Association of Serum Interleukin 6 and C-Reactive Protein in Childhood With Depression and Psychosis in Young Adult Life
A Population-Based Longitudinal Study

Golam M. Khandaker, PhD; Rebecca M. Pearson, PhD; Stanley Zammit, PhD; Glyn Lewis, PhD; Peter B. Jones, PhD

IMPORTANCE Longitudinal studies have linked the systemic inflammatory markers interleukin 6 (IL-6) and C-reactive protein (CRP) with the risk of developing heart disease and diabetes mellitus, which are common comorbidities for depression and psychosis. Recent meta-analyses of cross-sectional studies have reported increased serum levels of these inflammatory markers in depression, first-episode psychosis, and acute psychotic relapse; however, the direction of the association has been unclear.

OBJECTIVE To test the hypothesis that higher serum levels of IL-6 and CRP in childhood would increase future risks for depression and psychosis.

DESIGN, SETTING, AND PARTICIPANTS The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective general population birth cohort study based in Avon County, England. We have studied a subsample of approximately 4500 individuals from the cohort with data on childhood IL-6 and CRP levels and later psychiatric assessments.

MEASUREMENT OF EXPOSURE Levels of IL-6 and CRP were measured in nonfasting blood samples obtained in participants at age 9 years.

MAIN OUTCOMES AND MEASURES Participants were assessed at age 18 years. Depression was measured using the Clinical Interview Schedule–Revised (CIS-R) and Mood and Feelings Questionnaire (MFQ), thus allowing internal replication; psychotic experiences (PEs) and psychotic disorder were measured by a semistructured interview.

RESULTS After adjusting for sex, age, body mass index, ethnicity, social class, past psychological and behavioral problems, and maternal postpartum depression, participants in the top third of IL-6 values compared with the bottom third at age 9 years were more likely to be depressed (CIS-R) at age 18 years (adjusted odds ratio [OR], 1.55; 95% CI, 1.13-2.14). Results using the MFQ were similar. Risks of PEs and of psychotic disorder at age 18 years were also increased with higher IL-6 levels at baseline (adjusted OR, 1.81; 95% CI, 1.01-3.28; and adjusted OR, 2.40; 95% CI, 0.88-6.22, respectively). Higher IL-6 levels in childhood were associated with subsequent risks of depression and PEs in a dose-dependent manner.

CONCLUSIONS AND RELEVANCE Higher levels of the systemic inflammatory marker IL-6 in childhood are associated with an increased risk of developing depression and psychosis in young adulthood. Inflammatory pathways may provide important new intervention and prevention targets for these disorders. Inflammation might explain the high comorbidity between heart disease, diabetes mellitus, depression, and schizophrenia.

Published online August 13, 2014.
Depression is a leading cause of disability worldwide. It affects approximately 16% of Americans during their lifetime, with approximately 25% of these cases beginning before age 20 years. Psychotic disorders (including schizophrenia) are some of the most enduring neuropsychiatric illnesses, with a lifetime risk of 1% to 2% in the United States. Globally, schizophrenia is an important cause of disability among young people and is associated with premature death, largely from heart disease. Longitudinal studies have linked higher levels of circulating inflammatory markers such as interleukin 6 (IL-6) and C-reactive protein (CRP), with subsequent risk of type 2 diabetes mellitus and heart disease. These chronic physical conditions often occur concomitantly with depression and psychotic disorders and may share pathophysiologic mechanisms.

Mechanisms involving monoamines underpin contemporary pathophysiologic explanations and drug therapy for depression and psychosis. However, heterogeneity in presentation, course, and treatment responses suggest additional mechanisms. Supported by several observations, cytokine-mediated communication between the immune system and the brain has recently been implicated in the pathogenesis of these disorders. Animal models suggest that systemic inflammatory cytokines, such as IL-6, can communicate with the brain. An increase in cytokine serum levels may lead to decreased availability of serotonin and other neurotransmitters, activation of the hypothalamic-pituitary-adrenal axis, and increased oxidative stress in the brain. These effects could contribute to the impaired mood, cognition, and perception seen in depression and psychosis. Indeed, injection of IL-6 in healthy volunteers induces low mood, anxiety, and reduced cognitive performance. Epidemicologic studies have linked early-life infection and autoimmune disease with subsequent depression and psychosis in adult life. Currently used antidepressants and antipsychotics have anti-inflammatory properties. Finally, meta-analyses of cross-sectional studies have reported increased serum IL-6 and CRP levels in depression, first-episode psychosis, and acute psychotic relapse. However, there have been very few longitudinal studies of inflammatory markers and either psychosis or depression, and the inconsistent results may be the result of methodologic differences.

Using data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a general population birth cohort, we report a longitudinal study of serum IL-6 and CRP levels at age 9 years and the risks for depression or psychosis at age 18 years. We predicted that higher levels of these inflammatory markers at baseline would be associated with subsequent risks for depression and psychosis.

Methods

Description of Cohort

The ALSPAC birth cohort comprises 14,062 live births from women residing in Avon County, a geographically defined region in southwest England, with expected dates of delivery between April 1991 and December 1992 (http://www.bristol.ac.uk/alspac/). Parents completed regular postal questionnaires about all aspects of their child’s health and development from birth. From age 7 years, the children attended an annual assessment clinic during which they participated in various face-to-face interviews and physical tests.

The study received ethics approval from the ALSPAC Law and Ethics Committee and local research ethics committees. All participants provided written informed consent. There was no financial compensation.

The risk set for the present study included 4,585 individuals. Parents gave informed consent for venipuncture in 7,236 children aged 9 years. This was successfully completed on 5,880 children; 5076 provided enough blood to measure IL-6 and CRP levels. We were interested in baseline innate immune activity as reflected by inflammatory marker levels in healthy individuals. Thus, we excluded 491 children who reported an infection at the time of blood collection or in the preceding week because acute infection sharply increases IL-6 and CRP levels, which return to baseline after illness subsides. Of the risk set, 2,453 and 2,528 individuals, respectively, attended assessments for depression and psychosis at age 18; our main analyses were based on these samples. We subsequently repeated the analyses after imputation of missing outcome data (n = 4,415).

Laboratory Methods

Blood samples were collected from nonfasting participants and were immediately spun and frozen at −80°C. Inflammatory markers were assayed in 2008 after a median of 7.5 years in storage with no previous freeze-thaw cycles during this period. Interleukin 6 was measured by enzyme-linked immunosorbent assay (R&D Systems), and high-sensitivity CRP was measured by automated particle-enhanced immunoturbidimetric assay (Roche) (eMethods in the Supplement). All interassay coefficients of variation were less than 5%. No other inflammatory markers were measured.

Psychiatric Measures

Depression at Age 18

Depression was measured in 2 ways—interview and questionnaire—so as to allow internal replication. The computerized version of the Clinical Interview Schedule–Revised (CIS-R) was self-administered by cohort participants in assessment clinics. The CIS-R is a widely used standardized tool for measuring depression and anxiety in community samples. It includes core symptoms of depression based on International Statistical Classification of Diseases, 10th Revision, criteria and gives a total depression score of 0 to 21 comprising symptom scores for depression, depressive thoughts, fatigue, concentration, and sleep problems. Thus, the total depression score reflects the severity of depressive symptoms experienced in the past week. We used this continuous variable as the main outcome to make full use of variation in symptoms. We also created a binary outcome: individuals with scores of 7 or more were considered as cases of depression. In addition, we used 2 alternative cutoff scores (≥8 and ≥9).

Depression was also measured using the short version of the Mood and Feelings Questionnaire (MFQ) (eMethods in
We used logistic regression to calculate the odds ratios (ORs) and 95% CIs for psychiatric outcomes at age 18 among individuals in the middle and top thirds compared with the bottom third of inflammatory marker distribution at age 9. Linearity of association was tested by inspection of the ORs over the thirds of the inflammatory marker distribution. Using IL-6 levels as a continuous variable (z-transformed values), the ORs for psychiatric outcomes were calculated per SD increase in IL-6 levels; nonlinearity was examined by the inclusion of a quadratic term (IL-6^2) within these logistic regression models. Additionally for depression, linear regression calculated the coefficient for an SD increase in total CIS-R depression score at age 18 for each SD increase in IL-6 at age 9, using both depression and IL-6 as continuous variables (z-transformed values).

All regression models were adjusted for age at follow-up, body mass index (BMI) at baseline, sex, ethnicity, father’s social class, SDQ score at age 7, and maternal Edinburgh Postnatal Depression Scale score at 8 weeks postpartum.

We examined whether the direction of causality was childhood psychological problems (measured as SDQ score at age 7) leading to both increased IL-6 at age 9 and risk of psychiatric outcomes at age 18 rather than being an independent effect of IL-6 on later psychiatric risk. First, we separately assessed the associations between the SDQ score and IL-6 levels (linear regression) as well as psychiatric outcomes (logistic regression). Next, both the SDQ score and IL-6 values were included in the same regression model as predictors of psychiatric outcomes at age 18; if there was no direct effect of IL-6 on later psychiatric risk, we expected that the OR for psychiatric outcome for IL-6 would attenuate substantially.

To test whether sex modified any association between IL-6 and psychiatric outcomes, we tested for an interaction between IL-6 and sex in the logistic regression models of IL-6- and the binary outcomes of CIS-R depression, PE, and psychotic disorder.

Imputation of Missing Outcome Data
Of the 4585 individuals with inflammatory marker data at age 9 years, 4415 had provided data on depression and PEs in clinics or by questionnaires at some point between ages 10 and 16 years. For this sample of 4415, we used the depression, PE, and sociodemographic data to predict missing depression and PE information at age 18 with multiple imputation techniques used by the ice command in Stata, version 12 (Stata Corp) (eMethods in the Supplement).

Results
Baseline Comparisons and Characteristics of the Sample
Higher serum IL-6 levels at age 9 were associated with female sex, higher BMI, and father’s manual occupation (Table 1). Similar associations were also observed for CRP. After controlling for sex and BMI, IL-6 and CRP were significantly correlated (r = 0.04; P < .001).

Risk of Depression and Psychosis at Age 18
At follow-up, 422 of 2447 participants (17.2%) met the case definition for CIS-R depression; 121 of these individuals (4.9%) were
Higher baseline IL-6 levels were associated with an increased risk of depression (both CIS-R continuous and binary measures) and psychotic outcomes at follow-up. The Figure presents the proportion of individuals with depression and PE at age 18 by thirds of IL-6 at age 9.

Using the continuous measure of depression, the regression coefficient for an SD increase in the total CIS-R depression score at age 18 for each SD increase in serum IL-6 levels at age 9 was 0.03; the quadratic terms for potential confounders, this finding attenuated slightly to 0.06 (SE, 0.03; \( P = .02 \)). After adjustment for potential confounders, this finding attenuated slightly to 0.08 (SE, 0.03; \( P = .02 \)).

Using the CIS-R binary case definition, the adjusted OR for depression for participants in the top compared with the bottom third of IL-6 distribution was 1.55 (95% CI, 1.13-2.14) (Table 3). The adjusted OR for PEs was 1.81 (95% CI, 1.01-3.28) and that for psychotic disorder was 2.40 (95% CI, 0.88-6.22) (Table 4). There was no evidence of an association between psychiatric outcomes and CRP levels.

The associations between baseline IL-6 and subsequent depression and PEs were both consistent with a linear relationship. For each SD increase in IL-6, the OR for CIS-R binary depression was 1.14 (95% CI, 1.03-1.26; \( P = .01 \)) and the OR for PEs was 1.24 (95% CI, 1.06-1.46; \( P = .006 \)). The quadratic terms for IL-6 within these regression models were nonsignificant (\( P = .15 \) and \( P = .57 \), respectively).

We carried out additional analyses to examine the association between IL-6 and depression. Interleukin 6 was associated with depression using 2 alternative cutoff scores for CIS-R to define cases (eTable 1 in the Supplement), as well as the alternative measure of depression, MFQ (eResults, eTable 2 in the Supplement).

Effects of Previous Psychological and Behavioral Problems on Later IL-6 and Psychiatric Outcomes

The SDQ score at age 7 was associated with IL-6 levels at age 9, and both factors were independently associated with sub-
sequent risks for depression and, separately, PE at age 18. The ORs for these psychiatric outcomes for IL-6 after adjusting for the SDQ score were robust and remained statistically significant (Tables 3 and 4). Thus, our data suggest that the associations between IL-6 and later psychiatric outcomes are independent of the effect of early-life psychological and behavioral problems on IL-6 levels.

**Effect Modification by Sex**

No test results for the interaction between IL-6 and sex in the logistic regression models of IL-6 and CIS-R depression, PEs, and psychotic disorder approached statistical significance ($P = .94$, $P = .51$, and $P = .56$, respectively). These findings indicate that there were no sex differences in the associations between IL-6 and subsequent psychiatric outcomes.

**Results After Imputation of Missing Outcome Data**

The pattern of all results remained remarkably similar. Interleukin 6 was associated with depression (CIS-R and MFQ, both continuous and binary measures) and PE ($e$Results and $e$Tables 3, 4, and 5 in the Supplement). After imputation and adjustment for confounders, the ORs for CIS-R binary definition of depression and the ORs for PEs fell short of conventional statistical significance.

**Discussion**

Our findings demonstrate a longitudinal dose-response association between childhood IL-6 levels and future risk for depression in young adulthood. The association persisted after adjusting for several potential confounders including sex, BMI, social class, past psychological and behavioral problems, and maternal depression. Childhood IL-6 levels were associated with subsequent PEs, again in a dose-dependent manner. Interleukin 6 was also associated with the stricter phenotype of psychotic disorder at age 18.
Cross-sectional studies suggest that increased IL-6 and CRP levels occur in current depression and psychosis. However, there have been few longitudinal studies of inflammatory markers before depression, and these have yielded inconsistent results. One study based on hospital admission data found no evidence that CRP levels predicted later depression. Few patients with depression require hospitalization, so hospital registers alone are prone to ascertainment bias. A large study of London civil servants reported that IL-6 and CRP levels were associated with later cognitive symptoms of depression in men. One study involving perimenopausal women reported an association between CRP and later depression. Our findings in a general population birth cohort demonstrate associations between IL-6 and later depression and PEs independent of the effect of previous psychological problems on IL-6.

To our knowledge, this is the first longitudinal study of any inflammatory marker in childhood and subsequent psychotic disorder. We included the restricted category of psychotic disorder to assess whether the association became stronger when this category was applied. The operational definition for psychotic disorder, as described in the Methods section, has been used previously. The OR for psychotic disorder for higher IL-6 levels was slightly larger than that for PEs (Table 4). However, only 19 cases with complete data on confounders were included in this analysis; the 95% CI for the OR included unity, but the pattern of the results remained the same. We included participants with PEs or psychotic disorders who were not depressed in the comparison group in the analysis of depression and vice versa. This process is likely to have resulted in an underestimation of the true association between IL-6 and later psychiatric outcomes.

One limitation of the present study is attrition. Nearly half of the individuals with inflammatory marker data at baseline did not attend psychiatric follow-up sessions. However, the mean and median values of IL-6 were similar between the groups who did and did not attend assessments for depression and PEs. Compared with participants who attended the clinic at age 18, those who did not attend had more psychological problems at age 7 and lower socioeconomic status, and their mothers had higher postpartum depression scores. These factors were associated with an increased risk of psychiatric outcomes at age 18. Thus, it is likely that any bias from sample attrition has led to an underestimation of the true association between IL-6 and later psychiatric morbidity. Furthermore, we carried out multiple imputations to examine the effect of missing data. The pattern of all results remained remarkably similar after imputation of missing outcome data, increasing our confidence in the robustness of these results.

Subsequent blood samples from childhood were not analyzed to examine long-term, within-individual consistency of IL-6 levels. However, one study of healthy volunteers found no significant changes in IL-6 levels during a 3-year period. We acknowledge that nonfasting blood samples may be open to ascertainment bias. A large study of London civil servants reported that IL-6 and CRP levels were associated with later cognitive symptoms of depression in men. One study involving perimenopausal women reported an association between CRP and later depression. Our findings in a general population birth cohort demonstrate associations between IL-6 and later depression and PEs independent of the effect of previous psychological problems on IL-6.

<p>| Table 4. ORs for Psychotic Outcomes at Age 18 Years for Serum IL-6 and CRP Levels at Age 9 Years |
|---------------------------------|----------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Inflammatory Marker</th>
<th>Group</th>
<th>No.</th>
<th>Psychotic, No. (%)</th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Psychotic experiences at age 18 y</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Bottom third</td>
<td>887</td>
<td>27 (3.0)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>Middle third</td>
<td>842</td>
<td>32 (3.8)</td>
<td>1.25 (0.75-2.12)</td>
<td>1.23 (0.73-2.09)</td>
<td>1.21 (0.68-2.16)</td>
<td>1.12 (0.60-2.11)</td>
<td></td>
</tr>
<tr>
<td>Top third</td>
<td>793</td>
<td>42 (5.3)</td>
<td>1.78 (1.09-2.92)</td>
<td>1.73 (1.04-2.88)</td>
<td>1.89 (1.12-3.22)</td>
<td>1.81 (1.01-3.28)</td>
<td></td>
</tr>
<tr>
<td>Linear trend</td>
<td>2522</td>
<td>101 (4.0)</td>
<td>1.34 (1.04-1.71)</td>
<td>1.32 (1.02-1.70)</td>
<td>1.39 (1.06-1.81)</td>
<td>1.36 (1.01-1.84)</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>Bottom third</td>
<td>930</td>
<td>32 (3.4)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>Middle third</td>
<td>831</td>
<td>31 (3.7)</td>
<td>1.08 (0.66-1.80)</td>
<td>1.06 (0.64-1.76)</td>
<td>1.06 (0.61-1.85)</td>
<td>1.01 (0.55-1.83)</td>
<td></td>
</tr>
<tr>
<td>Top third</td>
<td>767</td>
<td>38 (5.0)</td>
<td>1.46 (0.90-2.36)</td>
<td>1.39 (0.82-2.34)</td>
<td>1.62 (0.96-2.71)</td>
<td>1.25 (0.67-2.34)</td>
<td></td>
</tr>
<tr>
<td>Linear trend</td>
<td>2528</td>
<td>101 (4.0)</td>
<td>1.21 (0.95-1.54)</td>
<td>1.18 (0.90-1.53)</td>
<td>1.28 (0.98-1.66)</td>
<td>1.12 (0.82-1.53)</td>
<td></td>
</tr>
<tr>
<td><strong>Psychotic disorder at age 18 y</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Bottom third</td>
<td>887</td>
<td>9 (1.0)</td>
<td>0.93 (0.36-2.43)</td>
<td>0.95 (0.36-2.48)</td>
<td>0.63 (0.18-2.16)</td>
<td>0.51 (0.13-2.14)</td>
</tr>
<tr>
<td>Middle third</td>
<td>842</td>
<td>8 (1.0)</td>
<td>2.26 (1.01-5.07)</td>
<td>2.36 (1.03-5.39)</td>
<td>2.39 (0.97-5.91)</td>
<td>2.40 (0.88-6.22)</td>
<td></td>
</tr>
<tr>
<td>Top third</td>
<td>793</td>
<td>18 (2.3)</td>
<td>1.58 (1.03-2.42)</td>
<td>1.61 (1.04-2.49)</td>
<td>1.69 (1.03-2.78)</td>
<td>1.73 (1.00-3.03)</td>
<td></td>
</tr>
<tr>
<td>Linear trend</td>
<td>2522</td>
<td>35 (1.4)</td>
<td>1.80 (0.81-4.00)</td>
<td>1.77 (0.79-3.98)</td>
<td>1.45 (0.57-3.70)</td>
<td>1.31 (0.47-3.65)</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>Bottom third</td>
<td>930</td>
<td>10 (1.1)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>Middle third</td>
<td>831</td>
<td>16 (1.9)</td>
<td>1.09 (0.44-2.70)</td>
<td>1.06 (0.40-2.80)</td>
<td>1.25 (0.46-3.36)</td>
<td>0.92 (0.28-2.99)</td>
<td></td>
</tr>
<tr>
<td>Top third</td>
<td>767</td>
<td>9 (1.2)</td>
<td>1.05 (0.70-1.58)</td>
<td>1.05 (0.67-1.63)</td>
<td>1.12 (0.70-1.79)</td>
<td>0.97 (0.55-1.70)</td>
<td></td>
</tr>
<tr>
<td>Linear trend</td>
<td>2528</td>
<td>35 (1.4)</td>
<td>1.05 (0.70-1.58)</td>
<td>1.05 (0.67-1.63)</td>
<td>1.12 (0.70-1.79)</td>
<td>0.97 (0.55-1.70)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; IL-6, interleukin 6; OR, odds ratio.
* Cutoff values for the top and bottom thirds of the distribution of IL-6 values in the total sample (cases and noncases combined) were 1.08 and 0.57 pg/mL, respectively. Cutoff values for the top and bottom thirds of the distribution of CRP values in the total sample were 0.33 and 0.13 mg/L, respectively.

Unadjusted Model 1* Model 2* Model 3*

* Adjusted for body mass index at baseline.
* Adjusted for psychological and behavioral problems at age 7 years.
* Adjusted for body mass index, past psychological and behavioral problems, age, sex, social class, ethnicity, and maternal postpartum depression.
Interleukin 6 and C-Reactive Protein as Predictors

Longitudinal associations between inflammatory markers and heart disease,7–11 diabetes,10 depression, and psychosis could all be linked with early-life factors influencing inflammatory regulation, such as impaired fetal development or childhood adversity. This view is consistent with Barker’s14–16 common-cause hypothesis, which postulates that alterations in physiologic systems from early-life adversity can increase the risk for several chronic diseases in adulthood. Low birth weight, a marker of suboptimal fetal development, is associated with increased circulating inflammatory markers16 and risks of heart disease, diabetes,15 depression,17 and schizophrenia in adults.47 Thus, inflammation might be a common cause for many chronic adult diseases.

Conclusions

Our study provides evidence for a longitudinal association between a circulating inflammatory marker in childhood and future risks for depression and psychosis. Inflammatory pathways may provide important new prevention and intervention targets for major mental illnesses while also undermining the helplessly persistent Cartesian division between the mind and body.

REFERENCES


