

Markers of Inflammation in Acute Coronary Syndromes: Association with Increased Heart Rate and Reductions in Heart Rate Variability

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Summary

Background: Systemic and vascular inflammation is at the heart of the thrombotic occlusion of coronary arteries.

Hypothesis: The study was undertaken to determine the relationship between established inflammatory markers (interleukin-6 [IL-6] and high-sensitivity C-reactive protein [hs-CRP]), neutrophil or white cell count, and concomitant autonomic tone in patients with coronary artery disease soon after occlusive events.

Methods: We tested the linkage between autonomic tone (as defined using both time domain and frequency domain estimates of heart rate variability [HRV]) and circulating markers of inflammation (white cell counts, hs-CRP, and IL-6) in a sample of 100 patients with proven acute coronary syndrome and compared these with healthy controls (n = 49) and the relationships on repeated measures at 4 months in recovery (n = 51).

Results: We demonstrated predictable depressed HRV in acute patients who tended to show recovery by 4 months. The acute changes in HRV indices (e.g., triangular index) showed modest negative correlation ($r = -0.2$ – -0.3) with the acute elevation of white cell count, IL-6, and hs-CRP. These associations did not persist on multivariate analysis of data gathered at 4 months post event.

Conclusion: These observational data, while limited, are the first to link autonomic tone and in particular sympathetic tone (as indicated by HRV), to the process of acute leukocytosis and systemic inflammation common in acute coronary syndromes.

Key words: Autonomic tone, heart rate variability, markers of inflammation, acute coronary syndromes, myocardial infarction

Introduction

Vascular inflammation plays a critical role in the initiation, evolution, and rupture of atherosclerotic plaque.¹ Circulating biomarkers of this process predict morbidity and mortality in patients with established coronary disease^{2–4} and the evolution of disease in healthy subjects.^{5–7} Furthermore, reductions in indices of heart rate variability (HRV), widely recognized as a marker of cardiac autonomic tone,⁸ also predict cardiovascular morbidity and mortality in both health and disease.^{9–11} Even simple increases in mean heart rate appear to be linked with the progression of atherosclerosis.^{12–14}

Recent studies have explored the link between altered autonomic tone and markers of inflammation (high-sensitivity C-reactive protein [hs-CRP] and white cell count [WCC]) in healthy individuals with no evidence of overt cardiovascular disease.^{15, 16} The cytokine response to mental stress in healthy individuals is associated with parallel changes in HR and HRV.¹⁷ In patients with systolic left ventricular (LV) impairment, there is evidence of concomitant change in both inflammatory markers such as neutrophil count and CRP and deteriorating autonomic tone in patients with systolic LV dysfunction (which is mostly due to chronic coronary artery disease [CAD]).¹⁸

Given the associations in this study, we believed it reasonable to test for a link between autonomic tone (as defined by HRV) and markers of inflammation during the evolution of an acute coronary syndrome (ACS).^{2,3,4,19} As reductions in HRV have been repeatedly demonstrated during the early phase of

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myocardial infarction, we hypothesized that a relationship may exist between established inflammatory markers (interleukin-6 [IL-6] and hs-CRP), neutrophil or WCC, and concomitant autonomic tone in patients with CAD soon after occlusive events.

Methods

We prospectively recruited patients admitted to our coronary care unit with ACS. Patients were eligible for the study if they had prolonged (> 30 min), typically ischemic chest pain at rest or during minimal effort, accompanied by elevated myocardial biomarkers (total creatine kinase and MB isoenzyme more than twice the upper limit of normal and/or raised cardiac troponin I (> 1 ng/ml) occurring with electrocardiographic (ECG) changes (ST-T segment elevation or depression) within 24 h of symptom onset.²⁰ All patients received standard treatment including appropriate reperfusion therapy (either by fibrinolysis alone and/or percutaneous coronary intervention), in combination with best medical treatment including aspirin, low-molecular-weight heparin, intravenous nitrate infusion, oral statin, beta blocker, and angiotensin-converting enzyme (ACE) inhibitor where appropriate.

Exclusion criteria were Killip class III or IV symptoms on admission, presence of significant valvular heart disease, cardiomyopathy, any chronic inflammatory disease, or history of or investigation for suspected malignancy. Patients were also excluded if they displayed significant rhythm disturbance that would alter the analysis of HRV (e.g., persistent heart block, paced rhythm, atrial fibrillation, frequent premature ventricular or supraventricular beats, idioventricular rhythm, recurrent ventricular tachycardia [VT] or ventricular salvos). Patients were excluded if beta-blocking drugs had been administered in either the emergency room or coronary care unit prior to initial HRV analysis.

Baseline parameters of the sample of patients with ACS were compared to age- and gender-matched healthy control subjects. These normal subjects (defined by history, examination, and basic blood tests) were recruited from members of the hospital staff and the visiting families of patients attending the hospital. No patients or controls had a history of renal or liver disease, malignancy, connective tissue disease, deep vein thrombosis or pulmonary embolism, recent infections, or inflammatory disorders, and none was taking regular non-steroidal anti-inflammatory drugs or anticoagulants. Blood pressure (phase V) was recorded using a semiautomated OMRON (Omron Europe B.V., Hoofddorp, The Netherlands) device supine from the nondominant arm in triplicate after a minimum 5-min rest. The local research and ethics committee approved the study and all subjects gave written and informed consent.

Repeat examinations were completed 4 months (\pm 5 days) after the date of index admission. Exclusion criteria to a follow-up assessment were readmission in the intervening period between discharge and follow-up (three patients) or the emergence of LV impairment (echo LV ejection fraction

<40%). Of the 100 patients studied acutely, 51 agreed to reattend for follow-up. All were taking a statin, beta blocker, ACE inhibitor (or angiotensin-2 receptor blocker), and aspirin. All patients who had undergone percutaneous intervention and stent insertion were still receiving clopidogrel in addition to aspirin.

Heart Rate Variability Analysis

Heart rate variability was estimated from a continuous 20-min sample recorded on a standard Holter system (Delmar-Reynolds, Ware, Hertfordshire, U.K.) applied at the time of admission to the coronary care unit, taking care to avoid delay or interference with the routine management of these patients. Patients were sampled supine during controlled breathing at 12–15 breaths/min in an enclosed cubicle without interruption or distraction. The follow-up recording at 4 months post event was completed using similar conditions in a quiet clinic room. A researcher blinded to clinical data (MS) analyzed the ECG data independent of knowledge of the patient data and visit schedule.

Heart rate variability studies were accepted only where >80% of RR intervals were suitable for analysis. Automated beat-to-beat analysis was verified as correct by visual inspection by the investigator. We calculated the following routine time domain measures of HRV: (1) the mean RR-interval duration in each recording; (2) the standard deviation (SD) of all normal RR intervals (SDNN); (3) the SD of the means of all normal RR intervals during each 5-min segment of the recording (SDANN); (4) the mean of the 5-min SD of NN intervals (SDNNi); and (5) the root-mean square of differences of successive RR intervals (RMSSD). In addition, the following frequency domain analyses were performed applying fast-Fourier transformation (RR Tools analytical software; Reynolds Delmar plc, Ware, Hertfordshire, U.K.) to isolate the following standardized power spectral bands: (1) High-frequency (HF) power (0.15–0.4 Hz); (2) low-frequency (LF) power (0.004–0.15 Hz); (3) very-low-frequency power (VLF) (0.0033–0.004 Hz); and (4) a ratio of LF/HF power.

Blood Sampling and Laboratory Analyses

Blood sampling from an antecubital vein was completed following Holter recordings. Samples from patients during follow-up and healthy control subjects were taken after overnight fast, and abstinence from tobacco and alcoholic or caffeine-containing beverages. Blood was drawn into chilled sodium citrate and centrifuged at 1000 g and 4° C for 20 min. Plasma was aliquoted and stored at –70° C for later batch analysis.

We analyzed plasma IL-6 by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Palo Alto, Calif., USA) using commercial kits and reagents. Ultra hs-CRP was analyzed using a Quantex CRP Ultra kit (Sensitive Biokit SA, Barcelona, Spain). Total white blood count and white blood count subcomponents were analyzed by routine Coulter counter. All assays had local intra- and interassay coefficients of variation of <5% and <10%, respectively.

Power Calculations

We performed power calculations for sample size estimated on previous studies examining the effects of inflammation^{19,21} and HRV^{22,23} in acute coronary syndromes. Seeking a difference of 0.8 SD, we needed data from a minimum of 30 patients in each group (i.e., ACS and healthy controls), for $p < 0.05$ and $1-\beta > 0.80$. Serial paired measurements in a minimum of 30 subjects provided the power to detect a difference of 0.5 SD at $p < 0.05$ and $1-\beta = 0.80$.

Statistical Analysis

Continuous data were subjected to a preliminary Anderson-Darling test to determine normality. Inflammatory and autonomic indices were non-normally distributed and were therefore compared using a nonparametric Mann-Whitney U test. Normally distributed data presented as mean and SD were analyzed by Student's unpaired *t*-test. Categorical data were analyzed by chi-square test. Correlations between variables were sought by Spearman's ranking method. The significance of changes in clinical and biochemical indices with therapy was evaluated by paired *t*-test or Wilcoxon's signed rank test for normal and non-normal data, respectively. A multivariate regression analysis of acute neutrophil count, IL-6, and CRP was completed accounting for age, gender, smoking habit, and the presence of hypertension and/or diabetes. All statistical analyses were performed using Minitab Software package Release 13 (Minitab Ltd., Coventry, U.K.).

Results

Data from 100 patients and 49 healthy controls were recruited (Table I). There were no differences in basic demography. It was not surprising that blood pressure and triglyceride levels were higher and high-density lipoprotein (HDL) cholesterol was significantly lower (by disease/treatment effects) in patients compared with controls. Similarly, total WCC, neutrophil count, monocytes, IL-6, and CRP levels were all higher in patients compared with controls (all $p < 0.001$). For HRV indices, mean RR interval, SDNN, SDNNi, RMSSD, VLF, and HF power were all significantly lower in patients than in controls (Table II).

Temporal Changes in Autonomic and Inflammatory Markers

In the subgroup of 51 patients who agreed to follow-up at 4 months following the initial event, there was a significant increase in both systolic and diastolic blood pressure and a reduction in total cholesterol with corresponding increase in statin, aspirin, beta-blocker, and ACE inhibitor/A2 blocker use (Table III). These changes were associated with significant reductions in total WCC to a median of 75% of baseline levels, neutrophil count to 64% of baseline, IL-6 to 22% of baseline, and CRP to 13% of baseline. Very-low-frequency power and LF/HF ratio decreased significantly, while mean RR interval, RMSSD, and HF power increased significantly at 4-month follow-up.

TABLE I Baseline characteristics (given as number n =; mean [SD] or median [interquartile range X-Y]) of patients and healthy age- and gender-matched controls

	Patients (n = 100)	Controls (n = 49)	p Value
Age, years	63 (12)	60 (10)	0.09
Male, n	77	32	0.130
Smokers, n	41		
Past medical history, n			
Ischemic heart disease	25		
Hypertension	45		
Diabetes	27		
Drug treatment, n			
Aspirin	31		
Diuretic	10		
Beta blocker	23		
ACE inhibitor/A2 blocker	25		
Calcium blocker	10		
Nitrate	9		
Statin	21		
Systolic BP, mmHg	127 (18)	140 (19)	<0.001
Diastolic BP, mmHg	66 (12)	84 (11)	<0.001
Total cholesterol, mmol/l	5.3 (4.4–5.9)	5.5 (5.0–5.7)	0.473
LDL cholesterol, mmol/l	4.0 (2.7–5.2)	4.0 (3.3–4.3)	0.666
HDL cholesterol, mmol/l	1.3 (1.1–1.5)	1.5 (1.3–1.8)	0.003
Triglycerides, mmol/l	1.7 (1.1–3.1)	1.2 (0.9–1.6)	<0.001

Abbreviations: n = number, ACE = angiotensin-converting enzyme, BP = blood pressure, LDL = low-density lipoprotein, HDL = high-density lipoprotein.

TABLE II Baseline differences in inflammatory and autonomic markers between patients (n = 100) and controls (n = 49)

	Patients (n = 100)	Controls (n = 49)	p Value patients vs. control
Inflammatory markers			
Total white cell count (x10 ⁹ /l)	10.0 (8.1–12.1)	5.8 (5.2–7.2)	<0.001
Neutrophils (×10 ⁹ /l)	7.0 (5.2–9.0)	3.5 (3.0–4.7)	<0.001
Lymphocytes (×10 ⁹ /l)	2.0 (1.4–2.5)	1.7 (1.5–2.2)	0.571
Monocytes (×10 ⁹ /l)	0.46 (0.34–0.61)	0.36 (0.30–0.42)	<0.001
Eosinophils (×10 ⁹ /l)	0.12 (0.06–0.22)	0.17 (0.11–0.31)	0.006
Basophils (×10 ⁹ /l)	0.04 (0.03–0.05)	0.04 (0.03–0.05)	0.939
IL-6	32 (6–54)	7 (4–80)	0.017
CRP	705 (365–1988)	95 (34–182)	<0.001
Autonomic markers			
Mean heart rate	74 (15)	67 (9)	0.003
Mean RR interval (ms)	853 (159)	936 (144)	0.001
SDNN (ms)	42 (27–58)	56 (43–69)	<0.001
SDNNi (ms)	34 (24–48)	51 (39–64)	<0.001
SDANN (ms)	16 (8–28)	17 (10–22)	0.881
RMSSD (ms)	22 (15–35)	34 (25–43)	<0.001
Triangular index	10.0 (7.0–13.0)	13.5 (11.0–17.3)	<0.001
VLF power (ms ²)	674 (241–1578)	1113 (721–1650)	0.005
LF power (ms ²)	202 (97–556)	591 (364–1017)	<0.001
HF power (ms ²)	140 (53–335)	346 (180–603)	<0.001
LF/HF	1.53 (0.80–2.59)	1.83 (1.20–3.14)	0.144

Abbreviations: SDNN, SDNNi, SDANN, RMSSD, see text. VLF = very low frequency, LF = low frequency, HF = high frequency.

TABLE III Temporal changes in clinical, inflammatory, and autonomic markers at baseline and repeated at 4 months following acute event in a sample of 51 patients. Data are mean (standard deviation) or median and IQR (X–Y)

	Baseline	4 Months	p Value (baseline vs. recovery)
Systolic BP, mmHg	124 (18)	131 (23)	0.049
Diastolic BP, mmHg	66 (12)	76 (10)	<0.001
Total cholesterol, mmol/l	5.5 (4.7–5.8)	3.5 (3.0–4.4)	<0.001
HDL cholesterol, mmol/l	1.3 (1.1–1.6)	1.2 (1.1–1.5)	0.721
Triglycerides, mmol/l	1.6 (1.0–2.7)	1.2 (0.8–2.0)	0.039
Statin, mmol/l	13	49	<0.001
Aspirin	16	46	<0.001
Beta blocker	16	39	<0.001
ACE inhibitor/A2 blocker	10	42	<0.001
Total white cell count			
Total white cell count	10.0 (8.1–11.7)	7.5 (6.1–8.7)	<0.001
Neutrophils	7.2 (5.8–8.8)	4.6 (3.5–5.5)	<0.001
Lymphocytes	1.9 (1.5–2.5)	1.8 (1.5–2.3)	0.672
Monocytes	0.46 (0.34–0.62)	0.41 (0.34–0.52)	0.06
Eosinophils	0.10 (0.006–0.18)	0.25 (0.13–0.41)	<0.001
Basophils	0.04 (0.03–0.06)	0.04 (0.03–0.05)	0.421
IL-6	22 (6–52)	5 (5–20)	<0.001
hs-CRP	532 (190–1646)	149 (45–331)	<0.001
Autonomic markers			
Mean heart rate	72 (15)	64 (14)	<0.001
Mean RR interval (ms)	876 (156)	969 (173)	<0.001
SDNN (ms)	32 (11–44)	44 (29–64)	0.422
SDNNi (ms)	39 (27–51)	40 (25–57)	0.900
SDANN (ms)	16 (9–30)	14 (10–20)	0.164
RMSSD (ms)	22 (17–32)	31 (21–48)	0.01
VLF power (ms ²)	862 (365–2051)	743 (389–1420)	<0.001
LF power (ms ²)	261 (137–621)	332 (108–874)	0.421
HF power (ms ²)	158 (64–357)	230 (106–562)	0.005
LF/HF	1.5 (1.1–2.4)	1.3 (0.8–2.3)	0.041

Abbreviations: IL-6 = interleukin-6, CRP = C-reactive protein. Other abbreviations as in Tables I and II.

Correlations

In the entire cohort of patients at baseline, there were only modest negative correlations ($r = -0.2$ – -0.3) between the measured markers of inflammation (WCC, neutrophil count, IL-6, and CRP) and some of the concomitantly measured HRV indices. These extended to some but not all time-domain (mean RR interval, SDNN, SDNNi) and frequency-domain (VLF and LF power) indices (Table IV). The strongest associations were seen between total neutrophil count and time domain HRV, mean RR interval ($r = -0.320$, $p = 0.001$) and triangular index ($r = -0.351$, $p < 0.001$) (Fig. 1).

Predictably during follow-up (where both disease and treatment effects were quiescent), there were fewer associations between inflammatory markers and HRV. There was a modest linear negative correlation between total WCC and HF power ($r = -0.322$, $p = 0.026$), CRP, and VLF power ($r = -0.337$, $p = 0.024$), as well as IL-6 and SDNN ($r = -0.332$, $p = 0.027$). However, changes in neutrophil count (baseline–follow-up), IL-6, and CRP were not associated with any changes in HRV parameters (mean HR, mean RR interval, RMSSD, VLF power, HF power, and LF/HF ratio) ($p = \text{NS}$, data not shown).

Multivariate Analysis

Stepwise logistic regression analysis revealed both triangular index ($p < 0.001$) and gender ($p = 0.017$) as significant independent predictors of measured acute neutrophil count. Both these variables accounted for 22% of the variation in neu-

TABLE IV Summary of main linear correlations between inflammatory and autonomic indices in patients with acute coronary syndrome

	WCC	Neut	IL6	CRP
Time domain				
Mean RR				
r	-0.314	-0.320	-0.274	—
p Value	0.001	0.001	0.007	—
SDNN				
r	-0.210	-0.214	-0.257	-0.224
p Value	0.036	0.033	0.011	0.032
SDNNi				
r	-0.220	-0.223	-0.276	-0.239
p Value	0.028	0.026	0.006	0.022
Triangle index				
r	-0.332	-0.351	-0.339	-0.259
p Value	0.001	<0.001	0.001	0.013
Frequency domain				
VLF				
r	-0.201	-0.232	-0.231	-0.282
p Value	0.046	0.021	0.024	0.007
LF				
r	—	—	-0.233	-0.226
p Value	—	—	0.023	0.031

Abbreviations as in Tables II and III.

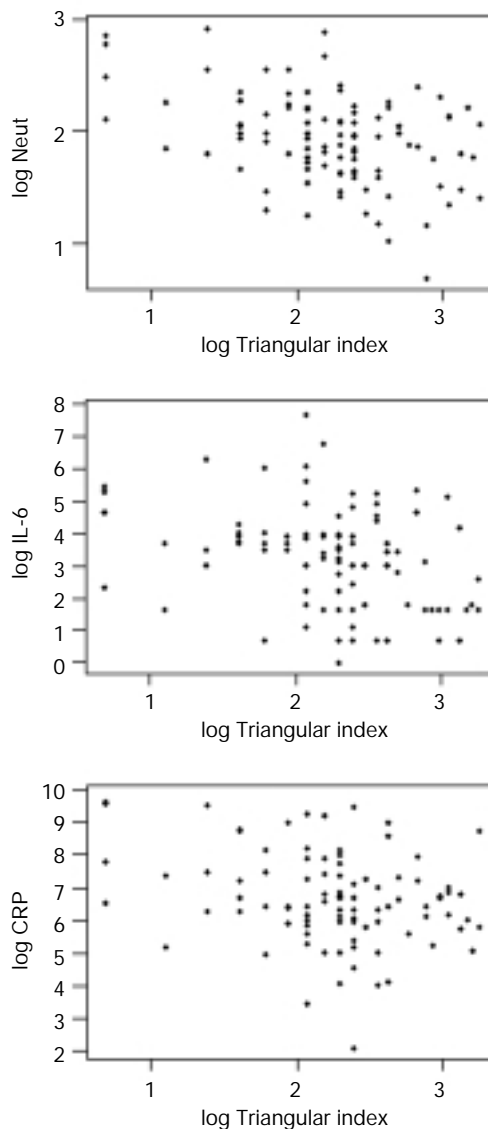


FIG. 1 Principal correlations between triangular index and neutrophil (Neut) count ($r = -0.351$, $p < 0.001$), IL-6 ($r = -0.339$, $p = 0.001$) and CRP ($r = -0.259$, $p = 0.013$). IL = interleukin, CRP = C-reactive protein.

trophil count, with triangular index accounting for 17% and gender accounting for 5% of the total variance; however, there were no statistically independent predictors of IL-6 or CRP in our sample. Analysis of the follow-up data revealed no independent predictors of each of the inflammatory variables ($p = \text{NS}$, data not shown).

Discussion

In this study we found a modest association between increased HR (reduced RR interval) and reduced HRV and circulating biomarker measures of systemic inflammation in patients with acute coronary disease. The modest association

between acute neutrophil count and initial triangular index remained significant even after correction by multivariate regression of potentially confounding factors.

Heart rate variability gives an estimate of cardiac autonomic tone, and reduced HRV in coronary disease reflects autonomic imbalance favoring sympathetic tone with or without a parallel reduction in parasympathetic activity.^{24, 25} The relationship between HRV and inflammation was apparent during the acute phase, and while this association remained at 4-month follow-up this was weak and did not persist on multivariate analysis. The associations were observed largely among HRV indices regarded as markers of cardiac sympathetic activity⁸ (LF power band, SDNN, SDNNi and triangular index), suggesting that the inflammatory response in acute coronary events may be associated with sympathetic activation rather than parasympathetic withdrawal.

Several studies have pointed to such an association between HRV and inflammation in a variety of settings, as well as to associations between HRV and total WCC or hs-CRP in healthy individuals.^{15, 25} In decompensated systolic heart failure, there is an inverse association between HRV and circulating levels of IL-6.²⁶ A population-based study in Japan has supported these observations with a strong correlation between basal HR and total WCC.²⁷ In healthy individuals subject to mental stress, a similar association was made between HR response and cytokine response.¹⁷

The present study extends these associations to patients with ACS. The systemic and localized inflammatory component of this state is well accepted.²⁻⁴ Our data suggest that this may in part be mediated by changes in autonomic tone. Both the marrow and lymphoreticular systems are responsive to circulating catecholamines and have direct innervation by the autonomic nervous system.^{28, 29} Sympathectomy reduces markers of inflammation,³⁰ and thus increased sympathetic activity and/or reduced parasympathetic activity during ACS may facilitate an inflammatory reaction. Conversely, inflammatory markers (in particular IL-6) may be an important direct central neuromodulator, and systemic changes may alter autonomic balance.³¹ It is interesting that the administration of exogenous IL-6 in healthy volunteers did result in modest increases in HR at 90 min, raising the possibility that IL-6 may modulate sympathetic activation or cardiac beta-receptor sensitivity directly.³²

Our data suggest that leukocyte count is the only independent association of triangular index. Leukocytes may be involved in the early stages of atherosclerosis and may be a potential source of proinflammatory cytokines, such as tumor necrosis factor- α , IL-1, and IL-6 within the atheromatous plaque.^{33, 34} Interleukin-6 is also the main stimulant for CRP production by the liver.³⁵ As such the leukocytosis observed in early ACS may mediate the production and release of secondary inflammatory markers such as IL-6 and CRP.

Study Limitations

A major limitation of our study is its cross-sectional observational design over two separate time periods. This does not

allow a secure causal relationship to be inferred. However, we believe that the data suggest that prospective experiments should address which is the initiating and which the controlling factor in this close relationship. Due to the conventional hierarchy of control systems, the role of autonomic tone should be closely scrutinized.

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