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Are there early inflammatory biomarkers that affect neurodevelopment in infancy?

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Are there early inflammatory biomarkers that affect neurodevelopment in infancy?

Abstract

Few studies have investigated the relationship between post-natal inflammatory biomarkers at early age and child neurodevelopment outcomes. The main aim of this study was to examine the relationship between IL-6, IL-1β, IL-4 cytokines, as well as cortisol at 6 and 12 months of age, and neurodevelopment and psychological problems at 30 months of age.

The study was conducted on a sample of 51 full-term newborns who were followed up at 6, 12, and 30 months of age. Infant neurodevelopment was assessed using the *Bayley Scales of Infant Development-II*, psychological problems were assessed with the *Child Behavior Checklist 1.5-5* (CBCL 1.5-5) and the mother's emotional symptoms were assessed with the *General Health Questionnaire-28*. When the infants were 6 and 12 months old, IL-6, IL-1β, IL-4 cytokines, and cortisol were measured in blood samples. The results showed that higher IL-6 at 12 months predicted higher scores in internalizing (emotionally reactive, anxious/depressed, withdrawn and attention problems) and externalizing problems (aggressive behavior) at 30 months. By contrast, high levels of IL-1β at 6 months were related to worse motor skills. Inflammatory biomarkers were not related to mental performance. IL-6 and IL-1β could be early markers of later psychological problems and psychomotor disabilities.

Keywords. Inflammatory biomarkers, IL-6, IL-1β, IL-4, cortisol, neurodevelopment, psychopathology, infants

1. Introduction

There is an increasing interest in studying the relationship between inflammation biomarkers and children's neurodevelopmental outcomes (Jiang et al., 2014; Krakowiak et al., in press). Inflammation is known to be a natural defense mechanism by body tissues in response to the recognition of injury, but this process may stop being protective for the organism and become harmful when it occurs chronically (Esteban-Cornejo et al., 2016; Tyrka, Parade, Valentine, Eslinger, & Seifer, 2015). Consequently, the inflammation processes may have important negative effects on neural plasticity and neurogenesis, and may also be related to gray matter atrophy as has been shown in animal models (Yirmiya & Goshen, 2011). Within the multiple range of inflammatory biomarkers that have been examined in the context of possible relationships with mental disorders, the most commonly measured are cytokines (Baumeister, Russell, Pariante, & Mondelli, 2014). There are pro-inflammatory cytokines, which are involved in the up-regulations of inflammatory reactions, and anti-inflammatory cytokines which control the pro-inflammatory cytokine response.

To our knowledge, few studies have examined the possible link between children's inflammatory biomarkers and their neurodevelopment and mental health, and most of these studies were conducted with samples of extremely premature infants or with clinical samples of children with autism spectrum disorders (ASD) (Businaro et al., 2016; Carlo et al., 2011; Leviton et al., 2016; Masi et al., 2015; Ricci et al., 2013). Furthermore, most of these studies have focused on the relationship between mothers' inflammatory cytokines during pregnancy and their children's neurodevelopmental outcomes, or the presence of mental illness disorders later in life (Ratnayke, Quinn, Walker, & Dickinson, 2013). In this regard, Jiang et al. (2014) stated that no studies have linked markers of inflammation during the post-neonatal period to child development. Those authors found that inflammation biomarkers in the first year of life

were related to neurodevelopment outcomes in infants living in conditions of poverty. In specific terms, elevated serum levels of pro-inflammatory cytokines (IL-1β and IL-6) were associated with poor scores for motor skills, whereas elevated serum levels of the T helper 2 (Th2) cytokine IL-4 were associated with elevated scores for cognitive skills at 12 and 24 months. Likewise, Tyrka et al. (2015) found that IL-1β was associated with adverse experiences such as child maltreatment or socioeconomic adversity and was involved in the neuropathology of psychiatric conditions in early childhood. Moreover, Pandey et al. (2012), using a sample of teenage suicide victims found high levels of IL-1β, IL-6, and TNF-α in the prefrontal cortex, thus linking cytokine levels with mood disorders, another issue that has also been widely investigated. Other studies have therefore corroborated that pro-inflammatory cytokines may be part of the pathophysiology of emotional disorders; in particular adolescent females with clinical anxiety and/or depression showed high plasma levels of IL-2, IL-1β, and IL-10 (Henje Blom, Lekander, Ingvar, Asberg, Mobarrez, & Serlachius, 2012). Furthermore, a study of patients between 8 and 17 years old who presented affective, anxiety, psychotic, obsessive-compulsive, tic or tourette's disorders showed that they presented higher levels of IL-1β, IL-6, IL-8, IFN-γ-induced protein-10 (IP-10), Monocyte Chemoattractant Protein-1 (MCP-1) and monocytes (Gariup et al., 2015). Meanwhile, in a community sample of Taiwanese children, the results showed that IL-1β was positively associated with anxiety and depression symptoms (Chung, Hu, Chen, Chiou, & Chen, 2014). In a case-control study, Yang et al. (2015) indicated that cortisol, IL-6 and TNF-α also had an association with ASD severity. In particular, subjects with ASD showed lower levels of cortisol, and high levels of IL-6 and TNF-α. Despite these studies, there is a lack of data from pediatric community samples.

Similarly, studies with adolescent and adult samples have also shown associations between alterations in serum levels of cytokines and the presence of schizophrenia, major depression,

post-traumatic stress disorder and bipolar disorder (Blom et al., 2012; Gola et al., 2013; Goldstein et al., 2015; Müller, Weidinger, Leitner, & Schwarz, 2015). Despite some controversial results, there therefore appears to be a relation between certain biomarkers and several psychiatric disorders, but further research is needed to identify the pathways that explain this relationship.

In the existing literature based on animal models and human subjects, few studies have investigated the influence of inflammatory markers on infant neurodevelopment. The main aim of this study was therefore to test the possible association between certain inflammatory biomarkers [IL-6, IL-1β, and IL-4 cytokines] and cortisol (which affects the immune response) measured at 6 and 12 months of age, and neurodevelopment and psychological problems at 30 months of age. We hypothesized that some of these early biomarkers may influence neurodevelopment and the presence of psychopathology.

2. Materials and methods

2.1 Procedure

The study was approved by the Research and Ethics Committee of Sant Joan University Hospital in Reus (Spain) and informed consent was obtained from all the participants. The sample was recruited at Sant Joan University Hospital between 2006 and 2009. The inclusion criteria for mothers were that they should be pregnant with no more than 11 weeks of gestation and over 18 years old, and the exclusion criteria were presenting with a chronic illness affecting nutritional status such as diabetes type I, Crohn's or celiac diseases, having a multiple pregnancy or having gone to another hospital to give birth. Meanwhile, the inclusion criteria for infants were that they should have a gestational age ≥ 37 weeks, be Caucasian, be from families that understand Catalan or Spanish, and have no medical problems; the

exclusion criteria were birth weight < 2,500g, the presence of an illness associated with iron metabolism, birth defects, immunodeficiency or hypothyroidism, and/or diseases requiring intensive care.

Socio-demographic, anthropometric, nutritional, psychopathological data, and blood samples were collected from these infants during the study phases. Data were also collected on the mothers' toxic habits during pregnancy (smoking, alcohol and/or drug consumption) and birth data. The follow-up study consisted of three visits: when the infants were 6 months, 12 months, and 30 months old (see the flow diagram of the study in Figure 1).

2.2 Participants

An initial sample of 158 newborns belonging to a larger project studying the effect of nutrition on psychological development was followed up at 6, 12, and 30 months. Of these, 51 infants completed all the psychological and biomedical assessments (25 boys) until 30 months of age. Thus, the follow-up sample contained 51 infants and the final statistical power was 98% according to the means for the total problems CBCL score and the distribution in IL groups.

The mean weight at birth was 3241.4 grams (SD = 471.7), the gestational mean age was 39.6 weeks (SD = 1.2), and their mothers had a mean age of 32.4 years (SD = 4.6). As regards the socioeconomic level of the families, 88.3 % were middle-high class. Apgar scores were 8.8 at 1-min (SD = 1.0) and 9.9 at 5-min (SD = 0.3). None of the pregnant women had consumed alcohol or illicit drugs during pregnancy, but 21.6 % smoked during pregnancy (11.8% smoked between 1 to 5 cigarettes per day, 3.9% between 6 to 10 cigarettes per day, and 5.9% more than 10 cigarettes) and 15.7% of these mothers were secondhand smokers (more data on the sample are shown in Table 1).

2.3 *Measures*

2.3.1 Biochemical measurements. At 6 and 12 months blood samples were collected by venipuncture into tubes with no anticoagulants to obtain serum early in the morning at Sant Joan University Hospital in Reus. The blood drawings were performed by hospital staff nurses from the Pediatrics service. Blood extractions were programed a few days before performing the child's assessment and interview with parents, or in some cases in the same day. The analyses were performed immediately, or aliquots of serum were stored in the Biobanc of the Institut d'Investigació Sanitària Pere Virgili at -80º for subsequent measurements. IL-6, IL-1β, IL-4 cytokines were measured by FlowCytomix^{TM} Multiplex assay (Bender MedSystems, Vienna, Austria) in accordance with the manufacturer's instructions. The kit allowed simultaneous quantification of three cytokines (IL-6, IL-1β and IL-4) by combining the human single cytokine and the Human Basic FlowCytomix kits. Samples were analyzed in a Coulter Epics XL-MLC flow cytometer (Beckman-Coulter). Concentration of each cytokine was obtained by interpolating fluorescence intensity to a 7-point dilution standard curve supplied by the manufacturer (ranged between 27 and 20000 pg/ml) using the FlowCytomix Pro 2.2 Software (Bender MedSystems). The minimum detectable concentrations of IL-6, IL-1β and IL-4 were 1.2, 4.2 and 20.8 pg/ml, respectively. Any cytokine value below the limits of detection was given zero. Plasma cortisol was measured by new electrochemiluminescence immunoassay technology, namely the Roche Elecsys 2010 Chemistry Analyzer. The lowest detection limits were 0.500 nmol/L. All the biochemical parameters were dichotomized into the two lowest tertiles and the highest tertile. In this sample we also measured ultrasensitive C-reactive protein values by using a CRP immunoturbidimetric reagents assay (Roche diagnostics, Mannheim, Germany) and the detection limits were 0.3 mg/L. The entire sample showed CRP levels in the normal range.

2.3.2 The Child Behavior Checklist 1.5-5 (CBCL 1.5-5; Achenbach & Rescorla, 2000) is a 110-item parent-report questionnaire that assesses behavioral and emotional problems. The CBCL 1.5-5 provides scores for seven scales: emotionally reactive, anxious/depressed, somatic complaints, withdrawn (internalizing problems), attention problems and aggressive behavior (externalizing problems), and sleep problems. The sum of the scores for all the problem items makes up the total psychological problems category. For this study, the mother and the father answered the CBCL 1.5-5 separately when the child was 30 months old. The results were obtained using the mean T score of the mother's and father's responses (CBCL scale scores for Spanish normative samples), or if only one of the two parents responded to the questionnaire we used that T score (n = 7 cases). In Spain, De la Osa, Granero, Trepat, Domènech, & Ezpeleta (2012) reported an internal reliability between .65 and .86.

2.3.3 The Bayley Scales of Infant Development (BSID-II; Bayley, 1993) were used to assess cognitive and psychomotor development at 30 months of age. Each scale provides a developmental quotient (raw score/chronological age), which generates a continuous Mental Developmental Index (MDI) and a corresponding Psychomotor Development Index (PDI). The infants were tested by trained psychologists under controlled conditions in a medical dispensary at the hospital. For this study, we used the quantitative scores of the two MDI and PDI indexes and the cognitive, language, social, and motor scales.

2.3.4 The General Health Questionnaire (GHQ-28; Goldberg & Hiller, 1979) is a 28-item self-administered questionnaire which determines general health status. The questionnaire includes four scales: somatic symptoms, anxiety and insomnia, social dysfunction, and depression. For this study, we used the Spanish version which had acceptable psychometric

properties (Lobo, Pérez-Echeverría, & Artal, 1986). Mothers answered the GHQ-28 when the infants were 30 months old.

2.3.5 Socioeconomic status of parents (SES, Hollingshead, 2011) was assessed by asking the parents about their educational level and occupation, and then combining the data obtained from the father and the mother. This index allows the social status of each individual to be determined by categorizing his or her occupation into one of nine categories (from unskilled work to highly skilled work) and his or her level of education into one of seven categories (from non-completed primary education to completed higher education). These data were collected at 11-12 weeks of gestation.

2.3.6 Anthropometric parameters were evaluated when the infants were 30 months of age. We obtained the Body Mass Index (BMI) (kg/m^2) using weight and height measurements. Weight was measured by nutritionists in the research group using the Tanita_TBF-300 scale, which has an accuracy of 100 g and a maximum weight of 200 kg. Height was measured using a "Perspective Enterprises Measuring Equipment" PE-RILB-STND model measuring rod, with a range of 12.5 to 99 cm and an accuracy of 1 mm.

2.4 Statistical analysis

SPSS software, version 23 for Windows was used for the statistical analysis.

First, all the variables were checked for normality of distribution before the analyses and square root transformations (SRT) were applied due to the skewed distributions of IL-6, IL-1β, IL-4, and cortisol variables. These variables were also dichotomized into the two lowest tertiles and the highest tertile, and were therefore analyzed as binary variables.

Table 1 presents the descriptive data for the socio-demographic, biological, and psychopathological characteristics of the sample. The values are expressed as the mean and

standard deviation for the quantitative variables, and as percentages for the qualitative variables. Student's *t*-test and chi-square analyses were used to find any significant differences between boys and girls.

Student's *t*-test analyses were also performed to compare the scores obtained in the CBCL 1.5-5 and in the BSID-II at 30 months. The analyses checked for low or high levels for the following biological parameters: IL-6, IL-1β, IL-4 cytokines, and cortisol analyzed at 6 months and 12 months. Because the descriptive results related to the CBCL 1.5-5 showed no differences between boys and girls, the analyses were not performed by gender.

Stepwise multiple linear regression models were used to predict CBCL 1.5-5 and BSID-II scores at 30 months. Taking into account the statistically significant results obtained by the Student's *t*-test, we considered the following biological risk predictors: IL-1β at 6 months and IL-6 at 12 months (one model for each biomarker) and the following covariables: socioeconomic status, breastfeeding during the first year, bottle feeding with one of two different types of iron-fortified formula (one containing more iron than the other), gestational age at birth, mother's age at birth, mother's health status, exposure to tobacco during pregnancy, and the infants' body mass index at 30 months. Before performing the regression models, collinearity between the variables introduced in the model was assessed by computing Pearson correlations; all the correlations were lower than 0.6.

As in other studies, statistical significance was defined as a p-value of <.05 (Esteban-Cornejo et al., 2016; Jiang et al., 2014).

3. Results

The descriptive data of the sample by gender are shown in Table 1. As can be seen, the only differences between boys and girls were for weight at birth and for IL-6 at 12 months, when the boys presented higher levels than the girls. A total of 37.3% of children scored within the

borderline and clinical range for the psychological problems measured with the CBCL 1.5-5 (40% boys and 34.6% girls).

Student's *t*-test analyses showed that subjects with low levels of IL-1β at 6 months presented higher PDI scores in the BSID-II than subjects with higher levels of this parameter. Moreover, scores for emotionally reactive, anxious depressed, withdrawn, internalizing and total problems at 30 months were found to be higher among infants with higher levels of IL-6 at 12 months than in infants with lower levels (see Tables 2 and 3 for all of these results). The regression models showed that high levels of IL-6 at 12 months predicted not only more internalizing symptoms such as emotionally reactive, anxious/depressed, withdrawn and attention problems, but also even more externalizing problems such as aggressive behavior. Moreover, higher levels of IL-1β at 6 months predicted lower motor skill performance at 30 months. Adjusted models showed that apart from the biological markers, the covariables were not related to the CBCL 1.5-5 and BSID-II scores (see Table 4).

4. Discussion

This study showed that the pro-inflammatory biomarkers IL-6 and IL-1β in the first period of life were related to the infant's later motor outcomes and the presence of psychological problems. Our data support the relationship between the inflammatory system and systems involved in the pathogenesis of mental disorders suggested in different periods of life. We found that elevated levels of IL-6 at 12 months predicted, in infants of non-clinical population, higher scores in the internalizing and the externalizing psychological problems at 30 months old. In this regard, it is known that cytokine dysregulation, and specifically systemic inflammation, may therefore have effects on neurodevelopment or neuronal activity that can adversely affect behavior (Ashwood et al., 2011; O'Shea et al., 2013). Elevated levels of IL-6 have been implicated in the pathophysiology of depression and autism spectrum

disorders (ASD) (Krakowiak et al., in press; Maes, Anderson, Kubera, & Berk, 2014). For depression, IL-6 was the highest replicated biomarker and this association could be explained by multiple potential pathways. Several studies suggested a relationship between IL-6 levels in plasma and responsiveness to antidepressant drugs, thus supporting the involvement of the inflammatory system in neurotransmitter systems related to depression; however, in this regard the specific relationship is not yet clear and further research is needed (Amitai et al., in press; Bay-Richter et al., 2015; Fonseka, McIntyre, Soczynska, & Kennedy, 2015; Kurosawa, Shimizu, & Seki, 2016). Furthermore, in cases with persistent inflammation, IL-6 may act on the locus coeruleus and this is also related to depressive symptoms. Specifically, in animal studies, Kurosawa et al. (2016), found that IL-6 activated noradrenergic neurons in the locus coeruleus, although the mechanisms behind IL-6- induced Tyrosine-Hidroxylase-positive neuronal activation in the locus coeruleus are still unclear. Meanwhile, in a community study conducted with 138 adults, Fagundes, Glaser, Hwang, Malarkey, and Kiecolt-Glaser (2013) found that individuals with more depressive symptoms had a greater IL-6 response to a stressor, thus supporting an interaction between stress, depression and immune dysregulation. On the other hand, several studies related ASD symptoms to brain inflammation, reflected by microglia activation and increased cytokine production (Krakowiak et al., in press; Tsilioni, Taliou, Francis & Theoharides, 2015). Ashwood et al. (2011) found significant increases in plasma levels of some cytokines such as IL-1β or IL-6 in a group of children with ASD in comparison with a control group, indicating greater impairment in communication, social interaction and repetitive behaviors (Ashwood et al., 2011; Mead & Ashwood, 2015). The relation found in our study between the withdrawn category and IL-6 supports a possible association between early inflammatory process and symptoms on the ASD spectrum. In contrast to our results, in a recent study, Krakowiak et al. (in press) found that in infants who were 2-5 years old, elevated levels of an anti-inflammatory cytokine (IL-4) were associated

with severe ASD. Those authors thereby demonstrated that peripheral cytokine profiles at birth were associated with the presence of ASD later in childhood and that cytokine profiles were also related to ASD severity. Tsilioni et al. (2015) also found that there were various different subgroups of children with ASD, which may be identifiable by serum levels of IL-6 and TNF-α. In short, research on ASD strengthens the evidence for an abnormal cytokine profile, as has been mentioned in relation to mood disorders. For ASD, it is still necessary to characterize the related immunological parameters and the possible explanation pathways, which has significant implications for early detection and treatment.

Moreover, as found Jiang et al. (2014) in Bangladeshi infants living in poverty, we observed that an early IL-1β dysregulation may affect at long-term psychomotor performance. In this regard, some studies have investigated the possible role of IL-1β in akinesia during Parkinson's disease (Cintia et al., 2006; Saghazadeh, Cintia, & Rezaei, 2016). Cintia et al. (2006) found that the chronic expression of IL-1β induced dopaminergic cell death in the substantia nigra and unilateral akinesia. Thus, more studies are needed to decipher the possible relationship between IL-1β and the brain mechanisms that govern motor skills. On the basis of the present results, we therefore suggest that a pro-inflammatory state (such as high levels of IL-6 or IL-1β) may be detrimental to neurodevelopment and brain maturity, and could be related to the presence of psychological problems. On the other hand, previous studies have demonstrated that mothers who have a child with a diagnosis of ASD showed higher levels of IL-4 in mid-pregnancy serum samples (Goines et al., 2011), suggesting that some anti-inflammatory cytokines such as IL-4, have a variety of neurological roles However, previous results suggest that whereas the number of T cells producing IL-4 was higher in children with ASD compared to controls (Gupta, Aggarwal, Rashanravan, & Lee, 1998), plasma levels of IL-4 levels appear to be normal (Ashwood et al., 2011). Thus, despite the fact that IL-4 has a neuroprotective role and could play a role in neurodevelopment, our

results showed that circulating IL-4 levels in children do not predict neurodevelopmental and psychological problems later in life.

As mentioned above, taking into account the possible relationship between immune regulation and systems involved in the pathogenesis of mental disorders, such as, hypothalamic pituitary adrenal axis (Dadds, Moul, Hawes, Mendoza-Diaz, & Brennan, 2015; Miller, Maletic, & Raison, 2009), this study also examined the infants' cortisol levels at 6 and at 12 months. The results did not show that cortisol levels were associated with mental or motor performance or with psychological problems later in life. This is understandable because the studied sample did not present early adversities which can lead to high and chronic levels of cortisol. According to other authors (Hawes, Brennan, & Dadds, 2009; Martínez-Torteya et al., 2015), it appears that the relationship between cortisol and behavior may depend on other variables, such as comorbidity patterns, the presence of early environmental adversities, the child's age and maturation of HPA-axis, the child's gender, etc. Many studies into the relationship between the mother's inflammatory cytokine levels during pregnancy and the child's outcomes in later life suggest that maternal levels of pro-inflammatory or anti-inflammatory cytokines could influence the development of multiple psychopathological disorders in their offspring (Allswede, Buka, Yolken, Torrey, & Cannon, 2016; Zerbo et al., 2015). However, our results indicate that neither maternal health or other maternal factors measured during the prenatal and perinatal periods were as important as the biological markers.

Our study had some limitations. These included the limited sample size, which was partially because blood sampling can be very difficult in patients in this age range. It was difficult to convince all the participants' parents, and some analyses did not go well. Moreover, because of the limited sample size, it was not possible to use the categorical variables of the CBCL 1.5-5.

It would also be more reliable to collect several cortisol measures throughout the day, and then use the variable area under the diurnal variation curves of cortisol (VAR), but it was not possible to carry out these measurements and analyses in our sample of infants. In regard to the socioeconomic status (SES) we think that changes may occur throughout gestation and until the child reached 30 months, but we only had the SES from the beginning of the study. However, this study has some strengths in that it assessed several biological markers at two ages of infants' lives. Moreover, the assessment of neurodevelopment and the presence of early psychological problems allows us detect possible pathologic trends at an early stage. Furthermore, it is one of the few studies which explores the possible association between inflammation biomarkers in the post-natal period and the child's subsequent development in a community sample. Moreover, we controlled the results with many relevant variables such as breastfeeding and smoking during pregnancy, and with socio-demographic variables such as the mother's age and socio-economic level.

The success of immunopsychiatry should be considered in terms of its ability to offer new therapies or possibilities for treatments that enhance the lives of people suffering from mental disorders or disabilities in their skills. However, according to Duka et al. (2016), it also can be considered a success when the study of the relationship between the inflammatory biomarkers and mental disorders leads to the detection of early biological markers, thus allowing experts to detect symptoms at an early stage and identify possible therapeutic targets.

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Figure 1. Flow diagram of study design

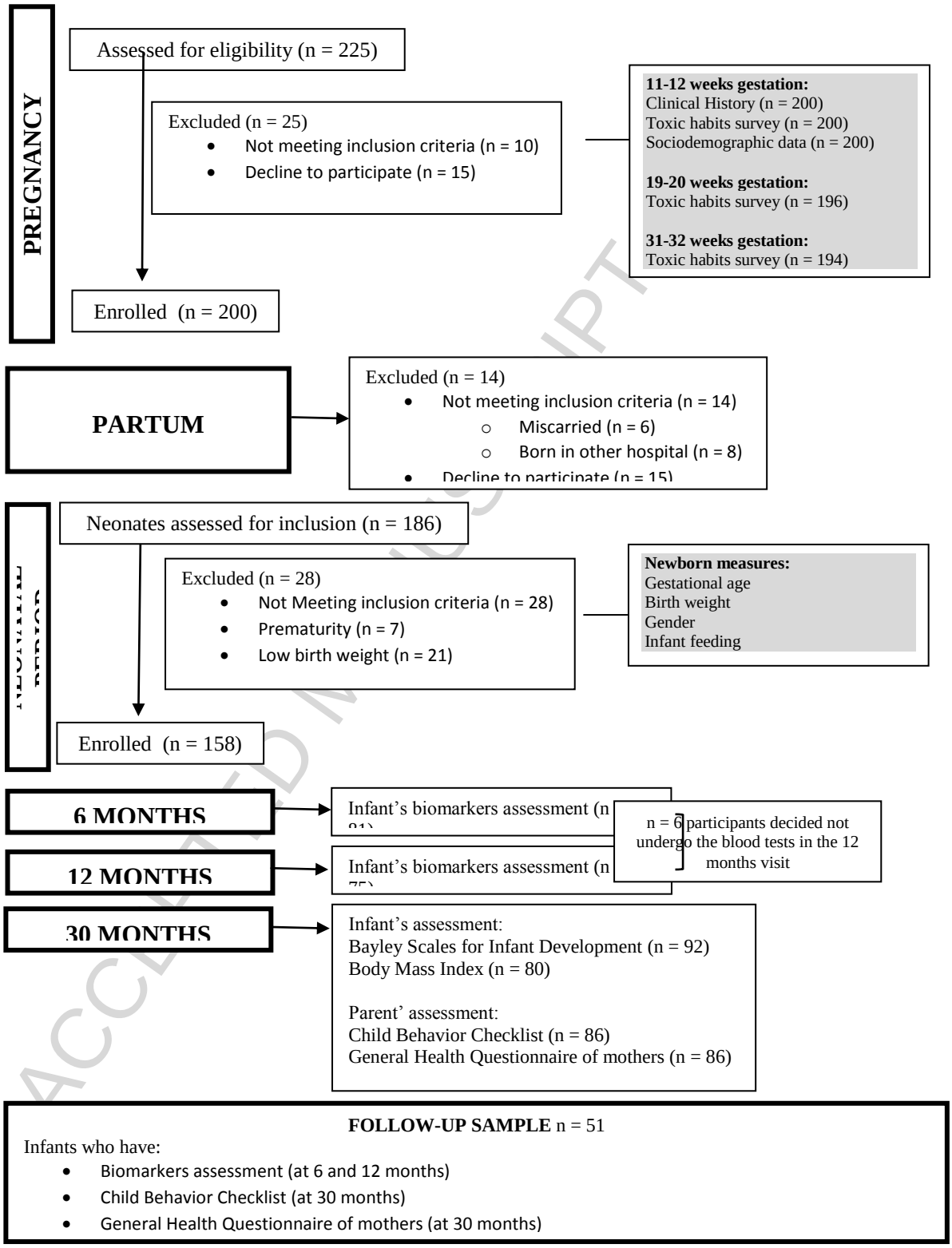

Table 1. Descriptive data of the sample by gender.

	Total (*N* = 51)	**Boys (*N* = 25)**	**Girls (*N* = 26)**	***p***
Mother's age at birth (years)	32.39 (4.6)	32.4 (5.6)	32.1 (3.5)	0.943
Gestational age (weeks)	39.57(1.2)	39.5 (1.2)	39.4 (1.3)	0.890
Socioeconomic status (%)				
Low	11.8	16	7.7	0.367
Middle	47.1	52	42.3	
High	41.2	32	50	
Weight at birth (g)	3241.4 (471.7)	3418.8 (476.5)	3070.8 (406.4)	**0.007**
Exposure to tobacco during pregnancy (%)				
Yes	37.3	44	30.8	0.329
No	62.7	56	69.2	
Type of feeding during first year (%)				
Formula	30	40	20	0.120
Breastfeeding 1 month	4	4	4	
Breastfeeding 3 months	14	4	24	
Breastfeeding 6 months	16	12	20	
Breastfeeding 9 months	4	0	8	
Breastfeeding 12 months	32	40	24	
6 months	**Total (*N* = 51)**	**Boys (*N* = 25)**	**Girls (*N* = 26)**	***p***
IL-6 (pg/mL)	.9 (1.2)	1.1 (1.2)	.8 (1.2)	0.407
IL-1β (pg/mL)	4.8 (5.9)	4.5 (5.1)	5.2 (6.6)	0.688
IL-4 (pg/mL)	11.2 (14.6)	11.5 (15.1)	10.8 (14.4)	0.870
	Total (*N* = 49)	**Boys (*N* = 23)**	**Girls (*N* = 26)**	***p***
CRP (mg/L)	2.7 (1.0)	2.7 (.6)	2.7 (1.3)	0.915
	Total (*N* = 46)	**Boys (*N* = 21)**	**Girls (*N* = 25)**	***p***
Cortisol (nmol/L)	17.6 (9.1)	15.9 (2.6)	19.1 (12.1)	0.241
12 months	**Total (*N* = 46)**	**Boys (*N* = 24)**	**Girls (*N* = 22)**	***p***
IL-6 (pg/mL)	.7 (1.1)	1.1 (1.3)	.4 (.8)	**0.021**
IL-1β (pg/mL)	5.1 (4.2)	4.5 (4.8)	5.7 (3.3)	0.351
IL-4 (pg/mL)	7.7 (9.5)	8.1 (10.3)	7.2 (8.7)	0.762
	Total (*N* = 40)	**Boys (*N* = 20)**	**Girls (*N* = 20)**	***p***
CRP (mg/L)	2.0 (.5)	2.1 (.7)	1.9 (.3)	0.149
	Total (*N* = 40)	**Boys (*N* = 20)**	**Girls (*N* = 20)**	***p***
Cortisol (nmol/L)	17.1 (3.1)	16.6 (3.1)	17.7 (3.1)	0.271
30 months				
CBCL	**Total (*N* = 51)**	**Boys (*N* = 25)**	**Girls (*N* = 26)**	***p***
Emotional reactivity (score)	1.8 (1.9)	2.1 (2.2)	1.5 (1.5)	0.237
Anxious / Depressed (score)	2.5 (2.0)	2.7 (2.3)	2.3 (1.6)	0.427
Somatic complaints (score)	1.7 (1.5)	1.7 (1.7)	1.7 (1.3)	0.986
Withdrawn (score)	1.6 (1.8)	2.0 (2.2)	1.3 (1.4)	0.227
Sleep Problems (score)	3.7 (2.8)	3.9 (2.8)	3.5 (2.7)	0.557
Aggressive behavior (score)	10.2 (4.8)	11.2 (5.0)	9.3 (4.5)	0.146
Attention Problems (score)	2.0 (1.5)	2.2 (1.6)	1.8 (1.3)	0.370
Internalizing problems (score)	7.6 (6.0)	7.2 (1.4)	4.7 (.9)	0.316
Externalizing problems (score)	12.2 (5.1)	6.2 (1.2)	5.5 (1.1)	0.159
Total	23.5 (12.6)	13.9 (2.8)	10.9 (2.1)	0.202
GHQ	**Total (*N* = 50)**	**Boys (*N* = 24)**	**Girls (*N* = 26)**	***p***
	47.7	48.5	47.1	0.673
BSID II	**Total (*N* = 50)**	**Boys (*N* = 22)**	**Girls (*N* = 28)**	***p***
MDI	99.2 (15.6)	95.7 (15.6)	101.9 (15.4)	0.163
PDI	95.6 (18.2)	93.9 (17.6)	97.0 (19.0)	0.547
Anthropometry	**Total (*N* = 44)**	**Boys (*N* = 23)**	**Girls (*N* = 21)**	***p***
BMI (kg/m^2)	16.8 (1.7)	17.0 (1.3)	16.5 (2.0)	0.252

CBCL, Child Behavior Checklist; BSID II, The Bayley Scales of Infant Development; GHQ, General Health Questionnaire MDI, Mental Developmental Index; PDI, Psychomotor Development Index; BMI, Body Mass Index; CRP, C-reactive protein

*Square root transformations (SRT) were applied for all the biochemical mesures

Table 2. Bivariate analyses to compare CBCL and BSID-II at 30 months with biological markers assessed at 6 months

	IL-6 6 months			IL-1β 6 months			IL-4 6 months			Cortisol 6 months		
	Low levels	High levels		Low levels	High levels		Low levels	High levels		Low levels	High levels	
	N=34/Mean (SD)	N=17/Mean (SD)	*p*	N=34/Mean (SD)	N=17/Mean (SD)	*p*	N=34/Mean (SD)	N=17/Mean (SD)	*p*	N=32/Mean (SD)	N=14/Mean (SD)	*p*
CBCL 30 months												
Emotionally reactive	55.2 (6.4)	55.8 (7.2)	.756	55.5 (6.3)	55.1 (7.4)	.859	55.3 (5.7)	55.6 (8.2)	.859	55.2 (7.4)	55.8 (5.4)	.793
Anxious / Depressed	55.9 (5.8)	58.4 (7.0)	.192	56.9 (5.9)	56.5 (7.1)	.840	56.6 (5.7)	57.0 (7.5)	.846	56.0 (6.5)	56.6 (5.5)	.763
Somatic complaints	55.4 (4.8)	56.1 (6.7)	.655	55.3 (4.9)	56.2 (6.5)	.579	55.1 (5.1)	56.6 (6.1)	.379	55.8 (6.0)	56.5 (4.4)	.697
Withdrawn	56.0 (6.9)	57.5 (8.3)	.498	56.4 (7.3)	56.6 (7.6)	.921	56.9 (7.4)	55.6 (7.4)	.577	56.0 (7.8)	57.7 (6.7)	.490
Sleep Problems	58.1 (7.5)	59.5 (6.4)	.513	58.5 (7.0)	58.7 (7.5)	.923	58.3 (6.8)	59.2 (7.9)	.685	57.3 (6.6)	60.6 (7.7)	.140
Aggressive behavior	57.1 (6.0)	58.6 (5.9)	.402	57.9 (6.1)	57.1 (5.9)	.456	57.8 (5.6)	57.4 (6.8)	.863	56.7 (6.4)	59.1 (5.5)	.217
Attention Problems	56.2 (5.2)	59.3 (8.5)	.110	57.7 (7.2)	56.3 (5.1)	.676	57.3 (6.8)	57.2 (6.1)	.959	56.5 (6.9)	58.2 (5.6)	.421
Internalizing problems	54.3 (9.0)	56.3 (10.4)	.477	55.5 (8.3)	53.9 (11.6)	.572	55.3 (8.2)	54.2 (11.8)	.694	53.9 (10.7)	56.8 (7.2)	.357
Externalizing problems	55.4 (9.7)	58.7 (7.1)	.215	56.7 (9.2)	55.9 (8.8)	.773	56.3 (9.1)	56.7 (8.9)	.879	54.5 (10.0)	59.4 (6.4)	.100
Total	56.5 (10.4)	59.3 (8.8)	.348	57.6 (9.5)	56.9 (11.0)	.806	57.4 (9.4)	57.5 (11.2)	.973	55.5 (11.2)	60.0 (7.2)	.169
BSID II 30 months	**N=33/Mean (SD)**	**N=16/Mean (SD)**	***p***	**N=32/Mean (SD)**	**N=17/Mean (SD)**	***p***	**N=32/Mean (SD)**	**N=17/Mean (SD)**	***p***	**N=31/Mean (SD)**	**N=13/Mean (SD)**	***p***
MDI	97.1 (14.9)	103.7 (16.9)	.170	100.4 (15.3)	97.1 (16.6)	.495	99.6 (16.4)	98.7 (14.7)	.848	99.2 (16.2)	97.5 (14.7)	.748
PDI	94.5 (18.5)	96.7 (19.3)	.698	99.7 (16.3)	86.8 (20.3)	**.019**	93.4 (19.9)	97.5 (16.3)	.526	95.7 (19.6)	92.9 (18.6)	.656
Cognitive scale	112.8 (8.8)	112.5 (7.2)	.910	113.2 (7.6)	111.7 (9.5)	.547	112.5 (8.9)	113.1 (7.3)	.824	113.1 (9.0)	112.0 (7.0)	.697
Language scale	42.9 (4.4)	43.4 (3.9)	.651	43.2 (4.1)	42.8 (4.5)	.795	43.2 (4.0)	42.8 (4.7)	.795	43.4 (4.3)	42.2 (4.4)	.390
Social scale	17.2 (1.0)	17.1 (1.0)	.858	17.3 (1.0)	16.9 (1.1)	.165	17.3 (1.1)	17.0 (0.9)	.423	17.2 (1.0)	17.0 (1.1)	.582
Motor scale	137.9 (4.6)	137.4 (4.5)	.721	138.7 (3.8)	136.1 (5.4)	.053	137.4 (5.0)	138.5 (3.6)	.441	137.9 (4.5)	137.2 (5.2)	.682

CBCL, Child Behavior Checklist; BSID II, The Bayley Scales of Infant Development; MDI, Mental Developmental Index; PDI, Psychomotor Development Index

Table 3. Bivariate analyses to compare CBCL and BSID-II at 30 months with biological markers assessed at 12 months

	IL-6 12 months			IL-1β 12 months			IL-4 12 months			Cortisol 12 months		
	Low levels	High levels		Low levels	High levels		Low levels	High levels		Low levels	High levels	
	N=34/Mean (SD)	N=12/Mean (SD)	*p*	N=29/Mean (SD)	N=17/Mean (SD)	*p*	N=32/Mean (SD)	N=14/Mean (SD)	*p*	N=28/Mean (SD)	N=12/Mean (SD)	*p*
CBCL 30 months												
Emotionally reactive	54.4 (5.7)	59.2 (8.7)	**.022**	55.4 (6.8)	56.0 (7.0)	.783	55.6 (6.8)	55.6 (7.1)	.988	56.1 (7.5)	55.7 (6.0)	.876
Anxious / Depressed	55.9 (6.0)	60.9 (6.2)	**.019**	57.8 (6.8)	56.2 (5.6)	.427	57.1 (6.6)	57.4 (6.1)	.872	57.2 (7.2)	56.3 (5.8)	.715
Somatic complaints	54.9 (4.6)	58.5 (7.5)	.138	55.5 (5.3)	56.3 (6.2)	.644	55.0 (5.6)	57.6 (5.5)	.164	56.3 (6.0)	54.7 (4.3)	.407
Withdrawn	54.8 (6.2)	61.1 (9.3)	**.029**	57.1 (8.3)	55.9 (6.6)	.642	56.4 (7.1)	57.2 (9.2)	.742	58.0 (7.7)	54.3 (6.8)	.156
Sleep Problems	58.1 (7.3)	61.2 (7.0)	.207	60.1 (7.7)	56.8 (6.1)	.135	59.2 (7.4)	58.2 (7.3)	.691	58.4 (7.1)	59.6 (7.8)	.561
Aggressive behavior	56.9 (5.9)	60.8 (6.2)	.058	58.6 (6.2)	56.8 (6.1)	.344	57.9 (6.3)	57.8 (6.1)	.954	58.2 (6.3)	57.3 (6.3)	.673
Attention Problems	55.9 (5.3)	60.5 (9.3)	.127	58.5 (7.6)	54.8 (4.2)	.072	57.7 (7.3)	55.8 (5.4)	.391	56.4 (4.9)	59.0 (9.7)	.394
Internalizing problems	52.9 (9.1)	62.0 (9.0)	**.005**	55.6 (9.8)	54.7 (10.1)	.780	54.8 (10.0)	56.4 (9.6)	.614	56.1 (10.2)	53.3 (9.9)	.438
Externalizing problems	54.9 (9.7)	60.9 (7.2)	.058	57.4 (9.7)	54.9 (9.0)	.405	56.5 (10.0)	56.5 (8.2)	.988	57.2 (8.3)	54.8 (12.3)	.484
Total	55.6 (10.5)	63.6 (7.8)	**.019**	58.4 (10.4)	56.3 (10.7)	.510	57.3 (10.9)	58.5 (9.6)	.723	58.7 (9.8)	55.5 (13.0)	.409
BSID II 30 months	**N=34/Mean (SD)**	**N=10/Mean (SD)**	***p***	**N=28/Mean (SD)**	**N=16/Mean (SD)**	***p***	**N=32/Mean (SD)**	**N=12/Mean (SD)**	***p***	**N=26/Mean (SD)**	**N=12/Mean (SD)**	***p***
MDI	97.8.4 (14.8)	100.17 (20.3)	.693	99.4 (17.2)	96.5 (13.8)	.574	98.3 (17.0)	98.3 (13.3)	.986	97.2 (14.3)	101.8 (17.5)	.396
PDI	95.5 (18.4)	95.7 (19.6)	.973	96.6 (16.8)	93.6 (21.5)	.600	96.9 (16.2)	91.9 (23.8)	.433	93.9 (17.1)	92.7 (18.2)	.843
Cognitive scale	113.4 (8.7)	110.1 (8.0)	.291	111.8 (8.1)	114.1 (9.3)	.409	112.8 (8.7)	112.2 (8.4)	.826	111.8 (7.1)	115.3 (10.8)	.233
Language scale	43.0 (4.3)	42.3 (4.3)	.656	42.6 (4.4)	43.2 (4.1)	.690	42.5 (4.6)	43.8 (3.4)	.354	42.7 (3.9)	44.0 (5.5)	.404
Social scale	17.2 (1.0)	17.1 (1.1)	.718	17.3 (1.0)	17.1 (1.1)	.494	17.2 (1.0)	17.3 (1.1)	.859	17.0 (.9)	17.3 (1.1)	.480
Motor scale	138.3 (4.5)	136.7 (4.7)	.329	138.1 (4.1)	137.7 (5.4)	.773	138.5 (3.7)	137.0 (6.3)	.346	137.5 (4.4)	138.1 (4.2)	.722

CBCL, Child Behavior Checklist; BSID II, The Bayley Scales of Infant Development; MDI, Mental Developmental Index; PDI, Psychomotor Development Index

Table 4. Stepwise multiple linear regression models to predict CBCL and BSID II scores at 30 months

CRITERION CBCL 30 months and BSID-II	**Predictors**	***Beta***	***t***	***p***	***R^2adjusted***	**Model**	
Emotional reactivity							
Non-adjusted	IL-6 12 months	.353	2.532	**.015**	10.5	$F_{1.46} = 6.411$	*p model* = .015
Adjusted	IL-6 12 months	.346	2.184	**.036**	9.5	$F_{1.36} = 4.768$	*p model* = .036
Anxious/Depressed							
Non-adjusted	IL-6 12 months	.367	2.644	**.011**	11.5	$F_{1.46} = 6.988$	*p model* = .011
Adjusted	IL-6 12 months	.366	2.330	**.026**	10.9	$F_{1.36} = 5.427$	*p model* = .026
Withdrawn							
Non-adjusted	IL-6 12 months	.427	3.164	**.003**	16.4	$F_{1.46} = 10.014$	*p model* = .003
Adjusted	IL-6 12 months	.446	2.952	**.006**	17.6	$F_{1.36} = 8.712$	*p model* = .006
Attention problems							
Non-adjusted	IL-6 12 months	.291	2.041	**.047**	6.4	$F_{1.46} = 4.166$	*p model* = .047
Adjusted	-	-	-	-	-	-	-
Agressive behavior							
Non-adjusted	IL-6 12 months	.332	2.363	**.022**	9.1	$F_{1.46} = 5.585$	*p model* = .022
Adjusted	IL-6 12 months	.353	2.233	**.032**	10.0	$F_{1.36} = 4.988$	*p model* = .032
Internalizing problems							
Non-adjusted	IL-6 12 months	.430	3.198	**.003**	16.7	$F_{1.46} = 10.229$	*p model* = .003
Adjusted	IL-6 12 months	.436	2.868	**.007**	16.7	$F_{1.36} = 8.224$	*p model* = .007
Externalizing problems							
Non-adjusted	IL-6 12 months	.314	2.221	**.031**	7.9	$F_{1.46} = 4.934$	*p model* = .031
Adjusted	IL-6 12 months	.347	2.188	**.035**	9.5	$F_{1.36} = 4.786$	*p model* = .035
Total problems							
Non-adjusted	IL-6 12 months	.382	2.775	**.008**	12.7	$F_{1.46} = 7.698$	*p model* = .008
Adjusted	IL-6 12 months	.401	2.589	**.014**	13.7	$F_{1.36} = 6.701$	*p model* = .014
BSID-II IDP							
Non-adjusted	IL-1β 6 months	.325	-2.402	**.020**	8.7	$F_{1.50} = 5.786$	*p model* = .020
Adjusted	IL-1β 6 months	-.334	-2.185	**.035**	8.8	$F_{1.39} = 4.773$	*p model* = .035

NON-ADJUSTED MODEL, risk variable: IL-6 at 12 months or IL-1β at 6 months

ADJUSTED MODEL, covariables: socioeconomic level (score); breastfeeding (score); gestational age (weeks + days); mother's age (score); GHQ mother
30 months (score); exposure to tobacco during pregnancy (1: non-smoker; 2: smoker); bottle fed iron fortified (0: high iron fortification; 1: low iron fortification), BMI 30 months (score)

CBCL, Child Behavior Checklist; BSID II, The Bayley Scales of Infant Development; MDI, Mental Developmental Index; PDI, Psychomotor Development Index; CRP, C-reactive protein; GHQ, General Health Questionnaire; BMI, Body Mass Index .

Highlights requested by the journal:

- We examined the relationship between inflammatory biomarkers and neurodevelopment.
- High levels of IL-6 predict internalizing, externalizing, and total problems.
- High levels of IL-1β predict worse motor skills performance.