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Published in:
The World Journal of Biological Psychiatry

DOI:
10.1080/15622975.2018.1433325

Publication date:
2018

The World Journal of Biological Psychiatry

ISSN: 1562-2975 (Print) 1814-1412 (Online) Journal homepage: http://www.tandfonline.com/loi/iwbp20

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To cite this article: Petrus J. W. Naudé, Annelieke M. Roest, Dan J. Stein, Peter de Jonge & Bennard Doornbos (2018) Anxiety disorders and CRP in a population cohort study with 54,326 participants: The LifeLines study, The World Journal of Biological Psychiatry, 19:6, 461-470, DOI: 10.1080/15622975.2018.1433325

To link to this article: https://doi.org/10.1080/15622975.2018.1433325

THE WORLD JOURNAL OF BIOLOGICAL PSYCHIATRY
2018, VOL. 19, NO. 6, 461–470
https://doi.org/10.1080/15622975.2018.1433325

ORIGINAL INVESTIGATION

Anxiety disorders and CRP in a population cohort study with 54,326 participants: The LifeLines study

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ABSTRACT

Objectives: Growing evidence indicates that inflammatory processes may play a role in the pathogenesis of anxiety disorders. Nevertheless, much remains to be learned about the involvement of inflammation, including C-reactive protein (CRP), in specific anxiety disorders. This study examines the relation between anxiety disorders and CRP.

Methods: Associations of serum CRP with anxiety disorders were determined in a large population study ($n = 54{,}326$ participants, mean age = 47 years; 59% female), the LifeLines cohort. Depressive and anxiety disorders (generalized anxiety disorder, social anxiety phobia, panic disorder with or without agoraphobia and agoraphobia without panic disorder) were assessed using the Mini-International Neuropsychiatric Interview.

Results: Anxiety disorders, with the exception of social anxiety disorder, were significantly associated with increased CRP. After adjusting for demographics, life style factors, health factors, medication use, depression, and psychological stressors, CRP remained significantly associated with panic disorder with agoraphobia ($\beta = 0.01$, $P = .013$). Moreover, CRP levels were significantly higher in people with panic disorder with agoraphobia compared to other anxiety disorders, independent of all covariates ($F = 3.00$, df = 4, $P = .021$).

Conclusions: Panic disorder with agoraphobia is associated with increased CRP, although the effect size of this association is small. This indicates that neuroinflammatory mechanisms may play a potential role in its pathophysiology.

ARTICLE HISTORY
Received 9 May 2017
Revised 11 December 2017
Accepted 17 January 2018

KEYWORDS
Generalized anxiety disorder; social anxiety disorder; panic disorder; inflammation; agoraphobia

1. Introduction

Anxiety disorders are the most prevalent of the mental disorders (Kessler et al. 2005). Generalized anxiety disorder, panic disorder with agoraphobia, social anxiety disorder and agoraphobia have a chronic clinical course, low rates of recovery and relatively high probabilities of recurrence (Bruce et al. 2005). Despite their high prevalence and negative impact on physical health and occupational functioning, much remains to be learned about their underlying psychopathological mechanisms. There is evidence of a possible role for inflammatory processes in the aetiology of clinical anxiety (Michopoulos et al. 2017) and potential differences in inflammatory regulation between anxiety disorders (Furtado and Katzman 2015). Our understanding of the role of endocrine and neuroinflammatory dysfunction in the pathophysiology and aetiology of anxiety is particularly advanced in posttraumatic stress disorder (PTSD) (Michopoulos et al. 2017). Findings from research on PTSD propose that a dysregulation of the hypothalamic-pituitary-adrenal axis may impact immune regulation (de Kloet et al. 2006), and account for symptoms of anxiety (Leonard 2005). General anxiety disorder, social anxiety disorder, panic disorder with or without agoraphobia and agoraphobia are highly co-morbid with each other as well as other psychiatric disorders including, depression, PTSD, and substance-use disorders, so determining specific associations requires large sample sizes (Kaufman and Charney 2000). Moreover, psychological stressors need to be controlled for since they are associated with increased circulating inflammatory markers, i.e., C-reactive protein (CRP) (Steptoe et al. 2007; Rohleder

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Supplemental data for this article can be accessed at https://doi.org/10.1080/15622975.2018.1433325

2014) and involved in the psychopathology of anxiety (Angst and Vollrath 1991; Breslau et al. 1995).

CRP is an important marker of inflammation (Pepys and Hirschfield 2003). Six large studies have investigated the relation between anxiety disorders and CRP (Pitsavos et al. 2006; Liukkonen et al. 2011; Copeland et al. 2012; Vogelzangs et al. 2013; Wagner et al. 2015; Tayefi et al. 2017); all found that anxiety disorders are significantly associated with increased CRP levels, indicative of a low-grade inflammation. These studies have, however, important limitations. All but two studies (Vogelzangs et al. 2013; Wagner et al. 2015) used self-report questionnaires to assess anxiety. These questionnaires are prone to confounding by physical health status and depression (Lovibond and Lovibond 1995). Five studies (Pitsavos et al. 2006; Liukkonen et al. 2011; Copeland et al. 2012; Wagner et al. 2015; Tayefi et al. 2017) did not examine CRP levels across different anxiety disorders. A third limitation is that four of the studies (Liukkonen et al. 2011; Copeland et al. 2012; Vogelzangs et al. 2013; Wagner et al. 2015) used birth cohorts or cohorts which over selected patients with anxiety or depression, restricting the generalizability of the results. The limitations of these studies include problems with generalization, and restricted adjustment for important variables that can explain the association between CRP and anxiety disorders.

To address these issues we investigated the relation between anxiety disorders and CRP with data from the LifeLines Cohort study. This study offers the opportunity to investigate the relation between different anxiety disorders, assessed by a clinical interview, and CRP as a marker for inflammation in a very large, representative population cohort, taking into account key determinants such as age, sex, lifestyle factors, general medical conditions, depression, psychological stressors and medication use. Our first aim was to evaluate the associations between anxiety disorders (generalized anxiety disorder, social anxiety disorder, panic disorder with or without agoraphobia and agoraphobia) and CRP as marker for inflammation in the general population. A second aim was to establish if the presence of comorbid anxiety disorders and the number of comorbid anxiety disorders affects this association. A third aim was to examine potential determinants i.e., socio-demographic variables (sex, age, education), life style factors (smoking status, alcohol use, BMI, frequency of activity), health status (cardiovascular disease and diabetes mellitus), medication use (anti-depressant, anti-inflammatory and statins) and depression and stress (major depression, Long-term Difficulties Inventory (LDI) and List of Threatening Experiences (LTE)) in these associations. The final aim was to compare serum CRP levels between specific anxiety disorders.

2. Materials and methods

2.1. Design and participants

In this cross-sectional study, we used data from subjects participating in the LifeLines Cohort Study between February 2007 and October 2012. The LifeLines Cohort Study is a multi-disciplinary prospective population-based cohort study with a unique three-generation design aiming to examine the health and health-related behaviours of 165,000 participants living in the north-eastern region of the Netherlands (Stolk et al. 2008; Scholtens et al. 2015). It employs a broad range of investigative procedures to assess the socio-demographic, biomedical, behavioural, and psychological factors that contribute to the health and disease of the general population, with a focus on multimorbidity. All survey participants were between 18 and 90 years old at the time of enrolment. All participants provided written informed consent before participating in the study. The study protocol was approved by the medical ethical review committee of the University Medical Centre Groningen. CRP was measured in 55,233 serum samples that were collected at three intervals, February 2007 to November 2008, February 2010 to September 2011 and June 2012 to November 2012. Participants for whom data for CRP and psychiatric assessments were available were selected for this study ($N = 54{,}326$).

2.2. Assessment of depression and anxiety disorders

Current (past 2 weeks) depression (major depressive episode) and anxiety disorders (social anxiety disorder, panic disorder with and without agoraphobia, and generalized anxiety disorder) were assessed according to the fifth edition of the Dutch translation of the MINI neuro-psychiatric interview by trained research assistants as previously described (Wanders et al. 2016). The MINI interview is a systematic interview based on DSM-IV and ICD-10 criteria, with excellent inter-rater reliability (Kappa, $k > 90$), and high retest reliability ($k = 0.87$ for MDD, $k = 0.78$ for anxiety disorders) (Sheehan et al. 1998). PTSD and specific phobia were not assessed in Lifelines. For anxiety disorders, variables for the individual disorders were used for analyses. In addition, the presence of at least one anxiety disorder (any anxiety disorder, $n = 5{,}649$) and a summative variable for the number of comorbid

disorders were created (two co-existing anxiety disorders, $n = 717$; three co-existing anxiety disorders, $n = 125$; four co-existing anxiety disorders, $n = 1$).

2.3. CRP measurement

Blood samples for all study participants were collected into serum tubes via venipuncture the morning after an overnight fast. Tubes were kept at room temperature for 30 min to allow for clotting and were subsequently centrifuged at 2,500 × *g*, aliquoted into cryo vials and immediately stored at −80 °C until analyses. High-sensitivity CRP was measured at the clinical chemistry laboratory of the University Medical Centre Groningen. Serum CRP concentrations were measured with an immunoturbidimetric assay (CRPL3, Roche Diagnostics, Indianapolis, IN, USA) the lower reference limit was 0.3 mg/dl and CardioPhase hs-CRP (Siemens Healthcare Diagnostics, Marburg, Germany) with a lower reference limit of 0.18 mg/dl. The intra-assay coefficient of variation (CV) for CRPL3 was 4.15% and for CardioPhase, 3.45%. Inter-assay CVs were 5.70% and 3.15%, respectively. The between assay CV was 6.42%.

2.4. Covariates

Sociodemographic factors included age, sex and education level. Education was assessed with a questionnaire asking for the highest level of education that the participant successfully finished. Based on these data three variables were generated: (1) no education or elementary school; (2) high school and applied education; (3) higher education. From this, two dummy variables were created with the no education/elementary school as reference group.

Life style is an important factor mediating the relation between anxiety and increased inflammation markers. Therefore markers indicating unhealthy life style where added as covariate in this study. Smoking status and alcohol use were assessed by self-report. Smoking status was categorized as non-smoker, former-smoker (did not smoke for more than 30 days prior to assessment), and current-smoker. For the regression analysis, two dummy variables were created with non-smokers as reference group. Alcohol use was transformed to a variable indicating the number of alcohol consumptions per week. Height was measured to the nearest 0.1 cm. Body weight was measured without shoes with a 0.1-kg precision. Body mass index (BMI) was calculated as weight in kg divided by the square of the measured height in m. Physical exercise was defined as the frequency per week in which the respondent typically engaged in moderate physical activities (e.g. walking, bicycling, gardening and household work) for at least half an hour, it was then categorized into high (twice or more per week), medium (once per week) and low (do not exercise/hardly per week) as previously described (Nigatu et al. 2016). Two dummy variables were created with high and medium activity for regression analysis.

Subjects were asked to complete a self-administered questionnaire on medical history and current diseases. Participants were considered to have a vascular disease if they answered affirmatively to the questions: 'do you/did you ever have a (1) myocardial infarction; (2) heart failure; (3) stroke; (4) obstruction of the carotid artery; (5) undergone an angioplasty?' Presence of diabetes (type I or type II) was assessed with the question 'do you have diabetes mellitus?'

For the assessment of current medication use participants were asked to bring the medication containers to interview. During the interview the research assistant noted the name and dose of the prescribed medication in a database where it was classified according to the World Health Organization Anatomical Therapeutic Chemical classification (ATC). For the analysis in this study, anti-inflammatory medication included amino salicylic acid and similar agents (A07EC), anti-allergic agents (A07EB), systemically applied corticosteroids (H02A), anti-inflammatory and anti-rheumatic products (M01), other analgesics and antipyretics (N02B) and statins (C10AA, C10B) as covariates. As different classes of antidepressants are found to influence immune function, medication included selective serotonin reuptake inhibitors (SSRIs) (N06AB), serotonin-norepinephrine reuptake inhibitors (SNRI) (N06AX16, N06AX21), tricyclic antidepressants (TCAs) (N06AA) and tetracyclic antidepressants (TeCA) (N06AX03, N06AX05, and N06AX11). Other anti-depressant medications were also included (N06AX12, N06AX18 and N06AX22).

Major depressive disorder and psychological stressors are associated with increased inflammatory markers, including CRP (Slavich and Irwin 2014). Stressful life events were assessed by means of the Dutch version of the LTE, a 12-item self-report questionnaire (Brugha and Cragg 1990). Chronic stress was measured with the LDI. The LDI is a self-report questionnaire intended to measure long-term difficulties (Hendriks et al. 1990). LTE and LDI possess adequate validity and stability for use in large epidemiological studies (Rosmalen et al. 2012).

2.5. Statistical analysis

All analyses were conducted using SPSS (version 22, IBM, USA). *P* values were considered statistically

significant for all analyses at a value of less than 0.05. Because of the skewed distribution of CRP, ln-transformed CRP levels were used for further statistical analyses. Data for smoking status and alcohol use had a relatively high number of missing values (alcohol use 14.4%; smoking 8.7%). Data were considered to be missing at random, an assumption when erroneous data tend to have only a minor impact on estimates (Collins et al. 2001). Therefore, a multiple imputation approach by chained equations was adopted to replace these missing values (Rubin and McHugh 1987). Missing values were replaced by imputed values estimated from all predictor variables. Statistical analyses were performed on ten imputed datasets.

Sex and age interactions between the association of anxiety disorders with CRP have been previously reported (Duivis et al. 2013; Vogelzangs et al. 2013). Therefore, analyses of covariance (ANCOVA) was performed to determine the interaction of sex, age and age^2 with anxiety disorders (social anxiety disorder, panic disorder with and without agoraphobia, and generalized anxiety disorder) with CRP as the dependent variable.

Multiple regression models were performed separately for individual anxiety disorders; generalized anxiety, panic disorder without agoraphobia, panic disorder with agoraphobia and agoraphobia without panic disorder, with CRP as dependent variable. Groups with any anxiety disorder and presence of comorbid anxiety disorders (>1) were included in this analysis. The following variables were added to the model: unadjusted; model (1) socio-demographic variables (sex, age, education); model (2) life style factors (smoking status, alcohol use, BMI, frequency of activity); model (3) health (cardiovascular disease and diabetes mellitus), and medication (anti-inflammatory and statins); model (4) adjusted for depression and stress (major depression, LDI and LTE) and anti-depressant medication; model (5) included all variables.

We next examined the differences of CRP levels between control group without anxiety disorders ($n = 48{,}677$) compared to people with anxiety ($n = 5{,}649$) with an independent samples *t*-test. Analyses of variance (ANOVA) with Tukey post hoc test for pair-wise comparisons was used to investigate CRP levels between anxiety disorders; generalized anxiety disorder, social anxiety disorder, and panic disorder with and without agoraphobia. For this analysis, individuals with co-existing anxiety disorders were excluded to adequately evaluate serum CRP levels between the different anxiety disorders. Exclusion of co-existing anxiety disorders resulted in generalized anxiety disorder ($n = 1{,}700$), social anxiety disorder ($n = 170$), panic disorder without agoraphobia ($n = 1050$), panic disorder with agoraphobia ($n = 259$), agoraphobia ($n = 1627$). Subsequently, analyses of covariance (ANCOVA) with post hoc test was performed to examine the potential effects of all determinants (socio-demographic variables, life style factors, health, medication use, depression and stress) in the comparison of serum CRP levels between control vs. presence of anxiety disorders and between the anxiety disorders.

3. Results

3.1. Baseline characteristics

Table 1 provides a description of the characteristics of the total study sample, the control group with participants without anxiety disorders and each anxiety disorder. The total study sample ($n = 54{,}326$) consisting of 22,189 male (40.8%) and 32,137 female participants (59.2%) with a mean age of 47.33 (SD = 11.92). Prevalence of any anxiety disorders was 10.4%, with prevalence rates of generalized anxiety disorder 4.5%, social anxiety disorder 0.9%, panic without agoraphobia 2.3%, panic disorder with agoraphobia 0.8%, agoraphobia without panic disorder 3.7% and major depression 2.7%. Exploration of the details of participants with missing data for alcohol use ($n = 7{,}079$), physical activity ($n = 2{,}057$) and smoking ($n = 4{,}753$) indicated that participants with anxiety disorder or depression were not significantly over-represented in this group; all other variables were comparable between participants with missing and non-missing data. As alcohol use, physical activity and smoking status are important covariates, missing data were replaced with multiple imputations as described in the materials and methods section. Moreover, this increased the sample size with 10,855 participants.

3.2. Interaction of sex and age with the association of anxiety disorders and CRP

No significant interaction effects were found for sex, age or age^2 with separate anxiety disorders (generalized anxiety, panic disorder without agoraphobia, panic disorder with agoraphobia and agoraphobia without panic disorder) and CRP as dependent variable, as $P < .05$ was considered significant for interaction terms (Table 2). Thus, stratified analyses for sex and age were not performed.

Table 1. Sample characteristics.

	Total Sample $N = 54,326$	No anxiety control $N = 48,677$	Generalized anxiety disorder $N = 2,420$	Social anxiety disorder $N = 508$	Panic disorder without agoraphobia $N = 1,260$	Panic disorder with agoraphobia $N = 438$	Agoraphobia without panic disorder $N = 1,993$
Sociodemographic variables							
Age years, mean (SD)	47.33 (11.92)	47.40 (12.01)	45.10 (10.52)	44.62 (11.01)	44.74 (9.95)	45.57 (9.77)	50.02 (11.74)
Sex, female *N* (%)	32,137 (59.2)	28,142 (57.8)	1,683 (69.5)	344 (67.7)	929 (73.7)	336 (76.7)	1,399 (70.2)
Education level							
No/primary school N (%)	9177 (16.89)	7,752 (15.9)	519 (21.5)	103 (20.3)	201 (16)	100 (22.8)	507 (25.4)
High school/applied education N (%)	29,685 (54.64)	26,652 (54.8)	1,371 (56.7)	294 (57.9)	737 (58.5)	258 (58.9)	1,080 (54.2)
Higher education/University N (%)	15,464 (28.47)	14,273 (29.3)	530 (21.9)	111 (21.9)	322 (25.6)	80 (18.3)	406 (20.4)
CRP mean (SD)	2.62 (4.66)	2.58 (4.62)	2.98 (5.01)	2.86 (4.43)	2.93 (5.37)	3.73 (5.80)	2.91 (4.67)
Lifestyle variables							
Smoking status *N* (%)	49,573 (91.3)	44,347 (91.1)	2,256 (93.2)	473 (93.1)	1,161 (92.1)	411 (93.8)	1,837 (92.2)
Never smoked N (%)	21,483 (39.5)	19,616 (40.3)	852 (35.2)	174 (34.3)	357 (28.3)	113 (25.8)	663 (33.3)
Former smoker N (%)	15,840 (29.3)	14,221 (29.2)	619 (25.6)	142 (28.0)	401 (31.8)	117 (26.7)	614 (30.8)
Current smoker N (%)	12,250 (22.5)	10,510 (21.6)	785 (32.4)	157 (30.9)	403 (32.0)	181 (41.3)	560 (28.1)
Alcohol intake *N* (%)	47,247 (87.0)	42,175 (86.6)	2,168 (89.6)	440 (86.6)	1,123 (89.1)	395 (90.2)	1,794 (90.0)
Drinks a week (SD)	1.29 (1.39)	1.30 (1.39)	1.12 (1.38)	1.09 (1.45)	1.19 (1.44)	0.86 (1.21)	1.11 (1.44)
Body Mass Index, mean (SD)	26.2 (4.3)	26.15 (4.24)	26.55 (5.13)	26.63 (5.36)	26.44 (4.79)	27.03 (5.37)	26.76 (4.72)
Frequency of activity per week N (%)	52,269 (96.2)	46,862 (96.3)	2,311 (95.5)	487 (95.9)	1,212 (96.2)	413 (94.3)	1,917 (96.2)
Low activity N (%)	4907 (9.0)	4,404 (9.0)	232 (9.6)	49 (9.6)	118 (9.4)	32 (7.3)	156 (7.8)
Medium activity N (%)	4661 (8.6)	4,232 (8.7)	179 (7.4)	41 (8.1)	98 (7.8)	35 (8.0)	146 (7.6)
High activity N (%)	42,701 (78.6)	38,226 (78.5)	1,900 (78.5)	397 (78.1)	996 (79.0)	346 (79.0)	1,615 (81.0)
Somatic conditions							
Presence of vascular disease *N* (%)	7761 (14.30)	6,642 (13.6)	514 (21.2)	114 (22.4)	231 (18.3)	94 (21.5)	398 (20.0)
Diabetes mellitus *N* (%)	1218 (2.23)	1,018 (2.1)	91 (3.8)	19 (3.7)	31 (2.5)	27 (6.2)	73 (3.7)
Stressful events							
Long term difficulties, mean (SD)	14.47 (2.46)	14.24 (2.24)	17.69 (3.40)	17.82 (3.77)	16.02 (2.97)	17.07 (3.53)	15.48 (3.12)
List of threatening experiences, mean (SD)	1.06 (1.30)	0.99 (1.23)	1.99 (1.81)	1.88 (1.91)	1.46 (1.65)	2.01 (2.02)	1.44 (1.68)
Medication use							
Antidepressants N (%)	1835 (3.4)	1,516 (3.1)	129 (5.3)	27 (5.3)	97 (7.7)	33 (7.5)	98 (4.9)
Anti-inflammatory medication N (%)	1695 (3.1)	1,508 (3.1)	76 (3.1)	10 (2.0)	37 (2.9)	15 (3.4)	69 (3.5)
Statins N (%)	2068 (3.8)	1,847 (3.8)	80 (3.3)	15 (3.0)	37 (2.9)	16 (3.7)	102 (5.1)
Depression (past 2 weeks)							
Major depression N (%)	1441 (2.7)	513 (1.1)	728 (30.1)	156 (30.7)	133 (10.6)	117 (26.7)	187 (9.4)

Table 2. Interaction of sex, age and age^2 with the association of anxiety disorders and CRP.

	Sex (P value)	Age (P value)	Age^2 (P value)
Generalized anxiety	.81	.61	.70
Social anxiety disorder	.79	.14	.27
Panic disorder without agoraphobia	.08	.14	.13
Panic disorder with agoraphobia	.39	.70	.71
Agoraphobia without panic disorder	.31	.30	.25

3.3. Anxiety disorders and CRP

Multiple linear regression analyses were conducted to examine the association of CRP with specific anxiety disorders. As shown in Table 3, generalized anxiety disorder was no longer significantly associated with CRP after correcting for major depression and stress. No significant association between symptoms of social anxiety disorder with CRP was found. Significant associations between CRP and panic disorder without agoraphobia were attenuated after adjustment for demographics (sex, age, education) and life style factors (smoking status, alcohol use, BMI, frequency of activity). The association between CRP with presence of panic disorder with agoraphobia remained significant after adjustment for all covariates (model 5, $B = 0.052$, SE = 0.021, $\beta = 0.010$, $P = .013$). Current symptoms of agoraphobia without panic disorder and the presence of anxiety disorders remained significantly associated with CRP after correction for demographics, life style factors, health, medication use, depression and stress, but significance was not present after adjustments for all covariates in the final model. Significance in associations between CRP and presence of any anxiety disorders and presence of comorbid anxiety disorders was lost after correcting for all variables in model 5. Because previous studies presented sex differences between anxiety disorders and CRP levels, we performed sex-stratified analyses to allow a comparison with previous findings (Supplementary Table 1).

3.4. Serum CRP levels in anxiety disorders

Figure 1(A,B) presents adjusted means of back-transformed CRP levels across different anxiety disorders. Serum CRP levels were significantly increased in people with anxiety disorders 2.96 (SE= 0.07) mg/l compared to people without anxiety disorders 2.58 (SE = 0.02) mg/l ($P < .001$) (Figure 1(A)). However, this significance was lost after controlling for all variables with ANCOVA ($F = 0.55$, df = 1, $P = .46$). Significant differences between the anxiety disorders were found (ANOVA, $F = 5.26$, df = 4, $P < .001$) (Figure 1(B)). Serum CRP levels were significantly elevated in people

Table 3. Determinants of the associations between serum CRP with characteristic of anxiety disorders.

	Generalized anxiety disorder			Social anxiety disorder			Panic disorder without agoraphobia			Panic disorder with agoraphobia			Agoraphobia without panic disorder			Presence of any Anxiety disorders			Comorbid anxiety disorders (>1)		
	B (SE)	β	*P*	*B* (SE)	β	*P*	*B* (SE)	*B*	*P*	*B* (SE)	β	*P*	*B* (SE)	β	*P*	*B* (SE)	β	*P*	*B* (SE)	β	*P*
Unadjusted	**0.051 (0.010)**	**0.022**	**<.001**	0.032 (0.021)	0.007	.124	**0.041 (0.013)**	**0.013**	**.003**	**0.140 (0.023)**	**0.027**	**<.001**	**0.061 (0.011)**	**0.024**	**<.001**	**0.059 (0.007)**	**0.038**	**<.001**	**0.031 (0.008)**	**0.018**	**<.001**
Model 1	**0.031 (0.010)**	**0.014**	**<.001**	0.015 (0.021)	0.003	.463	0.020 (0.013)	0.008	.75	**0.110 (0.022)**	**0.021**	**<.001**	**0.033 (0.011)**	**0.013**	**.002**	**0.034 (0.007)**	**0.022**	**<.001**	**0.018 (0.007)**	**0.011**	**.013**
Model 2	**0.021 (0.009)**	**0.005**	**.02**	0.002 (0.020)	0.000	.914	0.019 (0.012)	0.006	.126	**0.079 (0.021)**	**0.015**	**<.001**	**0.028 (0.010)**	**0.011**	**.005**	**0.029 (0.006)**	**0.019**	**<.001**	0.010 (0.007)	0.006	.154
Model 3	**0.044 (0.010)**	**0.019**	**<.001**	0.026 (0.021)	0.005	.223	**0.038 (0.013)**	**0.012**	**.005**	**0.130 (0.023)**	**0.025**	**<.001**	**0.056 (0.011)**	**0.022**	**<.001**	**0.053 (0.007)**	**0.034**	**<.001**	**0.027 (0.008)**	**0.016**	**<.001**
Model 4	0.011 (0.011)	0.005	.322	−0.007 (0.021)	−0.001	.746	0.025 (0.014)	0.008	.061	**0.104 (0.023)**	**0.020**	**<.001**	**0.048 (0.011)**	**0.019**	**<.001**	**0.039 (0.007)**	**0.025**	**<.001**	0.008 (0.008)	0.004	.331
Model 5	−0.004 (0.010)	−0.002	.646	−0.022 (0.020)	−0.005	.257	−0.004 (0.012)	−0.001	.736	**0.052 (0.021)**	**0.010**	**.013**	0.010 (0.010)	0.004	.371	0.005 (0.007)	0.003	.433	−0.004 (0.007)	−0.002	.638

Model (1): adjusted for demographics; age, age^2, sex and education.
Model (2): adjusted for life style factors; smoking, alcohol, BMI, physical activity.
Model (3): adjusted for health and medication use; cardiovascular disease, diabetes, anti-inflammatory and statins.
Model (4): adjusted for depression and stress; major depression, long-term difficulties, list of threatening experiences and anti-depressant medication.
Model (5): adjusted for all variables in models (2)–(4).
Bold values represent the significant findings.

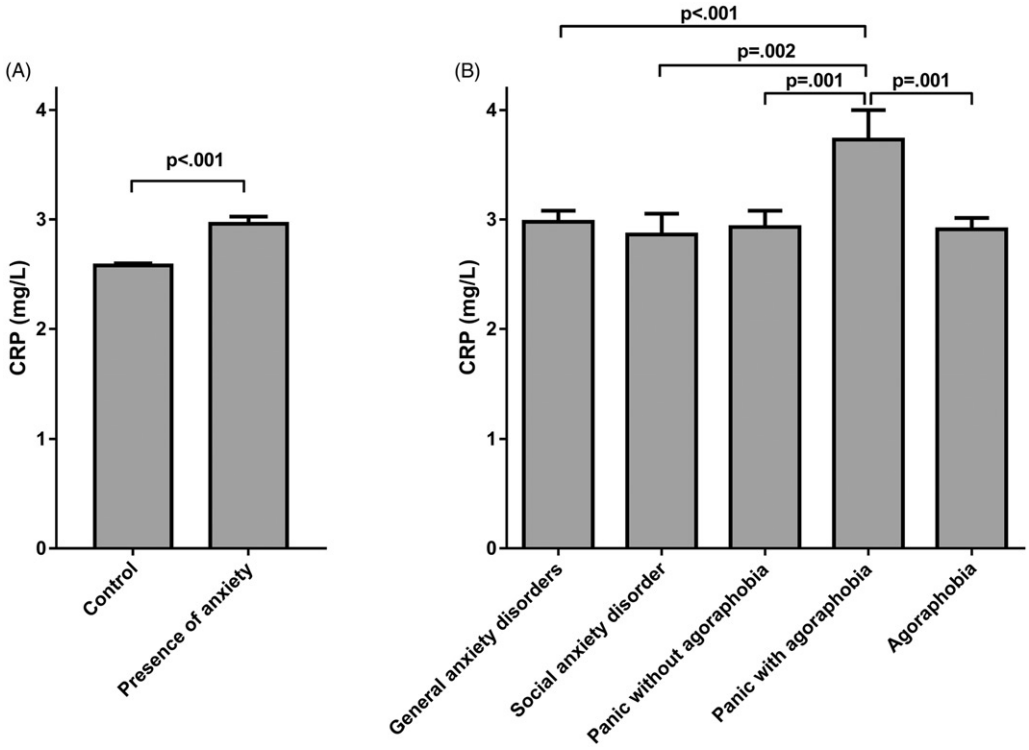

Figure 1. Adjusted marginal mean values of CRP levels in non-anxiety controls and anxiety disorders; generalized anxiety disorders, social anxiety disorder, panic disorder without agoraphobia, panic disorder with agoraphobia and agoraphobia without panic disorder (including *P* values of the ANOVA post hoc subgroup tests between anxiety disorders). The control group was included as a representation of serum CRP levels of individuals without anxiety disorders. Bars indicate the mean protein concentrations in the different study groups and are expressed as mean ± standard error of the mean (SEM).

with current panic disorder with agoraphobia 3.73 (SE = 0.27) mg/l compared to generalized anxiety disorder 2.98 (SE= 0.1) mg/l ($P < .001$), social anxiety disorder 2.8 (SE = 0.19) mg/l ($P = .002$), panic disorder without agoraphobia 2.93 (SE = 0.15) mg/l ($P = .001$) and agoraphobia without panic disorder 2.91 (SE = 0.11) mg/l ($P = .001$). Further analyses with ANCOVA adjusted for all variables ($F = 3.00$, df = 4, $P = .021$) and post hoc analyses showed that serum CRP levels remained significantly increased in current panic disorder with agoraphobia compared to generalized anxiety disorder ($P = .001$), social anxiety disorder ($P = .015$), panic disorder without agoraphobia ($P = .008$) and agoraphobia without panic disorder ($P = .017$).

4. Discussion

This is the largest study to date to investigate associations of CRP between different anxiety disorders. The results from this study show that no interaction of sex or age differences were found in the association of CRP with any anxiety disorders. Anxiety disorders, with the exception of social anxiety disorder, are associated with higher serum CRP levels. This association remained significant for panic disorder with agoraphobia after adjusting for all covariates. Serum CRP levels are significantly increased in people with panic disorder with agoraphobia.

No interaction of sex differences were found in the association of CRP with any anxiety disorders. Studies by Liukkonen et al. (2011) and Vogelzangs et al. (2013) reported associations between anxiety symptoms and CRP in men only, whereas the study by Pitsavos et al. (2006) and Tayefi et al. (2017) found significant associations in both men and women. Our finding supports the notion put forwards by Vogelzangs et al. that sex differences become less relevant in older individuals, as participants in our study (mean age 47 years; 19–92 years) and the population groups in Pitsavos et al. (mean age 45 years; 18–89 years) and Tyefi et al. (mean age 48 years), were older than the population groups of Liukkonen et al. (mean age 31 years) and Vogelzangs et al. (mean age 42 years; 18–65 years). Our results from sex-stratified analyses showed that the associations between CRP and anxiety disorders were generally similar between males and females. Higher significance in females was likely contributed to the higher ratio of females to males. However, CRP levels were particularly elevated in males with panic

disorder without agoraphobia and in females with current panic disorder with agoraphobia.

Our findings are consistent with the literature showing that higher CRP levels are associated with anxiety disorders (Pitsavos et al. 2006; Liukkonen et al. 2011; Copeland et al. 2012; Vogelzangs et al. 2013; Wagner et al. 2015; Tayefi et al. 2017), including generalized anxiety disorder (Copeland et al. 2012). Contrary to the findings of Copeland et al. we found that the significant association between CRP and generalized anxiety disorder was not attenuated by health-related variables but rather major depression and psychological stress, which were not included in the study of Copeland et al. (2012). The confounding effect of depression and psychological distress can be explained by the high comorbidity between depression with in particular generalized anxiety disorder (Bruce et al. 2001; Kessler et al. 2008, 1996) and its association with stressful life events (Moreno-Peral et al. 2014). Addition of psychological stress and depression as covariates may create an over-adjustment and thus an underestimation of the association between CRP and anxiety disorders in this study. However, the stepwise regression analyses illustrate that the correction for these variables did not materially change the associations between CRP and anxiety disorders.

No significant association between CRP and social anxiety disorder was observed in this study, which is in agreement with findings of Vogelzangs et al. (2013) showing that CRP levels were lowest among persons with social anxiety disorder in relation to anxiety disorders. The association between panic disorder without agoraphobia and CRP was no longer significant after adjusting for demographics, lifestyle factors, and co-existing depression, stress and anti-depressant medication. The loss of significance might be due to lifestyle factors such as smoking and sedentary behaviour frequently found in panic disorder (Broocks et al. 1997; Moreno-Peral et al. 2014) and high comorbidity of depression (Hirschfeld 2001) and stressful events (Klauke et al. 2010).

This is the first study to demonstrate the association between CRP and panic disorder with agoraphobia. Higher CRP levels were significantly associated with panic disorder with agoraphobia, which remained independent of all variables. To control whether this finding might be attributed to co-existing anxiety disorders, we further examined the associations between CRP levels with the presence of any anxiety disorders or number of comorbid anxiety disorders, which lost significant after controlling for all variables. Higher CRP levels were associated with agoraphobia but significance was lost after accounting for all variables. This is in accordance with the study by Wagner et al. (2015) showing that individuals with diagnosis of lifetime agoraphobia had higher CRP levels at follow-up (5.5 ± 0.4 years) in a random population sample of 2,890 persons with 124 individuals.

CRP levels were significantly elevated in panic disorder with agoraphobia compared to other anxiety disorders, which remained significant after adjustments for all variables, including depression and psychological stressors. Panic disorder with agoraphobia may be characterized by an exaggerated inflammatory status. It is intriguing why CRP levels were significantly higher in panic disorder with agoraphobia compared to panic disorder alone. This observation might be explained by the premise that the presence of agoraphobia increases the severity of panic disorder (Starcevic et al. 1993). Our data suggest that higher serum CRP levels in panic disorder with agoraphobia are not attributed to the presence of comorbid anxiety disorders or number of co-existing anxiety disorders. A recent study determined an abundance of up to 250 serum analytes in 120 people with panic disorder, with or without agoraphobia (Gottschalk et al. 2016). Increased CRP concentrations was identified as one of the 13 analytes that significantly differed in panic disorder without agoraphobia compared to panic disorder with agoraphobia (Gottschalk, Cooper, Chan, Bot, Penninx and Bahn 2016). These data further strengthen our results showing that higher levels of CRP is present in people with panic disorder with agoraphobia as compared to other anxiety disorders. The small effect size ($\beta = 0.01$) of the association between CRP and panic disorder with agoraphobia should be considered for interpretation of the data of this study and implications for future research. CRP used in this study is a general measure for inflammation. In this context, the findings of this study potentially indicate the involvement of specific (neuro)immune mechanisms that are involved in the pathophysiology of panic disorder with agoraphobia.

This study has some limitations that should be considered when interpreting the data. It is important to note that the percentages for depressive and anxiety disorders in the LifeLines study are lower than that previously reported for the general population (de Graaf et al. 2012). This might be explained by the fact that we used current diagnoses (past 2 weeks) for anxiety and depression, whereas earlier reports used past year diagnoses. PTSD, specific phobia and childhood trauma were not included and may affect the outcomes reported in this study. Further, the presence of general medical conditions, as co-morbidities diagnosis, has been established based on answers of a

self-administered questionnaire, which is less accurate than data based on medical records. Serum CRP levels were used as marker for inflammation. Additional pro- and anti-inflammatory cytokines can provide an improved understanding of immune status and specific immune mechanisms involved in anxiety disorders. A final, but important limitation is that the study has a cross-sectional design, limiting the conclusions on prospective relations between inflammation and anxiety disorders. Significant strengths of this study are; the population design, the large number of participants (the largest study published to date), the use of a clinical interview for the assessment of psychiatric diagnosis and the large number of covariates that is included.

In conclusion, in a population cohort of 54,326 people we found significant associations between higher serum CRP levels and anxiety disorders, except for social anxiety disorder. This association was most robust in panic disorder with agoraphobia, where it was independent of all variables that could explain its association. Indeed, our data suggest that panic disorder with agoraphobia is characterized by an exaggerated inflammatory status, in comparison to other anxiety disorders. The findings that panic disorder with or without agoraphobia and agoraphobia without panic disorder were associated with increased CRP, independent of depression and psychological stressors, suggests that these conditions possess a differential neuroinflammatory profile. The data from this study provides further insights in the differential immune regulation in anxiety disorders.

Acknowledgements

The authors wish to acknowledge the services of the Lifelines Cohort Study and Biobank, the contributing research centres delivering data and all the study participants. Lifelines adheres to standards for open data availability. The data catalogue of Lifelines is publicly accessible at www.lifelines.net. Researchers can apply for data at the Lifelines research office. The Lifelines system allows access for reproducibility of the study results.

Statement of interest

None to declare.

Funding

This work was supported by a VICI grant [no: 91812607] received by P. de Jonge from the Netherlands organization for Scientific research (NWO-ZonMW) and the Mandema Stipendium received by B. Doornbos. D. Stein is supported by the South African Medical Research Council.

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