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Elevated IL-27 in patients with acute coronary syndrome is associated with adverse ventricular remodeling and increased risk of recurrent myocardial infarction and cardiovascular death

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ABSTRACT

Background and aims: IL-27 is an immunoregulatory cytokine belonging to the IL-6/IL-12 family that was found to be elevated in acute coronary syndrome (ACS) patients. We investigated whether IL-27 is related to post-ischemic cardiac remodeling and long-term prognosis in this patient group.
Methods: We included 524 ACS patients, defined as acute myocardial infarction (AMI) or unstable angina (UA). A subgroup of 107 patients donated blood samples 6 weeks after the index event, and underwent a follow-up echocardiographical examination at 1 year. We measured plasma levels of IL-27, high sensitivity troponin T (hsTNT), C-reactive protein (hsCRP) and cystatin C at baseline and in the 6-week samples. The median follow-up period of the cohort was 2.2 years.
Results: The incidence of the combined end-point of AMI and cardiovascular death was higher in patients with plasma IL-27 within the top two tertiles both at baseline and after 6 weeks. After correction for cardiovascular risk factors, medication, hsTNT, hsCRP, and eGFR, patients with baseline IL-27 levels within the highest tertile had a significantly elevated risk for the combined end-point compared with the lowest tertile (hazard ratio 2.70, 95% CI 1.06–6.90, $p = .038$). Additionally, higher baseline IL-27 levels were associated with deleterious left ventricular remodeling and deterioration of systolic and diastolic function during the first year of follow-up.
Conclusions: Elevated IL-27 at the time of an ACS is independently related to impaired cardiac function and worse long-term prognosis. Our data warrants further mechanistic studies to elucidate the involvement of IL-27 in cardiac repair and remodeling after ACS.

1. Introduction

Interleukin 27 (IL-27) is a heterodimeric cytokine belonging to the IL-6/IL-12 family. IL-27 is a heterodimer of Epstein-Barr-induced gene 3 product (EBI3), an IL-12p40 related protein, and p28, an IL-12p35-related polypeptide [1]. It is mainly secreted by myeloid cells, including macrophages, dendritic cells and inflammatory monocytes, but it is also expressed in plasma cells, endothelial and epithelial cells [2]. IL-27 binds to a specific receptor, formed by the gp130 glycoprotein and the IL-27 receptor α chain (IL27Rα), signaling through the Janus kinase/signal transducer and activator (JAK/STAT) and mitogen activated protein kinase (MAPK) pathways [2]. IL-27 is a complex regulator of T-cell function, with both pro- and anti-inflammatory properties. The cytokine was initially thought to be pro-inflammatory, as it promotes naïve T cell shift towards the Th1 phenotype and stimulates interferon-γ (IFN-γ) secretion by activating signal transducer and activator of transcription (STAT) 1 and T-bet [2–4]. Additionally, IL-27 has stimulatory effects on the proliferation and functions of cytotoxic CD8 T-cells [5,6]. However, studies in various models of disease have also demonstrated potent immuno-regulatory properties of this cytokine. IL-27 inhibits

Abbreviations: AMI, acute myocardial infarction; CV, cardiovascular; HR, hazard ratio; CI, confidence interval; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; hsTnT, high-sensitive cardiac troponin T; hsCRP, high-sensitive C-reactive protein; ASA, acetylsalicylic acid; ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers; LVEF, LV ejection fraction; LVEDV, LV end diastolic volume; LVESV, left ventricular end systolic volume; LVID, left inner ventricular diameter; LVMI, left ventricular mass index; LAVI, left atrial volume index

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Th17 cell development [7,8] and promotes the generation and functions of regulatory T cells (Treg) [9,10]. Additionally, IL-27 is an important stimulus for the differentiation of the immunosuppressive Tr1 cell population [11,12] and for IL-10 production [13–15].

Previous animal studies and clinical reports have suggested an implication of IL-27 in cardiovascular (CV) disease. The exact mechanisms are unclear, as studies on IL-27 have so far generated opposing results in mice and humans. Atherosclerosis-prone mice transplanted with bone marrow deficient in IL-27 receptor developed larger atherosclerotic lesions characterized by increased accumulation of inflammatory cells, IL-17A and TNFα [16]. Similarly, knockout mice lacking IL-27 or its receptor were more susceptible to atherosclerosis and had increased infiltration of macrophages in the arterial wall, and administration of IL-27 was able to suppress disease development [17]. These results suggest that IL-27 may play an atheroprotective role in mice. However, the human data collected so far contradicts the experimental findings. IL-27 has been found to be present in carotid plaques [18] and was elevated in patients with angiographically-verified atherosclerotic lesions, correlating with lesion severity and with plasma levels of oxidized LDL [19]. Plasma levels of IL-27 are elevated in patients with UA and acute myocardial infarction (AMI) compared with patients with stable angina or chest pain without angiographically-detected lesions, and with healthy controls [20]. However, the role of IL-27 in the ischemic myocardial injury and post-AMI myocardial recovery is currently unknown, and the prognostic significance of the elevated IL-27 levels in AMI patients has not previously been explored.

In the present study, we investigated whether IL-27 levels at the time of the acute coronary event are related to recurrent AMI and death due to CV disease in a cohort of 524 ACS patients. We also examined the correlations between baseline IL-27 and cardiac structure and function measured by echocardiography at 1 year after the acute coronary event in a subgroup of 107 patients.

2. Materials and methods

2.1. Population

The study included 605 consecutive patients admitted at the Coronary Care Unit of Skåne University Hospital Malmö between October 2008 and December 2012. In order to be included into the study the patients had to be admitted for a suspected ACS, to understand the information and to provide a written informed consent for inclusion. No other exclusion criteria were applied. Fifty patients were later excluded because they did not fulfill the criteria for ACS at discharge (described below). Another 31 patients were excluded due to missing samples. The final study population included 524 individuals. ACS was defined as either AMI or UA. AMI was diagnosed when the patients had typical symptoms for myocardial ischemia associated with acute ischemic changes on electrocardiogram (ECG) and/or elevated troponin-levels with a typical rise and fall. UA was diagnosed when a patient had typical angina at rest, or a deterioration of previously known stable angina and normal or not significantly elevated troponin.

Within 24 h after admission, EDTA plasma samples were collected and centrifuged at 3000*g* for 10 min within an hour, and stored at −80 °C before analysis. An aliquot was used for IL-27 analysis (described below) and another was sent to the certified clinical laboratory of Skåne University Hospital Malmö for analyses of low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), high-sensitive cardiac troponin T (hsTnT), high-sensitive C-reactive protein (hsCRP) and cystatin C. The estimated glomerular filtration rate (eGFR) was calculated based on cystatin C levels, age and sex, using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [21]. Age, gender, body mass index (BMI), history of hypertension, history of diabetes or diabetes diagnosed during hospitalization, current smoking, medication and the results of coronary angiography were recorded. Follow-up of clinical events was performed by collecting data from the Swedish Hospital Discharge Register and the Swedish Cause of Death Register. The last follow-up date was 31 December 2012. The outcome was hospitalization due to a recurrent AMI episode or CV death during follow-up. The codes used to define AMI were ICD10 I21 and I22. CV death was defined as death due to ischemic heart disease or stroke, ICD10 codes I22, I21, I23, I25, I60, I61, I63 and I64. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been priorly approved by the ethical committee of Lund University for research on humans. Written informed consent was obtained from all patients.

2.2. IL-27 analysis

IL-27 was analyzed in plasma by the Proximity Extension Assay (PEA) technique [22] at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala, Sweden. Briefly, oligonucleotide-labeled antibody probe pairs were allowed to bind to their respective targets present in the plasma sample. Addition of a DNA polymerase led to an extension and joining of the two oligonucleotides and formation of a PCR template. Universal primers were used to pre-amplify the DNA template. Finally, the individual DNA sequence was detected and quantified using specific primers by microfluidic real-time quantitative PCR chip (96.96, Dynamic Array IFC, Fluidigm Biomark) on a Biomark HD instrument. Within-run coefficient of variation was 8% and between-run coefficient of variation was 19%. Data analysis was performed by a preprocessing normalization procedure using Olink Wizard for GenEx (Multid Analyses, Sweden). All data are presented as arbitrary units.

2.3. Echocardiography

Baseline echocardiograms were routinely performed during the index hospital stay in all participants. Patients aged 75 years or above were invited to complete a follow-up echocardiogram at 1 year after inclusion. The echocardiographical follow-up was focused on an elder population as the long-term changes in systolic and diastolic heart function following an acute coronary event is to a large extent unknown in this patient group [23]. All echocardiograms were performed at Skåne University Hospital by experienced sonographers, and were analyzed off-line using the Xcelera software (Philips) by a single examiner blinded to clinical data. A total of 108 patients donated further blood samples at 6 weeks after the acute coronary event, and completed the echocardiographic follow-up at 1 year. Data was missing in 1 subject due to technical failure, leaving 107 examinations for analysis of cardiac structure and function. Measurement of left ventricle (LV) ejection fraction (LVEF) was performed in 97 subjects, due to poor image quality or missing frames in 10 of the subjects. LVEF was measured according to Simpson's biplane method in the apical 4-chamber and 2-chamber views, based on LV end-systolic volume (LVESV) and LV end-diastolic volume (LVEDV). Delta values of LVEF (dLVEF) were calculated as LVEF at 1 year minus LVEF at inclusion. Chamber quantification, measurements of diastolic function and filling pressure were performed according to international literature and local established routines, as described below [24,25]. The left atrial volume index (LAVI) was assessed by using the area-length method in the 4-chamber view, indexed to body surface area (BSA). We used the Devereux formula to calculate the left ventricular mass index (LVMI) from diastolic linear dimensions of interventricular septum (IVS), inner left ventricular diameter (LVID) and LV posterior wall (PW), indexed to body surface area. Mitral valve (MV) flow velocity was assessed by pulsed wave Doppler in the apical 4-chamber view. We recorded the early maximal inflow velocity (E), late (atrial) maximal inflow velocity (A) and mitral valve deceleration time (MVDT). The early MV annular plane tissue velocity (e') was calculated using color-coded tissue Doppler [26]. The following criteria were used to indicate LV diastolic dysfunction: MVDT below 0.130 s; MV E/A ratio below 0.7 or above

1.5; and E/e' ratio above 15 [27].

2.4. Statistical analysis

Between-groups comparisons were made using the χ^2 test for categorical variables and the Mann-Whitney *U* test for continuous variables. Spearman's rank test was used to analyze correlations. A multivariate linear regression model was used to assess the associations between plasma IL-27 levels and traditional cardiovascular risk factors (age, gender, BMI, smoking, hypertension, diabetes, kidney function), laboratory parameters (LDL, HDL, hsTnT, hsCRP, eGFR) and statin treatment at baseline. Kaplan-Meier curves and log-rank tests were used to illustrate the incidence of the composite endpoint of AMI and CV death overtime in relation to IL-27 tertiles. Multivariate Cox proportional hazards analyses were used to assess the correlations between IL-27 and the considered end-points of AMI and CV death. Skewed variables were logarithmically transformed before the Cox analysis. A *p* value lower than 0.05 was considered to be statistically significant. All calculations were made using the SPSS 22.0 (IBM software, Armonk NY).

3. Results

3.1. Clinical characteristics of the study population

The clinical characteristics of the cohort are summarized in Table 1. After a median follow-up period of 2.2 years (interquartile range (IQR) 1.2–3.2), 75 patients (14%) reached the combined endpoint of AMI or CV death. Out of these patients, 63 suffered a new AMI and 12 died due to CV causes. The registered causes of death for these patients were: ischemic heart disease (7), atrial fibrillation (1), heart failure (1), stroke (2), and cardiac arrest (1). IL-27 levels were significantly higher in the group of patients who suffered an event compared to the subjects who remained event-free during follow-up [median (IQR) 12.57 (10.26–15.56) au vs 9.45 (7.57–11.79) au, $p < .001$] (Table 1). The subjects who suffered recurrent AMI or CV death were significantly older, had higher hsCRP and eGFR compared to the subjects who had no events during follow-up. A higher percentage of these patients were hypertensive and had been receiving statin treatment previously to admission, probably explaining the lower LDL levels in this subgroup. Left main coronary artery disease was present in a higher percentage of the patients who later suffered events, whereas the event-free controls had a higher frequency of 1-vessel disease (Table 1).

3.2. Determinants of plasma levels of IL-27

We studied the associations between IL-27 and age, sex, traditional CV risk factors (diabetes, hypertension, smoking, BMI, plasma lipids), hsTnT as a surrogate measure of infarction size, eGFR as a measurement of renal function, and hsCRP as a biomarker of systemic inflammation previously associated with prognosis in ACS patients. In a multivariate linear regression model including IL-27 as the dependent variable, age, eGFR and hsCRP were the only independent positive determinants of IL-27 levels in plasma, whereas LDL was negatively associated with IL-27 (Table 2).

3.3. IL-27 and the risk for recurrent AMI and CV death

Next, we tested whether plasma IL-27 at the time of the acute event is related with recurrent AMI and CV death. The incidence of recurrent AMI and CV death was higher with increasing baseline IL-27 tertiles (Fig. 1, $p < .001$). We evaluated the association between IL-27 tertiles and the composite outcome in Cox proportional hazard models consecutively adjusted for age, sex, diabetes, hypertension, smoking, LDL,

Table 1
Baseline characteristics of the study population.

	Whole cohort (n = 524)	AMI or CV death during follow-up (n = 75)	Event-free survivors (n = 449)	*p*[a]
Age	67 (59–77)	80 (72–85)	66 (58–74)	< 0.001
Male gender, n (%)	383 (73)	52 (69)	331 (74)	n.s.
BMI (kg/m^2)	27 (24–30)	26 (24–29)	27 (24–30)	n.s.
Current smoking, n (%)	132 (25)	15 (20)	117 (26)	n.s.
Hypertension, n (%)	284 (54)	49 (65)	235 (52)	0.040
Diabetes, n (%)	123 (24)	22 (29)	104 (23)	n.s.
Kidney function (eGFR)[b]	72 (53–95)	51 (34–68)	75 (57–96)	< 0.001
Statins on admission, n (%)	181 (35)	35 (47)	146 (33)	0.042
Discharge medication				
Statins, n (%)	504 (96.2)	62 (82.7)	442 (98.4)	< 0.001
ACEi/ARB, n (%)	463 (88.4)	56 (74.7)	407 (90.6)	< 0.001
Betablockers, n (%)	482 (92.0)	63 (84.0)	419 (93.3)	0.015
ASA, n (%)	504 (96.2)	69 (92.0)	435 (96.9)	n.s.
P2Y12 antagonists, n (%)	443 (84.5)	65 (86.7)	378 (84.2)	n.s.
Laboratory parameters				
LDL (mmol/L)	2.89 (2.09–3.80)	2.54 (1.86–3.25)	2.95 (2.16–3.88)	< 0.01
HDL (mmol/L)	1.05 (0.86–1.37)	1.07 (0.83–1.44)	1.05 (0.86–1.36)	n.s.
Triglycerides (mmol/L)	1.41 (1.02–1.93)	1.24 (0.97–1.73)	1.42 (1.03–1.96)	n.s.
Cystatin C (mg/L)	1.02 (0.85–1.25)	1.24 (1.06–1.73)	0.98 (0.84–1.21)	< 0.001
hsTNT (ng/L)	366 (63–1290)	331 (96–1235)	372 (55–1326)	n.s.
hsCRP (mg/L)	6.72 (2.88–17.85)	9.96 (4.44–29.60)	6.28 (2.58–16.35)	< 0.01
IL-27 (au)	9.85 (7.85–12.30)	12.57 (10.26–15.56)	9.45 (7.57–11.79)	< 0.001
Coronary angiography, n (%)				
Left main disease	44 (8.4)	11 (14.7)	33 (7.3)	0.019
1-vessel disease	148 (28.2)	12 (16.0)	136 (30.3)	0.045
2-vessel disease	104 (19.8)	13 (17.3)	91 (20.3)	n.s.
3-vessel disease	126 (24.0)	15 (20.0)	111 (24.7)	n.s.

Continuous variables are expressed as median (interquartile range) and categorical variables are expressed as n (%).
AMI, acute myocardial infarction, ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers; P2Y12 antagonists, platelet P2Y12 receptor antagonists; ASA, acetylsalicylic acid; hsTNT, high sensitive troponin T; hsCRP, high sensitive C-reactive protein; au, arbitrary units; n.s., not significant.
[a] Comparison between patients with an incident AMI or CV death during follow-up and event-free controls.
[b] Calculated using the CKD-EPI Cystatin C formula.

Table 2
Determinants of IL-27 concentration in plasma at the time of the acute coronary event.

Risk factors	Beta coefficient[a]	95% CI for the beta coefficient	*p*
Age (per year)	0.009	0.005–0.014	< 0.001
Male gender	0.032	−0.057–0.120	ns
BMI (kg/m^2)	−0.009	−0.049–0.32	ns
Current smoking	−0.029	−0.121–0.064	ns
Hypertension	0.003	−0.012–0.005	ns
Diabetes	0.026	−0.067–0.119	ns
eGFR	−0.069	−0.123 to −0.015	0.013
Statins at admission	−0.001	−0.007–0.005	ns
Laboratory parameters			
LDL (mmol/L)	−0.062	−0.105 to −0.019	0.005
HDL (mmol/L)	−0.011	−0.055–0.033	ns
Triglycerides (mmol/L)	−0.032	−0.076–0.012	ns
hsTnT (ng/L)	−0.026	−0.069–0.017	ns
hsCRP (mg/L)	0.166	0.121–0.212	< 0.001

CI, confidence interval; BMI, body mass index; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; hsTnT, high-sensitive cardiac troponin T; hsCRP, high-sensitive C-reactive protein; ns, not significant.

[a] Multivariate linear regression analysis with IL27 as the dependent variable and CV risk factors, cystatin C, hsTnT and hsCRP as independent variables. The values for BMI, LDL, HDL, triglycerides, eGFR, hsTnT and hsCRP were normalized by logarithmic transformation before entered in the analysis. The beta coefficient for the continuous variables is expressed per one standard deviation.

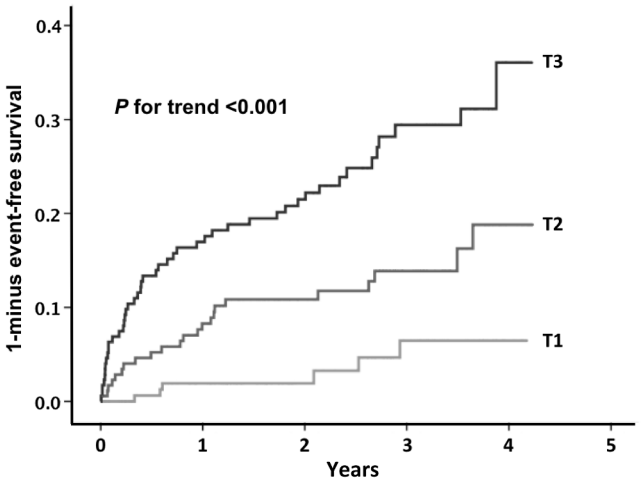

Fig. 1. Relationship between baseline IL-27 tertiles and incident major CV events during follow-up.
Kaplan-Meier 1-minus event-free survival curves showing incidence of AMI or CV death by baseline IL-27 tertiles. The *p* values for trend were calculated using the log-rank test.

HDL, triglycerides, hsTnT, hsCRP, eGFR and discharge medication (ASA, P2Y12, ACEi/ARB, BB, statin) (Table 3). Patients within the highest tertile of IL-27 had a significantly increased risk to suffer a new AMI or to die of CV causes compared with the lowest tertile, independently of all considered potential confounders [HR 2.70, 95% CI (1.06–6.90), $p = .038$] (Table 3, Models A-C). In a Cox proportional hazard regression analysis with forward selection of variables age, IL-27, eGFR, statin and betablocker treatment were the only factors retained in the final model (Supplementary Table 3). Increasing age, eGFR and IL-27 were correlated with a higher rate of events, whereas statin and betablocker treatment led to an improved prognosis.

In the 114 patients included in the follow-up group that donated blood samples at 6 weeks after the index acute coronary event, IL-27 levels at this time point correlated strongly with baseline IL-27 ($r = 0.458$, $p < .001$). This finding suggests that the increased IL-27 levels in certain individuals are not only present at the time of the acute event, but are maintained for a longer time period of at least several weeks. Similar to baseline values, individuals with high IL-27 at this time point had a significantly higher risk to suffer events (Table 3).

3.4. IL-27 and left ventricular function

Further, we studied the correlations between IL-27 at baseline and parameters of left ventricular remodeling, systolic and diastolic function in the subgroup of 107 patients that underwent echocardiographic control examinations at 1 year after the acute event. The characteristics of this sub-group are presented in Supplementary Table 1. In comparison with study participants that did not undergo echocardiographical follow-up, these patients were older and a larger proportion of them were males. Additionally, the patients included in the echocardiographical follow-up group had a lower BMI, lower prevalence of smoking, higher prevalence of hypertension, higher LDL, cystatin C and IL-27 levels, and lower triglycerides (Supplementary Table 1). In this patient subgroup baseline IL-27 correlated negatively with LVEF at 1 year, and higher IL-27 levels were associated with a more rapid deterioration of LV systolic function from discharge to the 1-year follow-up (dLVEF) (Table 4). Additionally, elevated levels of baseline IL-27 were associated with accelerated LV remodeling, as indicated by increased parameters of LV dilation and hypertrophy (LVID, LVESV, and LVMI) at the 1 year follow-up (Table 4). We also found a significant positive correlation between IL-27 and LAVI, indicative of LA dilation and overload (Table 4). In support of this data, patients with increased LV filling pressure determined by Doppler echocardiography at follow-up (as defined by the pre-specified criteria MVDT below 0.130 s and E/e′ ratio above 15), had significantly higher IL-27 levels at baseline compared with patients that had preserved diastolic function (Supplementary Table 2).

4. Discussion

To our knowledge, this is the first prospective study of the prognostic value of plasma IL-27 in ACS patients. We show that ACS patients with high IL-27 levels at baseline and during follow-up have a substantially increased risk to develop recurrent coronary events or to die due to CV disease, independently of other risk factors. Furthermore, our data indicates that IL-27 correlates with accelerated ventricular remodeling and deterioration of left ventricular systolic and diastolic function during the first year after the ischemic event.

4.1. Factors that influence IL-27 levels in plasma

In our cohort, plasma IL-27 levels were strongly and positively correlated with age, eGFR, hsCRP, and inversely correlated with LDL. The relationship between IL-27 and age has not been previously described in this clinical setting. As IL-27 is a regulator of T-cell function, this finding may be linked to the altered T-cell responses observed in elderly and warrants further study [28,29]. Renal failure is associated with an inflammatory state, which is considered to be one of the possible mechanisms behind the increased CV risk in patients with impaired renal function [30]. The mechanism is probably multifactorial, involving both increased production and decreased clearance of inflammatory mediators [31]. The strong relationship between IL-27 and hsCRP suggests that IL-27 production may be part of the systemic inflammatory response triggered by the acute coronary event. However, the relationship between IL-27 and incident events was independent of hsCRP, suggesting that the pathogenic pathways reflected by the two biomarkers overlap only partially. Finally, the inverse relation between IL-27 and LDL in our cohort might be explained by ongoing statin treatment in some patients, who had lower LDL and higher IL-27 levels at admission (data not shown). In support of this hypothesis, previous reports have shown that simvastatin induces IL-27 expression in monocytes and DCs [32,33].

Table 3
IL-27 levels and the risk for recurrent AMI or CV death.

		Model A		Model B		Model C	
	Events (N)	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
IL-27 levels at baseline (all patients, n = 524)							
Tertile 1	6	Reference		Reference		Reference	
Tertile 2	23	2.47 (0.99–6.12)	0.051	2.23 (0.90–5.54)	0.084	2.27 (0.90–5.69)	0.082
Tertile 3	46	3.31 (1.36–8.05)	0.008	2.54 (1.02–6.33)	0.045	2.70 (1.06–6.90)	0.038
IL-27 levels at 6 weeks after the AMI (only follow-up patients, n = 107)							
Tertile 1	6	Reference		Reference		Reference	
Tertile 2	10	1.73 (0.56–5.34)	0.336	1.76 (0.56–5.51)	0.333	1.53 (0.46–5.09)	0.484
Tertile 3	13	2.76 (0.93–8.21)	0.068	3.61 (1.12–11.65)	0.032	4.13 (1.10–15.45)	0.035

Cox proportional hazards analysis of the risk for the composite end-point of AMI or CV death during follow-up, according to tertiles of IL-27.
Model A: Adjusted for age, sex, diabetes, hypertension, smoking, LDL, HDL, triglycerides.
Model B: Adjusted for age, sex, diabetes, hypertension, smoking, LDL, HDL, triglycerides, hsTnT, hsCRP, eGFR.
Model C: Adjusted for age, sex, diabetes, hypertension, smoking, LDL, HDL, triglycerides, hsTnT, hsCRP, eGFR and discharge medication (ASA, P2Y12 antagonists, ACEi/ARB, beta-blockers, statins).
$p < .05$ was considered to be statistically significant. p values of $< .100$ are presented in the table to illustrate tendencies.
AMI, acute myocardial infarction; CV, cardiovascular; HR, hazard ratio; CI, confidence interval; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; hsTnT, high-sensitive cardiac troponin T; hsCRP, high-sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; ASA, acetylsalicylic acid; P2Y12 antagonists, platelet P2Y12 receptor blockers; ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers.

Table 4
Correlations between baseline IL-27 and echocardiographic measurements of cardiac structure and LV systolic function at 1 year after the index coronary event (N = 107).

Echocardiographic parameters	r[a]	*p*
LVEF	−0.203	0.047
dLVEF	0.252	0.039
LVEDV	0.134	ns
LVESV	0.229	0.019
LVID	0.216	0.026
LVMI	0.265	< 0.01
LAVI	0.370	< 0.001

LVEF, LV ejection fraction at 1-year follow-up; dLVEF, change in LVEF calculated as LVEF at baseline minus LVEF at 1 year; LVEDV, LV end diastolic volume; LVESV, Left ventricular end systolic volume; LVID, Left inner ventricular diameter; LVMI, Left ventricular mass index; LAVI, Left atrial volume index; ns = not significant.
[a] Spearman linear correlation coefficients.

4.2. IL-27 and cardiac remodeling

Several independent studies have demonstrated that plasma IL-27 is significantly higher in patients with coronary artery disease, UA and AMI compared with healthy controls [19,20,34]. Our data extend these results, showing that the high IL-27 levels at the time of the event are potentially involved in cardiac remodeling and have independent prognostic significance. In a cross-sectional study, Lin and colleagues have found an inverse correlation between IL-27 and LVEF in a population of coronary artery disease patients [34]. Here, we show that IL-27 is associated with deterioration of LV systolic function during the first year of follow-up after an acute coronary ischemic event. Additionally, high IL-27 was significantly linked with cardiac remodeling as reflected by LV dilation and hypertrophy, leading to diastolic dysfunction. These parameters have previously been associated with poor prognosis in MI survivors [35,36]. Cardiac fibroblasts play a key role in cardiac remodeling and development of heart failure, through deposition of fibrous proteins such as collagen in the extracellular matrix [37]. It has previously been shown that pressure-overloaded hearts have an increased amount of cardiac fibroblasts [37] and that IL-27 induces increased production of type I collagen in cultured human fibroblasts *in vitro* [38]. Consequently, it may be speculated that the interplay between immunoregulatory cells secreting IL-27 and cardiac fibroblasts may play an active role in cardiac remodeling, and that overproduction of IL-27 might contribute to the loss of LV systolic and diastolic function following coronary ischemia.

4.3. IL-27 and cardiovascular disease

It has previously been shown that IL-27 is correlated with atherosclerosis severity in patients with coronary artery disease, which might at least partially explain the correlation between IL-27 and the increased risk for progressive coronary plaque instability and recurrent AMI [19]. OxLDL induces IL-27 expression in dendritic cells *in vitro*, suggesting a potential active role for IL-27 in human atherosclerosis [19]. IL-27 is mainly secreted by dendritic cells and macrophages, but has also been found in endothelial cells and smooth muscle cells present in human atheromas [18]. Most of the experimental data generated so far on the role of IL-27 in CV disease is derived from studies in models of atherosclerosis, and there are no published studies on the role of IL-27 in animal models of AMI. While the cytokine seems to have anti-atherogenic properties in mice fed a high-fat diet [16,17], it might have a pro-inflammatory role in the context of acutely inflamed ischemic myocardium, as suggested by our findings and by other studies in humans [19]. This hypothesis is supported by *in vitro* data showing that IL-27 primes human macrophages for pro-inflammatory activation by upregulating TLR4 [39]. TLR4 is an innate immune receptor that has been demonstrated to mediate deleterious cardiac remodeling and loss of function in a mouse model of AMI [40]. Thus, IL-27-mediated overexpression of TLR4 in macrophages infiltrating the infarcted myocardium might promote an inflammatory over a reparatory phenotype, leading to increased myocardial damage and impaired post-ischemic recovery. Additionally, IL-27 has been shown to inhibit neutrophil function and recruitment to inflammatory sites [41,42], which might impair the beneficial role of these cells in myocardial repair and recovery after AMI [43]. The link between IL-27 and IL-10 also has to be considered in this context, as IL-27 is an important inducer of IL-10 secretion [15]. Intriguingly, despite its potentially beneficial immunosuppressive properties, elevated levels of IL-10 at the time of an AMI have also been found in several independent studies to predict higher 30-day mortality and a poor long-term prognosis [44–46]. Whether the elevated levels of IL-27 and IL-10 have a direct pathogenic effect in AMI patients with a poor prognosis, or whether secretion of these cytokines increases in an unsuccessful attempt to inhibit the ongoing potent inflammatory processes requires further investigation.

4.4. Study limitations

Our study has several limitations that have to be taken into account. Firstly, as this is an association study, we cannot prove causality

between the elevated levels of IL-27 in AMI survivors and cardiac remodeling. Mechanistic studies in animal models of AMI where IL-27 has been pharmaceutically blocked or genetically deleted will be required to address this question. Secondly, as our measurement method expresses IL-27 as arbitrary units, we cannot compare the absolute IL-27 concentrations measured in our cohort to those in other studies and cannot define the exact IL-27 cut-off levels that would define the different risk groups. Lastly, as the subgroup of patients that underwent 1-year echocardiographic follow-up was older than 75 years of age, the described associations between IL-27 and the deterioration of cardiac function cannot directly be extrapolated across other age categories without further verification.

4.5. Conclusions

In conclusion, we demonstrate for the first time that plasma IL-27 in ACS patients are associated with recurrent AMI and CV death, independently of traditional CV risk factors, infarction size, renal function and hsCRP. Excessive cardiac remodeling and deterioration of left ventricular function in patients with elevated IL-27 might partly explain the link between IL-27 and poor prognosis. Our results warrant further mechanistic studies on the role of IL-27 in ACS and its complications. Studies in animal models of AMI are required to investigate whether IL-27 has a negative influence on the immune mechanisms mediating cardiac repair after an ischemic injury. Testing in larger patient cohorts are needed to confirm whether IL-27 can be used as a clinical biomarker for risk stratification in AMI survivors.

Conflicts of interest

None.

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Author contributions

Helena Grufman participated in study conception and design, data analysis and interpretation, as well as manuscript drafting. Troels Yndigegn has performed all the measurements on the echocardiographical examinations included in the study, and has participated in data analysis and manuscript drafting. Isabel Goncalves and Jan Nilsson have participated in study conception and design and have critically revised the manuscript for important intellectual content. Alexandru Schiopu has participated in study conception and design, data interpretation and manuscript drafting.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cyto.2017.11.002.

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