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Brief Rapid Report

Serially Measured Cytokines and Cytokine Receptors in Relation to Clinical Outcome in Patients With Stable Heart Failure

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ABSTRACT

In this prospective cohort study of 250 stable heart failure patients with trimonthly blood sampling, we investigated associations of 17 repeatedly measured cytokines and cytokine receptors with clinical outcome during a median follow-up of 2.2 (25th-75th percentile, 1.4-2.5) years. Sixty-six patients reached the primary end point (composite of cardiovascular mortality, heart failure hospitalization, heart transplantation, left ventricular assist device implantation). Repeatedly measured levels of 8 biomarkers correlated with clinical outcomes independent of clinical characteristics. Rates of change over time

RÉSUMÉ

Dans cette étude prospective d'une cohorte de 250 patients atteints d' cardiaque stable, soumis à un prélèvement sanguin trimestriel, nous avons étudié les associations entre 17 cytokines et récepteurs aux cytokines mesurés de façon répétée et les conséquences cliniques au cours d'un suivi médian de 2.2 années (25^{e}-75^{e} percentile, 1,4-2,5). Soixante-six patients ont atteint le principal critère d'évaluation (indice composite prenant en compte la mortalité cardiovasculaire, l'hospitalisation pour insuffisance cardiaque, la transplantation cardiaque, l'implantation d'un dispositif d'assistance ventriculaire gauche). Les

During the course of chronic heart failure (HF), levels of numerous proinflammatory cytokines are elevated even without an acute stressor being present, which has led to the hypothesis that inflammation plays a central role in the progression of HF.[1] Many reports have suggested that cytokines predict adverse outcome in these patients, but most of these studies had limited sample size and lacked adjustment for traditional biomarkers like N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitivity troponin T, and C-reactive protein (CRP).[2] Moreover, to the best of our knowledge, the temporal patterns of inflammatory proteins other than CRP[3] have not yet been investigated in patients with stable HF. In the current study, we hypothesize that protein level changes occur in the time period before an incident adverse clinical event. To test this hypothesis, we measured a broad range of cytokines and cytokine receptors repeatedly with a multiplex assay in patients with stable HF, and investigated the association between their temporal patterns and clinical outcome.

Methods

Between October 2011 and June 2013, a total of 263 patients were prospectively enrolled at 2 tertiary medical centres in the **Bio**marker Mea**s**urements and New Echocardiographic Techniques in Chronic **H**eart **F**ailure Patients Result in **T**ailored Prediction of Prognosis (Bio-SHiFT) study. Stable HF patients were recruited during their regular outpatient clinic visit, as described previously.[4,5] In the current investigation, only the 250 patients with HF with a reduced ejection fraction were evaluated. Ambulatory patients were recruited during their regular outpatient clinic visit, and these patients were stable as defined by the fact that they had not been hospitalized for HF in the past 3 months. The study was approved by the responsible medical ethics committees and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. The trial is registered in ClinicalTrials.gov (NCT01851538; https://clinicaltrials.gov/ct2/show/NCT01851538).

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(slopes of biomarker evolutions) remained independently associated with outcome for 15 biomarkers. Thus, temporal patterns of cytokines and cytokine receptors, in particular tumour necrosis factor ligand superfamily member 13B and interleukin-1 receptor type 1, might contribute to personalized risk assessment.

niveaux de 8 biomarqueurs mesurés de manière répétée étaient corrélés avec les conséquences cliniques, indépendamment des caractéristiques cliniques. Les taux de changement au cours du temps (pentes d'évolution des biomarqueurs) sont restés indépendamment associés aux pronostics pour 15 biomarqueurs. Ainsi, les modèles temporels des cytokines et des récepteurs de cytokines, en particulier le membre 13B de la superfamille des ligands du facteur de nécrose tumorale et le récepteur de l'interleukine-1 de type I, pourraient contribuer à une évaluation personnalisée des risques.

An extended version of the *Methods* section with additional information is given in the Supplementary Material. We performed blood sampling and medical evaluation at baseline and this was repeated at each study follow-up visit. These visits were predefined and scheduled every 3 months (±1 month) with a maximum of 10 study follow-up visits. For the current investigation, follow-up lasted until November 2015. The primary end point (PE) was a composite of cardiac death, heart transplantation, left ventricular assist device implantation, and hospitalization for management of acute or worsened HF, whichever occurred first. We collected 1984 ethylenediaminetetraacetic acid (EDTA) plasma samples before occurrence of the PE or censoring (9 [25th-75th percentile, 5-10] blood samples per patient) during this first inclusion round of the Bio-SHiFT study. For reasons of efficiency, for the current investigation, we selected all samples drawn at baseline, the last sample available in patients in whom the PE did not occur during follow-up, and the 2 samples available before the PE (which, by design, were 3 months apart; Supplemental Fig. S1). This selection was on the basis of previous investigations in this cohort, which showed that levels of several biomarkers change in months before the incident adverse event, whereas event-free patients show stable biomarker levels.[4] Altogether, this resulted in 530 samples.

A total of 17 cytokines and cytokine receptors were measured using the Cardiovascular panel III of Olink Proteomics AB, Uppsala, Sweden, in a batch-wise analysis of the samples. Biomarkers were delivered in normalized protein expression (NPX) units, which are relative units expressed on a log2 scale in which 1 unit higher NPX thus represents a doubling of the measured protein concentrations.

We used linear mixed effect models to plot the average temporal pattern of the biomarkers for patients with and without a PE during follow-up, and freedom from composite end point was assessed using Kaplan−Meier analysis. To estimate the associations between patient-specific repeated biomarker measurements and the PE, we applied joint modelling (JM) analyses. JM combines linear mixed effect models for temporal evolution of the repeated measurements with relative risk models for the time-to-event data.[6] We studied the repeatedly measured biomarker levels (including baseline and follow-up), as well as their rates of change (ie, the slopes, which corresponds to the first derivative of the longitudinal biomarker trajectories). First, all JM analyses were performed univariably. Subsequently, we performed multivariable analyses to adjust for potential confounders. We applied a "clinical model," which was adjusted for age, sex, diabetes mellitus, atrial fibrillation, New York Heart Association class, use of diuretics, and systolic blood pressure, and a "cardiac biomarker model," which was adjusted for baseline NT-proBNP, high-sensitivity troponin T, and CRP. Adjustments were made in the relative risk and linear mixed effect model parts. For all JM analyses, we used the Z-score (ie, the standardized form) of the NPX values to allow for direct comparisons of different biomarkers. Results are given as hazard ratios (HRs) and 95% confidence intervals (CIs) per 1 SD difference of the repeatedly measured biomarker level and per 0.1 SD per year difference of the slope at any point in time during follow-up.

We used the conventional $P < 0.05$ threshold to conclude significance for the relation between patient characteristics and the occurrence of the PE during follow-up (Table 1). For the other analyses, we corrected for multiple testing using the Bonferroni correction (n = 17), which resulted in a significance level of $P < 0.0029$.

Results

Baseline characteristics and study end points

During a median follow-up of 2.2 (25th-75th percentile, 1.4-2.5) years, 66 patients (26%) reached the PE: 53 patients were rehospitalized for acute or worsened HF, 3 patients underwent heart transplantation, 2 patients underwent left ventricular assist device placement, and 8 patients died from cardiovascular causes. Overall, freedom from the composite end point was 76 ± 3% at 2 years of follow-up. Furthermore, freedom from cardiovascular death was 89 ± 2% at 2 years of follow-up and freedom from HF hospitalization ± standard error was 80± 3% at 2 years of follow-up (Supplemental Figure S2). Table 1 shows the patients' baseline characteristics and the differences between patients who reached the PE during follow-up and patients who did not. Overall, the median age was 68 (25th-75th percentile, 58-76) years, 74% were men, and median left ventricular ejection fraction was 30% (25th-75th percentile, 23%-37%).

Temporal patterns of circulating cytokine related biomarkers in relation to study end points

Supplemental Figure S3 shows the average temporal patterns of the biomarkers in patients with vs without the PE, on the basis of linear mixed models. Twenty-four months before occurrence of the end point, levels of C-C motif chemokine 15, tumour necrosis factor receptor 1, and tumour necrosis factor receptor superfamily member 14 were already higher in patients who ultimately reached the PE compared with patients who remained event-free. Furthermore, these biomarkers showed diverging patters as the end point drew closer. Also, levels of C-C motif chemokine 16, C-X-C motif chemokine 16, interleukin (IL)-1 receptor type 1 (IL-1RT1),

Table 1. Patient characteristics in relation to the occurrence of the primary end point

Variable	Total	Primary end point reached during follow-up		*P*
		Yes	No	
n	250 (100)	66 (26)	184 (74)	
Demographic characteristics				
Age, years	68 (58-76)	71 (60-79)	66 (58-74)	0.042*
Male sex	184 (74)	52 (79)	132 (71)	0.27
Clinical characteristics				
Body mass index	27 (24-30)	27 (24-30)	27 (24-30)	0.78
Heart rate, bpm	67 (60-74)	70 (60-76)	66 (60-72)	0.26
Systolic blood pressure, mm Hg	120 (108-132)	115 (104-128)	122 (110-136)	0.021*
Diastolic blood pressure, mm Hg	72 (62-80)	70 (60-78)	74 (65-80)	0.052
Features of HF				
Duration of HF, years	4.7 (1.7-9.8)	7.2 (3.2-13.1)	3.8 (1.1-7.9)	< 0.001*
NYHA class III or IV	62 (25)	29 (44)	33 (18)	< 0.001*
Left ventricular ejection fraction, %	30 (23-37)	25 (19-34)	30 (23-38)	0.035*
Traditional biomarkers				
NT-proBNP, pmol/L	133 (45-274)	297 (176-525)	94 (29-205)	< 0.001*
HsTnT, ng/L	18 (9-33)	30 (20-49)	14 (8-27)	< 0.001*
CRP, mg/L	2.2 (0.9-4.9)	3.0 (1.4-5.4)	1.8 (0.7-4.3)	0.016*
Etiology of heart failure				
Ischemic	116 (46)	36 (55)	80 (44)	0.097
Hypertension	31 (12)	8 (12)	23 (13)	
Secondary to valvular disease	10 (4)	5 (8)	5 (3)	
Cardiomyopathy	63 (25)	13 (20)	50 (27)	
Unknown or other	30 (12)	4 (6)	26 (14)	
Medical history				
Known coronary artery disease†	119 (48)	36 (55)	83 (46)	0.42
Previous percutaneous coronary intervention	81 (32)	26 (39)	55 (30)	0.16
Previous coronary artery bypass grafting	42 (17)	12 (18)	30 (16)	0.73
Previous CVA/TIA	39 (16)	14 (21)	25 (14)	0.14
Atrial fibrillation	97 (39)	33 (50)	64 (35)	0.030*
Diabetes Mellitus	77 (31)	29 (44)	48 (26)	0.007*
Hypercholesterolemia	94 (38)	29 (44)	65 (35)	0.22
Hypertension	113 (45)	34 (52)	79 (43)	0.23
COPD	31 (12)	12 (18)	19 (10)	0.097
Chronic inflammatory disease	23 (9)	9 (14)	14 (8)	0.26
Medication use				
β-Blocker	225 (90)	57 (86)	168 (91)	0.25
ACE-I or ARB	235 (94)	59 (89)	176 (96)	0.076
Diuretics	227 (91)	64 (97)	163 (89)	0.043*
Loop diuretics	226 (90)	64 (97)	162 (88)	0.035*
Thiazides	6 (2)	3 (5)	3 (2)	0.19
Aldosterone antagonist	174 (70)	50 (76)	124 (67)	0.21
Aspirin	45 (18)	9 (14)	36 (20)	0.30
Vitamin K antagonist	193 (77)	56 (85)	137 (75)	0.084
Nitrates	43 (17)	14 (21)	29 (16)	0.31
Antiarrhythmics	46 (18)	16 (24)	30 (16)	0.15
Statins	144 (58)	41 (63)	103 (56)	0.32
Anti-inflammatory agents	26 (11)	9 (14)	17 (9)	0.26
KDOQI classification				
eGFR ≥ 90 mL/min per 1.73 m^2	28 (11)	7 (11)	21 (11)	0.59
eGFR 60-89 mL/min per 1.73 m^2	92 (37)	20 (30)	72 (39)	
eGFR 30-59 mL/min per 1.73 m^2	110 (44)	33 (50)	77 (42)	
eGFR < 30 mL/min per 1.73 m^2	20 (8)	6 (9)	14 (8)	

Non-normally distributed continuous variables are expressed as median (25th-75th percentile). Categorical variables are expressed as n (%).

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blocker; bpm, beats per minute; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CVA, cerebrovascular accident; eGFR, estimated glomerular filtration rate; HF, heart failure, HsTnT, high-sensitivity troponin T; KDOQI, National Kidney Foundation **K**idney **D**isease **O**utcome **Q**uality **I**nitiative; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; TIA, transitory ischemic attack.

* $P < 0.05$.

† Known coronary artery disease was defined as stenosis ≥ 50%.

IL-1 receptor type 2, IL-17 receptor A, IL-18-binding protein, IL-2 receptor subunit alpha, lymphotoxin β receptor, tumour necrosis factor receptor 2, and tumour necrosis factor ligand superfamily member 13B (TNFSF13B) significantly increased as the end point approached, but remained stable or showed a divergent evolution in end point-free patients.

Figure 1A shows the associations of the levels of the 17 repeatedly measured biomarkers with the PE on the basis of JM analyses. In univariable analyses, repeatedly measured levels of 13 of the biomarkers were positively associated with the PE. Repeatedly measured levels of TNFSF13B showed the strongest association with a HR of 3.18 (95% CI, 2.26-4.71)

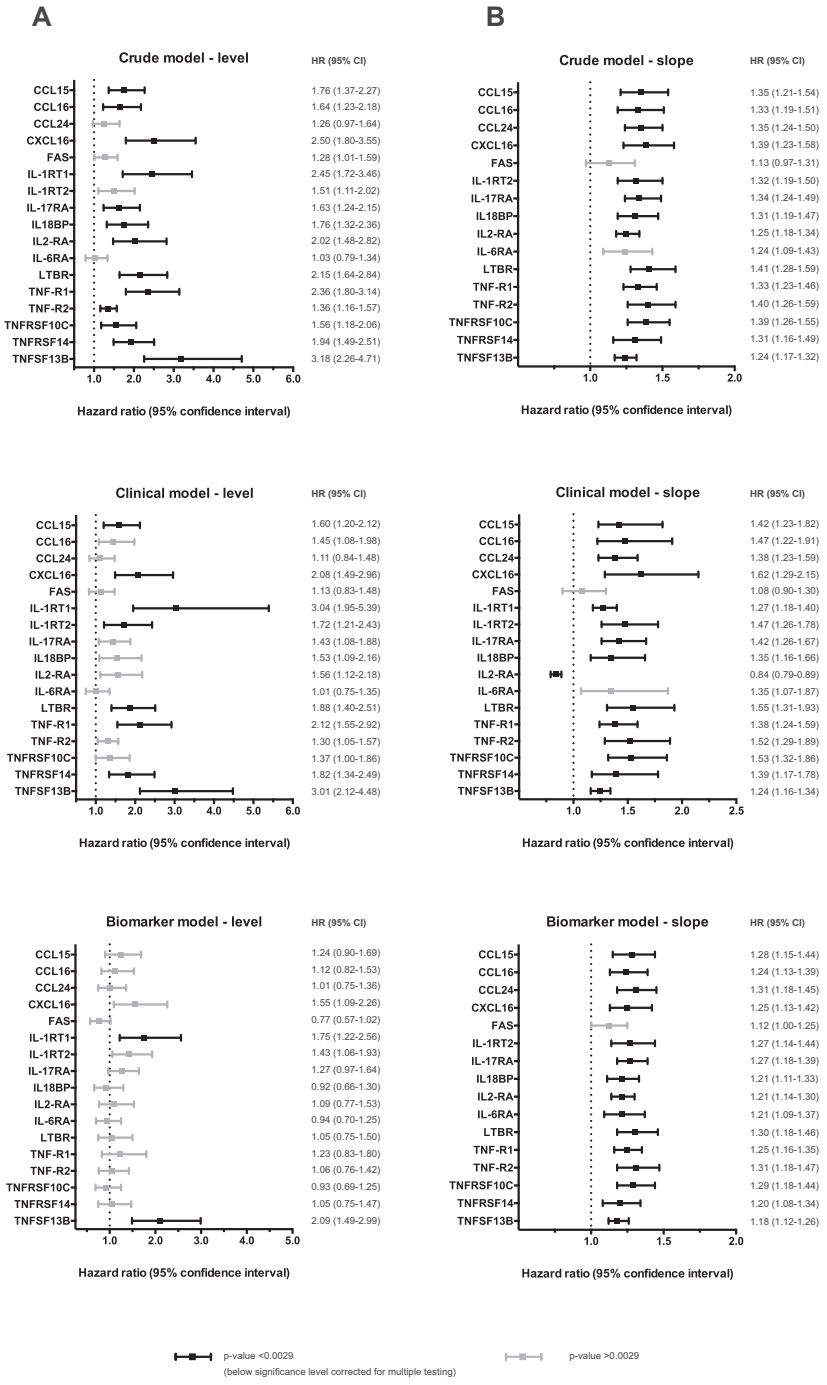

Figure 1. Associations of levels and slopes of cytokines and cytokine receptors with the primary end point. Hazard ratios (HRs) and 95% confidence intervals (CIs) are given per 1 SD change in repeatedly measured biomarker level at any point in time during follow-up (**A**), and per 0.1 SD of the annual slope at any point in time during follow-up (**B**). Crude model values were derived from Cox model unadjusted, and linear mixed effect (LME) model, unadjusted; clinical model values were derived from Cox and LME models adjusted for age, sex, diabetes, atrial fibrillation, baseline New York Heart Association class, diuretics, and systolic blood pressure, except for the model of TNFSF13B which was not adjusted for systolic blood pressure because of convergence problems; cardiac biomarker model values were derived from Cox and LME models adjusted for baseline N-terminal pro-B-type natriuretic peptide, high-sensitivity troponin T, and C-reactive protein. Slope analyses in crude and biomarker model were not available for IL-1RT1 because of computational difficulties. C-C motif chemokine 15, C-C motif chemokine 15; CCL16, C-C motif chemokine 16; CCL24, C-C motif chemokine 24; CXCL16, C-X-C motif chemokine 16; FAS, tumour necrosis factor receptor superfamily member 6; IL-18BP, interleukin-18-binding protein; IL-17RA, interleukin-17 receptor A; IL2-RA, interleukin-2 receptor subunit alpha; IL-6RA, interleukin-6 receptor subunit alpha; IL-1RT1, interleukin-1 receptor type 1; IL-1RT2, interleukin-1 receptor type 2; LTBR, lymphotoxin β receptor; TNF-R1, tumour necrosis factor receptor 1; TNF-R2, tumour necrosis factor receptor 2; TNFRSF14, tumour necrosis factor receptor superfamily member 14; TNFRSF10C, tumour necrosis factor receptor superfamily member 10C; TNFSF13B, tumour necrosis factor ligand superfamily member 13B.

per SD change at any point in time during follow-up. After adjustment for clinical characteristics, the level of 8 repeatedly measured biomarkers remained significantly associated with the PE. IL-1RT1 showed the strongest association (HR, 3.04; 95% CI, 1.95-5.39), followed by TNFSF13B: HR, 3.01(95% CI, 1.12-4.48), and tumour necrosis factor receptor 1: HR, 2.12 (95% CI, 1.55-2.92). The HR of TNFSF13B remained significant and the highest (HR, 2.09; 95% CI, 1.49-2.99) after adjustment for NT-proBNP, high-sensitivity troponin T, and CRP, followed by the HR of IL-1RT1 (Fig. 1A). The remaining biomarkers lost statistical significance after adjustment for traditional cardiac biomarkers. Performance measures of the models are shown in Supplemental Table S1.

The rates of change (ie, the slopes of the longitudinal biomarker trajectories) in relation to risk of the PE, showed significant, positive associations for all investigated biomarkers except tumour necrosis factor receptor superfamily member 6 and IL-2 receptor subunit alpha in univariable analyses (Fig. 1B). After adjustment for clinical characteristics, 15 of the biomarkers still showed significant associations. C-X-C motif chemokine 16 showed the numerically strongest association with the PE with a HR of 1.62 (95% CI, 1.29-2.15) per 0.1 SD change of the annual slope after adjustment for clinical factors.

Discussion

Temporal patterns of several cytokines and cytokine receptors were associated with adverse events in 250 stable patients with HF, even after adjustment for clinical factors. Proinflammatory cytokines are known for their important roles in the disease process of HF. In a recent meta-analysis, concentrations of tumour necrosis factor-α, IL-6, IL-1β, and CRP were significantly higher in chronic HF patients than in control participants, and serum IL-6 predicted mortality.[7] Recently, a study suggested that IL-32 is a novel predictor of adverse cardiac events in HF patients after myocardial infarction.[8] Nevertheless, clinical trials targeting different immune components have not resulted in improved clinical outcomes; some interventions even resulted in worsening of HF.[9] Previous studies in chronic HF patients have mostly described the value of single measurements of inflammatory markers (eg, at admission) for prognosis. To the best of our knowledge, the temporal patterns of cytokine-related biomarkers in patients with stable HF and their associations with clinical outcome have not yet been investigated in detail. Repeated measurements could provide more information on changes in marker levels over time and the association between these changes and adverse clinical events. Apart from the potential value for personalized risk assessment, when investigated in more detail, these temporal patterns might contribute to elucidation of aspects of immune activation that contribute to the progression of HF.

Some limitations of our study warrant consideration. Because of efficiency reasons, as described previously, we used a subset of all available plasma samples. However, our previous investigations using all samples showed that most of the examined biomarkers' levels change before the incident adverse event. Thus, we believe that with our approach we retain the most informative measurements while enhancing efficiency. Furthermore, the current investigation focused on HF with reduced ejection fraction patients only.

In conclusion, we showed that temporal patterns of several cytokines and cytokine receptors are independently associated with clinical adverse events in patients with stable HF. These results suggest that repeated measurements of these biomarkers, in addition to traditional cardiac biomarkers, might contribute to personalized risk assessment and better identify high-risk patients. Additionally, these findings might open new avenues for druggable targets. Further studies that measure absolute biomarker concentrations repeatedly in larger cohorts are needed to confirm and extend these findings.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at https://doi.org/10.1016/j.cjca.2020.08.010.