Research Article

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# MMP-2 and TIMP-2 in patients with heart failure and chronic kidney disease

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**Abstract**: The aim of the study was to assess MMP-2 (matrix metalloproteinase-2) and TIMP-2 (tissue inhibitor of metalloproteinase-2) serum levels in patients with diverse types of heart failure (HF) and chronic kidney disease (CKD).

101 patients with chronic HF were enrolled. Each patient has assessed the serum levels of MMP-2, TIMP-2, and NT-proBNP. Patients were initially classified into 2 groups based on their LVEF. 43 patients were classified into the HFREF group (HF with Reduced Ejection Fraction) and 58 characterized as HFPEF (HF with Preserved Ejection Fraction). Next, all patients were subdivided into 4 groups according to the degree of diastolic dysfunction.

38 patients with CKD were classified into HF/CKD(+) group. The HF/CKD(-) (HF without CKD) group comprised 61 patients.

This study provides original data on positive correlation between ejection fraction and MMP-2 levels in all patients with heart failure. Elevated levels of MMP-2 and TIMP-2 were found in serum from patients with chronic kidney disease; in addition, serum levels of MMP-2 were correlated with the degree of kidney failure. In all groups of patients there was positive correlation between MMP-2 and TIMP-2. Among patients with heart failure etiology was not related to MMP-2 and TIMP-2 serum levels.

**Keywords:** Cardiac extracellular matrix; Matrix metalloproteinases; Tissue inhibitors of metalloproteinases; Matrix metalloproteinase-2; Tissue inhibitor of metalloproteinase-2; Heart failure; Chronic kidney disease; Heart Failure with Reduced Ejection Fraction HFREF; Heart Failure with Preserved Ejection Fraction HFPEF

## 1 Introduction

The cardiac extracellular matrix (ECM) preserves the correct cardiac geometry and structural integrity of the myocardium by maintaining equilibrium between the deposition and degradation of matrix proteins. It forms a specific scaffold allowing the anchoring of these proteins and, therefore, aids heart cells to function properly [1, 2]. The ECM turnover influences a diverse range of physiological and pathological processes, i.e. cell proliferation and differentiation, and tissue morphogenesis [3]. The ECM is also responsible for the transduction of mechanical forces within the cardiac vessels and heart, and influences the diastolic compliance of arterial walls.

The main mediators of ECM remodeling are matrix metalloproteinases (MMPs): enzymes capable of degrading ECM structural proteins. Under normal conditions, these processes are tightly regulated. MMPs and their endogenous tissue inhibitors (TIMPs, tissue inhibitors of metalloproteinases) are also responsible for ECM remodeling, and the alternations in the balance between MMPs and TIMPs play an important role in pathological cardiac remodeling [1, 4]. Pathological, irreversible ECM remodeling contributes to both compensatory hypertrophy and congestive decompensated heart failure. Ventricular remodeling as a result of myocardial infarction or viral myocarditis is also mediated by MMPs [5,6]. In the study George et al matrix metalloproteinase-2 (MMP-2) serum level was an independent predictor of mortality in patients with chronic heart failure [7].

Based on their substrate specificity and structure, MMPs are divided into five main groups: matrilysins, collagenases, stromelysins, gelatinases, and membrane-type metalloproteinases/membrane-type MMPs. MMP-2 and MMP-9 (also named gelatinase A and B, respectively) are

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involved in the pathogenesis of atherosclerosis, coronary thrombosis, myocardial infarction, and heart failure [8,9]. These enzymes cleave structural proteins of the elastin and collagen networks. MMP-2 contributes to cell migration, differentiation, growth, and inflammation.

The known spectrum of substrates for MMP-2 contains not only the components of extracellular matrix such as collagens or elastin but it has been described that the MMP-2 can digest the components of contractile apparatus such as troponin I or light chain of myosin 1.

It has been demonstrated that during ischemia there is a general tendency for the proteolytic activity of MMPs in myocardium to increase, and thus alterations in the balance between MMPs and TIMPs may contribute to acute myocardial ischemia-reperfusion injury [10].

MMPs are produced throughout the body. MMP-2 has a widespread subcellular distribution in cardiomyocytes, including a significant presence in the nucleus [11]. Basal MMP production is normally very low in most tissues. Only additional signals such as tissue injury or growth factor signaling result in enhanced MMP gene transcription [12]. To date, upregulation of MMPs has been confirmed in the following pathological conditions: aneurysm, myocardial infarction, during myocardial stunning, left ventricular hypertrophy, acute lung injury, chronic obstructive pulmonary disease, cancer, arthritis, and eye or skin diseases [6,11,12]. In many pathological conditions there is not only an increased MMP serum level, but also a decreased level of TIMPs. This disturbed balance, in favor of the proteinases, is associated with excessive substrate turnover and disease progression [12]. In the study by Wilson et al changes in MMP/TIMP levels reflected the progression and/or acceleration of the left ventricle (LV) remodeling process [2].

The physiological balance between MMPs and TIMPs has become the basis for developing drugs aimed at the inhibition of MMPs in various diseases, in which excessive upregulation of MMPs contributes to the pathophysiology. Over the last 10 years, several drugs including KB-R7785 (Kanebo KK), MMP inhibitors (Nippon Organon KK), PD-66793, and PD-169469 (Parke-Davis & Co. – Pfizer) have been developed for therapeutic use in patients with heart failure.

The aim of the study was to assess MMP-2 and TIMP-2 (tissue inhibitor of metalloproteinase-2) serum levels in patients with diverse types of heart failure and concomitant disorders especially chronic kidney disease.

## 2 Patients and methods

One hundred and one patients with chronic stable heart failure (planned hospitalization), classified with New York Heart Association (NYHA) functional class II or III were enrolled in the study. There were 32 women and 69 men, aged between 35 and 87 years (mean age 65.2 ± 11.0 years). Detailed patient characteristics can be found in Table 1.

Patients with liver failure, malignancy, or acute inflammatory disease, as well as those receiving immunosuppressive treatment, steroid therapy, or patients with extremely elevated blood glucose levels (> 400 mg%) were excluded from the study.

Enrolled patients received the following medications: β-blockers (90%), statins (87%), angiotensin-converting enzyme inhibitors (80%), angiotensin receptor blockers (10%), aspirin (76%), diuretics (62%), aldosterone antagonists (44%), and clopidogrel (27%).

This study was approved by the local ethical committee and was conducted in accordance with the Helsinki Declaration.

Each enrolled patient had a venous blood sample drawn from an antecubital vein, within 24 hours of admission, to assess the serum levels of MMP-2, TIMP-2, and NT-proBNP. Blood samples were collected using a closed blood sampling system (BD Vacutainer®); 4 ml of blood was drawn into a tube containing EDTA (for plasma recovery) and 4 ml into a clot activator serum tube (for serum recovery). After centrifugation and fractionation, the separated serum and plasma samples were stored at -72°C, until analysis.

MMP-2 levels were determined by an enzyme immunoassay - Enzyme-linked Immunosorbent Assay Kit For Matrix Metalloproteinase 2 (Uscn Life Science Inc., Wuhan, China). The levels of the tissue inhibitor TIMP-2 were measured using the RayBio Human TIMP-2 ELISA (RayBiotech Inc., Norcross, USA). The levels of NT-proBNP were measured using a radioimmunoassay RK-011-24 BNP (1-46), Pro (Human) – RIA Kit (Phoenix Europe GmbH, Karlsruhe, Germany). Reference values provided by the manufacturers were as follows: 0.156-10 ng/ml, below 0.002 ng/ml, and 0.1-12.8 ng/ml for MMP-2, TIMP-2, and NT-proBNP, respectively.

Renal function was expressed as estimated glomerular filtration rate (eGFR). eGFR was calculated by the abbreviated Modification of Diet in Renal Disease (MDRD) formula:

$$eGFR = 186 \times SCr^{-1.154} \times (age)^{-0.203} \times (0.742 \text{ if female})$$

**Table 1:** Patient characteristics (classification according to the level of ejection fraction).

| Characteristic | Patients with HF n=101 | HF-REF group n=43 | HF-PEF group n=58 |
|---|---|---|---|
| Age, years | 65.2±11.0 | 62.7±12.7 | 67.3±9.5[a] |
| Males, %, (mean age, years) | 68 (62.9±11.0) | 84 (59.5±11.4) | 64 (65.9±9.9) |
| Females, %, (mean age, years) | 32 (70.1±9.5) | 26 (72.1±11.9) | 36 (69.1±8.2) |
| LVEF, % | 49±18 | 30.3±7.6 | 61.9±8.3[c] |
| Ischemic etiology of HF, % | 62 | 67 | 59 |
| Toxic etiology of HF, % | 1 | 0 | 2 |
| Inflammatory etiology of HF, % | 6 | 14 | 0[a] |
| Valve disorders, % | 60 | 83 | 44[c] |
| Type 2 diabetes mellitus, % | 33 | 26 | 37 |
| Hypertension, % | 81 | 64 | 95[c] |
| Hemoglobin, g/dl | 13.7±1.6 | 13.8±1.6 | 13.7±1.6 |
| Creatinine, mg/dl | 1.24±0.58 | 1.31±0.70 | 1.17±0.46 |
| Total cholesterol, mg/dl | 175.7±47.2 | 175.5±49.6 | 176.0±45.4 |
| Potassium, mmol/l | 4.36±0.46 | 4.36±0.54 | 4.35±0.39 |

Abbreviations: HF-PEF, heart failure with preserved ejection fraction; HF-REF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction
Data are presented as mean ± standard deviation.
[a] P<0.05, [c] P<0.0001

where: eGFR indicates estimated GFR (ml/min/1.73 $m^2$) and SCr – serum creatinine level.

Echocardiography was performed in all patients within 48 hours of admission using a General Electric Vingmed Echocardiography system with a 2.5 MHz phased-array transducer. Left ventricular ejection fraction (LVEF) was calculated using the universal formula: EF = (LVEDV – LVESV)/LVEDV x 100%, where LVEDV indicates left ventricular end-diastolic volume and LVESV, left ventricular end-systolic volume. LVEDV and LVESV were calculated using the Simpson biplane method, in which the machine computes the left ventricular volume based on perpendicular cross-sections of the left ventricle on the apical two- and four-chamber view, as determined by the operator.

Patients were initially classified into two groups based on their LVEF, according to the guidelines of the European Society of Cardiology for the diagnosis and treatment of acute and chronic heart failure. Reduced ejection fraction was defined as an LVEF level <45% [10, 12, 13]. 43 patients with chronic heart failure and significantly reduced LVEF with values below 45%, and typical symptoms and signs of heart failure (mean LVEF 30.3 ± 7.6%), were classified into the HF-REF group (Heart Failure with Reduced Ejection Fraction). There were 58 patients with preserved LVEF with levels above 45%, without LV dilatation and with diagnosis of heart damage (LV hypertrophy assessment based on interventricular septum thickness - IVSD ≥12 mm and/or left atrium enlargement established as the size of left atrium - LA >40 mm) and/or diastolic dysfunction evaluated by echocardiography and with typical symptoms and signs of heart failure (mean LVEF 61.9 ± 8.3%), characterized as HF-PEF (Heart Failure with Preserved Ejection Fraction) [13-16]. Detailed patient characteristics for both groups can be found summarized in Table 1.

Next, all patients were subdivided into four groups according to the degree of diastolic dysfunction [13]:

Group A – comprised 18 patients without diastolic dysfunction

Group B – comprised 63 patients with mild diastolic dysfunction (with impaired relaxation)

Group C + D – group C comprised 15 patients with moderate diastolic dysfunction ("pseudonormal mitral inflow") and group D comprised 5 patients with severe diastolic dysfunction (with restrictive filling)

In subsequent classification, patients were divided into two groups based on the presence of CKD.

Thirty-eight patients with heart failure and concomitant chronic kidney disease, with eGFR values between 26-59 ml/min/1.73m2 (mean eGFR 45±10 ml/min/1.73m2),

and stage 3 or 4 of CKD were classified into HF/CKD(+) group.

The HF/CKD(-) (Heart Failure without Chronic Kidney Disease) group comprised sixty-one patients with HF without concomitant CKD (mean eGFR value 79±15 ml/min/1.73m2). Two patients with end-stage kidney disease, with eGFR ≤ 17 ml/min/1.73m2 receiving hemodialysis were excluded from the analysis to maintain the homogeneity of the study group.

Patients were divided into subgroups based on LVEF level, degree of left ventricle diastolic dysfunction, or presence of concomitant diseases, i.e. type 2 diabetes mellitus, hypertension, ischemic heart disease, and chronic kidney disease (CKD).

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Bioethics Committee of the Wroclaw Medical University.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Multidimensional analysis was performed in reference to selected and independent classifications.

Quantitative data were summarized using means ± the standard deviation.

Differences between the two groups were tested using Student's t-test for unpaired data, once normality was demonstrated (by Kolmogorov-Smirnov or Shapiro-Wilk tests) and after the sample variances of the two groups were assessed using a Fisher's F-test to verify the homogeneity of variances. Since the null hypothesis of normal distribution for MMP-2 was rejected in the Kolmogorov-Smirnov test, this variable was normalized by logarithmic transformation prior to the analysis. Normal distribution of the log-transformed MMP-2 variable was demonstrated using the Kolmogorov-Smirnov test. Correlations between measurable parameters were estimated using the Pearson correlation coefficient (Pearson's r), once the assumption of normal data distribution was met. The statistical significance of differences between the groups was tested using Student's t-test, the $\chi 2$ test and analysis of variance (Kruskal-Wallis test) with post- hoc analysis, where appropriate. Statistical analyses were performed using STATISTICA 10 data analysis software system (StatSoft, Inc).

The statistical significance level was set at $P < 0.05$.

# 3 Results

The serum levels of MMP-2 and TIMP-2 did not differ between the HF-REF and HF-PEF groups. Compared to the HF-PEF group, the serum levels of NT-proBNP were significantly higher in HF-REF patients (Table 2).

No significant differences were observed in the serum levels of TIMP-2 and NT-proBNP among the groups (Table 3).

MMP-2 and TIMP-2 levels did not differ between patients with ischemic heart failure and patients with history of non-ischemic heart failure. However, NT-proBNP levels were significantly higher in patients with concomitant coronary artery disease (CAD) (Table 4).

The serum levels of MMP-2, TIMP-2, and NT-proBNP did not differ between patients with concomitant type 2 diabetes mellitus and patients without diabetes. No sig-

**Table 2:** The serum levels of MMP-2, TIMP-2 and NT-proBNP in patients with HF-REF versus HF-PEF (classification according to the level of ejection fraction).

| Parameter | Patients with HF n=101 | HF-REF group n=43 | HF-PEF group n=58 |
|---|---|---|---|
| MMP-2, ng/ml | 190.9±239.8<br>88.3<br>[22.0- 235.4] | 160.7±230.2<br>71.8<br>[11.7- 211.7] | 213.3±246.2<br>120.1<br>[34.7- 247.8] |
| TIMP-2, ng/ml | 151.6±43.9<br>155.2<br>[126.3- 176.0] | 150.1±48.1<br>155.2<br>[108.2- 182.2] | 152.8±40.8<br>153.8<br>[130.6- 174.2] |
| NT-proBNP, ng/ml | 1.20±0.52<br>1.08<br>[0.77- 1.64] | 1.51±0.52<br>1.64<br>[1.19- 1.89] | 0.96±0.38[c]<br>0.86<br>[0.68- 1.09] |

Abbreviations: HF-PEF, heart failure with preserved ejection fraction; HF-REF, heart failure with reduced ejection fraction
Data are presented as mean ± standard deviation. Median values are given in the second line with upper and lower quartile in parentheses.
[c] P<0.0001

**Table 3:** The serum levels of MMP-2, TIMP-2, NT-proBNP, and LVEF in A, B, and C groups (classification according to the degree of diastolic dysfunction, evaluated by ultrasonography).

| Parameter | group A n=18 | group B n=63 | group (C+D) n=20 |
|---|---|---|---|
| MMP-2, ng/ml | 219.7±303.6<br>45.7<br>[22.0- 247.8] | 180.9±228.8<br>88.8<br>[23.8- 211.7] | 196.6±219.5<br>(129.8) |
| TIMP-2, ng/ml | 149.4±39.6<br>157.3<br>[120.6- 171.8] | 152.9±43.8<br>156.6<br>[127.0- 182.2] | 149.6±49.6<br>(151.8) |
| NT-proBNP, ng/ml | 1.33±0.68<br>1.19<br>[0.77 -1.68] | 1.12±0.47<br>1.0<br>[0.72- 1.55] | 1.31±0.49<br>1.37 |
| LVEF, % | 50.4±16.9<br>60<br>[35-60] | 49.7±17.6<br>55<br>[35-65] | 43.7±18.8<br>38.0<br>[30 -70] |

Abbreviations: LVEF, left ventricular ejection fraction
Data are presented as mean ± standard deviation. Median values are given in the second line with upper and lower quartile in parentheses.

**Table 4:** The serum levels of MMP-2, TIMP-2 and NT-proBNP in HF patient subgroups by concomitant disease (ischemic heart disease, type 2 diabetes mellitus, hypertension).

| | Ischemic heart disease | | Type 2 diabetes mellitus | | Hypertension | |
|---|---|---|---|---|---|---|
| | Yes n=63 | No n=38 | Yes n=33 | No n=68 | Yes n=83 | No n=18 |
| MMP-2 ng/ml | 218.2±258.2<br>94.7<br>[30.0- 247.8] | 145.7±200.7<br>69.9<br>[14.0- 182.6] | 187.1±261.6<br>80.2<br>[18.9- 190.4] | 192.8±230.5<br>94.5<br>[25.1- 242.0] | 198.2±244.7<br>88.3<br>[26.3- 236.9] | 157.5±218.8<br>92.8<br>[16.9- 231.6] |
| TIMP-2 ng/ml | 154.9±44.7<br>158.7<br>[126.3- 182.2] | 146.2±42.4<br>142.3<br>[112.0- 173.6] | 152.9±36.8<br>158.9<br>[133.4- 172.8] | 151.0±47.1<br>150.2<br>[120.0- 183.9] | 154.2±42.8<br>156.6<br>[127.8- 182.2] | 139.6±48.1<br>139.7<br>[100.4- 169.2] |
| NT-proBNP ng/ml | 1.22±0.56<br>1.18<br>[0.71- 1.71] | 1.15±0.47[a]<br>1.04<br>[0.84- 1.55] | 1.16±0.54<br>1.00<br>[0.77- 1.46] | 1.21±0.51<br>1.20<br>[0.78- 1.71] | 1.15±0.51<br>1.02<br>[0.72- 1.57] | 1.42±0.51<br>1.47<br>[1.03- 1.88] |

Abbreviations: HF, heart failure
Data are presented as mean ± standard deviation. Median values are given in the second line with upper and lower quartile in parentheses.
[a] P<0.05

nificant differences in MMP-2, TIMP, and NT-proBNP levels were observed according to concomitant hypertension (Table 4).

Patients with HF/CKD(+) exhibited higher MMP-2, TIMP-2, and creatinine levels compared to the HF/CKD(-) group. No significant differences in NT-proBNP and LVEF levels were observed between the groups (Table 5).

The correlations for all patients (n = 101) are summarized in Table 6, whereas correlations in subgroups are described below.

Significant positive correlations between MMP-2 and TIMP-2 ($r$ = 0.39; $P$ = 0.01), and between TIMP-2 and NT-proBNP ($r$ = 0.31; $P$ = 0.046), were observed in the HF-REF group. Whereas, in HF-PEF group, a significant positive correlation between MMP-2 and TIMP-2 was observed ($r$ = 0.37; $P$ = 0.005).

In the HF/CKD(+) group, a positive correlation between MMP-2 and TIMP-2 ($r$ = 0.37; $P$= 0.02) and negative correlation between NT-proBNP and LVEF ($r$ = -0.61; $P$ < 0.0001) were found. Similarly, in the HF/CKD(-) group, a positive correlation between MMP-2 and TIMP-2 ($r$ = 0.35;

**Table 5:** The serum levels of MMP-2, TIMP-2, NT-proBNP, creatinine, and LVEF in HF/CKD(+) versus HF/CKD(-) group (classification according to eGFR).

| Parameter | HF/CKD(+) group n=38 | HF/CKD (-) group n=61 |
|---|---|---|
| MMP-2, ng/ml | 249.7±278.5<br>133.5<br>[41.7- 457.9] | 145.3±198.4[a]<br>71.8<br>[14.0- 190.4] |
| TIMP-2, ng/ml | 163.4±48.1<br>169.6<br>[149.0- 193.7] | 143.8±40.2[a]<br>140.9<br>[111.3- 169.2] |
| NT-proBNP, ng/ml | 1.28±0.52<br>1.31<br>[0.84- 1.83] | 1.11±0.47<br>0.99<br>[0.69- 1.53] |
| Creatinine, mg/dl | 1.47±0.35<br>1.36<br>[0.84- 1.83] | 0.99±0.17[c]<br>0.99<br>[0.89- 1.13] |
| LVEF, % | 49.3±17.9<br>56<br>[35- 60] | 48.2±17.9<br>50<br>[32-65] |

Abbreviations: HF/CKD(+), heart failure with chronic kidney disease; HF/CKD(-), heart failure without chronic kidney disease; LVEF, left ventricular ejection fraction
Data are presented as mean ± standard deviation. Median values are given in the second line with upper and lower quartile in parentheses.
[a] P<0.05; [c] P<0.0001

**Table 6:** The correlations between analyzed parameters in all patients (n=101).

| Parameter | MMP-2 | TIMP-2 | NT-proBNP |
|---|---|---|---|
| MMP-2 | --- | r= 0.39[c] | r= 0.06 |
| TIMP-2 | r= 0.39[c] | --- | r= 0.09 |
| NT-proBNP | r= 0.06 | r= 0.09 | --- |
| Creatinine | r= 0.34[b] | r= 0.24[a] | r= 0.30[b] |
| eGFR | r= -0.30[b] | r= -0.22[a] | r= -0.28[b] |
| LVEF | r= 0.20[a] | r= 0.03 | r= -0.53[c] |

Abbreviations: LVEF, left ventricular ejection fraction
[a] P<0.05; [b] P<0.005; [c] P<0.0001

$P = 0.005$), and negative correlation between NT-proBNP, and LVEF ($r = -0.51$; $P < 0.0001$) were observed. In addition, a positive correlation between creatinine and MMP-2 was observed in this group ($r = 0.34$; $P < 0.01$).

In all groups of patients HF-PEF, HF-REF, HF/CKD(+) and HF-CKD(-) there was positive correlation between MMP-2 and TIMP-2.

# 4 Discussion

Activities of MMPs are regulated at multiple levels, including: the synthesis of pro-MMP precursors, post-transcriptional conversion into active MMPs, and interactions with specific inhibitors. Gelatinases (MMP-2 is gelatinase A, and MMP-9 is gelatinase B) have various substrates which degrade elastin and collagens e.g. type IV, V, VII, and X [17-20].

This study provides the positive correlation between LVEF and MMP-2 levels in all patients with HF, but LVEF as a factor defining the type of HF was not associated with MMP-2 and TIMP-2 levels. Among patients with HF, the etiology was not related to MMP-2 and TIMP-2 serum levels. However, the association between MMP-2 and TIMP-2 was retained in both the HF/CKD(+) and (-) groups.

Patients with HF-PEF exhibited diastolic dysfunction with increased diastolic stiffness, but also non-diastolic abnormalities, induced by alternations in systolic velocity, and chronotropic incompetence. In spite of the increasing prevalence of HF-PEF in the last 15 years (the disease affects approximately half of all HF patients), knowledge about the molecular mechanisms underlying its pathophysiology remains uncertain because pathways leading to HF-PEF development are not restricted to a single pathology. Intracellular alterations associated with

elevated resting tension of cardiomyocytes are important in patients with severe HF-PEF. It was observed that excessive cardiac collagen deposition results in the deterioration of diastolic function. Increased migration of inflammatory cells from the endothelium to the myocardium may contribute to the development of these abnormalities, especially with regards to modifications in the ECM [17].

The level of MMP-2 in patients with the most advanced diastolic dysfunction was not different compared to group with less advanced dysfunction. Despite huge relative odds several differences are not statistically significant and this may raise the doubt that sample size might be underpowered to detect statistically significant differences.

In histopathology studies by Westremann et al. the activity of cardiac MMP-1, a key human collagenase, was downregulated, whereas TIMP-1 activity was upregulated in patients with HF-PEF, compared to the control group [21]. The endogenous collagen degradation system is regulated by increased activity of MMPs overcoming their tissue inhibitors [9]. Upregulation of TIMP-1 and downregulation of MMP-1 was found in biopsy samples from patients with HF-PEF, which results in a significant decrease in the MMP-1/TIMP-1 ratio. Inhibition of the collagen degradation system could be one of the mechanisms contributing to the accumulation of ECM in patients with HF-PEF, as well as initiation of the long-term development of diastolic dysfunction [22]. Increased cardiac expression of TIMP-1 and TIMP-2 is associated with cardiac fibrosis and dysfunction in a chronic pressure-overloaded heart [22]. Lopez et al. observed that this ratio was elevated in patients with systolic HF, whereas it remained unchanged in the hypertensive HF group [23]. Some studies reported MMP-2 serum levels in patients with hypertensive and diastolic HF, but the results were contradictory; some of them showed an increase, while others remained unchanged or even exhibited decreased levels of MMP-2 [24]. Therefore, further studies are required to confirm the results on larger populations of patients.

As an enzyme responsible for collagen degradation, MMP-2 may represent a response to excess myocardial fibrosis, loss of elastin and other components of the ECM, which in turn may promote ventricular stiffness. It is worth mentioning that the overall results confirm the increase of MMP-2 level in LV hypertrophy. In the study by Martos et al. MMP-2 levels were significant predictor of HF-PEF and diastolic dysfunction. MMP-2 provided 91% sensitivity and 76% specificity for predicting HF-PEF and was a better predictive marker than the best-known BNP [25]. Serum levels of NT-proBNP increase in systolic or diastolic dysfunction, so low levels of NT-proBNP effectively rule out systolic dysfunction. Very high levels of NT-proBNP will identify patients with heart failure, systolic or diastolic, with a high degree of certainty [26].

The heart and kidney are two strongly related organs in pathophysiological conditions. Patients with CKD were not different in regards to the correlation between MMP-2 and TIMP-2, as well as between NT-proBNP and LVEF. However, patients with CKD produced higher levels of MMP-2 and TIMP-2, although they were not significantly different in NT-proBNP and LVEF, from those groups without CKD. This shows an enhanced activation of MMPs, while retaining associations with their inhibitors. Until now, this activation has been observed in the cardiopulmonary field, and mainly in the case of enhanced inflammatory reactions. Our and some other studies reveal that CKD also results in the upregulation of MMPs [27,28].

Studies by Chen et al. demonstrated increased MMP-2, MMP-9, and TIMP-1 expression by reverse transcription polymerase chain reaction (RT-PCR) in aortas with progressive CKD and increased MMP-2 activity in the serum. This increase in enzyme expression was observed simultaneously with increased expression of RUNX-2 (runt-related transcription factor 2), an osteoblast transcription factor, thought to be important in the initial step of osteochondrocytic differentiation in vascular smooth muscle cells [29]. Moreover, increased tissue expression of MMP-2 and MMP-9 was demonstrated in rats with CKD. In the children study of Musial and Zwolinska MMP-2 concentrations kept growing from the beginning of renal failure progression [30]. Parallel studies conducted in patients with CKD undergoing a kidney transplant demonstrated increased expression of MMP-2 in arterial blood samples, with concomitant vascular calcification [31]. Circulating MMP-2, -3 and -9 are independently associated with kidney disease progression in non-diabetic CAD patients and add incremental predictive power to conventional risk factors [32]. Gluba-Brzózka et al in their study suggest that factors like MMP-2 and TIMP-2 may be involved in the pathogenesis of CAD in patients with CKD [33]. Nagano et al. observed a correlation between concentration of MMP-2 and kidney function parameters and confirmed that MMP-2 can be an indicator of the severity of atherosclerosis in CKD patients [34].

Increased levels of TIMP-2 were found in animals with CKD compared to controls, perhaps to compensate for the increased expression of MMPs. However, no progressive increase in TIMP-1 levels was found, as there was for MMP-2 activity in animals with CKD; this may imply that the compensatory mechanisms are unable to completely compensate with deteriorating CKD [28].

Marson et al. examined effects of hemodialysis on the concentrations of MMP-2 and TIMP-2 levels in of end-stage kidney disease (ESKD) patients. They found increased plasma MMP-2 and TIMP-2 levels in ESKD patients and hemodialysis decreased MMP-2 (but not TIMP-2) levels [28]. Variability in behavior of selected markers and correlations point at the complexity of relations between these elements [30].

Du et al. analyzed the role of MMP-2 and the effect of MMP inhibition on the development of renal fibrosis, which is typical for progressive kidney disease. While renal fibrosis was induced, progressive increase of both MMP-2 and MMP-9 was observed in the affected tubules. The results indicated that MMP-2 has a pathogenic role in renal interstitial fibrosis, possibly through the induction of EMT (epithelial-to-mesenchymal transition) and macrophage infiltration. Inhibition of MMPs may have therapeutic implications in renal fibrosis [35]. It has been hypothesized that degradation of the ECM is a necessary and early step preceding arterial calcification in CKD. The activity of MMPs is crucial for the normal bone remodeling that occurs throughout life [36]. Therefore, it is not surprising that MMPs are also essential in the pathogenesis of calcification in extra-osseous tissue. To conclude, in the presented study a progressive increase of MMP-2 activity was found in serum from patients with CKD. This led to changes in ECM and, in turn, could alter arterial structure and function. ECM accumulation, however, were not studied throughout this study. This direct effect is confirmed by studies, demonstrating that inhibition of calcification occurs ex vivo in cell culture models with the MMP inhibitors. However, in vivo studies in animal CKD models and in patients with CKD are warranted to determine if preventing ECM degradation also prevents arterial calcification in CKD patients.

HF has several negative effects on kidney function but, at the same time, CKD can significantly impair cardiac function. This process is due to hemodynamic alterations, inflammatory and endothelial activation, parenchymal damage, increased neuroendocrine activity and other factors, which can influence in different ways the improvement or the worsening of both HF and CKD [37].

Based on the diversity of biological actions of MMPs, it is likely that continued research of the relationship of LV remodeling to this family of proteases will yield new insights into the ECM remodeling process itself and relevant new therapeutic targets [38].

# 5 Conclusions

This study provides original data on positive correlation between ejection fraction and MMP-2 levels in all patients with heart failure.

Elevated levels of MMP-2 and TIMP-2 were found in serum from patients with chronic kidney disease; in addition, serum levels of MMP-2 were correlated with the degree of kidney failure.

In all groups of patients there was positive correlation between MMP-2 and TIMP-2.

Among patients with heart failure etiology was not related to MMP-2 and TIMP-2 serum levels.

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