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ORIGINAL RESEARCH ARTICLE

Semaphorin 4D levels in heart failure patients: a potential novel biomarker of acute heart failure?

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Abstract

Aims Semaphorin 4D (Sema4D) is expressed on platelets and T-cells and known to be involved in inflammation. The aims of this study include comparing Sema4D and N terminal pro brain natriuretic peptide (NT-proBNP) serum levels in heart failure (HF) patients to a control group, evaluating the correlation between Sema4D and NT-proBNP levels, and assessing Sema4D serum levels in HF patients during acute exacerbation and remission.

Methods and results Forty-five patients diagnosed with HF (based on echocardiographic findings, positive NT-proBNP levels, and normal C-reactive protein) and 11 healthy controls (declaring no chronic diseases or medications) comprised the study population. Demographic, clinical, laboratory, and echocardiographic data were used to create the study database. NT-proBNP and Sema4D serum samples were taken on admission and discharge. NT-proBNP levels were significantly higher in the HF group than in controls ($P < 0.001$). Sema4D levels were significantly higher in HF patients than in healthy controls (2143.04 ± 1253 vs. 762.18 ± 581.6 ng/mL, $P < 0.001$, respectively). Using linear regression, a higher creatinine level was found to predict both higher levels of NT-proBNP and Sema4D ($P = 0.05$ and $P < 0.014$, respectively), while a reduced ejection fraction was found to predict higher NT-proBNP levels only ($P < 0.001$ and $P = 0.87$, respectively). Average Sema4D levels reduced significantly at remission (3534.94 ± 1650.55 vs. 2455.67 ± 1424, $P = 0.03$), while mean NT-proBNP levels did not change significantly.

Conclusions Sema4D levels in HF patients' serum are significantly higher than in healthy controls. Clinical improvement caused rapid reduction in Sema4D levels, possibly reflecting the inflammatory aspect of HF. These findings might suggest that Sema4D can be used as a diagnostic biomarker of acute HF. Further studies of Sema4D and HF are warranted.

Keywords Heart failure; Inflammation; NT-proBNP; Semaphorin 4D

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Introduction

Semaphorins are transmembrane or soluble proteins, mainly involved in axonal guiding during embryogenesis.[1] In the context of cardiovascular diseases, semaphorins were found to be involved in the pathogenesis of arrhythmias, myocardial infarction, and heart failure (HF). Previous studies demonstrated down-regulation of tissue innervation in association with overexpression of Semaphorin 3A (Sema3A)[2] and vice versa: Down-regulation of semaphorin expression could result in a more amenable environment for axonal regeneration and sprouting. Genetically modified mice expressing higher levels of Sema3A were found to have reduced sympathetic innervation in heart biopsies, with lower thresholds for ventricular arrhythmias[3]; isoproterenol-induced HF rats expressed higher levels of secreted Sema3A in cardiomyocytes[4]; and lastly, a nonsynonymous polymorphism in Sema3A is a possible risk factor for human unexplained cardiac arrest with documented ventricular fibrillation.[5]

Semaphorin 4D (Sema4D) is a transmembrane glycoprotein expressed on platelets and T-cells, mainly involved in inflammation processes, axonal guidance during embryonal development, angiogenesis, and malignant cell proliferation. T-cell activation leads to proteolytic cleavage of Sema4D, creating its soluble form in the blood (CD100). Activated Sema4D influences immune system cells' migration, B-cell (CD40) proliferation, and cytokine and immunoglobulin production.[6] Elevated serum levels of Sema4D are related

to chronic inflammatory diseases,[7,8] malignancy,[9,10] and arrhythmias (i.e. atrial fibrillation).[11]

Heart failure prevalence in developed countries is 1–2% of the adult population.[12] Among people above the age of 65 presenting to primary care with breathlessness on exertion, one in six will have unrecognized HF.[13] The pathogenesis of the disease involves inflammatory aspects, neuro-hormonal changes, and biomechanical alterations.[14,15] Lu *et al.*[6] showed that in HF patients, plasma Sema4D levels were significantly higher than those in healthy controls, with the highest levels being in HF patients with diabetes mellitus. Additional findings of their study included the absence of correlation between patients' pre-admission chronic stable symptomology [New York Heart Association (NYHA) functional score] and Sema4D plasma levels on admission and that Sema4D increased levels were related to certain T-cell populations (CD3+, CD4+, and CD8+) rather than to B-cells or platelets.

Plasma concentration of natriuretic peptides has been used for a long time as an initial diagnostic test in the acute and non-acute settings, with high negative predictive values,[16] but only limited positive predictive value. Based on the aforementioned publications, we hypothesized that the Sema4D plasma level in HF patients may correlate with symptoms of HF and N terminal pro brain natriuretic peptide (NT-proBNP) levels. In this study, we aim to establish the possible role of Sema4D as a novel potential biomarker for acute HF exacerbation.

Methods

Study population

Sixty patients admitted to internal medicine departments in Bnai Zion Medical Center, Haifa, Israel, with the diagnosis of HF exacerbation were enrolled. Relevant demographic information, and clinical, and laboratory test results were collected from the patients or from their electronic medical records. Patients without a recent echocardiography had one performed during hospitalization. A peripheral blood sample was drawn on admission, and a second blood test was drawn before patients' discharge, after clinical improvement was verified by a senior physician. Only 45 patients were included in the final analysis, after excluding 10 patients for whom a diagnosis other than HF was found to be the aetiology for their complaints (based on normal echocardiographic measurements and normal NT-proBNP levels; one patient with an active infection and elevated C-reactive protein serum levels; and four patients later identified as having an active myocardial ischaemia with elevated troponin levels). A total of 11 healthy controls (random volunteers) with no history of any chronic diseases or treatments were included. Clinical improvement was defined by documented dyspnoea/oedema relieve (symptoms improvement) and the decision to discharge the patient by senior physician. All patients and healthy subjects signed an informed consent form. The study was approved by the institutional review board (approval number 134-14-BNZ).

N terminal pro brain natriuretic peptide and semaphorin 4D serum levels

Blood samples—at admission and discharge—from patients were centrifuged at 1300 *g* for 10 min, and serum was then stored at −80°C until laboratory analysis was performed. Serum samples were analysed for soluble CD100 (Sema4D soluble form in the blood) levels using a commercial kit according to the manufacturer's instructions (intra-assay precision: CV $\leq$ 8%; inter-assay precision: CV $\leq$ 10%; MyBioSource, cat. no. MBS2023012, San Diego, USA). Serum samples were also analysed for NT-proBNP levels in a routine laboratory, using a commercial kit according to the manufacturer's instructions (Elecsys proBNP II assay with cobas-e analyzer and Elecsys reagents, Roche, Mannheim, Germany).

Statistical analysis

Continuous data are presented as the mean ± standard deviation (SD). Categorical variables are presented as frequencies and percentages. Because NT-proBNP levels were found not to have a Gaussian distribution, we log-transformed the NT-proBNP levels. Correlation between any two continuous variables was determined using the Pearson correlation. Comparisons of Sema4D and log NT-proBNP levels from patients and healthy controls were performed using the unpaired Student's *t*-test. Levene's test for homogeneity of the variance was checked, and when significant, an unpaired unequal variances *t*-test was performed. The correlations between Sema4D, and similarly of log NT-proBNP, and other clinical and demographic data were analysed using a multivariate model that included only statistically significant clinical and demographic variables. A two-tailed *P* value of 0.05 or less was considered to be statistically significant. Statistical analysis was performed by a professional statistician using SPSS software version 22. Graphs were produced using the ggplot2 package[17] of the R software.[18]

Results

Study population—demographics and comorbidities

A total of 45 patients diagnosed with acute HF and 11 healthy controls were included. Patients' mean age was 76.5 (±12.5

SD) years old, compared with 72.2 (±3.5 SD) years old for healthy controls (P = 0.05). Of the HF patients, 57.8% were women compared with 27.3% for healthy controls (P = 0.07). The vast majority of HF patients had more than one comorbidity, with essential hypertension or valvular heart disease being the most prevalent. Mean haemoglobin A_1C of the diabetic patients was 7.5 (±1.8 SD, range 5.4–11). Most prevalent valvular heart disease was tricuspid regurgitation, followed by mitral regurgitation (33.3% and 27.7%, respectively). Among patients with cardiomyopathy or structural disease, 42.8% had left ventricular hypertrophy and 38.1% dilated cardiomyopathy. Of patients with arrhythmias, two-thirds were diagnosed with atrial fibrillation and 9.5% carried a pacemaker. Of the patients, 86.6% were treated with chronic medications: 81.4% with diuretics, 79.1% with beta-blockers, and 57.1% with angiotensin-converting enzyme inhibitors. Detailed percentages of comorbidities and chronic medications in the HF group are shown in *Table 1*.

Study population—heart failure clinical data

Of the patients, 77.8% were hospitalized for dyspnoea, and the remainder, for leg oedema or weakness, with 70% of patients having pulmonary congestion on chest X-ray. The vast majority were previously diagnosed with HF (86.7%), and their pre-admission NYHA scores varied (33% were NYHA IV, 17.8% NYHA III, 40% NYHA II, and 8.9% NYHA I). The left ventricular ejection fraction (EF) ranged from 15% to 65% (mean 44.1% ± 15.8% SD), and mean tricuspid annular plane systolic excursion (lower normal limit >20 mm) was 17.9 ± 4.9 mm (range 10–27). Subdivided by EF, 24 (53.3%) patients had HF with reduced EF (mean EF—32% ± 12.5%), and 21 (46.7%) patients had preserved EF (mean EF—57% ± 3.3%). Clinical data and HF characteristics are shown in *Table 2*.

Table 1 Demographic data and medical history of study population

Characteristics		Control (n = 11)	HF (n = 45)	P value
Age	Mean (SD)	72.2 (3.5)	76.5 (12.5)	0.05
	Range	68–78	34–90	
Gender, n (%)	Male	3 (27.3)	26 (57.8)	0.07
	Female	8 (72.7)	19 (42.2)	
Medical history, n (%)	Diabetes mellitus	0 (0)	22 (48.9)	
	Hypertension	0 (0)	39 (86.7)	
	Cardiomyopathy	0 (0)	21 (46.7)	
	Ischaemic heart disease	0 (0)	24 (53.3)	
	Valvular heart disease	0 (0)	36 (80)	
	Cardiac arrhythmia	0 (0)	22 (48.9)	
	Chronic kidney disease	0 (0)	19 (42.2)	
	Chronic medications	0 (0)	39 (86.6)	

HF, heart failure; SD, standard deviation.
Most HF patients had essential hypertension and valvular heart disease as a comorbidity.

Semaphorin 4D plasma level in heart failure and control groups

N terminal pro brain natriuretic peptide plasma levels, used for the diagnosis of HF, were obviously significantly higher in the HF group (3.5 ± 0.55 vs. 1.8 ± 0.26 pg/mL in controls, $P < 0.001$). In parallel, Sema4D plasma levels were 2143.04 ± 1253 ng/mL in HF patients vs. 581 ± 762 ng/mL in controls ($P < 0.001$, *Figure 1*). No difference in Sema4D levels was found between HF patients with or without diabetes melitus, hypertension, cardiomyopathy, ischaemic heart disease, valvular heart disease, arrhythmia, or chronic medications (P = 0.18, P = 0.95, P = 0.61, P = 0.96, P = 0.92, P = 0.72, and P = 0.11, respectively).

The correlation between semaphorin 4D and N terminal pro brain natriuretic peptide

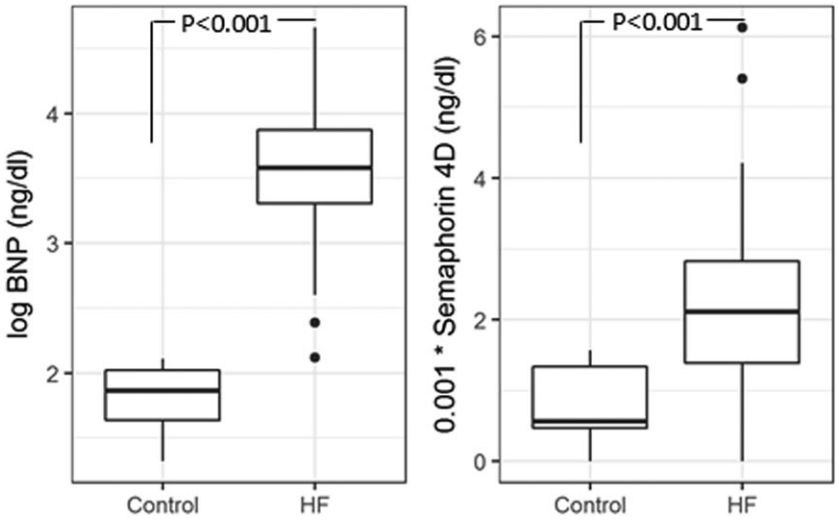
Based on previous literature and preliminary assumptions, correlations between Sema4D, NT-proBNP, and clinical and demographic data were analysed, and a multivariate model including significant correlations only was built. A significant correlation between Sema4D and NT-proBNP was found ($P < 0.001$). NT-proBNP correlated with gender, creatinine

Table 2 Clinical data and heart failure characteristics of study population

Clinical data		
Chief complaint, n (%)	Dyspnoea	35 (77.8)
	Leg oedema	9 (20)
	Weakness	1 (2.2)
HF diagnosis before admission?, n (%)	Yes	39 (86.7)
	No	6 (13.3)
Chest X-ray on admission, n (%)	Normal	6 (13.3)
	Pulmonary oedema	32 (71.1)
	Pleural effusion	7 (15.6)
NYHA, n (%)	1	4 (8.9)
	2	18 (40)
	3	8 (17.8)
	4	15 (33.3)
Echocardiographic data		
Left ventricle ejection fraction, mean (SD)	HFrEF (n = 24)	32% (12.5)
	HFpEF (n = 21)	57% (3.3)
Mean TAPSE, mm (SD)	17.9 (4.9)	

HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; NYHA, New York Heart Association; SD, standard deviation; TASPE, tricuspid annular plane systolic excursion.
Most study patients presented with dyspnoea were already diagnosed with HF prior to admission and were classified as NYHA 2 or 4. Echocardiography of patients revealed more than half of patients had HF with preserved EF.

Figure 1 Semaphorin 4D and NT-proBNP levels in heart failure (HF) group vs. healthy controls.



levels, and left ventricular EF (P = 0.001, P = 0.003, and $P < 0.001$, respectively). Sema4D correlated with age and creatinine only (P = 0.032 and P = 0.002, respectively). NYHA functional score and medical comorbidities (described previously) were not found to be correlated either to NT-proBNP or Sema4D and, therefore, were not included in the final model. In a multivariate logistic regression model, only creatinine and left ventricular EF were found to predict NT-proBNP levels (P = 0.006 and $P < 0.001$, respectively, model R^2 = 0.49, $P < 0.001$). In an identical multivariate model for the prediction of Sema4D levels, creatinine only and not left ventricle EF was found to be a significant predictor (P = 0.014 and P = 0.82, respectively, model R^2 = 0.29, P = 0.019). *Figures 2* and *3* demonstrate the differences between Sema4D and NT-proBNP levels found in the multivariate models.

Semaphorin 4D plasma levels on admission and after clinical improvement

Time between admission and discharge varied between 1 and 31 days (mean 7.83 ± 7.8 days). Mean Sema4D levels measured after clinical improvement were significantly lower than on admission—2455.67 ± 1424 vs. 3534.94 ± 1650 (for seven patients, P = 0.03). On the other hand, NT-proBNP levels did not change significantly during hospitalization

Figure 2 Semaphorin 4D levels by creatinine and left ventricle ejection fraction (EF).

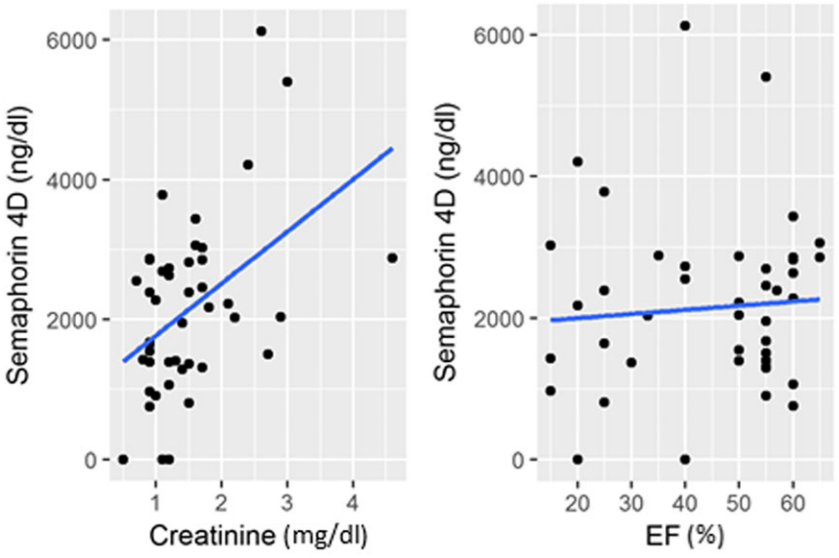
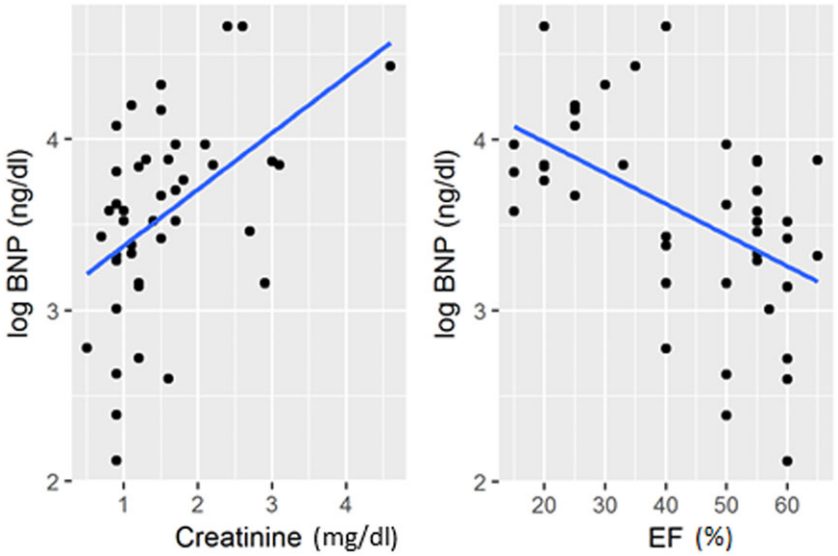


Figure 3 N terminal pro brain natriuretic peptide levels by creatinine and left ventricle ejection fraction (EF).



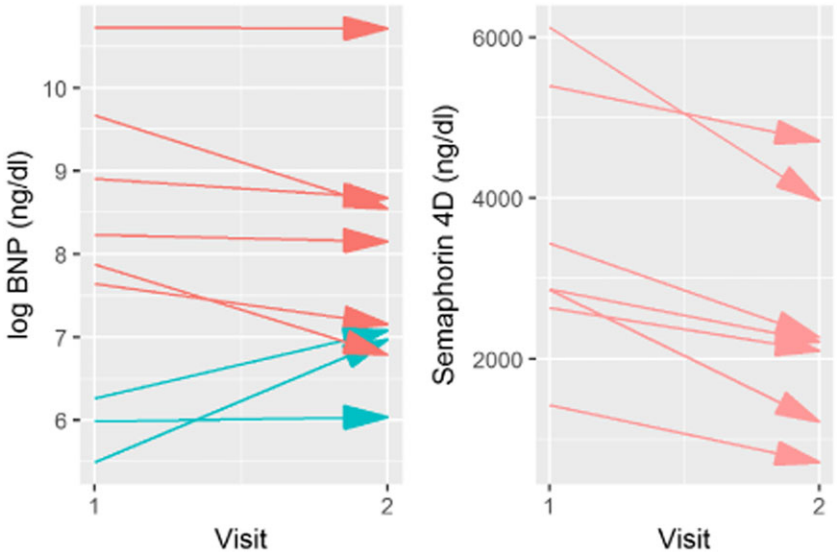
(3.41 ± 0.75 on admission vs. 3.38 ± 0.6 before discharge, for nine patients, $P = 0.79$). Analysis of patients' creatinine levels during hospitalization showed that Sema4D levels decreased regardless to changes in creatinine levels (data not shown). *Figure 4* demonstrates the differences in NT-proBNP and Sema4D per patient during hospitalization.

Discussion

This study is an exploratory study that aimed to compare Sema4D plasma levels in HF patient and healthy controls. Only patients with a confirmed acute HF diagnosis—based on echocardiographic data and elevated NT-proBNP levels—were included in the final analysis. Because of the previously proven correlation between Sema4D and infections or ischaemia, patients diagnosed with such conditions were excluded from the study.

A few minor gaps between the HF patients and healthy controls were uncovered. First, a borderline significant difference in mean age was found between the groups. The ages of the HF patient group (34–90) spanned a wide range compared with the more homogenous control group (68–78). Gender ratios were opposite between the groups, but this difference was not significant.

Figure 4 The change in Semaphorin 4D and N terminal pro brain natriuretic peptide plasma levels from admission to discharge. Red colour indicates lower levels on discharge; blue—higher or no change.



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Comparing Sema4D plasma levels in HF patients with healthy controls yielded few meaningful findings. First, the study's assumption was clearly confirmed—Sema4D levels in acute HF patients are higher than in healthy controls (without HF or any other comorbidity). Second, Sema4D plasma levels are creatinine dependent, and patients with higher creatinine levels have higher baseline levels of Sema4D. Our study was not designed to determine whether the reason for this is excessive production or disturbed clearance. Because kidney function varies between patients and different labs use different commercial kits for Sema4D, this is a key finding on the way to establish normal ranges for Sema4D plasma levels, controls selection, and correct interpretation of the results. The last finding is also well established for NT-proBNP[19] (i.e. NT-proBNP levels are creatinine dependent) and was similarly demonstrated in our study. Third, the increase in Sema4D in acute HF patients did not vary according to patients' EF, unlike NT-proBNP, which is more sensitive to HF with reduced EF.[20] Sema4D's involvement mainly with inflammation, angiogenesis, and axonal guiding mechanisms, compared with mechanisms of diuresis, hypertension, and the renin–angiotensin system (all of which strongly related to NT-proBNP), may be the reason for this contrast. Nevertheless, the strong significant correlation found between Sema4D and NT-proBNP levels is one of our study's pivotal findings.

To the best of our knowledge, the only previous study that addressed the issue of Sema4D and HF is that of Lu *et al*.[6] The researchers demonstrated elevated plasma levels of Sema4D in HF patients compared with healthy controls. Our study corroborated the aforementioned finding and also demonstrated that Sema4D is not related to pre-admission NYHA function score. Nonetheless, a few differences between the studies exist. First of all, the diagnosis of HF in our study was verified by the current official HF biomarker—NT-proBNP—facilitating the evaluation of NT-proBNP and Sema4D correlation. Second, in our study, diabetes mellitus was not found to influence Sema4D levels. Third, Lu *et al*.[6] gave no details regarding patients' kidney function, while according to our study, Sema4D is highly dependent on creatinine levels.

The 'clinical timeline' (admission to discharge) is an additional unique finding of our study. We proved that clinical improvement manifests as a decrease in Sema4D plasma levels, while NT-proBNP levels did not change during hospitalization. This finding might reflect higher clinical sensitivity of Sema4D compared with NT-proBNP for acute HF, but we restrict this possible conclusion for two reasons: (i) the number of patients having a second blood test was relatively low (due to technical issues) and (ii) per patient, NT-proBNP plasma levels did decrease for most patients (*Figure 4*); the mean levels were higher because of specific patients with extremely higher levels (reflecting misjudged clinical improvement or patients discharged too early).

Previous studies have emphasized specific cytokines involved in the development of chronic HF, among them are interleukin (IL)-1, IL-2, IL-6, and tumour necrosis factor alpha.[21] Several tissues and cells contribute to the inflammatory process, including leukocytes, platelets, tissue macrophages, and endothelial cells.[14] Chemokines affect T-lymphocytes by modulating different functions of this cells such as free radical generation and production of other cytokines.[21] This particular inflammatory pathway may explain increased Sema4D secretion in acute HF. Further laboratory research is needed to establish this assumption and to evaluate the discriminative qualities of Sema4D compared with NT-proBNP in the diagnosis of HF in the dyspnoeic patient.

Strengths of our study include meticulous methods and patient selection based on clear echocardiographic and laboratory (NT-proBNP) data that guaranteed inclusion of only patients with confirmed acute HF as the reason for their complaints; the multivariate model for predicting Sema4D levels was supported by detailed background and clinical information, thereby lowering the risk of hidden confounders; and finally, the original concept of comparing Sema4D plasma levels on admission and discharge. Main study limitations include a relatively small sample size of patients and controls and the exclusion of stable chronic HF patients for whom no information regarding Sema4D levels was available.

Summary

Semaphorin 4D is a transmembrane protein involved with and influencing processes of inflammation, angiogenesis, and axonal guidance—all considered to be important mechanisms of HF pathophysiology. Here, we report that Sema4D plasma levels of HF patients are increased compared with healthy controls, with significant and absolute correlation with NT-proBNP. Sema4D levels are higher in patients with elevated creatinine and do not differ in HF patients with decreased or preserved EF, unlike NT-proBNP levels. The decrease in Sema4D levels along with clinical improvement is an additional genuine finding of our study. Despite the previously mentioned limitations, our results may highlight a possible role for Sema4D as an acute HF biomarker for diagnosis and clinical follow-up, but further research and discussion on the matter are required.

Conflict of interest

None declared.

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