Circulating Interleukin-10: Association with Higher Mortality in Systolic Heart Failure Patients with Elevated Tumor Necrosis Factor-Alpha

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ABSTRACT: **Background:** Interleukin-10 is an anti-inflammatory cytokine and consequently is considered by many to have a protective role in heart failure, as opposed to the notorious tumor necrosis factor-alpha.

Objectives: To test the hypothesis of the possible beneficial impact of IL-10 on mortality in systolic heart failure patients in relation to their circulating TNFα levels.

Methods: We measured circulating levels of IL-10 and TNFα in 67 ambulatory systolic heart failure patients (age 65 ± 13 years).

Results: Mortality was or tended to be higher in patients with higher levels (above median level) of circulating TNFα (9/23, 39% vs. 6/44, 14%; $P = 0.02$) or IL-10 (10/34, 30% vs. 5/33, 15%; $P = 0.10$). However, mortality was highest in the subset of patients with elevation of both markers above median (7/16, 44% vs. 8/51, 16%; $P = 0.019$). Elevation of both markers was associated with more than a threefold hazard ratio for mortality (HR 3.67, 95% confidence interval 1.14–11.78).

Conclusions: Elevated circulating IL-10 levels in systolic heart failure patients do not have a protective counterbalance effect on mortality. Moreover, patients with elevated IL-10 and TNFα had significantly higher mortality, suggesting that the possible interaction in the complex inflammatory and anti-inflammatory network may need further study.

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KEY WORDS: heart failure, interleukin-10, tumor necrosis factor-alpha, inflammation, prognosis

The role of inflammation in the pathogenesis of heart failure is currently of considerable interest since a high serum level of cytokines, much like that of neurohormones, contributes to the clinical deterioration of these patients [1,2]. Tumor necrosis factor-alpha is a key pro-inflammatory cytokine. Chronic heart failure patients have high circulating levels of TNFα, which correlate with the severity of their disease [3]. TNFα has several deleterious effects, including myocardial cell apoptosis, blunted beta-adrenergic signaling, fetal gene activation, endothelial dysfunction, and collagen production [2,4]. These processes lead to cellular breakdown, decreased cardiac contractility and enhancement of the remodeling process [5-8]. Moreover, in patients with advanced heart failure, TNFα is associated with cardiac cachexia and rennin-angiotensin system activation and is an independent predictor of mortality [3]. TNFα levels provide a significant incremental increase in risk assessment above established indicators [9].

The anti-inflammatory cytokine interleukin-10 may down-regulate TNFα production and correlate with disease severity [10]. Unlike TNFα, its predictive relationship to outcome and prognosis is not clear. In the present study, we examined the possible protective value of IL-10 serum levels in patients with advanced systolic heart failure in relation to their circulating TNFα levels. We analyzed the association of these two markers with clinical and laboratory parameters, including mortality.

PATIENTS AND METHODS

We collected serum samples from 67 consecutive chronic heart failure patients (age 65 ± 13 years; 58 males, 9 females) for TNFα and IL-10 levels during a routine follow-up visit at our heart center. All patients had symptomatic heart failure symptoms of at least 3 months duration and were considered clinically stable in their condition. All suffered from systolic left ventricular dysfunction (echocardiographic LV ejection fraction < 40%). Patients were treated according to guidelines of the American Heart Association/American College of

IL = interleukin
TNFα = tumor necrosis factor-alpha
HR = hazard ratio
LV = left ventricular

Table 1. Patient characteristics (n=67)

Age (yrs, mean ± SD)	65 ± 13
Gender (male/female)	58/9
LV ejection fraction (%, mean ± SD)	25 ± 7
LV end-diastolic diameter (mm, mean ± SD)	61 ± 7
New York Heart Association class ≥ III (n, %)	38 (57%)
Ischemic etiology (n, %)	41 (61%)
History of systemic hypertension (n, %)	32 (48%)
Diabetes mellitus (n, %)	26 (39%)
Atrial fibrillation (n, %)	22 (33%)
Body mass index (kg/m², mean ± SD)	29 ± 6
Systolic blood pressure (mmHg, mean ± SD)	116 ± 21
Medication (n, %)	
Beta-blocker	64 (95%)
Angiotensin-converting enzyme inhibitor and/or angiotensin II receptor blocker	60 (89%)
Diuretics	67(100%)
Aldosterone antagonist	17 (25%)
QRS duration (msec, mean ± SD)	135 ± 48
Chronic renal failure (Cr › 2 mg/dl) (n, %)	7 (10%)
Mortality (n, %)	15 (22%)

Cardiology. Patients' characteristics are given in Table 1. The study conformed to the principles outlined in the Helsinki Declaration and was approved by the Institution Review Board (Helsinki Committee) of the Carmel Medical Center. All patients gave written informed consent before inclusion in the study.

Based on the median serum levels of both TNFα and IL-10, we compared patients' clinical parameters, including echocardiographic measurements of LV size and function, body mass index, New York Heart Association class, 6 minute walk test, QRS width, and mortality. We also examined the relationships to other laboratory markers such as matrix metalloprotease-9, highly sensitive C-reactive protein, troponin T, N-terminal pro-brain natriuretic peptide, serum hemoglobin level, serum total cholesterol level, and measurements of renal function (serum creatinine, calculated creatinine clearance).

Serum samples were drawn at the same time of day (8–10 a.m.). We used a commercial enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) for IL-10, TNFα and MMP9. For troponin T, we used the commercial immunoassay kit (Roche Diagnostics, Mannheim, Germany), in an ELECSYS 1010 analyzer. For NT-proBNP we used a commercial immunoassay kit (Roche), in an ELECSYS 1010 analyzer and for hs-CRP we used the commercial immuno-turbidimetric assay kit (Roche), in a Cobas Integra 800 analyzer.

MMP 9 = matrix metalloprotease-9
NT-proBNP = N-terminal pro-brain natriuretic peptide
hs-CRP = highly sensitive C-reactive protein

STATISTICAL ANALYSIS

Data were summarized and displayed as mean ± SD for continuous variables, and as number of patients plus percentage in each group for categorical variables. Since some variables – such as hs-CRP, NT-proBNP and MMP9 – have non-normal distribution, we used a logarithmic transformation that converts it to normal distribution for all statistical procedures like *t*-tests, correlations, etc. All results expressed as hs-CRP, NT-proBNP or MMP9 were back-transformed to geometrical mean and standard deviation. The one-sample Kolmogorov-Smirnov test was used to test for normal distribution. Variables not transformed to normal distribution (such as troponin T) are presented as median and interquartile range. For all normally distributed continuous variables, an independent Student *t*-test was performed to compare the various parameters between high and low serum levels of TNFα and IL-10, while the Mann-Whitney U analysis was used for non-normally distributed continuous variables. For categorical variables, chi-square statistics were used for assessing overall significance between the two groups of patients comparing the high and low serum levels of IL-10/TNFα. Correlations between markers were calculated using Spearman's correlation coefficient. For mortality, we used Cox linear regression analysis, and comparison of mortality between patients with high or low serum TNFα and/or IL-10 levels were computed by log-rank statistics with the Kaplan-Meier estimation. The level of significance for analysis was two-tailed, $P < 0.05$. The SPSS statistical package was used to perform the statistical evaluations (SSPS Inc., Chicago, IL, USA).

RESULTS

The clinical and laboratory profiles of patients whose circulating TNFα and IL-10 levels were lower than median (group 1) and higher (above median, group 2) are given in Tables 2 and 3. In 23 patients (34.3%) serum TNFα levels were above the median value (detectable levels). Since most patients had no detectable levels of TNF, almost every patient who had even a minimally positive level was above median. In 33 patients (49%), IL-10 was above the median value of 0.05 pg/ml. There was a significant correlation with IL-10 and TNFα serum levels ($r = 0.5$, $P < 0.001$). Both TNFα and IL-10 were above median in 16 patients (24%). In 51 patients neither of these cytokines was elevated above median (27 patients), while only one of them was elevated in 24 patients.

During a mean follow-up period of 21 months, 15 patients died: 13 due to progressive heart failure and 2 due to arrhythmia. Mortality was higher in patients with higher TNFα (39% vs. 14%, $P = 0.02$) and tended to be higher but not significantly so (30% vs. 15%, $P = 0.10$) for IL-10 levels. Combined higher IL-10 and TNFα levels were associated with a higher mortality rate (44% vs. 16%, $P = 0.019$). Differences in other

Table 2. Relation between TNFα, IL-10 and their combination and clinical parameters

	TNFα			IL-10			TNFα and IL-10 combined		
	Group 1 (n=44)	Group 2 (n=23)	*P* value	Group 1 (n=34)	Group 2 (n=33)	*P* value	Group 1 (n=51)	Group 2 (n=16)	*P* value
Age (yrs)	63 (4)*	68 (12)	0.1	64 (12)	65 (15)	0.8	64 (13)	68 (13)	0.3
New York Heart Association Class ≥ III (n, %)	25 (57)	13 (56)	0.9	19 (56)	19 (58)	0.9	29 (57)	9 (56)	1.0
Body mass index (kg/m^2)	29 (6)	29 (5)	0.6	28 (5)	30 (6)	0.4	29 (6)	30 (6)	0.6
Systolic blood pressure (mmHg)	117 (23)	114 (16)	0.6	119 (22)	113 (20)	0.2	118 (22)	110 (15)	0.2
LV ejection fraction (%)	25 (7)	25 (6)	0.7	26 (7)	25 (7)	0.5	26 (7)	23 (5)	0.08
LV end-diastolic diameter (mm)	62 (6)	61 (8)	0.5	61 (7)	63 (7)	0.3	61 (7)	62 (8)	0.7
QRS duration (msec)	134 (52)	137 (42)	0.8	130 (38)	140 (57)	0.4	132 (50)	143 (42)	0.5
Six minute walk (m)	244 (168)	224 (94)	0.5	230 (160)	244 (134)	0.7	235 (163)	243 (79)	0.8
Mortality n (%)	6(14)	9(39)	0.02	5(15)	10(30)	0.1	8(16)	7(44)	0.019

*Mean ± 1SD/*interquartile range

Table 3. Relation between TNFα, IL-10 and their combination and laboratory parameters

	TNFα			IL-10			TNFα – IL-10 combined		
	Group 1 (n=44)	Group 2 (n=23)	*P* value	Group 1 (n=34)	Group 2 (n=33)	*P* value	Group 1 (n=51)	Group 2 (n=16)	*P* value
NT-proBNP (pg/ml)	1139*	3666	0.001*	1403	2075	0.3	1292	4094	0.004
Matrix metalloprotease-9 (ng/ml)	467 (2)	79 (2)	0.006	53 (2)	592 (2)	0.6	508 (2)	762 (2)	0.06
Troponin T(ng/ml)	‹ 0.01 (‹ 0.001*)	0.02 (0.05*)	0.001	‹ 0.01 (‹ 0.001*)	‹ 0.01 (‹ 0.04*)	0.1	‹ 0.01 (‹ 0.001*)	0.02 (0.05*)	0.002*
Hs-CRP (mg/dl)	0.8(5)	0.6 (3)	0.6	0.8 (4)	0.7 (4)	1.0	0.8 (5)	0.6 (2)	0.3
Hemoglobin (g/dl)	13 (1.7)	11.8 (1.6)	0.007	12.8 (1.8)	12.4 (1.7)	0.3	12.7 (1.8)	12.2 (1.6)	0.3
Total cholesterol (mg/dl)	155 (36)	135 (32)	0.033	147 (37)	150 (36)	0.7	15 (37)	138 (33)	0.2
Serum creatinine (mg/dl)	1.1 (0.3)	1.9 (1.0)	0.002	1.2 (0.5)	1.5 (0.9)	0.1	1.2 (0.4)	1.9 (1.1)	0.026
Creatinine clearance (ml/min)	81 (34)	54 (32)	0.003	74 (32)	69 (38)	0.6	77 (34)	56 (34)	0.036

*Mean ± 1SD/*interquartile range

clinical parameters by cytokine group did not reach statistical significance, although LV ejection fraction was slightly lower in the group with combined higher TNFα /IL-10 (23 ± 5% vs. 26 ± 7%, *P* = 0.08) [Table 2].

The group of patients with elevated TNFα level (above median levels) also had higher NT-proBNP, troponin and creatinine, and lower MMP9, hemoglobin and creatinine clearance. Such differences were not seen in relation to elevated IL-10 levels; however, most of these prognostic parameters were also significantly abnormal in the combined IL-10/TNFα group.

Kaplan-Meier survival curves were calculated for high and low TNFα and TNFα/IL-10 patient groups. Mortality was higher in the elevated TNFα group (*P* = 0.02) [Figure 1A] and combined higher TNFα /IL-10 group (*P* = 0.02) [Figure 1B]. Using a Cox regression analysis, adjusted for age and gender, higher circulating TNFα level had a hazard ratio for mortality of 2.84 (95% confidence interval 0.98–8.24), while a combined elevated IL-10/TNFα profile was associated with a more than threefold hazard ratio for mortality (HR 3.67, 95% CI 1.14–11.78).

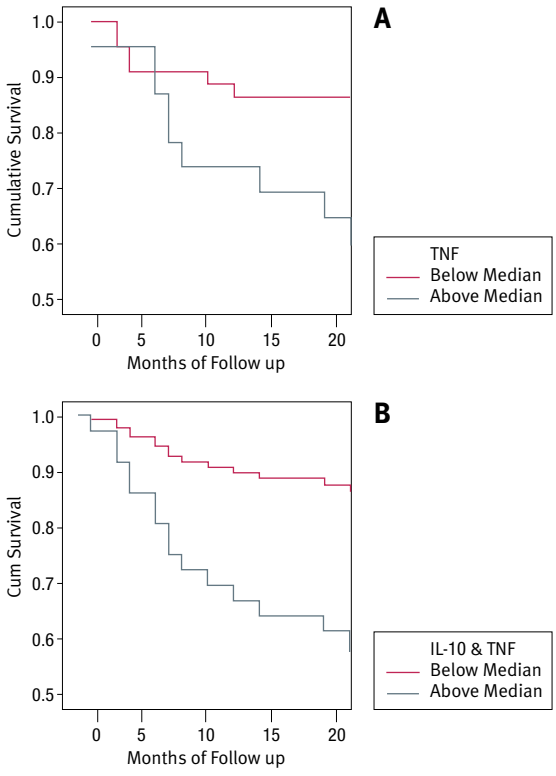
DISCUSSION

The essential findings of the study were that the hazard effects of elevated TNFα in our heart failure patients were not counterbalanced by a simultaneous increase in circulating IL-10, as patients with both elevated IL-10 and TNFα had more than a threefold mortality hazard ratio. Moreover, patients with higher IL-10 in addition to TNFα had significantly higher NT-proBNP and troponin levels, reduced measurements of renal function, and a tendency for higher serum MMP9, all of which are known markers of adverse prognosis in heart failure [11-15].

Although TNFα is a key factor in the inflammatory mediated process, the failure of the clinical therapeutic trials demonstrated the complexity of the cytokine network as a whole paradigm [10,16,17]. In our patients, there was a prominent association between TNFα and IL-10. IL-10 is considered to be a prototypic "anti-inflammatory" cytokine that decreases the production of TNFα from peripheral mononuclear cells and is traditionally assumed to blunt its deleterious effects in heart failure patients [18-20]. Of note, while IL-10 production is commonly associated with simultaneous TNFα increase [21], a rise in IL-10 level was found during phagocytosis of apoptotic cells, suggesting that IL-10 may also rise independently of TNFα [22]. IL-10 circulating levels are higher in heart failure patients compared with healthy controls

CI = confidence interval

Figure 1. [A] Kaplan-Meier survival curves according to circulating TNFα levels (below and above median). Survival was reduced in patients with higher TNFα levels ($P = 0.02$). **[B]** Cox proportional hazard ratio curves according to combined circulating TNFα and IL-10 levels (either below or above median). Survival was reduced in patients with higher TNF/IL-10 levels ($P = 0.03$)



[18]. However, IL-10 was claimed to have a protective anti-inflammatory effect as it was lower in patients with advanced compared with less advanced disease and was even considered as a potential therapy in heart failure [19,23].

The results of our current study question the "protective" role of IL-10 in heart failure patients. Although advanced heart failure and IL-10 were associated individually with increased mortality (IL-10 alone did not reach statistical significance), the combination of the two provided the most prominent prediction of mortality compared with elevation of either one alone. A few other studies have suggested that IL-10 may be linked to poor outcome. In post-myocardial infarction patients, elevated serum IL-10 was associated with increased mortality, and IL-10 was also found to be an important poor prognostic marker in patients with acute myocarditis [24,25]. Although one may argue that elevated levels of IL-10 and TNFα merely reflect disease severity rather than causing it, we believe that a cause-effect relationship may be a more realistic sequence.

The mechanism for the deleterious effects of combined elevation of IL-10 and TNFα in heart failure is not clear and beyond the scope of the current study. A possible explanation may be that there are interactions – as yet unmasked – which, via the simultaneous increase of IL-10 and TNFα, amplify the inflammatory process, leading to "ongoing myocardial damage," as supported by our patients' significantly high NT-proBNP and cardiac troponin levels, and renal dysfunction. These findings reflect once again the complexity of the inflammatory model in heart failure.

STUDY LIMITATIONS

The patient sample was rather small and homogenous as most patients suffered from already significant chronic heart failure. This may be the reason why neither elevated TNFα nor IL-10 was associated with other clinical prognostic parameters. The cytokine network is complex and our current understanding of its function and even more so of its dysfunction is limited. Accordingly, one should be cautious regarding network interactions and/or its clinical implications.

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