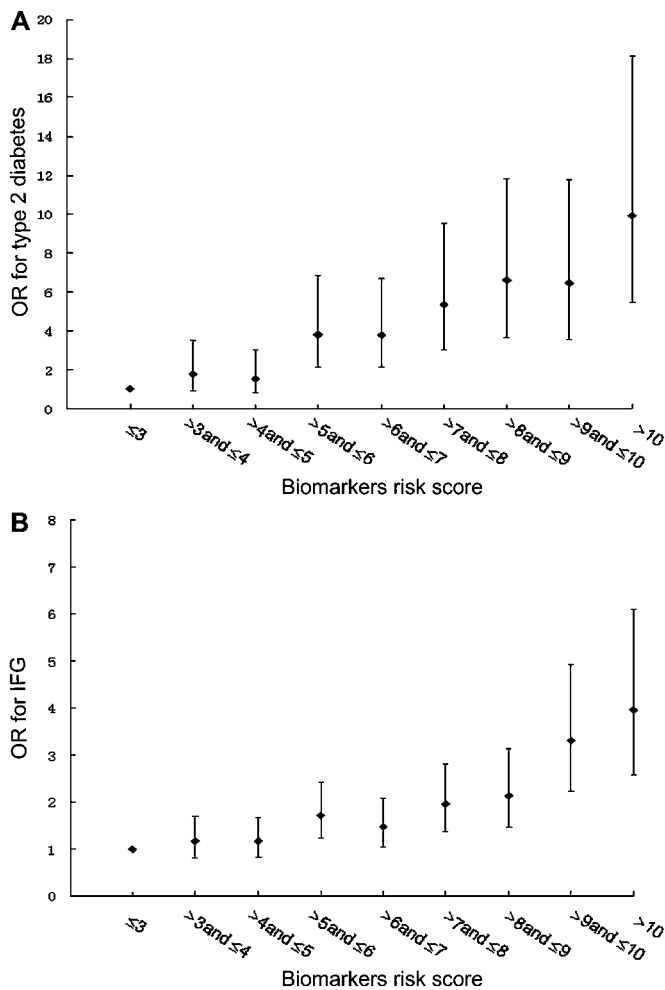
Figure 2 Risk of type 2 diabetes (A) and impaired fasting glucose (IFG) (B) in each biomarkers risk score category after adjustment for age, sex, region, residence, smoking, alcohol drinking, physical activity, family history of diabetes and body mass index.

Figure 3 Receiver operating characteristic curves for discriminating type 2 diabetes. Graphs show curves for biomarkers risk score alone (area under curve (AUC)=0.73, 95% CI 0.71 to 0.76), for conventional risk factors including age, sex, region, residence, smoking, alcohol use, physical activity, family history of diabetes and body mass index (AUC=0.76, 95% CI 0.74 to 0.78) and for biomarkers incorporated into conventional ones (AUC=0.81, 95% CI 0.79 to 0.83).

trend). Obese participants (BMI $\geq 28\,\mathrm{kg/m^2}$) in the highest BRS quintile had an OR of 7.08 (95% CI 3.98 to 12.59) compared with their normal-weight counterparts (BMI $<24\,\mathrm{kg/m^2}$) in the lowest quintile of BRS. However, no significant interaction was found between BRS and BMI (p=0.51 for interaction).

To explore the discriminatory ability of BRS, receiver operator characteristic curve analyses were performed (figure 3). The AUCs were 0.64, 0.64, 0.60 and 0.67 for adiponectin, PAI-1, IL-6 and ferritin, respectively. The AUCs were 0.73 (95% CI 0.71 to 0.76) for the BRS and 0.76 (95% CI 0.74 to 0.78) for conventional risk factors, including age, sex, region, residence, smoking, alcohol use, physical activity, family history of diabetes and BMI. Adding BRS to conventional risk factors significantly improved the AUC to 0.81 (95% CI 0.79 to 0.84; p<0.0001). Excluding the participants with previous diagnosis of diabetes did not appropriately change the identifying ability of BRS, with an increase of AUC from 0.74 (95% CI 0.71 to 0.78) to 0.82 (95% CI 0.80 to 0.85; p<0.0001) after combining the BRS with conventional

risk factors. On the other hand, adding the BRS into the model significantly improved the IDI (IDI=0.0669, p<0.0001). The NRI was estimated at 25.7% (p<0.0001), resulting from a total of 16.7% of cases classified upwards and a total of 9.0% of no cases classified downwards (table 3). If the cut off points <3%, 3%−8%, 8%−15% and >15% were used as in previous study,[22] the NRI was increased to 33.1% (p<0.0001).

DISCUSSION

In this study, we showed that a weighted BRS, summarising association of adiponectin, PAI-1, IL-6 and ferritin, was strongly associated with type 2 diabetes and IFG in a middle-aged and older Chinese population. The BRS substantially improved the discriminatory ability for type 2 diabetes beyond conventional risk factors.

Since lifestyle modification has been demonstrated to be effective in prevention of type 2 diabetes, several prediction models have been developed to detect high-risk individuals among apparently healthy people for early intervention. However, most of the previous scoring systems for early screening of type 2 diabetes were based on the risk factors related to demographic,[4 6] lifestyle,[4 6 7] anthropometric[4−6] or genetic parameters.[8 10] Additionally, most of these systems were derived from white populations. Until now no diabetes risk evaluation system has been developed for use in Asian populations, especially in Chinese people who have a lower than

Table 3 Net reclassification improvement due to the biomarkers risk score

Predicted risk without biomarkers risk score	Predicted risk with biomarkers risk score					
Participants with type 2 diabetes (n=434)	<10%	10−20%	20−35%	>35%	Up	Down
<10%	18 (40.9)	22 (50.0)	4 (9.1)	0 (0)		
10−20%	19 (16.2)	40 (34.2)	40 (34.2)	18 (15.4)	130 (30.0)	57 (13.1)
20−35%	3 (2.3)	17 (13.1)	64 (49.2)	46 (35.4)		
>35%	0 (0)	5 (3.5)	13 (9.1)	125 (87.4)		
Participants with NFG (n=1897)						
<10%	665 (84.8)	99 (12.6)	20 (2.6)	0 (0)		
10−20%	264 (42.0)	233 (37.0)	119 (18.9)	13 (2.1)	316 (16.7)	486 (25.6)
20−35%	36 (11.3)	120 (37.5)	99 (30.9)	65 (20.3)		
>35%	2 (1.2)	21 (12.8)	43 (26.2)	98 (59.8)		

Results are shown as number (%).
Net reclassification improvement: 25.7% (SE 0.012), p<0.0001.
NFG, normal fasting glucose.

average BMI but a similar or even higher prevalence of diabetes than Western populations.[23] In a cross-sectional sample of Taiwanese subjects, Lin *et al* compared the type 2 diabetes discriminatory power of 10 scoring systems such as QDScore, FINDRISC, ARIC and the Cambridge Risk Score derived from information obtained from questionnaire and physical examination without laboratory blood assays. It was found that all of these scores improved the ability to detect type 2 diabetes, with an AUC ranging from 0.67 to 0.74.[24]

In this middle-aged and older Chinese population, our data showed that a BRS based on the combined effect of adiponectin, PAI-1, IL-6 and ferritin, was closely associated with the prevalence of type 2 diabetes independently of conventional diabetes risk factors. The participants with a BRS>10 had about 10-fold increased risk of type 2 diabetes than those with BRS≤3.0. Both the BRS and conventional risk factors alone showed a reasonably good ability to distinguish individuals with type 2 diabetes, with AUCs of 0.73 and 0.76, respectively. More importantly, combining the BRS with conventional risk factors substantially enhanced the discriminatory ability for type 2 diabetes by increasing AUC to 0.81. Moreover, adding BRS into the model significantly improved the IDI and NRI. Similarly, in FINRISK97 cohort study, a score calculated as a linear combination of adiponectin, apolipoprotein B, CRP and ferritin was showed to improve the net reclassification and discrimination of diabetes risk in addition to the traditional risk factors.[22] Taken together, these findings emphasise that combining multiple circulating biomarkers might provide an additional objective tool in estimating the diabetes risk beyond the conventional risk assessments in clinical practice. These results might lead to use of these biomarkers in practice to assess diabetes risk, and benefit those individuals at high risk by allowing early preventive intervention. Nevertheless, more studies especially prospective studies are needed to verify these findings.

Unlike previous studies,[12] which emphasised individual biomarkers, we performed a combined analysis of multiple biomarkers related to different pathogenesis pathways of type 2 diabetes. A number of biomarkers secreted by adipose tissue or liver play important roles in the aetiology of type 2 diabetes via pathways which may not be greatly overlapping.[11] For instance, adiponectin is known to be an anti-inflammatory cytokine. However, a strong association between high circulating adiponectin levels and a lower risk of type 2 diabetes was found even after adjustment for inflammatory markers.[25] Indeed, adiponectin could also enhance lipid clearance, and stimulate insulin secretion.[26] The link between IL-6 levels and type 2 diabetes incidence in several prospective studies, however, was mainly through an inflammatory reaction that might modify glucose and lipid metabolism.[27−29] On the other hand, elevated PAI-1 concentration, a marker of endothelial dysfunction, was also an independent predictor of incident diabetes.[30 31] As an indicator of iron overload, which might result in β-cell oxidative stress, high circulating ferritin was established as a risk factor for type 2 diabetes.[32 33]

Consistent with previous studies,[23−31] plasma adiponectin, PAI-1, IL-6 and ferritin in our study were independently associated with the risk of diabetes. As each of these biomarkers may involve one or more specific pathways in the link with type 2 diabetes, it was not surprising to find a low value of the AUC for each separate biomarker in discriminating diabetes individuals. However, adding any one of these biomarkers to the conventional factors significantly improved the power of discriminating the participants with and without type 2 diabetes. Moreover, each of these biomarkers contributed independently and additively to risk assessment, and with the combined information of these biomarkers, the BRS appeared to provide much stronger discriminative power. In addition, with the advance of biomarker measurement technology, we can simultaneously assess multiple biomarkers with one blood sample; for example, adiponectin, resistin and PAI-1 were measured simultaneously in our study. Thus, the cost of testing these biomarkers has been reduced,

which makes it feasible to measure multiple biomarkers in primary care or large populations in the future. On the other hand, if we could prevent a person at high risk from developing clinically overt diabetes by early detection and intervention, the overall healthcare cost would be significantly reduced. Therefore, joint analysis for multiple potential predictors might be a potentially useful method for diabetes screening.

As far as we know, this is the first study to assess simultaneously the discriminatory ability of multiple biomarkers together with conventional risk factors for type 2 diabetes in a large population-based sample from the north and south of China. However, our study has some limitations. First, The cross-sectional nature of the study meant that we could not determine the extent to which diabetes incidence would be predicted by the BRS. Second, fasting glucose was measured only once. The oral glucose tolerance test, which is time-consuming and labour intensive, was not performed in such a large population sample size. However, HbA1c, which reflects long-term glycaemic control, was evaluated in the whole population. Another limitation is that we could not include more biomarkers involved in the pathophysiology of type 2 diabetes owing to limited blood samples. However, we have captured the well-established biomarkers in the major pathways related to insulin sensitivity, inflammation, endothelial function and oxidative stress.

In summary, a BRS derived from four circulating biomarkers, is strongly associated with the risk of type 2 diabetes. Combining the BRS and conventional risk factors substantially enhanced the discriminatory ability for the disease in middle-aged and older Chinese population. Whether the BRS could be used as an effective screening tool for diabetes needs to be verified in future studies.

REFERENCES

1. Yang W, Lu J, Weng J, et al. Prevalence of diabetes among men and women in China. N Engl J Med 2010;362:1090—101.
2. Kam Cheong W, Zhiqiang W. Prevalence of type 2 diabetes mellitus of Chinese populations in Mainland China, Hong Kong, and Taiwan. Diabetes Res Clin Pract 2006;73:126—34.
3. Hu FB, Manson JE, Stampfer MJ, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001; 345:790—7.
4. Lindstrom J, Tuomilehto J. The diabetes risk score: a practical tool to predict type 2 diabetes risk. Diabetes Care 2003;26:725—31.
5. Aekplakorn W, Bunnag P, Woodward M, et al. A risk score for predicting incident diabetes in the Thai population. Diabetes Care 2006;29:1872—7.
6. Schulze MB, Hoffmann K, Boeing H, et al. An accurate risk score based on anthropometric, dietary, and lifestyle factors to predict the development of type 2 diabetes. Diabetes Care 2007;30:510—15.
7. Thomas C, Hypponen E, Power C. Type 2 diabetes mellitus in midlife estimated from the Cambridge Risk Score and body mass index. Arch Intern Med 2006;166:682—8.
8. Meigs JB, Shrader P, Sullivan LM, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359:2208—19.
9. t Hart LM, Simonis-Bik AM, Nijpels G, et al. Combined risk allele score of eight type 2 diabetes genes is associated with reduced first-phase glucose-stimulated insulin secretion during hyperglycemic clamps. Diabetes 2009;59:287—92.
10. Qi Q, Li H, Wu Y, et al. Combined effects of 17 common genetic variants on type 2 diabetes risk in a Han Chinese population. Diabetologia 2009;53:2163—6.
11. Goldberg RB. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. J Clin Endocrinol Metab 2009;94:3171—82.
12. Sattar N, Wannamethee S, Forouhi N. Novel biochemical risk factors for type 2 diabetes: pathogenic insights or prediction possibilities? Diabetologia 2008;51:926—40.
13. Kolberg JA, Jorgensen T, Gerwien RW, et al. Development of a type 2 diabetes risk model from a panel of serum biomarkers from the Inter99 cohort. Diabetes Care 2009;32:1207—12.
14. Ye X, Yu Z, Li H, et al. Distributions of C-reactive protein and its association with metabolic syndrome in middle-aged and older chinese people. J Am Coll Cardiol 2007;49:1798—805.
15. Wang J, Li H, Franco OH, et al. Adiponectin and metabolic syndrome in middle-aged and elderly Chinese. Obesity 2007;16:172—8.
16. Qi Q, Wang J, Li H, et al. Associations of resistin with inflammatory and fibrinolytic markers, insulin resistance, and metabolic syndrome in middle-aged and older Chinese. Eur J Endocrinol 2008;159:585—93.
17. Qi Q, Yu Z, Ye X, et al. Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in Chinese people. J Clin Endocrinol Metab 2007;92:4827—34.
18. Ye X, Franco OH, Yu Z, et al. Associations of inflammatory factors with glycaemic status among middle-aged and older Chinese people. Clin Endocrinol 2009;70:854—62.
19. Sun L, Franco OH, Hu FB, et al. Ferritin concentrations, metabolic syndrome, and type 2 diabetes in middle-aged and elderly Chinese. J Clin Endocrinol Metab 2008;93:4690—6.
20. Lin X, Song K, Lim N, et al. Risk prediction of prevalent diabetes in a Swiss population using a weighted genetic score—the CoLaus Study. Diabetologia 2009;52:600—8.
21. Pencina MJ, D'Agostino RB, Vasan RS. Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157—72.
22. Salomaa V, Havulinna A, Saarela O, et al. Thirty-one novel biomarkers as predictors for clinically incident diabetes. PLoS One 2010;5:e10100.
23. Yoon KH, Lee JH, Kim JW, et al. Epidemic obesity and type 2 diabetes in Asia. Lancet 2006;368:1681—8.
24. Lin JW, Chang YC, Li HY, et al. Cross-sectional validation of diabetes risk scores for predicting diabetes, metabolic syndrome, and chronic kidney disease in Taiwanese. Diabetes Care 2009;32:2294—6.
25. Li S, Shin HJ, Ding EL, et al. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2009;302:179—88.
26. Scherer PE. Adipose tissue. Diabetes 2006;55:1537—45.
27. Pradhan AD, Manson JE, Rifai N, et al. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327—34.

28. Bertoni AG, Burke GL, Owusu JA, *et al*. Inflammation and the incidence of type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care* 2010;33:804—10.

29. Ley SH, Harris SB, Connelly PW, *et al*. Adipokines and incident type 2 diabetes in an Aboriginal Canadian population. *Diabetes Care* 2008;31:1410—15.

30. Festa A, D'Agostino R Jr, Tracy RP, *et al*. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 2002;51:1131—7.

31. Kanaya AM, Wassel Fyr C, Vittinghoff E, *et al*. Adipocytokines and incident diabetes mellitus in older adults: the independent effect of plasminogen activator inhibitor 1. *Arch Intern Med* 2006;166:350—6.

32. Jiang R, Manson JE, Meigs JB, *et al*. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA* 2004;291:711—17.

33. Forouhi NG, Harding AH, Allison M, *et al*. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. *Diabetologia* 2007;50:949—56.