

# Circulating adiponectin concentrations in patients with congestive heart failure

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**Objectives:** To determine concentrations of adiponectin and its predictive value on outcome in a cohort of patients with congestive heart failure (CHF).

**Methods:** Serum and clinical data were obtained for outpatients with clinically controlled CHF (n = 175). Serum concentrations of adiponectin, C reactive protein, N-terminal pro-brain natriuretic peptide (NT-proBNP), interleukin (IL) -1 $\beta$ , IL-6, IL-8, IL-10, IL-12, tumour necrosis factor  $\alpha$  and CD-40 ligand were determined. The association of adiponectin with the clinical severity of CHF was sought as well as the predictive value of this adipokine on mortality, CHF hospitalisations or the occurrence of each of these end points.

**Results:** Concentrations of adiponectin were significantly increased in patients with CHF. Patients with higher New York Heart Association class had significantly higher serum concentrations of adiponectin. Adiponectin serum concentrations were lower in patients with diabetes and CHF as well as in patients with ischaemic cardiomyopathy. Serum adiponectin concentration was positively associated with age and NT-proBNP but was negatively correlated with C reactive protein concentrations. Serum adiponectin above the 75th centile was found to be an independent predictor of total mortality, CHF hospitalisations or a composite of these end points over a two-year prospective follow up.

**Conclusion:** Adiponectin is increased in CHF patients and predicts mortality and morbidity.

Adiponectin is an adipocyte-derived cytokine with anti-inflammatory, antidiabetic and antiatherogenic properties.<sup>1</sup> It has been initially shown that circulating concentrations of adiponectin are reduced in patients with obesity and increase after weight loss.<sup>2</sup> Moreover, adiponectin concentrations negatively correlate with fractional body fat, waist to hip ratio and intra-abdominal fat.<sup>3</sup> Subsequent studies have shown that adiponectin serum concentrations are also reduced in patients with diabetes, atherosclerosis and acute coronary syndrome.<sup>4–8</sup> Of particular relevance is the correlation of adiponectin concentrations with markers of inflammation. It has been shown that adiponectin correlates negatively with C reactive protein (CRP), interleukin (IL) -6 and tumour necrosis factor (TNF)  $\alpha$ , suggesting adiponectin may antagonise some of their proinflammatory properties.<sup>9–10</sup> Experimentally, adiponectin has been shown to reduce atherosclerosis in apolipoprotein E-deficient mice and to suppress robust inflammatory responses within the vessel wall, supporting a protective role of this adipokine.<sup>11</sup>

Congestive heart failure (CHF) is a complex syndrome of neurohormonal inflammatory dysregulation resulting in progressive cardiac remodelling. CHF is still associated with significant morbidity and mortality.<sup>12</sup> A better understanding of factors governing the pathogenesis of CHF resulted in major advances in its treatment. Inhibition of both neurohormonal axes has accordingly become a mainstay in the management of patients with CHF.<sup>13</sup> Mounting evidence supports the role of immune system activation in the pathogenesis and progression of CHF, exemplified by raised circulating concentrations of proinflammatory cytokines (TNF $\alpha$ , IL-6) and acute phase reactants such as CRP (reviewed by Pugh *et al*).<sup>14</sup>

In the present study, we evaluated concentrations of the adipose tissue-derived cytokine adiponectin in patients with clinically controlled CHF of various degrees of severity. Furthermore, we determined the association of adiponectin

with various inflammatory and neurohormonal mediators known to be increased in patients with CHF and studied its prognostic value in the prediction of morbidity and mortality.

## METHODS

### Patient selection

One hundred and seventy-five consecutive patients with clinically controlled CHF attending the outpatient clinic of the Tel Aviv Sourasky Medical Center were recruited. Patients with CHF in New York Heart Association (NYHA) functional classes II–IV were included. The local research ethics committee approved the study protocol and all patients gave written informed consent.

At baseline, patients had a full medical history taken, clinical examination performed and NYHA class assigned. Patients were followed up every 1–3 months or more often as required. Baseline serum was drawn at the initial visit and frozen at  $-80^{\circ}\text{C}$  until assayed in the laboratory. Control, age-matched healthy participants (n = 43) were also recruited and blood was similarly drawn and preserved.

### Follow up

The study end points were all-cause death, hospitalisations caused by heart failure, or the combined occurrence of each. Patients were followed up for a minimum of 24 months. No patients were lost to follow up.

### Determination of circulating adiponectin

Fasting serum adiponectin was measured with the radioimmunoassay from Linco Research (St Charles, Missouri, USA) (human adiponectin sensitivity is 1 mg/l on a 100  $\mu\text{l}$

**Abbreviations:** CBA, cytometric bead array; CHF, congestive heart failure; CRP, C reactive protein; IL, interleukin; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; TNF, tumour necrosis factor

sample size.) Assay range is 1–200 mg/l and specificity is < 0.01%.

### Determination of N-terminal pro-brain natriuretic peptide concentrations

Serum N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured by automated immunoassay (Elecsys proBNP, Roche Diagnostics, Germany). The test uses two polyclonal antibodies directed against NT-proBNP: epitope 1, amino acids 1–21; and epitope 2, amino acids 39–50. The results are calibrated against a synthetic NT-proBNP (amino acids 1–76). Results range from 5–35 000 pg/ml.

### High sensitivity CRP concentrations

CRP was assayed according to the manufacturer's instructions (Dade Behring Inc). Briefly, the method uses polystyrene particles coated with monoclonal antibodies to CRP. These particles agglutinate with CRP. CRP concentration was determined according to the intensity of the scattered light in the nephelometer, compared with standards of known concentrations.

### Determination of circulating inflammatory cytokines

Serum cytokines were measured serially by cytometric bead array (CBA) with a four-colour FACSCalibur flow cytometer (Becton Dickinson, San Jose, California, USA). In CBA, six bead populations with distinct fluorescence intensities had been coated with capturing antibodies specific for the respective cytokines. These bead populations could be resolved in the fluorescence channels of the flow cytometer. The beads were incubated with 50 µl of serum and cytokines were captured by their corresponding beads. The cytokine-captured beads were then mixed with phycoerythrin-conjugated detection antibodies to form sandwich complexes. After incubation, washing and acquisition of fluorescence data, the results were generated in graphical format by the Becton Dickinson CBA software. The concentrations of IL-1β, IL-6, IL-10, IL-8, TNFα and IL-12p70 were measured by using the inflammatory cytokine CBA kits (BD PharMingen, San Diego, California, USA). The coefficients of variation for all cytokine assays were < 10%. Their respective normal ranges have been derived from measurement of healthy subjects.

### Determination of soluble CD-40 ligand concentrations

Serum concentrations of CD-40 ligand were determined by a commercial enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (R&D Systems).

### Statistical analysis

Data are presented as mean (SEM) unless otherwise stated. Serum adiponectin concentrations were stratified according to NYHA class and values within CHF patient groups were compared with those obtained for healthy controls by a one-way analysis of variance. Student's t test was used to compare concentrations of adiponectin between patients with ischaemic CHF, diabetes and chronic renal failure.

The association between baseline clinical characteristics and dichotomous adiponectin serum concentrations was analysed with the  $\chi^2$  test for categorical variables. Event rates were compared by Kaplan–Meier curves calculated for adiponectin serum concentrations above compared with below the 75th centile of 14 mg/l. The independent predictive power of serum adiponectin, left ventricular ejection fraction (LVEF), NT-proBNP, NYHA class, IL-1β, IL-6, IL-10, IL-8, TNFα and IL-12p70 and other clinical and demographic variables for death, hospitalisations for CHF or either of each of these end points was tested by stepwise Cox proportional hazards regression analyses. A value of  $p < 0.05$  was accepted as significant.

**Table 1** Clinical characteristics of the congestive heart failure outpatient cohort (n = 175)

Age (years)	71.1 (11.2)
Men	135 (77%)
NYHA	2.7 (0.53)
LVEF (%)	36.1 (12.1%)
Hyperlipidaemia	99 (55%)
Smoking	65 (37%)
Hypertension	101 (58%)
Diabetes mellitus	62 (35%)
Ischaemic heart disease	130 (74%)
Chronic atrial fibrillation	52 (30%)
TIA/CVA	20 (11%)
PTCA or CABG	92 (53%)
ACE inhibitors/ARBs	140 (80%)
Spironolactone	77 (44%)
β blockers	128 (73%)

Data are mean (SD) or number (%).

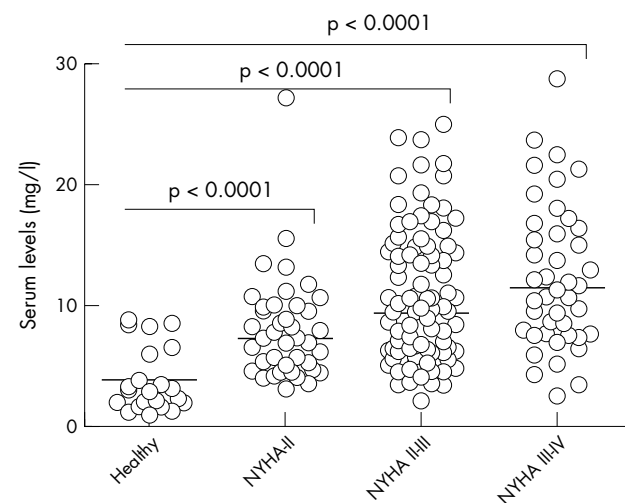
ACE, angiotensin-converting enzyme; ARB, angiotensinogen receptor blockers; CABG, coronary artery bypass surgery; CVA, cerebrovascular accident; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PTCA, percutaneous transluminal coronary angioplasty; TIA, transient ischaemic attack.

## RESULTS

Table 1 presents baseline clinical characteristics and use of drugs.

The more advanced the CHF was according to NYHA class, the higher the adiponectin concentrations were. Mean adiponectin serum concentrations in age-matched healthy controls were 3.7 (SEM 0.6) mg/l. Patients with NYHA class II, II–III and III–IV had significantly increased serum concentrations of adiponectin (8.0 (6.7) mg/l, 10.5 (0.5) mg/l and 12.3 (0.9) mg/l, respectively,  $p < 0.0001$  for all comparisons) (fig 1).

When patients with CHF were stratified according to whether they had ischaemic or non-ischaemic CHF by evidence of coronary heart disease, the ischaemic group had significantly lower adiponectin serum concentrations (9.7 (0.4) mg/l) than the non-ischaemic group (12.3 (0.9) mg/l,  $p = 0.009$ ) (fig 2A). The presence of diabetes mellitus in patients with CHF was also associated with reduced serum



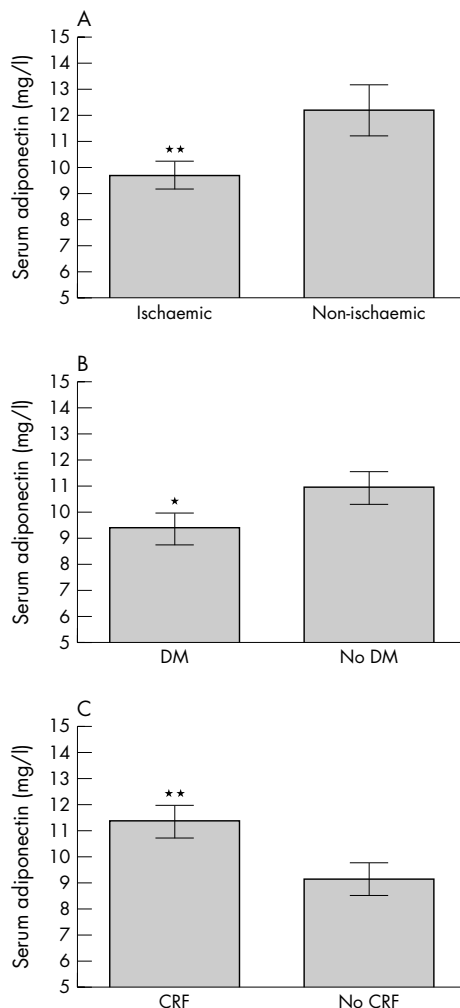
**Figure 1** Serum adiponectin according to the severity of congestive heart failure. Serum adiponectin concentrations were determined by radioimmunoassay. Patients were stratified by the severity of heart failure expressed by New York Heart Association (NYHA) functional class. Results were compared by a one-way analysis of variance. Horizontal lines indicate median serum concentrations.

concentrations of adiponectin (9.3 (0.6) mg/l) in patients with  $\nu$  10.9 (0.6) mg/l without diabetes,  $p < 0.05$ ) (fig 2B). Patients with CHF and chronic renal failure had significantly higher circulating adiponectin concentrations (11.4 (0.6) mg/l) than did patients with normal creatinine concentrations (9.1 (0.6) mg/l,  $p = 0.007$ ) (fig 2C).

Serum adiponectin concentrations significantly and positively correlated with the age of patients with CHF (table 2, fig 3). Whereas a positive correlation was also evident with the severity of CHF estimated by NYHA class, no significant association was found between adiponectin serum concentrations and LVEF.

Adiponectin serum concentrations were significantly correlated with NT-proBNP and negatively with CRP (table 2, fig 3). However, no association was found between adiponectin and either of the proinflammatory or anti-inflammatory cytokines (TNF $\alpha$ , IL-1 $\beta$ , IL-12, IL-10, IL-6 or IL-8), nor was adiponectin correlated with serum soluble concentrations of CD-40 ligand.

Of the total 175 patients, 36 (20.5%) died during the two-year follow up, whereas 40 (22.8%) were hospitalised for CHF deterioration and 64 (36.5%) reached the end point of either death or CHF hospitalisation within the follow-up period.



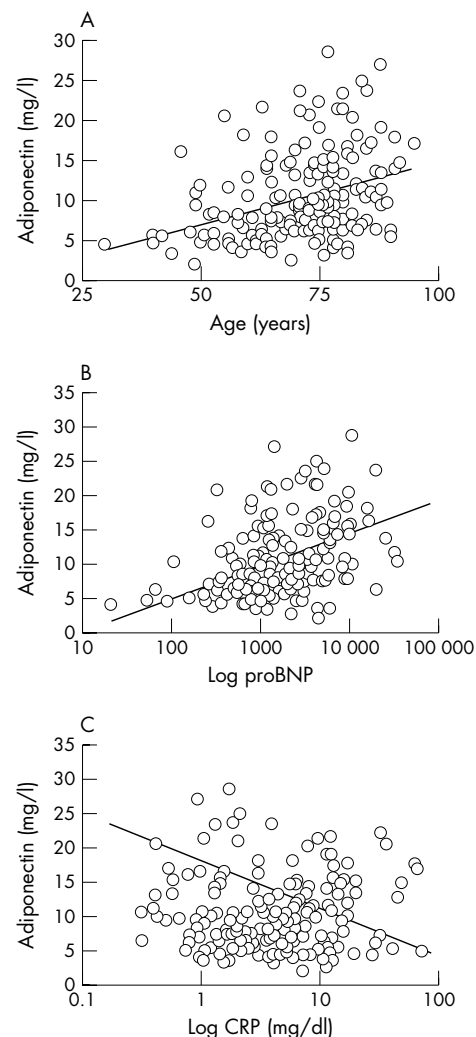
**Figure 2** Serum adiponectin according to the presence of comorbidities. Patients were divided according to whether they had (A) ischaemic or non-ischaemic cardiomyopathy by the presence of coronary artery disease, (B) diabetes (DM), or (C) chronic renal failure (CRF). \* $p < 0.05$ ; \*\* $p < 0.01$ .

**Table 2** Correlation of serum adiponectin with continuous clinical and laboratory variables

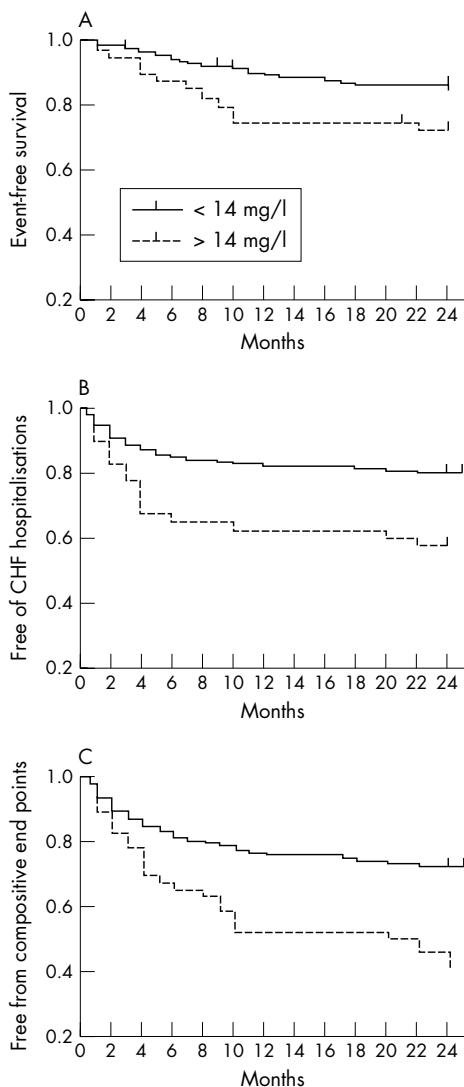
Parameter	Correlation coefficient (r)	p Value
Age	0.36	<0.0001
NYHA class	0.35	<0.0001
Ejection fraction	0.10	0.08
NT-proBNP	0.45	<0.0001
CD-40 ligand	0.008	0.45
C reactive protein	-0.24	<0.001
Interleukin 12	0.007	0.46
TNF $\alpha$	0.03	0.35
Interleukin 10	0.13	0.05
Interleukin 6	0.09	0.13
Interleukin 1 $\beta$	0.04	0.29
Interleukin 8	0.12	0.06

NYHA, New York Heart Association; NT-proBNP, N-terminal pro-brain natriuretic peptide; TNF, tumour necrosis factor.

Kaplan–Meier analysis indicated differences in event-free survival for death (fig 4A), hospitalisations caused by CHF (fig 4B), and the composite of death and CHF hospitalisation (fig 4C) when the group was divided



**Figure 3** Association of serum adiponectin with continuous determinants in patients with congestive heart failure (CHF). Adiponectin serum concentrations were correlated with (A) the age of patients with CHF, (B) their N-terminal pro-brain natriuretic peptide (NT-proBNP) concentration, and (C) their C reactive protein (CRP) concentration.



**Figure 4** Survival curves according to baseline adiponectin concentrations above or below the 75th centile of 14 mg/l. Patients with congestive heart failure (CHF) had their baseline serum adiponectin evaluated and were followed up for two years for the occurrence of (A) all-cause death, (B) hospitalisation for CHF, or (C) the first of the composite end points.

according to concentrations above and below the 75th centile of serum adiponectin (set at 14 mg/l).

Multivariate analysis by stepwise Cox proportional hazards regression analysis (incorporating as putative predictors age, NYHA class, sex, LVEF, diabetes and serum TNF $\alpha$ , IL-6, IL-10, IL-8, IL-12 CD-40 ligand, IL-1 $\beta$ , CRP and NT-proBNP) confirmed that serum adiponectin concentrations (determine dichotomously) remained independently predictive of death with a risk ratio of 2.2 (95% CI 1.1 to 5.9,  $p = 0.005$ ). Similarly, and more significantly, serum adiponectin concentration was an independent predictor of CHF hospitalisation during the 24-month follow up with a risk ratio of 2.4 (95% confidence interval (CI) 1.5 to 6.7,  $p = 0.001$ ) and remained so when the composite of death and CHF hospitalisation was studied as a separate end point with a risk ratio of 2.5 (95% CI 1.7 to 5.6,  $p < 0.0001$ ). Both NYHA class and NT-proBNP also were independent predictors of prespecified end points of death, CHF hospitalisations and the combined occurrence of each.

**DISCUSSION**

Adiponectin is an adipokine produced principally by adipose tissue that is found abundantly in plasma.<sup>1</sup> Recent literature suggests that adiponectin acts as an anti-inflammatory and antiatherogenic cytokine that is involved in vascular remodelling.<sup>1</sup> The purpose of this study was to determine the concentrations of adiponectin in the serum of patients with CHF and to study its predictive value. We have found that patients with CHF have significantly higher adiponectin concentrations than do healthy age-matched controls. Moreover, patients with more severe forms of CHF evident by higher NYHA class had higher adiponectin concentrations than patients with milder clinical presentations.

We have also found that serum adiponectin increased with age, yet it did not correlate with LVEF. The current study cannot answer why the concentrations of adiponectin are higher in patients with CHF. As adiponectin apparently is a protective agent, however, we may speculate that it is secreted by the adipocytes in an attempt to counteract the robust activation of neurohumoral and inflammatory responses. Recent publications report reduced concentrations of adiponectin in patients with obesity, diabetes and atherosclerosis.<sup>2-7</sup> Consistent with these reports, our study found that patients with evidence of obstructive coronary artery disease as the cause of CHF had lower serum concentrations of adiponectin than did patients with other forms of heart failure. Additionally, among the whole CHF cohort, patients with diabetes appeared to have lower concentrations of circulating adiponectin than patients without diabetes. Renal failure is a significant co-morbidity in patients with CHF. A recent study has shown that patients with chronic renal failure have higher serum concentrations of adiponectin that was associated with the degree of proteinuria, suggesting that adiponectin negates, by its anti-inflammatory properties, acute phase reactants. Herein, our results were similar in our CHF cohort—namely, that patients with chronic renal failure had significantly raised concentrations of adiponectin.

Interestingly, serum adiponectin concentrations were found to correlate significantly with serum NT-proBNP and negatively with CRP concentrations, suggesting that the production of adiponectin by adipocytes parallels the activation of neurohormonal and inflammatory axes. These results are consistent with a recent publication suggesting a reciprocal association between adiponectin and CRP.<sup>9</sup> However, unexpectedly, additional inflammatory cytokines such as TNF $\alpha$  and IL-6, which are known to be increased in patients with CHF,<sup>15,16</sup> did not correlate with adiponectin concentrations as has been shown in a recent study.<sup>17</sup> These findings may be related to the significant co-morbidities in this older cohort of patients with CHF that probably interact in a complex manner with innate and adaptive immune responses.

An additional interesting and potentially useful finding of this study was the predictive value ascribed to circulating adiponectin concentrations. We have found that at serum adiponectin concentrations above the 75th centile, patients with CHF are at a significantly increased risk of death, irrespective of other baseline clinical or laboratory findings. This independent predictive power was found to be even more significant with respect to “softer” end points such as either hospitalisation for CHF or a composite of death and CHF hospitalisation (fig 4).

In conclusion, we have shown that the serum concentrations of adiponectin, an antidiabetic, anti-inflammatory and antiatherogenic cytokine, are increased in patients with CHF and positively correlate with the severity of the clinical syndrome. Adiponectin concentrations can also predict a higher likelihood of death and morbidity and serve as a

potential marker for monitoring patients with CHF and predicting their outcome.

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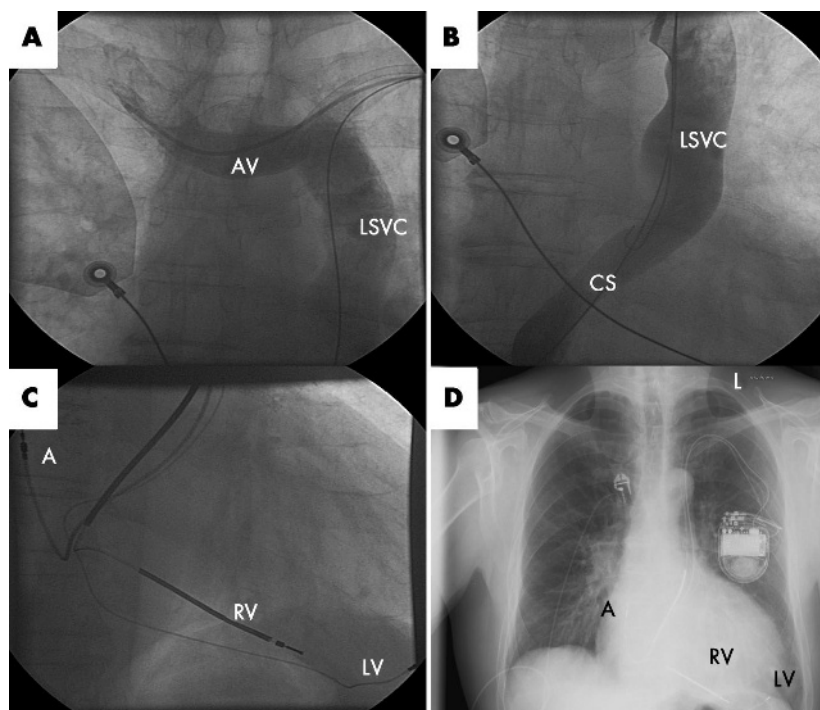
## IMAGES IN CARDIOLOGY

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### Implantation of a biventricular defibrillator system in a patient with persistent left and absent right superior vena cava

A 61-year-old man with a previous myocardial infarction experienced heart failure symptoms (New York Heart Association functional class III). An echocardiogram revealed a severely impaired left ventricular ejection fraction (15%) under optimal medical treatment. Since he also showed a complete left bundle branch block and pronounced dyssynchrony on echocardiography he was referred for implantation of a biventricular implantable cardioverter-defibrillator (ICD) system.

After access to the left subclavian vein, a persistent left superior vena cava (LSVC) was encountered. Subsequently, a venogram also revealed the absence of a right superior vena cava (panel A: AV, anomalous vein). The persistent LSCV drained into the notably enlarged coronary sinus (CS, panel B). Therefore, all three leads had to be implanted through the coronary sinus. The proximal shock coil was located within the coronary sinus. The left ventricular electrode (LV) had to be implanted through the posterior cardiac vein and could be positioned at a favourable position in a lateral branch (panel C: A, atrial lead in the right atrial appendage; RV, right ventricular defibrillation lead; panel D: x ray with the successfully implanted cardiac resynchronisation therapy-defibrillator system). The patient could be successfully defibrillated with 12 J and experienced significant improvement in heart failure symptoms briefly after the initiation of biventricular pacing. The persistence of the left superior vena cava is the most commonly encountered congenital anomaly of the venous system with a prevalence of 0.3-1%. This



case illustrates the feasibility of successfully implanting a biventricular ICD system through a persistent left superior vena cava and the coronary sinus.

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