Clinical implications of serum Mac-2-binding protein (M2BPGi) as novel biomarkers of advanced hepatic fibrosis in diabetes

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Abstract

Background Appropriate strategy for screening, identification, and linkage to care of patients with advanced fibrosis in general population is unsolved issue. The aim of this study was to find reference value and clinical role of Mac2 binding protein glycan isomer (M2BPGi) in health check-up setting.

Methods Adult subjects (n=1,073) who underwent a health check-up at the healthcare center were finally included for analysis, except for 952 subjects with risk factors for liver disease and insufficient data. Body composition analyzed by using the Bioelectrical Impedance Analysis (BIA). M2BPGi quantification based on a lectin antibody sandwich immunoassay. Fatty liver diagnosed by using abdominal sonography.

Results Reference value of M2BPGi was 0.5~1.0 C.O.I in the average risk group. Serum M2BPGi showed a positive correlation with metabolic parameters as well as age. Prevalence of abnormal M2BPGi (> 1.0) was higher in low muscle mass (4.7%, vs 17.4%, p=0.002), metabolic syndrome (14.2% vs. 30.4%, p=0.003) and hypertension (21.8%, vs. 58.7%, p<0.001) compare to healthy control. M2BPGi had a positive correlation with estimated fibrosis formulae such as FIB-4 (R=0.293, p< 0.001) and NAFLD fibrosis score (R=0.248, p<0.001). While the prevalence of advanced fibrosis in total population was just 1.6% (FIB-4 >2.65), the prevalence of advanced fibrosis increased to 50% in high M2BPGi (> 1.0) group with diabetes. It was 31.25 times higher than total population group.

Conclusions There was high possibility of advanced hepatic fibrosis in subjects with abnormal M2BPGi levels (> 1.0) in diabetes.

What Is Current Knowledge

√ Mac-2 binding protein glycosylation isomer (M2BPGi) has been recently studied as a useful serum marker for evaluating liver fibrosis in various chronic liver diseases

√ Studies to utilize M2BPGi as liver fibrosis marker on average risk population are insufficient.

√ NAFLD patients with high risk recommended to be evaluated for hepatic fibrosis burden.

What Is New Here

√ Reference value of M2BPGi was 0.5~1.0 C.O.I in the average risk group.

√ The prevalence of advanced fibrosis increased to 50% in high M2BPGi (> 1.0) group with diabetes.

√ The prevalence of advanced fibrosis was 31.25 times higher than total population group.
Background
Non-alcoholic fatty liver (NAFLD) is seemed to be a major cause of chronic liver disease in worldwide. [1, 2] It is important to select the advanced liver fibrosis in general population. The liver biopsy is a gold standard for assessing the fibrosis status, but it has several limitations, such as, invasiveness, sampling error and cost. Until now, various noninvasive approaches, which are transient elastography (FibroScan[3] and magnetic resonance elastography[4]), various scoring systems (AST-to-platelet ratio index (APRI)[5], FIB-4 index[6] and NAFLD Fibrosis Score[7]) and serologic markers (cytokeratin-18,[3] ASAGP[8] and Mac 2 binding protein), have been studied to predict advanced fibrosis subjects from general population. But none of single test or algorithm widely accepted in general population until now.

Glycoproteins are known to be synthesized, secreted and metabolized in the liver, and increased in liver disease.[9, 10] Among serum glycoproteins for liver fibrosis, Mac-2 binding protein glycosylation isomer (M2BPGi, Wisteria floribunda agglutinin-positive Mac-2 binding protein) has been recently studied as a useful serum marker for evaluating liver fibrosis in various chronic liver diseases such as chronic hepatitis C virus infection, autoimmune hepatitis, primary biliary cholangitis and NAFLD.[9-11] In biopsy proven cohort, M2BPGi discriminate from NASH at cut off 0.95 (AUROC 0.759) and significant fibrosis (>F2) at cut off 1.0 (AUROC 0.758).[11]

However, most of the studies so far focused on role of M2BPGi for NASH diagnosis or prediction of advanced fibrosis in patients with high risk group. Now we are called to make a strategy for screening patients with advanced liver fibrosis in the general population. There is few study on clinical characteristics of M2BPGi in average risk group. Moreover, cut off value of M2BPGi to predict advanced fibrosis was quite different according to etiology of liver fibrosis. There is still little research on whether MGBPGi can apply to screen advanced liver fibrosis patients in the general population.

The aim of this study was to find clinical role of M2BPGi in health check-up clinic setting

Methods
1. **Study design.**

The medical records of Hanyang University Hospital were collected for analysis, prospectively.
Koreans are required to get health checkups every year or every two years for all adults, aged over 20 years, under the Basic Health Checkup Act. The costs associated with the checkups are covered by the state. The Institutional Review Board (IRB) of Hanyang University Medical Center approved this study protocol (IRB No. No. 2018-12-020). The protocol was also registered at the Clinical Research Information Service. (https://cris.nih.go.kr/cris, Registration No. KCT0004462).

2. **Inclusion and exclusion criteria.**

In this study, a total of 2,025 of the adult subjects, who underwent a health check-up at International Healthcare Service at Hanyang University Medical Center between March 2019 and June 2019 were initially recruited into the study. We collected the data, excluding foreigners (n = 339), subjects with insufficient remnant blood sample (n=53), subjects with missing data for baseline physical measurement including skeletal muscle mass (n = 20), abdominal SONO (n=48), for alcohol consumption (n = 28) or serum M2BPGi level (n=270). And then we further excluded the high risk liver disease population, who had the positive serologic markers of hepatitis B virus or hepatitis C or who was self-reported HBV carrier (n = 65) or subjects whose weekly alcohol consumption was greater than 210 g for men or greater than 140 g for women (n = 160). Finally, 1,073 subjects were included in this study (Figure. 1).

3. **Clinical variables and laboratory evaluations.**

Personal medical and medication history, smoking history, exercise and alcohol consumption were collected through self-reported survey. Body weight and height were measured and body mass index (BMI) was calculated weight in kilograms (kg) divided by the height in meter squared (m²). Waist circumference (WC) was measured at the narrowest point between the iliac crest and the lower rib margin. Blood pressure was measured at rest in a sitting position. Body composition was analyzed by using Bioelectrical Impedance Analysis (BIA; InBody 720 body composition analysis). The skeletal muscle index (SMI) was calculated by dividing the total appendicular skeletal muscle (ASM), which is the sum of skeletal muscle in the bilateral upper and lower four limbs (kg), by the square of height (= total ASM/height²). The cutoff values for low muscle mass were defined by the hSMI (<6.58 kg/m² for
men and <4.56 kg/m2 for women).[12] In addition, the cutoff values for low muscle mass were defined by the wSMI (<29.1% for men and <23.0% for women).[12]

4. **Estimated hepatic fat and fibrosis formulae.**

Fatty Liver Index (FLI) was calculated descriptive statistic analysis by using this equation: 
\[ \frac{e^y}{1 + e^y} \times 100 \], where 
\[ y = 0.953 \times \ln(\text{Tg [mg/dL]}) + 0.139 \times \text{BMI [kg/m}^2\text{]} + 0.718 \times \ln(\text{GGT [U/L]}) + 0.053 \times \text{WC [cm]} - 15.745.\] [13] And the fibrosis-4 (FIB-4) index was calculated by using this equation: 
\[ \text{age} \times \text{AST [U/L]} / \text{platelet count [x 109/L]} / \text{ALT [U/L]} \]. [6] FIB-4 grades are divided into 0, 1, 2 by using cut off value at 1.3 and 2.67. The NAFLD fibrosis score (NFS) was calculated by using this equation: 
\[ -1.675 + 0.037 \times 3 \text{age [years]} + 0.094 \times \text{BMI [kg/m}^2\text{]} + 1.13 \times \text{impaired fasting glycemia/diabetes mellitus [yes = 1, no = 0]} + 0.99 \times \text{AST/ALT} - 0.013 \times \text{platelet count [x 109/L]} - 0.66 \times \text{Alb [g/dL]}.\] [7] NFS grades are divided into 0, 1, 2 by using cut off value at -1.455 and 0.675. Grade 0,1 and 2 of FIB-4 index and NFS mean advanced fibrosis excluded, needing further examination and advanced fibrosis, respectively. Impaired fasting glucose (IFG) was defined as FBS of 110–125 mg/dL. The presence of diabetes mellitus (DM) was defined as FBS ≥ 126 mg/dL, HbA1c ≥ 6.5 %, or treatment with anti-diabetic drugs.

5. **Measurement of M2BP**

M2BPGi quantification was based on a lectin antibody sandwich immunoassay performed using a fully automatic immune-analyzer (HISCL-2000i; Sysmex Co., Hyogo, Japan). The measured values of WFA1-M2BP conjugated to WFA were indexed with the obtained values using the following equation: Cutoff index \((\text{COI}) = \frac{([\text{WFA}^+\text{-M2BP}]_{\text{PC}} - [\text{WFA}^+\text{-M2BP}]_{\text{NC}})}{([\text{WFA}^+\text{-M2BP}]_{\text{sample}} - [\text{WFA}^+\text{-M2BP}]_{\text{NC}})}\) where \([\text{WFA}^+\text{-M2BP}]_{\text{sample}}\) was the WFA\(^+\)-M2BP count for the serum sample, PC was the positive control, and NC was the negative control. The PC was supplied as a calibration solution preliminarily standardized to yield a COI value of 1.0.

6. **The definition of Metabolic syndrome and NAFLD**

National Cholesterol Education Program’s Adult Treatment Panel III (NCEP-ATP III) criteria were followed, with the exception of abdominal obesity based on waist circumference for the diagnosis of
The waist circumference used was ≥85 for women and ≥90 for men, which are the standard measurement for Korean. Metabolic syndrome was diagnosed when at least three of the following five items were satisfied: (1) waist circumference: ≥85 for women and ≥90 for men (2) triglyceride: ≥150 mg/dL (3) high-density lipoprotein cholesterol: ≤50 mg/dL for women and ≤40 mg/dL for men (4) blood pressure: ≥130/85 mm Hg or taking a hypotensive agent and (5) fasting glucose: ≥100 mg/dL or taking a antidiabetic agents.

NAFLD was defined as people who have a liver fat, whose volume is greater than 5 % of total liver volume, without excessive alcohol consumption, viral or genetic liver disease. The degree of fatty liver was graded as normal, mild, moderate or severe fatty liver on a basis of sonographic hepatorenal index method.

### 7. Statistical analysis

Participants were divided into 2 groups (Abnormal M2BPGi/Normal groups) by using cut off 1.0 (>2SD). Continuous and categorical variables are presented as mean (SD) and number (percent), respectively. For comparing the mean values between the groups, T-test or ANOVA and Mann-Whitney U test or Kruskale Wallis test were used for continuous variables with and without a normal distribution respectively. Categorical variables were compared using chi-square tests. Fisher's Exact test was used for categorical variables, when 20% or more of the cells have an expected frequency of less than 5. Especially, odds ratios (OR) with 95% confidence intervals (CI) for risk factor analysis of high M2BPGi were calculated by using chi-square tests or Fisher's Exact test. For all analyses, p-values less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 26 for Windows (SPSS Inc., Chicago, IL).

### Results

**Characteristics of study population**

A thousand and seventy-three subjects were finally included for analysis, except for 952 subjects with risk factors for liver disease and insufficient data (Figure 1). The baseline characteristics, demographic data of the 1,073 study subjects according to the M2BPGi level and Pearson correlation between M2BPGi and other variables are presented in Table 1. The mean age of the subjects was...
47.11 years, and the proportion of males was 58.3%. The mean value and standard deviation of serum M2BPGi were 0.511 and 0.264, respectively. Upper normal limit (> 2SD) was 1.04 (Figure 2A and B).

**Clinical characteristics of M2BPGi**

Serum M2BPGi level increased, as the subjects were older (p for trend < 0.001) (**Figure 2C**). Age, BMI, blood pressure, cholesterol, triglyceride, fasting blood glucose, HbA1c level, AST and ESR were higher in abnormal M2BPGi group (> 1.0) than normal group and show a positive correlation with M2BPGi (**Table 1**). Interestingly, abnormal M2BPGi group had higher total fat mass and lower ASM. The prevalence of low muscle mass (4.7%, 48/1027 vs 17.4%, 8/46; p 0.002), metabolic syndrome (14.2%, 146/1027 vs 30.4%, 14/46; p 0.003) and hypertension (21.8%, 224/1027 vs 58.7%, 27/46; p <0.001) were higher significantly in abnormal M2BPGi group than control group (**Table 2**).

**Correlation with hepatic steatosis and hepatic fibrosis burden**

Serum M2BPGi level was higher in NAFLD than control group (**Figure 3A**). M2BPGi increased significantly, as the severity of hepatic steatosis and the grade of fibrosis formulae increased (all p for trend < 0.001). Pearson correlation analysis between M2BPGi and FIB-4 (R: 0.293, p <0.001) and NFS (R: 0.248, p <0.001) showed a positive relationship. The prevalence of abnormal M2BPGi (> 1.0 C.O.I) was higher in high cut-off of estimated fibrosis formulae (FIB-4 and NFS) compare to low or intermediated cut-off estimated fibrosis formulae (**Figure 3B and C**). Prevalence of abnormal M2BPGi in low cut-off of FIB-4 was 3%, and 52.9% in high cut-off of FIB-4.

**Clinical implication of M2BPGi in diabetes subjects**

The Pearson coefficients between of M2BPGi and estimated fibrosis formulae (FIB-4 and NFS) higher in diabetes group than general population (**Figure 4A and B**). But, Pearson coefficients between M2BPG1 and estimated hepatic fibrosis burden was similar in correlation coefficient was observed in the NAFLD and MetS groups.

The prevalence of advanced fibrosis (> 2.67 FIB-4) who had abnormal M2BPGi (> 1.0 C.O.I) was 19.6% in general population (**Figure 4C**). Prevalence of advanced fibrosis (> 2.67 FIB-4) who had abnormal M2BPGi (> 1.0 C.O.I) was 50% in diabetes. Sensitivity and specificity of abnormal M2BPGi
(> 1.0 C.O.I) in diabetes patients to predict advanced fibrosis was 50 and 95.6%, respectively. And positive and negative predictive value of M2BPGi in diabetes patients to predict advanced fibrosis was 50 and 95.6%, respectively.

**Discussion**

This data showed reference value of M2BPGi was 0.5 ~ 1.0 C.O.I in the average risk group. Serum M2BPGi showed a positive correlation with metabolic parameters as well as age. While the prevalence of advanced fibrosis in total population was just 1.6% (FIB-4 > 2.65), the prevalence of advanced fibrosis increased to 50% in high M2BPGi group with diabetes. There was high possibility of advanced hepatic fibrosis in subjects with abnormal M2BPGi levels (> 1.0) in diabetes. These results are also consistent with previous results such as, Kang et al’s reports in health check-up settings (1.3% in average risk group, 1.5% in NAFLD, 4.3% in DM) and Loomba et al’s reports in diabetes (7.1%) by using MRE for diagnosis advanced liver fibrosis (≥ F3).[2, 17] The M2BPGi test is seemed to be useful for advanced liver fibrosis screening, even if there are no risk factors for other liver diseases in subjects with impaired fasting glucose or diabetes.

To the best of our knowledge, this is the first study to compare the hepatic fibrosis burden according to underlying conditions, such as NAFLD and diabetes and M2BPGi abnormality in average risk group. Kamada et al also conducted a study in average risk group, but their study was focused only to predict the proportion of suspected advanced hepatic fibrosis patient in healthy cohort by cut-off value, resulted from biopsy proven cohort. However, our study was focused on clinical usage of M2BPGi in average risk group and further analyzed the hepatic fibrosis burden of subjects according to underlying condition and M2BPGi abnormality. As a result, we know patients identified as high M2BPGi group with diabetes in health checkup patients have a relatively high fibrosis burden and we recommend they should be evaluated immediately.

In the European NAFLD guidelines, the screening for insulin resistance, metabolic syndrome or T2DM was emphasized for NAFLD patients.[18] Many previous reports that diabetes strongly associates with NASH including mild and advanced fibrosis.[19, 20] and there are several studies to compare the prevalence of advanced liver fibrosis in patient with NAFLD and/or diabetes by using transient
elastography in primary care or health check up setting. The prevalence was much higher than the average risk group. However, the prevalences were less than 20%. [2, 17, 21, 22] The prevalence of advanced fibrosis by FIB-4 was highest in abnormal M2BPGi group with diabetes and the prevalence increased from 4.4–50% (11.36 times) in abnormal M2BPGi group with diabetes compared to normal M2BPGi group with diabetes and it’s odd ratio was 21.667 (CI: 3.007–156.141, p 0.006). These results indicate that M2BPGi well represents the hepatic fibrosis and subjects in abnormal M2BPGi with diabetes suspected to have higher fibrosis burden than control group. Moreover, the low cost and simple procedure to use a single blood sample make M2BPGi analysis for screening advanced hepatic fibrosis to be more suitable to primary clinical or health check-up setting.

This study has several limitations. Firstly, an estimated fibrosis formula such as FIB-4 and NFS was used to diagnose advanced fibrosis. Gold standard diagnostic tools such as liver biopsy or MRE would have been better for diagnosis for advanced fibrosis. Second, there were fewer abnormal M2BPGi subjects in the entire subject group. Large-scale studies will be needed for a more accurate analysis. Third, further study is needed to compare the performance of M2BPGi with performance of FIB-4 and NFS, which are frequently used for the screening of liver fibrosis, or merged method.

Conclusions
In conclusion, M2BPGi could discriminate high-risk patients with advanced fibrosis from the general population by cut off 1.0 (>2SD). Moreover, when abnormal M2BPGi group was accompanied by diabetes, the prevalence of advanced fibrosis increased up to 50%. Therefore, patients identified as high M2BPGi group with diabetes in health checkup patients have a relatively high fibrosis burden and should be evaluated.

Abbreviations
Nonalcoholic fatty liver disease (NAFLD), Nonalcoholic hepatic steatosis (NASH), Mac2 binding protein glycan isomer (M2BPGi), Fatty liver index (FLI), Fibrosis-4 (FIB-4), NAFLD fibrosis score (NFS), Appendicular skeletal muscle (ASM), weight or height adjusted skeletal muscle index (w/hSMI), European Working Group on Sarcopenia in Older People (EWGSOP), Health-enhancing physical activity (HEPA), Metabolic syndrome (MetS), Diabetes mellitus (DM)

Declarations
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Author's contributions: Dae Won Jun contributed to the study design, Huiyul Park wrote the manuscript, Mimi Kim contributed to general review and correction and Hoon-ki Park supervised manuscript and critical review.

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Availability of data and materials: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate: The Institutional Review Board of Hanyang University Medical Center approved this study protocol (IRB No 2018-12-020). The protocol was also registered at the Clinical Research Information Service. (https://cris.nih.go.kr/cris, Registration No. KCT0004462). Consent to participants was waived, because anticipated harm to participants was not exist or minimal.

Consent for publication: On behalf of co-author we agree with publication

Competing interests: The authors declare that they have no competing interests

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Tables

Table 1. Baseline and serological characteristics of study subjects according to M2BPGi level and correlation between M2BPGi level and other variables.

| Health check-up participants (n=1073) | M2BPGi level (Cut off 1.0) |
|--------------------------------------|----------------------------|
|                                      | Total (n=1073) | Normal n=1027(95.71) | High n=46(4.29) | p-value (Between) |
| Age (year)                           |               |                   |                |                   |
|                                      | 47.11(11.98)  | 45.56(11.53)      | 59.24(15.251)  |                   |
| Sex (Male)                           |               |                   |                |                   |
|                                      | 626(58.3)     | 602(58.6)         | 24(52.2)       |                   |
| Exercise Minimal Activity            |               |                   |                |                   |
|                                      | 353(32.9)     | 322(31.4)         | 31(67.4)       |                   |
|                                      | 431(40.2)     | 423(41.2)         | 8(17.4)        |                   |
| Moderate Activity (IPAQ) HEPA        |               |                   |                |                   |
|                                      | 289(26.9)     | 282(27.5)         | 7(15.2)        |                   |
| Smoking Ex smoker                    |               |                   |                |                   |
| Current smoker                       |               |                   |                |                   |
|                                      | 230(21.4)     | 2217(21.5)        | 9(19.6)        |                   |
|                                      | 151(14.1)     | 141(13.8)         | 10(21.7)       |                   |
| Height (cm)                          |               |                   |                |                   |
|                                      | 166.95(8.57)  | 167.11(8.50)      | 163.37(9.35)   |                   |
| Body weight (kg)                     |               |                   |                |                   |
|                                      | 65.94(12.61)  | 65.90(12.47)      | 66.89(15.45)   |                   |
| BMI (kg/m²)                          |               |                   |                |                   |
|                                      | 23.50(3.37)   | 23.44(3.34)       | 24.83(3.87)    |                   |
| WC (cm)                              |               |                   |                |                   |
|                                      | 78.56(9.47)   | 78.35(9.40)       | 83.26(10.06)   |                   |
| ASM (kg)                             |               |                   |                |                   |
|                                      | 20.39(4.85)   | 20.44(4.83)       | 19.33(5.31)    |                   |
| hSMI (ASM/HT²)                       |               |                   |                |                   |
|                                      | 7.21(1.13)    | 7.22(1.13)        | 7.10(1.22)     |                   |
| wSMI (ASM/BWx 100%)                  |               |                   |                |                   |
|                                      | 30.81(3.74)   | 30.90(3.70)       | 28.79(3.93)    |                   |
| Parameter                      | Value 1 (SD)       | Value 2 (SD)       | Value 3 (SD)       |
|-------------------------------|--------------------|--------------------|--------------------|
| Fat (kg)                      | 17.12(5.97)        | 16.98(5.87)        | 20.02(3.93)        |
| SBP (mmHg)                    | 120.46(17.02)      | 119.92(16.72)      | 132.50(19.38)      |
| DBP (mmHg)                    | 72.38(11.19)       | 72.11(11.11)       | 78.32(11.54)       |
| WBC count (x10^3/ul)          | 5.24(1.37)         | 5.22(1.36)         | 5.66(1.56)         |
| Hb (g/dl)                     | 14.42(1.48)        | 14.43(1.47)        | 14.09(1.50)        |
| Platelet count (x10^4/ul)     | 244.26(51.34)      | 244.74(50.46)      | 233.52(67.88)      |
| Fasting glucose (mg/dl)       | 97.35(15.53)       | 97.04(15.31)       | 104.21(18.70)      |
| HbA1c                         | 5.39(0.56)         | 5.38(0.56)         | 5.67(0.66)         |
| Triglyceride (mg/dl)          | 120.27(86.13)      | 117.98(78.87)      | 171.36(179.27)     |
| Total cholesterol (mg/dl)     | 195.72(35.36)      | 195.50(34.83)      | 200.58(45.82)      |
| LDL Cholesterol (mg/dl)       | 118.81(26.32)      | 117.73(25.99)      | 119.71(33.07)      |
| HDL Cholesterol (mg/dl)       | 56.17(12.34)       | 56.22(12.20)       | 55.19(15.27)       |
| AST (IU/L)                    | 26.39(12.19)       | 26.10(11.45)       | 32.93(22.51)       |
| ALT (IU/L)                    | 25.09(19.05)       | 24.86(18.78)       | 30.23(23.94)       |
| GGT (IU/L)                    | 26.23(26.26)       | 25.67(24.53)       | 38.71(50.34)       |
| ALP (IU/L)                    | 67.48(18.79)       | 67.05(18.43)       | 77.06(23.82)       |
| Total Bil (IU/L)              | 0.9015(0.3587)     | 0.9878(0.3595)     | 0.7620(0.3128)     |
| Direct Bil (IU/L)             | 0.1664(0.0616)     | 0.1675(0.0617)     | 0.1428(0.0555)     |
| Albumin(g/dl)                 | 4.41(0.25)         | 4.42(0.24)         | 4.19(0.36)         |
| NAFLD                         | 602(56.1)          | 570(55.5)          | 32(69.6)           |
| Fatty liver index             | 22.55(22.58)       | 22.01(22.13)       | 24.39(28.83)       |
| FIB-4                         | 1.12(0.54)         | 1.10(0.48)         | 1.79(1.15)         |
| NFS                           | -2.19(1.16)        | -2.24(1.12)        | -1.08(1.48)        |
| M2BPGi(C.O.I.)                | 0.5116(0.2642)     | 0.4744(0.1849)     | 1.3410(0.3862)     |

Data are presented as mean (SD) or number (percent). Mann-Whitney U test was used for continuous variables to compare (HEPA); body weight index (BMI); waist circumference (WC); appendicular skeletal muscle (ASM); weight or height adjusted (SBP/DBP); low/high density lipoprotein (LDL/HDL); aspartate aminotransferase (AST); alanine aminotransferase (ALT); g; (Bil); non-alcoholic fatty liver disease (NAFLD); fibrosis-4 index (FIB-4); NAFLD fibrosis score (NFS); Mac-2 binding protein
Table 2. Odd ratio (95% CI) of main categorical variables for high M2BPGi.

| Health check-up | M2BPGi (Cut off = 1.00) | Odds Ratio (95%CI) | p |
|-----------------|--------------------------|--------------------|---|
|                 | Low n=1027               | High n=46          |    |
| Sex. Male (58.3)| 602(58.6)                | 24(52.2)           | 0.386(0.426-1.392) |
| BMI ≥ 25 (29.5)| 297(28.9)                | 19(41.3)           | 1.730(0.947-3.159) |
| Low muscle mass by hSMI (1.2)| 9(0.9)  | 4(8.7)           | 10.772(3.188-36.399) |
| Low muscle mass by wSMI (5.2)| 48(4.7) | 8(17.4)           | 4.294(1.899-9.707) |
| MetS (14.9)     | 146(14.2)                | 14(30.4)           | 2.640(1.375-5.067) |
| High BP (34.9)  | 343(33.4)                | 31(67.4)           | 4.121(2.195-7.738) |
| Abdominal obesity (13.5)| 131(12.8) | 14(30.4)        | 2.992(1.556-5.756) |
| IFG (32.0)      | 320(31.2)                | 23(50.0)           | 2.209(1.221-3.997) |
| High Tg (22.6)  | 223(21.7)                | 20(43.5)           | 2.773(1.520-5.061) |
| Low HDL (16.3)  | 163(15.9)                | 12(26.1)           | 1.871(0.949-3.689) |
| HTN (23.4)      | 224(21.8)                | 27(58.7)           | 5.094(2.781-9.332) |
| DM (6.9)        | 68(6.6)                  | 6(13.0)            | 2.115(0.866-5.165) |
| NAFLD (56.1)    | 570(55.5)                | 32(69.6)           | 1.833(0.966-3.475) |

Data are presented as number (percent). Chi-square test or Fisher's Exact test was used for comparing each group. If there is a cell, which have expected count less than 5, Fisher's exact test was used. Abbreviation: body weight index (BMI); weight or height adjusted skeletal muscle index (w/h SMI); metabolic syndrome (MetS); Impaired fasting glucose (IFG); triglyceride; high density lipoprotein (HDL); hypertension (HTN); diabetes mellitus (DM); non-alcoholic fatty liver disease (NAFLD)

Figures
Figure 1

Study flow diagram.

Health check up participants (March 2019 ~ June 2019) 
n = 2,025

- Foreigner (n = 339)
- Insufficient blood samples (n = 53)
- Missing data for skeletal muscle (n = 20) or sonography (n=48)
- Missing data for alcohol consumption(n = 28)
- Missing data for M2BPGi level (n = 270)
  * Excluded subjects were counted multiply

Complete data 
n = 1269

- HBsAg or HCV antibody positive (n = 65)
- Alcohol consumption (>210 g for men and >140 g for women (n = 160)
  * Excluded subjects were counted multiply

Average risk population 
n = 1,073

- Normal n = 1,027
- High n = 46

M2BPGi cut off (> 2SD) = 1.04 ÷ 1.0
Figure 2

Histogram (a) and Box plot (b) of M2BPGi in average risk population. (n = 1073) (c) The graph of M2BPGi level of study subjects according to age.

**Descriptive statistics**

Total : 1073  
Mean : 0.5116  
SD : 0.2642  
Hi-M2BPGi (>2sd) : Cut off : 1.04 - > 1.0  
Normality : Kolmogorov-smirnov p-values <0.001
Box plot of M2BPGi versus hepatic steatosis status (a), FIB-4 (b) and NFS grade (c) Kruskale Wallis tests. was used for comparing each group. Fibrosis scoring systems such as FIB-4 and NFS are graded on a scale 0, 1, 2. Grade 0,1 and 2 mean advanced fibrosis excluded, needing further examination and advanced fibrosis, respectively.
The scatter plot of M2BPGi level versus (a) FIB-4 or (b) NFS for health check-up, NAFLD, metabolic syndrome or DM groups. Pearson correlation coefficients and p-values are presented in the graph. The prevalence of advanced fibrosis (FIB-4 index > 2.67) in subjects with and without NAFLD (a), MetS (b) or DM. Normal group (N) and High M2BPGi group (H) were divided at the cut-off value of 1.0. T indicate total subjects. Chi-square test or Fisher's Exact test was used for categorical variables to compare between groups.