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Association between high levels of inflammatory markers and cognitive outcomes at 4 years of age: The Rhea mother-child cohort study, Crete, Greece

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ABSTRACT

There is growing evidence associating inflammatory markers in complex, higher order neurological functions, such as cognition and memory. We examined whether high levels of various inflammatory markers are associated with cognitive outcomes at 4 years of age in a mother-child cohort in Crete, Greece (Rhea study). We included 642 children in this cross-sectional study. Levels of several inflammatory markers (IFN-γ, IL-1β, IL-6, IL-8, IL-17α, IL-10, MIP-1α, TNF-α and the ratios of IL-6 to IL-10 and TNF-α to IL-10) were determined in child serum via immunoassay. Neurodevelopment at 4 years was assessed by means of the McCarthy Scales of Children's Abilities. Multivariate linear regression analyses were used to estimate the associations between the exposures and outcomes of interest after adjustment for various confounders. Our results indicate that children with high TNF-α concentrations ($\geq 90^{th}$ percentile) in serum demonstrated decreased scores in memory (adjusted β = −4.0; 95% CI: −7.7, −0.2), working memory (adjusted β = −4.0; 95% CI: −8.0, −0.1) as well as in memory span scale (adjusted β = −4.0; 95% CI: −7.9, −0.1). We also found that children with high IFN-γ serum levels showed lower scores in memory span scale (adjusted β = −3.4; 95% CI: −7.3, −0.4). Children with elevated TNF-α/IL-10 ratio demonstrated decreased quantitative (adjusted β = −4.3; 95% CI: −8.2, −0.4), motor (adjusted β = −3.5; 95% CI: −7.5, −0.5), executive function (adjusted β = −4.8; 95% CI: −8.5, −1.1), general cognitive (adjusted β = −3.6; 95% CI: −7.3, −0.1), memory (adjusted β = −3.8; 95% CI: −7.6, −0), working memory (adjusted β = −3.5; 95% CI: −7.5, −0.5) and memory span scores (adjusted β = −5.3; 95% CI: −9.1, −1.4) The findings suggest that high levels of TNF-α may contribute to reduced memory performance at preschool age.

1. Introduction

Inflammation is identified as a natural defense mechanism by body tissues in response to injury, but this process may stop being protective for the organism and become harmful when it occurs chronically [1]. Inflammatory markers, such as cytokines, are proteins involved in normal aspects of neurodevelopment, including progenitor cell differentiation, cellular migration within the nervous system and synaptic

Abbreviations: IFN-γ, Interferon γ; IL-1β, Interleukin 1β; IL-6, Interleukin 6; IL-8, Interleukin 8; IL-17α, Interleukin 17α; IL-10, Interleukin 10; MIP-1α, Macrophage Inflammatory Protein 1α; TNF-α, Tumor Necrosis Factor α; ASD, autism spectrum disorders; DAGs, directed acyclic graphs; BMI, Body Mass Index; IQ, Intelligence Quotient; MSCA, McCarthy Scales of Children's Abilities; SD, Standard Deviation; 95% CI, 95% confidence interval; PCBs, polychlorinated biphenyls; GAMs, generalized additive models

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network formation [2–6] and there is growing evidence associating them in complex, higher order neurological functions, such as cognition and memory [7,8]. Imbalanced cytokine production, signaling and regulation may have various neurological consequences [9,10].

Insight into the link of inflammatory markers function and central nervous system processes has increased, mostly on the basis of animal models. As Voltas et al. [11] recently pointed out, few studies have examined the potential association between child inflammatory biomarkers and neurodevelopment, and most of these studies were carried out with samples of extremely premature infants or with clinical samples of children with autism spectrum disorders (ASD). In fact, a body of research has evolved around the role of prenatal cytokines as markers of risk for cognitive dysfunction in special populations, such as children born preterm [12–14], children with low birth weight [15], sickle cell disease [16] and chronic hepatitis C [17], indicating a potential role for inflammatory processes in neurodevelopmental outcomes for those vulnerable populations. Some clinical studies have linked cytokine imbalances during development and throughout life to ASD; case-control studies have found higher circulating IL-6, IL-1β, IL-8 and TNF-α levels in plasma of preschool [18,19] and school-age children with ASD [20–22] compared to typically developing controls. Moreover, plasma levels of IFN-γ and cerebrospinal fluid levels of TNF-α have been reported to be increased in autistic children [23–25] and, likewise, elevated concentrations of IFN-γ have been reported for subjects with ASD compared to controls [20].

However, to our knowledge, there are no available data discussing the relationship between inflammatory markers levels and neurodevelopment in a general population sample of children. The aim of the present study is to examine the role of various inflammatory markers (IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-17α, MIP-1α, TNF-α and two pro- to anti-inflammatory cytokine ratios, IL-6 to IL-10 and TNF-α to IL-10) measured in child serum at 4 years of age in neurodevelopmental scores assessed at 4 years of age in a cross-sectional study nested in the pregnancy cohort in Crete, Greece, after controlling for a range of confounders. It is hypothesized that increased levels of inflammation will be associated with elevated risk for inferior neurodevelopmental scores at 4 years of age.

2. Materials and methods

2.1. Study population

The Rhea study prospectively examines a population-based sample of pregnant women and their children at the prefecture of Heraklion, Crete, Greece. Methods are described in detail elsewhere [26]. Briefly, female residents (Greek and immigrants) who became pregnant during a period of one year starting in February 2007 were contacted and asked to participate in the study. The first contact was made at the time of the first major ultrasound examination (mean ± SD 12.0 ± 1.5 weeks) and several contacts followed (6th month of pregnancy, at birth, 6 months, 1st year and 4 years after birth). To be eligible for inclusion in the study, women had to have a good understanding of the Greek language and be older than 16 years of age. Face-to-face structured questionnaires along with self-administered questionnaires and medical records were used to obtain information on several psychosocial, dietary, and environmental exposures during pregnancy and early childhood. The study was conducted according to the guidelines of the Declaration of Helsinki and all procedures were approved by the ethical committee of the University Hospital in Heraklion, Crete, Greece. Written informed consent was obtained from all the participants after complete description of the study.

The present analysis is a cross-sectional study, nested within the Rhea cohort. Out of 1363 singleton live births, 879 singleton children participated at the 4 years follow-up of the study, during which inflammation markers were measured in 661 children. From those, complete data for neurodevelopment was available for 642 children. Of those, 59 children had incomplete information regarding pre-pregnancy BMI, smoking early in pregnancy, parity, birth weight, preterm birth, BMI at the age of 4 and passive smoking of the child at 4 years of age. Thus, full data was available for a total of 583 children (90.8% of the children with exposure data and outcome assessment). We observed differences in some of the exposures and outcome data ($p < 0.05$) between the children that had full data available (n = 583) and those that had incomplete covariate information (n = 59). Due to those differences, the incomplete covariate information was imputed.

2.2. Biological sample collection and exposure assessment

Following the completion of the 4-year-follow–up assessments, blood samples were collected by venipuncture for each child (10 ml) in SST gel separator vacutainer (BD vacutainers, UK), after written parental consent. For the reduction of pain and discomfort of the children, anesthetic cream 5% EMLA with composition 2.5% lidocaine and 2.5% prilocaine (AsraZeneca, UK) was used. Analyses were performed in the Laboratory of Clinical Nutrition and Epidemiology of Diseases of Medical School, University of Crete. Blood samples were centrifuged (Kubota4000, Japan) at 2500 rpm 10 min within 2 hrs after collection and stored at −80° C until assayed. The Milliplex Map human high sensitivity T cell magnetic bead panel (Cat. # HSTCMAG-28SK) from Millipore (Billerica, MA) was used for the simultaneous quantification of IFN-γ IL-1β, IL-6, IL-8, IL-10, IL-17α, MIP-1α and TNF-α in the supernatants. The principle of the assay is based on the quantification of multiple bio-molecules concurrently employing fluorescent-coded magnetic beads (MagPlex-C microspheres). The microspheres were incubated with the samples and then were allowed to pass rapidly through laser systems that distinguish the different sets of microspheres and the fluorescent dyes on the reporter bio-molecules. The sensitivity of the assay for every bio-molecule was: 0.3 pg/ml IFN-γ, 0.1 pg/ml IL-1β, 0.1 pg/ml IL-6, 0.1 pg/ml IL-8, 0.6 pg/ml IL-10, 0.3 pg/ml IL-17α, 0.9 pg/ml MIP-1α and 0.2 pg/ml TNF-α. We used a limit of 4 SD based on the statistical convention that observations 4 or more SD from the expected mean can be considered to be “extreme outliers” and thus, excluded from the statistical analyses. The intra-assay precision (%CV) for all biomolecules was < 5%. The inter-assay precision (%CV) for IFNγ, IL-6, IL-10 and IL-17α was < 20%, for IL-1β, IL-8, MIP-1α and TNF-α was < 15%. The above analyses were performed on an automated analyzer Luminex 100 connected with the Luminex xPONENT software.

2.3. Outcome assessment

2.3.1. McCarthy scales of Children’s Abilities (MSCA)

Children’s cognitive and motor development was assessed by two trained psychologists, with the McCarthy Scales of Children’s Abilities (MSCA), at the 4 year clinical visit at the University Hospital of Heraklion, Greece. In brief, MSCA test aims to identify possible developmental delay in different skills with the use of six scales: the Verbal, the Perceptual-Performance, the Quantitative, the General Cognitive, the Memory and the Motor scale [27]. Executive function, working memory and memory span are three additional scales derived from the MSCA test in accordance with their association with specific neurocognitive function areas [28].

The translation and cross-cultural adaptation of the MSCA was performed according to the internationally recommended methodology. Children were assigned to the two psychologists at random. The inter-observer variability was < 1%. Right after each MSCA assessment psychologists completed a brief report regarding difficulties encountered during administration, such as child’s behavior (bad moods, nervousness) and physical condition (tiredness, colds). This report was used for creating the quality of assessment index for the MSCA, which was flagged as “good”, “bad” or “very bad”. Additional information on children’s behavior was obtained via maternal report on standardized

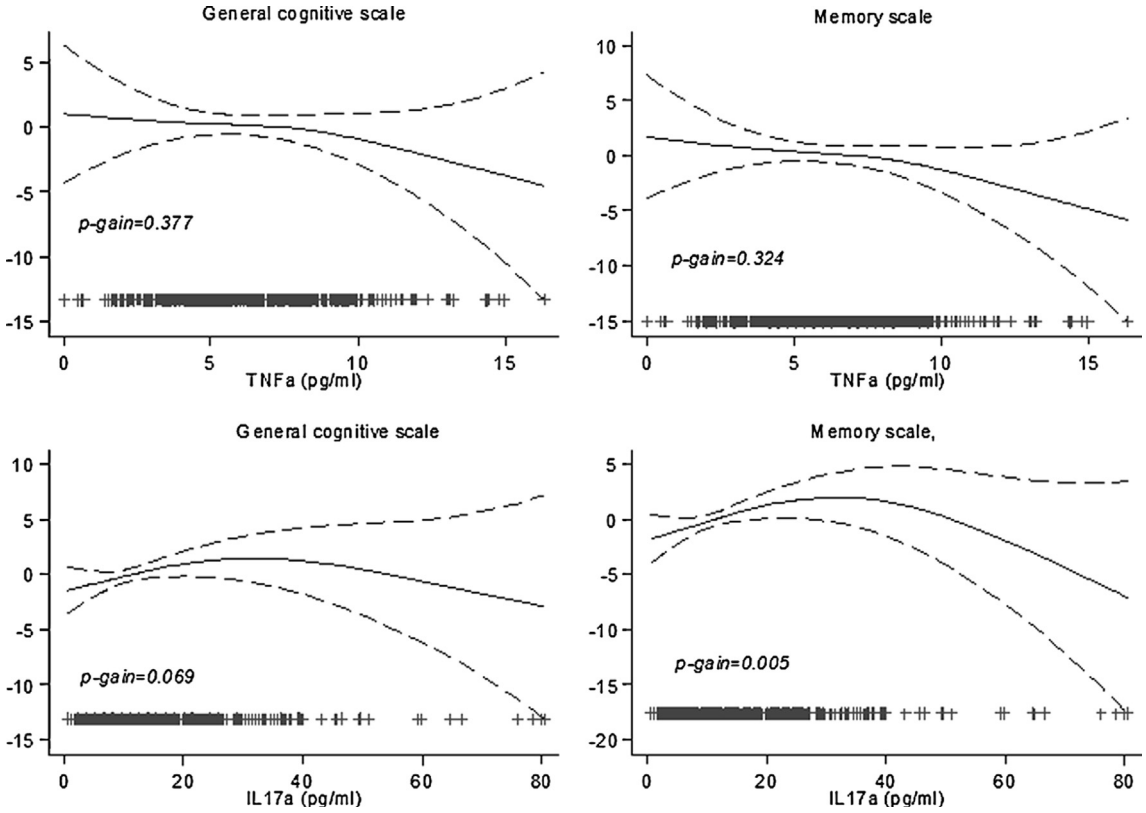

Fig. 1. GAMS for adjusted associations (95% CIs) of TNF-α and IL-17α with child general cognitive and memory scale at 4 years of age. All models were adjusted for examiner, quality of assessment, child sex, maternal age in pregnancy, maternal education, BMI pre-pregnancy, parity, passive smoking at 4 years, birth weight, preterm birth and BMI at 4 years. Plus symbols (+) represent observations.

child behavior scales which were administered at the 4 years of age follow-up.

Raw scores of the neurodevelopmental assessment scales were standardized for child's age at test administration using a method for the estimation of age-specific reference intervals based on fractional polynomials [29]. Standardized residuals were then typified having a mean of 100 points with a 15 SD to homogenize the scales (parameters conventionally used in psychometrics for IQ assessment). Scores were treated as continuous variables with higher scores representing better performance.

2.4. Statistical analysis

Descriptive analysis of the study population was conducted. We performed generalized additive models (GAMs) to assess the linear relationship between inflammatory markers and outcomes. Fig. 1 shows the GAMs for two main outcomes. Because GAMs did not show linearity (p-gain, defined as the difference in normalized deviance between the GAM model and the linear model for the same exposure and outcome > 0.1), we used serum concentrations of inflammatory biomarkers as categorical variables; the categories were defined as the "high exposure group" ($\geq 90^{th}$ percentile) and the reference group (< 90th percentile). This categorization was also decided upon graphical inspection of the relationship between outcomes and exposures after the application of a spline knot (Supplementary Fig. 1).

We estimated associations between serum concentrations of inflammatory marker levels and continuous scores in cognitive and motor development by performing multivariate linear models. Estimated associations were described in terms of β-coefficients and their 95% confidence intervals (CI). The significance of the β-coefficients was evaluated by the Wald's test. We assessed the association between serum concentrations of inflammatory markers and each neurodevelopmental outcome in an adjusted model for quality of MSCA assessment (good, bad, very bad), examiner (psychologist 1, psychologist 2), child sex (male, female), and maternal characteristics, such as maternal age in pregnancy (years), maternal education [low level: ≤ 9 years of mandatory schooling, medium level: > 9 years of schooling up to attending post-secondary school education and high level: attending university or having a university/technical college degree] parity (primiparous, multiparous) and maternal pre-pregnancy Body Mass Index (BMI) and child characteristics, such as passive smoking at 4 years (yes, no), birth weight (g), preterm birth (yes, no) and child BMI at 4 years (kg/m^2). For the fully adjusted model, we selected the covariates using directed acyclic graphs (DAGs) (Supplementary Fig. 2).

We performed various sensitivity analyses to assess the robustness of our results. First, in order to assess if our studied associations were modified by child sex, child BMI at 4 years (normal weight vs. overweight or obese), maternal pre-pregnancy BMI (normal weight vs. overweight or obese), Child nursery attendance (yes/no) or passive smoking exposure, appropriate interaction terms were included in the regression models. We stratified the sample in the cases that significant interactions were detected. Second, we repeated all analyses excluding preterm (< 37 gestational weeks) and low birth weight (< 2500 g) neonates. Third, because relations of inflammatory markers with cognitive outcomes could be confounded by chronic child health diseases and infections, we repeated the analysis after further adjusting for asthma occurrence at 4 years (yes/no), allergic rhinitis symptoms in the last 12 months at 4 years of age (yes/no), and helicobacter pylori seropositivity at 4 years of age (yes/no). We also included in the models exposures during pregnancy that have been previously shown to affect child neurodevelopment such as maternal serum concentrations of polychlorinated biphenyls (PCBs, pg/ml) [30] and maternal serum levels of Vitamin D (nmol/l) [31].

Due to the relatively high percentage of missing covariates (9.2%)

Table 1
Study participants characteristics.

	Total	
	N	% or Mean ± SD
Maternal characteristics		
Maternal age (years)	634	29.8 ± 5.0
Ethnic origin		
Greek	602	94.7
Other	34	5.3
Education		
Low	95	15.3
Medium	320	51.7
High	204	33.0
Parity		
Primiparous	298	46.5
Multiparous	343	53.5
BMI before pregnancy (kg/m^2)	608	24.5 ± 4.7
Child characteristics		
Sex		
Boy	336	52.3
Girl	306	47.7
Age at 4 years follow-up	642	4.2 ± 0.2
Birth weight (g)	618	3195.4 ± 449.2
Preterm birth	669	4.1 ± 4.3
Yes	74	11.8
No	551	88.2
BMI at age 4 (kg/m^2)	640	16.4 ± 1.8
Passive smoking at age 4		
Yes	280	43.8
No	359	56.2

we used multiple imputations with chained equations (MICE) in order to increase precision and reduce bias. The imputation model included exposures, outcomes, and covariates under study, as well as additional auxiliary variables [32]. In analytic models, we combined estimates from the 20 imputed data sets generated with the use of Rubin's rules [33]. Results were similar between multiple imputation and complete case analysis, and hence, we present effect estimates based on imputed data.

All hypothesis testing was conducted assuming a 0.05 significance level and a 2-sided alternative hypothesis. The standardization of the MSCA and all other statistical analyses were performed using Stata Software, version 13 (Stata Corp LP, College Station, TX, USA).

3. Results

Table 1 describes the study population characteristics. Participating mothers were predominantly Greek (94.7%) and had a mean (± SD) age of 29.8 (± 5.0) years. About half of them had medium educational level (51.7%) and were multiparous (53.5%). Before pregnancy, the mean maternal BMI was 24.5 (± 4.7) kg/m^2. About half of the children (52.3%) were boys, the mean (± SD) birth weight of the study population was 3195.4 (± 449.2) g and the mean age at assessment was 4.2 (± 0.2) years. A total of 280 (43.8%) were exposed to passive smoking and the mean (± SD) BMI at age 4 was 16.4 (± 1.8) kg/m^2.

Child inflammatory levels in serum at 4 years are presented in Table 2 and Supplementary Table 1 illustrates correlation coefficients calculated for all those markers.

Table 3 shows regression results for high inflammatory marker levels in child serum in relation to neurodevelopmental outcomes (MSCA scores) at 4 years of age. Children with high IFN-γ serum levels (≥90th percentile) showed lower scores in memory span scale (adjusted β = −3.4; 95% CI: −7.3, −0.4). Children with high TNF-α serum levels (≥90th percentile) demonstrated decreased scores in memory (adjusted β = −4.0; 95% CI: −7.7, −0.2), working memory (adjusted β = −4.0; 95% CI: −8.0, −0.1) as well as in memory span scale (adjusted β = −4.0; 95% CI: −7.9, −0.1). High TNF-α/IL-10 ratio was associated with decreased quantitative (adjusted β = −4.3; 95% CI: −8.2, −0.4), motor (adjusted β = −3.5; 95% CI: −7.5, −0.5), executive function (adjusted β = −4.8; 95% CI: −8.5, −1.1), general cognitive (adjusted β = −3.6; 95% CI: −7.3, −0.1), memory (adjusted β = −3.8; 95% CI: −7.6, −0), working memory (adjusted β = −3.5; 95% CI: −7.5, −0.5) and memory span scores (adjusted β = −5.3; 95% CI: −9.1, −1.4). No other association was detected between high inflammatory levels and other neurodevelopmental scores at 4 years of age.

Table 2
Child inflammatory levels at 4 years of age (pg/ml).

Inflammatory Marker	N	Median (IQR)	Geometric Mean (GSD)	Percentile	
				10th	90th
IFN-γ pg/ml	636	26.1 (22.7)	21.8 (2.3)	6.5	50.3
IL-1β pg/ml	637	1.3 (1.0)	1.1 (2.1)	0.5	2.3
IL-6 pg/ml	635	1.1 (0.8)	1.1 (1.8)	0.5	2.3
IL-8 pg/ml	635	3.5 (1.9)	3.6 (1.5)	2.2	5.7
TNF-α pg/ml	639	6.0 (3.2)	5.8 (1.5)	3.4	9.3
IL-17α pg/ml	634	11.0 (12.0)	10.7 (2.2)	4.1	26.8
MIP-1α pg/ml	641	13.4 (7.6)	12.8 (1.5)	6.9	21.1
IL-10 pg/ml	641	5.3 (5.0)	5.4 (2.1)	2.1	13.3

GSD: Geometric Standard Deviation.

Further analyses showed evidence for an interaction between child sex and IL-17α levels in response to neurodevelopmental scores (p for interaction < 0.05). Stratified analysis revealed reduced verbal (adjusted β = −4.2; 95% CI: −10.2, 1.7) scale scores for boys with high concentrations of IL-17a, whereas these associations in girls were in the opposite direction. Moreover, boys with high concentrations of IL-6 had lower motor (adjusted β = −0.2; 95% CI: −6.2, 5.8) scale scores (Supplementary Table 2). Further stratified analysis according to child BMI status, showed reduced scores in memory (adjusted β = −11.4; 95% CI: −20.6, −2.2) and memory span (adjusted β = −11.3; 95% CI: −20.2, −2.4) scores for overweight/obese children with high concentrations of TNF-α in serum compared to children with normal weight (Supplementary Table 3). We found no evidence for any significant interaction between maternal pre-pregnancy overweight/obesity, exposure to passive smoking or child nursery attendance and child high inflammatory biomarker levels at 4 years of age (p for interaction > 0.05). Sensitivity analyses excluding preterm newborns (< 37 gestational weeks) and low birth weight neonates (< 2500 g) did not meaningfully change our results (data not shown). After further adjustment for asthma, allergic rhinitis and helicobacter pylori seropositivity at 4 years, as well as exposure to environmental pollutants and pregnancy serum levels of Vitamin D, our results did not differ substantially from those derived from the main analysis (Supplementary Table 4).

4. Discussion

In the present analysis we examined for the first time the relationship between inflammatory marker levels and neurodevelopment in a general population sample of children and found that preschoolers with elevated TNF-α concentrations in serum demonstrated decreased scores in memory, memory span and working memory tasks. These associations persisted after the sequential adjustment for several maternal and child factors. We also found that children with high IFN-γ serum levels showed lower scores in memory span scale. Elevated levels of the rest inflammatory markers under examination (IL-1β, IL-6, IL-8, IL-17α, IL-10 and MIP-1α) were not associated with any other child neurodevelopmental scores.

Comparison with other studies is rather complex mainly because of different methodological approaches study design, type and size of study samples, age of the participants and outcomes examined. Studies with elderly populations have well-established the association between TNF-α levels with cognitive deficits. Elevated TNF-α serum

Table 3
Adjusted associations (β coefficients & 95% CIs) of child inflammatory markers levels with MSCA scales at 4 years of age (n = 634).

	Verbal beta (95% CI)	Perceptual beta (95% CI)	Quantitative beta (95% CI)	Motor beta (95% CI)	Exec. Function beta (95% CI)	General cognitive beta (95% CI)	Memory beta (95% CI)	Working memory beta (95% CI)	Memory span beta (95% CI)
Markers with pro-inflammatory activity									
IFN-γ[a]	−2.3 (−5.9, 1.3)	1.7 (−1.9, 5.4)	−1.0 (−4.8, 2.8)	2.5 (−1.5, 6.4)	−1.7 (−5.3, 2.0)	−1.0 (−4.6, 2.6)	−2.8 (−6.5, 1.0)	−2.1 (−6.1, 1.8)	**−3.4 (−7.3, 0.4.0)**
IL-1β[a]	−2.2 (−5.8, 1.4)	−2.1 (−5.7, 1.6)	1.2 (−2.7, 5.0)	−2.2 (−6.2, 1.7)	−0.8 (−4.5, 2.8)	−1.9 (−5.5, 1.7)	−2.6 (−6.3, 1.2)	1.3 (−2.6, 5.3)	−2.7 (−6.5, 1.2)
IL-6[a]	2.1 (−1.6, 5.7)	1.9 (−1.8, 5.5)	0.8 (−3, 4.7)	3.7 (−0.3, 7.6)	1.0 (−2.7, 4.6)	2.1 (−1.5, 5.8)	3.0 (−0.8, 6.8)	0.1 (−3.9, 4.1)	2.9 (−1.0, 6.8)
IL-8[a]	−1.0 (−4.6, 2.7)	−1.0 (−4.6, 2.7)	−0.9 (−4.8, 2.9)	0.8 (−3.2, 4.7)	−1.7 (−5.3, 2.0)	−1.2 (−4.8, 2.5)	−1.2 (−4.9, 2.6)	−2.0 (−5.9, 2.0)	−0.8 (−4.7, 3.1)
TNF-α[a]	−3.2 (−6.9, 0.4.0)	−1.7 (−5.3, 2.0)	−2.6 (−6.5, 1.2)	−2.4 (−6.3, 1.6)	−2.8 (−6.4, 0.9)	−3.0 (−6.6, 0.7)	**−4.0 (−7.7, −0.2)**	**−4.0 (−8.0, −0.1)**	**−4.0 (−7.9, −0.1)**
IL-17α[a]	1.4 (−2.3, 5.0)	−2.3 (−6.0, 1.4)	1.6 (−2.3, 5.5)	−1.5 (−5.5, 2.5)	0.3 (−3.4, 4.1)	0.1 (−3.5, 3.8)	0.9 (−2.9, 4.7)	1.9 (−2.1, 5.9)	0.3 (−3.6, 4.2)
MIP-1α[a]	−0.2 (−3.8, 3.4)	−0.5 (−4.2, 3.1)	0.7 (−3.1, 4.5)	0.8 (−3.1, 4.7)	1.3 (−2.3, 4.9)	−0.3 (−3.9, 3.3)	−1.5 (−5.2, 2.2)	1.1 (−2.8, 5.0)	−1.4 (−5.2, 2.4)
Markers with anti-inflammatory activity									
IL-10[a]	1.2 (−2.5, 4.8)	2.7 (−1.0, 6.3)	0.7 (−3.1, 4.6)	2.8 (−1.2, 6.7)	2.0 (−1.6, 5.7)	1.8 (−1.9, 5.4)	0.1 (−3.6, 3.9)	−0.4 (−4.4, 3.5)	1.0 (−2.8, 4.9)
Ratios									
IL-6/IL10[a]	−0.2 (−3.9, 3.5)	−1.5 (−5.2, 2.2)	−1.3 (−5.2, 2.6)	−2.6 (−6.6, 1.3)	−1.8 (−5.5, 1.9)	−0.9 (−4.6, 2.8)	0.8 (−3.0, 4.6)	−0.7 (−4.7, 3.3)	0.6 (−3.3, 4.5)
TNF-α/IL-10[a]	−3.0 (−6.7, 0.7)	−2.9 (−6.6, 0.8)	**−4.3 (−8.2, −0.4)**	**−3.5 (−7.5, 0.5)**	**−4.8 (−8.5, −1.1)**	**−3.6 (−7.3, 0.1)**	**−3.8 (−7.6, 0)**	**−3.5 (−7.5, 0.5)**	**−5.3 (−9.1, −1.4)**

All models are adjusted for examiner, quality of assessment, child sex, maternal age in pregnancy, maternal education, BMI pre-pregnancy, parity, passive smoking at 4 years, birth weight, preterm birth and BMI at 4 years.
Bold text indicates statistically significant associations at $p < 0.05$.
[a] ≥ 90th percentile.

concentrations have been detected in patients with cognitive decline, such as Alzheimer's disease [34–36] suggesting that TNF-α-driven processes may contribute to cognitive and memory deficits of the disease and that inhibition of TNF-α can be effective for treating it [37–40]. In addition, a study conducted with adult patients with depressive disorder demonstrated that elevated expression of TNF-α, TNFRSF1A and TNFRSF1B genes correlates negatively, among others, with working memory, direct and delayed auditory-verbal memory and effectiveness of learning processes and verbal fluency [41].

Available data on child inflammation and neurodevelopmental outcomes are mainly based on ASD samples; a recent cross-sectional study investigating the association between peripheral cytokine levels (including TNF-α) and cognitive profiles in children with ASD found negative correlations of IL-6 and IFN-γ serum levels with WISC verbal comprehension index and working memory index respectively, suggesting that cytokines may play a role in the neural development in ASD [42].

In general, inflammatory signaling is considered to be a critical contributor to the short-and long term regulation of mood and cognition, but the exact mechanisms by which cytokines may modulate memory remain unknown [43]. TNF-α concentrations are found elevated in various neuropathological states that are related to learning and memory deficits, highlighting a possible role in plasticity [44]. For this purpose, much work has been carried out in the hippocampus; in fact, animal studies provide evidence that mice over-expressing TNF-α demonstrate memory impairments and disrupted learning capabilities [45,46], supporting the notion that TNF-α activity at the hippocampus and the synaptic level may influence brain function and behavior [47,48]. Consistently, a negative effect of TNF-α was found following intra-hippocampal administration to rats, which lead to impaired hippocampal-dependent working memory, as shown by an increased number of errors and longer latencies regarding the runway task [49]. Moreover, increased TNF-α in rats following peripheral nerve injury may not only contribute to chronic pain, but also to memory deficits by dysfunction of hippocampus [50]. A study conducted in adults showed that higher concentrations of TNF-α are associated with smaller hippocampal volumes suggesting that the balance between the hypothalamic-pituitary adrenal axis and inflammation processes might explain hippocampal volume reductions [51]. Regarding high IFN-γ levels, animal models have shown that they can act as a negative regulator of neuroplastic changes such as hippocampus structure, cell density, neuronal morphology and synaptic plasticity and, therefore may be associated with poorer performance in learning and memory tasks [52]. We also found that high TNF-α/IL-10 ratio was associated with decreased quantitative, motor, executive function, general cognitive, memory, working memory and memory span scores. Since this is the first study illustrating the associations of these ratios with child cognitive domains, further data is required to validate these results and clarify the underlying mechanisms of these findings.

Our results showed greater risk for reduced verbal performance scores for boys with high IL-17α serum concentrations, as well as lower motor scores for boys with high IL-6 serum concentrations. To our knowledge, this is the first study revealing sex-related differences in inflammation-specific cognitive domain associations in children. Although further study is required to validate and clarify the underlying mechanisms and the clinical utility of these findings, increased male prevalence has been frequently reported in various neurodevelopmental disorders, highlighting the concept of a male vulnerability model [53]. It is hypothesized that this male susceptibility occurs partly because microglia and inflammatory molecules are involved in the normal developmental process of sexual differentiation [54] and also because males have more activated innate immune cells in the developing brain under normal conditions [55]. We also found greater risk for lower scores in memory and memory span scales for overweight/obese children with high TNF-α serum concentrations. This finding is in line with evidence from human clinical studies showing that obesity

may increase the risk of mild cognitive impairment, in the form of short-term memory and executive function deficits [56]. Obesity is considered to be a low-grade pro-inflammatory state, and studies have reported low-grade elevation of TNF-α in obese individuals [57–59]. Research in rodent models show that obesity-induced inflammation may directly interfere with synaptic communication in the hippocampus [60].

The results of this study should be interpreted in light of its limitations; the cross-sectional design of the study does not permit inferences on causality. Although we incorporated extensive information on potential child and social factors that are associated with child neurodevelopment, we acknowledge that residual confounding because of other unmeasured confounders may still occur. On the other hand, the strengths of the present study include the heterogeneous and relatively large sample size. In addition, we carefully assessed neurodevelopmental data using a robust instrument such as MSCA [27], a valid, standardized psychometric test which provides both a general level of child's intellectual functioning and an assessment of separate neurodevelopmental domains. Moreover, we used multiple imputations with chained equations in order to increase precision and reduce bias.

To our knowledge, this the first study conducted in a general population sample of children which highlights the significant role of increased TNF-α levels during preschool years in child memory performance. As there are no studies to this date that analyzed how inflammatory biomarkers relate to measures of neurodevelopmental scores in a general population sample, these results may shed some light in new pathways of investigation. Our findings reinforce the existing evidence that elevated inflammatory activity may be involved in early pathophysiological processes, such as memory deficits and further investigation on the meaning of these associations can provide new insights. The follow-up of this cohort could provide additional data about the potential predictive role of those biomarkers and elucidate some of the questions raised by the results.

5. Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.01.010.

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