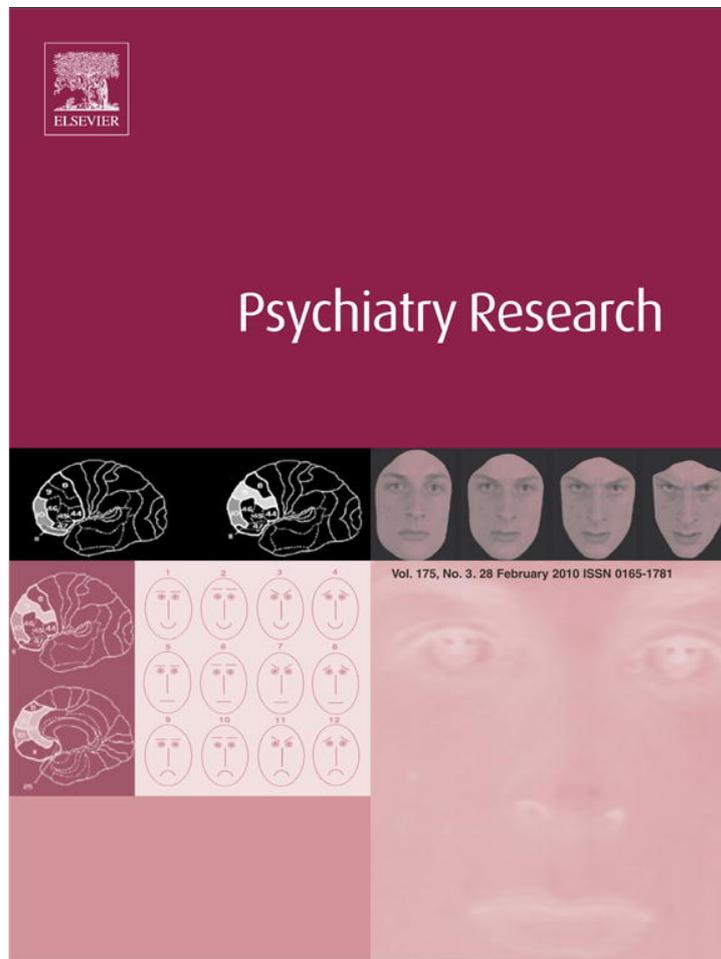


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Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Depression and platelet activation in outpatients with stable coronary heart disease: Findings from the Heart and Soul Study

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ARTICLE INFO

Article history:

Received 15 April 2008

Received in revised form 6 January 2009

Accepted 7 January 2009

Keywords:

Depression

Platelet activation

Coronary heart disease

Mechanisms

ABSTRACT

Depression is associated with increased morbidity and mortality in patients with coronary heart disease (CHD). Increased platelet activation has been proposed as a potential mechanism by which depression may lead to adverse cardiovascular outcomes. In this cross-sectional study, we measured platelet activation in 104 patients with stable CHD, including 58 with a current episode of major depression and 46 without past or current major depression. Participants were instructed not to take aspirin for 7 days prior to the study appointment. Platelet activation was measured by plasma concentrations of platelet factor 4 (PF4) and beta-thromboglobulin (β -TG), and by 24-h urinary concentrations of 11-dehydro-thromboxane B₂ (TBXB₂). We observed no differences in the mean levels of PF4, B-TG or TBXB₂ in patients with and without major depression. Results were unchanged after adjustment for age, smoking, use of aspirin, and use of any psychotropic medication. We found no evidence of an association between major depression and platelet activation as measured by plasma concentrations of PF4 and β -TG, or urinary TBXB₂ in 104 outpatients with stable CHD. These findings do not support a role for platelet activation in the association between depression and cardiovascular disease among patients with stable CHD.

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1. Introduction

Major depression is an established predictor of morbidity and mortality in patients with coronary heart disease (CHD) (Whooley, 2006). Several biological factors have been proposed as potential mechanisms by which depression may lead to adverse cardiovascular events (Carney et al., 2002; Ziegelstein, 2001). These biological factors include decreased heart rate variability (Carney et al., 2001), increased sympathetic nervous system activity (Otte et al., 2005), hyperactivity of the hypothalamic–pituitary–adrenocortical system (Otte et al., 2004), increased secretion of pro-inflammatory cytokines, and potential toxicity of psychotropic medications (Bingefors et al., 1996). Because serotonin dysregulation has a central role in both major depression and platelet activation (Willerson et al., 1989; Smith et al., 1997; Nemeroff and Musselman, 2000; Schins et al., 2003; von Kanel, 2004), increased

platelet activation has also been identified as a potential link between depression and CHD.

Previous studies have reported an association between major depression and increased plasma concentrations of the platelet-specific, alpha granule secretory proteins, platelet factor 4 (PF4) and β -thromboglobulin (β -TG) in a number of populations including patients with acute coronary heart syndrome (Kuijpers et al., 2002; Laghrissi-Thode et al., 1997; Musselman et al., 2000; Serebruanu et al., 2003c; Whyte et al., 2001). However, only one of these studies evaluated the association between depression and platelet activation in outpatients with stable CHD (Laghrissi-Thode et al., 1997). In an extensive review of the literature on depression and platelet activation, von Kanel concluded that “data on platelet activity in depression are inconclusive,” and that “studies in larger sample sizes controlling for confounders of platelet functioning are needed” (von Kanel, 2004).

We sought to determine whether depression is associated with platelet activation in outpatients with stable CHD. We assessed depression and measured three markers of platelet activation, plasma PF4, plasma β -TG, and 24-h urinary 11-dehydro-thromboxane B₂ (TBXB₂), in a cross-sectional study of 104 participants with stable CHD from the Heart and Soul Study.

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2. Methods

2.1. Participants

The Heart and Soul Study is a prospective cohort study of psychosocial factors and health outcomes in patients with CHD. Details regarding our recruitment procedures have been published previously (Ruo et al., 2003; Whooley et al., 2008). Briefly, we used administrative databases to identify stable outpatients with documented CHD at two Veterans Affairs Medical Centers (San Francisco and Palo Alto, California), one university medical center (University of California, San Francisco), and nine community health clinics in Northern California. Patients were eligible to participate if they had at least one of the following: a history of MI, angiographic evidence of at least 50% stenosis in one or more coronary vessels, prior evidence of exercise-induced ischemia by treadmill or nuclear testing, a history of coronary artery revascularization, or a diagnosis of CHD by an internist or cardiologist. Patients were excluded if they were unable to walk one block, reported a cardiac-related hospitalization or procedure in the prior 6 months, or were planning to move from the local area within 3 years.

Between September 2000 and April 2002, a total of 601 participants completed a daylong study appointment at the San Francisco Veterans Affairs Medical Center that included a comprehensive health interview, a medical history questionnaire, and an exercise treadmill with stress echocardiography. The protocol was approved by the appropriate institutional review boards, and all participants provided written informed consent. Although recruitment for the Heart and Soul Study continued through December 2002, with a total of 1024 participants enrolled, this substudy was limited to the first 601 participants.

Participants were instructed not to take aspirin for 7 days prior to the study appointment (Douketis et al., 2008). Because accurate measurement of platelet activation is influenced by a number of factors, we measured platelet activation only in those participants who fulfilled all of the following criteria: 1) had discontinued using aspirin as instructed (1 week before their study appointment), 2) had fasted for at least 12 h before the blood draw, 3) were non-smokers or had stopped smoking at least 4 h prior to the blood draw (Marasini et al., 1986), and 4) underwent a non-traumatic venipuncture using a minimum of tourniquet pressure (<40 mm Hg for <15 s). Of the 601 participants, 105 had current major depression (defined as below), and 58 of these met all of the above criteria. A random subset of 46 participants without past or current major depression who also met the above criteria served as the comparison group.

2.2. Major depression

We defined current major depression according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (American Psychiatric Association, 1994). To determine the presence of major depression, we utilized the Computerized National Institute of Mental Health Diagnostic Interview Schedule (CDIS-IV). The CDIS-IV is a validated, computerized version of the clinician-administered, structured clinical interview for the diagnosis of psychiatric disorders (Blouin et al., 1988; Levitan et al., 1991; Robins et al., 1981). Trained research assistants administered the computerized interview. Participants fulfilling diagnostic criteria for a current episode of major depression were informed that they were suffering from depression, instructed to discuss their symptoms with their primary care provider, and provided with a list of local resources available for further evaluation and treatment. We also assessed depressive symptoms as a continuous variable using the nine-item Patient Health Questionnaire (PHQ-9) (Spitzer et al., 1999). Participants reported the frequency of experiencing each of the nine DSM-IV symptoms of depression during the prior 2 weeks as not at all (0), several days (1), more than half the days (2), or nearly every day (3), yielding a total score range of 0 to 27.

2.3. Platelet activation

Platelet activation can be quantified by a number of different methods (Tsiara et al., 2003). Initiation of platelet activation induces expression of several surface proteins and receptors which can be detected by flow cytometry utilizing specific antibodies to detect activation-dependent epitopes (Nemeroff and Musselman, 2000; Schmitz et al., 1998). Several chemotactic factors, including platelet factor 4 (PF4) and β -thromboglobulin (β -TG), are subsequently released from the intracellular α -granules of the platelets. These proteins can be measured in plasma using an enzyme-linked immunosorbent assay (ELISA) (Nemeroff and Musselman, 2000). Thromboxane B_2 , a mediator of the hemostatic response, is activated by the enzyme cyclooxygenase. Urinary 11-dehydro-thromboxane B_2 , a major metabolite of platelet-derived thromboxane B_2 is thought to reflect platelet procoagulant activity (Perneby et al., 1999).

We measured platelet activation by plasma levels of PF4 and β -TG. Venous blood samples were collected from the antecubital vein with a flawless venipuncture using a 21-g butterfly needle. The first 10 ml of blood, collected in disodium EDTA, was used for determination of platelet concentration. The next 5 ml of blood was collected into a chilled polypropylene syringe containing a solution prepared immediately prior (1 ml ACD-A, 10 μ l PGE₁, and 80 μ l ASA/5 ml blood). Syringes were mixed well and placed on ice for at least 5 min but no more than 30 min. Blood was then transferred to glass Oakridge tubes and centrifuged in a Sorvall centrifuge (SS34 rotor) at 4 °C for 20 min at 19,000 rpm to obtain platelet-poor plasma (platelet count less than 10,000/ μ l). After centrifugation the center portion of platelet-poor plasma was removed and placed in cryovials then frozen at -80 °C until the assay.

Plasma concentrations of PF4 and β -TG were subsequently determined by ELISA (Asserachrom, American Bioproducts, Parsippany, NJ), with measurements in International

units per milliliter (IU/ml). Detection limit of the PF4 assay is 1 IU/ml with a range of 1–120 IU/ml. The intra-assay coefficient of variation for the PF4 assay was 9.09%, and the inter-assay coefficient of variation was 6.58%. Normal plasma concentrations of PF4 in the adult population range from 0–10 IU/ml (Files et al., 1981). We defined high PF4 plasma concentrations as >10 IU/ml. Detection limit of the β -TG assay is 1 IU/ml with a range of 5–220 IU/ml. The intra-assay coefficient of variation for the β -TG assay was 6.58%, and the inter-assay coefficient of variation was 8.39%. Normal plasma concentrations of β -TG in the adult population range from 10–50 IU/ml (van Hulsteijn et al., 1982). We defined high β -TG plasma concentration as >50 IU/ml.

We also sought to validate our measurement procedure by performing another established measure of platelet-activation, urinary 11-dehydro-thromboxane B_2 (TBXB2) (ng/mg/24 h), in a random sub-sample of 20 depressed and 14 non-depressed participants. Study participants were instructed to collect all urine for 24 h between the end of their study appointment and the time when a researcher visited their house the next day, and to keep the urine collection jugs refrigerated at all times. No preservatives were added to the urine jugs. Research personnel arrived at patient homes exactly 24 h after their appointment to ensure accurately timed specimens and to enhance compliance with the protocol. All patients were asked whether they were able to collect all urine or if some fraction had been inadvertently discarded. If the sample was reported to be incomplete, or if the volume was less than one liter, subjects were asked to repeat the collection, and research personnel returned 24 h later to re-collect the urine. Similarly, if the 3-liter collection jug was completely full, subjects were given two new jugs and asked to repeat the collection to insure that no urine was inadvertently discarded. If subjects were unable to collect all urine for any reason or had urinary incontinence, their samples were deemed inadequate and discarded.

Urinary excretion of TBXB2 was measured by immunoassay. The metabolite was extracted from urine by passage through C8-minicolumns (Cat# 6801-12-2, Whatman Inc., Clifton, NJ). Extracts were dried, redissolved in saline and assayed by ELISA using an antibody that lacks significant cross-reactivity with other commonly occurring eicosanoids (Cat # EA-30, Oxford Biomedical Research Inc., Rochester Hills, MI). Results are expressed as ng of 11-dehydro-thromboxane B_2 /mg creatinine/24 h to normalize the measurement across the study population. The ELISA has a sensitivity of 0.02 ng/ml urine, with an intra-assay coefficient of 5.6% and an inter-assay coefficient of 8.5%. We defined “high urinary TBXB2” as the top tertile of TBXB2 excretion.

2.4. Other patient characteristics

Age, sex, ethnicity, medical history, smoking status, and alcohol use were determined by questionnaire. Participants were instructed to bring their medication bottles to the study appointment, and study personnel recorded all current medications, which were categorized using Epocrates Rx (Epocrates, San Mateo, CA). We measured weight and height and calculated body mass index (kg/m^2). We assessed left ventricular ejection fraction using a resting echocardiogram (Schiller et al., 1989). We evaluated peak exercise capacity (METS achieved) using a symptom-limited, graded exercise treadmill test according to a standard Bruce protocol. To assess physical activity, we asked “Which of the following statements best describes how physically active you have been during the last month, that is, done activities such as 15–20 min of brisk walking, swimming, general conditioning, or recreational sports?” We considered participants physically active if they answered fairly, quite, very or extremely (vs. not at all or a little) active.

2.5. Statistical analysis

Differences in baseline characteristics between participants with and without depression were compared using two-tailed Student's *t*-tests for continuous variables and Chi-squared tests for dichotomous variables. We tested for agreement of plasma PF4 and β -TG with urinary TBXB2 levels using Pearson correlation coefficients. We used generalized linear models to calculate mean PF4, β -TG, and TBXB2 levels (log transformed because they were not normally distributed) in participants with and without current depression, adjusting for variables associated with depression at $P \leq 0.1$. Linear regression was used to evaluate the association of depressive symptoms as a continuous variable (score on Patient Health Questionnaire) with measures of platelet activation. We tested for interactions of depression with use of anti-coagulant and psychotropic medications. All analyses were performed using Statistical Analysis Software Version 9 (SAS Institute, Cary, N.C.).

3. Results

3.1. Characteristics of participants

Compared with participants who were never depressed, those with current major depression were younger, more likely to smoke, and more likely to be taking psychotropic medications (Table 1). There were otherwise no statistically significant differences in baseline characteristics between participants with and without depression. Pearson correlation coefficients for agreement with urinary TBXB2 were 0.67 ($P < 0.0001$) for PF4, and 0.24 ($P = 0.13$) for β -TG. Raw values ranged from 0.09 to 52 IU/ml for PF4, 1.9 to 112 IU/ml β -TG and 1.2 to 24.2 ng/mg/day for TBXB2.

3.2. Major depression and platelet activation

We found no association between current major depression and mean levels of plasma PF4, plasma β -TG, or urinary TBXB2 in unadjusted or adjusted analyses (Table 2). Although the lack of association between depression and platelet activation was similar in users and nonusers of antidepressant and other psychotropic medications (all *P* values for interaction >0.2), we verified that there was no significant association between depression and platelet activation after excluding participants who were taking these medications. When platelet activation was analyzed as a dichotomous variable, we also found no difference in the proportion of depressed and nondepressed participants with elevated PF4 >10 IU/ml (17% vs. 22%; *P*=0.56), elevated β -TG >50 IU/ml (17% vs. 20%; *P*=0.76), or TBXB2 in the highest tertile >7.6 ng/mg/24 h (30% vs. 36%; *P*=0.73).

3.3. Severity of depressive symptoms

When entered as a continuous variable, depressive symptoms (score from the 9-item Patient Health Questionnaire) were not associated with log PF4 (β coefficient -0.09 , *P*=0.83), log β -TG (β coefficient -0.04 , *P*=0.95), or log TBXB2 (β coefficient 0.19 , *P*=0.90) in age-adjusted linear regression models. Similarly, among the 58 participants with current depression, depressive symptoms were not associated with log PF4 (β coefficient 0.50 , *P*=0.37), log β -TG (β coefficient 0.95 , *P*=0.16), or log TBXB2 (β coefficient 0.36 , *P*=0.86). Further adjustment for age, smoking, use of psychotropic medication and use of aspirin did not change these results.

3.4. Platelet activation and characteristics of participants

Of the participants with increased platelet activation (PF4 >10 IU/ml), 95% (19/20) were male, compared with 70% (59/84) of participants without increased platelet activation (*P*=0.02). Otherwise, no Table 1 variables were associated with platelet activation. Specifically, increased platelet activation was not associated with diabetes, congestive heart failure, hypertension, myocardial infarction, stroke, exercise capacity, or left ventricular ejection fraction (all *P* values >0.3).

4. Discussion

Overall, we found no evidence of an association between current depression and measures of platelet activation in 104 outpatients with stable CHD. We observed no difference in mean levels of plasma PF4 or β -TG levels, and no difference in urinary TBXB2 excretion, in depressed and non-depressed participants. We also found no significant association between current depression and high plasma PF4, β -TG, or urinary TBXB2. These findings do not support the hypothesis that increased platelet activation, as measured by plasma PF4, β -TG or urinary TBXB2, is a likely mechanism linking depression with adverse outcomes in patients with stable CHD.

The potential association of depression with increased platelet activation has received considerable attention as a mechanism by which depression may lead to cardiovascular events. Treatment with selective serotonin reuptake inhibitors (SSRIs) decreases platelet activation in patients without CHD (Lederbogen et al., 2001), in patients with CHD (Serebruany et al., 2003b, 2001b), in patients with congestive heart failure (Serebruany et al., 2003a), and in vitro (Serebruany et al., 2001a). Although this reduction in platelet activation is probably due to the direct effects of SSRIs on platelet activity rather than to a decrease in depression, the potential influence of platelet activation on the association between depression and CHD remains intriguing.

Several small studies have examined the association of depression with PF4 and β -TG in patients with CHD. One study reported increased PF4 and β -TG in 8 depressed patients compared with 21

Table 1

Characteristics of 104 study participants with coronary heart disease, divided by the presence of major depression (by Computerized Diagnostic Interview Schedule).^a

Variables	Current depression (n = 58)	Never depressed (n = 46)	<i>P</i> value
<i>Demographic</i>			
Age, years	63.3 ± 0.7	70 ± 9.4	0.001
Male	41 (71%)	37 (80%)	0.25
White	40 (69%)	25 (54%)	0.12
Current smoking	13 (22%)	3 (7%)	0.02
Regular alcohol use	21 (36%)	14 (30%)	0.54
Body mass index, kg/m ²	29 ± 5.2	28.6 ± 4.7	0.73
Physically active	34 (59%)	31 (67%)	0.37
<i>Medical history</i>			
Congestive heart failure	10 (17%)	11 (24%)	0.39
Hypertension	37 (64%)	30 (65%)	0.88
Diabetes	15 (26%)	9 (20%)	0.44
Myocardial infarction	30 (53%)	25 (54%)	0.86
Stroke	11 (19%)	5 (11%)	0.25
<i>Psychotropic medications</i>			
Benzodiazepines	10 (17%)	6 (13%)	0.55
Anxiolytics/hypnotics	2 (3%)	0 (0%)	0.30
SSRI	16 (28%)	1 (2%)	<0.001
TCA	2 (3%)	0 (0%)	0.30
Other antidepressants	10 (17%)	1 (2%)	0.01
Anti-convulsants	12 (21%)	2 (4%)	0.01
Anti-histamines (sedating)	2 (3%)	1 (2%)	0.41
Muscle relaxants	1 (2%)	1 (2%)	0.49
Narcotics	13 (22%)	2 (4%)	0.006
Anti-psychotics	4 (7%)	0 (0%)	0.09
<i>Other medications</i>			
Aspirin	53 (91%)	37 (80%)	0.10
Beta blockers	29 (50%)	25 (54%)	0.65
Calcium blockers	18 (31%)	11 (24%)	0.42
ACE inhibitors	27 (47%)	27 (59%)	0.21
Anti-coagulants/thrombolytics	1 (2%)	2 (4%)	0.42
Diuretics	15 (26%)	12 (26%)	0.97
Nitrates	22 (38%)	18 (39%)	0.90
Diabetes medications	11 (19%)	7 (15%)	0.61
Statins	39 (67%)	32 (70%)	0.80
Nonsteroidal anti-inflammatory	8 (14)	7 (15%)	0.84
<i>Cardiac function</i>			
Exercise capacity, METS	7.9 ± 3.8	7.4 ± 3.13	0.45
Ventricular ejection fraction	0.64 ± 0.08	0.61 ± 0.09	0.12

^a Values expressed as *N* (% of column) or mean ± S.D.

nondepressed outpatients with stable CHD (Laghrissi-Thode et al., 1997). Although the PF4 and β -TG levels of the control subjects were similar to those in our study participants, we did not observe the 4-fold or 10-fold increase in PF4 or β -TG levels previously reported to be associated with depression. Two other studies examined PF4 and β -TG levels in post-MI patients. One reported an association of depression with PF4 but not β -TG (Kuijpers et al., 2002), and the other found no association of depression with either PF4 or β -TG (Schins et al., 2004). The largest study to date measured PF4 and β -TG in participants who were enrolled in several different clinical trials, including 64 depressed patients who were recovering from acute coronary syndrome, 41 patients presenting with MI, 126 patients presenting with unstable angina, and 50 healthy controls (Serebruany et al., 2003c). Although this study reported an association between depression and increased platelet activation, analyses were not adjusted for important patient characteristics, such as severity of coronary disease or use of anti-platelet agents. Thus, it was difficult to disentangle whether differences in platelet activation were due to depression or to other factors.

There are several potential explanations for the varied findings across studies. First, anti-platelet agents were not methodically withheld in prior studies. It is possible that anti-platelet agents have differing

Table 2

Mean levels of log plasma PF4 (IU/ml), log plasma β -TG (IU/ml), and log urinary TBXB2 (ng/mg/24 h), stratified by presence of major depression.

Measure	N (depressed, not depressed)	Age-adjusted mean (standard error)			Fully-adjusted mean (standard error) ^a		
		Depressed	Not depressed	P	Depressed	Not depressed	P
<i>Entire sample</i>							
Log PF4	58, 46	1.1 ± 0.17	1.3 ± 0.19	0.40	1.0 ± 0.26	1.4 ± 0.28	0.21
Log β -TG	58, 46	2.9 ± 0.13	3.2 ± 0.14	0.20	2.9 ± 0.19	3.3 ± 0.20	0.07
Log TBXB2	20, 14	0.16 ± 0.13	1.7 ± 1.6	0.45	2.0 ± 0.12	2.3 ± 0.27	0.14
<i>Excluding 17 participants taking SSRIs</i>							
Log PF4	42, 45	0.9 ± 0.20	1.3 ± 0.20	0.22	0.9 ± 0.29	1.3 ± 0.31	0.18
Log β -TG	42, 45	2.8 ± 0.15	3.2 ± 0.15	0.10	2.8 ± 0.21	3.2 ± 0.22	0.07
Log TBXB2	15, 14	1.7 ± 0.15	1.7 ± 0.15	0.71	2.0 ± 0.19	2.3 ± 0.26	0.19
<i>Excluding 23 participants taking any antidepressants</i>							
Log PF4	37, 44	0.8 ± 0.21	1.3 ± 0.19	0.10	0.6 ± 0.31	1.1 ± 0.32	0.10
Log β -TG	37, 44	2.8 ± 0.16	3.2 ± 0.15	0.06	2.6 ± 0.24	3.1 ± 0.24	0.05
Log TBXB2	14, 14	1.5 ± 0.13	1.8 ± 0.13	0.28	1.7 ± 0.23	2.0 ± 0.29	0.19
<i>Excluding 47 participants taking any psychotropic medication</i>							
Log PF4	24, 33	0.9 ± 0.29	1.2 ± 0.24	0.39	0.7 ± 0.42	1.1 ± 0.40	0.38
Log β -TG	24, 33	2.6 ± 0.20	3.2 ± 0.17	0.06	2.5 ± 0.30	3.0 ± 0.29	0.06
Log TBXB2	9, 11	1.5 ± 0.18	1.8 ± 0.16	0.31	1.4 ± 0.28	1.8 ± 0.34	0.23
<i>Excluding 3 participants taking anti-coagulant/thrombolytics</i>							
Log PF4	57, 44	1.1 ± 0.17	1.2 ± 0.20	0.53	0.9 ± 0.26	1.2 ± 0.29	0.28
Log β -TG	57, 44	2.9 ± 0.13	3.2 ± 0.15	0.14	3.0 ± 0.19	3.4 ± 0.21	0.05
Log TBXB2	20, 14	1.6 ± 0.13	1.7 ± 0.16	0.45	2.0 ± 0.20	2.3 ± 0.27	0.14

^a Adjusted for age, smoking, use of any psychotropic medication (except when these participants excluded), and use of aspirin.

effects in depressed and non-depressed patients that could account for differences in platelet activation as measured by PF4 and β -TG. Second, it is possible that anti-platelet agents may have attenuated the platelet activation (Gavaghan et al., 1990) of all the participants in our study so that differences in activation between depressed and non-depressed participants were not evident. However, our study participants were instructed to discontinue aspirin at least 1 week prior to their study appointment, and the study was conducted prior to the widespread use of clopidogrel. Third, beta-blockers which have been shown to potentially attenuate platelet activation (Mehta and Mehta, 1982) were not methodically withheld in prior studies or in our study.

Fourth, most of the prior studies examined the association between depression and platelet activation in patients with unstable coronary syndromes. Because stable and unstable CHD are pathophysiologically different entities with different hemostatic profiles (Chakhtoura et al., 2000; Patel et al., 2004), it is possible that an alteration in platelet activation in depressed patients may be the mechanism for adverse outcomes in patients with unstable but not stable CHD. Fifth, depression may not be associated increased PF4, β -TG or urinary TBXB2 in a stable situation (as the patients in this study) but might still demonstrate increased platelet activation and aggregation in response to vascular injury once platelets are activated at the local site of plaque rupture. Finally, acute emotional stress (Levine et al., 1985; Malkoff et al., 1993), phlebotomy procedure, and specimen handling (Gurney et al., 2002) can all affect platelet activation assays. However, all participants underwent exactly the same phlebotomy procedure, the quality of the procedure was repeatedly verified, and all specimens were handled with extreme care.

Several limitations must be considered when interpreting our findings. First, there was poor correlation between plasma β -TG levels and urinary TBXB2 levels in the 34 patients in whom both were measured. However, there was excellent correlation between plasma PF4 levels and urinary TBXB2 levels in these same 34 patients. This suggests that β -TG levels may not accurately reflect platelet activation in patients with stable CHD. Second, aspirin irreversibly blocks the

generation of thromboxane A2 in platelets, and the mean half-life of platelets is 4.4 (range of 2.9 to 5.9) days (Stuart et al., 1975). Thus, the anti-thrombotic effects of aspirin may last as long as 10 days. Although subjects were instructed not to take aspirin for 7 days prior to the study appointment, the lingering antithrombotic effects of aspirin may have made it difficult to detect any association of depression with platelet activation.

Third, our study may not have had a sufficient sample size to detect small differences in plasma PF4 and β -TG levels between study groups. However, our study was larger than most previously reported studies of PF4 and β -TG levels and, as discussed above, adequately powered to detect the large differences reported in these prior studies. Moreover, even if our sample size were larger, the point differences we observed suggest that depression would be associated with lower (not higher) platelet activation. Fourth, we did not perform stimulation testing or use fluorescence-activated flow cytometry, and these methods may be more sensitive than platelet releasing factors for measurement of platelet activation (Musselman et al., 1996). Finally, more than half of our study participants were recruited from VA medical centers, and thus the majority of the participants in our study were older men, so our results may not generalize to other patient populations.

In conclusion, we found that depression was not associated with increased platelet activation as measured by plasma PF4, plasma β -TG, or urinary TBXB2 levels. These findings raise questions about whether platelet activation is involved in the association of depression with adverse outcomes in patients with stable CHD. However, there are many other aspects of platelet activation that may be altered in depressed patients. Since platelet activation is a complex process, it will be important to understand the effect of depression on all such aspects of platelet activation so that therapy can be targeted appropriately.

Acknowledgements

This study was supported by grants from the Department of Veterans Affairs Health Services Research and Development Service, the Department of Veterans Affairs Epidemiology Merit Review program, the National Heart, Lung and Blood Institute, the American Federation for Aging Research (Paul Beeson Scholars Program), the Robert Wood Johnson Foundation (Faculty Scholars Program), and the Ischemia Research and Education Foundation.

References

- American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders 4th ed. APA, Washington, DC.
- Bingefors, K., Isacson, D., Knorrning, L.V., Smedby, B., Wicknertz, K., 1996. Antidepressant-treated patients in ambulatory care. Mortality during a nine-year period after first treatment. *British Journal of Psychiatry* 169, 647–654.
- Blouin, A.G., Perez, E.L., Blouin, J.H., 1988. Computerized administration of the Diagnostic Interview Schedule. *Psychiatry Research* 23, 335–344.
- Carney, R.M., Blumenthal, J.A., Stein, P.K., Watkins, L., Catellier, D., Berkman, L.F., Czajkowski, S.M., O'Connor, C., Stone, P.H., Freedland, K.E., 2001. Depression, heart rate variability, and acute myocardial infarction. *Circulation* 104, 2024–2028.
- Carney, R.M., Freedland, K.E., Miller, G.E., Jaffe, A.S., 2002. Depression as a risk factor for cardiac mortality and morbidity: a review of potential mechanisms. *Journal of Psychosomatic Research* 53, 897–902.
- Chakhtoura, E.Y., Shamon, F.E., Haft, J.I., Obiedzinski, G.R., Cohen, A.J., Watson, R.M., 2000. Comparison of platelet activation in unstable and stable angina pectoris and correlation with coronary angiographic findings. *American Journal of Cardiology* 86, 835–839.
- Douketis, J.D., Berger, P.B., Dunn, A.S., et al., 2008. The perioperative management of antithrombotic therapy: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 133, 299S–339S.
- Files, J.C., Malpass, T.W., Yee, E.K., Ritchie, J.L., Harker, L.A., 1981. Studies of human platelet alpha-granule release in vivo. *Blood* 58, 607–618.
- Gavaghan, T.P., Hickie, J.B., Krilis, S.A., Baron, D.W., Gebski, V., Low, J., Chesterman, C.N., 1990. Increased plasma beta-thromboglobulin in patients with coronary artery vein graft occlusion: response to low dose aspirin. *Journal of the American College of Cardiology* 15, 1250–1258.
- Gurney, D., Lip, G.Y., Blann, A.D., 2002. A reliable plasma marker of platelet activation: does it exist? *American Journal of Hematology* 70, 139–144.
- Kuijpers, P.M., Hamulyak, K., Strik, J.J., Wellens, H.J., Honig, A., 2002. Beta-thromboglobulin and platelet factor 4 levels in post-myocardial infarction patients with major depression. *Psychiatry Research* 109, 207–210.
- Laghrissi-Thode, F., Wagner, W.R., Pollock, B.G., Johnson, P.C., Finkel, M.S., 1997. Elevated platelet factor 4 and beta-thromboglobulin plasma levels in depressed patients with ischemic heart disease. *Biological Psychiatry* 42, 290–295.

- Lederbogen, F., Gilles, M., Maras, A., et al., 2001. Increased platelet aggregability in major depression? *Psychiatry Research* 102, 255–261.
- Levine, S.P., Towell, B.L., Suarez, A.M., Knieriem, L.K., Harris, M.M., George, J.N., 1985. Platelet activation and secretion associated with emotional stress. *Circulation* 71, 1129–1134.
- Levitan, R.D., Blouin, A.G., Navarro, J.R., Hill, J., 1991. Validity of the computerized DIS for diagnosing psychiatric inpatients. *Canadian Journal of Psychiatry* 36, 728–731.
- Malkoff, S.B., Muldoon, M.F., Zeigler, Z.R., Manuck, S.B., 1993. Blood platelet reactivity to acute mental stress. *Canadian Journal of Psychiatry* 55, 477–482.
- Marasini, B., Biondi, M.L., Barbesti, S., Zatta, G., Agostoni, A., 1986. Cigarette smoking and platelet function. *Thrombosis Research* 44, 85–94.
- Mehta, J., Mehta, P., 1982. Effects of propranolol therapy on platelet release and prostaglandin generation in patients with coronary heart disease. *Circulation* 66, 1294–1299.
- Musselman, D.L., Tomer, A., Manatunga, A.K., Knight, B.T., Porter, M.R., Kasey, S., Marzec, U., Harker, L.A., Nemeroff, C.B., 1996. Exaggerated platelet reactivity in major depression. *American Journal of Psychiatry* 153, 1313–1317.
- Musselman, D.L., Marzec, U.M., Manatunga, A., Penna, S., Reemsnyder, A., Knight, B.T., Baron, A., Hanson, S.R., Nemeroff, C.B., 2000. Platelet reactivity in depressed patients treated with paroxetine: preliminary findings. *Archives of General Psychiatry* 57, 875–882.
- Nemeroff, C.B., Musselman, D.L., 2000. Are platelets the link between depression and ischemic heart disease? *American Heart Journal* 140, 57–62.
- Otte, C., Marmar, C.R., Pipkin, S.S., Moos, R., Browner, W.S., Whooley, M.A., 2004. Depression and 24-hour urinary cortisol in medical outpatients with coronary heart disease: the Heart and Soul Study. *Biological Psychiatry* 56, 241–247.
- Otte, C., Neylan, T.C., Pipkin, S.S., Browner, W.S., Whooley, M.A., 2005. Depressive symptoms and 24-hour urinary norepinephrine excretion levels in patients with coronary disease: findings from the Heart and Soul Study. *American Journal of Psychiatry* 162, 2139–2145.
- Patel, P.B., Pfau, S.E., Cleman, M.W., Brennan, J.J., Howes, C., Remetz, M., Cabin, H.S., Setaro, J.F., Rinder, H.M., 2004. Comparison of coronary artery specific leukocyte-platelet conjugate formation in unstable versus stable angina pectoris. *American Journal of Cardiology* 93, 410–413.
- Perneby, C., Granstrom, E., Beck, O., Fitzgerald, D., Harhen, B., Hjemdahl, P., 1999. Optimization of an enzyme immunoassay for 11-dehydro-thromboxane B(2) in urine: comparison with GC-MS. *Thrombosis Research* 96, 427–436.
- Robins, L.N., Helzer, J.E., Croughan, J., Ratcliff, K.S., 1981. National Institute of Mental Health Diagnostic Interview Schedule. Its history, characteristics, and validity. *Archives of General Psychiatry* 38, 381–389.
- Ruo, B., Rumsfeld, J.S., Hlatky, M.A., Liu, H., Browner, W.S., Whooley, M.A., 2003. Depressive symptoms and health-related quality of life: the Heart and Soul Study. *JAMA* 290, 215–221.
- Schiller, N.B., Shah, P.M., Crawford, M., DeMaria, A., Devereux, R., Feigenbaum, H., Gutgesell, H., Reichek, N., Sahn, D., Schnittger, I., et al., 1989. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *Journal of the American Society of Echo Cardiography* 2, 358–367.
- Schins, A., Honig, A., Crijns, H., Baur, L., Hamulyak, K., 2003. Increased coronary events in depressed cardiovascular patients: 5-HT_{2A} receptor as missing link? *Psychosomatic Medicine* 65, 729–737.
- Schins, A., Hamulyak, K., Scharpé, S., Lousberg, R., Van Melle, J., Crijns, H., Honig, A., 2004. Whole blood serotonin and platelet activation in depressed post-myocardial infarction patients. *Life Sciences* 76, 637–650.
- Schmitz, G., Rothe, G., Ruf, A., Barlage, S., Tschöpe, D., Clemetson, K.J., Goodall, A.H., Michelson, A.D., Nurdén, A.T., Shankey, T.V., 1998. European working group on clinical cell analysis: consensus protocol for the flow cytometric characterisation of platelet function. *Thrombosis and Haemostasis* 79, 885–896.
- Serebruany, V.L., Glassman, A.H., Malinin, A.I., Atar, D., Sane, D.C., Oshrine, B.R., Ferguson, J.J., O'Connor, C.M., 2003a. Selective serotonin reuptake inhibitors yield additional antiplatelet protection in patients with congestive heart failure treated with antecedent aspirin. *European Journal of Heart Failure* 5, 517–521.
- Serebruany, V.L., Glassman, A.H., Malinin, A.I., Nemeroff, C.B., Musselman, D.L., van Zyl, L.T., Finkel, M.S., Krishnan, K.R., Gaffney, M., Harrison, W., Califf, R.M., O'Connor, C.M., Sertraline AntiDepressant Heart Attack Randomized Trial Study Group, 2003b. Platelet/endothelial biomarkers in depressed patients treated with the selective serotonin reuptake inhibitor sertraline after acute coronary events: the Sertraline AntiDepressant Heart Attack Randomized Trial (SADHART) Platelet Substudy. *Circulation* 108, 939–944.
- Serebruany, V.L., Glassman, A.H., Malinin, A.I., Sane, D.C., Finkel, M.S., Krishnan, R.R., Atar, D., Lekht, V., O'Connor, C.M., 2003c. Enhanced platelet/endothelial activation in depressed patients with acute coronary syndromes: evidence from recent clinical trials. *Blood Coagulation and Fibrinolysis* 14, 563–567.
- Serebruany, V.L., Gurbel, P.A., O'Connor, C.M., 2001a. Platelet inhibition by sertraline and N-desmethylsertraline: a possible missing link between depression, coronary events, and mortality benefits of selective serotonin reuptake inhibitors. *Pharmacological Research* 43, 453–462.
- Serebruany, V.L., O'Connor, C.M., Gurbel, P.A., 2001b. Effect of selective serotonin reuptake inhibitors on platelets in patients with coronary artery disease. *American Journal of Cardiology* 87, 1398–1400.
- Smith, K.A., Fairburn, C.G., Cowen, P.J., 1997. Relapse of depression after rapid depletion of tryptophan. *Lancet* 349, 915–919.
- Spitzer, R.L., Kroenke, K., Williams, J.B., 1999. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. *Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. JAMA* 282, 1737–1744.
- Stuart, M.J., Murphy, S., Oski, F.A., 1975. A simple nonradioisotope technic for the determination of platelet life-span. *New England Journal of Medicine* 292, 1310–1313.
- Tsiara, S., Elisaf, M., Jagroop, I.A., Mikhailidis, D.P., 2003. Platelets as predictors of vascular risk: is there a practical index of platelet activity? *Clinical and Applied Thrombosis/Hemostasis* 9, 177–190.
- van Hulsteijn, H., Bertina, R., Briet, E., 1982. A one-year follow-up study of plasma fibrinopeptide A and beta-thromboglobulin after deep vein thrombosis and pulmonary embolism. *Thrombosis Research* 27, 225–229.
- von Kanel, R., 2004. Platelet hyperactivity in clinical depression and the beneficial effect of antidepressant drug treatment: how strong is the evidence? *Acta Psychiatrica Scandinavica* 110, 163–177.
- Whooley, M.A., 2006. Depression and cardiovascular disease: healing the broken-hearted. *JAMA* 295, 2874–2881.
- Whooley, M.A., de Jonge, P., Vittinghoff, E., Otte, C., Moos, R., Carney, R.M., Ali, S., Dowray, S., Na, B., Feldman, M.D., Schiller, N.B., Browner, W.S., 2008. Depressive symptoms, health behaviors, and risk of cardiovascular events in patients with coronary heart disease. *JAMA* 300, 2379–2388.
- Whyte, E.M., Pollock, B.G., Wagner, W.R., Mulsant, B.H., Ferrell, R.E., Mazumdar, S., Reynolds, C.F. III., 2001. Influence of serotonin-transporter-linked promoter region polymorphism on platelet activation in geriatric depression. *American Journal of Psychiatry* 158, 2074–2076.
- Willerson, J.T., Golino, P., Eidt, J., Campbell, W.B., Buja, L.M., 1989. Specific platelet mediators and unstable coronary artery lesions. Experimental evidence and potential clinical implications. *Circulation* 80, 198–205.
- Ziegelstein, R.C., 2001. Depression in patients recovering from a myocardial infarction. *JAMA* 286, 1621–1627.