Association between C-reactive protein and generalized anxiety disorder in stable coronary heart disease patients

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Aims
Comprehensive evaluation of major depressive disorder (MDD), anxiety disorder, and MDD in conjunction with anxiety disorder in stable coronary heart disease (CHD) patients, including cardiac biomarkers such as C-reactive protein (CRP), troponin T (TnT), and amino-terminal pro-B-type brain natriuretic peptide (NT-proBNP).

Methods and results
Cross-sectional study of a consecutive series of 120 stable CHD outpatients (n = 30 with MDD, n = 30 with anxiety disorder, n = 30 with MDD and anxiety disorder, n = 30 with no psychiatric disorder). Psychiatric diagnoses were established by using the structured clinical interview for DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV). Binomial logistic regression analyses using cut-off scores of biomarkers as dependent variables showed associations between CRP and generalized anxiety disorder (GAD) (P = 0.04), and education (P = 0.004), whereas MDD, and MDD and anxiety disorder did not reach the significance level. TnT showed relationships with hyperlipidaemia (P = 0.009), history of obesity or overweight (P = 0.04), and education (P = 0.04). NT-proBNP was associated with type II diabetes (P = 0.005).

Conclusion
After adjusting for relevant demographic, medical, and psychiatric co-variables, CRP was associated with GAD.

Keywords
C-reactive protein • Generalized anxiety disorder • Stable CHD

Introduction
Previous studies underlined an association between C-reactive protein (CRP) and depression in otherwise healthy adults as well as in coronary heart disease (CHD) patients,1–4 potentially indicating a biological link between depression and cardiovascular morbidity and mortality.

However, previous research seemed to mainly focus on depression and biomarkers in patients with and without CHD, not taking into account anxiety and specific anxiety disorders. Several, mostly epidemiological studies underlined the impact of anxiety on CHD incidence and re-occurrence. In particular, generalized anxiety, generalized anxiety disorder (GAD), and phobic anxiety showed significant associations with CHD.5–11 The results on the relationship between anxiety and CHD seem more mixed as compared with the relationship between depression and CHD, according to specific anxiety disorders.

In this study, we aimed for a comprehensive evaluation of major depressive disorder (MDD), anxiety disorder, and MDD in conjunction with anxiety disorder in stable CHD patients, including meticulous psychiatric assessment and cardiac biomarkers such as CRP, a marker for inflammation, troponin T (TnT), a marker for myocardial injury, and amino-terminal pro-B-type brain natriuretic peptide (NT-proBNP), a marker for left ventricular dysfunction. We hypothesized (i) that significant associations would exist between MDD and one or more cardiac biomarkers in stable CHD patients, that (ii) significant associations would exist between one or more anxiety disorders and these...
biomarkers as well, and (iii) in the sense of a potentiating effect, significant relationships would exist between MDD in conjunction with anxiety disorder and one or more biomarkers studied.

Methods

Design and setting
We performed a cross-sectional study of a consecutive series of 120 stable CHD outpatients ($n = 30$ with MDD, $n = 30$ with anxiety disorder, and $n = 30$ with no psychiatric disorder, used as Control group) between July 2005 and June 2006 at the Outpatient Cardiology Clinic of the Massachusetts General Hospital (MGH). The Institutional Review Board (IRB) of the MGH approved a power analysis included in the detailed research protocol in considering the sample size of the four psychiatric diagnostic groups of stable CHD patients. Stable CHD outpatients of one cardiologist (J.L.J.) were included in the study. Psychiatric consultation and treatment referral was offered in accordance with psychiatric diagnoses determined by the interview.

Recruitment procedure
The study recruitment overture was made at the front desk at the Outpatient Cardiology Clinic in the form of a questionnaire, which asked outpatients in an anonymous fashion whether they would be interested in being enrolled in the study. If patients agreed to participate and met study criteria, the cardiologist requested their inclusion before the cardiac outpatient visit. The interviews were conducted immediately before or immediately after the outpatient visit. Patients who were included in the study signed a detailed written informed consent. No incentives or reimbursements were given to the patients.

Stable CHD patients who met study inclusion criteria and who gave informed consent were consecutively included in the study, until the sample size of $n = 30$ in each group has been reached. Accordingly, we had to include more stable CHD patients in two psychiatric diagnostic groups, in the group of stable CHD patients with no psychiatric disorder ($n = 42$), and in the group with anxiety disorder ($n = 43$), since the psychiatric diagnostic interview took place after inclusion of patients. According to the research study protocol, we only used the sample sizes of $n = 30$ for each psychiatric diagnostic group for statistical analyses. The continuous presence of the first author (B.B.) might have contributed to the high enrolment rate of this study, since there were no waiting or scheduling procedures. In sum, $n = 49$ stable CHD patients refused study inclusion because of blood collection ($n = 15$), lack of time for the psychiatric diagnostic interview ($n = 16$), or feeling uncomfortable with inclusion in a study ($n = 18$).

Medical inclusion and exclusion criteria
There was no restriction regarding the age of the participants in the study. The medical inclusion criteria for stable CHD outpatients were: (i) CHD diagnosed via a positive stress test (included treadmill stress test, infusion stress test, stress echo, as well as thallium or nuclear imaging testing), or (ii) history of documented myocardial infarction (M) by electrocardiogram (ECG) and creatine phosphokinase isoenzymes/troponins, or (iii) coronary atherosclerosis (documented by coronary angiography), with or without revascularization procedures including percutaneous coronary intervention or coronary artery bypass graft surgery.

The medical exclusion criteria were: (i) active unstable angina, (ii) active congestive heart failure with Class IV symptoms by New York Heart Association Symptom Severity estimates, (iii) severe co-morbid cardiac disease, e.g. advanced valvular heart disease, and (iv) prior valve replacement therapy. We also excluded subjects with unstable medical disorders (unstable neurological, endocrine, pulmonary, gastrointestinal, hepatic, renal, immunological or haematological disease, organic brain disease, or cancer) as determined by history, physical, ECG, and laboratory examination.

Cardiac biomarker assessment
At the time of enrolment, we obtained a sample of blood, which was tested for CRP, using a high-sensitivity assay (Dade-Behring, Inc., Newark, DE, USA). This assay is a turbidometric immunoassay with a dynamic range of 0.05–25.0 mg/L. Generally speaking, <1 mg/L is considered as ‘low risk’ for acute MI, while >3 mg/L is considered ‘high risk’. In addition, we determined concentrations of TnT using a standard immunoassay (Roche Diagnostics, Inc., Indianapolis, IN, USA). In healthy reference populations, the 99th percentile for this assay is considered to be <0.01 ng/mL, while the cut-point yielding acceptable analytic precision is 0.03 ng/mL. However, the range for TnT is not well established for ambulatory cardiac patients. Lastly, NT-proBNP concentrations were determined by using standard immunochemical methods (Roche Diagnostics, Inc., Indianapolis, IN, USA). The lower limit for detectable with this assay is >5 ng/L. Normal outpatient reference ranges for NT-proBNP are <125 ng/L for patients <75 years, and <450 ng/L for patients 75+ years.

Psychiatric assessment
Psychiatric diagnoses were established by using the structured clinical interview for DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV). Psychiatric assessment was performed on Axis I (clinical disorders), Axis III (general medical conditions), Axis IV (psychosocial and environmental problems), and Axis V (global assessment of functioning). The first author (B.B.) conducted all structured clinical interviews, and was blinded to all psychiatric information about the cardiac patients before the study interview.

Psychiatric exclusion criteria were significant risk of suicide or homicide, or the presence of any one of the following DSM-IV diagnoses: (i) substance use disorders, including alcohol, active within the last 3 months, (ii) schizophrenia, (iii) delusional disorder, (iv) psychotic disorders not elsewhere classified, (v) bipolar disorder, (vi) delirium, and (vii) dementia. In particular, actively suicidal and psychiatric patients were not included in the study, since the IRB of the MGH discourages recruitment of subjects who are clinically unstable, e.g. actively suicidal or psychotic, in studies that offer no direct treatment benefit.

Data analyses
Concentrations of biomarkers within the four psychiatric diagnostic groups were generated using means plus standard deviations (SD), and comparisons between groups were performed by using analysis of variance. We used the Bonferroni correction for multiple comparisons of means (significance level alpha = 0.008).

Binomial logistic regression analyses were performed to separately assess the relationship between the biomarkers CRP, TnT, and NT-proBNP (used as categorical dependent variables) and the independent variables (categorical and continuous variables). The cardiac biomarkers used as categorical dependent variables had the following cut-off scores: CRP >3 mg/L ($yes = 1, no = 0$), TnT >0.01 ng/mL ($yes = 1, no = 0$), and NT-proBNP >125 ng/L for patients <75 years, and >450 ng/L for patients 75+ years ($yes = 1, no = 0$).

Independent variables included age, education, MDD, anxiety disorder, MDD in conjunction with anxiety disorder, past smoking, obesity, current weight or obesity, past smoking
status, current smoking status, type II diabetes mellitus, hypertension, and hyperlipidaemia.

Data collection and calculations were performed using the SPSS 15.0 for Windows program.18 We used the significance level of alpha = 0.05, and included the Hosmer and Lemeshow goodness of fit test for model assessment.

Results

Demographic characteristics

Age and SD in the four groups of MDD, anxiety, MDD in conjunction with anxiety, and no psychiatric disorder respectively were 67 years (SD = 11), 68 years (SD = 8), 68.5 years (SD = 9.5), and 68 years (SD = 13). In the four groups of MDD, anxiety, MDD in conjunction with anxiety, and no psychiatric disorder, respectively, 67% (n = 20), 67% (n = 20), 60% (n = 18), and 80% (n = 24) were married. Table 1 summarizes demographic and medical characteristics of the study participants in all four groups including age, gender, marital status, children, ethnicity, educational attainment, family history of CHD, past and current smoking status, type II diabetes mellitus, hypertension, and hyperlipidaemia.

Psychiatric diagnoses, psychopharmacological treatment, psychosocial and environmental problems, and global assessment of functioning

Of 30 stable CHD outpatients with MDD (n = 30), 11 patients had a single past major depressive episode, 11 patients had recurrent episodes in the past, and eight patients had recurrent major depressive episodes with a current major depression. Of stable CHD patients with anxiety disorder (n = 30), four patients had GAD, one patient had posttraumatic stress disorder (PTSD), seven had social phobia, nine had specific anxiety disorder, one had panic disorder with agoraphobia, two had agoraphobia alone, and 11 patients had anxiety disorder not otherwise specified, in all cases heightened generalized anxiety which did not fulfil all clinical criteria for GAD. If a patient fulfilled all clinical criteria for more than one anxiety disorder, then both anxiety diagnoses were given. In the group of patients with MDD in conjunction with anxiety disorder (n = 30), 11 patients had GAD, nine had PTSD, four patients had social phobia, 12 had specific phobia, four had agoraphobia, and four had anxiety disorder not otherwise specified.

Of stable CHD patients with MDD, 67% (n = 20) had past antidepressant treatment, whereas 50% (n = 15) had current antidepressant medication. Of CHD patients with MDD in conjunction with anxiety disorder, 67% (n = 20) had past antidepressant treatment, 53% (n = 16) had current antidepressant medication, 57% (n = 17) had past benzodiazepine treatment, and 50% (n = 15) had present benzodiazepine treatment. Present SSRI (serotonin reuptake inhibitor) treatment was mentioned by 47% (n = 14) of patients with MDD, and 47% (n = 14) of patients with MDD in conjunction with anxiety disorder.

CHD patients with MDD in conjunction with anxiety disorder most often had the problems with the primary support group, e.g. recent divorce (70%, n = 21), and occupational problems, e.g. unemployment (33%, n = 10). Not surprisingly, stable CHD patients with no psychiatric disorder had the highest mean score of the global assessment of functioning score (72, SD = 8.4), whereas CHD patients with MDD, anxiety disorder, and MDD in conjunction with anxiety disorder, respectively, had the mean scores of 63.4 (SD = 6.2), 65.5 (SD = 5), and 56.3 (SD = 6.5).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic characteristics and medical background of stable coronary heart disease (CHD) outpatients with major depressive disorder (MDD) (n = 30), anxiety disorder (n = 30), MDD in conjunction with anxiety disorder (n = 30), and without psychiatric disorder (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable CHD patients</td>
<td>MDD (n = 30)</td>
</tr>
<tr>
<td>Age, mean ± standard deviation</td>
<td>67.2 ± 11.2</td>
</tr>
<tr>
<td>Gender [male] n (%)</td>
<td>24 (80)</td>
</tr>
<tr>
<td>Marital status [married] n (%)</td>
<td>20 (67)</td>
</tr>
<tr>
<td>Children, mean ± standard deviation</td>
<td>2.3 ± 1.4</td>
</tr>
<tr>
<td>Caucasians, n (%)</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Completed high school, n (%)</td>
<td>10 (33)</td>
</tr>
<tr>
<td>Completed college, 4 years, n (%)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Family history of CHD, n (%)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Past overweight or obesity, n (%)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Current overweight or obesity, n (%)</td>
<td>8 (27)</td>
</tr>
<tr>
<td>Smoking history, n (%)</td>
<td>19 (63)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Type II diabetes mellitus, n (%)</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>19 (63)</td>
</tr>
<tr>
<td>Hyperlipidaemia, n (%)</td>
<td>24 (80)</td>
</tr>
</tbody>
</table>
Levels of C-reactive protein, troponin T, and amino-terminal pro-B-type brain natriuretic peptide in coronary heart disease patients with major depressive disorder, anxiety disorder, major depressive disorder in conjunction with anxiety disorder, and no psychiatric disorder

Means of CRP were generally higher in CHD patients with MDD (8.5 ± 12 mg/L), MDD in conjunction with anxiety disorder (7.6 ± 11.4 mg/L), and anxiety disorder (6.7 ± 10 mg/L), but none of these differences were statistically significant by using the Bonferroni correction for multiple comparisons of means (significance level alpha = 0.008). With regard to markers of necrosis or haemodynamic stress, the highest means of TnT (0.01 ± 0.03 ng/mL) were found in the group of CHD patients with MDD in conjunction with anxiety disorder, who also had higher mean levels of NT-proBNP (1315 ± 3173 ng/L) as compared with the other groups. Table 2 summarizes all results.

Associations between C-reactive protein, troponin T, and amino-terminal pro-B-type brain natriuretic peptide and major depressive disorder, anxiety disorder, and major depressive disorder in conjunction with anxiety disorder

Table 3 shows the results of binomial logistic regression analyses separately using CRP, TnT, and NT-proBNP as dependent categorical variables, with cut-off values of >3 mg/L (CRP), >0.01 ng/mL (TnT), and >125 ng/L for age <75 years and >450 ng/L for age 75+ years. The cut-off scores for NT-proBNP for both age groups have been integrated in the indicator variable.

Independent variables included age, education, MDD, anxiety disorder, MDD in conjunction with anxiety disorder, no psychiatric disorder, past overweight or obesity, current overweight or obesity, smoking history, current smoking status, type II diabetes mellitus, hypertension, and hyperlipidaemia. Since the association between CRP and the more mixed group of anxiety disorders (n = 30) came close to a significant level (P = 0.06), the specific anxiety disorder GAD (n = 15) has been entered as additional independent variable for CRP, after removing the more mixed anxiety disorder group from the logistic regression model. All independent variables, all B coefficients and P-values were indicated, with a significance level alpha = 0.05.

For each of the four logistic regression models, a Hosmer and Lemeshow goodness of fit test assessed adequate fit, and showed no significance (logistic regression with CRP as dependent categorical variable P = 0.4, model with CRP as dependent variable and including GAD as independent variable, while removing the variable of the anxiety disorders group P = 0.7, and P = 0.9 for the models with TnT as well as NT-proBNP as categorical dependent variable).

The binomial logistic regression analyses showed a significant association between CRP levels and GAD (P = 0.04), and education (P = 0.004), whereas MDD and MDD in conjunction with anxiety disorder did not reach the significance level. TnT levels showed a significant relationship with hyperlipidaemia (P = 0.009), a history of obesity or overweight (P = 0.04), and education (P = 0.04). NT-proBNP levels were significantly associated with type II diabetes (P = 0.005).

Discussion

Our findings show a significant association between CRP and GAD in stable CHD patients. Stable CHD patients included in our study, who fulfilled the clinical criteria for GAD, belonged to the group of anxiety disorders and the group of MDD in conjunction with anxiety disorder. According to the DSM-IV, the psychiatric diagnostic construct of GAD is defined by specific symptoms and a concrete symptom count, as compared with the psychological construct of generalized anxiety, which is not strictly defined. In particular, the essential feature of GAD is excessive anxiety and worry, occurring more days than not for a period of at least 6 months, about a number of events or activities. Although individuals with GAD may not always identify the worries as ‘excessive’, they report subjective distress due to constant worry, have difficulty controlling the worry, or experience related impairment in social, occupational, or other important areas of functioning.

Table 2 Means and standard deviations (SD) of C-reactive protein (CRP) (mg/L), troponin T (TnT) (ng/mL), and amino-terminal pro-B-type brain natriuretic peptide (NT-proBNP) (ng/L) in stable coronary heart disease (CHD) outpatients with major depressive disorder (MDD) (n = 30), anxiety disorder (n = 30), MDD in conjunction with anxiety disorder (n = 30), and with no psychiatric disorder (n = 30)

<table>
<thead>
<tr>
<th>Stable CHD patients n = 120</th>
<th>CRP mean (SD) &gt;3 mg/L, n = 58</th>
<th>TnT mean (SD) &gt;0.01 ng/mL, n = 12</th>
<th>NT-proBNP mean (SD) &gt;125 ng/L age &lt;75 years and &gt;450 ng/L age 75+ years, n = 78</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD, n = 30</td>
<td>8.5 (12)</td>
<td>0.002 (0.006)</td>
<td>447 (637)</td>
</tr>
<tr>
<td>Anxiety disorder, n = 30</td>
<td>6.7 (10)</td>
<td>0.002 (0.006)</td>
<td>375 (416)</td>
</tr>
<tr>
<td>MDD and anxiety disorder, n = 30</td>
<td>7.6 (11.4)</td>
<td>0.01 (0.03)</td>
<td>1315 (3173)</td>
</tr>
<tr>
<td>No psychiatric disorder, n = 30</td>
<td>5.1 (4.4)</td>
<td>0.007 (0.02)</td>
<td>643 (1117)</td>
</tr>
</tbody>
</table>

Reference levels: CRP >3 mg/L, TnT >0.01 ng/mL, NT-proBNP >125 ng/L for age <75 years, and >450 ng/L for age 75+ years. Significance level alpha = 0.008 with Bonferroni correction for multiple comparisons of means.
The intensity, duration, or frequency of the anxiety and worry is far out of proportion to the actual likelihood or impact of the feared event.

We hypothesized that one or more anxiety disorders would have a significant relationship with one or more cardiac biomarkers. This finding is in line with previous research underlining the impact of generalized anxiety symptoms on CHD re-occurrence. In particular, Grace et al. underscored that over and above the effects of depressive symptoms, generalized anxiety symptoms appeared to have a negative effect on self-reported outcome following an ischaemic coronary event, and the authors concluded that symptoms of heightened anxiety are under-recognized and under-treated. Accordingly, high prevalence of depressive as well as specific anxiety disorders has been detected in stable CHD patients.

In contrast to previous research, we did not find significant associations of MDD with the cardiac biomarkers included in the analyses, specifically with CRP. In addition, surprisingly, a hypothesized potentiating effect of MDD in conjunction with anxiety disorder in relationship to the cardiac biomarkers studied could not be confirmed by our analyses. On one hand, this finding might be related to the inclusion of a distinct anxiety disorders group and specific anxiety disorders in our study. On the other hand, our results need to be replicated in further studies with larger sample sizes. In particular, future research needs to shed light on a possible potentiating effect of heightened levels of anxiety and specific anxiety disorders in conjunction with MDD in larger groups of stable CHD patients.

The biomarkers studied, CRP, TnT, and NT-proBNP, have each been shown to be of use for stratifying risk for future cardiac events among ambulatory patients such as the ones enrolled in this study. In particular, Ridker et al. underlined the use of CRP levels for monitoring cardiovascular risk, showing that CHD patients after statin therapy with low CRP levels had better clinical outcome as compared with those with higher CRP levels. With respect to TnT, in a large population study, detectable levels were correlated with clinical variables or structural abnormalities on cardiac imaging. Lastly, NT-proBNP concentrations have been found to be powerfully prognostic for CHD events among subjects with stable CHD, independent of left ventricular dysfunction.

The significance of our study is two-fold. First, to our knowledge, to date, our study is the most comprehensive evaluation of associations between MDD, anxiety disorders, and MDD in conjunction with anxiety disorder and cardiac biomarkers in ambulatory CHD patients. Psychiatric variables were determined by the use of the DSM-IV clinical nomenclature, and not a questionnaire cut-off or a construct, such as e.g. psychological distress, that is not easily translated into the DSM code. Secondly, our results suggest that the current main focus on depressive disorders and cardiac biomarkers in CHD patients should be widened to include anxiety disorders.

**Methodological limitations**

First, the inclusion of stable CHD outpatients of one cardiologist represents a possible selection bias, although homogeneity in treatment from a single practitioner would be expected to reduce the confounding effects of variable treatment patterns. Secondly, CHD patients with psychiatric disorders are higher users of clinics, and hence they are more likely to be represented in this study. Thirdly,
since most of the patients included in this study are Caucasians, the findings cannot be necessarily generalized for other ethnicities. Fourthly, we aimed to present comparable groups of CHD patients considering gender. However, for allowing a gender specific analysis, future research needs to include male and female CHD patients in distinct groups. Fifthly, since the mean age of study participants in the groups is 67–68 years, we cannot exclude a potential impact of unmeasured general medical conditions on the biomarker levels. In addition, our findings are limited by the small sample size of the four groups. The inclusion criteria for stable CHD are very broad, and resulting bias cannot be excluded. Finally, the value of the results is limited by the fact that the material of this study represents stable CHD outpatients, and, therefore, the results cannot be generalized for all CHD patients.

In conclusion, our findings show a significant association between CRP and GAD, despite adjusting the models for MDD, MDD in conjunction with anxiety disorder, a Control group with no psychiatric disorder, and demographic and medical variables known to influence the biomarkers studied. Accordingly, our findings suggest that the current focus on depression with respect to biomarkers and CHD outcomes should be broadened to include anxiety disorders as well.

**Conflict of interest:** None declared.

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**References**


