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ORIGINAL RESEARCH

Interleukin 6 and Development of Heart Failure With Preserved Ejection Fraction in the General Population

Yook Chin Chia, MBBS, FRCP; Lyanne M. Kieneker, PhD; Gaston van Hassel, BSc; S. Heleen Binnenmars, MD; Ilja M. Nolte, PhD; Jelmer J. van Zanden, PhD; Peter van der Meer, MD, PhD; Gerjan Navis, MD, PhD; Adriaan A. Voors, MD, PhD; Stephan J. L. Bakker, MD, PhD; Martin H. De Borst, MD, PhD*; Michele F. Eisenga, MD, PhD*

BACKGROUND: The cause of heart failure with preserved ejection fraction (HFpEF) is poorly understood, and specific therapies are lacking. Previous studies suggested that inflammation plays a role in the development of HFpEF. Herein, we aimed to investigate in community-dwelling individuals whether a higher plasma interleukin 6 (IL-6) level is associated with an increased risk of developing new-onset heart failure (HF) over time, and specifically HFpEF.

METHODS AND RESULTS: We performed a case-cohort study based on the PREVEND (Prevention of Renal and Vascular End-Stage Disease) study, a prospective general population-based cohort study. We included 961 participants, comprising 200 participants who developed HF and a random group of 761 controls. HF with reduced ejection fraction or HFpEF was defined on the basis of the left ventricular ejection fraction of ≤40% or >40%, respectively. In Cox proportional hazard regression analyses, IL-6 levels were statistically significantly associated with the development of HF (hazard ratio [HR], 1.28; 95% CI, 1.02–1.61; *P*=0.03) after adjustment for key risk factors. Specifically, IL-6 levels were significantly associated with the development of HFpEF (HR, 1.59; 95% CI, 1.16–2.19; *P*=0.004), whereas the association with HF with reduced ejection fraction was nonsignificant (HR, 1.05; 95% CI, 0.75–1.47; *P*=0.77). In sensitivity analyses, defining HFpEF as left ventricular ejection fraction ≥50%, IL-6 levels were also significantly associated with the development of HFpEF (HR, 1.47; 95% CI, 1.04–2.06; *P*=0.03) after adjustment for key risk factors.

CONCLUSIONS: IL-6 is associated with new-onset HFpEF in community-dwelling individuals, independent of potential confounders. Our findings warrant further research to investigate whether IL-6 might be a novel treatment target to prevent HFpEF.

Key Words: general population ■ heart failure ■ heart failure with preserved ejection fraction ■ interleukin 6

The global prevalence of heart failure (HF) is increasing, affecting nearly 1% to 2% of the general population.[1,2] On the basis of the left ventricular ejection fraction (LVEF), HF is being subdivided into HF with reduced ejection fraction (HFrEF) or HF with preserved ejection fraction (HFpEF). Although effective treatment has been fairly well established for patients with HFrEF,[3–5] no specific therapies for patients with HFpEF have been discovered.[6,7]

The cause of HFpEF is poorly understood, but previous studies have suggested that inflammation plays a role in the development of HFpEF.[8,9] It has been well appreciated that because of the chronic inflammatory state that HF comprises, an upregulation of

Correspondence to: Michele F. Eisenga, MD, PhD, Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, the Netherlands. E-mail: m.f.eisenga@umcg.nl

*M. H. De Borst and M. F. Eisenga are co–senior authors.

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CLINICAL PERSPECTIVE

What Is New?

- Higher interleukin 6 levels in the general population are associated with an increased risk of developing heart failure (HF), specifically HF with preserved ejection fraction, independent of potential confounders and variables that could potentially lie in the causal path, including iron deficiency and fibroblast growth factor 23.

What Are the Clinical Implications?

- Interleukin 6 may be a promising biomarker in community-dwelling individuals for the development of HF with preserved ejection fraction.
- The currently identified strong relationship between interleukin 6 and HF with preserved ejection fraction might form the basis to investigate whether interleukin 6 could be a potential therapeutic target in the prevention of HF with preserved ejection fraction.

Nonstandard Abbreviations and Acronyms

BIOSTAT-CHF	Biology Study to Tailored Treatment in Chronic Heart Failure
CHS	Cardiovascular Health Study
FGF23	fibroblast growth factor 23
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
MESA	Multi-Ethnic Study of Atherosclerosis
PREVEND	Prevention of Renal and Vascular End-Stage Disease

inflammatory cytokines ensues, which have been associated with adverse outcomes and cardiac remodeling.[10,11] One of the major upregulated cytokines is interleukin 6 (IL-6).[10–12] IL-6 is a multifunctional proinflammatory cytokine that mediates both immune and inflammatory responses. Increased levels of IL-6 have been identified in patients with HF compared with patients without HF, and interestingly, IL-6 levels continue to increase progressively, with worsening of the New York Heart Association classification.[11,13] In fact, a recent article from Markousis-Mavrogenis et al showed that elevated IL-6 levels were present in more than half of the population of patients with HF, in particular in patients with HFpEF, defined as LVEF >40%, and that increased IL-6 levels were independently associated with death and/or HF hospitalization.[9]

Previous studies have shown that IL-6 levels are closely related to the induction of iron deficiency through upregulation of hepcidin, the master regulator of the iron homeostasis.[14,15] Similarly, increased levels of IL-6 are strongly related to increased levels of fibroblast growth factor 23 (FGF23), an osteocyte-derived hormone crucial in vitamin D and phosphorus homeostasis.[16] This ensues both directly and indirectly through the induction of iron deficiency, which upregulates total FGF23 levels. More important, iron deficiency, anemia, and increased FGF23 levels have previously been shown to be involved in the pathophysiological features of HF,[17–21] and could therefore be crucial factors involved in the relationship between IL-6 and HF.

To date, it is unknown whether levels of IL-6 in the general healthy population are associated with an increased risk of development of HF. Hence, in this case-cohort study, we aimed to investigate the association between IL-6 and development of HF, including subdivision into HFrEF and HFpEF, and whether a putative association between IL-6 and new-onset HF could be explained by induction of iron deficiency, anemia, or elevated FGF23 levels.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request. For current study, we performed a case-cohort study of 1003 participants from the PREVEND (Prevention of Renal and Vascular End-Stage Disease) study. The PREVEND study has been described in detail previously.[22] In brief, from 1997 to 1998, all inhabitants of the city of Groningen, the Netherlands, who were aged 28 to 75 years (n=85 421) received a questionnaire on demographics, disease history, smoking habits, and use of medication; and they also received a vial to collect an early morning urinary sample. Of these individuals, 40 856 (47.8%) responded. After exclusion of subjects with insulin-dependent diabetes mellitus and pregnant women, participants with a urinary albumin concentration ≥10 mg/L (n=6000) and a randomly selected control group with a urinary albumin excretion <10 mg/L (n=2592) completed the screening protocol and as such formed the baseline PREVEND study cohort (n=8592). We used data from the second survey, which took place between 2001 and 2003, consisting of 6894 participants because for this visit data on iron metabolism and FGF23 levels were available. For current case-cohort study, all participants were free from HF at inclusion (baseline), and we measured IL-6 levels only once from stored frozen samples gathered during

the second visit of the PREVEND study, which we consider baseline for the current analyses. We measured IL-6 levels in all participants who had developed new-onset HF (n=213) over time during follow-up and in a randomly selected control group of participants (n=790) to have a case-control ratio of 1 case with up to 3 or 4 controls.[23,24] Furthermore, we excluded participants with missing data on FGF23 (n=42), resulting in 961 participants eligible for analyses. The PREVEND study protocol was approved by the institutional medical review board and was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Exposures and Definitions

Fasting blood samples were drawn in the morning from all participants from April 24, 2001, to December 3, 2003. All hematologic measurements were measured in venous blood. Aliquots of these samples were stored immediately at −80°C until further analysis. The IL-6 assay for this study was performed on the stored serum samples from the second visit using the e601 module of the cobas 6000 (Roche Diagnostics, Mannheim, Germany; IL-6 reagent Roche reference number 05109442190). IL-6 measurements had intra-assay and interassay coefficients of variation <3.5% and <4.3%, respectively, measured in our own hands. The lower limit of detection was 1.5 pg/mL, and as such, levels of IL-6 ≤1.5 pg/mL were categorized at the lower limit of detection. Serum creatinine was measured using an enzymatic method on a Roche Modular analyzer (Roche Diagnostics). Detailed explanation of the measurement of NT-proBNP (N-terminal pro-B-type natriuretic peptide) and hs-CRP (high-sensitivity C-reactive protein) has been described elsewhere.[25,26] Serum ferritin levels were measured using immunoassay (Roche Diagnostics). FGF23 levels were measured in plasma EDTA samples with a human FGF23 ELISA (Quidel Corp, San Diego, CA), the details of which have been described elsewhere.[27] The intra-assay and interassay coefficients of variation of the FGF23 ELISA were <5% and <16%, respectively, in blinded replicated samples.[28] The FGF23 measurement that we used in our current study is directed against 2 different epitopes within the C-terminal part of the FGF23 molecule. Hence, the currently used assay measures both the intact hormone and the C-terminal fragments, and as such measures total FGF23 levels. For estimated glomerular filtration rate (eGFR), the Chronic Kidney Disease Epidemiology Collaboration formula was applied.[29] Presence of type 2 diabetes mellitus was defined as a fasting glucose level ≥7 mmol/L (>126 mg/dL), a nonfasting glucose level of ≥11.1 mmol/L (>200 mg/dL), or use of antidiabetic medication. Information on alcohol consumption and smoking behavior was gathered using a questionnaire. Smoking behaviour was classified as never, former, or current smoker.

HF Definitions

The definitions of HF have been well described previously.[30] Briefly, in the PREVEND study cohort, information on dates and causes of death for every participant was obtained from Statistics Netherlands until January 1, 2011, and coded according to the *International Classification of Diseases, Tenth Revision* (*ICD-10*).[30]

Patient files were checked in the 2 hospitals serving Groningen for the presence of HF at baseline and for new-onset HF, by recording signs, symptoms, and objective evidence of HF. Permission to access hospital records was granted by the local Ethics Committees of both hospitals. Patients were diagnosed with HF using criteria in accordance with the Heart Failure Guidelines of the European Society of Cardiology, as described elsewhere.[31] An end point independent adjudication committee involving 7 independent experts in the field of HF evaluated all cases suspected for the diagnosis of new-onset HF. Each case was validated by 2 different experts by reviewing anonymized clinical charts, hospitalization, and physician office records to ascertain the incidence of HF. In case of consensus, patients were classified as "definite new-onset HF," "definite no new-onset HF," or "definite HF, with date of onset before time of recruitment in PREVEND study." In case of difference of opinion about an individual case, the committee made a joint decision. The experts further adjudicated the cases as HFrEF, when the LVEF was ≤40%, HFpEF (LVEF ≥50%), or HF with midrange ejection fraction when the LVEF was between 41% and 49%. On the basis of the LVEF at the time of diagnosis, HF in our study was classified as HFrEF or HFpEF based on a LVEF ≤40% or >40%, respectively, in line with the previous BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure), which identified a LVEF >40% to be strongly associated with IL-6 in patients with HF.[9] In primary analysis, we therefore included the midrange LVEF of 41% to 49% HF (n=7) included into the HFpEF category. As a sensitivity analysis, we also excluded subjects in the gray area, HF with midrange ejection fraction (ie, LVEF of 41%–49% [n=7]), to obtain HFpEF as defined by LVEF >50%, in line with the original article from the PREVEND study assessing the onset of HF.[30] The cause and the date of onset of HF were also derived from clinical charts. Data on LVEF were available in 98.4% of cases with new-onset HF. In 6 cases, diagnosis was confirmed through joint decision, because of insufficient data on LVEF.

Statistical Analysis

Data were analyzed using IBM SPSS software, version 23.0 (SPSS Inc, Chicago, IL), R version 3.2.3 (Vienna,

Austria), and STATA 14.1 (STATA Corp, College Station, TX). Baseline characteristics are described as means with SD for normally distributed variables, as medians with interquartile range for skewed variables, or as numbers with corresponding percentages for categorical variables. Differences in baseline characteristics across groups of IL-6 were tested with a 1-way ANOVA for normally distributed variables, a Kruskal-Wallis test for skewed variables, and a χ^2 test for categorical variables. We specifically categorized the participants into 4 groups, with the first category being individuals with an IL-6 level under the detection limit (ie, ≤1.5 pg/mL) and categorized as category 1 nondetectable, with the remainder of IL-6 measurements split into equally distributed tertiles. This way of categorizing participants prevents large imbalance between categorizing the participants solitarily into tertiles because a considerable part of included participants have IL-6 levels under the detection limit. In addition, we described differences in baseline characteristics between the group that developed HF and did not develop HF. Between-group differences were tested using *t* test, Mann-Whitney *U* test, or χ^2 test, as appropriate. Hereafter, we performed Cox proportional hazard regression analyses to assess the association of IL-6 with development of new-onset HF over time. Skewed distributed variables (ie, IL-6, ferritin, and FGF-23) were naturally log transformed before inclusion into Cox regression analyses on the association between IL-6 and new-onset HF, and subsequently its subdivided forms (ie, HFrEF and HFpEF). Nonlinearity was tested by using the likelihood ratio test, comparing nested models with linear or linear and cubic spline terms. To visualize the association, we generated restricted cubic splines to illustrate the association between naturally log-transformed IL-6 levels and the subdivided forms of HF. Three knots were placed at the 10th, 50th, and 90th percentiles of the IL-6 levels.

In the Cox regression analyses, we adjusted for potential confounders based on univariate associations or for factors of known biologic importance. After univariable analysis, we adjusted the association between IL-6 and development of new-onset HF for age, sex, and race (model 2). Because of the limited number of events (n=87 in continuous analysis, and n=40 in the upper category of IL-6), we performed subsequently additive instead of cumulative models to avoid overadjustment for potential confounders. Subsequent models were generated to combine variables to fully adjust for kidney function, body composition, diabetes mellitus, hypertension, cardiovascular risk factors, or variables that could potentially lie in the causal path. Hence, further adjustment was performed for eGFR and 24-hour urinary albumin excretion as kidney function parameters (model 3); for body mass index (BMI), 24-hour urinary creatinine excretion (as marker for muscle mass), presence of diabetes mellitus, and use of antidiabetic agents (model 4); and for history of cardiovascular disease and hypertension, use of antihypertensive agents, smoking status, and alcohol consumption (model 5). Finally, we adjusted for variables that could potentially lie in the causal path of the association between IL-6 and development of HF (ie, adjustment for hemoglobin and ferritin levels [model 6]); and last, for FGF23 (model 7).

Hereafter, we subdivided development of new-onset HF into HFrEF and HFpEF and reassessed the association between IL-6 and development of HFrEF and HFpEF with the same models. Subsequently, we specifically assessed the association between IL-6 divided into categories with the development of HFpEF. We evaluated potential effect modification by age, sex, race, and eGFR of the association of IL-6 with risk of HF by each time, fitting models that contain both main effect terms and their cross-product terms. As sensitivity analyses, we performed propensity score matching to as fully as possible account for the difference in baseline characteristics between patients who did and did not develop HF. We matched the controls to the treatment group (1:1) based on propensity scores calculated by logistic regression for variables significantly related to the outcome. Hereafter, we reran the Cox regression analyses between natural log-transformed IL-6 and HF. As another sensitivity analysis, we excluded the patients with HF with midrange ejection fraction (n=7), and reran the Cox regression analysis between natural log-transformed IL-6 and HFpEF. Furthermore, we performed competing risk analysis with death as competing risk event as a sensitivity analysis. For the analyses of subcategories, development of the other category of HF (HFrEF versus HFpEF) was also regarded as competing risk. We applied the competing risk analysis according to the method of Fine and Gray.[32] Overall, 1.3% of the demographic data were missing and were imputed using regressive switching. In all analyses, a 2-sided significance level <0.05 was considered significant.

RESULTS

Baseline Characteristics

We included 961 participants, of whom the mean±SD age was 54±12 years, 45% were men, and the mean BMI was 26.8±4.5 kg/m^2. Median (interquartile range) IL-6 levels in the total population were 1.9 (1.5–3.2) pg/mL. Baseline characteristics according to categories of IL-6 levels are shown in Table 1. A total of 367 (38.2%) participants had IL-6 levels of ≤1.5 pg/mL. The remaining participants with detectable IL-6 levels were subdivided according to tertiles. Of these, the participants had median (interquartile range) IL-6 levels of 1.9

Table 1. Baseline Characteristics of the Included 961 Participants Divided by the Different Categories of IL-6 Levels

Characteristics	All (n=961)	Categories of IL-6 (pg/mL)				*P* Value
		Category 1 (n=367) (Nondetectable)	Tertile 1 (n=196) (1.51–2.28)	Tertile 2 (n=201) (2.29–3.35)	Tertile 3 (n=197) (≥3.36)	
Age, y	54±12	49±10	52±11	58±12	60±13	<0.001
Male sex, n (%)	432 (45)	162 (44)	90 (46)	83 (41)	97 (49)	0.44
Race/ethnicity, n (%)						0.44
White	925 (96.3)	354 (96)	185 (94)	198 (98)	188 (95)	
Black	5 (0.5)	3 (1)	1 (1)	0 (0)	1 (1)	
Asian	22 (2)	6 (2)	8 (4)	3 (2)	5 (2)	
Others*	9 (1)	4 (1)	2 (1)	0 (0)	3 (2)	
BMI, kg/m^2	26.8±4.5	24.9±3.1	26.9±3.9	28.4±4.2	28.7±5.8	<0.001
Alcohol use, n (%)						0.003
No alcohol use	249 (26)	76 (21)	47 (24)	58 (29)	68 (35)	
1–4 Units/mo	165 (17)	60 (16)	37 (19)	37 (18)	31 (16)	
2–7 Units/wk	297 (31)	127 (35)	62 (32)	55 (27)	53 (27)	
>1–3 Units/d	200 (21)	94 (26)	38 (19)	38 (19)	30 (15)	
>3 Units/d	39 (4)	6 (2)	8 (4)	12 (6)	13 (7)	
Missing	11 (1)	4 (1)	4 (2)	1 (1)	2 (1)	
Smoking, n (%)						0.03
Never smoker	298 (32)	127 (35)	67 (35)	54 (27)	50 (26)	
Ex-smoker	382 (40)	140 (39)	79 (42)	89 (44)	74 (38)	
Current smoker	267 (28)	96 (26)	44 (23)	57 (29)	70 (36)	
Diabetes mellitus, n (%)	59 (6)	7 (2)	11 (6)	14 (7)	27 (14)	<0.001
Use of antidiabetic agents, n (%)	32 (3)	3 (1)	5 (3)	9 (5)	15 (8)	<0.001
Hypertension, n (%)	313 (33)	57 (16)	60 (31)	89 (44)	107 (54)	<0.001
Systolic blood pressure, mm Hg	126±19	120±15	125±18	130±21	134±22	<0.001
Diastolic blood pressure, mm Hg	72±9	71±8	73±9	74±9	74±9	<0.001
Use of antihypertensive agents, n (%)	208 (22)	35 (10)	36 (18)	60 (30)	77 (39)	<0.001
Laboratory parameters						
IL-6, pg/mL	1.9 (1.5–3.2)	1.5	1.9 (1.7–2.0)	2.8 (2.5–3.2)	5.2 (4.2–7.1)	<0.001
Hemoglobin, g/dL	13.6±1.2	13.5±1.2	13.7±1.2	13.7±1.3	13.7±1.2	0.30
Ferritin, µg/L	89 (48–166)	68 (38–132)	90 (43–165)	112 (61–211)	115 (59–207)	<0.001
FGF23, RU/mL	70 (57–88)	66 (56–80)	68 (54–87)	75 (61–93)	81 (64–109)	0.02
Erythropoietin, IU/L	7.8 (6.0–10.5)	7.3 (5.5–9.5)	7.5 (5.9–10.0)	8.3 (6.2–11.0)	8.6 (6.9–12.1)	<0.001
Total cholesterol, mg/dL	209.5±40.5	207.6±39.2	209.3±44.2	214.5±39.3	208.1±40	0.25
Glucose, mg/dL	91±22	87±17	89±16	93±21	99±33	<0.001
Serum creatinine, mg/dL	0.96±0.32	0.91±0.14	0.97±0.16	0.95±0.17	1.03±0.65	<0.001
eGFR, mL/min per 1.73 m^2	91.9±17.0	99.2±13.4	92.0±14.2	84.4±16.4	83.7±20.2	<0.001
24-h Urinary albumin, mg/L	7.9 (5.9–11.9)	7.4 (5.7–10)	7.3 (5.5–11.6)	8.7 (6.2–14.3)	9.0 (6.4–18.1)	<0.001
Plasma NT-proBNP, ng/L	44 (23–89)	36 (19–66)	46 (25–90)	51 (23–104)	59 (30–168)	<0.001
hs-CRP, mg/L	1.3 (0.6–3.1)	0.7 (0.3–1.3)	1.2 (0.7–2.3)	2.2 (1.1–4.2)	3.9 (1.9–7.5)	<0.001
Calcium, mg/dL	9.19±0.45	9.15±0.54	9.20±0.36	9.23±0.43	9.23±0.33	0.18
Phosphate, mg/dL	3.18±0.99	3.25±1.12	3.09±0.47	3.08±0.46	3.25±1.41	0.18
25-OH vit D, ng/mL	23.0±9.9	24.4±14.8	24.3±10.4	21.8±9.	19.4±12.3	0.003
PTH, pg/mL	47.4 (38.8–56.3)	45.5 (38.0–53.2)	44.5 (37.6–56.2)	50.0 (40.9–60.0)	49.7 (42.1 –58.1)	<0.001

Data are given as mean±SD or median (interquartile range), unless otherwise indicated. System of units conversion factors: to convert hemoglobin from g/dL to mmol/L, multiply by 0.6206; to convert total cholesterol from mg/dL to mmol/L, multiply by 0.0259; to convert glucose from mg/dL to mmol/L, multiply by 0.0555; to convert phosphate from mg/dL to mmol/L, multiply by 0.3229; to convert calcium from mg/dL to mmol/L, multiply by 0.2495; to convert PTH from pg/mL to pmol/L, multiply by 0.105; to convert 25-OH vit D from ng/mL to nmol/L, multiply by 2.496; to convert creatinine from mg/dL to µmol/L, multiply by 88.42; to convert plasma NT-proBNP from ng/L to pmol/L, multiple by 0.1182; to convert FGF23 from RU/mL to pg/mL, multiple by 21 and then minus 14. BMI indicates body mass index; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; 25-OH vit D, 25-hydroxy vitamin D; PTH, parathyroid hormone; and RU, relative units.

*Others refers to Hispanic, mixed race, or unknown.

Table 2. Baseline Characteristics of the Included Participants, Subdivided Into Those Who Did and Who Did Not Develop HF

Characteristics	All (n=961)	HF (n=200)	Without HF (n=761)	*P* Value
Age, y	54±12	67±9	50±11	<0.001
Male sex, n (%)	432 (45)	122 (61)	310 (41)	<0.001
BMI, kg/m^2	26.8±4.5	28.6±4.3	26.3±4.4	<0.001
Alcohol use, n (%)	712 (74)	133 (67)	579 (76)	0.004
Smoking, n (%)	267 (28)	64 (33)	203 (27)	0.08
Diabetes mellitus, n (%)	59 (6)	36 (18)	23 (3)	<0.001
Hypertension, n (%)	313 (33)	159 (80)	154 (20)	<0.001
Systolic blood pressure, mm Hg	126±19	140±22	122±16	<0.001
Diastolic blood pressure, mm Hg	72±9	76±10	71±8	<0.001
Laboratory parameters				
IL-6, pg/mL	1.9 (1.5–3.2)	3.2 (2.2–3.2)	1.7 (1.5–1.7)	<0.001
FGF23, RU/mL	70 (57–88)	82 (56–68)	68 (65–82)	0.005
Hemoglobin, g/dL	13.6±1.2	14.0±1.3	13.6±1.2	<0.001
Ferritin, µg/L	89 (48–166)	129 (65–129)	82 (42–82)	<0.001
TSAT, %	24.5±9.0	24.5±8.2	24.5±9.2	0.96
eGFR, mL/min per 1.73 m^2	91.9±17	76.1±18.0	96.1±14.0	<0.001
hs-CRP, mg/L	1.3 (0.6–3.1)	2.3 (1.2–2.3)	1.1 (0.5–1.1)	<0.001

Data are given as mean±SD or median (interquartile range), unless otherwise indicated. System of units conversion factors: to convert hemoglobin from g/dL to mmol/L, multiply by 0.6206; to convert FGF23 from RU/mL to pg/mL, multiple by 21 and then minus 14. BMI indicates body mass index; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HF, heart failure; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; RU, relative units; and TSAT, transferrin saturation.

(1.7–2.0), 2.8 (2.5–3.2), and 5.2 (4.2–7.1) pg/mL across tertiles, respectively.

Across categories of IL-6, we observed significant positive associations between IL-6 levels and plasma glucose, serum creatinine, hs-CRP, NT-proBNP, FGF23, and ferritin levels. In contrast, inverse associations were identified between IL-6 levels and alcohol use, eGFR, and vitamin D. Furthermore, individuals in the upper category of IL-6 were older, had a higher BMI, were more likely to smoke or to have diabetes mellitus, had higher systolic and diastolic blood pressure, and were more likely to use antihypertensive and antidiabetic drugs (Table 1).

IL-6 and Incident HF

During a median (interquartile range) follow-up of 8.2 (7.7–8.8) years, 200 participants developed HF: 87 (43.5%) developed HFpEF, and 113 (56.5%) developed HFrEF. The association between IL-6 and new-onset HF was nonlinear ($P_{nonlinearity}$<0.001). Table 2 shows the baseline characteristics of those who developed HF and those who did not. Those who developed HF were older, more men, had a higher BMI, had more diabetes mellitus and hypertension, but consumed less alcohol compared with those who did not develop HF. Similarly, those who developed HF had higher systolic and diastolic blood pressure and had higher IL-6, FGF23, hs-CRP, hemoglobin, and ferritin levels. Conversely, levels of eGFR were lower in those who developed HF (Table 2).

In unadjusted Cox regression analysis, IL-6 was significantly associated with an increased risk of developing new-onset HF (hazard ratio [HR], 2.32 per 1 ln pg/mL increase; 95% CI, 1.95–2.76; *P*<0.001; Table 3). In univariable analysis, IL-6 was similarly associated with both HFrEF and HFpEF. In multivariable Cox regression analyses, the association between IL-6 and new-onset HF remained independently of adjustment for potential confounders (Table 3). The association between IL-6 and new-onset HF remained independent of adjustment for hemoglobin and ferritin levels (HR, 1.47; 95% CI, 1.19–1.83; *P*<0.001). The association between IL-6 and new-onset HF remained also independent of further adjustment for FGF23 (HR, 1.28; 95% CI, 1.02–1.61; *P*=0.03).

When assessing the association of IL-6 with the subdivided forms of HF, it became apparent that IL-6 was significantly associated with development of HFpEF independent of potential confounders, whereas IL-6 was not significantly associated with HFrEF (Table 3 and Figure). The association between IL-6 and HFpEF remained independent of adjustment for hemoglobin and ferritin levels (HR per 1 ln pg/mL increase, 1.75; 95% CI, 1.29–2.37; *P*<0.001). In addition, the association between IL-6 and HFpEF remained also independent of adjustment for FGF23 (HR, 1.59; 95% CI, 1.16–2.19; *P*=0.004). When divided into IL-6 categories,

Table 3. Cox Proportional Hazard Regression Analyses on the Association Between IL-6 and Development of HF in the PREVEND Study

	IL-6 (pg/mL)					
	HF (n=200)		HFrEF (n=113)		HFpEF (n=87)	
	HR (95% CI)*	*P* Value	HR (95% CI)*	*P* Value	HR (95% CI)*	*P* Value
Model 1	2.32 (1.95–2.76)	<0.001	2.08 (1.64–2.65)	<0.001	2.64 (2.06–3.39)	<0.001
Model 2	1.45 (1.18–1.78)	<0.001	1.25 (0.93–1.68)	0.13	1.72 (1.28–2.30)	<0.001
Model 3	1.31 (1.05–1.64)	0.02	1.12 (0.82–1.53)	0.48	1.58 (1.16–2.16)	0.004
Model 4	1.35 (1.08–1.70)	0.01	1.18 (0.86–1.63)	0.31	1.61 (1.16–2.23)	0.005
Model 5	1.45 (1.13–1.84)	0.003	1.15 (0.82–1.61)	0.43	1.84 (1.29––2.63)	0.001
Model 6	1.47 (1.19–1.83)	<0.001	1.26 (0.93–1.72)	0.14	1.75 (1.29–2.37)	<0.001
Model 7	1.28 (1.02–1.61)	0.03	1.05 (0.75–1.47)	0.77	1.59 (1.16–2.19)	0.004

Model 1: univariable analysis. Model 2: model 1+adjustment for age, sex, and race. Model 3: model 2+adjustment for estimated glomerular filtration rate and 24-hour urinary albumin excretion. Model 4: model 2+adjustment for body mass index, 24-hour urinary creatinine excretion, presence of diabetes mellitus, and use of antidiabetic agents. Model 5: model 2+adjustment for history of cardiovascular disease, hypertension, use of antihypertensive agents, smoking status, and alcohol consumption. Model 6: model 2+adjustment for hemoglobin and ferritin levels. Model 7: model 2+adjustment for fibroblast growth factor 23 levels. HF indicates heart failure; HFpEF, HF with preserved ejection fraction; HFrEF, HF with reduced ejection fraction; HR, hazard ratio; IL-6, interleukin 6; and PREVEND, Prevention of Renal and Vascular End-Stage Disease.

*HRs are shown per 1 ln pg/mL increase.

we identified similar findings, with participants in the upper tertile of IL-6 being at an increased risk of developing HFpEF, independent of potential confounders or variables that could potentially lie in the causal path (Table 4).

There was no evidence for effect modification by age, sex, race, or eGFR in the association between IL-6 and development of HF ($P_{interaction}$>0.1 for all). As sensitivity analyses, we performed propensity score matching. We identified that age, sex, and BMI were all statistically significantly associated with HF. Hence, we performed matching of the patients with and without HF for the propensity scores for these covariates (Table S1), after which baseline characteristics were similar among the 2 patient subgroups. After propensity score matching, the association between IL-6 and HF remained statistically significant (Table S1). As another sensitivity analysis, we identified similar results as the primary analysis for the association between IL-6 and HFpEF (HR, 1.47; 95% CI, 1.04–2.06; *P*=0.03 after adjustment for key risk factors) when excluding patients with HF with midrange ejection fraction (Table S2). In addition, we performed competing risk analysis, which rendered similar results (sub-HR per 1 ln pg/

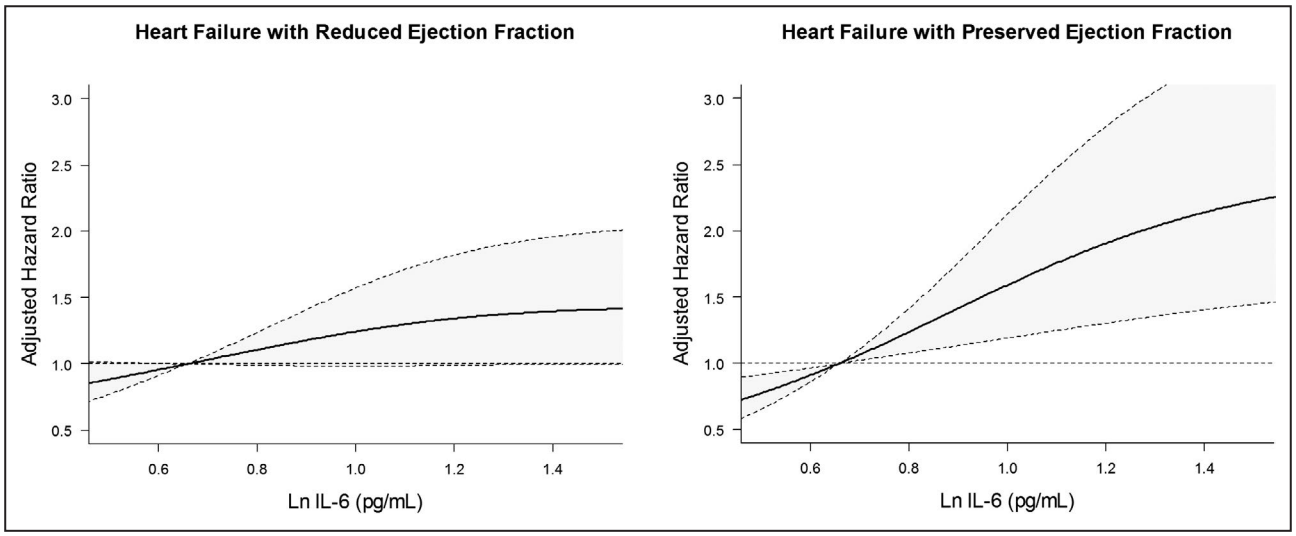

Figure 1. Prospective associations between interleukin 6 (IL-6) levels and development of heart failure with reduced ejection fraction vs heart failure with preserved ejection fraction.

Adjustment has been performed for age, sex, and race, according to model 2. Knots have been placed at 10th, 50th, and 90th percentiles of IL-6 levels. Line represents hazard ratio, and dotted lines with the gray area represent the 95% CI. Ln IL-6 indicates naturally log-transformed IL-6.

Table 4. Association Between Categories of IL-6 and Development of HFpEF

Category (n=Events)	Category 1 (n=11)	Tertile 1 (n=8)	Tertile 2 (n=28)	Tertile 3 (n=40)
Model	**Reference**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Model 1	1.00	1.40 (0.59–3.51)	4.85 (2.19–10.74)	6.74 (2.27–19.96)
Model 2	1.00	0.92 (0.37–2.30)	2.00 (0.96–4.17)	2.82 (1.38–5.74)
Model 3	1.00	0.92 (0.37–2.33)	1.90 (0.91–3.97)	2.46 (1.20–5.05)
Model 4	1.00	0.83 (0.33–2.10)	1.68 (0.79–3.58)	2.26 (1.06–4.81)
Model 5	1.00	0.85 (0.34–2.14)	1.62 (0.76–3.44)	2.15 (1.03–4.51)
Model 6	1.00	0.94 (0.37–2.35)	2.01 (0.96–4.21)	2.81 (1.37–5.76)
Model 7	1.00	0.90 (0.36–2.26)	1.90 (0.91–3.98)	2.41 (1.16–5.03)

Model 1: univariable analysis. Model 2: model 1+adjustment for age, sex, and race. Model 3: model 2+adjustment for estimated glomerular filtration rate and 24-hour urinary albumin excretion. Model 4: model 2+adjustment for body mass index, 24-hour urinary creatinine excretion, presence of diabetes mellitus, and use of antidiabetic agents. Model 5: model 2+adjustment for history of cardiovascular disease, hypertension, use of antihypertensive agents, smoking status, and alcohol consumption. Model 6: model 2+adjustment for hemoglobin and ferritin levels. Model 7: model 2+adjustment for fibroblast growth factor 23 levels. HFpEF indicates heart failure with preserved ejection fraction; HR, hazard ratio; and IL-6, interleukin 6.

mL increase, 1.45; 95% CI, 1.14–1.83; *P*=0.002; model 2) for the association between IL-6 and new-onset HF as the primary analysis. Similarly, the results remained materially unchanged when performing competing risk analysis on the association between IL-6 and HFpEF (sub-HR, 1.61; 95% CI, 1.16–2.22; *P*=0.004; model 2).

DISCUSSION

In this study, we show that higher IL-6 levels are associated with an increased risk of developing HF. Interestingly, we found that IL-6 is statistically significantly associated with development of HFpEF, after adjustment for potential confounders and variables that could potentially lie in the causal path, including iron deficiency and FGF23. The current study confirms previous studies highlighting the importance of IL-6 in patients with HF, and extends this to the general population, revealing that IL-6 may be a promising biomarker in community-dwelling individuals for the development of HFpEF.

Our results confirm the important role of IL-6 with respect to HFpEF, as recently identified in the BIOSTAT-CHF cohort, where HFpEF was an independent predictor of elevated IL-6 levels in a large and heterogeneous cohort involving patients with HF.[9] We now extend this finding to the general population, unraveling that IL-6 levels are associated with the development of HFpEF over time. To date, only 2 studies have assessed the association between IL-6 and development of incident HF over time. Kalogeropoulos et al have shown in the Health, Aging, and Body Composition Study, a cohort involving elderly patients, that IL-6 was associated with incident HF and that this association was especially pronounced in patients with HFpEF.[8] On the other hand, de Boer et al have shown using general population cohorts (ie, the MESA [Multi-Ethnic Study of Atherosclerosis] and CHS [Cardiovascular Health Study]) that IL-6 was associated with new-onset HF overall, with IL-6 being suggestive for HFrEF, but not for HFpEF.[33] The discrepancy between our findings most likely results from the cohorts used. The MESA cohort comprises a heterogeneous population involving multiple ethnicities, likely influencing the results of IL-6.[34,35] Similarly, the CHS included a different population than in our study by including much older participants with a high prevalence of comorbidities, which possibly resulted in more complex multifactorial HF phenotypes.[36]

One of the possible mechanisms underlying the association of IL-6 with HF is that IL-6 has pleiotropic effects on both cardiomyocytes and noncardiomyocytes. Infusion of IL-6 has been shown to induce cardiac hypertrophy, fibrosis, and diastolic dysfunction, which can eventually lead to HFpEF.[37,38] Furthermore, another study showed that patients with HFpEF had higher levels of IL-6 than controls, and that IL-6 and tumor necrosis factor-α downregulated the expression of sarcoplasmic reticulum Ca^{2+} ATPase channels in the cardiomyocytes of patients with HFpEF.[39] Sarcoplasmic reticulum Ca^{2+} ATPase mediates the reabsorption of calcium in the sarcoplasmic reticulum, which, in turn, affects diastolic relaxation of the cardiomyocytes. These findings correlated with the echocardiographic indexes of diastolic dysfunction. We extend the importance of IL-6 to the general population, implying that IL-6 is not only relevant in the setting of HF, but also before occurrence of HF because our cohort constitutes community-dwelling subjects without HF at baseline. Several studies have also shown that elevated IL-6 levels were seen in patients with left ventricular dysfunction even in the absence of the clinical syndrome of HF.[40,41] Taken together, these data suggest that IL-6 may be involved in the progression from asymptomatic or subclinical left ventricular dysfunction to symptomatic left ventricular dysfunction and clinical HF. Hence, IL-6 may be a biomarker of patients at risk

for progression to clinical HF, mainly HFpEF. However, these findings should be replicated and confirmed in a larger independent cohort with more individuals who develop HFpEF.

Interestingly, we have found that IL-6 is statistically significantly associated with new-onset HF, even after adjusting for levels of ferritin, hemoglobin, and FGF23, implying that the association seems not primarily attributable to occurrence of iron deficiency, anemia, or FGF23 upregulation. This is intriguing as IL-6 is known to be strongly involved in regulation of iron deficiency and FGF23 physiological features.[42,43] Iron deficiency, anemia, and FGF23 upregulation have previously been shown to be involved in the pathophysiological features of HF.[17–20]

Although the treatment of HFrEF has been well established, there is currently not an evidence-based therapeutic strategy for HFpEF.[7] Because we found a strong relationship between IL-6 and HFpEF, it seems warranted to investigate whether monoclonal antibodies against IL-6 receptors could potentially be beneficial in the treatment of HFpEF. A compound currently used for rheumatoid arthritis, such as tocilizumab, a monoclonal antibody against the IL-6 receptor, might be a possible therapeutic agent when given in a low dose. Of course, the beneficial effect of reducing IL-6 levels needs to be weighted against an increased theoretical chance of infections and malignancies. However, results from the STREAM study in patients with rheumatoid arthritis are promising in that tociluzimab was considered a long-term safe and efficacious treatment, with almost no infections or malignancies observed.[44] Furthermore, a recent meta-analysis showed that tocilizumab was associated with a lower hazards of myocardial infarction compared with other disease-modifying biologics against rheumatoid arthritis. However, there were not enough data available to perform an analysis for HF.[45] Notwithstanding all this, the use of such therapy needs to be tested in well-designed randomized controlled clinical trials. Furthermore, several studies are currently ongoing to try to elucidate biomarkers of HFpEF,[46,47] and our findings can add on to the body of knowledge and evidence for HFpEF.

Our study has strengths and limitations. Our study comprises a large general population-based cohort with simultaneous IL-6 and FGF23 levels and several other biomarkers and extensive demographic data available, with a long follow-up of the included participants. A possible limitation of our study could be that IL-6 levels were undetectable in a substantial part of the population; however, most likely, this represents the difference in low-grade inflammation, which is present in the included participants. Another limitation is that we did not have data available on other inflammatory cytokines, such as tumor necrosis factor-α, interleukin 1β, or tumor growth factor-β. Another limitation of the current study is the large difference in baseline characteristics, particularly in age, between patients who did and who did not develop HF. As sensitivity analysis, we therefore performed propensity score matching in which the association of IL-6 with HF remained. Although we adjusted in our primary analysis for numerous confounders (eg, age), we cannot exclude the possibility of residual confounding. The possibility of residual confounding combined with the observational design of the study means that no conclusions about causality can be drawn. This hampers also the possibility to carefully dissect the potential origin of the increased IL-6 levels leading to new-onset HF over time. IL-6 is known to be produced among others by adipocytes and as such adipose tissue might be a possible origin and trigger for the IL-6 production in our cohort. However, the association between IL-6 and new-onset HF remained in our cohort independent of adjustment for BMI. Similarly, diabetes mellitus and hypertension can be regarded as states with endothelial dysfunction combined with low-grade inflammation; however, also, adjustment for these factors materially unaltered the association between IL-6 and new-onset HF. Most likely, the origin of the IL-6 in our study is a state of low-grade inflammation, which is also reflected by the increased hs-CRP levels across the IL-6 categories.

In conclusion, we show that increased levels of IL-6 are statistically significantly associated with new-onset HF in community-dwelling individuals, independent of adjustment for key risk factors. More important, this association seems to be mainly attributable to the association between IL-6 and new-onset HFpEF. The current strong relationship between IL-6 and HFpEF might form the basis to investigate whether IL-6 might be a potential therapeutic target in the prevention of HFpEF.

ARTICLE INFORMATION

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Affiliations

Division of Nephrology, Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands (Y.C.C., L.M.K., G.v.H., S.H.B., G.N., S.J.B., M.H.D.B., M.F.E.); ; Department of Medical Sciences, School of Medical and Life Sciences, Sunway University, Bandar Sunway, Selangor, Malaysia (Y.C.C.); ; Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands (I.M.N.); ; Certe, Department of Clinical Chemistry, Martini Hospital, Groningen, Netherlands (J.J.v.Z.); and Department of Cardiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands (P.v.d.M., A.A.V.)..

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manuscript; Dr Kieneker, Dr Nolte, G. van Hassel, Dr Binnenmars, Dr Nolte, Dr van Zanden, Dr van der Meer, Dr Navis, Dr Voors, Dr Bakker, Dr De Borst, and Dr Eisenga reviewed and edited the manuscript; Drs Bakker, De Borst, and Eisenga supervised the manuscript.

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Disclosures

None.

Supplementary Material

Tables S1–S2

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SUPPLEMENTAL MATERIAL

Table S1. Propensity score matching analysis of the association between IL-6 and HF.

Variable		Before matching	After matching
Age	Mean treatment	66.579	66.579
	Mean control	50.497	66.702
Female sex	Mean treatment	38%	38%
	Mean control	59%	42%
BMI	Mean treatment	28.623	28.623
	Mean control	26.325	28.269

The association between IL-6 and HF also remained statistically significant in propensity scored matching with an estimate of 1.37 (standard error 0.32), $P<0.001$.

Table S2. Association between IL-6 and HFpEF with exclusion of HFmrEF patients.

	HFpEF (n=80)	
	HR* (95% CI)	P value
Model 1	2.52 (1.93-3.28)	<0.001
Model 2	1.60 (1.17-2.20)	0.003
Model 3	1.47 (1.05-2.06)	0.02
Model 4	1.48 (1.04-2.11)	0.03
Model 5	1.66 (1.12-2.44)	0.01
Model 6	1.61 (1.16-2.23)	0.004
Model 7	1.47 (1.04-2.06)	0.03

*Hazard ratios are shown per 1 ln pg/mL increase

Model 1: univariate analysis

Model 2: Model 1 + adjustment for age, sex, and race

Model 3: Model 2 + adjustment for estimated glomerular filtration rate and 24-hour urinary albumin excretion

Model 4: Model 2 + adjustment for body mass index, 24-hour urinary creatinine excretion, presence of diabetes, use of anti-diabetics

Model 5: Model 2 + adjustment for history of cardiovascular disease, hypertension, use of anti-hypertensives, smoking status, and alcohol consumption

Model 6: Model 2 + adjustment for hemoglobin and ferritin levels

Model 7: Model 2 + adjustment for fibroblast growth factor 23 levels

CI, confidence interval; HR, hazard ratio; HFpEF, heart failure with preserved ejection fraction.