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Published in final edited form as:

Title: The bidirectional relationship between anxiety disorders and circulating levels of inflammatory markers: Results from a large longitudinal population-based study.

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Journal: Depression and anxiety

Year: 2017

DOI: [10.1002/da.22710](https://doi.org/10.1002/da.22710)

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The bidirectional relationship between anxiety disorders and circulating levels of inflammatory markers: results from a large longitudinal population-based study

Running title: Anxiety disorders and inflammation

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Keywords: anxiety disorders, agoraphobia, social phobia, panic disorder, cytokines, C-Reactive Protein, body mass index.

CONFLICT OF INTEREST DISCLOSURE AND FINANCIAL SUPPORT

P. Vollenweider, G. Waeber and M. Preisig received two unrestricted grants from GlaxoSmithKline Clinical Genetics to build the CoLaus|PsyCoLaus study. J. Glaus received support from the Swiss National Science Foundation and from the Intramural Research Program of the National Institute of Mental Health through a Visiting Fellowship. A. Lasserre received support from the Swiss National Science Foundation. The remaining authors report no biomedical financial interests or potential conflicts of interest.

ABSTRACT

Background: Whereas there has been abundant research on chronic low-grade inflammation as a potential mechanism underlying the link between mood disorders and cardiovascular risk, less is known about the role of inflammatory factors and anxiety disorders. The aim of this paper is to evaluate the bi-directional associations between inflammatory markers including Interleukin (IL)-6, Tumor Necrosis Factor (TNF)- α and high sensitivity C-reactive protein (hsCRP) with anxiety disorders and its subgroups.

Methods: The sample consisted of 3,113 participants (53.7% women; mean age: 51.0, s.d. 8.8 years), randomly selected from the general population, who underwent comprehensive somatic and psychiatric evaluations at baseline and follow-up (mean follow-up duration = 5.5 years, s.d. 0.6). Anxiety disorders were assessed with semi-structured diagnostic interviews. Inflammatory biomarkers were analyzed in fasting blood samples.

Results: After adjustment for potential confounders, current anxiety disorders ($\beta=0.09$, 95%CI 0.00-0.17) and agoraphobia ($\beta=0.25$, 95% CI: 0.07-0.43) at baseline were associated with a steeper increase of hsCRP levels over the follow-up period. Current post-traumatic stress disorder (PTSD) was associated with a lower increase of IL-6 levels over the follow-up period ($\beta=-0.52$, 95% CI: -1.00/-0.04). There was no evidence for an association between inflammation markers at baseline and anxiety disorders at follow-up.

Conclusions: The prospective association between agoraphobia at baseline and hsCRP levels over the follow-up period suggests that chronic low-grade inflammation may be a consequence of this condition. The decrease in IL-6 in PTSD also requires further investigation. No evidence was found for chronic low-grade inflammation as a predictor of future anxiety disorders.

INTRODUCTION

There is consistent evidence for a potent association between anxiety disorders and medical conditions, particularly cardiovascular risk factors (CVRFs) and diseases (CVD) (Furtado, & Katzman, 2015; Glaus et al., 2013; Tully, Cosh, & Baune, 2013; Van der Kooy et al., 2007). Medical comorbidity is associated with substantially greater disability than anxiety disorders alone (Furtado, & Katzman, 2015). Recent research has examined the role of chronic low-grade inflammation as a potential mechanism underlying the association between anxiety disorders and CVRFs/CVD (Furtado, & Katzman, 2015; Salim, Chugh, & Asghar, 2012). Insight into the role of inflammation in anxiety disorders could inform novel treatments and reduce the risk of anxiety disorders through the prevention of chronic inflammation. Compared to depression, studies on the role of the immune system in anxiety disorders are scarce, and it is still unclear to which degree chronic inflammation is implicated in their etiology and consequences (Furtado, & Katzman, 2015; Salim, Chugh, & Asghar, 2012; Turna, Grosman Kaplan, Anglin, & Van Ameringen, 2016). Psychological stress related to anxiety disorders has been shown to dysregulate the hypothalamic-pituitary-adrenal (HPA) axis with an activation of the innate inflammatory response, evidenced by increases in Interleukin (IL)-1 β , IL-6 and Tumor Necrosis Factor (TNF)- α , as well as the C-Reactive Protein (CRP) (Furtado, & Katzman, 2015). Moreover, increased oxidative stress could lead to chronic activation of pro-inflammatory markers which could alter anxiety disorders, such as generalized anxiety disorder (GAD), social phobia and panic disorder (Salim, Chugh, & Asghar, 2012).

Existing literature on the associations between aggregate anxiety disorders and specific subtypes thereof with increased inflammation is limited and comorbidity with other mental disorders has not always been taken into consideration (Gill, Saligan, Woods, & Page, 2009; Khandaker, Zammit, Lewis, & Jones, 2016; Liukkonen et al., 2011; Pitsavos et al., 2006; Turna, Grosman Kaplan, Anglin, & Van Ameringen, 2016; Vogelzangs, Beekman, de Jonge, & Penninx, 2013). With respect to specific subtypes of anxiety, the most consistent evidence has been found for increased levels of inflammatory markers

with post-traumatic stress disorder (PTSD) (Gill, Saligan, Woods, & Page, 2009; Spitzer et al., 2010; von Kanel et al., 2007). Moreover, a recent cross-sectional study of adolescents found an association between GAD and increased CRP levels (Khandaker, Zammit, Lewis, & Jones, 2016). Evidence for an association between inflammatory markers and obsessive-compulsive disorder (OCD) has also been found, even though results of a series of studies are inconsistent (Turna, Grosman Kaplan, Anglin, & Van Ameringen, 2016). Although previous research consistently shows increased levels of inflammation in anxiety disorders, longitudinal studies that can inform the ordinal patterns of inflammation with respect to the onset and recurrence of anxiety disorder subtypes are necessary to inform potential mechanisms for these associations. The only prospective study of inflammatory factors and anxiety in a community sample of youth showed a bidirectional association between GAD and CRP that was attenuated after controlling for the mediating effect of BMI and medication use (Copeland et al., 2012). Our previous analyses of inflammation and agoraphobia showed that a history of agoraphobia was associated with subsequent increase in CRP and TNF- α levels (Wagner et al., 2015).

Accordingly, the aims of the present study were: (1) to evaluate the specificity of 5-year prospective associations between aggregate anxiety disorders and specific subtypes, (i.e., GAD, social phobia, panic disorder, agoraphobia, OCD and PTSD), and changes in circulating levels of specific inflammatory markers (IL-6, TNF- α , hsCRP); (2) to assess the directional associations between inflammatory markers and anxiety disorders and subtypes; (3) to examine whether the markers comprise state versus trait indices by comparing remitted versus current disorders, as well as incident cases; and (4) to examine the role of health behaviors, CVRFs, and treatment factors that may influence these associations.

MATERIALS AND METHODS

Study sample

The data of the present investigation were drawn from CoLaus|PsyCoLaus (Firmann et al., 2008; Preisig et al., 2009), a cohort study designed to prospectively assess the associations of mental disorders with CVD and CVRFs in the community. The sample was randomly selected from the civil register of the city of Lausanne (Switzerland) in 2003. Sixty-seven percent of the 35 to 66 year-old subjects ($n = 5,535$) who underwent the physical exam between 2003 and 2006 also participated in a psychiatric evaluation, which resulted in a sample of 3,719 individuals (Preisig et al., 2009) (see Figure 1, flow chart). Five years later, 3,191 participated in a follow-up physical exam (85.8% participation). Among these, 61 participants were excluded due to missing information on inflammatory markers both at baseline and follow-up, 14 subjects were excluded due to missing information on mental disorders and 3 subjects were excluded due to missing information on CVRFs. The final sample used to examine associations between anxiety disorders at baseline and inflammation at follow-up consisted of 3,113 participants in total (53.7% women; mean age: 51.0, s.d. 8.8 years): 2,791 participants were analyzed for cytokine levels, and 2,891 for hsCRP levels. The mean follow-up duration was 5.5 years (s.d. 0.6 years). The number of participants with data from the psychiatric evaluation at follow-up was 2,833. Among these, 258 participants were excluded due to missing information on inflammatory markers and 2 participants were excluded due to missing information on CVRFs. The resulting number of participants used to determine the association between inflammation at baseline and anxiety disorders at follow-up was 2,573. The Institutional Ethics' Committee of the University of Lausanne approved the CoLaus and the PsyCoLaus study. All participants provided written informed consent for the study protocol.

Measurements

Anxiety disorders and comorbid disorders

Mental disorders at baseline and follow-up were assessed using the French version (Leboyer et al., 1995) of the semi-structured Diagnostic Interview for Genetic Studies (DIGS), which was developed and

validated by the National Institute of Mental Health (Nurnberger et al., 1994). The French version of this instrument (Leboyer et al., 1995) revealed adequate inter-rater reliability in terms of kappa and Yule's coefficients for major mood and psychotic disorders (Preisig et al., 1999) as well as substance use disorders (Berney et al., 2002), whereas the 6-week test-retest reliability was slightly lower (Berney et al., 2002; Preisig et al., 1999). The DIGS was completed with sections on GAD, PTSD and phobia disorders using questions from the Schedule for Affective Disorders and Schizophrenia - Lifetime and Anxiety disorder version (SADS-LA (Endicott, & Spitzer, 1978)), which also revealed satisfactory test-retest reliability for panic disorder/agoraphobia (Yule's $Y = 0.43$), GAD (Yule's $Y = 0.61$) and phobic disorders (Yule's $Y = 0.66$). The Yule coefficient for the overall category of anxiety disorders was 0.49. (Leboyer et al., 1991). In our own reliability study, we found excellent or perfect inter-rater reliability for all specific anxiety disorders except for agoraphobia (Yule's $Y = 0.96$), whereas the 6-week test-retest reliability estimates were 0.58 for panic disorder, 0.55 for agoraphobia, 0.44 for social phobia and 0.77 for specific phobia. (Rougemont-Buecking et al., 2008). Interviewers were required to be masters-level psychologists and were trained over a two-month period. In order to provide ongoing supervision throughout the study, each interview and diagnostic assignment was reviewed by an experienced senior psychologist. Diagnoses were assigned according to the DSM-IV. Anxiety disorders included GAD, social phobia, panic disorder, agoraphobia, OCD and PTSD. For this study, anxiety disorders were defined as current if they were present at the time of the physical evaluation.

Inflammatory markers

HsCRP was assessed during baseline and follow-up physical evaluations using immunoassay and latex HS (IMMULITE 1000-High, Diagnostic Products Corporation, LA, CA, USA), with maximum intra- and interbatch coefficients of variation of 1.3% and 4.6%, respectively (Firmann et al., 2008). Subjects with a hsCRP level higher than 10 mg/l were excluded as an elevation of this magnitude is likely to be

attributable to acute infection (Pearson et al., 2003). For the baseline and follow-up cytokine measurements, serum was preferred to plasma, as it has been shown that different anticoagulants may differentially affect absolute cytokine levels (Skeppholm, Wallen, Blomback, & Kallner, 2008). Serum samples were stored at -80°C before assessment and sent on dry ice to the laboratory. Cytokine levels were measured using a multiplexed particle-based flow cytometric cytokine assay (Marques-Vidal et al., 2011). Lower detection limits (LOD) for IL-6 and TNF- α were 0.2 pg/ml. Good agreement between signal and cytokine was found within the assay range ($R^2 \geq 0.99$).

Covariates

Data were collected on age, race (Caucasian versus non-Caucasian), marital status (living alone versus living with someone) and health-related behaviors at baseline including smoking (never, former, current) and physical inactivity (no or low versus at least 20 minutes twice a week). Information on socio-economic status (SES) and on psychotropic drug treatment was derived from the DIGS. The level of SES was assessed using the Hollingshead scale (Hollingshead, 1975). The section on psychotropic drug treatment covered all antidepressants, mood stabilizers (lithium and anti-epileptics), antipsychotics and anxiolytics. Moreover, information on aspirin, statin and nonsteroidal anti-inflammatory use was collected during the baseline physical evaluation (never versus occasional versus regular use). The following biological variables were measured during the baseline physical evaluation: BMI (weight in kilograms divided by height in meters squared), diabetes (fasting blood glucose ≥ 7 mmol/l or treatment for diabetes), dyslipidemia (HDL-cholesterol < 1 mmol/l, or LDL-cholesterol ≥ 4.1 mmol/l, or triglycerides ≥ 2.2 mmol/l, or treatment with a lipid-lowering drug), hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or treatment for hypertension).

Statistical analysis

Statistical analyses were conducted using the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA), version 9.3 for Windows. Age was standardized. Descriptive data for demographic characteristics, comorbid disorders, medication, health-related behaviors, physical risk factors for CVD and inflammatory marker levels were derived by lifetime anxiety disorders, and Chi-square and Kruskal-Wallis tests were used to determine the difference between anxiety disorders. The tests were two-tailed and p-values were not adjusted for multiple testing because the hypothesized associations between anxiety disorders and inflammatory markers were specified a priori.

Associations between current and remitted anxiety disorders at baseline and levels of inflammatory markers at follow-up with adjustment for the respective levels at baseline were determined using multiple linear regression models. Because we adjusted 5-year inflammatory markers for the corresponding inflammatory marker level at baseline, the follow-up variables represent change scores (e.g., 5-year change in hsCRP). Box-Cox transformation was applied to the response variable whenever a deviation from fundamental assumptions was observed. Values below the LOD of 0.2 [pg/ml] (i.e., 5% of values for IL-6, 0.5% of values for TNF- α) were considered as censored observations. IL-6 and TNF- α were analyzed using the qualitative and limited dependent variable model (QLIM) with the threshold of -1.65 [log (pg/ml)] as the meaningful lower bound of observed values.

A multiple linear regression model was performed on log-transformed and standardized observed values. Two models of increasing complexity were computed. Model 1 included one single anxiety disorder (lifetime, current and remitted) at a time as the independent variable, adjusted for the corresponding inflammatory marker at baseline. Model 2 included current and remitted anxiety disorders simultaneously in order to determine the associations of specific anxiety disorder with inflammatory markers at follow-up, adjusting for the length of follow-up, sociodemographic characteristics (sex, age, SES, race and marital status), comorbid disorders (major depressive disorder, bipolar disorder and substance use disorders), medications (psychotropic medication, aspirin, statins

and nonsteroidal anti-inflammatories), health-related behaviors and physical risk factors for CVD at baseline and the covariate of Model 1. We did not adjust for steroid medications because of the low proportion of people taking immunosuppressants ($n = 4$) or steroid inhalers ($n = 61$).

Associations between inflammatory markers at baseline and a history of anxiety disorders during the follow-up period were assessed using logistic regression models. Two models were computed. Model 1 included all inflammatory markers simultaneously, adjusting for the corresponding history of anxiety disorder at baseline. Model 2 was further adjusted for the length of follow-up, sociodemographic characteristics, medications, health-related behaviors and physical risk factors for CVD.

RESULTS

Sample characteristics

The description of the cohort of 3,113 participants used for the assessment of the prospective associations between anxiety disorders at baseline and the concentrations of inflammatory markers at follow-up is presented in Table 1. The groups (GAD, social phobia, panic disorder, agoraphobia, OCD, PTSD and no history of anxiety disorders) differed with respect to sex, SES, ethnicity, marital status, comorbid disorders (except for substance use disorders), psychotropic medication and BMI.

HsCRP levels for current agoraphobia (median 1.05 [IQR 0.60-2.15]), remitted agoraphobia (0.95 [0.45-2.05]) and no anxiety disorders (1.00 [0.50-2.10]) at baseline did not differ among the 3 groups (p -value = 0.781), whereas hsCRP levels for current agoraphobia (1.60 [0.70-3.35]), remitted agoraphobia (0.95 [0.65-2.40]) and no anxiety disorders (1.10 [0.60-2.10]) at follow-up were statistically different among the 3 diagnostic groups (p -value = 0.023).

Since no significant sex interactions were found, models were run for males and females together.

Associations between anxiety disorders at baseline and inflammatory markers at follow-up

Associations between current and remitted anxiety disorders at baseline and levels of inflammatory markers at follow-up with adjustment for the respective level at baseline are presented in Table 2 and 3. When models were only adjusted for the corresponding inflammatory marker at baseline (Table 2), current anxiety disorders, as well as current agoraphobia were associated with a steeper increase of hsCRP levels over the follow-up period. In contrast, current PTSD was associated with a lower increase of IL-6 levels over the follow-up period. No significant associations were found for remitted anxiety disorders with inflammatory markers. When the model was further adjusted for potential confounders, including depression, current anxiety disorders were still significantly associated with hsCRP levels (Table 3). Moreover, current agoraphobia was associated with a steeper increase of hsCRP levels and remitted agoraphobia was newly associated with a steeper increase of TNF- α levels over the follow-up period in the fully adjusted model. Finally, the association between current PTSD and a lower increase of IL-6 levels over the follow-up period remained significant.

Associations between inflammatory markers at baseline and anxiety disorders at follow-up

Levels of the 3 inflammatory markers at baseline were not associated with any lifetime anxiety disorder at follow-up in either model (Table 4).

We also tested the associations between inflammatory markers at baseline and incident cases of anxiety disorders excluding subjects that already had a lifetime history of these disorders at baseline. However, no significant associations were found (results not shown).

DISCUSSION

Based on a large adult cohort with comprehensive physical and psychiatric evaluations at baseline and follow-up, we found significant associations between current anxiety disorders in general, and current

agoraphobia specifically, at baseline with a steeper increase of hsCRP levels over a more than five year follow-up period. Moreover, current PTSD at baseline was associated with a lower increase of IL-6 levels over the follow-up period. These associations remained significant after adjustment for a comprehensive array of potential confounders, including major depressive disorder. No evidence was found for associations between the other anxiety disorder subtypes with any pro-inflammatory markers.

Consistent with previous literature, anxiety disorders were associated with a steeper increase of hsCRP levels over the follow-up period after adjustment for multiple covariates (Liukkonen et al., 2006; Pitsavos et al., 2006; Vogelzangs, Beekman, de Jonge, & Penninx, 2013). Our further evaluation of specific subtypes of anxiety disorders that may explain this association revealed that the findings regarding hsCRP were specifically attributable to agoraphobia, confirming our previous analyses of lifetime anxiety disorders with inflammatory markers (Wagner et al., 2015). Similar to previous findings (Khandaker, Zammit, Lewis, & Jones, 2016; Pitsavos et al., 2006; Vogelzangs, Beekman, de Jonge, & Penninx, 2013), these results were not confounded by depressive disorders. The link between hsCRP and agoraphobia but not panic disorder may be attributable to the trait-like manifestations of agoraphobia versus the episodic fluctuations of panic attacks.

To our knowledge, this study is the first to examine the association between inflammatory markers with the full range of anxiety disorder subtypes. Our findings do not confirm those of a recent cross-sectional population-based study that showed an association between GAD and increased CRP levels in adolescents (Khandaker, Zammit, Lewis, & Jones, 2016). However, the latter study did not adjust for the potentially confounding influences of psychotropic or inflammatory medications. Indeed, a prospective study of children found that the association between GAD and elevated CRP levels was mediated by health-related behaviors, such as BMI and medication (Copeland et al., 2012). Moreover, consistent with

our investigations, they found no association between CRP levels and subsequent GAD (Copeland et al., 2012). This finding implies that inflammation may be a consequence of anxiety or its correlates, rather than the opposite. Our findings are also inconsistent with the only other study of a population-based sample of older adults that found that CRP was associated with increased psychosocial distress, including anxiety (Das, 2016). Therefore, future studies are needed to confirm our findings and to explore the potential mechanisms for this association.

Our finding of a lower increase of IL-6 levels over the follow-up period in participants with PTSD at baseline is not consistent with results from several previous cross-sectional studies that showed either increased (Baker et al., 2001; von Kanel et al., 2010) or similar (Miller, Sutherland, Hutchison, & Alexander, 2001; Vidovic et al., 2011) levels of IL-6 in patients with PTSD compared to non-PTSD controls. Nevertheless, one previous study also revealed lower IL-6 levels in military veterans with lifetime history of PTSD than in their counterparts without PTSD, after adjustment for sociodemographic factors, health behaviors, traditional cardiovascular risk factors and medications (Plantinga et al., 2013). As IL-6 may also act as an anti-inflammatory cytokine through stimulation of the HPA axis, the authors of the study argued that low IL-6 levels could reflect long-term suppression of the anti-inflammatory IL-6 response due to a hypersensitive HPA axis (Plantinga et al., 2013). In support of such reasoning, we found that current but not remitted PTSD was associated with lower IL-6 levels during the follow-up period compared to non-PTSD controls.

Current, but not remitted, anxiety disorders in our sample were associated with a steeper increase of hsCRP levels over the follow-up period after adjustment for multiple covariates, while only remitted agoraphobia was associated with a steeper increase of TNF- α levels over the follow-up period. In cross-sectional analyses, remitted agoraphobia at baseline was not associated with hsCRP levels at baseline, and the same for cross-sectional analyses at follow-up. Further analyses among the remitted

agoraphobia group at baseline ($n = 34$) showed that these participants had only one episode in their lifetime and the average time in remission was around 14.5 years (s.d. 9.8 years). For hsCRP, but not necessarily for TNF- α , these findings suggest that once anxiety has waned, inflammation may also decrease. Therefore, with respect to anxiety disorders, inflammation seems to be a "scar" which may be persistent for a certain duration of time even after remission. However, after sufficient time, this "scar" may "heal". Subsequently, future studies should investigate consequences of having had high inflammatory levels and the amount of agoraphobia remission time needed in order to "heal" and not to be anymore at increased risk of consequent cardiovascular diseases. Consequently, the anxiety status should be taken into consideration for the prevention and treatment of chronic inflammation. However, repeated assessments over time of different inflammatory markers would be needed to substantiate such a temporal relationship. Interestingly, in our previous cross-sectional analysis, anxiety disorders were associated with lower levels of hsCRP (Glaus et al., 2014). At the time, we suggested that this association may have been attributable to the low BMI of subjects with anxiety disorders (Glaus et al., 2014). This may be less the case in this longitudinal study since we controlled for BMI. However, further longitudinal analysis of the associations between anxiety disorders and BMI and other measures of adiposity are needed to better elucidate these contradictory results.

The finding of a prospective association of anxiety disorders with CRP in our study may provide one explanation for the link between anxiety and an increased risk of future coronary heart disease (CHD) and CVD mortality in the general population reported in a recent meta-analysis of more than two millions of participants (Emdin et al., 2016). The acute phase reactant CRP is arguably the most established circulating inflammatory biomarker of increased CVD (Vlachopoulos et al., 2015), which has been shown to add prognostic information on future CVD risk above and beyond the Framingham CHD risk score (Pearson et al., 2003). Regarding the clinical relevance of our findings, cut points of CRP for

low risk (<1.0 mg/L), average risk (1.0-3.0 mg/L), and high risk (>3.0 mg/L) of CHD have been defined, with the high-risk category showing a 2-fold higher relative risk of CHD than the low risk group (Pearson et al., 2003). Therefore, a 78% increase in the CRP median level in individuals with agoraphobia compared with the average CRP level in our cohort may seem a clinically meaningful difference in the relative risk of CHD.

One candidate mechanism that may facilitate inflammation and elevated hsCRP in anxiety disorders is autonomic nervous system dysfunction as reflected in reduced heart rate variability (Chalmers, Quintana, Abbott, & Kemp, 2014) that is associated with heightened inflammation (Haensel et al., 2008). Although we did not investigate potential mechanisms, such as autonomic nervous system and HPA axis dysfunction, that might play a specific role in increased inflammation in agoraphobia as opposed to the other anxiety disorders, as noted above, these systems may be more chronically dysregulated in more stable conditions such as agoraphobia. As a consequence, sympathetic arousal and stress hormone production could be more sustained in agoraphobia through kindling of greater chronic low-grade inflammation (Elenkov, 2008). However, this remains speculative as, to our knowledge, systematic investigations on autonomic, neuroendocrine, and immune function in patients with agoraphobia (without panic disorder) in comparison with other anxiety disorders, including PTSD and OCD, have not previously been performed.

Our results should be interpreted in the context of several limitations. First, there was an interval of almost one year between the physical and the psychiatric evaluations. Although the timing of anxiety episodes was elicited in our diagnostic interviews, we cannot exclude misclassification regarding the "current" status of disorders at the time of the physical evaluation. Second, similar to most prior studies, inflammatory markers were only assessed once at baseline and again at follow-up, and thus did not

reflect the dynamic changes of inflammatory markers over time. However, the majority of previous studies also collected only a single measure per assessment. Third, low-grade inflammation was measured by hsCRP and cytokine levels, but other potential biomarkers of inflammation, such as anti-inflammatory cytokines and white blood cells were not included. Despite these limitations, this is one of the largest prospective studies of a community sample, with the hitherto longest follow-up period. It is the first study to investigate the full range of anxiety disorder subtypes, measuring both inflammatory markers and anxiety disorders at both times which design permitted us to disentangle some of the bi-directional associations. Moreover, this study is the first that distinguished anxiety as a state versus a trait marker. To our knowledge, no previous studies looked at both current and remitted anxiety disorders in association with subsequent inflammation.

CONCLUSION

The results of this study suggest a direct association between current anxiety disorders at baseline and chronic low-grade inflammation at follow-up. In particular, current agoraphobia was prospectively associated with a steeper increase of hsCRP levels and PTSD was associated with a lower increase of IL-6 levels over the follow-up period. Because anxiety disorders were prospectively associated with changes in these inflammatory markers that were not associated in remitted cases, these findings suggest that inflammation may be a consequence rather than risk factor for anxiety disorders. Further prospective studies with repeated assessments of inflammatory markers earlier in development are required to confirm potential mechanisms for the potential causal link between anxiety disorders and inflammation.

ACKNOWLEDGEMENTS

The CoLaus|PsyCoLaus study was and is supported by research grants from GlaxoSmithKline, Verona, Italy, the Faculty of Biology and Medicine of Lausanne (Switzerland), the Swiss National Science Foundation (Switzerland) (grants 3200B0-105993, 3200B0-118308, 33CSCO-122661, 33CS30-139468 and 33CS30-148401), and in part by the Intramural Research Program of the National Institute of Mental Health (ZIAMH002932). The funders had no role in the design of the study; the collection, management, analysis, and interpretation of the data; the preparation, review or approval of the manuscript; or the decision to submit the manuscript for publication.

The authors would like to express their gratitude to the Lausanne inhabitants who volunteered to participate in the PsyCoLaus study and to the collaborators who contributed to the coordination of the study and the collection of data. We would also like to thank all the investigators of the CoLaus study, who made the psychiatric study possible, as well as many GSK employees who contributed to the execution of this study.

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Table 1: Sociodemographic, Medication, Health-Related Behaviors, Physical Risk Factors for Cardiovascular Disorders and Inflammatory markers by lifetime anxiety disorders at baseline (n=3,113)

	Anxiety disorders n = 652		GAD n = 74		Social Phobia n = 384		Panic Disorder n = 78		Agoraphobia n = 116		OCD n = 43		PTSD n = 118		No Anxiety n = 2461		p-value ^a	
	Current n = 412		Current n = 39		Current n = 255		Current n = 34		Current n = 82		Current n = 22		Current n = 50					
	Remitted n = 240		Remitted n = 35		Remitted n = 129		Remitted n = 44		Remitted n = 34		Remitted n = 21		Remitted n = 68					
Sociodemographics																		
Length of follow-up [years], mean (SD)	5.49	(0.62)	5.53	(0.62)	5.53	(0.63)	5.53	(0.57)	5.47	(0.61)	5.47	(0.59)	5.46	(0.59)	5.53	(0.61)	0.439 ^b	
Sex, n (%)																		
Female	427	(65.49)	47	(63.51)	243	(63.28)	57	(73.08)	90	(77.59)	29	(67.44)	82	(69.49)	1245	(50.59)	<.0001	
Male	225	(34.51)	27	(36.49)	141	(36.72)	21	(26.92)	26	(22.41)	14	(32.56)	36	(30.51)	1216	(49.41)		
Age [years], mean (SD)	50.69	(8.70)	53.02	(8.87)	50.06	(8.64)	50.72	(8.81)	51.96	(8.54)	48.60	(9.15)	51.08	(8.82)	51.08	(8.85)	0.173 ^b	
Socio-economic status ^c , mean (SD)	3.31	(1.27)	3.19	(1.35)	3.33	(1.24)	3.22	(1.26)	3.17	(1.29)	3.37	(1.33)	3.08	(1.35)	3.45	(1.27)	0.037 ^b	
Ethnicity, n (%)																		
Caucasian	611	(93.71)	70	(94.59)	362	(94.27)	73	(93.59)	114	(98.28)	42	(97.67)	103	(87.29)	2265	(92.04)	0.014	
Other	41	(6.29)	4	(5.41)	22	(5.73)	5	(6.41)	2	(1.72)	1	(2.33)	15	(12.71)	196	(7.96)		
Marital status, n (%)																		
Living alone	252	(38.65)	25	(33.78)	148	(38.54)	23	(29.49)	45	(38.79)	21	(48.84)	50	(42.37)	748	(30.39)	0.004	
Living in couple	400	(61.35)	49	(66.22)	236	(61.46)	55	(70.51)	71	(61.21)	22	(51.16)	68	(57.63)	1713	(69.61)		
Comorbid disorders, n (%)																		
Bipolar Disorders	24	(3.68)	5	(6.76)	16	(4.17)	1	(1.28)	5	(4.31)	4	(9.30)	4	(3.39)	30	(1.22)	0.000	
Major Depressive disorders	402	(61.66)	49	(66.22)	235	(61.20)	47	(60.26)	65	(56.03)	27	(62.79)	88	(74.58)	963	(39.13)	<.0001	
Atypical MDD	63	(9.66)	4	(5.41)	38	(9.90)	2	(2.56)	10	(8.62)	1	(2.33)	17	(14.41)	142	(5.77)	0.001	
Substance use disorders	105	(16.10)	9	(12.16)	60	(15.63)	10	(12.82)	17	(14.66)	10	(23.26)	29	(24.58)	364	(14.79)	0.065	
Medication, n (%)																		
Antidipressants	217	(33.28)	31	(41.89)	121	(31.51)	30	(38.46)	44	(37.93)	23	(53.49)	43	(36.44)	421	(17.11)	<.0001	
Mood stabilizers	11	(1.69)	4	(5.41)	3	(0.78)	1	(1.28)	4	(3.45)	2	(4.65)	2	(1.69)	10	(0.41)	0.003	
Antipsychotics	27	(4.14)	5	(6.76)	14	(3.65)	6	(7.69)	8	(6.90)	5	(11.63)	6	(5.08)	23	(0.93)	<.0001	
Anxiolytics	273	(41.9)	42	(56.8)	139	(36.2)	46	(59.0)	71	(61.2)	22	(51.2)	59	(50.0)	540	(21.9)	<.0001	
Aspirin	58	(8.90)	9	(12.16)	26	(6.77)	6	(7.69)	10	(8.62)	4	(9.30)	12	(10.17)	202	(8.21)	0.963	
Statins	55	(8.44)	7	(9.46)	28	(7.29)	10	(12.82)	15	(12.93)	3	(6.98)	11	(9.32)	176	(7.15)	0.172	
NSAIDs	107	(16.41)	10	(13.51)	59	(15.36)	12	(15.38)	26	(22.41)	5	(11.6)	15	(12.7)	384	(15.6)	0.472	
Health-related behaviors, n (%)																		
Former smoking	231	(35.43)	31	(41.89)	126	(32.81)	30	(38.46)	49	(42.24)	10	(23.26)	42	(35.59)	798	(32.43)	0.195	
Current smoking	176	(26.99)	15	(20.27)	102	(26.56)	20	(25.64)	31	(26.72)	17	(39.53)	40	(33.90)	683	(27.75)	0.285	
Physical inactivity	293	(44.94)	29	(39.19)	165	(42.97)	39	(50.00)	59	(50.86)	16	(37.21)	59	(50.00)	1063	(43.19)	0.334	
Biological risk factors																		
Body mass index, mean (SD)	24.94	(4.41)	25.74	(4.22)	24.64	(4.19)	24.90	(5.03)	24.99	(4.65)	23.89	(3.92)	25.86	(4.84)	25.44	(4.34)	0.004 ^b	
Diabetes, n (%)	33	(5.06)	5	(6.76)	17	(4.43)	2	(2.56)	10	(8.62)	3	(6.98)	5	(4.24)	115	(4.67)	0.169	
Dyslipidemia, n (%)	194	(29.75)	27	(36.49)	115	(29.95)	26	(33.33)	36	(31.03)	8	(18.60)	36	(30.51)	795	(32.30)	0.141	
Hypertension, n (%)	174	(26.69)	23	(31.08)	95	(24.74)	24	(30.77)	41	(35.34)	4	(9.30)	31	(26.27)	727	(29.54)	0.037	
Inflammatory markers at baseline, Median (IQR)																		
Interleukin-6	1.22	(0.50-3.11)	1.28	(0.74-3.20)	1.08	(0.45-3.12)	1.28	(0.70-3.43)	1.25	(0.49-3.62)	0.81	(0.40-2.34)	1.35	(0.73-3.11)	1.28	(0.56-3.17)	0.219	
Tumor Necrosis Factor-α	2.65	(1.63-4.41)	3.28	(2.01-4.60)	2.70	(1.62-4.49)	2.56	(1.55-4.66)	2.50	(1.69-4.40)	2.43	(1.78-3.90)	2.60	(1.61-4.04)	2.82	(1.79-4.39)	0.271	
High-sensitivity C-Reactive protein	1.00	(0.50-2.10)	1.20	(0.60-3.00)	0.90	(0.40-1.90)	0.90	(0.50-1.70)	1.00	(0.50-2.15)	0.60	(0.30-1.70)	1.10	(0.50-2.50)	1.00	(0.50-2.10)	0.130	
Inflammatory markers at follow-up, Median (IQR)																		
Interleukin-6	2.41	(0.92-7.30)	1.95	(0.79-5.61)	2.48	(0.88-8.25)	2.40	(0.89-8.05)	3.01	(1.05-8.09)	2.36	(0.90-8.75)	2.06	(0.87-6.56)	2.53	(0.93-8.44)	0.693	
Tumor Necrosis Factor-α	4.66	(2.56-8.49)	4.46	(2.76-8.66)	4.75	(2.58-8.83)	4.32	(2.82-7.43)	4.76	(2.98-9.21)	4.09	(1.89-6.71)	4.00	(1.88-9.36)	4.56	(2.44-8.00)	0.768	
High-sensitivity C-Reactive protein	1.1	(0.60-2.40)	1.50	(0.70-2.30)	1.00	(0.60-2.35)	0.90	(0.40-1.90)	1.50	(0.70-3.15)	0.80	(0.40-1.60)	1.20	(0.70-2.40)	1.10	(0.60-2.10)	0.051	

Abbreviations: SD = standard deviation; GAD = Generalized Anxiety Disorder; MDD = Major Depressive Disorder; OCD = Obsessive-Compulsive Disorder; PTSD = Post-Traumatic Stress Disorder;

IQR=Interquartile range (the 25% and 75% are provided); NSAIDs = Nonsteroidal anti-inflammatories

Median and IQR of inflammatory markers were not logarithmically transformed (n IL-6, TNF-α = 2791, n hsCRP = 2891).

^a Chi-square Test; ^b Kruskal-Wallis Test; ^c A value of 3 represents a socio-economic status of III (middle class) on the Hollingshead scale.

TABLE 2: Associations between current and remitted anxiety disorders at baseline and inflammatory markers at follow-up

	Interleukin 6 [pg/ml]			Tumor Necrosis Factor- α [pg/ml]			hs C-Reactive Protein [mg/l]		
	β^a	(95% CI)	<i>p-value</i>	β^a	(95% CI)	<i>p-value</i>	β^a	(95% CI)	<i>p-value</i>
Current diagnoses									
Anxiety disorders	-0.05	(-0.22-0.12)	<i>0.572</i>	0.05	(-0.06-0.16)	<i>0.387</i>	0.08	(-0.01-0.16)	<i>0.069</i>
Generalized Anxiety Disorder	-0.30	(-0.83-0.24)	<i>0.274</i>	-0.13	(-0.47-0.20)	<i>0.436</i>	-0.00	(-0.26-0.25)	<i>0.971</i>
Social Phobia	0.00	(-0.21-0.22)	<i>0.963</i>	0.07	(-0.07-0.20)	<i>0.314</i>	0.08	(-0.02-0.18)	<i>0.129</i>
Panic Disorder	-0.02	(-0.58-0.54)	<i>0.937</i>	-0.15	(-0.50-0.20)	<i>0.390</i>	-0.14	(-0.40-0.12)	<i>0.292</i>
Agoraphobia	0.01	(-0.36-0.38)	<i>0.959</i>	0.12	(-0.11-0.35)	<i>0.289</i>	0.24	(0.07-0.42)	0.005
Obsessive-Compulsive Disorder	-0.29	(-1.00-0.41)	<i>0.419</i>	-0.35	(-0.79-0.09)	<i>0.116</i>	0.10	(-0.23-0.43)	<i>0.550</i>
Post-Traumatic Stress Disorder	-0.57	(-1.04/-0.10)	0.018	0.02	(-0.27-0.31)	<i>0.885</i>	0.00	(-0.22-0.22)	<i>0.992</i>
Remitted diagnoses									
Anxiety disorders	0.14	(-0.08-0.36)	<i>0.211</i>	0.02	(-0.12-0.16)	<i>0.778</i>	0.05	(-0.05-0.15)	<i>0.330</i>
Generalized Anxiety Disorder	-0.28	(-0.82-0.27)	<i>0.319</i>	0.06	(-0.28-0.39)	<i>0.745</i>	0.09	(-0.18-0.37)	<i>0.515</i>
Social Phobia	0.17	(-0.12-0.46)	<i>0.241</i>	-0.01	(-0.19-0.17)	<i>0.945</i>	0.06	(-0.07-0.20)	<i>0.365</i>
Panic Disorder	-0.04	(-0.57-0.50)	<i>0.890</i>	0.09	(-0.24-0.43)	<i>0.580</i>	0.02	(-0.20-0.25)	<i>0.843</i>
Agoraphobia	0.13	(-0.43-0.69)	<i>0.657</i>	0.35	(-0.00-0.69)	<i>0.052</i>	0.04	(-0.23-0.30)	<i>0.779</i>
Obsessive-Compulsive Disorder	0.26	(-0.48-1.01)	<i>0.490</i>	-0.12	(-0.58-0.35)	<i>0.618</i>	-0.24	(-0.56-0.09)	<i>0.150</i>
Post-Traumatic Stress Disorder	0.04	(-0.38-0.47)	<i>0.841</i>	0.00	(-0.26-0.27)	<i>0.976</i>	-0.02	(-0.21-0.17)	<i>0.806</i>

Abbreviations: β = β -estimator; CI = confidence interval.

Models adjusted for the corresponding inflammatory marker at baseline.

^a Multiple regression with logarithmically transformed cytokine (n = 2791) or hsCRP concentrations (n = 2891).

Statistically significant results are in bold.

p-values are in italic.

Table 3: Adjusted associations between current and remitted anxiety disorders at baseline and inflammatory markers at follow-up

	Interleukin 6 [pg/ml]			Tumor Necrosis Factor- α [pg/ml]			hs C-Reactive Protein [mg/l]		
	β^a	(95% CI)	<i>p-value</i>	β^a	(95% CI)	<i>p-value</i>	β^a	(95% CI)	<i>p-value</i>
Current Diagnoses									
Anxiety disorders	-0.03	(-0.21-0.16)	<i>0.783</i>	0.06	(-0.05-0.18)	<i>0.280</i>	0.09	(0.00-0.17)	0.040
Generalized Anxiety Disorder	-0.26	(-0.80-0.28)	<i>0.349</i>	-0.21	(-0.55-0.13)	<i>0.228</i>	-0.03	(-0.29-0.23)	<i>0.827</i>
Social Phobia	0.02	(-0.20-0.25)	<i>0.827</i>	0.10	(-0.04-0.24)	<i>0.154</i>	0.10	(-0.01-0.20)	<i>0.064</i>
Panic Disorder	-0.02	(-0.61-0.56)	<i>0.943</i>	-0.28	(-0.65-0.08)	<i>0.127</i>	-0.25	(-0.53-0.02)	<i>0.067</i>
Agoraphobia	0.07	(-0.31-0.45)	<i>0.720</i>	0.15	(-0.09-0.39)	<i>0.222</i>	0.25	(0.07-0.43)	0.005
Obsessive Compulsive Disorder	-0.16	(-0.88-0.56)	<i>0.664</i>	-0.40	(-0.85-0.05)	<i>0.079</i>	0.05	(-0.29-0.39)	<i>0.766</i>
Post-Traumatic Stress Disorder	-0.52	(-1.00/-0.04)	0.033	0.06	(-0.23-0.36)	<i>0.676</i>	-0.04	(-0.26-0.19)	<i>0.738</i>
Remitted Diagnoses									
Anxiety disorders	0.16	(-0.06-0.39)	<i>0.158</i>	0.04	(-0.10-0.18)	<i>0.598</i>	0.08	(-0.03-0.18)	<i>0.153</i>
Generalized Anxiety Disorder	-0.27	(-0.82-0.28)	<i>0.338</i>	0.07	(-0.28-0.41)	<i>0.704</i>	0.06	(-0.21-0.34)	<i>0.645</i>
Social Phobia	0.20	(-0.09-0.49)	<i>0.185</i>	0.01	(-0.17-0.19)	<i>0.943</i>	0.09	(-0.05-0.22)	<i>0.216</i>
Panic Disorder	-0.09	(-0.63-0.46)	<i>0.755</i>	0.00	(-0.34-0.34)	<i>0.977</i>	-0.00	(-0.23-0.23)	<i>0.982</i>
Agoraphobia	0.15	(-0.43-0.73)	<i>0.613</i>	0.42	(0.06-0.78)	0.024	0.09	(-0.18-0.36)	<i>0.498</i>
Obsessive Compulsive Disorder	0.25	(-0.50-1.01)	<i>0.515</i>	-0.13	(-0.60-0.34)	<i>0.577</i>	-0.18	(-0.51-0.14)	<i>0.275</i>
Post-Traumatic Stress Disorder	0.07	(-0.36-0.50)	<i>0.749</i>	0.01	(-0.26-0.27)	<i>0.962</i>	-0.05	(-0.24-0.14)	<i>0.577</i>

Abbreviations: β = β -estimator; CI = confidence interval.

Models adjusted for the corresponding inflammatory marker at baseline, length of follow-up, socio-demographic variables (age, gender and socio-economic status), comorbid disorders (mood and substance use disorders), medications (antidepressants, mood stabilizers, antipsychotics, anxiolytics, aspirin, statin, nonsteroidal anti-inflammatories), behavioral cardiovascular risk factors (physical activity and smoking status) and physical cardiovascular risk factors (BMI, diabetes, hypertension and dyslipidemia) at baseline.

^a Multiple regression with logarithmically transformed cytokine (n = 2791) or hsCRP concentrations (n = 2891).

Statistically significant results are in bold.

p-values are in italic.

Table 4: Associations between inflammatory markers at baseline and anxiety disorders at follow-up (n=2,573)

Levels of Inflammatory marker at baseline	Anxiety Disorders n = 186			GAD n = 48			Social Phobia n = 85			Panic Disorder n = 21			Agoraphobia n = 45			OCD n = 14			PTSD n = 14		
	OR	(95% CI)	<i>p-value</i>	OR	(95% CI)	<i>p-value</i>	OR	(95% CI)	<i>p-value</i>	OR	(95% CI)	<i>p-value</i>	OR	(95% CI)	<i>p-value</i>	OR	(95% CI)	<i>p-value</i>	OR	(95% CI)	<i>p-value</i>
Model 1																					
IL-6 [pg/ml]	1.09	(0.94-1.28)	<i>0.257</i>	1.21	(0.91-1.59)	<i>0.184</i>	1.23	(0.98-1.55)	<i>0.070</i>	0.87	(0.56-1.37)	<i>0.557</i>	0.80	(0.57-1.12)	<i>0.184</i>	0.83	(0.47-1.47)	<i>0.526</i>	0.98	(0.57-1.66)	<i>0.925</i>
TNF-α [pg/ml]	1.10	(0.95-1.27)	<i>0.192</i>	1.02	(0.77-1.36)	<i>0.884</i>	1.11	(0.91-1.37)	<i>0.302</i>	0.98	(0.63-1.53)	<i>0.920</i>	0.92	(0.68-1.26)	<i>0.602</i>	0.92	(0.54-1.57)	<i>0.752</i>	1.07	(0.64-1.79)	<i>0.792</i>
hsCRP [mg/l]	0.98	(0.84-1.14)	<i>0.766</i>	0.89	(0.67-1.20)	<i>0.450</i>	0.99	(0.78-1.24)	<i>0.908</i>	1.07	(0.70-1.65)	<i>0.752</i>	0.88	(0.64-1.20)	<i>0.422</i>	0.93	(0.55-1.55)	<i>0.771</i>	1.42	(0.84-2.42)	<i>0.192</i>
Model 2																					
IL-6 [pg/ml]	1.05	(0.89-1.25)	<i>0.576</i>	1.25	(0.93-1.67)	<i>0.142</i>	1.16	(0.89-1.50)	<i>0.267</i>	0.88	(0.54-1.44)	<i>0.604</i>	0.76	(0.52-1.10)	<i>0.145</i>	0.84	(0.45-1.58)	<i>0.595</i>	0.88	(0.47-1.62)	<i>0.671</i>
TNF-α [pg/ml]	1.06	(0.90-1.25)	<i>0.459</i>	1.02	(0.76-1.37)	<i>0.892</i>	1.04	(0.82-1.32)	<i>0.767</i>	0.98	(0.60-1.61)	<i>0.942</i>	0.94	(0.66-1.31)	<i>0.722</i>	0.97	(0.54-1.76)	<i>0.928</i>	1.13	(0.64-2.00)	<i>0.683</i>
hsCRP [mg/l]	0.99	(0.83-1.19)	<i>0.935</i>	0.99	(0.71-1.37)	<i>0.937</i>	1.06	(0.81-1.40)	<i>0.662</i>	0.98	(0.61-1.57)	<i>0.922</i>	0.90	(0.62-1.32)	<i>0.601</i>	0.91	(0.51-1.62)	<i>0.745</i>	1.28	(0.71-2.32)	<i>0.408</i>




Abbreviations: IL-6 = Interleukin-6; TNF- α = Tumor Necrosis Factor- α ; hsCRP = high sensitivity C-Reactive Protein; GAD = Generalized Anxiety Disorder; OR = odds ratio; CI = confidence interval.

Model 1: adjusted for the corresponding anxiety disorder at baseline.

Model 2: adjusted for the corresponding anxiety disorder at baseline, length of follow-up, socio-demographic variables (gender, age, socio-economic status, ethnicity and marital status), medications (antidepressants, mood stabilizers, antipsychotics, anxiolytics, aspirin, statin, nonsteroidal anti-inflammatories), health-related behaviors (former smoker, current smoker and physical inactivity), and physical cardiovascular risk factors (body mass index, diabetes, dyslipidemia and hypertension) at baseline.

p-values are in italic.

FIGURE LEGEND

Figure 1. Flow chart of the study for the association between anxiety disorders at baseline and inflammatory markers at follow-up and for the association between inflammatory markers at baseline and anxiety disorders at follow-up. Abbreviation: hsCRP, high-sensitivity C-Reactive Protein.  Baseline assessment;  Follow-up assessment for the association between anxiety disorders at baseline and inflammatory markers at follow-up;  Follow-up sample for the association between inflammatory markers at baseline and anxiety disorders at follow-up.

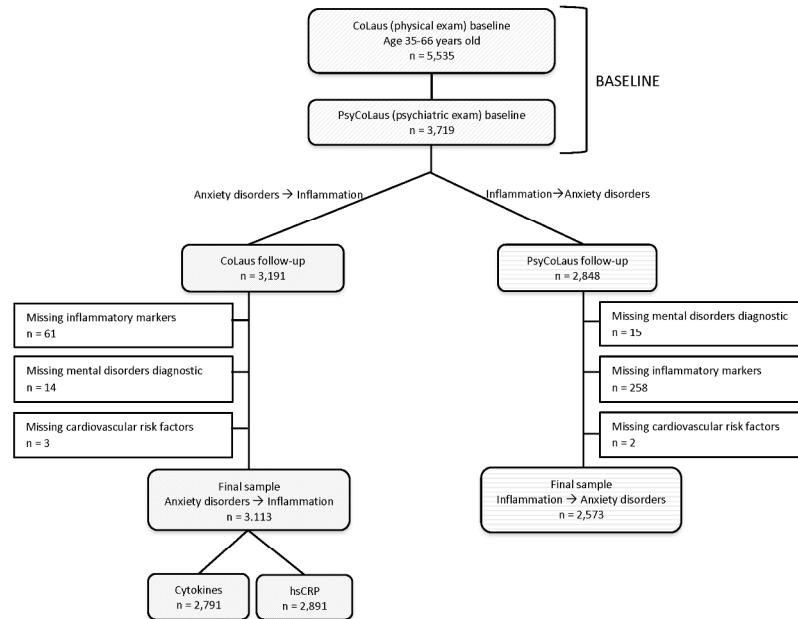


Figure 1. Flow chart of the study for the association between anxiety disorders at baseline and inflammatory markers at follow-up and for the association between inflammatory markers at baseline and anxiety disorders at follow-up. Abbreviation: hsCRP, high-sensitivity C-Reactive Protein. Baseline assessment; Follow-up assessment for the association between anxiety disorders at baseline and inflammatory markers at follow-up; Follow-up sample for the association between inflammatory markers at baseline and anxiety disorders at follow-up.

215x279mm (300 x 300 DPI)