Elevated levels of serum sialic acid and high-sensitivity C-reactive protein: markers of systemic inflammation in patients with chronic heart failure

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Accepted: 18 December 2013

ABSTRACT

Heart failure (HF) is a common, debilitating disorder in which the heart is unable to pump an adequate blood supply to the tissues. Although it has been shown that inflammation occurs in HF, inflammatory markers have yet to be defined. Inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP), cytokines and serum sialic acid (SA) have been suggested as cardiovascular risk biomarkers. This study aims to assess the serum levels of inflammatory markers such as sialic acid and hs-CRP in chronic heart failure (CHF). Forty-eight patients with CHF and 30 healthy controls were recruited. Total sialic acid (TSA) and lipid-associated sialic acid (LASA), and the inflammatory marker hs-CRP, were assayed in all study subjects. *N*-terminal pro-brain natriuretic peptide (NT-proBNP) was assayed in the patient group only. Serum mean TSA and LASA were significantly higher in CHF patients when compared to healthy controls ($P<0.01$). Mean hs-CRP levels in CHF patients showed a significant elevation compared with healthy controls ($P<0.01$). There was a significant positive correlation between TSA and hs-CRP. Thus, TSA and hs-CRP would appear to be stable markers of systemic inflammation in chronic heart failure.

KEY WORDS: C-reactive protein.
Heart failure.
Inflammation.
Sialic acid.

Introduction

Chronic heart failure (CHF) is a debilitating disorder in which a patient exhibits symptoms such as breathlessness, fatigue and signs of fluid retention with high morbidity and mortality, inflicting a significant economic burden on the healthcare system.[1] Coronary artery disease (CAD) is one of the major causes found in most cases of CHF, while other causes such as hypertension, valvular heart disease and cardiomyopathy also attribute to CHF.[2,3]

Inflammatory mechanisms have been shown to cause myocardial damage, while inflammatory agents contribute to the progression of CHF.[4] Various inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP) and cytokines have been studied in relation to cardiovascular diseases, including HF, where inflammation-induced damage is seen.

Sialic acid (SA), an *N* or *O* derivative of neuraminic acid (monosaccharide), is present both in lipoproteins and glycolipids found in plasma and in cellular membranes.[5,6] Previous study has shown that elevation of serum SA indicates higher risk of developing cardiovascular diseases,[7–9] and that measurement of SA helps to identify inflammation in the disease.[10] Using discrimination ratio analysis, use of CRP with large within-individual variability, will underestimate the relationship between inflammation and disease, and it also suggests that measurement of serum SA may be the most useful estimate of an individual's habitual inflammatory status.[11]

The SA content of low-density lipoprotein (LDL) has not been associated with clinical signs of atherosclerosis, but the catabolic rate of dense LDL apolipoprotein B is positively related to the SA content of the respective lipoproteins, suggesting that lipid-associated sialic acid (LASA) may contribute to raised TSA in CHF.[12] *N*-terminal pro-brain natriuretic peptide (NT-proBNP) is also a strong marker of cardiovascular disease, with recent evidence showing that inflammation may influence levels of this peptide.[13]

The aim of this study is to assess serum levels of hs-CRP, TSA, LASA and NT-pro-BNP in patients with CHF, and to compare results with those obtained from healthy controls.

Materials and methods

Study subjects

Forty-eight patients with CHF (left ventricular ejection fraction [LVEF] less than 40%) and 30 apparently healthy controls were included in this hospital-based cross-sectional study. Clearance was obtained from the Institute Ethics Committee (Human Studies) and written informed consent was obtained from all study subjects prior to participation. Any CHF patient with systemic inflammatory disease including rheumatic heart disease, cancer, hepatic

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dysfunction, connective tissue disorder and any systemic infection was excluded from the study. The control group included apparently healthy subjects with no history of diabetes, hypertension or smoking, and with normal clinical examination and electrocardiogram (ECG). A detailed clinical history was taken and clinical examination, ECG and body mass index (BMI) was performed in all study subjects.

Sample analysis

A fasting blood sample (5 mL) was collected from each study subject. A 1 mL sample was transferred to a heparinised vial for NT-proBNP estimation, with the remainder collected in a plain vial for estimation of routine parameters (e.g., fasting blood glucose, uric acid and lipid profile, SA and hs-CRP). The serum was separated by centrifugation (2000 x*g* for 10 min) and stored at –20°C. Fasting blood glucose, total cholesterol, triglyceride and high-density lipoprotein (HDL)-cholesterol (after LDL precipitation with heparin–$MnCl_2$) were estimated on the same day using commercial kits on a fully automated random-access clinical chemistry analyser.

Intra-assay and inter-assay coefficients of variation (CVs) were 4–6%, within the recommended range suggested by the National Cholesterol Education Program.[14] Calculation of LDL cholesterol levels was performed using the Friedwald formula.[15] Quantitative determination of NT-proBNP was performed by immunoassay in heparinised venous blood using the cobas H232 point-of-care (POC) system (Roche Diagnostics, Switzerland) with measurement range of 60–9000 pg/mL.[16]

Total SA was determined by a modified Aminoff method[17] where the SA moiety reacts with thiobarbituric acid (TBA) to form a TBA-SA complex, giving a pink colour when boiled. The colour was extracted using an acid–butanol mixture and then measured spectrophotometrically at 549 nm (Systronics, India). Intra-assay variations were <2% and the inter-assay variations were <5%.

Lipid-associated SA was determined spectrophotometrically by the modified method of Katopodis *et al*.[18] Intra-assay and inter-assay variations were 3.6% and 7.5%, respectively. *N*-acetyl neuraminic acid (Sigma CA, USA) was used for the calibration. High-sensitivity CRP was assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Diagnostics Biochem, Ontario, Canada).

Statistical analysis

Statistical analysis was performed using IBM SPSS Version 20 for Windows. The sample size was estimated based on a previous study by Canan *et al*.[20] with expected mean difference in TSA of 0.24 mmol/L with standard deviation (SD) 0.33 mmol/L at 80% power and 5% level of significance. The minimal sample size required was estimated as 30 in each group. Baseline characteristics were analysed using descriptive statistics. The normality of continuous data was assessed by the Kolmogrov-Smirnov test. Normally distributed data were described as mean±SD and compared by independent Student's *t*-test. Non-normally distributed data were described as median±interquartile range (IR) and analysed by the Mann Whitney U test. χ^2 test was performed to study the association of risk factors. Correlation between biochemical parameters was studied using Spearman correlation. $P<0.05$ was considered statistically significant.

Table 1. Comparison of the demographic and baseline characteristics in the study groups.

	CHF (n=48)	Controls (n=30)	P value
Age (years)	52.4±12.3	49.6±9.7	0.251
BMI (kg/m^2)	23.9±5.1	25.8±4.0	0.139
W:H ratio	0.95±0.07	0.98±0.05	0.356
Male:female	40:8	22:8	0.287
Smoker (%)	33 (69)	10 (33)	0.002
Hypertension (%)	14 (29)	–	0.01
Diabetes mellitus (%)	11 (23)	–	0.05
Scorpion sting history (%)	24 (50)	5 (17)	0.01
Regular alcohol use >5 years (%)	27 (56)	–	–
Non-ischaemic cardiomyopathy (%)	17 (35)	–	–
LVEF (mL)	40.0±5.7	–	
Anaemia (%)	26 (54)	5 (17)	0.01
NYHA Class II (%)	35 (73)	–	–
NYHA Class III (%)	13 (27)	–	–

Data presented as mean±SD (Student's *t*-test) or number of people (%) (χ^2 test).

Results

Baseline characteristics of the study subjects are shown in Table 1. There was no significant difference in age, BMI or waist:hip (W:H) ratio between the two groups.

Mean serum TSA and LASA were significantly higher in CHF patients when compared to healthy controls ($P<0.01$). Median hs-CRP levels in CHF patients showed significant elevation when compared with healthy controls ($P<0.01$). Median NT-proBNP was 627 ng/L in CHF patients. Serum TSA levels showed a significant positive correlation with hs-CRP levels (Spearman correlation coefficient 0.392; $P<0.01$). In the ischaemic heart failure (IHF) and non-ischaemic heart failure (NIHF) subgroups there was no significant difference in serum TSA, LASA and hs-CRP.

Discussion

A growing body of evidence suggests that systemic inflammation in CHF may play a role in its development and progression, not only by promoting myocardial dysfunction but also by inducing pathogenic consequences in other organs and tissues, thereby contributing to additional aspects of the HF syndrome (e.g., cachexia, endothelial dysfunction and anaemia).[19] This would suggest that the inflammation may play major role in this disorder. Total SA and LASA have been found to be elevated in patients with atherosclerosis. A study by Canan *et al*. has shown that these are elevated in patients with heart failure,[20] and the present study supports these findings

A localised inflammatory response associated with the atherosclerotic plaque may lead to a systemic acute-phase response.[21] In relation to increases in serum TSA and LASA, CAD could be a factor in CHF patients; however, analysis of

Table 2. Comparison of biochemical parameters in the study groups.

	CHF (n=48)	Controls (n=30)	P value
Fasting blood glucose (mmol/L)	4.8±1.4	4.4±0.5	0.026
Total cholesterol (mmol/L)	4.1±1.1	5.6±0.8	0.030
Triglycerides (mmol/L)	1.3±0.6	1.6±0.8	0.194
HDL (mmol/L)	1.00±0.27	1.29±0.18	0.075
LDL (mmol/L)	2.4±0.9	3.5±0.8	0.341
VLDL (mmol/L)	0.60±0.29	0.74±0.37	0.194
UA (mmol/L)	0.36±0.08	0.31±0.09	0.236
TSA (mmol/L)	5.60±2.12	2.33±0.50	<0.01
LASA (mmol/L)	0.64±0.21	0.49±0.109	<0.01
NT-proBNP (ng/L)	627 (380.5–1650.0)	–	–
hs-CRP (mg/L)	9.1 (6.9–15.9)	5.3 (2.7–7.2)	<0.01

Data presented as mean±SD (Student's *t*-test) or as median (IQR)(Mann-Whitney U test).

inflammatory markers between the IHF and NIHF groups did not show any significant differences. In the NIHF group, therefore, elevation may be due to an inflammatory response such as that seen in heart failure. Lipid parameters such as total cholesterol and LDL cholesterol are decreased in CHF, whereas serum HDL levels are elevated in CHF.

One reason for an elevated serum SA level might be in order to act as a substrate for resialylation of SA-deficient structures and thereby counteract the atherosclerotic process. Gracheva *et al*. reported that sialyltransferase activity in human plasma and aortic intima is enhanced in atherosclerosis.[22] Lindberg *et al*. recently suggested that SA plays a protective role in the endothelium, the desialylation of which is the initial step in the atherosclerotic process. Thereafter, cytokines from macrophages that reach the damaged endothelium trigger the hepatic production of SA-rich glycoproteins in order to provide a substrate for resialylation of the endothelium.[23] The increase in serum TSA seems to reflect the existence of accelerated atherosclerosis. In cell culture studies and in the serum of CAD patients, desialylated LDL increased intracellular cholesterol accumulation, compared to SA-rich LDL or serum levels in healthy individuals.[24,25]

This LASA elevation may contribute to a rise in TSA. Another possible source of increased serum SA is increased output of serum proteins by the liver due to an acute-phase reaction. As many acute-phase proteins are glycoproteins with SA residues at the terminal position of their oligosaccharide side chains,[26] the increase in serum TSA seen in the present study may be an inflammatory response in heart failure, independent of atherogenesis. This is supported by the increase in sialyltransferase activity, an enzyme catalysing the transfer of SAs to an asialoglycoprotein-like lipoprotein (a).

An important biomarker for left ventricular systolic dysfunction and left ventricular stress in the general population,[27] NT-proBNP has been found to capture some aspects of a chronic inflammatory state (e.g., rheumatoid arthritis).[28] In the present study, elevation in CHF of ischaemic and non-ischaemic origin indicates that it is independent of the atherosclerotic process. A recent study by Sanchez-Lazaro *et al*. has shown that increased inflammatory marker levels relate to functional classification,[29] and the results of the present study support this finding.

A positive correlation between hs-CRP and serum TSA levels is supported by previous work by Sathiapriya et al., in which elevated levels of these inflammatory markers were shown to interact to increase cardiovascular morbidity and mortality in hypertension.[30] Using a discrimination ratio analysis, Browning and co-workers reported that hs-CRP could underestimate inflammation and SA measurement could be used as an inflammatory marker for chronic diseases;[11] findings that are supported by the work reported here.

In conclusion, elevated serum SA and hs-CRP levels in patients with CHF parallel the systemic inflammatory response in CHF; however, raised LASA may have contributed to the rise in TSA level. It has been shown that sialic acid and hs-CRP can be used as stable inflammatory markers, and are of use as biomarkers for cardiovascular risk and prognosis, especially in heart failure. □

The study was supported by the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) intramural research fund.

References

1 Dickstein K, Cohen-Solal A, Filippatos G *et al*.; ESC Committee for Practice Guideleines. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur Heart J* 2008; **29** (19): 2388–442.

2 Fox KF, Cowie MR, Wood DA *et al.* Coronary artery disease as the cause of incident heart failure in the population. *Eur Heart J* 2001; **22** (3): 228–36.

3 McMurray JJ, Pfeffer MA. Heart failure. *Lancet* 2005; **365** (9474): 1877–89.

4 Oikonomou E, Tousoulis D, Siasos G, Zaromitidou M, Papavassiliou AG, Stefanadis C. The role of inflammation in heart failure: new therapeutic approaches. *Hellenic J Cardiol* 2011; **52** (1): 30–40.

5 Silllanaukee P, Ponnio M, Jaaskelainen IP. Occurrence of sialic acids in healthy humans and different disorders. *Eur J Clin Invest* 1999; **29** (5): 413–25.

6 Schauer R. Sialic acids and their role as biological masks. *Trends in Biochemical Sciences* 1985; **10** (9): 357–60.

7 Nigam PK, Narain VS, Ajay Kumar. Sialic acid in cardiovascular diseases. *Indian J Clin Biochem* 2006; **21** (1): 54–61.

8 Gopaul KP, Crook MA. Sialic acid: a novel marker of cardiovascular disease? *Clin Biochem* 2006; **39** (7): 667–81.

9 Nanettib L, Vignini A, Raffaelli F *et al*. Sialic acid and sialidase activity in acute stroke. *Dis Markers* 2008; **25** (3): 167–73.

10 Gokmen SS, Kilicli G, Ozcelik F, Ture M, Gulen S. Association between serum total and lipid-bound sialic acid concentration and the severity of coronary atherosclerosis. *J Lab Clin Med* 2002; **140** (2): 110–8.

11 Browning LM, Krebs JD, Jebb SA. Discrimination ratio analysis of inflammatory markers: implications for the study of inflammation in chronic disease. *Metabolism* 2004; **53** (7): 899–903.

12 Melaji N, Cylliing H, Miettinen TA. Sialic acids and the metabolism of low density 1ipoprotein. *J Lipid Res* 1996; **37** (8): 1625–31.

13 Moraes JC, Ribeiro AC, Saad CG, Lianza AC, Silva CA, Bonfa E. NT-proBNP levels may be influenced by inflammation in active ankylosing spondylitis receiving TNF blockers: a pilot study. *Clin Rheumatol* 2013; **32** (6): 879–83.

14 National Cholesterol Education Program. Second report of the expert panel on detection evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel II). *Circulation* 1994; **89** (3): 1333–445.

15 Friedwald WT, Laby RI, Frederickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18** (6): 499–502.

16 Blick KE. Economics of point-of-care (POC) testing for cardiac markers and B-natriuretic peptide (BNP). *Point of Care* 2005; **4**: 11–4.

17 Aminoff D. Methods for the quantitative estimation of *N*-acetyl neuraminic acid and their application to hydrolysates of sialomucoids. *Biochem J* 1961; **81**: 384–92.

18 Katopodis N, Hirshaut Y, Geller NL, Stock CC. Lipid-associated sialic acid test for the detection of human cancer. *Cancer Res* 1982; **42** (12): 5270–5.

19 Yndestad A, Damas JK, Oie E, Ueland T, Gullestad L, Aukrust P. Systemic inflammation in heart failure – the whys and wherefores. *Heart Fail Rev* 2006; **11** (1): 83–92.

20 Topcuoglu C, Yilmaz FM, Sahin D *et al*. Total and lipid associated sialic acid in serum and thrombocytes in patients with chronic heart failure. *Clin Biochem* 2010; **43** (4–5): 447–9.

21 Crook M, Haq M, Haq S, Tutt P. Plasma sialic acid and acute phase proteins in patients with myocardial infarction. *Angiology* 1994; **45** (8): 709–15.

22 Gracheva EV, Samovilova NN, Golovanova NK *et al*. Sialyltransferase activity of human plasma and aortic intima is enhanced in atherosclerosis. *Biochim Biophys Acta* 2002; **1586** (1): 123–8.

23 Lindberg G, Eklund GA, Gullberg B, Rastam L. Serum sialic acid concentration predicts both coronary heart disease and stroke mortality: multivariate analysis including 54385 men and women during 20.5 years follow up. *Int J Epidemiol* 1992; **21** (2): 253–7.

24 Tertov VV, Sobenin IA, Gabbasov ZA, Popov EG, Orekhov AN. Lipoprotein aggregation as an essential condition of intracellular lipid accumulation caused by modified low density lipoproteins. *Biochem Biophys Res Commun* 1989; **163** (1): 489–94.

25 Orekhov AN, Tertov VV, Pokrovsky SN *et al*. Blood serum atherogenicity associated with coronary atherosclerosis. Evidence for nonlipid factor providing atherogenicity of low density lipoproteins and an approach to its elimination. *Circ Res* 1988; **62** (3): 421–9.

26 Taniuchi K, Chifu K, Hayashi N *et al*. A new enzymatic method for the determination of sialic acid in serum and its application for a marker of acute phase reactants. *Kobe J Med Sci* 1981; **27** (3): 91–102.

27 Groenning BA, Raymond I, Hildebrandt PR, Nilsson JC, Baumann M, Pedersen F. Diagnostic and prognostic evaluation of left ventricular systolic heart failure by plasma *N*-terminal pro-brain natriuretic peptide concentrations in a large sample of the general population. *Heart* 2004; **90** (3): 297–303.

28 Crowson CS, Myasoedova E, Davis JM *et al*. Use of B-type natriuretic peptide as a screening tool for left ventricular diastolic dysfunction in rheumatoid arthritis patients without clinical cardiovascular disease. *Arthritis Care Res (Hoboken)* 2011; **63** (5): 729–34.

29 Sanchez-Lazaro IJ, Almenar L, Reganon E *et al*. Inflammatory markers in stable heart failure and their relationship with functional class. *Int J Cardiol* 2008; **129** (3): 388–93.

30 Sathiyapriya V, Nandeesha H, Selvaraj N, Bobby Z, Agrawal A, Pavithran P. Association between protein-bound sialic acid and high-sensitivity C-reactive protein in essential hypertension: a possible indication of underlying cardiovascular risk. *Angiology* 2009; **59** (6): 721–6.