Mast cell specific immunological biomarkers and metabolic syndrome among middle-aged and older Chinese adults

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Abstract. The main aim of this study is to explore whether these mast cell specific immunological biomarkers [immunoglobulin E (IgE), chymase and tryptase] is an independent risk factor of MetS and whether the combined action of these biomarkers increased the associations with MetS. Three mast cell-specific immunological biomarkers were measured using enzyme linked immunosorbent assay (ELISA). One-way analysis of covariance and logistic regression models were used for analyzing the associations between immunological biomarkers with MetS. A total of 340 participants, 82 (24.1%) individuals had diabetes mellitus, 31 (9.1%) had MetS (without diabetes mellitus) and 110 had MetS plus diabetes mellitus. After adjusting by multivariable (age, gender, smoking, and family history for hypertension), compared with no diabetes mellitus or MetS group (reference group), hs-CRP was associated with diabetes mellitus [OR (odds ratio): 2.29 (1.15-4.57, 95% CI (confidence interval), p=0.019] and MetS plus diabetes mellitus [OR: 2.20 (1.05-4.61, 95% CI), p=0.036]. IgE was associated with MetS plus diabetes mellitus [OR: 2.38 (1.13-5.02, 95% CI), p=0.023]. After adjusting by multivariable, compared with reference group, most of combined elevated inflammatory or immunological biomarkers were significantly associated with diabetes mellitus or MetS with or without diabetes mellitus. Patients with established diabetes mellitus or MetS had different inflammatory or immunological cytokine profile (such as hs-CRP, IgE, chymase, tryptase), which indicated that there is an alteration in the function of the immune system in diabetes mellitus or MetS patient. But these results are requested to be further demonstrated for large sample population-based cohort study.

Key words: Metabolic syndrome, C-reactive protein, Immunoglobulin E, Chymase, Tryptase

METABOLIC SYNDROME (MetS) is defined by a constellation of interconnected physiological, biochemical, clinical, and metabolic factors [1-3]. The pathophysiology of MetS is very complex and has been only partially elucidated [2]. A number of markers of systemic inflammation, including C-reactive protein (CRP), are often increased, as are fibrinogen, interleukin 6, tumor necrosis factor-alpha (TNF-α), and others in MetS individuals [3-5]. Definition for MetS is also different in different organizations, but almost all definitions included diagnosed diabetes. Recently, more and more literature indicated that MetS was associated with the risk of developing cardiovascular disease and diabetes, and all cause mortality [1-3]. According to this view, it may be more meaningful to separate diagnosed diabetes mellitus from the components of MetS in the analysis of the relationship between some exposures or risk factors and MetS.

Mast cells (MCs) had long been known as critical effectors in the development of allergic diseases and in many immunoglobulin E (IgE)-mediated immune responses [6-11]. In the past two decades, MCs have gained increased recognition and prominence for their involvement in both immunological and inflammatory diseases [12-16]. MCs exert their physiological and pathological activities by releasing granules containing proteases including MCs specific chymase and tryptase [17, 18]. Recent studies have revealed that chymase and tryptase directly participated in insulin resistance, cardiovascular diseases and metabolic disorders, although the mechanisms are not fully understood [19-27].
of MCs in diet-induced obesity and diabetes, MCs inhibitory medications used in obesity and diabetes in experimental models offer hope to patients with these common chronic inflammatory diseases [19-25].

In our previous articles, we have explored the association between these inflammatory and mast cell-specific immunological biomarkers (CRP, IgE, chymase and tryptase) with pre-diabetes and type 2 diabetes by binary or order logistic regression model and found that both plasma MC proteases and IgE levels are significant risk factors for human pre-diabetes and diabetes mellitus [26, 27].

Based on the above analysis, the action of CRP on MetS has been widely discussed and confirmed, and the action of specific immunological biomarkers on hyperglycemia (pre-diabetes and diabetes) has been discussed in our previous articles, in the present article, we try to focus on the following two questions: 1) whether these mast cell specific immunological biomarkers (IgE, chymase and tryptase) or inflammatory biomarkers CRP is an independent risk factor of MetS, especially diagnosed diabetes separated from the components of MetS; 2) whether the combined action of these biomarkers increased the associations with MetS.

Materials and Methods

Study population
A cross-sectional study aiming at screening pre-diabetes subjects was conducted between 2008 and 2009 at three communities in the city of Huzhou, Zhejiang province, China. Because our research purpose is screening pre-diabetes individuals, considering the availability of study, our research subjects were selected mainly from diabetes high-risk groups. The inclusion criteria were: 1) all subjects were aged 55-75 years old; 2) without or unknown diabetic mellitus; 3) without or unknown cardiovascular and cerebrovascular diseases; 4) overweight or obese 5) with impaired glucose tolerance, including fasting blood glucose (FPG) 5.7 ~ 7.0 mmol/L or 2 hour postprandial blood glucose (2h-OGTT) 7.8 ~ 11.1 mmol/L or glycated hemoglobin 5.7 ~ 6.5%; 6) having a family history of diabetes; 7) hyperlipidemia; 8) high blood pressure. The exclusion criteria were: 1) known type 2 diabetic mellitus (T2DM); 2) known cardiovascular and cerebrovascular diseases; 3) malignant diseases, chronic liver diseases or kidney failure. A total of 340 participants had carried screening test. This study was approved by the Huzhou City Ethics Committee and all subjects gave written, informed consent prior to participating in the study.

In this article, according to the MetS and hyperglycemia criteria of Chinese Diabetes Society (CDS) [28], we categorized 340 participants as one of four groups based on diagnostic criteria of metabolic and diabetes mellitus: 1) 117 (34.41%) were No diabetes mellitus and MetS, 2) 82 (24.12%) diabetes mellitus only, 3) 31 (9.12%) MetS without diabetes mellitus, and 4) 110 (32.35%) MetS plus diabetes mellitus.

Data collection
Demographic data (age and sex), anthropometric measurements (body weight and height, waist and hip circumferences), and blood pressure were collected from each participant when testing fasting glucose and 2h-OGTT. The biochemical parameters were measured in the Clinical Biochemistry Unit of Huzhou First Hospital, a teaching hospital of the School of Medicine. Plasma chymase and tryptase levels were determined as described previously [24]. Details of the study data collection have been reported elsewhere [27].

Clinical criteria
MetS and diabetes mellitus were defined according to the criteria of CDS [28]. Individuals were considered to have MetS if they had three or more of the following risk factors: 1) overweight and obesity was defined when a body mass index (BMI) ≥25 kg/m²; 2) Pre-diabetes was defined as FPG ≥6.1 mmol/L and <7.0 mmol/L and 2h-OGTT ≥7.8 mmol/L and <11.1 mmol/L; diabetes mellitus was defined when fasting glucose ≥7.0 mmol/L, and/or 2h-OGTT ≥11.0 mmol/L and/or diagnosed as diabetes; 3) elevated blood pressure was defined when systolic blood pressure (SBP) ≥130 mmHg or diastolic blood pressure (DBP) ≥85 mmHg or diagnosed as hypertension; 4) elevated triglycerides (TG) (≥1.70 mmol/L) or received lipid lowering therapy, and 5) reduced HDL cholesterol (<0.9 mmol/L in men or <1.0 mmol/L in women).

Statistical analysis
Descriptive statistics for demographic variables, components of MetS, and inflammation and immune-related biomarkers were calculated separately for the four groups. The mean and standard deviation (mean ± SD) of continuous and normal distributional variables and median and interquartile range of con-
Data were analyzed using one-way analysis of covariance, chi-square test, Kruskal-Wallis test, Mann-Whitney U test or Binary logistic model. All p-values were 2-tailed and a significance level of 0.05 was used. All statistical analysis was conducted using SPSS statistical software (version 12.0).

Results

The descriptive characteristics of 340 study participants are presented separately for the four categories according to metabolic or diabetes mellitus (Table 1).

Table 1  Descriptive characteristics of 340 participants according to category of metabolic or glyceric abnormality

|                               | No diabetes mellitus and MetS | Diabetes mellitus | MetS without diabetes mellitus | MetS with diabetes mellitus |
|-------------------------------|-------------------------------|-------------------|--------------------------------|-----------------------------|
| n                             | 117                           | 82                | 31                             | 110                         |
| Age (y)                       | 65 (59, 70)                   | 65 (61, 71)       | 65 (64, 70)                    | 65 (61, 79)                 |
| Female (%)                    | 76.9                          | 73.2              | 74.2                           | 64.5                        |
| Current smoker (%)            | 16.2                          | 20.7              | 22.5                           | 25.5                        |
| Alcohol consumption (%)       | 20.8                          | 24.2              | 25.3                           | 27.6                        |
| Family history of hypertension (%) | 49.2                         | 52.8              | 48.6                           | 54.7                        |
| WC (cm)                       | 80.17 (8.58)                  | 80.26 (8.23)      | 87.81 (8.74) ‡                 | 86.42 (8.14) ‡              |
| AC (cm)                       | 90.86 (7.47)                  | 90.53 (6.29)      | 96.16 (8.17) ‡                 | 95.63 (8.03) ‡              |
| WHR                           | 0.87 (0.83, 0.91)             | 0.88 (0.84, 0.92) | 0.89 (0.85, 0.90) ‡            | 0.91 (0.88, 0.96) ‡         |
| TC (mmol/L)                   | 4.80 (0.86)                   | 4.95 (0.88)       | 5.46 (1.13) ‡                  | 5.25 (1.15) ‡               |
| LDL-c (mmol/L)                | 2.46 (0.58)                   | 2.64 (0.62) †     | 2.45 (0.73)                     | 2.60 (0.65)                 |
| Fasting insulin (mU/L)        | 5.29 (1.17)                   | 5.11 (1.67)       | 8.14 (1.53) †                  | 7.88 (1.62) †               |
| HOMA-β index                  | 85.07 (1.78)                  | 62.21 (1.88)      | 60.86 (2.03) †                 | 40.09 (2.06) †              |
| HOMA-IR index                 | 1.24 (1.83)                   | 1.40 (1.63)       | 1.99 (1.54) †                  | 2.18 (1.66) †               |
| **Components of the Metabolic Syndrome** |                               |                   |                                |                             |
| BMI (kg/m²)                   | 23.31 (3.51)                  | 22.71 (2.45)      | 26.69 (2.82) ‡                 | 25.66 (2.93) ‡              |
| FPG (mmol/L)                  | 5.48 (4.99, 5.74)             | 6.13 (5.44, 6.57) ‡ | 5.64 (5.18, 5.85) ‡            | 6.14 (5.40, 6.83) ‡         |
| 2h-OGTT (mmol/L)              | 5.90 (5.16, 6.72)             | 9.71 (7.97, 11.49) † | 6.44 (5.22, 7.10)               | 9.83 (8.27, 13.40) ‡        |
| SBP (mmHg)                    | 132.55 (15.74)                | 137.55 (24.25)    | 144.87 (14.85) ‡               | 143.32 (14.85)              |
| DBP (mmHg)                    | 77.09 (8.48)                  | 76.78 (9.57)      | 79.48 (8.82)                   | 81.78 (9.53) †              |
| TG (mmol/L)                   | 1.11 (0.86, 1.56)             | 1.17 (0.86, 1.46) | 2.28 (1.88, 3.65) ‡            | 1.97 (1.52, 2.79) †         |
| HDL-c (mmol/L)                | 1.27 (1.10, 1.47)             | 1.27 (1.11, 1.52) | 0.99 (0.89, 1.33) ‡            | 1.12 (0.97, 1.25) †         |
| **Inflammation and immune-related biomarkers** |                               |                   |                                |                             |
| hs-CRP (mg/L)                 | 3 (2, 5)                      | 5 (3, 8) †        | 3 (2, 9)                       | 5 (2, 9) †                  |
| IgE (IU/L)                    | 10.0 (5.0, 32.0)              | 23.50 (6.75, 48.50) † | 17.00 (6.00, 51.00)            | 19.00 (5.00, 81.75) †        |
| Chymase (µg/mL)               | 18.33 (12.99, 25.79)          | 21.77 (13.02, 26.60) | 23.66 (10.17, 27.99)           | 22.73 (14.52, 26.46)        |
| Tryptase (>2.74 ng/mL)        | 22 (19.3)                     | 20 (24.4)         | 8 (25.8)                       | 33 (30.8) †                 |

Abbreviations: MetS, Metabolic Syndrome; WC, waist circumference; AC, abdominal circumference; WHR, waist hip ratio; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA, homeostasis model assessment; BMI, body mass index; FPG, fasting plasma glucose; 2h-OGTT, 2 hour oral glucose tolerance test; SBP, systolic blood pressure; DBP, diastolic blood pressures; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; IgE, immunoglobulin E; hs-CRP, hypersensitivity C-reactive protein. For normally distributed variables, values are presented as means [standard deviation (SD)] or frequencies (%) and as median (interquartile range) for variables that did not follow a normal distribution. p-values were computed for each category versus the reference category using one-way analysis of covariance (ANOVA) or chi-square for normally distributed variables or Wilcoxon nonparametric rank test for variables that did not follow a normal distribution, † p < 0.05, compared with the No diabetes and MetS group; ‡ p < 0.01, compared with the No diabetes and MetS group.
A total of 340, 82 (24.1%) individuals had diabetes mellitus, 31 (9.1%) had MetS (without diabetes mellitus), and 110 had MetS plus diabetes mellitus. Overall, except age, sex, smoking status, alcohol use, and family history of hypertension, some traditional vascular risk factors, components of the MetS and inflammation and immune-related biomarkers were worse in diabetes mellitus and MetS individuals than without diabetes mellitus or MetS. Compared with no diabetes mellitus and MetS group (reference group), diabetes mellitus group have elevated LDL-c, FPG, 2h-OGTT, hs-CRP and IgE level; MetS without diabetes mellitus have elevated WC, AC, WHR, TC, Fasting insulin, HOMA-IR index, BMI, SBP, TG, HDL-c level and decreased HOMA-β index; MetS with diabetes mellitus group have elevated WC, AC, WHR, TC, Fasting insulin, HOMA-IR index, BMI, FPG, 2h-OGTT, SBP, DBP, TG, HDL-c, hs-CRP, IgE, tryptase level and decreased HOMA-β index.

Table 2 displays the average levels of individual components of the MetS presented upper quartile of biomarker in a model adjusted for age and gender. Hs-CRP was associated with 2h-OGTT, WC, SBP, IgE were associated with 2h-OGTT and TG. Chymase was only associated with SBP but tryptase was not associated with any components of the MetS.

### Table 2

| Upper quartile of biomarker | FPG (mmol/L) | 2h-OGTT (mmol/L) | WC (cm) | TG (mmol/L) | HDL-c (mmol/L) | SBP (mmHg) | DBP (mmHg) |
|-----------------------------|--------------|------------------|---------|-------------|---------------|------------|------------|
| hs-CRP (mg/L)              |              |                  |         |             |               |            |            |
| <8                         | 5.67 (5.17, 6.22) | 7.33 (5.90, 9.75) | 82.31 (81.18, 83.44) | 1.41 (1.01, 2.05) | 1.20 (1.05, 1.18) | 138.00 (124.25, 146.00) | 78.00 (70.00, 87.00) |
| ≥8                         | 5.85 (5.43, 6.37) | 8.73 (6.52, 11.02) | 84.47 (82.69, 86.26) | 1.53 (1.03, 1.94) | 1.22 (0.98, 1.39) | 141.00 (130.00, 160.00) | 80.00 (72.00, 85.50) |
| p values                   | 0.069        | 0.025            | 0.038   | 0.902       | 0.996         | <0.001     | 0.820      |
| IgE (IU/L)                 |              |                  |         |             |               |            |            |
| <47.5                      | 5.68 (5.18, 6.23) | 7.30 (5.85, 9.93) | 82.64 (81.52, 83.75) | 1.40 (0.96, 1.93) | 1.20 (1.05, 1.18) | 138.32 (136.12, 140.53) | 78.99 (77.84, 80.14) |
| ≥47.5                      | 5.80 (5.43, 6.30) | 8.26 (6.30, 10.85) | 83.83 (81.90, 85.76) | 1.59 (1.05, 2.59) | 1.20 (1.01, 1.42) | 138.22 (133.67, 142.76) | 78.01 (75.95, 80.07) |
| p values                   | 0.136        | 0.049            | 0.271   | 0.03        | 0.873         | 0.866      | 0.287      |
| Chymase (μg/mL)            |              |                  |         |             |               |            |            |
| <26.39                     | 5.69 (5.20, 6.21) | 7.82 (5.98, 10.01) | 83.07 (81.96, 84.18) | 1.43 (1.01, 2.00) | 1.20 (1.04, 1.37) | 137.01 (134.70, 139.33) | 78.48 (77.33, 79.64) |
| ≥26.39                     | 5.80 (5.29, 6.34) | 8.71 (5.93, 10.52) | 82.38 (80.42, 84.34) | 1.49 (1.05, 2.25) | 1.23 (1.04, 1.41) | 142.59 (138.51, 146.66) | 79.71 (77.61, 81.80) |
| p values                   | 0.540        | 0.971            | 0.568   | 0.345       | 0.375         | 0.018      | 0.312      |
| Tryptase (ng/mL)           |              |                  |         |             |               |            |            |
| <2.74                      | 5.70 (5.22, 6.18) | 7.52 (5.90, 10.07) | 82.68 (81.55, 83.81) | 1.41 (1.03, 1.92) | 1.05 (0.93, 1.20) | 138.63 (136.52, 140.74) | 79.06 (77.91, 80.21) |
| ≥2.74                      | 5.77 (5.23, 6.45) | 8.17 (6.24, 10.88) | 83.54 (81.67, 85.42) | 1.57 (1.01, 2.41) | 1.00 (0.86, 1.19) | 137.77 (132.66, 142.88) | 77.99 (75.88, 80.10) |
| p values                   | 0.342        | 0.212            | 0.494   | 0.253       | 0.636         | 0.714      | 0.755      |

### Effects of single inflammation and immune-related factors on MetS and diabetes mellitus

After adjusting confounding factors by multivariable (age, gender, smoking, and family history for hypertension), compared with the reference group, hs-CRP was associated with diabetes mellitus [odds ratio (OR): 2.29 (1.15-4.57, 95% CI), p=0.019] and MetS with diabetes mellitus [OR: 2.20 (1.05-4.61, 95% CI), p=0.036], IgE was associated with MetS with diabetes mellitus [OR: 2.38 (1.13-5.02, 95% CI), p=0.023]. But only in unadjusted model, tryptase was associated with MetS with diabetes mellitus [OR: 2.86 (1.00-3.47, 95% CI), p=0.049], compared with the reference group (Table 3).

### Combined effects of inflammation and immune-related factors on MetS and diabetes mellitus

Table 4 showed combined effects of inflammation and immune-related factors on diabetes mellitus. After adjusting confounding factors by multivariable (age, gender, smoking, and family history for hypertension), compared with the lowest level group, combined higher hs-CRP and IgE or chymase or tryptase were significantly associated with diabetes mellitus [OR: 3.12 (1.41-6.90, 95% CI), p=0.005; OR: 9.66 (1.97-42.43, 95% CI), p=0.005; OR: 2.31 (1.06-5.06, 95% CI), p=0.005, respectively]; combined higher IgE and tryptase were significantly associated with diabetes mellitus [OR: 4.27 (1.09-16.73, 95% CI), p=0.037].
Table 3  Odds ratios for metabolic or glycemic abnormality associated with elevated biomarkers of inflammation and immune-related factors

| No diabetes mellitus and MetS | Diabetes mellitus only | p | MetS without diabetes mellitus | p | MetS with diabetes mellitus | p |
|-----------------------------|------------------------|---|-------------------------------|---|-----------------------------|---|
| hs-CRP (mg/L)               |                        |   |                               |   |                             |   |
| Unjustment                  | 1                      | 2.37 (1.20, 4.66) | 0.012 | 2.43 (0.99, 2.58) | 0.053 | 2.48 (1.32, 4.68) | 0.005 |
| Age, gender                 | 1                      | 2.34 (1.18, 4.46) | 0.015 | 2.36 (0.96, 5.85) | 0.063 | 2.59 (1.36, 4.94) | 0.004 |
| Multivariable               | 1                      | 2.29 (1.15, 4.57) | 0.019 | 1.86 (0.66, 5.18) | 0.238 | 2.20 (1.05, 4.61) | 0.036 |
| IgE (IU/L)                  |                        |   |                               |   |                             |   |
| Unjustment                  | 1                      | 1.57 (0.79, 3.12) | 0.194 | 1.59 (0.63, 4.04) | 0.330 | 2.13 (1.15, 3.96) | 0.017 |
| Age, gender                 | 1                      | 1.56 (0.79, 3.10) | 0.203 | 1.56 (0.61, 3.98) | 0.353 | 2.04 (1.09, 3.82) | 0.025 |
| Multivariable               | 1                      | 1.53 (0.76, 3.09) | 0.237 | 1.90 (0.67, 5.35) | 0.225 | 2.38 (1.13, 5.02) | 0.023 |
| Chymase (μg/mL)             |                        |   |                               |   |                             |   |
| Unjustment                  | 1                      | 1.54 (0.79, 3.00) | 0.201 | 1.62 (0.66, 3.98) | 0.295 | 1.40 (0.75, 2.63) | 0.292 |
| Age, gender                 | 1                      | 1.50 (0.77, 2.94) | 0.238 | 1.59 (0.65, 3.94) | 0.312 | 1.45 (0.77, 2.74) | 0.254 |
| Multivariable               | 1                      | 1.34 (0.67, 2.67) | 0.412 | 1.50 (0.56, 3.99) | 0.421 | 1.28 (0.61, 2.65) | 0.512 |
| Tryptase (ng/mL)            |                        |   |                               |   |                             |   |
| Unjustment                  | 1                      | 1.35 (0.68, 2.68) | 0.392 | 1.45 (0.57, 3.68) | 0.429 | 1.86 (1.00, 3.47) | 0.049 |
| Age, gender                 | 1                      | 1.35 (0.67, 2.72) | 0.406 | 1.35 (0.52, 3.51) | 0.544 | 1.79 (0.94, 3.43) | 0.078 |
| Multivariable               | 1                      | 1.31 (0.64, 2.66) | 0.462 | 1.57 (0.55, 4.49) | 0.400 | 1.75 (0.82, 3.76) | 0.148 |

The multivariable models are adjusted for age, gender, smoking, and family history for hypertension.

Table 4  Odds ratios for diabetes mellitus only group associated with interaction between elevated biomarkers of inflammation and immune-related factors

| Unjusted analysis | Age- and sex adjusted analysis | Multivariable adjusted analysis |
|-------------------|-------------------------------|--------------------------------|
|                  | OR (95% CI)                   | p                             | OR (95% CI)                   | p                             | OR (95% CI)                   | p                             |
| Interaction hs-CRP (mg/L) / IgE (IU/L) |                        |                               |                               |                               |                               |                               |
| hs-CRP < 8 / IgE < 47.5 | 1                      | 1.66 (0.43, 6.36) | 0.459 | 1.66 (0.43, 6.36) | 0.459 | 1.66 (0.43, 6.36) | 0.459 |
| hs-CRP ≥ 8 / IgE ≥ 47.5 | 3.04 (1.40, 6.59) | 0.005 | 3.01 (1.38, 6.56) | 0.006 | 3.12 (1.41, 6.90) | 0.005 |
| Interaction hs-CRP (mg/L) / Chymase (μg/mL) |                        |                               |                               |                               |                               |                               |
| hs-CRP < 8 / Chymase < 26.39 | 1                      | 1.25 (0.55, 2.82) | 0.595 | 1.25 (0.55, 2.82) | 0.595 | 1.25 (0.55, 2.82) | 0.595 |
| hs-CRP ≥ 8 / Chymase ≥ 26.39 | 9.73 (2.08, 45.51) | 0.004 | 9.88 (2.06, 47.39) | 0.004 | 9.66 (1.97, 42.43) | 0.005 |
| Interaction hs-CRP (mg/L) / Tryptase (ng/mL) |                        |                               |                               |                               |                               |                               |
| hs-CRP < 8 / Tryptase < 2.74 | 1                      | 2.62 (0.68, 10.09) | 0.160 | 2.62 (0.68, 10.09) | 0.160 | 2.62 (0.68, 10.09) | 0.160 |
| hs-CRP ≥ 8 / Tryptase ≥ 2.74 | 2.41 (1.12, 5.20) | 0.025 | 2.40 (1.11, 5.19) | 0.026 | 2.31 (1.16, 5.06) | 0.036 |
| IgE (IU/L) / Chymase (μg/mL) |                        |                               |                               |                               |                               |                               |
| IgE < 47.5 / Chymase < 26.39 | 1                      | 1.63 (0.71, 3.77) | 0.250 | 1.63 (0.71, 3.77) | 0.250 | 1.63 (0.71, 3.77) | 0.250 |
| IgE ≥ 47.5 / Chymase ≥ 26.39 | 2.36 (0.71, 7.89) | 0.162 | 2.33 (0.69, 7.79) | 0.171 | 1.88 (0.54, 6.54) | 0.323 |
| IgE (IU/L) / Tryptase (ng/mL) |                        |                               |                               |                               |                               |                               |
| IgE < 47.5 / Tryptase < 2.74 | 1                      | 1.01 (0.43, 2.34) | 0.985 | 1.01 (0.43, 2.34) | 0.985 | 1.01 (0.43, 2.34) | 0.985 |
| IgE ≥ 47.5 / Tryptase ≥ 2.74 | 0.87 (0.38, 1.98) | 0.737 | 0.86 (0.37, 2.00) | 0.720 | 0.81 (0.34, 1.91) | 0.628 |
| Chymase (μg/mL) / Tryptase (ng/mL) |                        |                               |                               |                               |                               |                               |
| Chymase < 26.39 / < Tryptase < 2.74 | 1                      | 1.40 (0.60, 3.29) | 0.436 | 1.40 (0.60, 3.29) | 0.436 | 1.40 (0.60, 3.29) | 0.436 |
| Chymase ≥ 26.39 / ≥ Tryptase ≥ 2.74 | 1.65 (0.55, 5.01) | 0.375 | 1.64 (0.54, 5.05) | 0.386 | 1.44 (0.46, 4.49) | 0.530 |

The multivariable models are adjusted for age, gender, smoking, and family history for hypertension.
Table 5 showed combined effects of inflammation and immune-related factors on MetS without diabetes mellitus. After adjusting confounding factors by multivariable (age, gender, smoking, and family history for hypertension), compared with the lowest level group, combined higher hs-CRP and chymase were significantly associated with MetS without diabetes mellitus [OR: 12.25 (1.78-84.44, 95% CI), \( p = 0.011 \)] and combined higher IgE and tryptase were significantly associated with MetS without diabetes mellitus [OR: 6.59 (1.17-37.10, 95% CI), \( p = 0.033 \)].

Table 6 showed combined effects of inflammation and immune-related factors on MetS plus diabetes mellitus group. After adjusting confounding factors by multivariable (age, gender, smoking, and family history for hypertension), compared with the lowest level group, combined higher hs-CRP and IgE or tryptase were significantly associated with MetS with diabetes mellitus [OR: 3.28 (1.07-11.17, 95% CI), \( p = 0.017 \); OR: 3.94 (1.10-7.10, 95% CI), \( p = 0.035 \), respectively]; and combined higher IgE and chymase or tryptase were significantly associated with MetS without diabetes mellitus.

| Interaction | Unadjusted | Age- and sex adjusted | Multivariable adjusted |
|-------------|------------|-----------------------|-----------------------|
| hs-CRP (mg/L) / IgE (IU/L) | | | |
| hs-CRP < 8 / IgE < 47.5 | 1 | 1 | 1 |
| hs-CRP ≥ 8 / IgE < 47.5 | 2.04 (0.69, 6.07) 0.199 | 1.97 (0.66, 5.92) 0.225 | 1.55 (0.43, 1.60) 0.505 |
| hs-CRP < 8 / IgE ≥ 47.5 | 1.19 (0.35, 4.01) 0.778 | 1.17 (0.35, 3.96) 0.798 | 1.59 (0.43, 5.83) 0.485 |
| hs-CRP ≥ 8 / IgE ≥ 47.5 | 3.81 (0.93, 15.69) 0.064 | 3.72 (0.90, 15.38) 0.070 | 3.15 (0.65, 15.14) 0.153 |
| Chymase (μg/mL) / Tryptase (ng/mL) | | | |
| Chymase < 26.39 / Tryptase < 2.74 | 1 | 1 | 1 |
| Chymase ≥ 26.39 / Tryptase < 2.74 | 0.71 (0.19,2.69) 0.617 | 0.68 (0.18, 2.61) 0.572 | 0.56 (0.16, 3.29) 0.673 |
| Chymase < 26.39 / Tryptase ≥ 2.74 | 0.83 (0.25, 2.73) 0.760 | 0.76 (0.22, 2.56) 0.654 | 0.84 (0.22, 3.21) 0.800 |
| Chymase ≥ 26.39 / Tryptase ≥ 2.74 | 5.26 (1.09, 25.54) 0.039 | 4.87 (0.99, 24.00) 0.052 | 6.59 (1.17, 37.10) 0.033 |

The multivariable models are adjusted for age, gender, smoking, and family history for hypertension.
with diabetes mellitus [OR: 3.97 (1.61-9.77, 95% CI), p=0.003; OR: 4.49 (1.03-19.52, 95% CI), p=0.045, respectively]. After adjusting confounding factors by age and gender, compared with the lowest level group, combined higher hs-CRP and chymase and combined higher chymase and tryptase were significantly associated with MetS with diabetes mellitus [OR: 7.78 (1.60-37.90, 95% CI), p=0.011; OR: 2.23 (1.04-4.78, 95% CI), p=0.039, respectively].

### Table 6

| Interaction hs-CRP (mg/L) / IgE (IU/L) | Unjusted analysis | Age- and sex adjusted analysis | Multivariable adjusted analysis |
|--------------------------------------|-------------------|-------------------------------|--------------------------------|
|                                      | OR (95% CI)       | p                             | OR (95% CI)       | p                             | OR (95% CI)       | p                             |
| hs-CRP < 8 / IgE < 47.5               | 1                 | 1                             | 1                 | 1                             | 1                 | 1                             |
| hs-CRP ≥ 8 / IgE < 47.5               | 2.56 (1.21, 5.42) | 0.014                         | 2.64 (1.23, 5.69) | 0.013                         | 2.59 (1.06, 6.33) | 0.037                         |
| hs-CRP < 8 / IgE ≥ 47.5               | 2.14 (1.03, 4.45) | 0.041                         | 2.01 (0.96, 4.23) | 0.065                         | 2.79 (1.14, 6.85) | 0.025                         |
| hs-CRP ≥ 8 / IgE ≥ 47.5               | 4.05 (1.36, 12.03)| 0.012                         | 4.08 (1.36, 12.28)| 0.012                         | 3.28 (1.07, 11.17)| 0.017                         |

| Interaction hs-CRP (mg/L) / Chymase (μg/mL) | Unjusted analysis | Age- and sex adjusted analysis | Multivariable adjusted analysis |
|---------------------------------------------|-------------------|-------------------------------|--------------------------------|
|                                      | OR (95% CI)       | p                             | OR (95% CI)       | p                             | OR (95% CI)       | p                             |
| hs-CRP < 8 / Chymase < 26.39               | 2.11 (1.04, 4.27) | 0.039                         | 2.21 (1.08, 4.53) | 0.031                         | 1.89 (0.82, 4.34) | 0.135                         |
| hs-CRP ≥ 8 / Chymase ≥ 26.39               | 1.18 (0.57, 2.43) | 0.652                         | 1.22 (0.59, 2.54) | 0.593                         | 1.08 (0.47, 2.53) | 0.851                         |
| hs-CRP < 8 / Chymase ≥ 26.39               | 6.89 (1.45, 32.74)| 0.015                         | 7.78 (1.60, 37.90)| 0.011                         | 5.13 (0.97, 26.97)| 0.054                         |

| Interaction hs-CRP (mg/L) / Tryptase (ng/mL) | Unjusted analysis | Age- and sex adjusted analysis | Multivariable adjusted analysis |
|----------------------------------------------|-------------------|-------------------------------|--------------------------------|
|                                      | OR (95% CI)       | p                             | OR (95% CI)       | p                             | OR (95% CI)       | p                             |
| hs-CRP < 8 / Tryptase < 2.74                | 2.14 (1.02, 4.52) | 0.045                         | 2.37 (1.11, 5.08) | 0.026                         | 1.97 (0.81, 4.79) | 0.137                         |
| hs-CRP ≥ 8 / Tryptase ≥ 2.74                | 1.54 (0.74, 3.22) | 0.248                         | 1.58 (0.74, 3.38) | 0.242                         | 1.48 (0.60, 3.68) | 0.394                         |
| hs-CRP < 8 / Tryptase ≥ 2.74                | 5.12 (1.59, 16.41)| 0.006                         | 5.00 (1.51, 16.54)| 0.008                         | 3.94 (1.10, 14.08)| 0.035                         |

| IgE (IU/L) / Chymase (μg/mL) | Unjusted analysis | Age- and sex adjusted analysis | Multivariable adjusted analysis |
|------------------------------|-------------------|-------------------------------|--------------------------------|
|                              | OR (95% CI)       | p                             | OR (95% CI)       | p                             | OR (95% CI)       | p                             |
| IgE < 47.5 / Chymase < 26.39 | 2.34 (0.75, 7.55) | 0.155                         | 2.28 (0.70, 7.49) | 0.173                         | 1.47 (0.38, 5.71) | 0.577                         |
| IgE ≥ 47.5 / Chymase ≥ 26.39 | 1.62 (0.78, 3.36) | 0.192                         | 1.70 (0.81, 3.55) | 0.161                         | 1.95 (0.82, 4.63) | 0.128                         |
| IgE ≥ 47.5 / Chymase < 26.39 | 2.63 (1.28, 5.42) | 0.009                         | 2.57 (1.24, 5.33) | 0.011                         | 3.97 (1.61, 9.77) | 0.003                         |

| IgE (IU/L) / Tryptase (ng/mL) | Unjusted analysis | Age- and sex adjusted analysis | Multivariable adjusted analysis |
|------------------------------|-------------------|-------------------------------|--------------------------------|
|                              | OR (95% CI)       | p                             | OR (95% CI)       | p                             | OR (95% CI)       | p                             |
| IgE < 47.5 / Tryptase < 2.74 | 2.21 (1.03, 4.34) | 0.040                         | 2.16 (1.05, 4.46) | 0.037                         | 2.66 (1.11, 6.35) | 0.028                         |
| IgE ≥ 47.5 / Tryptase ≥ 2.74 | 1.74 (0.85, 3.54) | 0.128                         | 1.77 (0.85, 3.68) | 0.128                         | 1.80 (0.75, 4.30) | 0.186                         |
| IgE ≥ 47.5 / Tryptase < 2.74 | 5.50 (1.46, 20.71)| 0.012                         | 4.63 (1.19, 18.03)| 0.027                         | 4.49 (1.03, 19.52)| 0.045                         |

| Chymase (μg/mL) / Tryptase (ng/mL) | Unjusted analysis | Age- and sex adjusted analysis | Multivariable adjusted analysis |
|-------------------------------|-------------------|-------------------------------|--------------------------------|
| Chymase < 26.39 / Tryptase < 2.74 | 1                 | 1                             | 1                             | 1                             | 1                             | 1                             |
| Chymase ≥ 26.39 / Tryptase ≥ 2.74 | 1.76 (0.84, 3.70) | 0.137                         | 1.79 (0.84, 3.80) | 0.130                         | 1.73 (0.73, 4.09) | 0.216                         |
| Chymase < 26.39 / Tryptase ≥ 2.74 | 1.61 (0.55, 4.70) | 0.385                         | 1.66 (0.56, 4.93) | 0.365                         | 1.32 (0.37, 4.67) | 0.668                         |
| Chymase ≥ 26.39 / Tryptase ≥ 2.74 | 2.35 (1.13, 4.86) | 0.022                         | 2.23 (1.04, 4.78) | 0.039                         | 2.42 (0.98, 5.97) | 0.055                         |

The multivariable models are adjusted for age, gender, smoking, and family history for hypertension.

Combinations of hs-CRP and mast cell specific immunological biomarkers on the sensitivity for diabetes mellitus and MetS with diabetes mellitus

Tables 3-6 showed that both hs-CRP alone and combinations of hs-CRP and mast cell-specific immunological biomarkers increased the risk for diabetes mellitus and MetS with diabetes mellitus. We further analyzed combination effectors of hs-CRP and mast cell specific immunological biomarkers on the sensitivity for diabetes mellitus and MetS with diabetes mellitus, and indi-

Mast cell and metabolic syndrome 251
cated that the sensitivity for diabetes mellitus is 37.8% (hs-CRP alone), 19.5% (combined higher hs-CRP and higher IgE), 29.3% (combined higher hs-CRP and higher chymase) and 20.7% (combined higher hs-CRP and higher tryptase), respectively, and the sensitivity for MetS with diabetes mellitus is 30.6% (hs-CRP alone), 20.0 % (combined higher hs-CRP and higher IgE), 18.7% (combined higher hs-CRP and higher chymase) and 18.7% (combined higher hs-CRP and higher tryptase), respectively. These analyses suggest that combinations of hs-CRP and mast cell-specific immunological biomarkers don’t increase the sensitivity for diabetes mellitus and MetS with diabetes mellitus.

**Discussion**

The characteristic elements of the MetS, including abdominal obesity, high blood glucose, hypertension, dyslipidemia and so on, are considered common risk factors for human cardiovascular diseases [2]. Some studies have suggested that MCs participate in the pathogenesis of inflammatory diseases such as cardiovascular diseases and metabolic disorders, MCs dysfunction has been viewed as the main offenders in some chronic inflammatory disorders, but the exact mechanisms of MCs in the pathogenesis of these diseases are not fully understood [19-28]. Participating of MCs in inflammatory diseases can be activated by non-allergic triggers, such as cytokines, often having synergistic effects among them [20]. Our study found that both plasma MCs proteases and IgE levels are significant risk factors for human pre-diabetes and diabetes mellitus [26, 27]. A study indicated that MCs specific serum tryptase concentration was significantly higher in obese subjects than in lean individuals ($p=0.001$) [30]. Another study demonstrated that serum tryptase levels associated strongly with BMI ($p<0.0001$, $p=0.037$) before and after adjustment for age, sex, serum HDL, alcohol consumption, smoking, and atopy status [31].

In this cross-sectional study, we demonstrated that, after adjusting by multivariable (age, gender, smoking, and family history for hypertension), hs-CRP was associated with diabetes mellitus [OR: 2.29 (1.15-4.57, 95% CI (confidence interval), $p=0.019$] and MetS plus diabetes mellitus [OR: 2.20 (1.05-4.61, 95% CI), $p=0.036$], IgE was only associated with MetS plus diabetes mellitus [OR: 2.38 (1.13-5.02, 95% CI), $p=0.023$]. We further analyzed combinations of hs-CRP and mast cell specific immunological biomarkers on the sensitivity for diabetes mellitus and MetS with diabetes mellitus. Our analysis indicates that combinations of hs-CRP and mast cell-specific immunological biomarkers don’t increase the sensitivity for diabetes mellitus and MetS with diabetes mellitus. It may be possible that CRP and MCs specific immunological biomarkers act on diabetes mellitus or MetS through different pathways. It also may be possible that small sample size leads to reduced efficiency of the test, and thus the sensitivity of synergy when analyzing the interaction of the various factors.

In conclusion, this study provided the epidemiological evidence that both hs-CRP alone and MCs specific immunological biomarkers increased the risk for diabetes mellitus and MetS with diabetes mellitus, but combined effect of these biomarkers don’t increase the sensitivity for diabetes mellitus and MetS with diabetes mellitus. IgE also increased the risk for MetS with diabetes mellitus. But it was very unfortunate that we didn’t consider potential autoimmune diseases and allergic diseases [e.g., rheumatoid arthritis (RA) or osteoarthritis (OA)], which might lead to bias. Further large population-based prospective and rigorously designed studies will give full consideration to potential autoimmune diseases and allergic diseases by a well-designed questionnaire and some inflammatory and immunological indicators and are warranted to clarify independent predictive values of mast cell-specific immunological biomarkers for MetS and its associated diseases.

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**Disclosure**

None of the authors have any potential conflicts of interest associated with this research.
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