The Importance of Pro-inflammatory Cytokines in Patients with Chronic Heart Failure and Chronic Kidney Disease and Type 2 Diabetes

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Abstract

Background: Heart failure (HF) often coincides with diabetes type 2 (DM) and chronic kidney disease (CKD). In the course of these disorders markers of inflammation such as tumour necrosis factor α (TNFα) and interleukin 6 (IL-6) are elevated. Inflammatory cytokines are markers of bad prognosis. The aim of the study was to assess the severity of inflammation in the course of HF, DM and CKD. We also evaluated the prognostic value TNFα and IL-6 serum concentration.

Methods: 129 patients with HF were enrolled. 50% of the patients had DM, 50% CKD of the stage 3 and 30% patients had both diseases. The serum concentration of TNF-α and IL-6 were assessed by ELISA method. Endpoints are death and hospitalization due to worsening of HF during the 12 months of follow-up.

Results: The lowest serum concentration of IL-6 was in patient with only HF, the highest in patients with HF and DM The highest serum concentration of TNF-α was found in patients with HF, DM and CKD and the lowest in patients with only HF. IL-6 was the predictor of the composite end point in multivariate analysis. TNF-α was not found to be a predictive factor.

Conclusions: Inflammatory condition presented by concentration of pro-inflammatory cytokines seems to be the lowest in patients with heart failure only and increases when comorbidities appear. Only IL6 has the prognostic value. Elevated concentrations of IL-6 increases the risk of the composite end point.

Keywords: Diabetes type 2; Heart failure; Chronic kidney disease

Introduction

In the XXI-st century we can observe higher incidence and frequency of chronic heart failure that is taking on epidemic proportions. Symptomatic stage of chronic heart disease (stage C according to ACC/AHA; functional class II and III according to NYHA) is estimated in 0.4-2% of the European population, which means that the problem concerns about 6.5-10 million Europeans. This condition is progressive and has poor prognosis. Annual mortality rate for NYHA stage II and III is up to 40%. If chronic heart failure coincides with diabetes type 2 and chronic kidney disease, the prognosis for patients gets even worse. Chronic kidney disease and diabetes are recognized cardiovascular risk factors. They can contribute to further myocardial damage and progression of heart failure and makes prognosis worse.

In case of heart failure, inflammation begins with the increase in concentration of pro-inflammatory cytokines. TNF-α (tumor necrosis factor-α) is a pleiotropic cytokine [1]. TNF-α, apart from being cytotoxic and cytostatic, has also influence on the growth and differentiation and activity of almost all types of cells, including cardiomyocytes [2]. Receptors for TNFα type 1 (tumor necrosis factor receptor 1) and type 2 (tumor necrosis factor receptor 2) are present both in a failing and in a healthy cardiac muscle. Majority of adverse effects- mainly negative inotropic effect, are attributed to TNFR1 [3,4]. Interleukin-6 (IL-6) is a multifunctional cytokine that acts as a mediator in inflammatory reactions. Presence of IL-6 was found after infusion of TNF-α, as well as after stunned myocardium [5,6]. Experimental research shows that chronic IL-6 receptor stimulation causes significant concentric left ventricular hypertrophy [7,8]. Increased concentration of IL-6 causes negative chronotropic and inotropic effects and leads to decrease of intercellular calcium during cardiomyocyte contraction [9,10].

It has been found that pro-inflammatory cytokines are present in early stages of myocardium damage and as the disease progresses the concentration of these cytokines gradually increases [11,12]. Activation mechanisms of the immune system in chronic heart failure are not completely clear. The source of cytokines lies in a number of pathophysiological processes that increase gradually together with the progression of myocardial dysfunction.

Type 2 diabetes occurs in 30% of patients with chronic heart failure, as often as chronic kidney disease. There is no accurate data on the

coexistence of these three diseases. There is pathophysiological relationship between chronic heart failure, chronic kidney disease and diabetes. Type 2 diabetes is the cause of chronic kidney disease and chronic heart failure. In turn, chronic kidney disease can lead to cardiovascular diseases. Myocardial dysfunction often leads to the deterioration of kidney function. Inflammatory process gets also activated in the course of a chronic kidney disease and diabetes [13,14].

Probably patients with chronic heart failure, chronic kidney disease and diabetes - reach the highest level of pro-inflammatory cytokines concentration which are markers of disease severity. Both IL-6 as well as TNF-α, can also influence the prognosis of patients with heart failure, but the prognostic impact of cytokines in patients with three diseases has not been proved by clinical research yet.

Materials and Methods

The study included prospective 134 patients hospitalized in the year 2012 due to exacerbation of chronic heart failure with impaired systolic function. The patients were included in the research when in progress of after hemodynamic parameters stabilization a day before or on the day of leaving the hospital. All participants provided written consents. Local Bio-ethic Committee approved the protocol (KB/ 34/2012).

According to the study protocol part of patients included in the research have also type 2 diabetes and chronic kidney failure in stage 3.

Inclusion criteria in the research were as follows: age>18 years and <80 years, chronic heart failure diagnosed at least three months earlier, irrespectively of etiology with ejection fraction EF<45% in functional class NYHA II or NYHA III, chronic kidney disease in stage 3 with estimated GFR<60 and $\geq$ 30 ml/min/1.73 m^2 and/or diabetes type 2.

Exclusion criteria were: lack of patient's consent, acute coronary syndrome < 3 months, serious comorbidities: decompensated cirrhosis, cancer, serious chronic obstructive pulmonary disease, autoimmune diseases, anemia with Hgb<11.0 g/dl, thyroid disorders and other endocrynological diseases and acute infections.

The patients were observed for 12 months. The end points included: death for any reason, first hospitalization due to decompensation of heart failure. Data was collected by means of phone contacts, and during subsequent hospitalization.

Initially, 134 patients were enrolled in the study. In the course of research three patients withdrew their consent and two others could not continue due to occurrence of other serious diseases (malignant lung cancer and central nervous system tumor). Finally, 129 patients were analyzed (38 women and 91 men) and divided into 4 groups:

1. patients with heart failure only (n=32),
2. patients with heart failure and diabetes type 2 (n=33),
3. patients with heart failure and chronic kidney disease in stage 3 (n=32),
4. patients with heart failure, chronic kidney disease in stage 3 and diabetes type 2 (n=32).

On the day of enrollment information on reasons for chronic heart failure, comorbidities, pharmacological and invasive treatment was obtained. Heart failure was classified according to functional classification NYHA. BMI (body mass index) and WHR (waist-hip ratio) were calculated. Blood tests were made in order to assess biochemical parameters– pro-inflammatory cytokines.

For assessment of glomerular filtration rate formula CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) was used as recommended by KDIGO.

IL-6 and TNF-α were measured by means of enzyme-linked immunosorbent assay (ELISA) with the use of sets produced by R&D Systems Minneapolis, USA, according to producer's recommendations. For IL-6 the applied set was Quantikine ELISA Human IL-6 Immunoassay and for TNF-α Immunoassay.

Statistics

Methods that have been used in descriptive statistics: mean values, standard deviation, median. Shapiro-Wilk test was used to examine the normality of distribution features. For the analysis of links between factors correlation coefficient was used. Comparison of analyzed parameters among groups was made by means of analysis of variance (for normal variables) and Wilcoxon test (for variables that do not meet normal frequency). Predictive values of the analyzed variables and the occurrence of hospitalization or death were defined on the basis of logistic regression with the support of odds ratio (Odds Ratio, OR) with 95% confidence interval on OR. Predictive value of the analyzed variable was depicted by means of ROC (receiver operating characteristic) and a calculated AUC (area under curve). In time analysis until hospitalization or death, assessed together and separately, Kaplan-Meier method was used.

Statistical tests were based on two-sided statistical hypothesis with assuming the level of statistical significance P<0.05. Calculations were made by means of statistical package SAS ver. 9.2.

Results

The patients did not show significant differences in terms of etiology of heart failure. Similar frequency was observed for the occurrence of atrial fibrillation, hypertension and heart valve disease in all the groups of patients. The most dominant group among all the patients were patients of functional class NYHA III (n=89; 68.9% vs. n=40; 31.0%; p=0.002). The biggest number of patients with functional class NYHA III was found in group 4 (87.5%; p=0.001), the smallest in group 1 (43.7%). Ejection fraction was similar in all groups of patients, average EF was between 25% and 31% (p-0.3) (Table 1).

The groups did not differ in applied treatment for heart failure, except for hipolipemic therapy by statins that was common in patients with diabetes type 2. Over 90% of patients in all groups were prescribed β- blockers, from 68% to 81% patients took angiotensin-converting enzyme inhibitors and from 56% to 75% aldosterone antagonists. Cardioverter-defibrillator and cardiac resynchronization therapy were used in 12%-30% of patients (Table 1).

	Group 1	Group 2	Group 3	Group 4	p
	CHF	CHF +DM	CHF +CKD	CHF+CKD +DM	
Number	n=32	n=33	n=32	n=32	
Sex (female)	7 (21.8%)	9 (27.7%)	11(34.3%)	11(34.3%)	0.6
Age	57 ± 10	65 ± 7	69 ± 9	71±±7	<0.001
BMI	27.4 ± 5.3	30.5 ± 6.3	27.1 ± 6.0	29.8 ± 5.1	0.01

WHR	1.00 ± 0.09	1.03 ± 0.1	0.97 ± 0.09	1.02 ± 0.08	0.04
NYHA III	14 (43.7%)	22 (66%)	25 (78%)	28 (87.5%)	0.001
Valve disease	5 (15.6%)	1 (3%)	6 (18.7%)	4 (12.5%)	0.2
IHD	19 (59.4%)	26 (78.7%)	20 (62.5%)	27 (84.5%)	0.06
DCM	11 (34.3%)	6 (18.1%)	9 (28.1%)	4 (12.5%)	0.1
CABG	0	5 (15.1%)	1 (3.1%)	4 (12.5%)	0.06
PCI	9 (28.1%)	16 (48.4%)	12 (37.5%)	15 (46.8%)	0.3
Atrial fibrillation	15 (46.8%)	18 (54.5%)	16 (50%)	14 (43.7%)	0.8
Hypertension	16 (50%)	25 (75.7%)	21 (65.6%)	23 (71.8%)	0.1
EF %	28 ± 6	30 ± 8	30 ± 6	31 ± 6	0.3
NTproBNP (pg/ml)	3772 ± 260	3528 ± 575	4445 ± 989	4808 ± 1213	0.5
Creatinine (mg/dl)	1.12 ± 0.24	1.02 ± 0.24	1.45 ± 0.25	1.57 ± 0.32	<0.001
eGFR (ml/min/1.73m2)	72.5 ± 10.2	74.3 ± 10.5	50.1 ± 6.5	43.2 ± 7.3	<0.001
ICD/CRT	4 (12.5%)	10 (30.3%)	8 (25%)	7 (21.8%)	0.3
ACE-I	22 (68.7%)	27 (81.8%)	24 (75%)	25 (78.1%)	0.6
ARB	2 (6.25%)	0	1 (3.13%)	1 (3.13%)	0.5
- adrenolytics	31 (96.8%)	30 (90.9%)	31 (96.8%)	30 (93.7%)	0.6
MRA	24 (75%)	25 (75.7%)	21 (65.6%)	18 (56.2%)	0.2
Diuretics	28 (87.5%)	31 (93.4%)	31 (96.8%)	31 (96.8%)	0.3
Digitalis	12 (37.5%)	14 (42.4%)	11 (34.3%)	7 (21.8%)	0.3

Table 1: Basic characteristic of patients; ACE-I- Angiotensin-Converting Enzyme Inhibitors; ARB-Angiotensin Receptor Blocker II; BMI- Body Mass Index; CABG0- Coronary Artery Bypass Surgery; CKD- Chronic Kidney Disease in stage 3; CRT- Cardiac Resynchronization Therapy; DCM- Dilated Cardiomyopathy; DM-Diabetes type 2; EF- Ejection Fraction; eGFR-Glomerular Filtration Rate; CHF- Chronic Heart Failure; ICD- Implantable Cardioverter-Defibrillator; MRA- Mineralocorticoid Receptor Antagonists; WHR-Waist-Hip Ratio; PCI-Precutaneous Coronary Intervention; VKA-Vitamin K Antagonists.

Studied groups of patients significant differences were found concerning plasma concentration of pro-inflammatory cytokines (Table 2). TNF-α was of the highest level in group 4. (106.9 ± 28.5 pg/ml), in relation to the lowest level found in group 1. (22.4 ± 7.8 pg/ml;

p<0,05). IL-6 was of the highest level in patients in group 2. (11.49 ± 5.6 pg/ml), then in group 4. (10.8 ± 8.6 pg/ml), with the lowest level in patients with heart failure only (group 1)- 7.08 ± 6.5 pg/ml (p<0.05).

	Group 1	Group 2	Group 3	Group 4	p
	CHF	CHF+DM	CHF+CKD	CHF+CKD+DM	
IL-6 (pg/ml)	7.08 ± 6.5	11.49 ± 5.6	10.31 ± 6.9	10.8 ± 8.6	<0.05
TNF-a (pg/ml)	22.4 ± 7.8	70.3 ± 16.2	97.9 ± 25.3	106.9 ± 28.5	<0.05

Table 2: Results of pro-inflammatory cytokines tests; CKD- Chronic Kidney Disease in stage 3; DM- Diabetes type 2; CHF- Chronic Heart Failure; IL-6- Interleukin 6; TNF-α: Tumor Necrosis Factor Type α.

Correlation between pro-inflammatory cytokines and other parameters was assessed (Table 3). Interleukin-6 showed positive correlation with NT pro BNP (p=0.007; r=0.23) and negative correlation with left ventricular ejection fraction (p=0.03; r=-0.18) and fT3 (p=0.02; r=-0.19). No correlations were found between IL-6 and parameters of kidney function, BMI, insulin resistance index. The research did not show any correlation between TNF-α and parameters of kidney function, BMI and level of ejection fraction (Table 3).

|	Death	Hospitalization	Death+Hospitalization
EF	-	OR 0.94	OR 0.94
		95% CI 0.90-0.98	95% CI 0.91-0.97; p=0.001
		p=0.007	
BMI	-	OR 1.04	-
		95%CI 1.00-1.09	
		p=0.03	
IL-6	-	-	OR 1.01
			95% CI 1.00-1.03; p=0.01
Creatinine	-	-	OR 2.63
			95%CI 1.26-5.26; p=0.01
NTproBNP	OR 2.18	-	-
	95% CI 1.40-3.41 p<0.001		

Table 3: Multivariate analysis of end points; BMI- Body Mass Index; EF- Ejection Fraction; IL-6- Interleukin 6; NTproBNP- N-terminal pro B-type Natriuretic Peptide; NS- Non-Significant for Statistics.

During the study 22 people died (17%), 44 (34%) patients were hospitalized due to decompensation of heart failure, altogether end point occurred in 51% of patients. Frequency of end points occurrence did not differ significantly between the groups

In univariate analysis TNF-α was not found to be a predictive factor in the univariate analysis Il-6 increased the probability of death (OR

1.02; 95% CI 1.00-1.04, p=0.03), hospitalization (OR 1.01; 95% Cl 1.00-1.03 p=0.03) and composite end point (p=0.002; OR 1.02; 95% CI 1.00-1.03). Predictive factors for death in the univariate analysis included: heart valve disease, HOMA-IR, creatinine, IL-6, NTproBNP, fraction HDL-cholesterol. Predictors of hospitalization were: female, diabetes type 2, left ventricular ejection function, body mass index. Predictive factors for composite end point were: WHR, EF, creatinine, NTproBNP, HDL-cholesterol.

Multivariate analysis of end points was performed for independent variables: NTproBNP, IL-6, BMI, creatinine level, EF. An independent predictive factor for death was plasma NTproBNP level, while for hospitalization concentration of creatinine and high BMI. IL-6 was an independent factor of composite end point similar to left ventricular ejection fraction and condensation of creatinine. Increase of IL-6 by 1 pg/ml increased the probability of end point occurrence by 1% in the course of observation (OR 1.01; 95% CI 1.00-1.03). Cut-off point for IL-6 as a predictive factor for composite end point was 4.20 pg/ml with AUC amounting to 0.533 (Figure 1).

Figure 1: ROC for IL-6 as a composite predictive factor for composite end point, x-cut-off point, specificity, sensitivity–AUC 0.56.
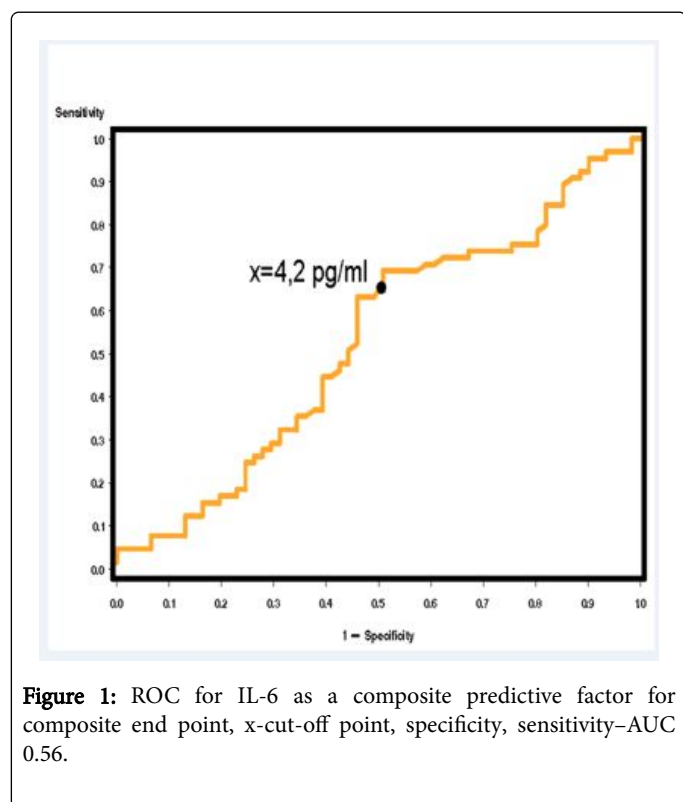
Discussion

Results of our study prove the hypothesis, that with the increase of the number of diseases in patients with heart failure, inflammation is developed. There are some publications on to this subject, but there is no publication that would assess increase of inflammation coinciding with heart failure, chronic kidney disease and diabetes type 2. In research by Lassus et al. higher concentration of IL-6 and TNF-α was shown in patients with heart and kidney dysfunctions than in patients with heart failure only [15]. The study of Lassus at al. did not concern abnormalities in glucose metabolism.

In the present research, IL-6 showed positive correlation with diabetes type 2, so it can be assumed that diabetes is connected with the activation of inflammation, similarly to dysfunction of kidneys and myocardium. Similar results were presented by Marques-Vidal at al., who revealed that diabetes type 2 shows a link with some inflammatory parameters IL-6, CRP – C - reactive protein) [16]. They did not observe, however, such relations for TNF-α, which is in accord with my research. Having analyzed the population of Framingham, a relation was found between IL-6, as well as TNF-α with diabetes type 2 [17]. However, according to the researchers, activation of immune system is the result of diabetes type 2, not the reason for it. Higher concentration of pro-inflammatory cytokines probably is linked with chronic complications of the disease, while does not constitute a factor for its occurrence [18]. It seems that these conclusions apply to the present research, because one of the main complications of diabetes is reduced glomerular filtration, and the highest concentration of TNF-α was found in group 4.

The present research has shown that concentration of IL-6 increases together with the decrease of left ventricular ejection fraction, however, no dependencies were shown for kidney functioning parameters and insulin resistance. TNF-α does not show any relation with any of the assessed parameters. Most researches do not a present simple and explicit relation between inflammation and the level of kidney and myocardium dysfunctions. Oberg at al. proved that patients with chronic kidney disease in stages 3-5, in comparison with healthy patients, have higher concentration of IL-6, which does not correlate with eGFR [19], as in the present research. Borrayo-Sánchez et al. stated that IL-6 as a marker for inflammation was higher in patients with ischemic heart failure after, especially in a group of patients with eGFR<60 ml/min/1.73 m^2 [20]. While Koller-Strametz at al. showed a relation between the concentration of pro-inflammatory cytokines (IL-6 and TNF-α) and severity of heart failure defined by functional class NYHA. They found relation between TNF-α and creatinine concentration but did not prove such a dependency for IL-6 [21]. Ambiguous relation between cytokines and functioning of kidney and heart probably results from a lack of organ specificity of these inflammatory substances. In case of TNF-α presence of two receptor isoforms of different activity may additionally influence the final effect of cytokine. It may be assumed that a project that would apply soluble receptor proteins in blood serum, sTNFR1 and sTNFR2 would give more accurate data.

Data from specialist literature shows similar results concerning the relations between pro-inflammatory cytokines and echocardiographic parameters. Petretta et al. proved that IL-6 correlates with left ventricular ejection fraction in contrast to TNFα [22]. Romeo at al. did not prove the relation between TNF-α and EF either [23].

Previous publications also prove influence of IL-6 on prognosis. Jug at al. showed that this cytokine is an independent factor that determines death in patients with chronic heart failure basing on a 2-year observation [24]. Also a randomized clinical research Vesnarinone trial (VEST) showed that IL-6 is an independent predictor of death (p=0.002) basing on a 12-month observation of patients with systolic heart failure [25]. Nakagomi et al., in a their study showed that IL-6 is an independent predictor of adverse cardiovascular events in patients with heart failure with a cutoff 160 pg/ml [26]. While Cabassi et al. in his project did not confirm prognostic value IL-6, that was assessed as an additional biomarker in Seattle Heart Failure Model, in patients of advanced age and with heart failure [27]. The differences in results can be accounted for by the size of average ejection fraction, that was significantly higher in Cabassi's project than in the present research. In the present research IL-6 was

an independent prognostic factor only for a composite end point. This kind of dependency was not found in case of deaths and hospitalization that were assessed separately and did not constitute a big number over the 12-month observation.

In the present research, predictive value TNF-α was not found in patients with heart failure, similarly to projects by Bielecka-Dabrowa et al., Cabassi et al. and Lassus et al., Gottlieb et al. [15, 27-29].

In the present research over a 12-month observation period 17% of patients died, 34% were hospitalized due to exacerbated heart failure, altogether 51% of patients experienced end point. The frequency of occurrence of end point did not differ much statistically among all the four groups.

In the analysis of DIG research (Digitalis Investigation Group Trial) published in 2009 patients with chronic heart failure were divided into subgroups according to the diabetes type 2 and chronic kidney disease with GFR<60 ml/min/1.73m^2 [30]. Over a 36-month observation it was found that total death rate was significantly higher in the group of patients with diabetes and impaired glomerular filtration in comparison to patients with diabetes type 2 only. The risk of death for patients with three diseases (HF+DM+CKD) was 34% higher than for patients with two diseases (CHF+DM). In the subgroup of patients with three diseases there was also a greater risk of hospitalization due to exacerbation of heart failure. The difference between DIG research and the present research may result from the three times longer observation time.

Data from ESC-HF Trial from 2013, concerning patients with heart failure with an average EF=38% showed that annual total death rate in patients with acute heart failure amounts to 17.4% (patients included during hospitalization), while in patients with chronic stable heart failure amounted to 7.2% (outpatients) [31]. Annual frequency of hospitalization due to exacerbation of heart failure amounted to 24.8% and 13.3% respectively. The above results are close to the present research, which included patients during hospitalization due to exacerbation of chronic heart failure.

Limitations

A potential limitation to the research is the number of patients in groups, which included 32-33 patients. Only 30% of patients in the project were women. Particular groups differed in demographic parameters such as: age, body mass index and WHR, frequency of hypolipidemic treatment. Diabetes was responsible for differences in anthropometric measurements (BMI, WHR) among groups, while age differences were a result of the applied enrollment to study- patients who agreed to be included in the research. The group of patients with three diseases was the oldest due to the fact that connections between diseases are of chronic character and GFR reduction appears in elderly and often after many years of diabetes or chronic heart failure. Functional class NYHA III was more often found in patients of group 4. Due to more advanced age and a greater number of diseases.

Conclusions

Inflammatory condition presented by concentration of proinflammatory cytokines seems to be the lowest in patients with heart failure only and increases when comorbidities appear. Pro-inflammatory cytokines influence the prognosis, but only IL-6 is an independent prognostic factor. An elevated concentration of IL-6 increases the risk of the composite end point.

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