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ORIGINAL ARTICLE

# IL-17 is associated with disease severity and targetable inflammatory processes in heart failure

Lukas Baumhove[1], Bart J. van Essen[1], Martin M. Dokter[1], Sietske N. Zijlstra[1], Frederik E. Deiman[1], Jon D. Laman[2], Chim C. Lang[3], Gwenny M.P.J. Verstappen[4], Dirk J. van Veldhuisen[1], Peter van der Meer[1], Nils Bomer[1] and Adriaan A. Voors[1]*

[1]*Department of Cardiology, University Medical Center Groningen, Groningen, The Netherlands;* [2]*Department of Pathology & Medical Biology, University Medical Center Groningen, Groningen, The Netherlands;* [3]*Division of Molecular and Clinical Medicine, School of Medicine, Ninewells Hospital & Medical School, University of Dundee, Dundee, UK; and* [4]*Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*

## Abstract

**Aims** Heart failure (HF) is recognized as an inflammatory disease in which cytokines play an important role. In animal HF models, interleukin-17A (IL-17) has been linked to deterioration of cardiac function and fibrosis, whereas knock-out of IL-17 showed beneficial cardiac effects. However, there is limited evidence of IL-17 involvement in patients with HF. This study aims to investigate the clinical characteristics, outcomes, and pathophysiological processes associated with circulating IL-17 concentrations in patients with HF.

**Methods and results** IL-17 was measured by ELISA in 2082 patients diagnosed with HF along with 363 circulating proteins using proximity extension assay technology for differential expression and pathway analysis. Data were validated in an independent cohort of 1737 patients with HF. Patients with elevated IL-17 concentrations had more severe HF, as reflected by more frequent current or previous hospitalizations for HF, higher New York Heart Association functional class (NYHA) and higher levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP). High IL-17 concentrations were independently associated with an increased risk of hospitalization for HF and mortality. In both cohorts, the most strongly up-regulated proteins in patients with high IL-17 were fibroblast growth factor 21 (FGF-21), interleukin-6 (IL-6), C-X-C motif chemokine ligand 13 (CXCL13), tumour necrosis factor receptor superfamily member 6B (TNFRSF6B) and interleukin-1 receptor antagonist (IL-1RA). Pathway over-representation analysis showed increased activity of pathways related to lymphocyte-mediated immunity, leukocyte activation and regulation of the immune response.

**Conclusions** In patients with HF, elevated IL-17 concentrations indicate more severe HF and increased activity of inflammatory processes known to be involved in the pathophysiology of HF. IL-17 might hold potential for identifying and targeting inflammation in HF.

**Keywords** heart failure; inflammation; cytokines

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**Correspondence to:*
*Prof. dr. A. A. Voors, Department of Cardiology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.*
*Email: a.a.voors@umcg.nl*
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## Introduction

Heart failure is recognized as an inflammatory disease in which immune activation is strongly involved in its pathophysiology. Substantial experimental and clinical evidence suggests that heart failure, regardless of aetiology, involves induction of cytokines that contribute to the pathogenesis of adverse myocardial remodelling and systolic and diastolic dysfunction. In particular, the inflammatory cytokines tumour necrosis factor (TNF), interleukin (IL)-1 and IL-6 have been shown to play an important role in the pathogenesis of heart failure as they modulate phenotype and function of all myocardial cells, suppress contractile function in cardiomyocytes and promote microvascular dysfunction.[1,2] However, despite the suggested role of inflammation in the pathophysiology of heart failure, no anti-inflammatory treatments have yet

been proven to reduce mortality or morbidity in patients with heart failure.

In patients with prior myocardial infarction and elevated C-reactive protein (CRP), IL-1β inhibition reduced hospitalizations for heart failure.[2,3] A phase 3 randomized clinical trial investigating the effects of the IL-6 inhibitor ziltivekimab in patients with heart failure with preserved ejection fraction (HFpEF) is currently ongoing.[4] These developments have increased interest in better understanding the underlying mechanisms and specificities of inflammation in heart failure and in more precisely identifying patients with an inflammatory phenotype and cardiac-specific inflammation, which may lead to the identification of novel therapeutic targets.

The inflammatory cytokine interleukin-17A (IL-17) is predominantly released by T lymphocytes and induces the production of inflammatory cytokines such as IL-1, IL-6 and TNF.[5–7] In murine models, IL-17 has been linked to deterioration of cardiac function and fibrosis and has been shown to promote the production of these inflammatory cytokines in the heart.[8,9] Conversely, in IL-17 knockout mice, cardiac function has been observed to improve, with enhanced calcium handling and contractility, while cardiac hypertrophy has been reduced.[10] Moreover, IL-17 has been demonstrated to be a driver of cardiac dysfunction in immune checkpoint inhibitor-induced cardiotoxicity in mice.[11] Notably, a number of anti-IL-17 therapies are already available or in development for the treatment of immunological diseases.[12] However, there are little data on the cytokine IL-17 in patients with heart failure. Therefore, this study aims to investigate the clinical characteristics, outcomes and pathophysiological processes associated with elevated IL-17 concentrations in patients with heart failure.

## Methods

IL-17 concentrations were measured in serum by means of an enzyme-linked immunosorbent assay (ELISA) in 2082 heart failure patients enrolled in the BIOSTAT-CHF registry, which has been described earlier.[13] In brief, BIOSTAT-CHF is a multinational, prospective, observational cohort study that included patients with worsening heart failure from 11 European countries. Inclusion criteria were age $>$18 years, symptoms of new-onset or worsening heart failure, either a left ventricular ejection fraction $\leq$40% or elevated levels of B-type natriuretic peptide and/or N-terminal pro-B-type natriuretic peptide (NT-proBNP) and treatment with either oral or intravenous furosemide $\geq$40 mg/day or equivalent at the time of enrolment. In addition, patients had to be suboptimally treated with guideline-recommended therapy with the prospect of uptitration to guideline-recommended therapy. The data were validated in an independent cohort of 1737 patients with heart failure from six centres in Scotland. The research protocol was approved by the local ethics committee, and all patients provided informed consent, in compliance with the Declaration of Helsinki.

Measurements of IL-17 were performed using IL-17A Human ELISA kits (catalogue# BMS2017, Thermo Fisher Scientific Inc.) in the laboratory of the Department of Experimental Cardiology, UMCG, Groningen. IL-17 assays had a detection limit of 0.5 pg/mL. Circulating concentrations of 363 proteins were measured using four Olink Proseek Multiplex panels. This method uses a proximity extension assay (PEA) technology providing normalized protein expression data.

Patients were stratified into three groups according to their IL-17 concentrations: low (below 0.5 pg/mL), intermediate (0.5 pg/mL up to the median of concentrations above 0.5 pg/mL) and high IL-17 (above this median). The mean (standard deviation) is presented for normally distributed continuous variables, the median (interquartile range) for non-normally distributed continuous variables and the number (percentage) for categorical variables. Baseline characteristics were compared using one-way analysis of variance (ANOVA), Kruskal–Wallis test and $\chi^2$ test where appropriate. To investigate the association between IL-17 and clinical outcomes of heart failure hospitalization or mortality and mortality alone, Kaplan–Meier analysis and Cox regression analysis were performed. Cox regression was adjusted for potential confounders and for the established BIOSTAT-CHF risk model consisting of the strongest predictors of each clinical outcome.[14] A multivariable logistic regression model was conducted in the index cohort to examine independent associations with high IL-17 concentrations. Variables that differed significantly among the baseline characteristics were included in a multivariable model, with backward selection carried out using a *P* value cut-off of 0.1.

The differential expression analysis was conducted through implementation of the Limma package in R. Patients exhibiting high IL-17 concentrations were compared with those with low IL-17 concentrations (IL-17 high vs. low) in both cohorts. A false discovery rate (Benjamini–Hochberg) $<$5% was considered statistically significant. Subsequently, exclusively proteins found differentially expressed in both the index and validation cohort were employed for pathway analysis. Pathway over-representation analysis was conducted with the web-based tool gProfiler, using the Gene Ontology Biological Process (GO) database. All proteins included for differential expression analysis were used as the background and Bonferroni correction was used to correct for multiple testing.

## Results

*Tables* 1 and 2 present the baseline characteristics for the index and validation cohort, respectively. Patients with elevated IL-17 concentrations exhibited more severe heart

failure, as reflected by more frequent current and previous heart failure hospitalizations, more frequent New York Heart Association functional class III or IV and higher plasma concentrations of NT-proBNP. Furthermore, patients with high IL-17 had a higher prevalence of chronic kidney disease and lower estimated glomerular filtration rate and haemoglobin levels. Elevated IL-17 concentrations in the index cohort were also associated with a higher prevalence of chronic obstructive pulmonary disease (COPD) and higher levels of CRP and IL-6. No significant differences in the occurrence of HFpEF were observed in either cohort. From multivariable logistic regression, the strongest variables associated with elevated IL-17 were higher NT-proBNP, higher IL-6, history of COPD, previous heart failure hospitalization and lower haemoglobin levels (*Figure* S1).

Kaplan–Meier analyses showed that high concentrations of IL-17 d endpoint of heart failure hospitalization and mortality (index cohort *Figure* 1A and validation cohort *Figure* 1B). Furthermore, after adjustment for potential confounders in multivariable Cox regression analyses, the association between high IL-17 concentrations and both clinical outcomes remained significant in comparison with low IL-17 concentrations (*Table* 3). After adjusting for the BIOSTAT-CHF risk model, this association remained significant for mortality, but not for the combined endpoint.

Differential protein expression analyses between patients with high versus low IL-17 concentrations are shown in *Figure* 2. In patients with high serum concentrations of IL-17, 81 proteins were consistently up-regulated in both the index and validation cohort. In both cohorts, the most strongly up-regulated proteins in patients with high IL-17 were fibroblast growth factor 21 (FGF-21), IL-6, C-X-C motif chemokine ligand 13 (CXCL13), tumour necrosis factor receptor superfamily member 6B (TNFRSF6B) and interleukin-1 receptor antagonist (IL-1RA). Pathway over-representation analysis showed that patients with high IL-17 concentrations had

**Table 1** Baseline characteristics according to IL-17 concentrations in the index cohort.

| | Low | Intermediate | High | *P* value |
|---|---|---|---|---|
| | *N* = 1659 | *N* = 212 | *N* = 211 | |
| Age (years) | 68.2 (12.1) | 70.0 (11.3) | 69.7 (12.8) | **0.039** |
| Women (%) | 436 (26.3%) | 58 (27.4%) | 66 (31.3%) | 0.301 |
| Caucasian (%) | 1643 (99.0%) | 211 (99.5%) | 207 (98.1%) | 0.337 |
| New York Heart Association class III/IV (%) | 982 (61.0%) | 123 (59.4%) | 146 (70.5%) | **0.023** |
| Hospitalized (%) | 1131 (68.2%) | 140 (66.0%) | 159 (75.4%) | 0.072 |
| Hospitalized in the previous year (%) | 505 (30.4%) | 62 (29.2%) | 83 (39.3%) | **0.026** |
| Heart failure with preserved ejection fraction (%) | 94 (6.39%) | 14 (7.33%) | 15 (7.98%) | 0.656 |
| Left ventricular ejection fraction (%) | 30.0 [25.0;36.0] | 30.0 [25.0;38.0] | 30.0 [23.0;36.2] | 0.773 |
| BMI (kg/m$^2$) | 27.9 (5.38) | 27.0 (5.36) | 27.1 (5.33) | **0.012** |
| Obesity (BMI > 30%) | 486 (29.7%) | 48 (23.0%) | 53 (26.0%) | 0.084 |
| Systolic BP (mmHg) | 125 (22.0) | 122 (20.7) | 124 (23.9) | 0.126 |
| Diastolic BP (mmHg) | 74.7 (13.4) | 74.0 (12.8) | 73.8 (13.4) | 0.517 |
| Heart rate (bpm) | 79.4 (19.5) | 78.2 (16.6) | 81.8 (20.7) | 0.137 |
| Active smoking (%) | 238 (14.4%) | 32 (15.1%) | 27 (12.8%) | 0.777 |
| Myocardial infarction (%) | 636 (38.3%) | 91 (42.9%) | 77 (36.5%) | 0.347 |
| Hypertension (%) | 1041 (62.7%) | 133 (62.7%) | 117 (55.5%) | 0.117 |
| Diabetes Mellitus (%) | 544 (32.8%) | 62 (29.2%) | 72 (34.1%) | 0.513 |
| Atrial fibrillation (%) | 710 (42.8%) | 95 (44.8%) | 107 (50.7%) | 0.088 |
| Stroke (%) | 154 (9.28%) | 20 (9.43%) | 22 (10.4%) | 0.866 |
| Chronic obstructive pulmonary disease (%) | 269 (16.2%) | 43 (20.3%) | 54 (25.6%) | **0.002** |
| Chronic kidney disease (%) | 460 (27.7%) | 54 (25.5%) | 75 (35.5%) | **0.038** |
| Haemoglobin (g/dL) | 13.4 [12.0;14.5] | 13.0 [11.5;14.2] | 12.6 [11.0;14.1] | <**0.001** |
| Sodium (mmol/L) | 140 [137;142] | 140 [137;142] | 139 [137;141] | 0.065 |
| Potassium (mmol/L) | 4.20 [3.90;4.60] | 4.20 [3.90;4.60] | 4.10 [3.80;4.40] | **0.005** |
| Serum creatinine (mmol/L) | 100 [82.0;129] | 101 [84.0;132] | 106 [83.5;141] | 0.251 |
| Estimated glomerular filtration rate (mL/kg/min) | 61.9 (23.6) | 59.8 (21.8) | 57.8 (24.6) | **0.034** |
| NT-proBNP (ng/L) | 2596 [1104; 5336] | 2732 [1296; 6238] | 4060 [1710; 9990] | <**0.001** |
| Troponin T (ng/L) | 30.9 [18.9;53.3] | 34.3 [19.5;59.3] | 36.7 [23.9;77.0] | **0.001** |
| Interleukin-6 (pg/mL) | 5.00 [2.73;9.50] | 5.50 [3.10;10.9] | 8.30 [4.00;16.4] | <**0.001** |
| C-reactive protein (mg/L) | 12.6 [5.55;24.7] | 13.4 [5.88;29.1] | 19.8 [7.20;40.0] | <**0.001** |
| ACEi/ARB (%) | 1189 (71.7%) | 156 (73.6%) | 151 (71.6%) | 0.839 |
| Beta-blockers (%) | 1392 (83.9%) | 170 (80.2%) | 172 (81.5%) | 0.302 |
| Loop diuretics (%) | 1652 (99.6%) | 210 (99.1%) | 209 (99.1%) | 0.224 |
| Aldosterone antagonist (%) | 872 (52.6%) | 116 (54.7%) | 101 (47.9%) | 0.332 |
| Digoxin (%) | 263 (15.9%) | 37 (17.5%) | 25 (11.8%) | 0.236 |
| IL-17 (pg/mL) | <0.5 | 1.87 [1.09;2.82] | 11.3 [6.16;20.9] | |

*Note*: Normally distributed continuous data are presented as means and standard deviation, not normally distributed data as medians and 25th until 75th percentile, categorical variables as percentages and frequencies.
Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

**Table 2** Baseline characteristics according to IL-17 concentrations in the validation cohort.

| | Low | Intermediate | High | *P* value |
|---|---|---|---|---|
| | *N* = 1312 | *N* = 213 | *N* = 212 | |
| Age (years) | 72.4 (10.8) | 75.5 (10.2) | 75.9 (10.1) | **<0.001** |
| Women (%) | 435 (33.2%) | 83 (39.0%) | 75 (35.4%) | 0.233 |
| Caucasian (%) | 1304 (99.4%) | 212 (99.5%) | 211 (99.5%) | 1.000 |
| New York Heart Association class III or IV (%) | 716 (54.6%) | 141 (66.5%) | 150 (70.8%) | **<0.001** |
| Hospitalized (%) | 660 (50.3%) | 123 (57.7%) | 151 (71.2%) | **<0.001** |
| Hospitalized in the previous year (%) | 354 (27.3%) | 51 (24.4%) | 54 (26.1%) | 0.657 |
| Heart failure with preserved ejection fraction (%) | 319 (27.0%) | 60 (31.4%) | 62 (31.6%) | 0.234 |
| Left ventricular ejection fraction (%) | 40.0 [35.0;50.0] | 42.0 [33.5;52.0] | 43.0 [35.0;50.0] | **0.025** |
| BMI (kg/m$^2$) | 29.2 (6.42) | 29.2 (6.32) | 28.3 (6.44) | 0.190 |
| Obesity (BMI > 30%) | 501 (39.0%) | 83 (39.9%) | 72 (34.6%) | 0.447 |
| Systolic BP (mmHg) | 126 (22.1) | 127 (25.8) | 125 (21.9) | 0.796 |
| Diastolic BP (mmHg) | 69.6 (13.2) | 67.6 (13.3) | 67.9 (12.7) | **0.040** |
| Heart rate (bpm) | 73.7 (16.6) | 73.6 (17.0) | 77.6 (16.0) | 0.007 |
| Myocardial infarction (%) | 629 (48.0%) | 113 (53.1%) | 106 (50.5%) | 0.352 |
| Hypertension (%) | 734 (56.2%) | 138 (65.1%) | 134 (63.5%) | **0.012** |
| Diabetes mellitus (%) | 416 (31.8%) | 69 (32.5%) | 75 (35.9%) | 0.508 |
| Atrial fibrillation (%) | 578 (44.4%) | 88 (41.5%) | 94 (44.8%) | 0.715 |
| Stroke (%) | 227 (17.4%) | 36 (17.2%) | 51 (24.2%) | 0.059 |
| Chronic obstructive pulmonary disease (%) | 226 (17.4%) | 47 (22.2%) | 46 (22.2%) | 0.086 |
| Chronic kidney disease (%) | 573 (44.4%) | 99 (47.1%) | 112 (53.6%) | **0.043** |
| Haemoglobin (g/dL) | 13.4 [12.0;14.7] | 12.7 [11.4;13.9] | 12.4 [11.0;13.8] | **<0.001** |
| Sodium (mmol/L) | 139 [137;141] | 139 [138;141] | 139 [137;142] | 0.628 |
| Potassium (mmol/L) | 4.30 [4.00;4.60] | 4.30 [4.00;4.60] | 4.30 [3.90;4.50] | 0.316 |
| Serum creatinine (mmol/L) | 96.0 [79.0;123] | 99.0 [79.0;124] | 100 [82.0;136] | 0.293 |
| Estimated glomerular filtration rate (mL/kg/min) | 61.7 (22.7) | 58.9 (21.9) | 57.7 (24.2) | **0.024** |
| NT-proBNP (ng/L) | 1238 [480; 3179] | 1757 [578; 4312] | 1768 [726; 5118] | **<0.001** |
| ACEi/ARB (%) | 961 (73.2%) | 138 (64.8%) | 126 (59.4%) | **<0.001** |
| Beta-blockers (%) | 967 (73.7%) | 144 (67.6%) | 147 (69.3%) | 0.102 |
| Loop diuretics (%) | 1297 (98.9%) | 208 (97.7%) | 212 (100%) | 0.085 |
| Aldosterone antagonist (%) | 444 (33.8%) | 60 (28.2%) | 59 (27.8%) | 0.082 |
| IL-17 (pg/mL) | <0.5 | 1.96 [1.24;3.01] | 8.84 [5.96;21.1] | |

*Note*: Normally distributed continuous data are presented as means and standard deviation, not normally distributed data as medians and 25th until 75th percentile, categorical variables as percentages and frequencies.
Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

increased activity of pathways related to several different types of inflammatory processes compared with patients with low IL-17 concentrations. These included adaptive immune response, leukocyte activation, lymphocyte-mediated immunity, regulation of the adaptive immune response and immune effector processes (*Figure* 3).

## Discussion

In two large and independent cohorts of patients with heart failure, we found that high serum concentrations of IL-17 were consistently associated with more severe heart failure. Furthermore, the pattern of up-regulated proteins in patients with high IL-17 concentrations reflected increased activity of distinct inflammatory pathways.

Previous studies have demonstrated an association between severity of heart failure and inflammatory cytokines, including IL-1, IL-6, TNF and CRP.[15] Although we also found that high concentrations of IL-17 were associated with elevated levels of CRP and IL-6, in multivariable regression analysis only IL-6 was independently associated with increased IL-17 concentrations. IL-6 is known to be substantially involved in the inflammatory mechanisms of heart failure pathophysiology based on experimental and clinical evidence whereas CRP appears to be less specific in this context.[15,16] This suggests that IL-17 might more selectively reflect inflammatory processes in heart failure. Moreover, while IL-17 promotes the release of IL-6, IL-6 signalling is also involved in the maintenance and function of Th17 cells, which are one of the known producers of IL-17.[17,18] Thus, our results might also reflect the strong interaction between IL-17 and IL-6, which could be of particular importance given the well-known role of IL-6 in the inflammatory mechanisms underlying heart failure pathophysiology. In addition, in this study, patients with high concentrations of IL-17 also had lower levels of haemoglobin. Anaemia is common in patients with heart failure and is associated with more severe symptoms, and increased rates of hospitalization and mortality.[19] It is known that TNF, IL-6 and several other proinflammatory cytokines are inversely correlated with haemoglobin levels in heart failure and that an activated proinflammatory state is probably

**Figure 1** Cumulative incidence curves and *P* value of log-rank test for all-cause mortality and the combined endpoint of HF hospitalization and mortality at 2 years in the index cohort (A) and the validation cohort (B) stratified according to levels of IL-17.

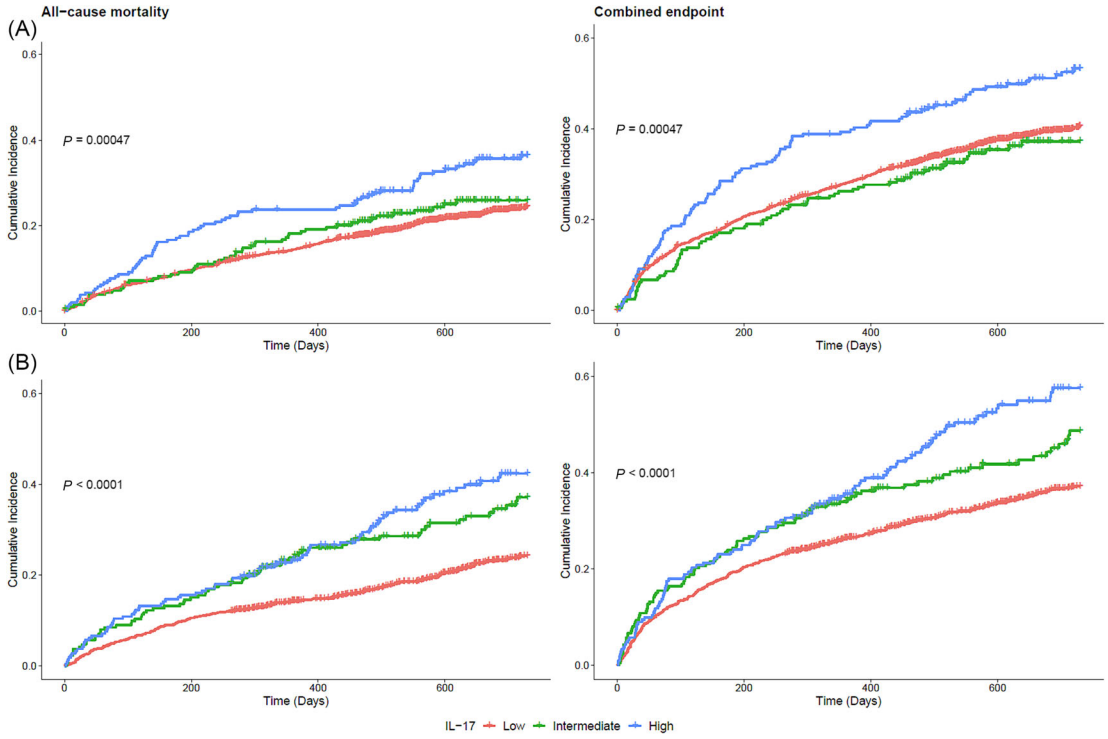



**Table 3** Cox regression analyses comparing heart failure patients with high IL-17 concentrations to patients with low IL-17 concentrations adjusted for age, sex and COPD or the BIOSTAT CHF risk model.

| | Index cohort | | Validation cohort | |
|---|---|---|---|---|
| | HR (95%CI) | *P* value | HR (95%CI) | *P* value |
| Mortality | | | | |
| Univariable | 1.64 (1.28–2.11) | <0.001 | 1.96 (1.53–2.51) | <0.001 |
| Adjusted | 1.55 (1.21–2.00) | <0.001 | 1.64 (1.27–2.12) | <0.001 |
| BIOSTAT-CHF risk model | 1.29 (1.00–1.66) | 0.049 | 1.78 (1.39–2.29) | <0.001 |
| Combined endpoint | | | | |
| Univariable | 1.47 (1.2–1.8) | <0.001 | 1.68 (1.36–2.07) | <0.001 |
| Adjusted | 1.45 (1.18–1.77) | <0.001 | 1.47 (1.18–1.82) | <0.001 |
| BIOSTAT-CHF risk model | 1.12 (0.91–1.38) | 0.27 | 1.15 (0.93–1.43) | 0.19 |

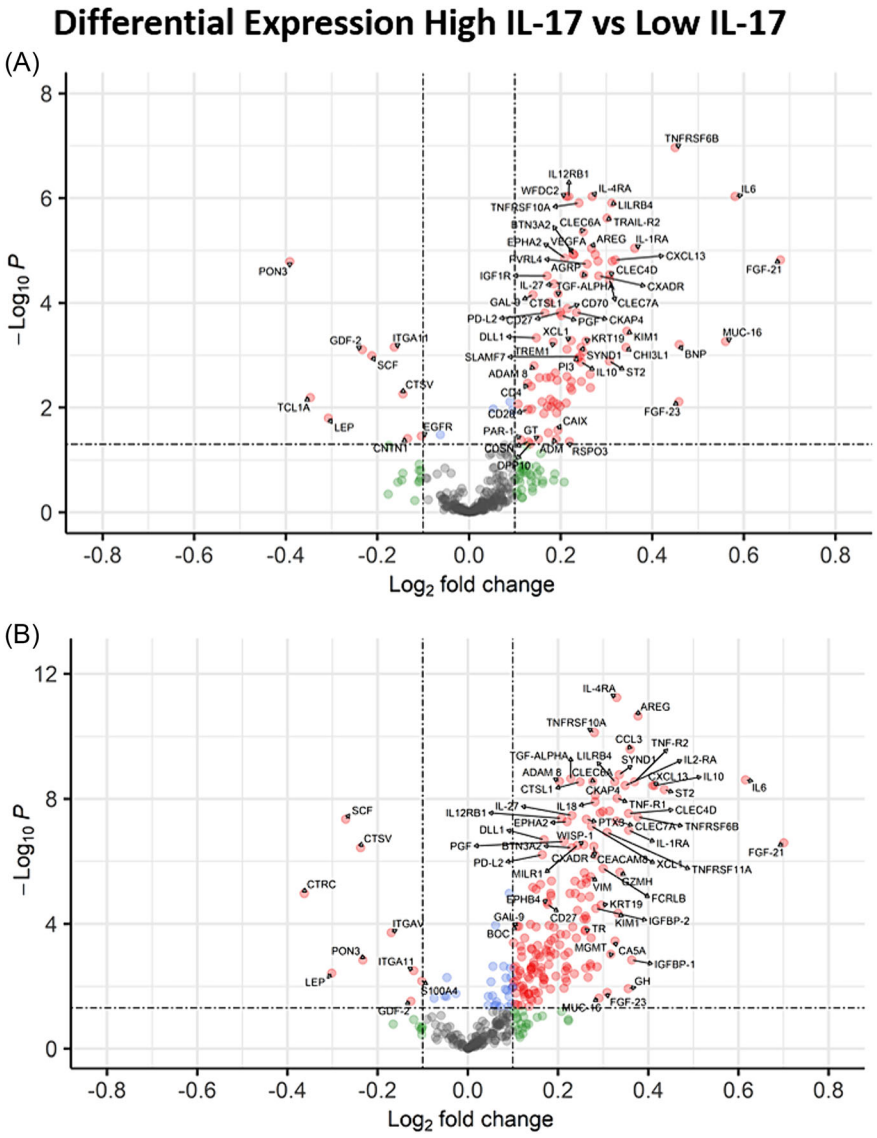
*Note*: Variables included in the BIOSTAT risk model for the combined endpoint of HF hospitalization or mortality: age, previous HF hospitalization, peripheral oedema, systolic blood pressure, N-terminal pro-B-type natriuretic peptide (NT-proBNP), haemoglobin, high-density lipoprotein, sodium, and use of beta-blockers. Variables included in the BIOSTAT risk model for mortality:age, blood urea nitrogen, NT pro-BNP, haemoglobin and failure to prescribe a beta-blocker.
Abbreviations: CI, confidence interval; HR, hazard ratio.

one of the most frequent underlying causes of anaemia in heart failure.[20]

In both cohorts, the strongest up-regulated proteins in patients with high IL-17, alongside IL-6, were CXCL13, FGF-21, TNFRSF6B and IL-1RA. CXCL13 is one of the CXC chemokines that promote immune response and recruit inflammatory cells to inflamed tissue.[21] It is associated with chronic inflammation and was found to be particularly involved in local inflammation in the heart, contributing to myocardial remodelling.[22,23] Similarly, FGF-21 is a peptide hormone that is specifically implicated in cardiomyocyte inflammation.[24,25] It has been shown to be involved in both inflammation and fibrosis, contributing to cardiac remodelling.[26] Up-regulation of IL-1RA and TNFRSF6B observed in our analysis can be considered as indicative of increased IL-1 and TNF activity, both of which are well-known to be central drivers of inflammation in the pathophysiology of heart failure.[15]

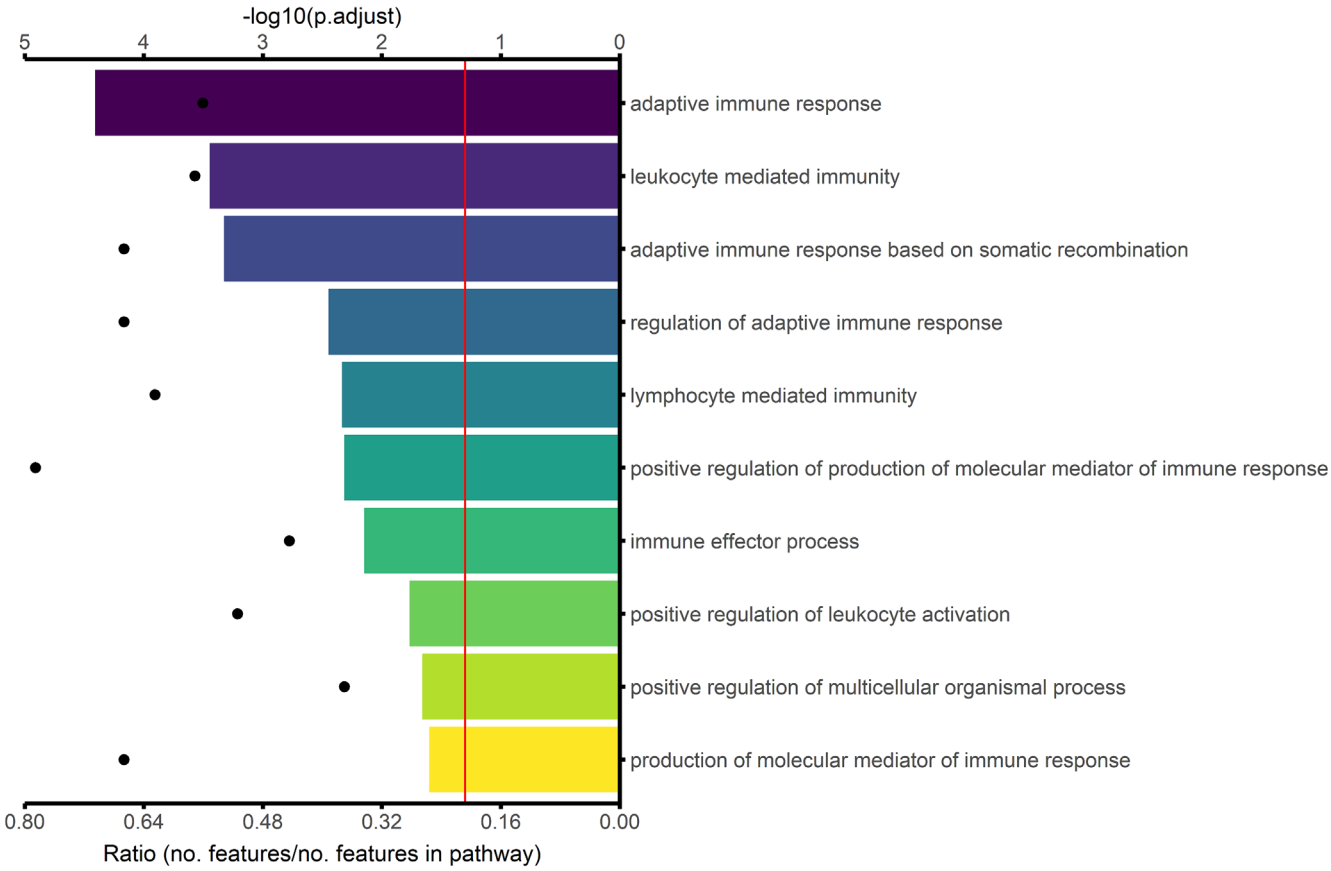
Pathway over-representation analysis conducted in this study showed that patients with elevated IL-17 concentrations had increased activity of pathways associated with

**Figure 2** Volcano plots presenting differentially expressed proteins in heart failure patients with high IL-17 concentrations compared with heart failure patients with low IL-17 concentrations corrected for age and sex in(A) the index cohort, (B) the validation cohort. Proteins labelled grey (not significant), green (log2fold change >0.1), blue (significant) and red (significant and log2fold change >0.1). Adjusted *P* value cut-off 0.05.




various inflammatory processes, such as the adaptive immune response, leukocyte activation, lymphocyte-mediated immunity and immune effector processes suggesting a strong link between IL-17 concentrations and inflammatory responses. In particular, the identified pathways, including the adaptive immune response, leukocyte activation and lymphocyte-mediated immunity, suggest a role for IL-17 not only in modulating general inflammation but notably also in modulating adaptive immunity in patients with heart failure. Adaptive immunity is known to be involved in various aspects of the pathophysiology of heart failure through the recruitment of lymphocytes to the myocardium and is particularly relevant in chronic and acutely decompensated heart failure.[2,27] These infiltrating adaptive immune cells substantially contribute to altering the structural and functional properties of the heart and kidney, often also promoting disease progression.[28] Also, the increased activation of pathways related to the regulation of the adaptive immune response and to immune effector processes underlines the potential of IL-17 in modulating the immune response in heart failure. This suggests that IL-17's influence may extend beyond participation in inflammatory processes to potentially guiding the response of the immune system to cardiac stress. Interestingly, certain pathways identified as up-regulated in this analysis, such as leukocyte-mediated immunity or regulation of the adaptive immune response, were

**Figure 3** Pathway over-representation analyses for heart failure patients with high IL-17 concentrations compared withheart failure patients with low IL-17 concentrations. The ratio represents the proportion of up-regulated proteins in the pathway analysis, divided by the total number of proteins present.




also associated with worse outcomes, as shown in another study in the same cohort.[29] This convergence of findings emphasizes the potential role of IL-17 in harmful immune processes in heart failure. Targeting IL-17 may provide a multimodal approach, potentially mitigating the effects of other important inflammatory cytokines and processes and providing a more comprehensive and tailored therapeutic approach.

Several therapies targeting the IL-17 pathway are already available and in development for a number of immunological diseases.[12] For instance, both secukinumab and ixekizumab are monoclonal antibodies that inhibit IL-17 from binding and interacting with its receptor, thereby suppressing the downstream production of inflammatory cytokines. Both drugs have received approval from the United States Food and Drug Administration (FDA) for the treatment of patients with psoriasis, and secukinumab has also been reported to improve myocardial function in these patients, demonstrating the clinical potential of IL-17 targeting therapies.[30–32]

Limitations of this study include the retrospective study design. Results of this study cannot provide proof of causality and should therefore be regarded hypothesis-generating. Additionally, because the analysed proteins come from panels of selected categories (cardiovascular, oncological and immunological), any up-regulated protein or pathway may be biased towards one of these categories. Nevertheless, we accounted for this by including all proteins as a background in pathway over-representation analyses. Furthermore, no data are available on the occurrence of infections or autoimmune diseases in this cohort. Although the design of the study implies a lower probability of infections, it is possible that it could have influenced the results. Furthermore, there is currently a lack of reference values or thresholds for IL-17 concentrations. A straightforward approach based on the detection limit of the ELISA was chosen, despite the lack of additional evidence to support this. A considerable proportion of the measurements fell below the limit of detection, which is a common challenge in quantitative analysis, particularly when dealing

with low abundance molecules. However, it is important to note that IL-17 appears more often at lower concentrations, so measurements below the limit of detection are not unreasonable.[33] Lastly, the patients enrolled were predominantly Caucasian, which limits generalizability. Further studies in more diverse populations will have to be performed to replicate our findings.

## Conclusion

In patients with heart failure, elevated IL-17 concentrations indicate more severe heart failure and increased activity of inflammatory processes known to be involved in the pathophysiology of heart failure. IL-17 might hold potential for identifying and targeting inflammation in heart failure.

## Conflict of interest statement

C. C. L. received research grants from Applied Therapeutics, Anacardia, Astra Zeneca, Boehringher Ingelheim, Eli Lilly, Moderna, Roche Diagnostics, Novo Nordisk, Novartis, BHF, UKRI, NIHR-HTA, CSO, Horizon 2020 EU funding and JDRF. Consultancy/speaking fees from Amarin, Astra Zeneca, Boehringheringelheim, Novartis and Vifor; A. A. V. received consulting fee payments to his institution from AstraZeneca, Bayer AG, Boehringer Ingelheim, AnaCardio, Cytokinetics, Eli Lilly, Merck, Moderna, Novartis, Novo Nordisk and Pfizer; P. v. d. M. received institutional payments for consultancy fees and/or grants from AstraZeneca, Boehringer Ingelheim, BridgeBio, Ionis, Novartis, Novo Nordisk, Pfizer, Pharmacosmoc, Pharma Nord and Vifor.

## Permission note

All material is original to this submission.

## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Forest plot with a multivariable logistic regression model for high IL-17 levels.
**Table S1.** List of consistently upregulated proteins in both index and validation cohort.

## References


1. Hanna A, Frangogiannis NG. Inflammatory cytokines and chemokines as therapeutic targets in heart failure. *Cardiovasc Drugs Ther* **34**:849-863. doi:10.1007/s10557-020-07071-0
2. Adamo L, Rocha-Resende C, Prabhu SD, Mann DL. Reappraising the role of inflammation in heart failure. *Nat Rev Cardiol* 2020;**17**:269-285. doi:10.1038/s41569-019-0315-x
3. Everett BM, Cornel JH, Lainscak M, Anker SD, Abbate A, Thuren T, *et al*. Anti-inflammatory therapy with canakinumab for the prevention of hospitalization for heart failure. *Circulation* 2019;**139**:1289-1299. doi:10.1161/CIRCULATIONAHA.118.038010
4. A research study to look at how ziltivekimab works compared to placebo in people with heart failure and inflammation - Full Text View - ClinicalTrials.gov https://classic.clinicaltrials.gov/ct2/show/NCT05636176 (18 December 2023)
5. Hu Y, Shen F, Crellin NK, Ouyang W. The IL-17 pathway as a major therapeutic target in autoimmune diseases. *Ann N Y Acad Sci* 2011;**1217**:60-76. doi:10.1111/j.1749-6632.2010.05825.x
6. Chen J, Liao MY, Gao XL, Zhong Q, Tang TT, Yu X, *et al*. IL-17A induces pro-inflammatory cytokines production in macrophages via MAPKinases, NF-κB and AP-1. *Cell Physiol Biochem* 2013;**32**:1265-1274. doi:10.1159/000354525
7. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, *et al*. IL-17 stimulates the production and expression of Proinflammatory cytokines, IL-β and TNF-α, by human macrophages. *J Immunol* 1998;**160**:3513-3521. doi:10.4049/JIMMUNOL.160.7.3513
8. Zhou SF, Yuan J, Liao MY, Xia N, Tang TT, Li JJ, *et al*. IL-17A promotes ventricular remodeling after myocardial infarction. *J Mol Med* 2014;**92**:1105-1116. doi:10.1007/s00109-014-1176-8
9. Baldeviano GC, Barin JG, Talor MV, Srinivasan S, Bedja D, Zheng D, *et al*. Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. *Circ Res* 2010;**106**:1646-1655. doi:10.1161/CIRCRESAHA.109.213157
10. Xue GL, Li DS, Wang ZY, Liu Y, Yang JM, Li CZ, *et al*. Interleukin-17 upregulation participates in the pathogenesis of heart failure in mice via NF-κB-dependent suppression of SERCA2a and Cav1.2 expression. *Acta Pharmacol Sin* 2021;**42**:1780-1789. doi:10.1038/s41401-020-00580-6
11. Gergely TG, Kucsera D, Tóth VE, Kovács T, Sayour NV, Drobni ZD, *et al*. Characterization of immune checkpoint inhibitor-induced cardiotoxicity reveals interleukin-17A as a driver of cardiac dysfunction after anti-PD-1 treatment. *Br J Pharmacol* 2023;**180**:740-761. doi:10.1111/bph.15984
12. Huangfu L, Li R, Huang Y, Wang S. The IL-17 family in diseases: from bench to bedside. *Signal Transduct Target Ther* 2023;**8**:1-22. doi:10.1038/s41392-023-01620-3
13. Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, *et al*. A systems BIOlogy study to TAilored treatment in chronic heart failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail* 2016;**18**:716-726. doi:10.1002/ejhf.531
14. Voors AA, Ouwerkerk W, Zannad F, van Veldhuisen DJ, Samani NJ, Ponikowski P, *et al*. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. *Eur J Heart Fail* 2017;**19**:627-634. doi:10.1002/ejhf.785

15. Murphy SP, Kakkar R, McCarthy CP, Januzzi JL. Inflammation in heart failure: JACC state-of-the-art review. *J Am Coll Cardiol* 2020;**75**:1324-1340. doi:10.1016/j.jacc.2020.01.014
16. Kalogeropoulos A, Georgiopoulou V, Psaty BM, Rodondi N, Smith AL, Harrison DG, *et al*. Inflammatory markers and incident heart failure risk in older adults: the health ABC (health, aging, and body composition) study. *J Am Coll Cardiol* 2010;**55**:2129-2137. doi:10.1016/j.jacc.2009.12.045
17. Sisto M, Lisi S. Targeting Interleukin-17 as a novel treatment option for fibrotic diseases. *J Clin Med* 2023;**13**:164. doi:10.3390/jcm13010164
18. Harbour SN, DiToro DF, Witte SJ, Zindl CL, Gao M, Schoeb TR, *et al*. TH17 cells require ongoing classic IL-6 receptor signaling to retain transcriptional and functional identity. *Sci Immunol* 2020;**5**: 2262. doi:10.1126/sciimmunol.aaw2262
19. Grote Beverborg N, van Veldhuisen DJ, van der Meer P. Anemia in heart failure: still relevant? *JACC Heart Fail* 2018;**6**: 201-208. doi:10.1016/j.jchf.2017.08.023
20. Anand IS, Gupta P. Anemia and iron deficiency in heart failure. *Circulation* 2018;**138**:80-98. doi:10.1161/CIRCULATIONAHA.118.030099
21. Romagnani P, Lasagni L, Annunziato F, Serio M, Romagnani S. CXC chemokines: the regulatory link between inflammation and angiogenesis. *Trends Immunol* 2004;**25**:201-209. doi:10.1016/j.it.2004.02.006
22. Waehre A, Halvorsen B, Yndestad A, Husberg C, Sjaastad I, Nygård S, *et al*. Lack of chemokine signaling through CXCR5 causes increased mortality, ventricular dilatation and deranged matrix during cardiac pressure overload. *PLoS ONE* 2011;**6**:e18668. doi:10.1371/journal.pone.0018668
23. Heinrichs M, Ashour DED, Siegel J, Büchner L, Wedekind G, Heinze KG, *et al*. The healing myocardium mobilizes a distinct B-cell subset through a CXCL13-CXCR5-dependent mechanism. *Cardiovasc Res* 2021;**117**:2664-2676. doi:10.1093/cvr/cvab181
24. Tucker W, Tucker B, Rye KA, Ong KL. Fibroblast growth factor 21 in heart failure. *Heart Failure Rev* 2022;**28**: 261-272. doi:10.1007/s10741-022-10268-0
25. Planavila A, Redondo-Angulo I, Ribas F, Garrabou G, Casademont J, Giralt M, *et al*. Fibroblast growth factor 21 protects the heart from oxidative stress. *Cardiovasc Res* 2015;**106**:19-31. doi:10.1093/cvr/cvu263
26. Zhao Z, Cui X, Liao Z. Mechanism of fibroblast growth factor 21 in cardiac remodeling. *Front Cardiovasc Med* 2023; **10**:1202730. doi:10.3389/fcvm.2023.1202730
27. Markousis-Mavrogenis G, Baumhove L, Al-Mubarak AA, Aboumsallem JP, Bomer N, Voors AA, *et al*. Immunomodulation and immunopharmacology in heart failure. *Nat Rev Cardiol* 2024;**21**:119-149. doi:10.1038/s41569-023-00919-6
28. Ilatovskaya DV, Halade GV, DeLeon-Pennell KY. Adaptive immunity-driven inflammation and cardiovascular disease. *Am J Physiol Heart Circ Physiol* 2019; **317**:H1254-H1257. doi:10.1152/ajpheart.00642.2019
29. Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, Ferreira JP, Anker SD, Cleland JG, *et al*. Multimarker profiling identifies protective and harmful immune processes in heart failure: findings from BIOSTAT-CHF. *Cardiovasc Res* 2022;**118**:1964-1977. doi:10.1093/cvr/cvab235
30. Frieder J, Kivelevitch D, Menter A. Secukinumab: a review of the anti-IL-17A biologic for the treatment of psoriasis. *Ther Adv Chronic Dis* 2018;**9**:5-21. doi:10.1177/2040622317738910
31. Markham A. Ixekizumab: first global approval. *Drugs* 2016;**76**:901-905. doi:10.1007/s40265-016-0579-y
32. Makavos G, Ikonomidis I, Andreadou I, Varoudi M, Kapniari I, Loukeri E, *et al*. Effects of interleukin 17A inhibition on myocardial deformation and vascular function in psoriasis. *Can J Cardiol* 2020;**36**:100-111. doi:10.1016/j.cjca.2019.06.021
33. Todd J, Simpson P, Estis J, Torres V, Wub AHB. Reference range and short- and long-term biological variation of interleukin (IL)-6, IL-17A and tissue necrosis factor-alpha using high sensitivity assays. *Cytokine* 2013;**64**:660-665. doi:10.1016/j.cyto.2013.09.018