Aim: Apoptosis has been implicated in the development of various cardiovascular diseases. Caspase-cleaved cytokeratin 18 (M30) is released during apoptotic cell death. The concentrations of M30 and the correlation with asymmetric dimethylarginine (ADMA) in heart failure (HF) are not known. The objectives of this study were to determine the possible association between M30 and ADMA and the potential use of M30 as an apoptotic marker in patients with HF. Material and Method: In this study M30 and ADMA concentrations were evaluated in 30 patients with heart failure and 30 healthy control subjects. Results: Increased M30 (p=0.01) and ADMA (p = 0.012) concentrations were found in the patients and a positive correlation was determined between ADMA and M30 in the patient group (p < 0.001, r= 0.627). No correlation was determined between M30, N terminal pro brain natriuretic peptide (NT-pro-BNP), and ejection fraction. Discussion: These results demonstrate that M30 can be used as a novel apoptotic serum marker in patients with heart failure. The apoptotic cascade activated by increased ADMA concentrations can be considered to contribute to the molecular mechanism of HF.

Keywords
Asymmetric Dimethylarginine; M30; Cytokeratin-18 Peptide; Heart Failure; Apoptosis
**Introduction**

Cardiovascular diseases are the leading cause of death in developing countries [1]. Heart failure (HF) is a systemic disease in which the heart cannot pump enough blood to meet the body's requirement [2]. The prevalence of the disease is on the rise, with approximately two million new cases of HF diagnosed per year worldwide [3]. Different pathophysiological mechanisms such as oxidative stress, neurohormonal changes, and inflammatory activation lead to myocyte death by promoting apoptosis, necrosis, and autophagic cell death in HF [4,5]. Cytokeratin 18 (CK-18) is an intermediate filament of epithelial cells. CK 18 fragments such as caspase cleaved cytokeratin 18 (M30) are released into the extracellular space due to caspase digestion during apoptosis and serve as markers of apoptosis [6]. The elevation of plasma M30 level is involved in different diseases including colon cancer [7], sepsis [8], and chronic hepatitis B [9]. To the best of our knowledge, no previous study has investigated the changes of M30 concentrations in heart failure.

Asymmetric dimethylarginine (ADMA) is one of three circulating endogenous analogues of L-arginine derived by methylation of arginine residues catalyzed by a family of proteins known as protein arginine methyltransferases [10]. ADMA is an endogenous competitive inhibitor of NO synthase and is eliminated from the body by a combination of renal excretion and metabolism by the dimethylarginine dimethylaminohydrolase (DDAH) enzymes [10,11]. Increased levels of ADMA have been found to be associated with atherosclerosis [12], coronary artery disease [9], peripheral arterial occlusive disease, hypertension [13], and HF [14]. However, the role of ADMA in heart failure has not been well investigated.

The hypothesis of our study is that increased serum ADMA concentrations may trigger the apoptotic process in HF. Therefore, an assessment was made of the ADMA and M30 concentrations and the possible association between these biomarkers in patients with HF. The potential use of M30 as an apoptotic marker in patients with HF was also investigated. This study provided an important opportunity to advance the understanding of the role of ADMA in HF.

**Material and Method**

**Patients and controls**

The study included 30 consecutive patients [17 males and 13 females; mean age: 68 ± 12 years (range, 24-83 years)] with chronic HF with reduced ejection fraction (HFrEF) who were hospitalized for acute decompensated HF and 30 healthy control subjects [10 males and 20 females; mean age: 65 ± 13 years (range, 28-78 years)]. HF had been diagnosed in the patients group at least 12 months previously. Data was collected from the patients’ records in the Cumhuriyet University Medical Faculty, Department of Cardiology. Overnight fasting blood samples were collected from all participants into red top tubes (Becton Dickinson, UK) during the admission. The serum samples were allowed to clot before centrifugation. After centrifugation at 4°C for 15 minutes at 3500 rpm, the serum was aliquoted and immediately frozen at -20°C. The study protocol was approved by the Ethics Committee of Cumhuriyet University Medical Faculty (Approval number: 2016-04/06).

**Biochemical analysis**

Caspase cleaved cytokeratin 18 and ADMA concentrations were determined using commercially available ELISA kits. The Complete Blood Count analysis was performed using a hematology system (Mindray BC 6800, China). CK-MB, TG, TCHOL, HDL-C, and LDL-C concentrations were determined by the enzymatic colorimetric method (Beckman Coulter AU5800, USA). Serum NT-pro-BNP was measured using immunoassay (AQT90 flex Radiometer, Denmark). PT and aPTT concentrations were determined using a coagulation system (ACL TOP 700, Italy).

**Echocardiographic examination**

Echocardiographic examinations were performed by experienced operators. Patients were imaged in the left lateral decubitus position with commercially available systems (Vivid systems, GE Healthcare, Wauwatosa, USA). Left ventricular dimensions, volumes, and ejection fraction (EF) [by modified Simpson's method] were measured according to the European Association of Echocardiography (EAE)/American Society of Echocardiography (ASE) recommendations [15]. LV diastolic functions were evaluated according to EAE/ASE standards [16]. The diagnoses of HFrEF were made according to guidelines [17].

**Statistical analysis**

Sample size was determined as 30 observations for each group, based on α=0.05 and β=0.10. The power of the actual performed test was calculated as 90%. Analyses were conducted using PASS 11.0 (Power Analysis Statistical System) software. The Shapiro-Wilk test was used to determine the distribution characteristics of the variables. The Student t test and Mann-Whitney U test were applied to compare the differences of the parametric and nonparametric variables between the groups, respectively. Spearman correlation coefficients were calculated to evaluate the relationship between M30, ADMA, NT-pro BNP,
Discussion

Recent evidence suggests that apoptosis is involved at multiple points in HF, although the molecular biology and biochemistry of the apoptotic death machinery are far from being completely resolved in HF [5]. In this study, M30 concentrations were found to be higher in patients than in the healthy control group. There have been few studies conducted on the concentrations of M30 in cardiovascular disease [18-19]. It has been revealed that cardiac lipofuscin-laden lysosomes obtained from patients with ischemic, congestive, and hypertrophic cardiomyopathy contain M30 [19]. Adlbrecht et al. [18] reported elevated levels of M30 in patients with acute myocardial infarction. Recent evidence has suggested that caspsases, which are a group of cysteine proteases, play a crucial role in the apoptosis of myocyte and the formation of M30 and thereby have a crucial role in HF [20,21]. In previous research of sheep fitted with variable aortic constriction devices, it was indicated that activated cardiomyocyte caspase enzymes play an important role during the transition to heart failure [20]. M30 concentrations can be considered an additional biomarker in the monitoring of myocardial damage associated with apoptosis in patients with heart failure. In the present study, no associations were found between the concentrations of NT-pro-BNP, EF, troponin I, and M30. Natriuretic peptides,  

Table 1. Laboratory parameters of patients and controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=30)</th>
<th>Control (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>25.68 ± 9.79</td>
<td>23.97 ± 5.94</td>
<td>0.556</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>22 ± 14.26</td>
<td>22.97 ± 14.02</td>
<td>0.360</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>34.72 ± 10.16</td>
<td>44.89 ± 9.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>96.66 ± 32.21</td>
<td>86.20 ± 15.15</td>
<td>0.116</td>
</tr>
<tr>
<td>TCHOL (mg/dL)</td>
<td>157.10 ± 49.50</td>
<td>140.55 ± 20.82</td>
<td>0.100</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>108.60 ± 49.17</td>
<td>99.55 ± 40.58</td>
<td>0.445</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m2)</td>
<td>75.41 ± 30.40</td>
<td>99 ± 9.57</td>
<td>0.014</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.08 ± 0.85</td>
<td>0.93 ± 0.17</td>
<td>0.457</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>11.78 ± 6.09</td>
<td>11.3 ± 0.32</td>
<td>0.392</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>29 ± 2.81</td>
<td>31 ± 1.25</td>
<td>0.285</td>
</tr>
<tr>
<td>INR</td>
<td>1.02 ± 0.5</td>
<td>1.03 ± 0.2</td>
<td>0.427</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>134.50 ± 4.50</td>
<td>136 ± 2</td>
<td>0.642</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>3.79 ± 0.65</td>
<td>4.07 ± 0.30</td>
<td>0.846</td>
</tr>
<tr>
<td>WBC (10^3 mcL)</td>
<td>7.70 ± 1.65</td>
<td>8.10 ± 1.5</td>
<td>0.153</td>
</tr>
</tbody>
</table>

Table 2. Clinical features of heart failure patients (n=30)

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA functional class III/IV</td>
<td>24/6</td>
</tr>
<tr>
<td>Diabetes mellitus (yes/no)</td>
<td>11/19</td>
</tr>
<tr>
<td>Hypertension (yes/no)</td>
<td>22/8</td>
</tr>
<tr>
<td>Hyperlipidemia (yes/no)</td>
<td>6/24</td>
</tr>
<tr>
<td>Obesity (yes/no)</td>
<td>11/19</td>
</tr>
<tr>
<td>COPD (yes/no)</td>
<td>7/23</td>
</tr>
<tr>
<td>CEID (yes/no)</td>
<td>9/21</td>
</tr>
<tr>
<td>Chronic kidney disease (yes/no)</td>
<td>6/24</td>
</tr>
<tr>
<td>Ischemic/non-ischemic etiology</td>
<td>12/18</td>
</tr>
<tr>
<td>SPAP (mm/Hg)</td>
<td>39 ± 16</td>
</tr>
<tr>
<td>EF (%)</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Concomitant right ventricular systolic dysfunction (yes/no)</td>
<td>10/20</td>
</tr>
</tbody>
</table>

uretic peptides including BNP and NT-pro-BNP, an amino-terminal pep-
epropetide equivalent to BNP, are reliable biomarkers for the diagnosis, prognosis determination, and treatment of heart failure [23]. From the results of the current study, it was con-
mcluded that M30 may not be used for the diagnosis and prog-
nosis determination of HF as there was no correlation between M30 and NT-pro-BNP and EF. However, caution must be applied in the evaluation of this conclusion because of the low sample size. Further studies are required with larger sample sizes to evaluate the diagnostic performance of M30 in patients with HF.

Although an association has been reported in literature be-

 tween ADMA concentrations and heart failure [14], the role of ADMA has not been completely defined. The ADMA concentra-
tions were found to be higher in the patients than in the control group in the present study. Previous studies have reported el-
vated ADMA concentrations in patients with heart failure [24-27]. The findings of the current study are in accordance with these previous studies. A positive correlation between ADMA and M30 concentrations was determined in the current study. It is a well-known fact that there is an association between ADMA and apoptosis. Different molecular mechanisms such as the p38MAPK-dependent signaling pathway, accumulation of cytochrome c, and activation of endoplasmic reticulum stress have been described in ADMA-related apoptotic processes in different conditions [22,28,29]. The current study results sug-
ggest that increased concentrations of ADMA trigger the cas-
pases activation in HF. Small sample size was major limitation of our study. In conclusion, M30 can be used as an apoptotic serum marker in patients with heart failure. In addition, the activated apoptotic cascade caused by increased ADMA concentrations can con-
tribute to the molecular mechanism of the progression and pro-
gression of HF. Further, studies are warranted with respect to the potential therapeutic utility of the regulating of ADMA concentrations and caspase inhibition with potential DDAH activity and caspase inhibitors and its improved delivery system to the heart in patients with HF.

Competing interests

The authors declare that they have no competing interests.

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Journal of Clinical and Analytical Medicine  | 283

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