Polymorphism of the ITGAM gene (rs7193943) and bioelectric impedance analysis as potential predictors of cachexia in chronic heart failure

Grzegorz Sobieszek1, Radosław Mlak2, Tomasz Powrózek2, Marcin Mazurek2, Aneta Skwarek-Dziekanowska1, Piotr Terlecki3 & Teresa Małecka-Massalska2

Cardiac cachexia (CC) is an unfavorable metabolic syndrome leading to exacerbation of chronic heart failure (CHF) and a higher risk of death. The main factor contributing to the development of cachexia is the ongoing inflammatory process mediated by genes (e.g. Integrin Subunit Alpha M—ITGAM). The study aimed to assess the relationship between a single nucleotide polymorphism (SNP) -323G > A of the ITGAM and the occurrence of nutritional disorders in patients with CHF. 157 CHF patients underwent clinical and nutritional screening. Body composition was evaluated by bioelectrical impedance analysis (BIA). Patients with cachexia were characterized by significantly lower weight, body mass index (BMI), lower fat mass (FM), albumin, and hemoglobin. Lower values of BIA parameters: capacitance of membrane (Cm), phase angle (PA), and impedance ratio (Z200/Z5) were noted in women. Those patients demonstrated significantly higher values of creatinine, c-reactive protein (CRP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), and pulmonary artery systolic pressure (PASP). A significantly higher risk of cachexia was reported in patients: aged ≥ 74 years (OR 3.55), with renal failure (OR 3.75), New York Heart Association classification (NYHA) III-IV (OR 2.83), with moderate or severe malnutrition according to the score of subjective global assessment (SGA) (OR 19.01) and AA genotype of ITGAM gene (OR 2.03). Determination of the -323G > A SNP in the ITGAM may prove to be a useful marker (after confirmation in further studies and appropriate validation) in the assessment of the risk of nutritional disorders in patients with CHF.

The incidence of chronic heart failure (CHF) in adults is growing in highly developed countries. The factors associated with the growing number of newly diagnosed cases of CHF are: age (> 70 years) and concomitant diastolic heart failure. As a result of metabolic, anabolic and catabolic disorders associated with CHF, a large number of patients with suspected CHF are diagnosed with left ventricular dysfunction. Patients with CHF are also often diagnosed with cardiac cachexia (CC). Cachexia is defined as ≥ 5% weight loss over 3–12 months. It is typically accompanied by characteristic symptoms. These symptoms include fatigue, decrease of muscle strength and mass, anorexia, anemia, low albumin level and severe inflammation. The inflammatory process triggers CC pathogenesis and the progression of cachexia. The mortality rate in patients with CC is 20–30% per year. The mechanism of cachexia progression in patients with CHF, in whom the incidence of CC ranges from 8 to 42%, is associated with reduced myocardial perfusion, depletion of high energy stores, cardiomyocyte decline and accumulation of water and lactates in cardiac tissue, accompanied by inflammatory response. The inflammatory process is mediated by genes, including Integrin Subunit Alpha M—ITGAM gene (also known as CD11b, Mac-1 integrin alpha chain or complement receptor 3), located on the 16p11.2 chromosome. Its protein product influences interferon gamma (INF-γ) receptor functioning and inflammatory mediator.
secretion regulation. In the case of low ITGAM expression on the surface of antigen presenting cells (APCs), there occurs an increase in the production of interleukin (IL), including IL-6 and IL-17. In patients with systemic lupus erythematosus (SLE), studies have demonstrated the effect of rs114367 single nucleotide polymorphism (SNP) on the level of both the ITGAM transcript and monocyte surface protein. The level of mRNA was dependent on the genotype (and the protein amount was proportional to the transcript level). Over twofold decrease in the level of protein was observed in patients with AA genotype (higher risk patients) compared to GG genotype (lower risk group). These differences in the protein expression may be the result of the allele-specific decrease in transcription repression. However, as far as we know, no studies have been performed regarding the correlation between the occurrence of nutritional disorders in patients with CHF and the ITGAM gene status. In this study, we have aimed at the assessment of the relationship between ITGAM gene regulatory region (−323G>A) SNPs and the incidence of nutritional disorders in cachectic patients with CHF.

Materials and methods

Study group. This study included 157 patients (mean age: 69.5 ± 14 years) with the diagnosis of CHF. Enrolled patients were diagnosed and treated at the Clinic of Cardiology and Internal Medicine, Department of Cardiology, Military Hospital in Lublin, Poland in the period of 2013 and 2014. Patients were enrolled in the study consecutively (all newly diagnosed patients with CHF admitted to the cardiology department fulfilling the inclusion and exclusion criteria, respectively were considered). Diagnosis of CHF was made on the latest clinical guidelines issued by European Society of Cardiology (ECS), and the applied criteria involve patients’ clinical screening, echocardiographic assessment (ejection fraction—EF%; left ventricular end-diastolic and end-systolic diameters—LVEDd and LVEpSd; left atrial diameter—LAD; tricuspid annular plane systolic excursion—TAPSE; right ventricular outflow tract—RVOT) followed by laboratory tests (serum concentration of c-reactive protein—CRP, N-terminal prohormone of brain natriuretic peptide—NT-proBNP, lipid profile, creatinine and hemoglobin concentration). New York Heart Association (NYHA) functional classification was used to assess extent of the disease by qualifying patients to group I–IV according to the disease severity. The inclusion and exclusion criteria for the study participants were defined, as follows: the inclusion criteria—(a) age > 18 years and polish ethnicity; (b) newly diagnosed CHF; (c) signed informed consent to participate in the study; (d) stable patients without rapid changes in body weight and diuretic treatment for a minimum period of 10 days, and the exclusion criteria—(a) extreme renal failure (according to National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) criteria—glomerular filtration rate (eGFR) below 15 mL/min or dialysis); (b) acute coronary syndrome; (c) presence of metallic implants, pacemakers, implanted cardioverter defibrillator or cardiac resynchronization systems; (d) hyper- or hypothyroidism; (e) recent coronary artery bypass grafting (within last 6 months); (f) an active neoplastic process; (g) amputation in the upper or lower limbs. In terms of smoking, the patients were classified as non-smokers, ex-smokers and current smokers. Non-smokers are those who have never smoked or have smoked fewer than 100 cigarettes in their lifetime. Ex-smokers are people who, at the time of the interview, declared that they did not smoke, but had smoked over 100 cigarettes during their lives. The current smoker is what he was smoking at the time of the interview. The patients were followed up for 60 months (5 years) from January 2015 (baseline) until the January 2020. Baseline characteristic of the study group is presented in Table 1. Bioethical Commission in Medical University of Lublin approved the study protocol (no of consent: KE-0234/64/2017).

Nutritional assessment and cachexia detection. Cachexia was diagnosed according to the criteria of Evans et al., as follows: a weight loss ≥ 5% or more in 12 months or less in the presence of underlying illness, plus three of the following criteria: decreased muscle strength, fatigue, anorexia, low fat-free mass index, abnormal biochemistry (increased inflammatory markers: CRP > 5.0 mg/L), anemia (hemoglobin < 12 g/dL) and low concentration of serum albumin (< 3.2 g/dL). The time points were 15 months at which we measured the parameters. Patients enrolled in this study met the above criteria for cachexia.

Anthropometric measurements (body mass index—BMI, body weight) were taken from all patients and they were also asked to fill in the subjective global assessment (SGA) questionnaire. The bioelectrical impedance analysis (BIA) was used to establish the parameters reflecting the nutritional status and body composition, including: fat mass (FM) and fat-free mass (FFM). The phase angle (PA) (at 50 kHz frequency) and capacitance of membrane (Cm) values were also established using BIA. All study participants had similar conditions of BIA measurements. The measurements were performed in patients lying on the bed in the supine position (patients’ extremities did not come into contact with the torso or each other). The patients rested for at least 5 min before BIA to allow for the equalization of the body fluid level. The body composition parameters were measured using ImpediMed bioimpedance analysis SFB7 Biolmp v1.55 device (Pinkenba, QLD, Australia).

Prior to the study, peripheral blood was collected from each study subject in the amount of 5 mL. Before further laboratory analysis, the samples were stored at – 80 °C. The isolation of DNA was conducted using the column method with a dedicated kit according to the manufacturer’s recommendations (DNA Blood Mini Kit, Qiagen, Canada). The concentration and quality of the obtained DNA was spectrophotometrically evaluated using NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, USA). The Real-Time polymerase chain reaction (PCR) technique was used for genotyping reaction on StepOnePlus device (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer’s instructions (using Genotyping Master Mix and TaqMan probes specific for ITGAM SNP: rs7193943) (Thermo Fisher Scientific, USA). The above methods were also described in detail in our previous studies.

Statistical analysis. Available data were statistically analyzed with the MedCalc 15.8 program (MedCalc Software, Belgium). Results p < 0.05 were considered statistically significant. Comparisons of the values of inde-
dependent continuous variables were performed using the Mann–Whitney U test (comparison of 2 groups) or ANOVA Kruskal–Wallis test (comparison of more than 2 groups). All selected continuous variables had distribution different than the normal—it was verified by the D’Agostino-Pearson test). To assess the risk cachexia depending on selected demographic, clinical, and genetic factors the odds ratio test (OR) with 95% Confidence Interval (95% CI) (univariate analysis) and logistic regression analysis (multivariate analysis, the model took into account factors significant from the univariate analysis) was used.

**Ethical approval.** All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved of Bioethical Commission in Medical University of Lublin approved the study protocol (no of consent: KE-0254/64/2017).

**Results**

**Patients characteristics.** The study group consisted of 157 patients diagnosed with CHF. The study group was dominated by men (58.6%), patients with CHF degree II (29%) and III (25.8%) (according to NYHA). The patient median age was 74 years (range: 27–109 years). Patients were mostly well-nourished (SGA A: 45.5%) or in moderate malnutrition (SGA B: 44.9%). Median BMI was 29.3 (range 18.7–53.2). Most of the patients did not

### Table 1. Characteristics of the study group.

| Variable                        | Study group (n=157) |
|---------------------------------|--------------------|
| Sex                             |                   |
| Men                             | 92 (58.6%)         |
| Women                           | 65 (41.4%)         |
| NYHA (missing data: n = 2)      |                   |
| I                               | 35 (22.6%)         |
| II                              | 45 (29.0%)         |
| III                             | 40 (25.8%)         |
| IV                              | 35 (22.6%)         |
| SGA (missing data: n = 1)       |                   |
| A                               | 71 (45.5%)         |
| B                               | 70 (44.9%)         |
| C                               | 15 (9.6%)          |
| Diabetes mellitus (missing data: n = 1) |       |
| Yes                             | 62 (39.7%)         |
| No                              | 94 (60.3%)         |
| Renal failure (missing data: n = 1) |                  |
| Yes                             | 59 (37.8%)         |
| No                              | 97 (62.2%)         |
| Smoking (missing data: n = 1)   |                   |
| Current smoker                  | 67 (42.9%)         |
| Ex-smoker                       | 34 (21.8%)         |
| Non-smoker                      | 55 (35.3%)         |
| Continuous variables            | Mean ± SD; median (range) |
| Age (years)                     | 72.6 ± 13.4; 74.0 (27.0–109.0) |
| Weight (kg)                     | 82.0 ± 19.0; 80 (42.0–162.0) |
| BMI (kg/m²)                     | 29.3 ± 6.1; 29.3 (18.7–53.2) |
| Albumin (g/dL)                  | 3.4 ± 0.6; 3.5 (1.4–4.8) |
| Triglycerides (mg/dL)           | 110.5 ± 63.2; 90.4 (33.0–369.0) |
| Total cholesterol (mg/dL)       | 156.1 ± 43.1; 153.5 (60.0–333.0) |
| HDL (mg/dL)                     | 49.8 ± 17.5; 49.8 (7.0–110.0) |
| LDL (mg/dL)                     | 85.2 ± 34.2; 82.0 (9.8–217.0) |
| Creatinine (mg/dL)              | 1.3 ± 0.5; 1.1 (0.6–3.7) |
| Hemoglobin (g/dL)               | 13.0 ± 2.2; 13.2 (8.8–19.1) |
| CRP (mg/L)                      | 16.8 ± 24.2; 6.0 (0.0–16.8) |
| Systolic blood pressure (mmHg)  | 131.4 ± 23.3; 130.0 (80.0–200.0) |
| Diastolic blood pressure (mmHg) | 75.6 ± 13.6; 75.0 (40.0–130.0) |
| EF%                             | 44.0 ± 14.6; 47.7 (0.0–58.6) |
| NT-proBNP (pg/mL)               | 4809.1 ± 7964.4; 2788.0 (138.0–84,919.0) |
| LVESe (cm)                      | 4.4 ± 1.0; 4.2 (2.2–7.0) |
| LVESe (cm)                      | 5.6 ± 2.4; 5.4 (3.4–33.0) |
| LAD (cm)                        | 4.5 ± 0.6; 4.5 (3.0–6.1) |
| RVOT (cm)                       | 3.5 ± 0.5; 3.4 (2.3–6.1) |
| TAPSE (cm)                      | 2.1 ± 2.5; 1.9 (0.7–25.0) |
| PASP (mmHg)                      | 40.9 ± 12.7; 40.0 (3.4–80.0) |
have diabetes (39.7%) or renal failure (37.8%). Patients were mostly current smokers (42.9%). Patients classified as non-smokers were in the minority (35.3%). The remaining patients were ex-smokers (21.8%). Detailed data on demographic-clinical and laboratory variables are presented in Table 1.

Relationships between demographic and clinical factors and ITGAM gene genotypes. In carriers of the GG genotype of the ITGAM gene, compared to other variants of studied SNP (AA or GA, respectively), significantly higher values of weight (94 vs 80 or 81 kg), low-density lipoprotein (LDL) (107.7 vs 83.3 or 71.6 mg/dL) and diastolic blood pressure (90 vs 72.5 or 70 mmHg) were found. In addition, a significantly lower incidence of diabetes mellitus was observed in these patients (7% vs. 64.9% or 28.1%). Detailed data of the comparison of selected variables depending on the occurrence of the ITGAM genotypes were presented in Table 2.

Comparison of selected variables depending on the occurrence of cachexia. Patients with cachexia were characterized by significantly older age (78.5 vs 68.5 years), obviously lower weight and BMI, as well as lower FM (22.9 vs 26.4 kg), albumin (3 vs 3.7 g/dL) and hemoglobin values (12.5 vs 13.8 g/dL), moreover, lower but only in women values of parameters derived from the BIA: Cm (0.7 vs 1.3 nF), PA (2.8 vs 4.2) and impedance ratio—Z200/Z5 (1.1 vs 1.2). On the other hand, in those patients significantly higher values of creatinine (1.2 vs. 1.1 mg/dL), CRP (14.2 vs. 3.6 mg/dL), NT-proBNP (4255.5 vs. 1806 pg/mL) and pulmonary artery systolic pressure—PASP (45 vs. 35 mmHg) were observed (Table 3).

Risk of the cachexia depending on selected demographic, clinical and genetic factors. A significantly higher risk of cachexia was reported in elderly patients (≥74: OR 3.55), people with renal failure (OR 3.75), with a higher degree of CHF according to NYHA (III-IV: 2.83), with moderate or severe malnutrition according to SGA (B or C: OR 19.01) and AA genotype of ITGAM gene (2.03) (Table 4).

Discussion

CHF is becoming an ever more prevalent public health issue. The incidence of CHF increases with age, so taking into account the problem of the aging society, the prevalence of CHF is likely to grow in the future largely due to such age-related risk factors as hypertension or chronic inflammation. The inflammatory process taking place in CHF patient is responsible for the nutritional disorders that eventually result in chronic cachexia.

The mechanism of the development of nutritional disorders is significantly affected by the development of inflammation15–18. Subsequently, there occurs an increase in the production of the inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), INF-γ, IL-6 and IL-1β. They constitute mediators with confirmed involvement in the mechanisms leading to muscle fiber breakdown in the Ubiquitin–Proteasome Pathway (UPP) through the activation of the muscle ring-finger 1 (MuRF1) and muscle atrophy F box (MAFbx) ligase genes. It has also been confirmed that the inflammatory process is important in the adipose tissue breakdown. The increase in the number of apoptotic fat cells and decrease in the rate of lipogenesis is associated with increased production of TNF-α. It reduces the glucose and free fatty acid (FFA) transport into cells by interacting with IL-1, which is associated with decreased adipose tissue synthesis. The catabolism of both adipose and muscle tissue also takes place with IL-6 involvement. It may be of great importance for the assessment of the nutritional status and further prognosis to determine the level of this IL6.7

CC is associated with skeletal myopathy due to genetic changes in a few key regulatory pathways19. ITGAM gene is of key importance in the activation, migration and adhesion of leukocytes, its main task being the coding of the αMβ2-integrin chain. B-cell receptors, and Fcγ receptors are regulated with the involvement of CD11b. It is an essential molecule in the activation of the αMβ2-integrin chain. B-cell signaling and the function of INF-γ receptors, Toll-like receptors (TLR), and in the subpopulation of macrophages, monocytes (p = 0.001) and polymorphonuclear cells (p = 0.002). An influence was demonstrated between the advancement of CHF (NYHA) and CD11b expression on the surface
of monocytes, macrophages and polymorphonuclear cells (p = 0.001). The increase in the level of this integrin on the surface of these cells is used as a marker of cellular activity23. In our study a significantly higher risk of cachexia was reported in elderly patients (≥ 74: OR 3.55), people with renal failure (OR 3.75), with a higher degree of CHF according to NYHA (III-IV: OR 2.83), with moderate or severe malnutrition according to SGA (B or C: OR 19.01) and AA genotype of ITGAM gene (OR 2.03). In that aspect AA genotype of ITGAM gene may be a

Table 2. Comparison of selected variables depending on the occurrence of the ITGAM genotypes.

| Variable                        | ITGAM genotypes | Median (interquartile range) | (missing data: n = 17) | p     |
|---------------------------------|-----------------|-------------------------------|------------------------|-------|
|                                 | AA (1) (n = 72) | GA (2) (n = 60)               | GG (3) (n = 8)         |       |
| Age (years)                     | 77.0 (39.0–109.0)| 73.0 (27.0–95.0)              | 67.5 (49.0–82.0)       | 0.153 |
| Weight (kg)                     | 80.0 (48.0–162.0)| 81.0 (42.0–150.0)             | 94.0 (75.0–130.0)      | 0.047* 2 vs 3, 1 vs 3 |
| BMI (kg/m²)                     | 28.3 (19.5–53.2) | 27.7 (18.7–46.9)              | 33.6 (23.0–37.2)       | 0.191 |
| FM (kg)                         | 24.9 (2.0–68.6)  | 22.9 (0.0–65.6)               | 32.8 (21.9–43.2)       | 0.339 |
| FFM (kg)                        | 50.8 (0.0–93.3)  | 52.7 (0.0–85.1)               | 57.1 (38.1–65.8)       | 0.090 |
| Albumin (g/dL)                  | 3.4 (1.4–4.6)    | 3.6 (1.9–4.6)                 | 3.6 (2.4–4.0)          | 0.311 |
| Triglycerides (mg/dL)           | 91.8 (40.0–349.0)| 84.0 (37.0–369.0)             | 165.0 (73.9–241.0)     | 0.065 |
| Total cholesterol (mg/dL)       | 159.0 (81.0–261.0)| 150.0 (60.0–333.0)            | 181.0 (95.0–235.0)     | 0.173 |
| HDL (mg/dL)                     | 51.1 (7.0–87.0)  | 49.0 (9.1–110.0)              | 49.0 (11.0–65.4)       | 0.404 |
| LDL (mg/dL)                     | 83.3 (26.0–178.0)| 71.6 (9.8–217.0)              | 107.7 (35.6–149.0)     | 0.020* 1 vs 2 |
| Creatinine (mg/dL)              | 1.1 (0.6–2.8)    | 1.1 (0.7–2.7)                 | 1.2 (0.7–3.7)          | 0.743 |
| Hemoglobin (g/dL)               | 13.0 (8.0–18.1)  | 12.9 (8.8–19.1)               | 13.6 (11.9–16.2)       | 0.194 |
| CRP (mg/L)                      | 4.8 (1.1–123.0)  | 8.3 (0.0–117.8)               | 3.2 (0.6–56.3)         | 0.256 |
| Systolic blood pressure (mmHg)  | 130.0 (80.0–200.0)| 130.0 (90.0–200.0)            | 165.0 (115.0–180.0)    | 0.074 |
| Diastolic blood pressure (mmHg) | 72.5 (40.0–100.0)| 70.0 (45.0–100.0)             | 90.0 (70.0–130.0)      | 0.026* 2 vs 3, 1 vs 3 |
| EF%                             | 41.5 (15.0–65.0) | 45.0 (10.0–65.0)              | 45.0 (20.0–60.0)       | 0.171 |
| NT-proBNP (pg/mL)               | 3073.5 (138.0–84919.0)| 2501.0 (285.0–22534.0)       | 3039.5 (639.1–18628.0)| 0.830 |
| LVESEd (cm)                     | 4.2 (2.7–6.8)    | 4.0 (2.2–7.0)                 | 4.8 (3.1–7.0)          | 0.137 |
| LVEDd (cm)                      | 5.3 (3.5–7.5)    | 5.2 (3.4–33.0)                | 5.6 (4.8–7.8)          | 0.135 |
| LAD (cm)                        | 4.5 (3.0–6.1)    | 4.4 (3.3–5.5)                 | 4.9 (4.0–5.7)          | 0.149 |
| RVOT (cm)                       | 3.4 (2.3–4.6)    | 3.3 (2.6–5.0)                 | 3.6 (3.0–6.1)          | 0.135 |
| TAPSE (cm)                      | 1.9 (0.7–3.0)    | 1.9 (0.9–9.0)                 | 1.9 (1.4–16.0)         | 0.800 |
| PASP (mmHg)                     | 40.0 (3.4–80.0)  | 40.0 (10.0–74.0)              | 36.0 (22.0–55.0)       | 0.551 |
| NYHA (missing data: n = 2)      |                |                               |                        | 0.784 |
| I                               | 14 (46.7%)       | 14 (46.7%)                    | 2 (6.6%)               |       |
| II                              | 19 (50.0%)       | 17 (44.7%)                    | 2 (5.3%)               |       |
| III                             | 23 (62.2%)       | 13 (35.1%)                    | 1 (2.7%)               |       |
| IV                              | 15 (45.5%)       | 15 (45.5%)                    | 3 (9.0%)               |       |
| SGA (missing data: n = 1)       |                |                               |                        | 0.102 |
| A                               | 27 (43.5%)       | 29 (46.8%)                    | 6 (9.7%)               |       |
| B                               | 34 (54.0%)       | 27 (42.9%)                    | 2 (3.1%)               |       |
| C                               | 11 (78.6%)       | 3 (21.4%)                     | 0 (0.0%)               |       |
| Diabetes mellitus (missing data: n = 1) | 37 (64.9%) | 16 (28.1%) | 4 (7.0%) | 0.017* |
| Renal failure (missing data: n = 1) | 31 (59.6%) | 17 (32.7%) | 4 (7.7%) | 0.184 |
| Smoking (missing data: n = 1)   |                |                               |                        | 0.870 |
| Current smoker                  | 30 (52.6%)       | 24 (42.1%)                    | 3 (5.3%)               |       |
| Ex-smoker                       | 13 (43.3%)       | 15 (50%)                      | 2 (6.7%)               |       |
| Non-smoker                      | 29 (55.8%)       | 20 (38.5%)                    | 3 (5.7%)               |       |
| Cm (nF)                         |                |                               |                        | 0.753 |
| Men                             | 1.4 (0.5–3.6)    | 1.0 (0.4–3.6)                 | 1.1 (0.8–2.0)          | 0.377 |
| Women                           | 1.2 (0.0–2.7)    | 0.9 (0.0–3.6)                 | 0.9 (0.9–9.0)          |       |
| Pa (*)                          |                |                               |                        | 0.227 |
| Men                             | 3.2 (1.9–9.6)    | 3.8 (1.5–6.6)                 | 4.8 (4.1–5.1)          |       |
| Women                           | 3.6 (0.0–6.7)    | 4.0 (0.0–6.6)                 | 3.5 (3.5–3.5)          |       |
| Z200/Z5                         |                |                               |                        | 0.972 |
| Men                             | 1.1 (1.1–1.4)    | 1.2 (1.1–1.3)                 | 1.2 (1.2–1.2)          | 0.371 |
| Women                           | 1.2 (1.1–1.3)    | 1.2 (1.1–1.3)                 | 1.2 (1.1–1.2)          | 0.671 |

*Statistically significant results.
useful marker assessing the risk of nutritional disorders in patients with CHF. However, these results cannot be compared with any others as there are none.

It is known that one of the main causes of left ventricular dysfunction and CHF is acute coronary syndrome (AMI) in the form of ST-elevation myocardial infarction (STEMI). In one of the studies, a significantly increased expression of Bone Marrow Stromal Cell Antigen 1—BST1 (sensitivity 77.7778%, specificity 75%) and ITGAM (sensitivity 100%, specificity 75%) was found already in the acute phase of STEMI in which CHF developed at 6 months of follow-up. It was a factor that differentiated patients with post-STEMI CHF from those without CHF. This makes it possible to use new tools for the early prognosis of patients with post-STEMI CHF and the search for genetic potentiality to the evolution of CHF and LV remodeling after STEMI24.

The studied SNP’s presence in the regulatory region of the ITGAM gene may significantly impact the expression of the gene and, hence, specific protein production. This may lead to the regulation of the production of pro-inflammatory cytokines such as IL-6. Thus, the chronic inflammatory process occurring due to the increased cytokine production, which is characteristic of patients with CHF, may result in the development of nutritional disorders and lead to irreversible conditions like cachexia. Further studies aimed at finding new markers of inflammation involved in the occurrence of malnutrition leading to CC, as well as explaining their mechanisms of action, may facilitate more efficient diagnosis of nutritional disorders, faster implementation of nutritional treatment and the development of new therapies.

Our study has certain limitations, including no assessment of the influence of the diet or of the food intake problems. What is more, we have not investigated the impact of individual genotypes on the expression of the studied gene (and the protein encoded by it). Despite the above limitations, this is, as far as we know, the first study demonstrating that the evaluation of ITGAM SNP (−323G > A) may, after confirmation of its value in further studies, constitute a useful marker in assessing the risk of occurrence of nutritional disorders in CHF patients.

| Variable                        | Study group (n = 157) Median (interquartile range) | Cachectic (n = 74) | Non-cachectic (n = 83) | p      |
|---------------------------------|---------------------------------------------------|--------------------|------------------------|--------|
| Age (years)                     | 78.5 (70.0–86.0)                                  | 68.5 (59.0–78.0)   | < 0.0001*              |        |
| Weight (kg)                     | 75.5 (64.8–83.0)                                  | 83.0 (75.0–95.0)   | 0.0001*                |        |
| BMI (kg/m²)                     | 26.9 (24.2–30.1)                                  | 30.0 (26.4–33.5)   | 0.003*                 |        |
| FM (kg)                         | 22.9 (9.4–34.6)                                   | 26.4 (19.5–32.9)   | 0.047*                 |        |
| FFM (kg)                        | 53.6 (39.2–62.8)                                  | 56.4 (41.8–59.1)   | 0.901                  |        |
| Albumin (g/dL)                  | 3.0 (2.7–3.4)                                     | 3.7 (3.5–4.0)      | < 0.0001*              |        |
| Triglycerides (mg/dL)           | 91.7 (69.0–130.0)                                 | 90.5 (68.0–143.0)  | 0.815                  |        |
| Total cholesterol (mg/dL)       | 148.2 (118.0–176.0)                               | 162.0 (128.0–183.0)| 0.258                  |        |
| HDL (mg/dL)                     | 45.0 (37.0–60.0)                                  | 49.5 (37.0–63.0)   | 0.414                  |        |
| LDL (mg/dL)                     | 81.0 (61.8–97.9)                                  | 82.8 (61.6–105.4)  | 0.571                  |        |
| Creatinine (mg/dL)              | 1.2 (1.0–1.5)                                     | 1.1 (0.9–1.3)      | 0.042*                 |        |
| Hemoglobin (g/dL)               | 12.5 (11.0–14.0)                                  | 13.8 (12.1–14.6)   | 0.0020*                |        |
| CRP (mg/L)                      | 14.2 (5.1–33.5)                                   | 3.6 (1.6–8.4)      | < 0.0001*              |        |
| Systolic blood pressure (mmHg)  | 130.0 (110.0–140.0)                               | 130.0 (120.0–150.0)| 0.408                  |        |
| Diastolic blood pressure (mmHg) | 130.0 (70.0–85.0)                                 | 75.0 (70.0–85.0)   | 0.832                  |        |
| EF%                             | 40.0 (25.0–52.7)                                  | 45.0 (30.0–55.0)   | 0.184                  |        |
| NT-proBNP (pg/mL)               | 4255.5 (1927.0–8015.0)                            | 1806.0 (1018.0–3333.0) | < 0.0001* |
| LVESd (cm)                      | 4.0 (3.6–5.2)                                     | 4.3 (3.8–5.0)      | 0.435                  |        |
| LVEDd (cm)                      | 5.3 (4.5–6.2)                                     | 5.5 (4.8–6.1)      | 0.379                  |        |
| LAD (cm)                        | 4.6 (4.0–5.0)                                     | 4.5 (4.0–5.0)      | 0.365                  |        |
| RVOT (cm)                       | 3.5 (3.1–3.8)                                     | 3.4 (3.1–3.8)      | 0.765                  |        |
| TAPSE (cm)                      | 1.8 (1.4–2.0)                                     | 1.9 (1.6–2.1)      | 0.109                  |        |
| PASP (mmHg)                     | 45.0 (35.0–50.5)                                  | 35.0 (30.0–45.0)   | 0.003*                 |        |
| Cm (nF)                         | Men 1.2 (0.8–1.6)                                 | 1.4 (0.7–2.0)      | 0.687                  |        |
| PA (*)                          | Women 0.7 (0.0–1.3)                               | 1.3 (0.9–1.8)      | 0.009*                 |        |
| Z200/Z5                         | Men 1.2 (1.1–1.2)                                 | 1.2 (1.1–1.2)      | 0.169                  |        |
|                                 | Women 1.1 (1.1–1.2)                               | 1.2 (1.1–1.2)      | 0.015*                 |        |

Table 3. Comparison of selected variables depending on the occurrence of cachexia. *Statistically significant results.
Conclusion
Until now, there are lack of established criteria or clinical guidelines allowing detection and management of CC in CHF patients. However, assessment of genetic markers, such as ITGAM (-323G > A) can serve (after appropriate validation) as a putative promising marker for nutritional disorders screening in CHF patients, because of its correlation with patients' clinical and nutritional features. Prospective clinical utility of the proposed diagnostic marker requires confirmation in large cohort studies.

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| Variable                  | Study group (n = 157) |          |          |          |
|---------------------------|-----------------------|----------|----------|----------|
|                           | Univariate            | Multivariate* |
|                           | Cachectic (n = 74)    | Non-cachectic (n = 83) | OR (95% CI) | p        | OR (95% CI) | p        |
| Sex                       |                       |          |          |          |
| Men                       | 40 (43.5%)            | 52 (56.5%) | 0.70 (0.37–1.33) | 0.36 (0.12–1.12) |
| Women                     | 34 (52.3%)            | 31 (47.7%) | 0.276    | 0.077    |
| Age                       |                       |          |          |          |
| ≥74                       | 51 (63%)              | 30 (37%)  | 3.92 (2.01–7.62) | 3.55 (1.63–7.76) |
| <74                       | 23 (30.3%)            | 53 (69.7%) | 0.001*   | 0.0015*  |
| Smoking                   |                       |          |          |          |
| Smoker (current or ex)    | 46 (45.5%)            | 55 (54.5%) | 0.81 (0.42–1.56) | 0.87 (0.44–1.70) |
| Non-smoker (missing data: n = 1) | 28 (50.9%)        | 27 (49.1%) | 0.522    | 0.454    |
| Diabetes mellitus         |                       |          |          |          |
| Yes                       | 33 (53.2%)            | 29 (46.8%) | 1.47 (0.77–2.80) | 1.01 (0.41–2.46) |
| No (missing data: n = 1)  | 41 (43.6%)            | 53 (56.4%) | 0.2403   | 0.9849   |
| Renal failure             |                       |          |          |          |
| Yes                       | 38 (64.4%)            | 21 (35.6%) | 3.07 (1.56–6.01) | 3.75 (1.52–9.26) |
| No (missing data: n = 1)  | 36 (37.1%)            | 61 (62.9%) | 0.0011*  | 0.004*   |
| NYHA                      |                       |          |          |          |
| II–IV                     | 58 (48.3%)            | 62 (51.7%) | 1.24 (0.58–2.66) | 1.37 (0.40–4.54) |
| I (missing data: n = 2)   | 15 (42.9%)            | 20 (57.1%) | 0.568    | 0.615    |
| NYHA                      |                       |          |          |          |
| III–IV                    | 46 (61.3%)            | 29 (38.7%) | 3.05 (1.58–5.89) | 2.83 (1.04–7.73) |
| I–II                      | 27 (34.2%)            | 52 (65.8%) | 0.001*   | 0.042*   |
| NYHA                      |                       |          |          |          |
| IV                        | 24 (68.6%)            | 11 (31.4%) | 3.16 (1.42–7.04) | 1.63 (0.47–5.58) |
| I–III (missing data: n = 2) | 49 (40.8%)         | 71 (59.2%) | 0.005*   | 0.437    |
| SGA                       |                       |          |          |          |
| B and C                   | 63 (75%)              | 21 (25%)  | 16.36 (7.27–36.81) | 19.01 (7.51–48.11) |
| A (missing data: n = 1)   | 11 (15.5%)            | 60 (84.5%) | < 0.0001* | < 0.0001* |
| SGA                       |                       |          |          |          |
| C                         | 14 (93.3%)            | 1 (6.7%)  | 18.90 (2.42–147.71) | 2.90 (0.31–26.96) |
| A and B (missing data: n = 1) | 60 (42.6%)        | 81 (57.4%) | 0.005*   | 0.350    |
| ITGAM                     |                       |          |          |          |
| AA                        | 42 (58.3%)            | 30 (41.7%) | 2.13 (1.08–4.17) | 2.03 (1.01–4.06) |
| GA and GG (missing data: n = 17) | 27 (39.7%)      | 41 (60.7%) | 0.029*   | 0.048*   |
| ITGAM                     |                       |          |          |          |
| GG                        | 2 (25%)               | 6 (75%)   | 0.32 (0.06–1.66) | 0.35 (0.04–2.63) |
| AA and GA (missing data: n = 17) | 67 (50.8%)        | 65 (49.2%) | 0.176    | 0.306    |

Table 4. Risk of the cachexia depending on selected demographic, clinical and genetic factors. *Statistically significant results. †In multivariate analysis, all statistically significant results from univariate analysis were included.
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Correspondence and requests for materials should be addressed to G.S. or R.M.

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