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Mood disorders and circulating levels of inflammatory markers in a longitudinal population-based study

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Abstract: Background There has been increasing evidence that chronic low-grade inflammation is associated with mood disorders. However, the findings have been inconsistent because of heterogeneity across studies and methodological limitations. Our aim is to prospectively evaluate the bi-directional associations between inflammatory markers including interleukin (IL)-6, tumor necrosis factor (TNF)- and high sensitivity C-reactive protein (hsCRP) with mood disorders. Methods The sample consisted of 3118 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). Current and remitted mood disorders including bipolar and major depressive disorders (MDD) and its subtypes (atypical, melancholic, combined atypical and melancholic, and unspecified) were based on semi-structured diagnostic interviews. Inflammatory biomarkers were analyzed in fasting blood samples. Associations were tested by multiple linear and logistic regression models. Results Current combined MDD [$\beta = 0.29$, 95% confidence interval (CI) 0.03–0.55] and current atypical MDD ($\beta = 0.32$, 95% CI 0.10–0.55) at baseline were associated with increased levels of hsCRP at follow-up. There was little evidence for inflammation markers at baseline predicting mood disorders at follow-up. Conclusions The prospective unidirectional association between current MDD subtype with atypical features and hsCRP levels at follow-up suggests that inflammation may be a consequence of this condition. The role of inflammation, particularly hsCRP that is critically involved in cardiovascular diseases, warrants further study. Future research that examines potential influences of medications on inflammatory processes is indicated.

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1 **Mood disorders and circulating levels of inflammatory markers in a longitudinal population-**
2 **based study**

3 **Running title: Mood disorders and inflammatory markers**

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31 **ABSTRACT**

32 **Background:** There has been increasing evidence that chronic low-grade inflammation is associated with
33 mood disorders. However, the findings have been inconsistent because of heterogeneity across studies
34 and methodological limitations. Our aim is to prospectively evaluate the bi-directional associations
35 between inflammatory markers including Interleukin (IL)-6, Tumor Necrosis Factor (TNF)- α and high
36 sensitivity C-reactive protein (hsCRP) with mood disorders.

37 **Methods:** The sample consisted of 3,118 participants (53.7% women; mean age: 51.0, s.d. 8.8 years),
38 randomly selected from the general population, who underwent comprehensive somatic and psychiatric
39 evaluations at baseline and follow-up (mean follow-up duration = 5.5 years, s.d. 0.6). Current and
40 remitted mood disorders including bipolar and major depressive disorders (MDD) and its subtypes
41 (atypical, melancholic, combined atypical and melancholic, and unspecified) were based on semi-
42 structured diagnostic interviews. Inflammatory biomarkers were analyzed in fasting blood samples.
43 Associations were tested by multiple linear and logistic regression models.

44 **Results:** Current combined MDD ($\beta=0.29$, 95% CI: 0.03-0.55) and current atypical MDD ($\beta=0.32$, 95% CI:
45 0.10-0.55) at baseline were associated with increased levels of hsCRP at follow-up. There was little
46 evidence for inflammation markers at baseline predicting mood disorders at follow-up.

47 **Conclusions:** The prospective unidirectional association between current MDD subtype with atypical
48 features and hsCRP levels at follow-up suggests that inflammation may be a consequence of this
49 condition. The role of inflammation, particularly hsCRP that is critically involved in cardiovascular
50 diseases, warrants further study. Future research that examines potential influences of medications on
51 inflammatory processes is indicated.

52

53 **Key-words:** mood disorders; atypical depression; pro-inflammatory cytokines; C-Reactive protein;
54 prospective study; cardiovascular risk factors.

55 INTRODUCTION

56 Mood disorders and cardiovascular diseases (CVD) are among the highest sources of morbidity and
57 premature mortality worldwide (GBD 2015 DALYs & HALE Collaborators, 2016). Moreover, their high
58 comorbidity (Van der Kooy *et al.* 2007; Glaus *et al.* 2013) is associated with even greater disability
59 adjusted life years than either disorder alone (Charlson *et al.* 2013). An increasingly recognized
60 mechanism to explain comorbidity of CVD with mood disorders is common underlying low-grade
61 inflammation. Specifically, there is a consistent association between circulating levels of pro-
62 inflammatory cytokines, such as Interleukin (IL)-1 β , IL-6 and Tumor Necrosis Factor (TNF)- α , and the
63 acute phase reactant C-Reactive Protein (CRP) with both mood disorders (Howren *et al.* 2009; Dowlati *et*
64 *al.* 2010; Modabbernia *et al.* 2013; Glaus *et al.* 2014) and CVD (Baune *et al.* 2012). Alternatively, such
65 inflammatory processes could also provoke mood disorders or CVD through, for instance, stress-
66 induced perturbation of the Hypothalamic-Pituitary-Adrenal (HPA) axis (Rosmond & Bjorntorp, 2000;
67 Watson, 2006). Conversely, mood disorders could induce inflammation through similar mechanisms
68 (Rosenblat *et al.* 2014).

69 Although the bulk of prospective research on inflammatory markers and CVD has focused on major
70 depression, comparably few studies have examined the extent to which associations extend to other
71 mood disorders, such as bipolar disorder (BPD)(Van der Kooy *et al.* 2007; Modabbernia *et al.* 2013). In a
72 recent meta-analysis of 25 cross-sectional studies and 2 longitudinal analyses (changes in CRP levels
73 before and after treatment for mania and depression), BPD disorder was associated with increased
74 levels of CRP (Fernandes *et al.* 2016). In another meta-analysis of 30 cross-sectional studies, BPD
75 disorder was associated with increased levels of IL-6 and TNF- α (Modabbernia *et al.* 2013).
76 Moreover, few longitudinal studies have examined depressive subtypes (Rothermundt *et al.* 2001;
77 Kaestner *et al.* 2005; Yoon *et al.* 2012; Lamers *et al.* 2013; Rudolf *et al.* 2014; Glaus *et al.* 2014) that have
78 been shown to be highly heterogeneous (Schmidt *et al.* 2011; Lopresti *et al.* 2014) and even fewer

79 studies have considered the well-established comorbidity among mental disorders (Stewart *et al.* 2009;
80 Kivimaki *et al.* 2014) or current versus past disorders (Whooley *et al.* 2008).

81

82 With respect to directionality, longitudinal studies of the links between mood disorders and
83 inflammation have predominantly been performed in patients with a major depressive disorder (MDD)
84 (Valkanova *et al.* 2013; Chocano-Bedoya *et al.* 2014; Kivimaki *et al.* 2014; Tully *et al.* 2015). Recent meta-
85 analyses (Valkanova *et al.* 2013) and more recent studies (Chocano-Bedoya *et al.* 2014; Tully *et al.* 2015)
86 have shown that these associations between inflammatory factors and mood disorders may be partially
87 attributable to potential confounders, including body mass index (BMI) and smoking. Similar findings
88 regarding mediation by BMI and behavioral cardiovascular risk factors (CVRFs) have also emerged from
89 prospective studies of inflammation as a potential consequence of mood disorders (Matthews *et al.*
90 2007; Stewart *et al.* 2009; Gimeno *et al.* 2009; Duivis *et al.* 2011). However, to date, the few studies that
91 have examined the bi-directional associations simultaneously have yielded conflicting results (Stewart *et al.*
92 *et al.* 2009; Gimeno *et al.* 2009; Matthews *et al.* 2010; Duivis *et al.* 2011). The aggregate findings from
93 these studies highlight the importance of incorporating CVRFs including diabetes (Marques-Vidal *et al.*
94 2012a), overweight (Marques-Vidal *et al.* 2012b; Kiecolt-Glaser *et al.* 2015), smoking (Yanbaeva *et al.*
95 2007), chronic alcohol consumption (Achur *et al.* 2010) and physical inactivity (Hamer, 2007; Kiecolt-
96 Glaser *et al.* 2015) along with depression and anxiety (e.g. (Agosti & Levin, 2006; Mezuk *et al.* 2008;
97 Chaiton *et al.* 2009; Luppino *et al.* 2010; Anthenelli, 2010; Glaus *et al.* 2013; Lasserre *et al.* 2014; Kiecolt-
98 Glaser *et al.* 2015)), when examining the association with inflammation.

99

100 Our previous cross-sectional findings from a large community cohort revealed that there were
101 significantly elevated levels of high sensitivity CRP (hsCRP) among those with the atypical subtype of
102 depression and bipolar disorders, but these associations were explained by concurrent CVRFs, such as
103 BMI, diabetes and hypertension (Glaus *et al.* 2014). In the present study, we extend these earlier cross-

104 sectional analyses from our large community-based cohort in Lausanne, Switzerland, to examine the
105 directionality, specificity, timing, and potential causal versus common etiologic mechanisms for
106 associations between mood disorders and markers of a chronic low-grade inflammation by addressing
107 the following aims: (1) to evaluate the 5-year prospective associations between mood disorders
108 including their subtypes, and changes in circulating levels of specific inflammatory markers (IL-6, TNF- α ,
109 hsCRP) to determine the specificity of the associations; (2) to assess the directionality of links between
110 inflammatory markers and mood disorders; (3) to examine whether the markers comprise state versus
111 trait indices by comparing remitted versus current disorders, as well as incident cases; and (4) to
112 examine the role of health behaviors, CVRFs, and treatment factors that may influence this association.
113 Based on our previous cross-sectional findings (Glaus *et al.* 2014) as well as those of other studies
114 (Lamers *et al.* 2013; Hickman *et al.* 2014; Rudolf *et al.* 2014), we hypothesized that there would be an
115 association between the atypical subtype of depression and increased levels of inflammatory markers,
116 whereas other subtypes of major depressive disorders were not expected to be associated with
117 increased inflammation.

118

119

120 **METHODS AND MATERIALS**

121 **Study sample**

122 We drew the data for the present investigation from CoLaus|PsyCoLaus (Firmann *et al.* 2008; Preisig *et*
123 *al.* 2009), a cohort study designed to prospectively assess the associations between mental disorders
124 and CVD or CVRFs in the community. The sample was randomly selected from the civil register of the
125 city of Lausanne (Switzerland) in 2003. Sixty-seven percent of participants between 35 and 66 years-of
126 age ($n=5,535$), who underwent the physical exam between 2003 and 2006, also accepted the psychiatric
127 evaluation, resulting in a sample of 3,719 individuals at baseline (Preisig *et al.* 2009) (see Figure 1, flow
128 chart). The mean interval between the physical and psychiatric evaluations at baseline was 1.3 years

129 (s.d. 0.5). Five years later, 3,191 participated in the physical exam (85.8% participation). Among them, 61
130 participants were excluded due to missing information on inflammatory markers both at baseline and
131 follow-up, 9 subjects were excluded due to missing information on mental disorders and 3 subjects were
132 excluded due to missing information on CVRFs. The final sample used to examine associations between
133 mood disorders at baseline and inflammation at follow-up consisted of 3,118 participants in total (53.7%
134 women; mean age: 51.0, s.d. 8.8 years): 2,796 participants were analyzed for cytokines levels, and 2,896
135 for hsCRP levels. The mean follow-up duration for the physical examination was 5.5 years (s.d. 0.6
136 years). The mean follow-up duration for the mental health assessment was also 5.5 years (s.d. 0.8). The
137 number of participants with data on the psychiatric evaluation at follow-up was 2,840. Among them,
138 258 participants were excluded due to missing information on inflammatory markers and 2 participants
139 due to missing information on CVRFs. The resulting number of participants used to determine the
140 association between inflammation at baseline and mental disorders at follow-up was 2,580. The
141 Institutional Ethics' Committee of the University of Lausanne approved the CoLaus and the PsyCoLaus
142 study. All participants provided written informed consent for the study protocol.

143

144 **Measurements**

145 *Mood disorders and comorbid disorders*

146 Mental disorders at baseline and follow-up were assessed using the French version (Leboyer *et al.* 1995)
147 of the semi-structured Diagnostic Interview for Genetic Studies (DIGS), which was developed and
148 validated by the National Institute of Mental Health (Nurnberger *et al.* 1994). The French version of this
149 instrument revealed adequate inter-rater and test-retest reliability for major mood (Preisig *et al.* 1999)
150 and substance use disorders (Berney *et al.* 2002). The DIGS was completed with sections on generalized
151 anxiety and phobia disorders using questions from the Schedule for Affective Disorders and
152 Schizophrenia-Lifetime and Anxiety disorder version (SADS-LA (Endicott & Spitzer, 1978)), which also
153 revealed satisfactory test-retest reliability (Leboyer *et al.* 1991; Rougemont-Buecking *et al.* 2008).

154 Interviewers were required to be masters-level psychologists and were trained over a two-month
155 period. In order to provide ongoing supervision throughout the study, each interview and diagnostic
156 assignment was reviewed by an experienced senior psychologist. Diagnoses were assigned according to
157 the DSM-IV. Following Angst et al. (Angst *et al.* 2006), MDD was subtyped into: 1) MDD with at least one
158 atypical and one melancholic episode or MDD with atypical and melancholic features simultaneously
159 (combined type); 2) MDD with at least one atypical (but no melancholic) episode; 3) MDD with at least
160 one melancholic (but no atypical) episode; and 4) MDD with neither atypical nor melancholic episodes
161 (unspecified type). We defined MDD as current if it was present at the time of the physical evaluation.

162

163 *Inflammatory markers*

164 HsCRP was assessed during baseline and follow-up physical evaluations using immunoassay and latex HS
165 (IMMULITE 1000-High, Diagnostic Products Corporation, LA, CA, USA), with maximum intra- and
166 interbatch coefficients of variation of 1.3% and 4.6%, respectively (Firmann *et al.* 2008). Subjects with a
167 hsCRP level higher than 10 mg/l were excluded as such an elevation is likely to be attributable to acute
168 infection (Pearson *et al.* 2003). For the baseline and follow-up cytokine measurements, serum was
169 preferred to plasma, as it has been shown that different anticoagulants may differentially affect
170 absolute cytokine levels (Skeppholm *et al.* 2008). Serum samples were stored at -80°C before
171 assessment and sent on dry ice to the laboratory. As previously described, cytokine levels were
172 measured using a multiplexed particle-based flow cytometric cytokine assay (Marques-Vidal *et al.* 2011).
173 Lower detection limits (LOD) for IL-6 and TNF- α were 0.2 pg/ml. Good agreement between signal and
174 cytokine was found within the assay range ($R^2 \geq 0.99$).

175

176 *Covariates*

177 Data were collected on age, race (Caucasian versus non-Caucasian), marital status (living alone versus
178 living with someone) and health-related behaviors at baseline including smoking (never, former,

179 current) and physical inactivity (no or low versus at least 20 minutes twice a week). Information on
180 socio-economic status (SES) and on psychotropic drug treatment was derived from the DIGS. The level of
181 SES was assessed using the Hollingshead scale (Hollingshead, 1975). The section on psychotropic drug
182 treatment covered all types of antidepressants, mood stabilizers (lithium and anti-epileptics) and
183 antipsychotics. Moreover, information on aspirin and statin use was collected during the baseline
184 physical evaluation (never versus occasional versus regular use). The following biological variables were
185 measured during the baseline physical evaluation: BMI (weight in kilograms divided by height in meters
186 squared), diabetes (fasting blood glucose ≥ 7 mmol/l or treatment for diabetes), dyslipidemia (HDL-
187 cholesterol < 1 mmol/l, or LDL-cholesterol ≥ 4.1 mmol/l, or triglycerides ≥ 2.2 mmol/l, or treatment with
188 a lipid-lowering drug), hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure \geq
189 90 mmHg, or treatment for hypertension).

190

191 **Statistical analysis**

192 Statistical analyses were conducted using the Statistical Analysis System (SAS Institute Inc., Cary, NC,
193 USA), version 9.3 for Windows. Age was standardized. Descriptive data for demographic characteristics,
194 comorbid disorders, medication, health-related behaviors, physical risk factors for CVD and
195 inflammatory marker levels were derived by lifetime mood disorders and Chi-square and Kruskal-Wallis
196 tests were used to determine the difference between mood disorders. The Benjamini-Hochberg false
197 discovery method (Benjamini & Hochberg, 1995) was employed to correct for multiple tests for all
198 depressive subtypes except the pre-specified association with the atypical subtype of depression.
199 Associations between current and remitted mood disorders at baseline and changes in inflammatory
200 markers at follow-up were determined using multiple linear regression models. Box-Cox transformation
201 was applied to the response variable whenever a deviation from fundamental assumptions was
202 observed. All three inflammatory markers were transformed: for the dependent variables, inflammatory

203 markers were log-transformed, whereas for the independent variables, inflammatory markers were
204 normalized and log-transformed. Values below the LOD of 0.2 [pg/ml] (i.e., 5% of values for IL-6, 0.5% of
205 values for TNF- α) were considered as censored observations. IL-6 and TNF- α were analyzed with the
206 qualitative and limited dependent variable model (QLIM) with the threshold of -1.65 [log (pg/ml)] as the
207 meaningful lower bound of observed values.

208 A multiple linear regression model was performed on naturally log-transformed and standardized
209 observed values. Three models of increasing complexity were computed. Model 1 included one single
210 mood disorder (current and remitted) at a time as the independent variable, adjusted for the
211 corresponding inflammatory marker at baseline. Model 2 included current and remitted mood disorders
212 simultaneously in order to determine the associations of specific mood disorder with inflammatory
213 markers at follow-up, adjusting for the length of follow-up, sociodemographic characteristics (sex, age,
214 SES, race and marital status), comorbid disorders (anxiety and substance use disorders), health-related
215 behaviors and physical risk factors for CVD at baseline and the covariate of Model 1. In case of
216 significant associations, additional models also adjusted for the occurrence of new episodes in order to
217 test whether the associations persist after the offset of depressive episodes. Similar models adjusted for
218 the occurrence of new physical risk factors for CVD during the follow-up in order to test whether the
219 prospective associations between mood disorders and inflammation marker levels were independent of
220 the occurrence of these new risk factors.

221 Associations between inflammatory markers at baseline and a history of mood disorders during the
222 follow-up period were determined using logistic regression models. Two models were computed. Model
223 1 included all inflammatory markers simultaneously, adjusting for the corresponding history of mood
224 disorder at baseline. Model 2 was further adjusted for the length of follow-up, sociodemographic
225 characteristics, health-related behaviors and physical risk factors for CVD.

226

227 **RESULTS**

228 **Sample characteristics**

229 The description of the cohort of 3,118 participants used for the assessment of the prospective
230 associations between mood disorders at baseline and the concentrations of inflammatory markers at
231 follow-up is presented in Table 1. The groups (bipolar disorder, MDD subtypes and no history of mood
232 disorders) differed with respect to sex, age, marital status, comorbid disorders, medication, current
233 smoking status, BMI and hypertension. Moreover, the groups also differed with respect to hsCRP levels
234 at baseline and at follow-up.

235 **Associations between mood disorders at baseline and inflammatory markers at follow-up**

236 Associations between current and remitted mood disorders at baseline and changes in levels of
237 inflammatory markers are presented in Table 2 and 3. When models were only adjusted for the
238 corresponding inflammatory marker at baseline (Table 2), current MDD, as well as the current combined
239 and atypical MDD subtypes were associated with increased hsCRP levels. Moreover, current combined
240 MDD was associated with decreased IL-6 levels. No significant associations were found for remitted
241 mood disorders with inflammatory markers. When the model was further adjusted for potential
242 confounders, MDD was no longer significantly associated with hsCRP levels ($\beta=0.09$, 95%CI -0.02-0.21).
243 In contrast, current combined MDD was associated with increased hsCRP levels and decreased IL-6
244 levels and current atypical MDD was associated with increased hsCRP levels at follow-up (Table 3).
245 Moreover, remitted melancholic MDD was associated with decreased IL-6 levels at follow-up in the fully
246 adjusted model (Table 3). Additional analyses revealed that the occurrence of new mood episodes
247 during the follow-up did not affect the size of the associations between current mood disorders at
248 baseline and the hsCRP at follow-up. Similarly, the occurrence of incident physical risk factors for CVD
249 did not account for the associations between current combined MDD or current atypical MDD at
250 baseline and the hsCRP at follow-up.

251 Since an association was found between the atypical MDD subtype and increased hsCRP levels at follow-
252 up, we also looked at the correlation between the age of onset of atypical MDD and inflammatory
253 markers. Interestingly, later atypical MDD onset was correlated with increased hsCRP levels (Pearson
254 correlation = 0.16, p-value = 0.0214).

255 **Associations between inflammatory markers at baseline and mood disorders at follow-up**

256 Regarding the associations between inflammatory markers at baseline and mood disorders at follow-up,
257 higher levels of TNF- α were associated with a decreased risk of MDD and of the MDD atypical and
258 unspecified subtypes after controlling for a history of depression at baseline (Table 4). After controlling
259 for all the covariates (Model 2), higher levels of TNF- α were still associated with a decreased risk of MDD
260 and of the MDD unspecified subtype, but not with the MDD atypical subtype. Moreover, levels of IL-6
261 and hsCRP at baseline were not associated with any mood disorders at follow-up.

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

265 However, none of these associations remained significant after correction for multiple testing that was
266 conducted on all subtypes of depression except the pre-specified atypical subtype.

267 **Additional analyses**

268 In order to examine the potential influence of concurrent medication use on these associations, we
269 examined the effects of mood stabilizers and antidepressants on changes in inflammatory marker levels
270 over time. We found that lower IL-6 levels were associated with mood stabilizer use in those with BPD
271 (β =-1.33, p-value=0.036), but no differences were found for antidepressant use in the MDD subgroups.

272

273 **DISCUSSION**

274 The present study prospectively assessed the bi-directional 5-year associations between mood disorder
275 and its subtypes with several inflammatory markers with serial adjustments for sociodemographic,
276 comorbid mental disorders, medication use, health behaviors, and CVRFs, in a large community sample
277 of adults. Our results showed differential associations depending on temporal ordering, current versus
278 remitted status of the mood disorder, the specific type of mood disorder and different indices of
279 inflammation. The major finding is that there is an association between the current atypical subtype of
280 MDD at baseline with increased levels of hsCRP at follow up, whereas inflammatory levels at baseline
281 were not associated with subsequent atypical MDD at follow-up. This unidirectional association
282 indicates that this disorder may be causally related to increased inflammation, rather than inflammation
283 comprising a vulnerability factor for mood disorders. Future studies with more closely timed follow-up
284 assessments may inform potential mechanisms for elevation of hsCRP among those with this condition.
285 Our finding of unidirectional associations between MDD and elevated hsCRP levels appear to be specific
286 to the atypical subtype of MDD, which is characterized by somatic symptoms including sleep, energy and
287 eating behavior (Rothermundt *et al.* 2001; Kaestner *et al.* 2005; Stewart *et al.* 2009; Duvis *et al.* 2011;
288 Copeland *et al.* 2012; Lamers *et al.* 2013; Hickman *et al.* 2014; Rudolf *et al.* 2014; Schmidt *et al.* 2014;
289 Glaus *et al.* 2014). This finding suggests that the atypical MDD is associated with more detectable
290 immune dysfunction. This could be a primary characteristic of this disorder or reflect other indirect
291 factors, such as greater disturbance of circadian systems, HPA systems or other relevant immune
292 modulators. A similar link between cumulative depressive episodes and CRP at follow-up in a
293 prospective study of a community sample of youth would confirm this explanation and further imply
294 that the associations observed herein may be present across the life span (Copeland *et al.* 2012). The
295 specificity of the findings with respect to the atypical subtype rather than BPD (Modabbernia *et al.*
296 2013), as shown in previous studies of adults and youth, further suggests that the somatic
297 manifestations of depression may be etiologically related to inflammation. However, the power to test
298 the association between BPD and inflammation was reduced because of the relatively low numbers of

299 people with BPD in this sample. Interestingly, a previous case-control study (Cunha *et al.* 2008) that
300 evaluated BPD patients in different phases of their illness found increased hsCRP levels in patients with a
301 current manic episode compared to depressed, euthymic patients and healthy controls. Moreover,
302 recent studies have shown that mania, and to a lesser extent depression, in BPD patients are associated
303 with pro-inflammatory cytokines (Muneer, 2016). To our knowledge, no previous studies have examined
304 depressive subtypes in BPD compared to MDD patients and healthy controls. Future prospective studies
305 should further investigate the different state-related associations between mood disorder subtypes and
306 inflammation.

307 The lack of association between inflammatory markers at baseline and subsequent mood disorders at
308 follow-up suggests that inflammation may not be a risk factor for the onset of mood disorders. This
309 result extends the findings of two previous longitudinal studies, which found that depression predicted
310 higher inflammation, whereas inflammation did not predict depression (Stewart *et al.* 2009; Duivis *et al.*
311 2011). However, our results do not confirm findings from previous studies that focused on depressive
312 symptoms rather than disorder (Gimeno *et al.* 2009; Matthews *et al.* 2010; Valkanova *et al.* 2013; Tully
313 *et al.* 2015). These discrepancies could be due to methodological differences. Moreover, no depressive
314 subtypes were analyzed.

315
316 Our findings were restricted to the hsCRP, rather than the pro-inflammatory cytokines. CRP has been
317 shown to lead to broader manifestations of the metabolic syndrome, particularly among those with
318 current disorders rather than symptoms alone (Gimeno *et al.* 2009; Matthews *et al.* 2010; Valkanova *et al.*
319 *et al.* 2013). This further suggests that the physical symptoms of these conditions may induce a stress-like
320 physiologic reaction in affected individuals. Accordingly, depression may contribute to a chronic low-
321 grade inflammatory state, which, in turn, might lead to a vascular pathology and ultimately to
322 atherosclerotic vascular disease (Wagner *et al.* 2015). Several potential pathophysiological mechanisms
323 have been suggested to underlie the association between depression and higher inflammation, including

324 HPA axis activation and oxidative stress (Rosenblat *et al.* 2014). However, future longitudinal studies are
325 needed to confirm these potential mechanisms.

326 Our evaluation of medication use at index evaluation as a possible explanation for changes in
327 inflammatory markers revealed that mood stabilizer use was associated with lower IL-6 levels at follow-
328 up. This suggests that future studies should examine the specific influences of mood stabilizers not only
329 on symptom relief but also on concomitant inflammatory factors. However, no such changes emerged
330 for inflammatory markers and other mood disorder subtypes.

331 Our results should be interpreted in the context of several limitations. First, there was an interval of
332 almost one year between the physical and the psychiatric evaluations. Although the timing of depressive
333 episodes was elicited in our diagnostic interviews, we cannot exclude misclassification regarding the
334 "current" status of disorders at the time of the physical evaluation. Second, inflammatory markers were
335 only assessed twice, once at baseline and 5.5 years later at follow-up, which may only partially reflect
336 the dynamic temporal relationship between mood disorders and inflammatory markers over time.
337 However, despite this limitation, our findings still indicate that there may be an enduring increase of
338 inflammation in individuals with specific subtypes of mood disorders. Third, low-grade inflammation was
339 assessed through hsCRP and the two pro-inflammatory cytokines IL-6 and TNF- α . We did not include
340 additional pro-inflammatory (i.e. IL-1 β , IL-2, IFN- γ) and anti-inflammatory (i.e. IL-4, IL-8, IL-10) cytokines,
341 and other potential biomarkers of inflammation, such as cortisol, leucocytes and thrombocytes. Fourth,
342 with only 55 subjects with a lifetime history of BPD at baseline we did not have sufficient power to
343 detect differences in inflammation levels at follow-up as a function of the BPD status at baseline.
344 Despite these limitations, this is one of the largest prospective studies of a community sample, with the
345 hitherto longest follow-up period. It is the first study to investigate the full range of mood disorder
346 subtypes.

347 These prospective data from a large community sample show that unidirectional association between
348 current MDD subtypes with atypical features and increased hsCRP levels at a more than 5-year follow-up
349 may be a consequence of this condition. Further prospective studies with repeated assessments of
350 inflammatory markers earlier in the development of mood disorders are required to examine potential
351 influences of medications on inflammatory processes.

352

353

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360

361 **CONFLICTS OF INTEREST:**

362 None.

363

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544

TABLE 1: Sociodemographic, Medication, Health-Related Behaviors, Physical Risk Factors for Cardiovascular Disorders and Inflammatory markers by lifetime mood disorders at baseline (n=3118)

	Bipolar disorder		MDD		Major depressive disorder subtypes						No Mood Disorders		p-value ^a		
					Combined MDD		Atypical MDD		Melancholic MDD					Unspecified MDD	
	n = 55		n = 1367		n = 171		n = 206		n = 362		n = 628			n = 1696	
	Current n = 2	Remitted n = 53	Current n = 213	Remitted n = 1154	Current n = 36	Remitted n = 135	Current n = 47	Remitted n = 159	Current n = 49	Remitted n = 313	Current n = 81	Remitted n = 547			
Sociodemographics															
Length of follow-up, mean (SD)	5.62	(0.71)	5.51	(0.59)	5.46	(0.61)	5.51	(0.55)	5.53	(0.57)	5.51	(0.61)	5.54	(0.63)	0.568 ^b
Sex, n (%)															
Female	26	(47.27)	899	(65.76)	123	(71.93)	148	(71.84)	245	(67.68)	383	(60.99)	748	(44.10)	<.0001
Male	29	(52.73)	468	(34.24)	48	(28.07)	58	(28.16)	117	(32.32)	245	(39.01)	948	(55.90)	
Age, mean (SD)	51.15	(8.43)	50.33	(8.71)	50.64	(8.84)	49.56	(8.68)	50.60	(8.69)	50.35	(8.70)	51.53	(8.87)	0.007 ^b
Socio-economic status, mean (SD)	3.33	(1.29)	3.42	(1.26)	3.31	(1.24)	3.34	(1.21)	3.39	(1.34)	3.50	(1.23)	3.42	(1.28)	0.381 ^b
Ethnicity, n (%)															
Caucasian	55	(100.00)	1251	(91.51)	160	(93.57)	191	(92.72)	331	(91.44)	569	(90.61)	1575	(92.87)	0.119
Other	0	(0.00)	116	(8.49)	11	(6.43)	15	(7.28)	31	(8.56)	59	(9.39)	121	(7.13)	
Marital status, n (%)															
Living alone	25	(45.45)	547	(40.01)	65	(38.01)	93	(45.15)	153	(42.27)	236	(37.58)	429	(25.29)	<.0001
Living in couple	30	(54.55)	820	(59.99)	106	(61.99)	113	(54.85)	209	(57.73)	392	(62.42)	1267	(74.71)	
Comorbid disorders, n (%)															
Anxiety disorders	22	(40.00)	340	(24.87)	56	(32.75)	51	(24.76)	94	(25.97)	139	(22.13)	204	(12.03)	<.0001
Substance use disorders	13	(23.64)	218	(15.95)	31	(18.13)	22	(10.68)	60	(16.57)	105	(16.72)	240	(14.15)	0.060
Medication, n (%)															
Antidepressants	28	(50.91)	522	(38.19)	86	(50.29)	91	(44.17)	176	(48.62)	169	(26.91)	90	(5.31)	<.0001
Mood stabilizers	12	(21.82)	8	(0.59)	1	(0.58)	1	(0.49)	5	(1.38)	1	(0.16)	2	(0.12)	<.0001
Antipsychotics	10	(18.18)	32	(2.34)	8	(4.68)	4	(1.94)	9	(2.49)	11	(1.75)	9	(0.53)	<.0001
Aspirin	10	(18.18)	121	(8.85)	17	(9.94)	24	(11.65)	33	(9.12)	47	(7.48)	130	(7.67)	0.030
Statins	6	(10.91)	93	(6.80)	12	(7.02)	14	(6.80)	23	(6.35)	44	(7.01)	132	(7.78)	0.819
Health-related behaviors, n (%)															
Former smoking	21	(38.18)	441	(32.26)	49	(28.65)	64	(31.07)	112	(30.94)	216	(34.39)	569	(33.55)	0.557
Current smoking	21	(38.18)	417	(30.50)	62	(36.26)	54	(26.21)	102	(28.18)	199	(31.69)	423	(24.94)	0.001
Physical inactivity	21	(38.18)	600	(43.89)	78	(45.61)	97	(47.09)	148	(40.88)	277	(44.11)	737	(43.46)	0.681
Biological risk factors															
Body mass index, mean (SD)	26.46	(4.99)	25.13	(4.47)	25.66	(5.21)	26.46	(4.83)	24.56	(4.16)	24.87	(4.20)	25.46	(4.23)	0.000 ^b
Diabetes, n (%)	3	(5.45)	57	(4.17)	11	(6.43)	9	(4.37)	9	(2.49)	28	(4.46)	88	(5.19)	0.293
Dyslipidemia, n (%)	20	(36.36)	406	(29.70)	54	(31.58)	60	(29.13)	104	(28.73)	188	(29.94)	563	(33.20)	0.379
Hypertension, n (%)	13	(23.64)	364	(26.63)	44	(25.73)	67	(32.52)	95	(26.24)	158	(25.16)	525	(30.96)	0.036
Inflammatory markers at baseline, Median (IQR)															
Interleukin-6	1.24	(0.35-2.64)	1.19	(0.50-3.04)	1.25	(0.63-3.37)	1.29	(0.56-3.25)	1.22	(0.52-2.73)	1.13	(0.45-3.04)	1.34	(0.60-3.36)	0.106
Tumor Necrosis Factor-α	2.52	(1.65-3.91)	2.69	(1.62-4.29)	2.54	(1.62-4.17)	2.89	(1.71-4.81)	2.72	(1.63-4.26)	2.61	(1.57-4.26)	2.87	(1.85-4.47)	0.067
High-sensitivity C-Reactive protein	1.30	(0.70-3.10)	1.00	(0.50-2.10)	0.90	(0.50-2.20)	1.10	(0.60-2.60)	1.00	(0.50-2.20)	1.00	(0.50-2.00)	1.00	(0.50-2.20)	0.030
Inflammatory markers at follow-up, Median (IQR)															
Interleukin-6	2.49	(1.25-6.10)	2.31	(0.89-7.79)	2.44	(0.73-5.54)	2.51	(0.80-8.92)	2.05	(0.85-7.27)	2.35	(0.93-8.02)	2.82	(0.98-8.83)	0.100
Tumor Necrosis Factor-α	5.35	(2.98-7.86)	4.48	(2.43-8.07)	4.52	(2.17-7.51)	4.25	(2.33-9.14)	4.56	(2.80-7.89)	4.53	(2.42-8.09)	4.72	(2.52-8.27)	0.826
High-sensitivity C-Reactive protein	1.60	(0.90-3.40)	1.10	(0.60-2.50)	1.20	(0.70-2.60)	1.40	(0.70-3.20)	1.10	(0.60-2.70)	1.10	(0.50-2.35)	1.20	(0.60-2.50)	0.018

Abbreviations: SD = standard deviation; MDD = major depressive disorder; IQR=Interquartile range (the 25% and 75% are provided).

Median and IQR of inflammatory markers were not logarithmically transformed (n IL-6, TNF- α = 2796, n hsCRP = 2896).

^a Chi-square Test; ^b Kruskal-Wallis Test.

TABLE 2: Associations between current and remitted mood 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									
Bipolar Disorder	-1.29	(-3.51-0.93)	<i>0.256</i>	0.17	(-1.22-1.56)	<i>0.810</i>	-0.61	(-1.66-0.43)	<i>0.251</i>
Major Depressive Disorder	-0.10	(-0.33-0.14)	<i>0.429</i>	-0.06	(-0.21-0.09)	<i>0.427</i>	0.12	(0.01-0.22)	0.037
MDD Combined Subtype	-0.69	(-1.23/-0.14)	0.014	-0.27	(-0.61-0.08)	<i>0.127</i>	0.33	(0.07-0.59)	0.011
MDD Atypical Subtype	-0.12	(-0.62-0.37)	<i>0.627</i>	0.04	(-0.27-0.35)	<i>0.809</i>	0.36	(0.14-0.58)	0.002
MDD Melancholic Subtype	-0.07	(-0.55-0.42)	<i>0.785</i>	-0.18	(-0.48-0.12)	<i>0.247</i>	-0.09	(-0.31-0.13)	<i>0.406</i>
MDD Unspecified Subtype	0.19	(-0.19-0.56)	<i>0.322</i>	0.06	(-0.17-0.29)	<i>0.622</i>	-0.02	(-0.19-0.16)	<i>0.855</i>
Remitted diagnoses									
Bipolar Disorder	0.14	(-0.33-0.60)	<i>0.567</i>	0.09	(-0.20-0.38)	<i>0.550</i>	0.18	(-0.04-0.39)	<i>0.111</i>
Major Depressive Disorder	-0.07	(-0.19-0.06)	<i>0.300</i>	-0.00	(-0.08-0.07)	<i>0.925</i>	-0.03	(-0.08-0.03)	<i>0.384</i>
MDD Combined Subtype	-0.05	(-0.34-0.24)	<i>0.740</i>	0.00	(-0.18-0.19)	<i>0.984</i>	-0.08	(-0.22-0.05)	<i>0.226</i>
MDD Atypical Subtype	-0.02	(-0.29-0.24)	<i>0.856</i>	-0.11	(-0.28-0.06)	<i>0.189</i>	-0.03	(-0.16-0.10)	<i>0.640</i>
MDD Melancholic Subtype	-0.19	(-0.38-0.01)	<i>0.069</i>	0.06	(-0.07-0.18)	<i>0.379</i>	0.01	(-0.08-0.10)	<i>0.781</i>
MDD Unspecified Subtype	0.03	(-0.13-0.19)	<i>0.691</i>	-0.00	(-0.10-0.10)	<i>0.954</i>	-0.02	(-0.09-0.06)	<i>0.675</i>

Abbreviations: β = β -estimator; CI = confidence interval; MDD = major depressive disorder.

Models adjusted for the corresponding inflammatory marker at baseline, "No mood disorders" considered as the comparison group.

^a multiple regression with logarithmically transformed cytokine (n = 2796) or hsCRP concentrations (n = 2896).

Statistically significant results are in bold.

p-values are in italic.

TABLE 3: Adjusted associations between current and remitted mood 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									
Bipolar Disorder	--	--	--	--	--	--	--	--	--
MDD Combined Subtype	-0.74	(-1.30\ -0.18)	0.010	-0.26	(-0.61-0.09)	0.141	0.29	(0.03-0.55)	0.026
MDD Atypical Subtype	-0.17	(-0.67-0.34)	0.521	0.04	(-0.28-0.35)	0.814	0.32	(0.10-0.55)	0.005
MDD Melancholic Subtype	-0.14	(-0.64-0.35)	0.573	-0.13	(-0.44-0.18)	0.412	-0.10	(-0.33-0.12)	0.362
MDD Unspecified Subtype	0.14	(-0.25-0.52)	0.478	0.08	(-0.16-0.32)	0.521	-0.01	(-0.19-0.16)	0.889
Remitted diagnoses									
Bipolar Disorder	0.04	(-0.46-0.55)	0.872	-0.01	(-0.32-0.30)	0.954	0.13	(-0.10-0.37)	0.261
MDD Combined Subtype	-0.13	(-0.44-0.18)	0.415	-0.01	(-0.20-0.18)	0.916	-0.09	(-0.23-0.05)	0.202
MDD Atypical Subtype	-0.10	(-0.38-0.19)	0.499	-0.10	(-0.28-0.08)	0.268	-0.04	(-0.17-0.09)	0.552
MDD Melancholic Subtype	-0.23	(-0.45\ -0.02)	0.036	0.06	(-0.07-0.20)	0.372	0.03	(-0.07-0.13)	0.517
MDD Unspecified Subtype	-0.04	(-0.21-0.13)	0.649	0.00	(-0.10-0.11)	0.970	0.00	(-0.08-0.08)	0.983

Abbreviations: β = β -estimator; CI = confidence interval; MDD = major depressive disorder.

Models adjusted for the corresponding inflammatory marker at baseline, length of follow-up, socio-demographic variables (age, gender, socio-economic status, ethnicity and marital status), medications, comorbid mental disorders (anxiety and substance use disorders), behavioral cardiovascular risk factors (physical activity and smoking status) and physical cardiovascular risk factors (BMI, diabetes, hypertension and dyslipidemia) at baseline. "No mood disorders" considered as the comparison group.

Associations between inflammatory markers and a current diagnosis of bipolar disorder could not be assessed due to small sample size.

^a Multiple regression with logarithmically transformed cytokine (n = 2796) or hsCRP concentrations (n = 2896).

TABLE 4: Associations between inflammatory markers at baseline and mood disorders at follow-up (n=2580)

Levels of Inflammatory marker at baseline	Bipolar disorder n = 13			MDD n = 608			MDD Combined Subtype n = 67			MDD Atypical Subtype n = 129			MDD Melancholic Subtype n = 155			MDD Unspecified Subtype n = 257		
	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]	0.68	(0.37-1.25)	0.215	0.96	(0.88-1.06)	0.436	0.95	(0.74-1.22)	0.703	0.87	(0.73-1.05)	0.153	1.04	(0.88-1.22)	0.674	0.96	(0.84-1.09)	0.497
TNF-α [pg/ml]	0.75	(0.40-1.41)	0.372	0.86 (0.78-0.95)	0.002		1.02	(0.80-1.30)	0.889	0.83 (0.69-1.00)	0.046		0.90	(0.76-1.06)	0.214	0.86 (0.75-0.98)	0.023	
hsCRP [mg/l]	0.98	(0.56-1.69)	0.929	0.93	(0.85-1.03)	0.148	1.03	(0.81-1.32)	0.786	0.98	(0.82-1.18)	0.849	0.87	(0.74-1.03)	0.101	0.94	(0.82-1.07)	0.312
Model 2																		
IL-6 [pg/ml]	0.74	(0.35-1.56)	0.431	1.01	(0.91-1.12)	0.857	0.94	(0.72-1.23)	0.642	0.88	(0.72-1.08)	0.221	1.08	(0.91-1.28)	0.386	1.02	(0.88-1.17)	0.826
TNF-α [pg/ml]	0.75	(0.34-1.63)	0.467	0.85 (0.77-0.95)	0.003		1.05	(0.81-1.36)	0.713	0.85	(0.69-1.04)	0.117	0.88	(0.74-1.05)	0.162	0.86 (0.74-0.99)	0.034	
hsCRP [mg/l]	1.10	(0.57-2.12)	0.772	0.90	(0.81-1.00)	0.054	1.05	(0.80-1.38)	0.704	0.88	(0.72-1.08)	0.219	0.94	(0.79-1.14)	0.544	0.90	(0.78-1.04)	0.143

Abbreviations: IL-6 = Interleukin-6; TNF-α = Tumor Necrosis Factor-α; hsCRP = high sensitivity C-Reactive Protein; OR = odds ratio; CI = confidence interval; MDD = major depressive disorder.

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

Model 2: adjusted for the corresponding mental disorder at baseline, length of follow-up, socio-demographic variables (gender, age, socio-economic status, ethnicity and marital status), medications, health-related behaviors (former smoker, current smoker and physical inactivity), and physical cardiovascular risk factors (body mass index, diabetes, dyslipidemia and hypertension) at baseline.

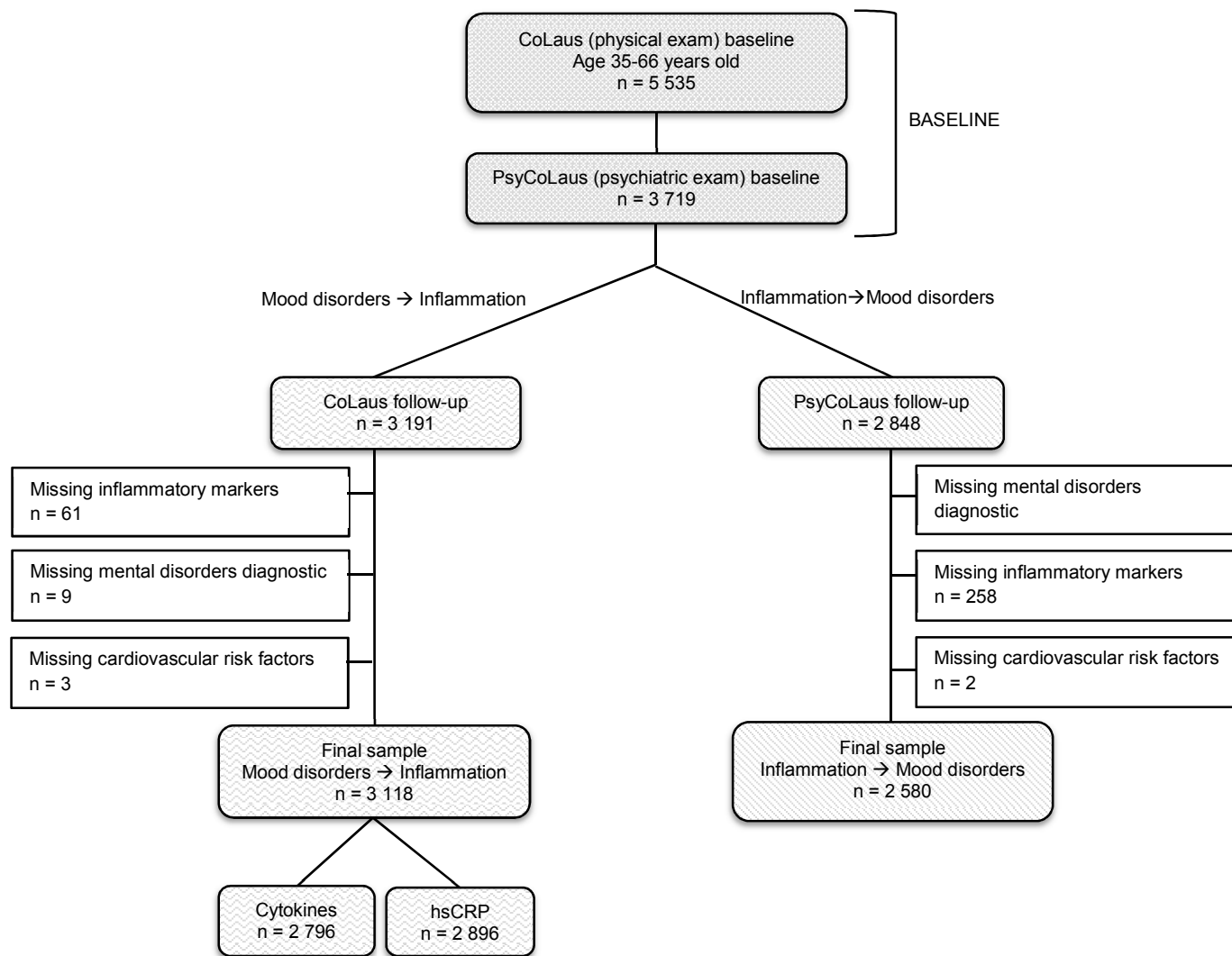





Figure 1. Flow chart of the study for the association between mood disorders at baseline and inflammatory markers at follow-up and for the association between inflammatory markers at baseline and mood disorders at follow-up. Abbreviation: hsCRP, high-sensitivity C-Reactive Protein.  Baseline assessment;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