Relation of Depressive Symptoms to C-Reactive Protein and Pathogen Burden (Cytomegalovirus, Herpes Simplex Virus, Epstein-Barr Virus) in Patients With Earlier Acute Coronary Syndromes

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Despite mounting evidence that depressive symptoms increase the risk of morbidity and mortality in patients who have coronary artery disease, little is known about the biologic mechanisms that underlie this association. This study examined whether depressive symptoms are associated with markers of infection and inflammation that have been implicated in the pathogenesis of coronary artery disease. Sixty-five patients who were recovering from an acute coronary syndrome were enrolled (63% men; mean age 61 years, 90% white). Depressive symptoms were assessed through self-report and observer ratings; the inflammatory molecules C-reactive protein, interleukin-6, and tumor necrosis factor-α were measured in serum, as were antibody titers to 3 latent viruses associated with atherosclerosis. Patients who had more severe depressive symptoms exhibited higher levels of C-reactive protein ($r = 0.27, p = 0.03$) and higher rates of seropositivity to the latent viruses ($r = 0.41, p = 0.001$). These effects were large in magnitude: patients in the highest tertile of the depression distribution had C-reactive protein levels >50% higher than did patients in the middle and lowest tertiles; they also were 2 times as likely to show evidence of infection with all 3 latent viruses. Disparities in the extent, severity, or management of cardiac disease were not responsible for these associations. These findings provide evidence that depressive symptoms are associated with increases in C-reactive protein and pathogen burden in patients who have coronary artery disease. In doing so, they highlight a mechanism through which depressive symptoms might foster morbidity and mortality among patients who have cardiac disease.

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epression is a common feature of coronary artery disease (CAD). About 20% of patients who have CAD meet diagnostic criteria for major depression, and an even larger percentage develops difficulties with mood that are below the diagnostic threshold. Among patients who recover from an acute coronary syndrome (ACS), the presence of depressive symptoms is associated with a twofold to fourfold increase in cardiac morbidity and mortality rates, independent of disease severity, treatment regimen, and standard risk factors. This is true even when the symptoms are of a subclinical nature, i.e., not sufficiently persistent or disabling to merit a diagnosis of major depression. Despite this robust pattern of findings, little is known about the underlying mechanisms. There has been much speculation that depression accelerates CAD progression by fostering long-term infections and activating inflammatory processes. We tested this hypothesis by examining whether depressive symptoms, even of a subclinical nature, are associated with infectious and inflammatory processes implicated in the pathogenesis of CAD.

METHODOLOGY

Patients: The sample consisted of 65 patients who had been recruited from cardiology practices at the Barnes-Jewish Hospital at Washington University School of Medicine (St. Louis, Missouri). All patients were recovering from an ACS and had myocardial infarction, bypass surgery, or coronary angioplasty ≥3 months previously. Patients who agreed to participate were contacted and, provided they were stable, took part in an eligibility screening during which a nurse collected medical history. Candidates were excluded if they had (1) severe cognitive impairment, excessive substance/alcohol use, or psychiatric conditions other than depression or anxiety; (2) severe co-morbid medical conditions including advanced malignancy, diabetic neuropathy, pulmonary disease, or sleep disorder; or (3) valvular heart disease, active congestive heart failure, or an implantable pacemaker. Patients who met eligibility criteria were scheduled...
for a 2-night stay at the Washington University Sleep Medicine Center as part of a larger project. The protocol was approved by the institutional review board of Washington University School of Medicine.

Depressive symptoms: Depressive symptoms were assessed by self-report and observer ratings. During their visit to the sleep laboratory, patients completed the Beck Depression Inventory, a widely used 21-item measure of depressive symptoms. Observer ratings were obtained at the eligibility screening, when a nurse administered the 17-item Hamilton Rating Scale for Depression as part of a diagnostic interview. These measurements showed high levels of internal consistency in this sample (α = 0.94, 0.89). Because scores on the Beck Depression Inventory and Hamilton Rating Scale for Depression were strongly related (r = 0.86, p < 0.001), we z-transformed and averaged the scores to form an index that reflected the severity of depressive symptoms. This procedure diminishes random error and therefore boosts statistical power. Although the results presented in the following were produced with this composite index of symptoms, virtually identical findings emerge when the Beck Depression Inventory and Hamilton Rating Scale for Depression are used as stand-alone measures.

Inflammatory and infectious markers: We assessed the expression of 3 inflammatory molecules, C-reactive protein (CRP), interleukin-6, and tumor necrosis factor-α, implicated in the development and progression of CAD. Blood was drawn through antecubital venipuncture at 1 hour of awakening at the Sleep Medicine Center. After the blood had been centrifuged for 25 minutes at 1,000g, the serum was aspirated, divided into aliquots, and frozen at −70°C. At the end of the study, samples were thawed and run in a single batch. CRP was quantitated by high-sensitivity immunnoassay on a BN-100 nephelometer (Dade Behring, Deerfield, Illinois). This assay has a sensitivity of 0.175 mg/L and intra- and interassay coefficients of variation of <10%. Interleukin-6 and tumor necrosis factor-α were measured with a commercially available immunnoassay (Linco Research, St. Louis, Missouri) on a Luminex 100. These assays have a sensitivity of <3.2 pg/ml and intra- and inter-assay coefficients of variation of <12%.

To evaluate latent infections that might accelerate CAD, we used thawed serum to quantify antibodies against 3 pathogens. Immunoglobulin-G antibodies to cytomegalovirus and herpes simplex virus were measured with commercially available immunnoassays (cytomegalovirus immunnoassay, Vidas; herpes simplex virus immunnoassay, Dade Behring) on a Vitex Immunodiagnostic System (bioMerieux Corp., Durham, North Carolina). Immunoglobulin-G antibodies to Epstein-Barr virus were quantified with a commercially available immunofluorescence technique (Merilflour, Meridian Diagnostics, Cincinnati, Ohio). Intra-assay coefficients of variation for all assays were <10%. Patients were categorized as having recently active infections when they showed values of ≥6 Bodansky’s units for cytomegalovirus, ≥10 Bodansky’s units for Epstein-Barr virus, and net absorbance ≥0.20 for herpes simplex virus. Because the total number of latent infections is a stronger determinant of inflammation than any single infection, we created a “pathogen burden” score for each patient that reflected the number of organisms for which that patient was seropositive.

Demographic and medical characteristics: Relations between depressive symptoms and biologic outcomes can arise from residual confounding with demographic characteristics, medical history, disease severity, or treatment received. To examine this possibility, we gathered data on these variables by chart reviews and interviews about medical history. Because inflammatory molecules can be increased due to acute infection, a complete blood count was done on blood drawn during visits to the Sleep Medicine Center. One patient who showed evidence of active infection was excluded from the analyses.

Statistical analyses: Because markers of inflammation and pathogen burden were distributed in a non-normal fashion, rank-order correlations were used to quantify relations between depression and outcomes. To clarify the nature of significant associations, the sample was later divided into 3 evenly sized groups that corresponded to patients who did not have depressive symptoms (Beck Depression Inventory median score 1, Hamilton Rating Scale for Depression median score 1) and those who had symptoms of mildly severe depression (median scores 14 and 10) or moderately severe depression (median scores 25 and 19). To evaluate disparities between these groups, Mann-Whitney U tests (for continuous outcomes) and Kendall’s τ (for categorical outcomes) were used. Two-tailed tests of significance were employed. All data are presented as mean ± SEM, unless otherwise noted.

RESULTS

Demographic, psychiatric, and cardiovascular characteristics of patients are presented in Table 1.

Depressive symptoms and markers of inflammation and infection: To the extent that they had more severe depressive symptoms, patients showed higher levels of CRP (r = 0.27, p = 0.03). Further analyses showed that patients in the highest symptom tertile had significantly more CRP than did patients in the middle tertile (z = 2.0, p = 0.04) and lowest tertile (z = 2.2, p = 0.03). The latter groups did not differ from each other (z = 0.8, p = 0.78). The median CRP values for the highest, middle, and lowest tertiles were 3.6, 2.4, and 2.4 mg/L, respectively. Thus, patients who had moderately severe symptoms had CRP levels >50% than did patients who had mild or negligible symptoms (Figure 1). Depressive symptoms were unrelated to levels of interleukin-6 (r = 0.05, p = 0.72) and tumor necrosis factor-α (r = 0.17, p = 0.21). There was no association between psychiatric diagnosis (major depression vs minor depression vs no diagnosis) and levels of inflammatory molecules (p > 0.37).

There was also a relation between depressive symptoms and pathogen burden. To the extent that they endorsed more severe depressive symptoms, patients were seropositive for a larger number of latent viruses (r...
Further analyses showed that 100% of patients in the highest symptom tertile were seropositive to all 3 viruses (Figure 2). This pattern was much less common among patients in the middle tertile (55%; p = 0.001) and lowest tertile (43%; p = 0.001). In terms of pathogens (Figure 3), patients in the highest tertile were more likely to be seropositive for cytomegalovirus than were patients in the middle tertile (Kendall’s τ = 0.008) and lowest tertile (p = 0.001). They also were more likely to be seropositive for herpes simplex virus than were patients in the lowest tertile (p = 0.005). The 3 groups were similar with respect to Epstein-Barr virus (p > 0.30 for all groups). There were no disparities in pathogen burden according to the presence of clinical depression (p > 0.10).

The clustering of pathogen burden and systemic inflammation confers a potent risk of morbidity and mortality. To determine whether depressive symptoms are associated with this pattern, we classified patients as clustered if they were seropositive to all 3 viruses assessed. This pattern was much less common among patients in the middle tertile (55%; p = 0.001) and lowest tertile (43%; p = 0.001). In terms of pathogens (Figure 3), patients in the highest tertile were more likely to be seropositive for cytomegalovirus than were patients in the middle tertile (Kendall’s τ = 0.008) and lowest tertile (p = 0.001). They also were more likely to be seropositive for herpes simplex virus than were patients in the lowest tertile (p = 0.005). The 3 groups were similar with respect to Epstein-Barr virus (p > 0.30 for all groups). There were no disparities in pathogen burden according to the presence of clinical depression (p > 0.10).

To evaluate alternative explanations for these findings, we conducted analyses to control for potential confounders. Relations between depressive symptoms and biologic outcomes were independent of demographics, such as age, gender, ethnicity, and marital status (p < 0.05). They also were independent of medical history (current smoker, obesity, sleep apnea, diabetes, hypertension, angina, myocardial infarction, congestive heart failure, atrial fibrillation, peripheral vascular disease, and stroke) and disease severity as indexed by Killip’s class and time since infarction (p < 0.04). Controlling for receipt of angioplasty, coronary bypass surgery, age, gender, ethnicity, and marital status (p < 0.05). They also were independent of medical history (current smoker, obesity, sleep apnea, diabetes, hypertension, angina, myocardial infarction, congestive heart failure, atrial fibrillation, peripheral vascular disease, and stroke) and disease severity as indexed by Killip’s class and time since infarction (p < 0.04). Controlling for receipt of angioplasty, coronary bypass surgery,
angiotensin-converting enzyme inhibitors, antiarrhythmic medication, antidepressants, anticoagulants, aspirin, β blockers, calcium channel blockers, diuretics, digitalis, nitrates, statins, and vasodilators did not attenuate the associations (p <0.05). Collectively, these findings indicate that disparities in demographics or the extent, severity, and management of disease are responsible for little (if any) of the systemic inflammation or pathogen burden evident in patients who have depressive symptoms.

**DISCUSSION**

We found evidence of a graded relation between depressive symptoms and systemic inflammation in patients who were recovering from an ACS. Follow-up analyses showed that this association was strongest in patients who had depressive symptoms in the highest tertile. These patients had CRP levels >50% than did those in the middle or lowest tertile. This disparity was not simply a result of clinical depression because no association emerged between diagnosis and inflammation. Thus, high levels of depressive symptoms, regardless of where they are with respect to the diagnostic threshold, are associated with a marked increase in CRP. Although the clinical significance of this finding is unclear, CRP predicts mortality in a roughly dose-response fashion in patients who have cardiac disease. Patients who have CRP levels ≥3.0 mg/L, like those in the highest tertile of depressive symptoms, represent a high-risk group for adverse cardiac outcomes.

These findings contribute to a growing corpus of evidence that links depression with inflammation. In healthy adults there is a graded, linear relation between depressive symptoms and inflammatory molecules and evidence that clinical depression is accompanied by marked increases in CRP (41%) and interleukin-6 (54%). Similar evidence is emerging in patients who have cardiac disease. One recent study of patients who had an ACS-associated major depression with increased levels of soluble intercellular adhesion molecule-1. Another associated exhaustion, which is common in depression, with increased levels of interleukin-1β and tumor necrosis factor-α. Collectively, these findings highlight inflammation as a potential mechanism that underlies the excessive morbidity and mortality in patients who have an ACS and depressive symptoms.

There also was evidence of a graded relation between depressive symptoms and pathogen burden. This effect was most evident among patients who had symptoms in the highest tertile and was independent of whether these patients met diagnostic criteria for clinical depression. The size of this effect was notable: 100% of patients in the highest symptom tertile were seropositive to all 3 viruses compared with only a few patients in the middle and lowest tertiles. These disparities in pathogen burden suggest another plausible mechanism that underlies the excessive morbidity and mortality among patients who have an ACS and depressive symptoms. Although the evidence linking infection with cardiac outcomes is mixed, studies have suggested that pathogen burden, when coupled with systemic inflammation, confers a strong risk of morbidity and mortality. Our findings suggest that these processes cluster in patients who have depressive symptoms. More than 60% of patients in the highest symptom tertile showed evidence of systemic inflammation and pathogen burden, whereas the comparable figure was 19% among patients in the other tertiles.

Although they reliably clustered in patients who had depressive symptoms, pathogen burden and CRP expression were unrelated in this sample, suggesting that latent infection does not act as an inflammatory stimulus. What else could underlie the depression–inflammation association? We examined the roles of smoking and adiposity, which promote inflammation, but they did not explain the high levels of CRP. These
findings are somewhat surprising because, in healthy young adults, adiposity explains most of the excessive CRP and interleukin-6 associated with depression. We also examined whether medical history, disease severity, or management might underlie associations between depression and inflammation. Findings showed that disease characteristics do not simply foster a spurious association between these processes. Thus, the reason depressive symptoms become associated with CRP in patients who have an ACS is not clear, and it will be important for future research to identify the underlying mechanisms.

The cross-sectional design of this study precludes causal inferences regarding the associations between depression, pathogen burden, and inflammation. Because each of these processes is capable of eliciting the others, it is tempting to speculate that patients enter a vicious positive-feedback circuit, the long-term result of which is increased vulnerability to morbidity and mortality from CAD. To evaluate this hypothesis, future studies will need to repeatedly assess depression, pathogen burden, and inflammation in a longitudinal protocol and determine their relations with clinical outcomes over time. This sort of work is likely to yield critical insights into the mechanisms through which depression contributes to morbidity and mortality from CAD.