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C-REACTIVE PROTEIN, INTERLEUKIN-6, SOLUBLE TUMOR NECROSIS FACTOR α RECEPTOR 2 AND INCIDENT CLINICAL DEPRESSION

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Abstract

Background—Despite an extensive literature on the role of inflammation and depression, few studies have evaluated the association between inflammatory biomarkers and depression in a prospective manner, and results are inconclusive.

Methods—We conducted a prospective analysis of blood levels of CRP, IL-6 and TNFα-R2 in 4,756 women participating in the Nurses' Health Study who donated blood in 1990 and were depression-free up to 1996. Participants were followed between 1996 and 2008 for reports of clinical diagnosis depression or antidepressant use. Additionally, we conducted cross-sectional analyses for CRP, IL-6 and TNFα-R2 and antidepressant use at time of blood draw.

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CONTRIBUTORS The authors' responsibilities were as follows:
Dr. Chocano-Bedoya, Dr. O'Reilly and Dr. Ascherio designed research; Dr. Hu, Dr. Rimm and Dr. Ascherio contributed to data acquisition; Dr. Chocano-Bedoya conducted statistical analysis and Dr. Mirzaei, Dr. O'Reilly, Dr. Lucas, Dr. Okereke, Dr. Rimm and Dr. Ascherio contributed to data analysis and interpretation; Dr. Chocano-Bedoya wrote the first draft of the manuscript and Dr. Mirzei, Dr. O'Reilly, Dr. Lucas, Dr. Okereke, Dr. Hu, Dr. Rimm and Dr. Ascherio critically reviewed the manuscript for important intellectual content; Dr. Chocano-Bedoya, Dr. O'Reilly and Dr. Ascherio had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have contributed to and approved the final manuscript.

Results—After adjustment for body mass index, menopause status, use of anti-inflammatory drugs and other covariates, no significant associations between CRP, IL-6 and TNFα-R2 and incident depression were observed after a follow-up of 6-18 years. However, menopause status appears to modify the association between IL-6 and depression risk. In cross-sectional analyses, TNFα-R2 was associated with antidepressant use (OR=1.96, 95% CI=1.23-3.13, P-trend=0.001), but no significant associations were found for CRP and IL-6.

Limitations—Depression diagnosis was first assessed in 1996, 6 years after blood draw. However the biomarkers have high within-person correlations with measurements 4 years apart.

Conclusions—Blood levels of CRP, IL-6 and TNFα-R2 were not associated with incident depression over a follow-up of 6-18 years. In cross-sectional analyses, antidepressant use may be associated with higher levels of TNFα-R2 but no associations with depression or antidepressant use were observed in the prospective analysis.

Keywords

Inflammation; Depression; Prospective study; C-reactive protein; Interleukin-6; Soluble tumor necrosis factor – receptor 2

1. INTRODUCTION

Depression is the fourth leading cause of disability worldwide, yet its pathophysiology is still uncertain (Ustun et al., 2004). A role of inflammation in depression has been proposed based on several lines of evidence, including emergence of depressive symptoms following interferon-α treatment (Raison et al., 2005) and higher levels of inflammatory markers among people with depression (Dowlati et al., 2010; Howre et al., 2009; Liu et al., 2012).The mechanisms by which inflammation may affect depression include activation of the hypothalamic-pituitary –adrenal axis, depletion of tryptophan for serotonin conversion, and decreased neuroplasticity (Miller et al., 2009; Felger and Lotrich, 2013).

Among the inflammatory markers investigated in depression are C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor α (TNFα). CRP, produced by the liver, interacts with IL-6 and it is a marker of inflammation previously associated with cardiovascular (Pai et al., 2004) and diabetes risk (Hu et al., 2004). TNFα and IL-6 are acute-phase proteins secreted into the bloodstream in response to immunologic challenges; elevations in the absence of infection or injury are abnormal.

Recent meta-analyses of cross-sectional studies have reported increased levels of CRP (Howren et al., 2009), IL-6 (Dowlati et al., 2010; Howren et al., 2009; Liu et al., 2012) and TNFα (Dowlati et al., 2010; Liu et al., 2012) among people with depression compared to healthy controls (Howren et al., 2009; Liu et al., 2012). However, the results from prospective studies are inconsistent. While some prospective studies have observed that CRP is associated with a higher risk of depressive symptoms (Gimeno et al., 2009; Matthews et al., 2010; Pasco et al., 2010; van den Biggelaar, et al, 2007)others have not found significant associations (Baune et al., 2012; Matthews et al., 2007; Milaneschi et al., 2009; Stewart et al., 2009). With regard to IL-6, two prospective studies observed a significant association with depressive symptoms (Baune et al., 2012; Gimeno et al., 2009),

while three did not (Milaneschi et al., 2009; Stewart et al., 2009; van den Biggelaar et al., 2007). Only three prospective studies evaluated TNFα and depression risk, with no significant results (Baune et al., 2012; Milaneschi et al., 2009; van den Biggelaar et al., 2007). Moreover, most of the current prospective studies have used short-term questionnaires to evaluate change in depression symptoms as the outcome of interest, and only one study(Pasco et al., 2010) evaluated incident depression diagnosis.

Therefore, we prospectively studied the role of the inflammatory biomarkers CRP, IL-6, and the soluble TNFα-receptor 2 (TNFα-R2) and incident clinical depression among participants in the Nurses' Health Study (NHS) without depressive symptoms at the time of blood collection.

2. METHODS

The NHS was established in 1976 when 121,700 female US registered nurses replied to a mailed questionnaire about lifestyle factors and medical history. Participants update their information in biennial questionnaires, with a follow-up rate greater than 90% to date. Between 1989 and 1990, 32,826 participants (aged 43 to 70 years) free of diabetes, coronary heart disease, stroke, and cancer provided blood samples in heparin-containing tubes. Women shipped their samples overnight with an icepack and a questionnaire including information about time of blood draw. Upon arrival, blood samples were aliquoted into plasma, WBC, and RBC components and stored in liquid nitrogen freezers with an electronic alarm system; the majority of samples arrived for processing within 24 hours of draw.

Blood levels of CRP, IL-6 and TNFα-R2 have been measured in subsets of the population (overall 11,709 participants) for 15 nested case-control studies and one validation study (supplementary table 1). Our study is restricted to the 6892 controls (disease-free participants) from the aforementioned studies. Among these women, we excluded those who reported history of depression (lifetime depression before 1996, n=345), antidepressant medication use (assessed in the blood sample questionnaire and since 1996 in the biennial questionnaires, n=271) or severe depressive symptoms in 1992 or 1996 (a Mental Health Index score ≤52 (Berwick et al., 1991) as reported in the biennial questionnaires, n=335), or those with incomplete information (n=1026). We additionally excluded 159 women who had CRP levels higher than 10 mg/L as such high levels are potentially associated with acute inflammation or trauma, and thus may not appropriately represent chronic inflammation (Pearson et al., 2003).

Our baseline population comprised a total of 4,756 apparently healthy women who were depression free up to 1996 and who had information on CRP, IL-6, and/or TNFα-R2 blood levels because they were selected as controls in other studies. Participants were followed until June 2010 for the development of clinical depression. This protocol has been approved by the Institutional Review Board at Brigham and Women's Hospital, Boston, MA.

2.1 Assessment of depression status

Starting in 1996, participants were asked about their antidepressant use in the biennial questionnaires. In 2000, participants reported if they ever had a physician diagnosis of depression and the year of diagnosis (≤1996, 1997-1998, 1999 or on 2000). Women who reported depression diagnosis in 1996 or earlier were not considered incident depression cases in our study, because they may have had depression before blood draw in 1989-1990. After 2000, information on physician diagnosed depression was updated biennially.

We considered a “strict” and a “broad” definition of a first lifetime clinical depression based on clinical diagnosis and antidepressant use. The strict definition included only women who reported both a clinical diagnosis of depression and antidepressant use. The broad definition comprised women who reported a diagnosis of depression but did not take antidepressants and those who took antidepressant but did not report a diagnosis.

2.2 Measurement of CRP, IL-6, and TNFα-R2

The laboratories that conducted all of the analyses have undergone rigorous blinded pilot testing. Aliquots from pooled quality control (QC) specimens, indistinguishable from study specimens, were randomly interspersed among case-control samples to monitor quality. Coefficients of variation ranged from 1.6–3.0% for CRP, 0.07–6.1% for IL-6, and 4.9–11.6% for TNFα-R2.

CRP (mg/L) was measured using a high-sensitivity latex-enhanced immunonephelometric assay on a BNII analyzer. High sensitivity CRP (hs-CRP) was measured instead of CRP in 1503 participants (38% of the sample). As most of the TNFα had likely degraded after shipping and processing, soluble TNFα-R2 was measured instead. IL-6 (pg/ml) and TNFα-R2 (pg/ml) were measured by an ultra-sensitive quantitative sandwich enzyme immunoassay from R&D Systems. CRP and TNFα-R2 stability were assessed in 17 fresh blood samples from the NHS, at baseline and after a delay in processing of 24 and 36 hours. The intraclass correlation coefficient (ICC) was >75% for the comparison of 0 to 36 hours(Pai et al., 2002). The day-to-day assay variabilities of TNFα-R2 at the concentrations of 54.8, 252 and 356 pg/mL were 8.8, 3.7 and 5.8%, respectively. The intraclass correlations of these biomarkers measured 4 years apart were 0.67 for CRP, 0.47 for IL-6 and 0.78 for TNFα-R2 among 84 men from the Health Professionals Follow-up study, a cohort similar to the NHS (Pischon et al., 2003).

2.3 Assessment of covariates

The blood questionnaire included information on antidepressant use, menopausal status, hormonal replacement therapy, and on weight, which was used to estimate body mass index [BMI; weight (in kg)/height^2 (in m)]. Physical activity information was collected in 1988 and 1992 on the general biennial questionnaires using validated questions (Wolf et al., 1994) and metabolic equivalent task (METs) hours per week were calculated. Because the questionnaire in 1990 (most recent to time of blood draw) did not include physical activity, we used the average of 1988 and 1992. Information on smoking, medication and multivitamin use, and diagnosis of chronic disorders was collected in the biennial questionnaires. Diet, including alcohol and caffeine intake, was assessed using a validated

semiquantitative food frequency questionnaire in 1990 (Rimm et al., 1992; Willett et al., 1985) and an alternative healthy eating score (AHEI)was calculated on the bases of existing dietary guidelines with additional scoring for qualitative dietary guidance (a maximum possible score of 87.5 represents healthiest diet, and a minimum of 2.5 represents least healthy)(McCullough et al., 2002). Information on personal education (bachelor, registered nurse, master's or doctoral degree) and education level of husband (complete high school, college degree or higher) was collected in 1992. Neighborhood socioeconomic status summary scores were estimated from participants' addresses based on US Census information about wealth and income, educational levels, and occupation (Kim et al., 2010).

2.4 Statistical analyses

Because participants were originally controls from 16 previous studies, CRP, IL-6, and TNFα-R2blood levels were adjusted by batch according to the methods described before (Rosner et al., 2008). Briefly, all biomarkers were first regressed on age, BMI, menopause status and study of origin and predicted values were subtracted from the average study effect. Then, participants were divided into groups according to quartiles of biomarker blood levels. We additionally categorized CRP levels into cutoff points of low, moderate and high risk of cardiovascular disease (<1 mg/L, 1-3 mg/L, and >3 mg/L respectively) as reported in the American Heart Association guidelines (Ridker, 2003). Age-adjusted baseline characteristics were compared among groups using ANOVA. The correlation between CRP, IL-6, TNFα-R2, and BMI was assessed with Spearman correlation coefficients.

To evaluate whether CRP, IL-6 or TNFα-R2 levels were associated with development of a first clinical depression over the follow-up time (6 to 18 years) we used Cox proportional hazards models to estimate relative risks (RR) and 95% confidence intervals (CI) using the lowest category as the reference and stratifying for age in months. Person-years of follow-up for each participant were computed from baseline (1996) to the date of first diagnosis of depression, date of reported start of antidepressant medication, death, end of total follow-up (June 2008) or the last questionnaire received, whichever came first. In multivariable analyses, we adjusted our models for BMI, smoking, alcohol intake, physical activity, menopausal status, hormone therapy use, use of non-steroidal anti-inflammatory drugs (NSAIDS) or aspirin, and socioeconomic status (represented by personal education, husband's education and neighborhood socioeconomic status) at the time of the blood draw as well as development of comorbidities (myocardial infarction or angina, diabetes, hypertension or cancer diagnosis) during the follow-up. To evaluate linear trends across categories in the regression models, we used a Mantel extension test for trend, modeling the medians of each quartile or category as a continuous variable. These analyses were conducted for both definitions of depression. We also assessed potential effect modification of BMI, menopause status, and smoking status in the association between inflammatory markers and depression. In sensitivity analyses, we restricted our follow-up time to June 2002 to evaluate a shorter term effect of these biomarkers (total follow-up time of 12 years instead of 18 years).

Finally, we conducted cross-sectional analyses of biomarker levels and antidepressant use at the time of blood draw among all women with information on any of these biomarkers

(n=6425). Using logistic regression, we estimated odds ratios (OR) and 95% CIs, adjusting for age and the same covariates as for the prospective models.

3. RESULTS

3.1 Baseline characteristics and correlation between biomarkers

Compared to women in the lowest quartile for CRP, IL-6, and TNFα-R2, those in the highest quartile were older, had higher BMI, lower physical activity, lower alcohol intake, less healthy diet (i.e. lower AHEI), were more likely to be smokers and were less likely to have an advanced degree (masters or doctorate). Interestingly, women in the highest quartile of CRP reported a higher intake of hormone therapy, while women in the highest quartile of IL-6 and TNFα-R2 had a lowest intake of hormones, compared to the lowest quartiles (Table 1). All biomarkers were moderately correlated with each other (range Spearman correlation coefficients=0.25-0.40, P<0.0001), and with BMI (range of correlation coefficients=0.21-0.39, P<0.0001) (Table 2).

3.2 Prospective analyses of CRP, IL-6 and TNFα-R2 levels and incident clinical depression

Between 1997 and 2010, there were 703 new cases under the broad definition (diagnosis of depression or antidepressant use) and 292 new cases under the strict definition (diagnosis of depression and antidepressant use) among 4403 women who had CRP blood levels measured. Using the broad definition, we observed a significant association between CRP levels and depression risk in the age- and BMI-adjusted models but no significant associations after additional multivariate-adjustment (RR=1.18, 95% CI=0.91-1.53, P trend=0.29). Results were similar but slightly attenuated for the analyses using the strict definition of depression (Table 3). No significant associations were observed when CRP was categorized in <1, 1-3, and >3 mg/L (Ridker, 2003) (multivariate-adjusted RR =1.06, 95% CI=0.73-1.53, P trend=0.95, for highest vs. lowest category or CRP and depression under the strict definition).

Among 2661 participants with measured IL-6 blood levels, there were 419 new cases of clinical depression under the broad definition and 166 new cases under the strict definition between 1997 and 2010. Although a significant increased risk of clinical depression among women in the highest quintile of IL-6 was observed under the broad definition in the age-adjusted models (RR=1.40, 95% CI=1.04-1.87, P trend=0.04). However, this association was no longer significant in the multivariate-adjusted models or using the strict definition. Among 3028 women who had information on TNFα-R2 blood levels, there were 463 and 186 new clinical depression cases under the broad and the strict definition respectively. No significant associations were observed between TNFα-R2 and incident depression under either definition (Table 3).

3.3 Effect modification

There was a significant interaction between IL-6 and menopausal status (likelihood ratio test, p-value=0.01). Among postmenopausal women that were users of hormone replacement therapy at the time of blood draw (n=1041), IL-6 was associated with a higher risk of depression under the broad definition (RR=1.93, 95% CI=1.08-3.43, P trend 0.07 for the

multivariate adjusted model, Supplementary Table 2). No significant associations were observed between IL-6 and depression among postmenopausal women who were not users of hormone replacement therapy (n=1089) or among premenopausal women (n=449). No significant interactions were observed between menopausal status and the other two biomarkers, CRP and TNFα-R2, and depression. BMI and smoking were not significant effect modifiers of the relationships between CRP, IL-6, and TNFα-R2 and depression risk.

3.4 Cross-sectional analyses of CRP, IL-6 and TNFα-R2 and prevalent use of antidepressants at time of blood draw

In an additional cross-sectional analysis, TNFα-R2 levels were significantly positively associated with use of antidepressant use at time of blood draw in all models (multivariate-adjusted Odds Ratio Q4 vs. Q1 =1.96, 95% CI= 1.23-3.13, P trend=0.001, table 4). CRP blood levels were associated with antidepressant use only in age- and BMI-adjusted models but not in the multivariate-adjusted models. No significant associations were observed between IL-6 levels and antidepressant use at time of blood draw.

3.5 Sensitivity analyses

To evaluate shorter term effects of these biomarkers on depression risk, we conducted a sensitivity analysis restricting the follow-up period to 6- 12 years after blood draw. Under the broad definition, CRP levels were associated with a higher risk of depression. Women in the highest quartile of CRP had 50% higher risk of depression (RR=1.50, 95%CI=1.05-2.13, P trend=0.12) compared to those in the lowest quartile. However this association was not significant when we used the strict definition of depression. For IL-6 and TNFα-R2, no significant associations were observed with depression under either definition.

4. DISCUSSION

In this prospective study of inflammatory markers and depression risk, blood levels of CRP, IL-6, and TNFα-R2 were not associated with incident clinical depression over a period of 6-18 years. Although in age adjusted models high levels of CRP were associated with a higher risk of depression, this association was no longer significant after adjustment for BMI and other important covariates, including hormone therapy and NSAID use. In the additional cross-sectional analyses, we observed an increased likelihood of antidepressant use among women with highest levels of TNFα-R2, but no significant associations with CRP or IL-6.

From 8 previous prospective studies conducted on CRP and depression (Baune et al., 2012; Gimeno et al., 2009; Matthews et al., 2007; Matthews et al., 2010; Milaneschi et al., 2009; Pasco et al., 2010; Stewart et al., 2009; van den Biggelaar et al., 2007), four of them found no significant results (Baune et al., 2012; Matthews et al., 2010; Milaneschi et al., 2009; Stewart et al., 2009). One of the studies that observed significant associations was conducted in the Leiden-85 plus cohort, with a population significantly older than ours (van den Biggelaar et al., 2007). Another study conducted among 3,339 participants (mean age 50 years at baseline) from the Whitehall cohort (Gimeno et al., 2009) reported a significant association between CRP levels and depression symptoms (General Health Questionnaire; β=0.04, S.E.=0.01, P=0.004), but when restricted to women (n=951)the association was not

significant (β=0.02, S.E.=0.03, P=0.52). More recently, a prospective study (Pasco, et al, 2010) was conducted among 1,494 Australian women aged 20-84 years followed for over 10 years to evaluate the role of CRP on incident depression using the Structured Clinical Interview for DSM-IV disorders questionnaire. The authors observed a 44% risk increase in depression risk for each standard deviation increase in log-transformed hs-CRP. Other two studies (Matthews et al., 2007; Matthews et al., 2010) were based in the U.S. SWAN study (Study of Women's Health Across the Nation), where participants aged 42-52 years were followed for five years and had measurements of depressive symptoms using the Center for Epidemiologic Studies questionnaire (CES-D). In the 2007 paper (Matthews et al., 2007), the authors observed a significant association between fibrinogen, plasminogen activator inhibitor Type 1, and tissue-type plasminogen activator antigen and depression risk but no significant associations of CRP with depression over a 5 year period (total participants=3249). However, in additional analyses (Matthews et al., 2010) excluding women with comorbidities at baseline (total participants=1781), the authors observed that CRP predicted higher CES-D depression scores in the following year (p=0.03).

With regards to IL-6, three previous prospective studies have observed no association with depression (Milaneschi et al., 2009; Stewart et al., 2009; van den Biggelaar et al., 2007), while two have found a higher risk of depressive symptoms associated with higher levels of IL-6 (Baune et al., 2012; Gimeno et al., 2009). In the study conducted among the Whitehall cohort (Gimeno et al., 2009) the association between IL-6 and depression symptoms was significant in the total population (β=0.05, S.E=0.02, p=0.01), but not significant when restricted to women (β=0.03, S.E.=0.03, p=0.26) in consistency with their CRP results. Another study (Baune et al., 2012) conducted among 1037 participants of the Sydney Memory and Aging Study (age 70-90 years) reported a significant positive association between IL-8 and IL-12p70 and Geriatric Depression Scale (GDS) scores at follow-up, while the association between IL-6 and GDS scores was only marginally significant (β=0.04, S.E.=0.02, P=0.09). CRP and TNFα were not associated with depression, similarly to our results.

We additionally observed that results varied according to menopausal status for IL-6 and depression. Given the age of our population, the percentage of premenopausal women in our study was small (15.5%). Levels of IL-6 have been shown to vary across the menstrual cycle (Gorai et al., 1998) and increase after natural or surgical menopause (Yasui et al., 2007). The effect of hormone replacement therapy on IL-6 levels has been studied extensively with inconsistent results (Koh and Byung-Koo 2006; Lakoski and Herrington, 2005), particularly because of differences in treatments. For example, in a study (Vitale et al., 2005) conducted among 205 postmenopausal women, estrogen alone was associated with lower levels of IL-6 (−14% percent change, p<0.01) but higher IL-6 levels were observed among women taking estrogen-progestogen associations or tibolone. Future studies among younger women are needed to explain the role of menopause and hormone replacement therapy in the association between inflammation and depression risk.

We used soluble TNFα-R2 as a proxy for TNFα, because TNFα is easily degraded during shipping and processing of blood samples, and TNFα-R2 has a longer half-life and higher sensitivity than TNFα (Aderka, 1996) . Similarly to our results, three prospective studies

that evaluated the role of TNFα and depression risk did not observe a significant association (Baune et al., 2012; Milaneschi et al., 2009; van den Biggelaar et al., 2007). However, in two meta-analyses (Dowlati et al., 2010; Liu et al., 2012) of cross-sectional studies of cytokines and major depression TNFα was significantly higher among participants with depression. Two more recent cross-sectional studies using TNFα-R2 as a proxy reported increased levels of TNFα-R2 among patients with depression compared to healthy controls (Diniz et al., 2010; Grassi Oliveira et al., 2009).

While antidepressants have been associated with lower levels of CRP and IL-6 (Hiles et al., 2012), we observed no significant associations of CRP and IL-6 with antidepressant use at time of blood draw. In a previous cross-sectional study (Narita et al., 2006), patients with remitted depression receiving low-maintenance antidepressant therapy had lower levels of TNFα. In contrast, we observed significantly higher levels of TNFα-R2 among participants taking antidepressants. While antidepressants may have an anti-inflammatory role, TNFα-R2 could also be involved in neuroprotection (Marchetti et al., 2004) and the direction of this association was not clear.

An important limitation of our study is the lag time between blood draw and depression assessment. While the follow up period of the previous prospective studies is on average 5 or 6 years, our follow up starts 6 years after blood draw. Thus it is possible that significant associations between these biomarkers and depression have been observed with a shorter follow-up term. After restriction of the follow-up period to 6-12 years (instead of 6 to 18 yrs.) we observed that CRP was associated with higher risk of depression using the broad definition but we did not observe a substantial difference in our results using our strict definition of depression. In addition, CRP, IL-6 and TNFα-R2 appear to be stable over time. In a validation study conducted among 84 men from the Health Professionals Follow-up study, a cohort of men similar to the NHS, the intraclass correlations for plasma cytokine levels measured 4 years apart were high (CRP=0.67, IL-6=0.47, TNFα-R2 =0.78)(Pischon et al., 2003). In the NHS, one time measurements of inflammatory biomarkers have been associated with long-term risk diabetes (Hu et al., 2004), heart disease(Pai et al., 2004) and rheumatoid arthritis (Karlson et al., 2009) among others.

Other limitations are noteworthy. First, we used a convenience sample based on controls from previous studies in which CRP, IL-6 and/or TNFα-R2 were measured. To minimize the variation between studies, we adjusted our categories by batch as described before. Second, some degree of measurement error in the laboratory may have affected the ability to detect modest associations. However levels of inflammatory markers were largely unaffected by transport conditions and had high reproducibility within subjects over time (Pai et al., 2004; Pischon et al., 2003). Finally, because we relied on self-reported data of depression diagnosis and antidepressant use, some misclassification of depression is also possible (e.g., depression is often under-diagnosed, antidepressants – especially non-serotonergic types – may be used for non-depression indications, etc.). To minimize this potential misclassification we used two definitions for clinical depression, one more sensitive (broad definition) and one more specific (strict definition). Our results were similar for both definitions, but underdiagnosis and undertreatment of depression could have limited the number of cases included in our study.

The strengths of this study include its large sample size, a long period of follow-up of a population apparently healthy and without depression at time of blood draw, and a detailed assessment of lifestyle, medication use and further adjustment for comorbidities during follow-up. Our study design allowed us to evaluate the effect of inflammation in middle age with 6 to 18 years of follow up for depression diagnosis, a time frame that has not been evaluated before. Although we did not observe a significant association between CRP, IL-6, and TNFα-R2 and depression risk during this time, an association in a shorter time frame cannot be discarded.

While these biomarkers are the most commonly studied and most reliable (Miller et al., 2009), they cannot reflect all the possible biological interactions in the human immune response. Other markers of inflammation include IL-2 (Liu et al., 2012) and IL-1β receptor antagonists (Milaneschi et al., 2009) which were associated with depression in previous studies. To clarify the role of inflammation in the development of clinical depression, future prospective studies need to be conducted including these and other biomarkers involved in the immune response. In addition, future studies should also aim to clarify shorter-term vs. longer-term effects as well as the role of menopause and hormone use. Finally, the association between antidepressants and inflammatory biomarkers needs further clarification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Age adjusted characteristics of the population at time of blood draw. NHS 1989-1990.

	CRP (mg/L), n=4403				IL-6 (pg/L), n=2661				TNFα-R2 (pg/L), n=2993			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Median	0.36	0.97	1.90	4.55	0.58	0.93	1.42	2.61	1855	2283	2694	3406
Range	0.01-0.64	0.65-1.29	1.30-2.83	2.84-10.00	0.03-0.75	0.75-1.15	1.15-1.81	1.81-98.67	82-2080	2080-2481	2481-2973	2974-8185
Age	56.5(7.2)	58.5(6.4)	59.2(6.4)	59.0(6.3)	56.3(6.8)	58.0(6.8)	58.4(6.5)	59.0(6.5)	56.2(6.7)	57.8(6.7)	58.6(6.6)	60.0(6.3)
BMI	22.8(2.7)	24.3(3.5)	25.5(3.9)	27.3(5.3)	23.2(3.0)	24.5(4.0)	25.6(4.3)	26.8(5.5)	23.8(3.2)	24.4(3.8)	25.2(4.6)	26.8(5.6)
Physical Activity (MET/wk)	24.9(26.7)	22.7(23.5)	20.2(20.8)	19.2(21.1)	25.1(26.0)	21.7(23.2)	21.1(22.0)	18.2(21.1)	23.4(26.5)	20.9(22.4)	21.7(22.4)	19.9(21.7)
Alcohol intake (mg/d)	6.0(9.9)	5.8(9.7)	6.0(10.2)	5.5(10.3)	6.3(9.2)	6.0(10.3)	5.4(10.0)	5.9(11.2)	8.2(11.6)	5.8(10.0)	5.1(9.3)	3.9(8.1)
Caffeine intake (mg/d)	258(216)	259(226)	248(212)	238(208)	246(215)	248(210)	259(219)	237(209)	265(209)	246(217)	253(216)	232(212)
AHEI	45.6(11.5)	44.0(10.9)	43.3(10.9)	42.7(10.7)	45.5(10.9)	43.7(11.1)	43.8(11.2)	41.7(11.1)	45.0(11.0)	44.1(10.6)	44.0(11.3)	42.1(11.3)
MHI-5	81.6(9.8)	81.5(10.0)	81.0(10.3)	81.6(9.9)	81.5(9.9)	81.3(9.8)	81.1(10.2)	81.6(10.0)	81.8(9.9)	81.1(9.8)	81.2(10.0)	81.9(9.8)
Postmenopausal, %	80	83	84	88	81	81	82	83	81	83	84	84
Current HRT users, %	28	35	39	51	44	39	38	35	41	40	38	31
Current smokers, %	10	13	14	15	9	11	12	15	9	10	14	12
Ever smokers, %	52	55	57	56	54	55	55	54	52	54	53	54
NSAID users, %	30	31	36	38	47	49	50	47	48	48	49	47
Aspirin user, %	48	50	47	48	32	35	32	35	32	31	32	36
Personal Education: Master/doctorate%	13	12	8	9	13	11	9	10	12	10	11	9
Husband:												
College/Grad	53	50	46	48	54	45	48	48	51	49	50	43
school%												

Abbreviations: BMI, Body Mass Index; MET, Metabolic Equivalent Task-hours; AHEI, Healthy Eating Index; MHI-5, Mental Health Index; HRT, Hormonal Replacement Therapy; NSAID, Non-steroidal anti-inflammatory drugs.

BMI, menopause status and HRT use information are from blood questionnaire (1989-1990). All other variables are from 1990 questionnaire, except for physical activity and MHI which are from the 1992 questionnaire.

Values are means and standard errors, except for age where means and standard deviation was presented instead.

Table2

Spearman correlations between biomarkers and body mass index (BMI). NHS 1989-1990.

	CRP	IL-6	TNFaR2	BMI
CRP	1.00			
IL-6	0.40	1.00		
TNFα-R2	0.25	0.28	1.00	
BMI	0.39	0.29	0.21	1.00

All correlations were statistically significant at <.0001, except for IL-6 and BMI where the p-value was 0.01

Table 3

Association between CRP, IL-6 and TNFα-R2 blood levels and incident clinical depression. NHS 1989-2010

		CRP			P trend
Broad definition	**Q1**	**Q2**	**Q3**	**Q4**	
Number of events	145	182	178	198	
Person-years	13,639	12,886	12,688	12,625	
Age adjusted RR	1.00	1.34 (1.07-1.69)	1.33 (1.05-1.67)	1.42 (1.14-1.78)	0.0 04
Age and BMI adjusted RR	1.00	1.33 (1.05-1.67)	1.31 (1.03-1.66)	1.41 (1.11-1.80)	0.01
Multivariate adjusted RR[1]	1.00	1.23 (0.97-1.56)	1.16 (0.91-1.49)	1.18 (0.91-1.53)	0.29
Strict definition					
Number of events	53	79	79	81	
Person-years	13,730	12,988	12,789	12,742	
Age adjusted RR	1.00	1.55 (1.08-2.23)	1.62 (1.13-2.34)	1.48 (1.03-2.12)	0.04
Age and BMI adjusted RR	1.00	1.51 (1.04-2.17)	1.56 (1.07-2.29)	1.39 (0.94-2.06)	0.12
Multivariate adjusted RR	1.00	1.48 (1.01-2.17)	1.48 (1.00-2.21)	1.26 (0.83-1.92)	0.34
		IL-6			
Broad definition					
Number of events	104	109	87	119	
Person-years	8,080	7,919	7,994	7,592	
Age adjusted RR	1.00	1.11 (0.83-1.48)	0.98 (0.72-1.34)	1.40 (1.04-1.87)	0.04
Age and BMI adjusted RR	1.00	1.09 (0.82-1.47)	0.96 (0.70-1.31)	1.35 (0.99-1.83)	0.07
Multivariate adjusted RR	1.00	1.11 (0.82-1.51)	1.04 (0.75-1.44)	1.31 (0.95-1.80)	0.12
Strict definition					
Number of events	43	44	34	45	
Person-years	8,141	7,985	8,046	7,669	
Age adjusted RR	1.00	1.03 (0.66-1.62)	0.98 (0.61-1.59)	1.23 (0.78-1.95)	0.39
Age and BMI adjusted RR	1.00	0.98 (0.62-1.55)	0.89 (0.54-1.46)	1.09 (0.67-1.77)	0.76
Multivariate adjusted RR	1.00	0.92 (0.56-1.51	0.94 (0.55-1.60)	1.15 (0.68-1.93)	0.56
		TNFα-R2			
Broad definition					
Number of events	116	116	97	134	
Person-years	9,080	8,946	8,987	8,476	
Age adjusted RR	1.00	0.98 (0.75-1.30)	0.83 (0.62-1.10)	1.17 (0.89-1.54)	0.35
Age and BMI adjusted RR	1.00	0.98 (0.74-1.29)	0.82 (0.61-1.09)	1.13 (0.86-1.49)	0.53
Multivariate adjusted RR	1.00	1.04 (0.78-1.39)	0.78 (0.58-1.05)	1.08 (0.80-1.45)	0.91
Strict definition					
Number of events	44	49	40	53	
Person-years	9,154	9,012	9,047	8,556	
Age adjusted RR	1.00	0.98 (0.63-1.52)	0.79 (0.50-1.24)	1.05 (0.67-1.62)	0.97
Age and BMI adjusted RR	1.00	0.92 (0.59-1.43)	0.75 (0.47-1.18)	0.96 (0.61-1.51)	0.77
Multivariate adjusted RR	1.00	0.94 (0.59-1.51)	0.69 (0.42-1.14)	0.96 (0.59-1.56)	0.72

[1] Adjusted for age, BMI (<20, 20-23, 23-25, 25-28 or ≥28 kg/m^2), physical activity (METs/week in quintiles), menopause (yes vs.no), hormonal replacement therapy use (never, past or current user), smoking (never, past 1-24, past ≥25, current 1-24, current ≥25 packs per year), use of NSAIDs or aspirin (no use, use 1-4 days, 5-14 days, 15-21 days, ≥ 22 days/month) caffeine intake (quintiles), alcohol intake (never, <5, 5-10, 10-15 or >15 mg/day), neighborhood socioeconomic status (quintiles), husband education (college/graduate school vs. completed high school or less), and personal education (completed master or doctorate program vs. registered nurse or bachelor) at time of blood draw and presence of comorbidities (diabetes, hypertension, stroke, myocardial infarction, angina, or cancer) during follow-up.

Table 4

Associations between inflammatory biomarkers and antidepressant use at time of blood draw. NHS 1989-1990

	Q1	Q2	Q3	Q4	P Trend
CRP (n=5645)					
Case:Control	49:1365	50:1363	53:1350	69:1326	
Age adjusted OR	1.00	1.06 (0.71-1.00)	1.15 (0.77-1.72)	1.53 (1.05-2.23)	0.03
Age and BMI adjusted OR	1.00	1.05 (0.67-1.58)	1.14 (0.75-1.73)	1.50 (1.00-2.25)	0.05
MultivariateadjustedOR[1]	1.00	0.98 (0.65-1.54)	1.00 (0.65-1.54)	1.14 (0.74-1.76)	0.52
IL-6 (n=3377)					
Case:Control	33:809	40:795	31:815	31:801	
Age adjusted OR	1.00	1.26 (0.78-2.02)	0.96 (0.58-1.59)	1.08 (0.66-1.77)	0.99
Age and BMI adjusted OR	1.00	1.22 (0.76-1.97)	0.90 (0.54-1.51)	0.99 (0.59-1.66)	0.71
Multivariate adjusted OR	1.00	1.19 (0.72-1.96)	0.88 (0.51-1.50)	0.90 (0.52-1.55)	0.48
TNFaR2 (n=3784)					
Case:Control	37:914	24:933	30:913	65:868	
Age adjusted OR	1.00	0.69 (0.41-1.17)	0.90 (0.55-1.48)	2.21 (1.44-3.40)	<0.0 001
Age and BMI adjusted OR	1.00	0.69 (0.41-1.17)	0.90 (0.55-1.48)	2.21 (1.42-3.44)	<0.0001
Multivariate adjusted OR	1.00	0.64 (0.37-1.09)	0.81 (0.48-1.36)	1.96 (1.23-3.13)	0.001

[1] Adjusted for age, BMI (<20, 20-23, 23-25, 25-28 or ≥28 kg/m^2), physical activity (METs/week in quintiles), menopause (yes vs.no), hormonal replacement therapy use (never, past or current user), smoking (never, past 1-24, past ≥25, current 1-24, current ≥25 packs per year), use of NSAIDs or aspirin (no use, use 1-4 days, 5-14 days, 15-21 days, >≥22 days/month) caffeine intake (quintiles), alcohol intake (never, <5, 5-10, 10-15 or >15 mg/day), neighborhood socioeconomic status (quintiles), husband education (college/graduate school vs. completed high school or less), and personal education (completed master or doctorate program vs. registered nurse or bachelor) at time of blood draw.