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Elevated Plasma Homocysteine Level in Chronic Heart Failure is an Important Predictive Factor for Cardiovascular Mortality in Males with Coronary Artery Disease

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Abstract

Previous reports have consistently showed that high Homocysteine (Hcy) levels were associated with high risk for cardiovascular disease. Furthermore, there is emerging epidemiological evidence that Hyperhomocysteinemia (HHcy) has been reported as an independent risk factor for fatal and non-fatal cardiovascular disease. Increased Hcy levels have been identified as a crucial clinical issue in Chronic Heart Failure (CHF) which is probably one of the most important areas of interest for HHcy. The aim of this study was to determine the prognostic importance of Hcy levels in female and male patients with CHF as well as relationship between Coronary Artery Disease (CAD) and Hcy levels in males and females in CHF.

A total of 602 patients were included into the study in retrospective cohort design. The median follow-up duration was 38 ± 15 months. The mean age of the cohort was 63 ± 13 years with mean ejection fraction of 26 ± 10%. There were 233 cardiac deaths among the study population which means an overall cardiac mortality rate of 38.7%. It was demonstrated that the male patients with CAD who had higher Hcy levels died more than male patients with CAD whose Hcy levels were lower (20.2 ± 8.6 μmol/L *vs.* 17.8 ± 8.2 μmol/L, p=0.02). The female patients who had CAD had no statistical difference between survivors and non-survivors in terms of Hcy. Patients without CAD there was no statistical difference between survivors or non-survivors in terms of Hcy levels in both genders.

Our study showed that serum Hcy levels were significantly elevated in male non-survivors compared to male survivors who had CAD with severe systolic dysfunction while the difference did not reach statistical significance between female groups.

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Introduction

Previous reports have consistently showed that high Homocysteine (Hcy) levels were associated with high risk for cardiovascular disease [1]. Furthermore, there is emerging epidemiological evidences that Hyperhomocysteinemia (HHcy) has been reported as an independent risk factor for fatal and non-fatal cardiovascular disease [2,3]. Hcy has pro-oxidative and pro-inflammatory properties which promotes vasoconstriction and endothelial dysfunction [4,5]. All of these features may lead to more rapid development of atherosclerosis with subsequent atherosclerotic and thrombotic vascular complications [6-8].

Recently, increased Hcy levels have been identified as a new clinical issue in Chronic Heart Failure (CHF) which is probably one of the most important areas of interest for HHcy [9,10]. Unfortunately, CHF is a major health care problem and prevention of CHF is still a most important unsolved problem [11-13].

It was shown that HHcy resulted in increased perivascular collagen levels and coronary arteriolar wall thickness due to smooth muscle hyperplasia and mast cells accumulation in an experimental

study [14]. Echocardiographic evaluation of Hcy-treated animals showed significant increases in left ventricular diastolic and systolic dimensions and decreases in posterior wall thickness which shows that HHcy has direct adverse effect on cardiac structure and function [15].

The aim of this study was to determine the prognostic importance of Hcy levels in female and male patients with CHF as well as relationship between Coronary Artery Disease (CAD) and Hcy levels in males and females in CHF.

Methods

Study population

This retrospective cohort design study was conducted at the Kocaeli University hospital which had a detailed clinical database. Patients who were hospitalized due to decompensated heart failure between 2003 and 2010 were screened from the hospital data base to include in this study. The study population consisted of patients with depressed left ventricular systolic function (Left Ventricular Ejection Fraction (LVEF) <45%) and were treated according to the guidelines valid at the time of hospitalization. Thirty-two patients were excluded due to missing Hcy values. Thus, the analyzed study group consisted of 387 males and 215 females' patients with New York Heart Association (NYHA) functional class II-IV heart failure. Exclusion criteria were as follows: Malignancy, right heart failure due to chronic obstructive pulmonary disease, severe aortic stenosis and mitral stenosis with patients who had symptoms of decompensated heart failure, methylenetetrahydrofolate reductase deficiency, hypothyroidism, and patients who were taken certain medications (such as antiepileptic drugs and methotrexate). All patients had diagnostic coronary angiography for determining the etiology of the heart failure. Patients' survival information was retrieved from the hospital records, from telephone interview with the patients or their family members.

The study was conducted in accordance with the Declaration of Helsinki and approved by local institutional ethics committee.

Clinical evaluation and echocardiographic examination

Each patient had a detailed medical history, physical examination, baseline electrocardiogram and a transthoracic echocardiography using an echocardiograph equipped with a broadband transducer (Vivid 7, GE Vingmed, Horten, Norway). Measurements of the left atrium, left ventricle and right ventricle were obtained from parasternal long axis and apical four-chamber view according to standard criteria. LVEF was calculated using the modified Simpson's rule in the apical 2- and 4-chamber views. Mitral flow was measured from the apical 4-chamber view with Pulsed Wave (PW)- Doppler by placing the sample volume at the tips of mitral leaflets.

Laboratory testing

Fasting blood samples were drawn from a large antecubital vein in each patient for determination of laboratory testing during the first 1 to 3 days of hospitalization. Brain Natriuretic Peptide (BNP) levels were measured by the commercially available assay kit (Biosite Inc., San Diego, California, USA) which is a fluorescence immunoassay for the quantification of BNP in 24 h. Serum highly sensitive C-Reactive Protein (hs-CRP), was measured by a sensitive nephelometric assay. The samples were centrifuged for 10 min and serum FT3 (free-T3), FT4 (free-T4), and TSH levels were measured by means of an advanced immunoassay system (Siemales Medical Solutions USA, Inc.; Malvern, Pa). Uric acid levels were evaluated by automated biochemistry analyzer (Aeroset, Abbot, Minnesota, USA). Hcy level was determined by a High-Pressure Liquid Chromatography (HPLC) method by using commercial kit (Recipe, Chemicals & Instruments, GmbH, Labortechnik, Munich, Germany) during the first 24 h to 72 h. Sedimentation, albumin, creatinine, hemoglobin and lipid levels were measured according to standard methods.

Statistical methods

Continuous variables were given as mean ± SD and categorical variables were defined as percentages. Normality of continuous variables distribution was tested using Kolmogorov-Smirnov test. Normally distributed continuous variables were compared using independent-samples t test and unequally distributed variables were analyzed with Mann-Whitney U test. Categorical data and proportions were analyzed using Chi-square ($\chi 2$) test. Univariate Cox proportional hazards models were used to evaluate the association of different variables between survivors and non-survivors. Measure of Hcy levels were entered as continuous variables or categorized in dichotomous fashion. In multivariate analysis, Hcy levels adjusted for all variables with p-value <0.05 in univariate analysis. The outcome was evaluated by Kaplan-Meier survival analysis using the determined cut-off value. Pearson correlation analysis was used to evaluate related parameters with homocysteine. Subgroup analysis was also performed for females and males depending on whether the patients have CAD or not. Clinical events were defined as cardiac death including sudden death and death attributable to advanced heart failure in male and female patients with CHF. A p value less than 0.05 was considered as statistically significant. All statistical work-including graphical representations- were performed with the SPSS 13.0 (SPSS Inc., Chicago, IL, USA) statistical software package program.

Results

A total of 602 patients were included into the study. The median follow-up duration was 38 ± 15 months. The mean age of the cohort was 63 ± 13 years with mean ejection fraction of 26 ± 10%. All the patients had CHF with NYHA class II to IV. The baseline characteristics that were analyzed according to patients' genders were almost similar, except that females had a higher LVEF, higher prevalence of hypertension and lower prevalence of CAD. The ratios of diabetes mellitus and chronic renal disease were comparable between the groups (Table 1).

There were 233 cardiac deaths amongst the study population which means an overall cardiac mortality rate of 38.7%. 1-year and 3-year cardiac mortality rates of the study population were 10.8% and 30.2%, respectively. Cardiac death rate was 41% (88 patients) among the females and 37% (145 patients) among the males that demonstrated no statistically significant difference. Survivors were younger, had lower NYHA functional class, reduced hs-CRP levels and decreased urea levels compared to non-survivors due to cardiac mortality in females. Male patients who died due to cardiac reasons were older and had higher NYHA functional class. Male non-survivors had higher Hcy levels, increased BNP levels, increased urea levels, higher creatinine levels, decreased sodium levels and reduced LVEF than the male survivors in the cohort (Table 2).

While Hcy levels were not statistically different between survivors and non-survivors in female patients (17.2 ± 7.8 μmol/L *vs.* 17.5 ± 7.7 μmol/L, p=NS), male non-survivors had significantly higher Hcy

Table 1: Baseline characteristics of female and male patients with chronic heart failure.

Characteristic	Female (n=215)	Male (n=387)	p value
Mean age (yrs)	63 ± 14	63 ± 12	NS
BMI	28 ± 11	25 ± 9	NS
LVEF (%)	28.1 ± 11.7	25.7 ± 10.5	0.002
Mean NYHA	2.8 ± 0.5	2.8 ± 0.5	NS
Blood Pressure (mmHg)	126/77	124/76	NS
Homocysteine (micromol/L)	17.3 ± 7.7	18.1 ± 8.0	NS
Sodium (mmol/L)	138 ± 5	137 ± 5	NS
BNP (pg/mL)	1153 ± 972	1151 ± 1023	NS
hs-CRP (pg/mL)	2.67 ± 4.85	2.60 ± 3.97	NS
Creatinine (mg/dL)	1.4 ± 1.3	1.5 ± 1.1	NS
Urea (mg/dL)	63 ± 38	61 ± 35	NS
Medication			
Beta Blocker (%)	155 (72%)	271 (70%)	NS
ACE-I/ARB (%)	166 (77%)	333 (86%)	0.006
Spironolactone (%)	113 (53%)	165 (43%)	0.019
Loop diuretics (%)	180 (84%)	306 (79%)	NS
Statin (%)	125 (58%)	245 (63%)	NS
Co-morbidities			
CAD	105 (49%)	280(72%)	<0.001
Hypertension (%)	172 (80%)	283 (70%)	0.008
Diabetes mellitus (%)	86 (40%)	132 (34%)	NS
Chronic Renal Disease (%)	56 (26%)	101 (26%)	NS

ACE-I/ARB: Angiotensin Converting Enzyme Inhibitors/Angiotensin Receptor Blockers; BNP: Brain Natriuretic Peptide; BMI: Body Mass Index; hs-CRP: Highly sensitive C-Reactive Protein; LVEF: Left Ventricular Ejection Fraction; NYHA: New York Heart Association; NS: Not Significant

levels compared to male survivors (19.4 ± 8.1 μmol/L *vs.* 17.4 ± 7.8 μmol/L, p=0.015) (Table 2).

In statistical analysis it was found that while homocysteine had a positive correlation with age, urea and creatinine, hs-CRP and LVEF in females, it showed negative correlation with sodium in female group. In male patients, homocysteine had a positive correlation with age, urea, creatinine, and hs-CRP (Table 3).

Table 3: Correlated parameters with homocysteine in female and male patients in chronic heart failure.

	Female patients (n=215)		Male Patients (n=387)	
Homocysteine	R	p	R	P
NYHA	0.04	NS	0.1	0.044
Age	0.49	<0.001	0.39	<0.001
Urea	0.38	<0.001	0.34	<0.001
Creatinine	0.29	<0.001	0.23	<0.001
Sodium	-0.14	0.03	-0.05	NS
Hs-CRP	0.22	0.001	0.18	<0.001
Ejection Fraction	0.14	0.043	0.06	NS
BNP	0.14	NS	0.06	NS

BNP: Brain Natriuretic Peptide; Hs-CRP: Highly sensitive-C Reactive Protein; NYHA: New York Heart Association; NS: Not Significant

The patients were further separated to the 4 groups. Group 1: female patients with CAD; Group 2: female patients without CAD; Group 3: male patients with CAD and Group 4: male patients without CAD. When the analyses were repeated according to these groups as mentioned above female patients who had CAD (group 1) had no any statistical difference between survivors and non-survivors in terms of Hcy levels (17.1 ± 7.0 μmol/L *vs.* 18.3 ± 8.5 μmol/L, p=NS) but there was still a statistically significant difference between male non-survivors and survivors (group 3) in terms of Hcy levels (20.2 ± 8.6 μmol/L *vs.* 17.8 ± 8.2 μmol/L, p=0.02). Patients without CAD in both genders (group 2 and group 4) no statistical differences were identified between survivors or non-survivors in terms of Hcy levels.

In multivariate analysis, when Hcy levels were adjusted for the other variables (age, NYHA class, CAD, urea level, creatinine level, sodium level, BNP level), age (hazard ratio (HR): 1.041; 95% Confidence Interval (CI), 1.025-1.056; p<0.001), sodium level (HR: 0.952; 95% CI, 0.925-0.978; p<0.001) and Hcy level (HR: 1.026; 95% CI, 1.008-1.046; p=0.006) appear to be an independent predictor for cardiac death in male patients with CHF (Table 4). In female patients, age (HR: 1.020; 95% CI, 1.001-1.039; p=0.042), NHYA class (HR: 2.091; 95% CI, 1.232-3.551; p=0.006) and hs-CRP level (HR: 1.056; 95% CI, 1.010-1.104; p=0.017) emerged as an independent factor of cardiac death in female CHF patients.

Optimal prognostic cut-off values for Hcy levels were considered in sensitivity and specificity at ROC curves. A 12.65 mg/dL cut-off

Table 2: Baseline characteristics of survival and non-survival patients.

	Female			Male		
	Survival Patients (n=125)	Non-survival Patients (n=88)	P value	Survival Patients (n=242)	Non-survival Patients (n=145)	P value
Age (years)	59 ± 14	70 ± 11	<0.001	61 ± 12	68 ± 11	<0.001
NYHA Class	2.7 ± 0.5	3.0 ± 0.4	<0.001	2.7 ± 0.5	3.1 ± 0.5	<0.001
BMI (kg/m^2)	27 ± 6	26 ± 4	NS	27 ± 4	26 ± 4	0.06
Homocysteine (mg/dL)	17.2 ± 7.8	17.5 ± 7.7	NS	17.4 ± 7.8	19.4 ± 8.1	0.015
BNP (pg/mL)	1141 ± 1012	1170 ± 916	NS	972 ± 852	1445 ± 1200	<0.001
Hs-CRP (mg/dL)	1.6 ± 2.5	3.8 ± 6.0	0.002	2.3 ± 3.4	3.0 ± 4.7	NS
Urea (mg/dL)	58 ± 39	70 ± 36	0.029	56 ± 32	71 ± 38	<0.001
Creatinine (mg/dL)	1.4 ± 0.9	1.4 ± 1.5	NS	1.4 ± 1.0	1.6 ± 1.2	0.06
Sodium (mEq/L)	138 ± 5	138 ± 5	NS	138 ± 4	136 ± 6	<0.001
LVEF (%)	29 ± 12	27 ± 12	NS	26 ± 10	23 ± 10	0.002

BNP: Brain Natriuretic Peptide; BMI: Body Mass Index; Hs-CRP: Highly sensitive C-Reactive Protein; LVEF: Left Ventricular Ejection Fraction; NYHA: New York Heart Association; NS: Not Significant

Table 4: Cox Regression analysis for cardiovascular death in male patients with chronic heart failure patients.

Male patients	Hazard Ratio	95% CI	p Value
Age	1.041	(1.025-1.058)	<0.001
Homocysteine	1.025	(1.006-1.045)	0.011
Sodium	0.952	(0.925-0.978)	<0.001

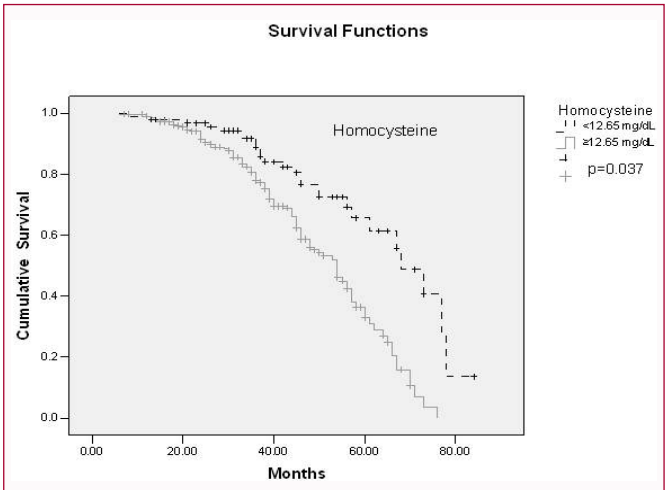

Figure 1: Event- free survival for patients with homocysteine <12.65 mg/dl (dash line) and those with ≥ 12.65 (gray line) survival curve is significantly different between the two groups in male patients (p=0.037).

value for Hcy level predicted cardiac death with a sensitivity of 82% and a specificity of 73% in ROC curve analysis for male patients. Cumulative survival curves (Kaplan–Meier Curves) showed more cardiac events in patients with a higher Hcy levels (≥ 12.65 μmol/L) than lower Hcy levels (<12.65 μmol/L) (Figure 1).

Discussion

This study showed that in hospitalized patients with heart failure with reduced ejection fraction, Hcy levels were higher in non-survivor male patients compared to survivor male patients. However, there was no statistically significant difference between survivor and non-survivor female patients in terms of Hcy levels. Further analysis showed that there was only statistically significant difference between survivor's Hcy levels and non-survivor's Hcy levels in males if the patients had CAD. In multivariate analysis Hcy level was one of the independent predictors for cardiac mortality in male gender.

Gueant-Rodriguez et al. [16] determined that Hcy level was significantly higher in patients with a decreased LVEF than in those without ventricular dysfunction. In addition, a positive association between Hcy level and the serum N-terminal pro-B type natriuretic peptide (NT-proBNP), serum creatinine, uric acid and C-reactive protein was observed in the same study [16]. Previous study shows that in patients with CHF, whom 72% of them were male, increased Hcy levels were common with a mean plasma level of 12.5 ± 5.5 μmol/L.

Furthermore, the patients with increased Hcy levels were older and had advanced NYHA functional class [17]. In another study it was shown that Hcy was an important marker for increased cardiovascular risk in patients with CHF and a Hcy level of >/= 20 μmol/L was associated with a high risk to decompensation or mortality in these group of patients. It was also found a positive correlation between increased Hcy levels and the severity of the disease, BNP and uric acid [18]. In our study Hcy levels positively correlated with functional class, age, serum urea, and creatinine and hs-CRP levels in males and there was also a positive correlation between serum Hcy levels and age, serum urea, creatinine, sodium levels in females.

High homocysteine levels cause a reduction of vessel radius by thickening the arterial wall [10,19]. In addition, it increases matrix metalloproteinase activity resulting in an alteration of the elastin/collagen ratio and reduced compliance of the arterial walls [11,20]. Hcy reduces nitric oxide bioavailability by stimulating the formation of reactive oxygen species [9,21]. It is known that Hcy also increases the platelets' adhesion and aggregation, favoring the formation of the arterial thrombi [12,13,22,23]. Furthermore, it also stimulates LDL oxidization as well as smooth-muscle cell proliferation [13,14,24,25]. Jamaluddin et al. demonstrated that HHcy exerts highly selective inhibitory effects on cyclin A transcription and endothelial cell growth through a hypomethylation related mechanism which blocks cell cycle progression and endothelium regeneration which happens by DNA methyltransferase 1 activity reduction [26]. It is known that cyclin A levels are increased in a variety of conditions such as tumors, in normal cardiac myocyte growth, in cardiac hypertrophy and in atherosclerosis. Cyclin A suppression appears as a mechanism responsible for growth-inhibition in endothelial cells contributing to cardiovascular diseases [27]. In the present study we determined that Hcy levels were higher in non-survivor male patients. Further analysis of our data showed that only male patients with CAD have statistically significant difference between Hcy levels of survivors and non-survivors but we did not find any significant statistical difference between survivors and non-survivors in male patients without CAD. This is an interesting finding because it could help save patients' life if we may manage to reduce Hcy levels successfully in this group of patients. It is also possible to speculate that increased Hcy levels might be related to CAD and death in males with CHF or at least there may be a causal relationship between CAD and increased Hcy levels.

Many randomized trials have tried to show effects of folic acid and/or vitamins B6 and B12 supplementation on multiple markers of cardiovascular disease. Homocysteine Studies Collaboration has demonstrated a reduced risk of ischemic heart disease by 11% and stroke by 19% per 3 μmol/L reductions in homocysteine concentration in patients with HHcy treated with folic acid and B vitamins [27]. The Swiss Heart Study has shown that Hcy-lowering therapy with folic acid, vitamin B12, and vitamin B6 has significantly decreased the rate of target lesion revascularization and a non-significant trend toward has been seen in terms of fewer deaths and nonfatal myocardial infarctions with Hcy-lowering therapy [28]. Contrary to previous findings, Lange [29] reported that the administration of folate, vitamin B6, and vitamin B12 after coronary stenting may increase the risk of in-stent restenosis and the need for target-vessel revascularization in their study. Folate therapy had adverse effects on the risk of restenosis in all subgroups except for women, patients with diabetes, and patients with markedly elevated Hcy levels (15 μmol/L or more) at baseline [29]. Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) Collaborative Group concluded that folic acid/vitamin B12 supplementation did not have any beneficial effects on vascular outcomes. There were no significant differences in the numbers of deaths attributed to vascular causes or nonvascular causes or in the incidence of any cancer [30]. Although Hcy lowering therapies failed to show benefit to reduce Hcy level with folic acid, vitamin B6 and vitamin B12, many evidences showed that increased Hcy levels were both a marker and a risk factor of

atherosclerosis [31]. Available evidence did not support the routine use of B vitamins to prevent cardiovascular disease. Folic acid and vitamin B supplementation did not show a significant reduction in regard to the rate of myocardial infarction, stroke and peripheral disease in previous studies in spite of decreased Hcy levels [32,33].

There are conflicting results between the observational and cohort vitamin trials about the prevention of cardiovascular disease. While the results of the vitamin trials may be confounded by concomitant treatments and folic acid fortification, their results do not allow reliable estimation of vitamin therapy for cardiovascular prevention [34]. Besides, the meta-analysis and stratified analysis of the interventional vitamin trials revealed that several the trials were effective in reducing the risk of stroke and cardiovascular disease [35]. Though some previous disappointed results, a recent study have showed an inverse relationship between B-vitamins intake and DNA-methylation of the candidate genes that are related to the One-Carbon Metabolism (OCM) and in the Hcy pathways. Authors have concluded that DNA-methylation patterns in specific regions of OCM and Hcy pathways genes may modulate the cardiovascular disease risk conferred by folic acid and B-vitamins low intake [36].

The use of B-vitamins for secondary prevention may be an option if statin therapy is accompanied by serious adverse effects and should be omitted [35]. There is no clear data about the effects of using B vitamins and folic acid on mortality and morbidity in CHF. But it is accepted that the statins are not beneficial as an adjunctive therapy when prescribed solely for the diagnosis of heart failure in the absence of other indications for their use [37]. The third update of the Cochrane review showed that there were no differences in effects of homocysteine-lowering interventions in the form of supplements of vitamins B6, B9 or B12 given alone or in combination comparing with placebo on myocardial infarction, death from any cause or adverse events [38]. A recent research demonstrated that selective homocysteine lowering gene transfer improves infarct healing, attenuates remodeling, and significantly enhances diastolic function post-MI in female mice's. Authors concluded that the study has corroborated the view that hyperhomocysteinemia exerts direct effects on the myocardium and may potentiate the development of heart failure [39].

Conclusion

Our study showed that serum Hcy levels were significantly increased in male non-survivors compared to male survivors who had heart failure with severe systolic dysfunction while the difference did not reach statistical significance between female groups. Low prevalence of CAD in women could partly explain the absence of an association between plasma Hcy level and mortality in females. Previous studies did not evaluate the relationship between Hcy levels and gender in patients with severe systolic dysfunction such in our cohort. So, our study is unique as it shows the effect of higher Hcy levels on the mortality of female and male genders in CHF. The study also showed that females and males could have different risk factors or predictors for cardiac mortality in severe systolic heart failure. It is difficult to conclude that either Hcy was a causal risk factor or it is a marker of cardiovascular disease and CHF. It seemed that higher Hcy levels were associated with a higher risk of cardiac death in male patients with CHF and CAD. Elevated Hcy levels might be both a risk factor and a marker of CAD in males with CHF. It is not clear if homocysteine lowering therapies and measures were started earlier for example when the patients were diagnosed with heart failure and/or CAD it would be more effective. Since Hcy levels were positively correlated with urea and creatinine renal protection measures seem especially important to manage Hcy levels in patients. Prospective randomized studies in similar populations would provide more precise results and a response as to the predictive value of Hcy levels in males and females patients with heart failure.

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