

Neonates with reduced neonatal lung function have systemic low-grade inflammation

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1 **Reduced Neonatal Lung Function Associates with Systemic Low-grade**
2 **Inflammation in Early Life**

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44 design and conduct of the study; collection, management, and interpretation of the data; or
45 preparation, review, or approval of the manuscript.

46 **Abbreviations:** COPSAC₂₀₀₀ = COpenhagen Prospective Study on Asthma in Childhood; CXCL8
47 (IL-8) = Chemokine (C-X-C motif) Ligand 8; FEV_{0.5} = Forced Expiratory Volume at 0.5 seconds;
48 FEF₅₀ = Forced Expiratory Flow at 50% of the forced vital capacity; hs-CRP = high-sensitivity C-
49 reactive protein; IL-1 β = Interleukin-1 β , IL-6 = Interleukin-6; MMEF = Maximal Mid-Expiratory
50 Flow; PtcO₂ = transcutaneous oxygen saturation; PD₁₅ = Provocative Dose of methacholine causing
51 a 15% drop in PtcO₂; PD₂₀ = Provocative Dose of methacholine causing a 20% drop in FEV₁ from
52 baseline; TNF- α = tumor necrosis factor- α ; TROLS = TROublsome Lung Symptoms.

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54 table of content online at www.atsjournals.org.

55 **At a Glance Commentary:**

56 *Scientific Knowledge on the Subject*

57 Elevated hs-CRP as a proxy of systemic low-grade inflammation has been demonstrated in
58 asthmatic children and adults with diminished pulmonary function. It is however unknown whether
59 asymptomatic reduced neonatal lung function is associated with systemic inflammation.

60 *What this Study Adds to the Field*

61 This study shows that children with impaired respiratory capacity as neonates are characterized by
62 elevated hs-CRP and an up-regulated blood inflammatory profile suggesting presence of systemic
63 low-grade inflammation in early life.

64 **Data from this manuscript has not been presented before in abstract or any other form.**

65 **ABSTRACT**66 *Rationale*

67 Previous studies indicate presence of systemic inflammation in children and adults with asthma and
68 impaired lung function, but it is unknown whether asymptomatic reduced infant lung function is
69 associated with low-grade inflammation in early life.

70 *Objective*

71 To investigate the possible association between infant lung function indices and biomarkers of
72 systemic inflammation in early life.

73 *Methods*

74 Serum levels of high-sensitivity C-reactive protein (hs-CRP), interleukin-1 β (IL-1 β), IL-6, tumor
75 necrosis factor- α (TNF- α) and CXCL8 (IL-8) were measured at age 6 months in 300 children of the
76 Copenhagen Prospective Study on Asthma in Childhood₂₀₀₀ (COPSAC₂₀₀₀) birth cohort, who
77 completed infant lung function testing at age 1 month, spirometry at 7yrs, and fulfilled a respiratory
78 day-to-day diary from 0-7yrs. Associations between lung function indices, asthmatic symptoms and
79 inflammatory biomarkers were investigated by conventional statistics and unsupervised principle
80 component analysis.

81 *Measurements and Main Results*

82 Infant's forced expiratory volume at 0.5s (FEV_{0.5}) was inversely associated with hs-CRP (β -
83 coefficient, -0.12; 95% CI, -0.21 to -0.04; p=0.004) and with a uniform up-regulated inflammatory
84 signature (p=0.02). hs-CRP at 6mo was elevated in children with asthmatic symptoms at 0-6mo
85 compared to children without asthmatic symptoms (median, 1.79mg/L vs. 1.19mg/L; p=0.05), but
86 was not associated with asthma or lung function at age 7yrs. Adjusting for older children in the
87 home, infections 14d prior to blood sampling, birth BMI, and maternal smoking did not affect the
88 associations.

89 *Conclusion*

90 Diminished infant lung function associates with elevated hs-CRP and an up-regulated blood

91 inflammatory response suggesting linkage between lung function and systemic low-grade

92 inflammation in early life.

93 **Abstract Word Count:** 252 words

94 **Key-words:** Asthma, Children, high-sensitivity C-reactive protein, pro-inflammatory cytokines,

95 spirometry.

96 **INTRODUCTION**

97 C-reactive protein (CRP) is an acute-phase reactant found in the blood in response to acute and
98 chronic inflammatory conditions and has a broad clinical application in the screening for infectious
99 and immune-mediated diseases¹. CRP harbors important innate immunity properties and is released
100 from the liver triggered by pro-inflammatory cytokines such as interleukin 6 (IL-6), IL-1 β , and
101 tumor necrosis factor α (TNF- α)². Newer CRP assays³ has enabled assessment of previously
102 immeasurable low levels of CRP, termed high sensitivity CRP (hs-CRP), which is now increasingly
103 recognized as a marker of low-grade inflammation in e.g. cardiovascular disease⁴, obesity⁵, and
104 diabetes mellitus⁶.

105 Recently, elevated hs-CRP has also been demonstrated in manifest airway diseases such as asthma⁷
106 and chronic obstructive pulmonary disease⁸. In addition, previous studies indicate that impaired
107 lung function in asthmatic children and adults is associated with presence of systemic low-grade
108 inflammation^{9,10}. It is however unknown whether asymptomatic neonates with reduced pulmonary
109 function are characterized by systemic low-grade inflammation in early life.

110 We hypothesized that children with reduced neonatal lung function may have biochemical signs of
111 systemic low-grade inflammation in infancy. The objective of the current study was therefore to
112 investigate the possible association between lung function indices measured in asymptomatic
113 neonates of the Copenhagen Prospective Study on Asthma in Childhood₂₀₀₀ (COPSAC₂₀₀₀) birth
114 cohort and serum levels of hs-CRP, IL-1 β , IL-6, TNF- α , and CXCL8 (formerly IL-8) at age 6
115 months.

116 **METHODS**117 *Study Cohort*

118 The study participants were 411 infants born of mothers with a history of asthma enrolled at 1
119 month of age in the COPSAC₂₀₀₀ prospective birth cohort study¹¹⁻¹³. Exclusion criteria were any
120 respiratory symptoms or respiratory support prior to inclusion, gestational age <36 weeks, and any
121 congenital abnormality or systemic illness. The children attended the COPSAC research clinic at
122 age 1 month for assessment of infant lung function and subsequently at 6-monthly intervals till age
123 7 years for scheduled clinical investigations, collection of medical history since last visit supported
124 by a day-to-day lung symptom diary, and for detailed exposure assessments. Additional acute visits
125 were arranged upon occurrence of any respiratory symptoms.

126 *Ethics*

127 The study was conducted in accordance with the guiding principles of the Declaration of Helsinki
128 and was approved by the Local Ethics Committee (KF 01-289/96), and the Danish Data Protection
129 Agency (2008-41-1754). Both parents gave written informed consent before enrolment.

130 *Inflammatory Biomarkers*

131 At age 6 months blood was drawn from a cubital vein, centrifuged to separate serum and serum
132 cells, and immediately stored at -80° C until analyses. The samples were transported on dry ice to
133 the laboratory, where levels of the selected biomarkers were determined by high-sensitivity ELISA
134 assays based on electrochemiluminescence in a 4-plex setting for IL-1 β , IL-6, CXCL8 and TNF- α
135 and as a single assay for hs-CRP. Samples were read in duplicates using the Sector Imager 6000
136 (MesoScale Discovery®, Gaithersburg, MD, USA). The limit of detection (mean signal from blanks
137 +3SD) was 9.54 pg/mL for hs-CRP, 0.15 pg/mL for IL-1 β , 0.17 pg/mL for IL-6, 0.09 pg/mL for
138 CXCL8 and 0.08 pg/mL for TNF- α .

139 *Lung Function*

140 Infant spirometry was measured at age 1 month applying the raised volume rapid thoraco-
141 abdominal “squeeze”-jacket compression technique¹⁴. Repeated ventilations to predefined mouth-
142 pressures assured expansion of the lung volume before an instant inflation of the jacket caused a full
143 exhalation during which the flow was measured by a pneumotachograph with an aircushion
144 facemask^{15,16}. The software identified the Forced Vital Capacity (FVC) as the first plateau on the
145 volume-time curve and measurements with FVC appearing after 0.5s and with the Forced
146 Expiratory Volume at 0.5s (FEV_{0.5}) being smaller than or equal to FVC were accepted. Three to
147 five acceptable curves were obtained for each infant and the curve containing the median value of
148 FEV_{0.5} was used for the analyses of FEV_{0.5} and Forced Expiratory Flow at 50% of FVC (FEF₅₀).

149 Spirometry at age 7yrs was performed as previously detailed¹⁷ using a pneumotachograph,
150 Masterscope Pneumoscreen, system 754,916 spirometer (Erich Jaeger, Wurtzburg, Germany) for
151 assessing FEV₁ and maximal mid-expiratory flow (MMEF).

152 Infant bronchial responsiveness: After an initial saline inhalation, methacholine was given in
153 quadrupling dose-steps via a dosimeter attached to a nebulizer (SPIRA 08 TSM 133; Respiratory
154 Care Center; Hämeenlinna, Finland)¹⁶. Bronchial responsiveness was determined by continuous
155 assessment of transcutaneous oxygen saturation (PtcO₂) (TCM3; Radiometer; Copenhagen,
156 Denmark). The provocative dose causing a 15% drop in PtcO₂ (PD₁₅) was estimated from the dose
157 response curves fitted with a logistic function.

158 Bronchial responsiveness at age 7yrs was defined as the provocative dose of methacholine causing a
159 20% drop in FEV₁ from baseline (PD₂₀)¹⁷.

160 *Clinical Investigator-diagnosed End-points*

161 Troublesome lung symptoms (TROLS) were defined as significant cough or wheeze or dyspnea

162 severely affecting the well-being of the child and recorded by the parents in a daily diary chart as a

163 dichotomized score (yes/no) from birth till age 7yrs¹⁸. *Recurrent TROLS* was defined from the
164 diaries as five episodes within 6 months, each episode lasting at least three consecutive days, or
165 daily symptoms for four consecutive weeks^{19,20}.

166 Asthma at age 7yrs was diagnosed according to international guidelines and was based on recurrent
167 TROLS as defined above, symptoms judged by the COPSAC pediatricians to be typical of asthma,
168 in need of intermittent inhaled β_2 -agonist, responding to a 3-month trial of inhaled corticosteroids
169 and relapsing when stopping treatment^{12,13}.

170 *Covariates*

171 Covariates included *heredity* (father's history of asthma, eczema or allergy [yes/no]);
172 *anthropometrics* (birth BMI [7-12, 12-13, 13-14, 14-17m/kg²]); *demographics* (gender, older
173 children in the home at birth [yes/no], yearly household income [low (<53.000 €), medium (53.000-
174 80.000 €), high (>80.000 €)]; *pre- and antenatal exposures* (maternal smoking during 3rd
175 pregnancy trimester [yes/no], caesarean section [yes/no]); *postnatal exposures* (solely breastfeeding
176 length [0-3, 3-6, >6mo], age at start in daycare [0-9, 9-12, >12mo], pets in the home in the 1st year
177 of life: cat [yes/no], dog [yes/no]); and *infections 14 days prior to biomarker assessment* (upper and
178 lower respiratory tract infections, gastroenteritis or fever with unknown cause [yes/no]).

179 *Statistics*

180 Biomarker null values were set to half of the lowest detected value for the specific biomarker,
181 values were log-transformed, and the mean of the duplicate measurements were used for association
182 analyses. Z-scores were calculated for FEV_{0.5}, FEV₁, FEF₅₀ and MMEF, and PD₁₅ and PD₂₀ were
183 log-transformed to obtain normality. The associations between lung function, asthmatic symptoms,
184 and inflammatory biomarkers were tested by conventional statistics and by unsupervised pattern
185 recognition using principal component analysis (PCA).

186 The relation between continuous lung function indices and continuous levels of inflammatory
187 biomarkers at age 6 months was tested with general linear models. The association between
188 biomarker levels and time to recurrent TROLS was modeled using Cox-regression. Logistic
189 regression was used to compute the odds ratio of asthma at age 7yrs.

190 For the pattern recognition analyses, we extracted underlying orthogonal components that described
191 the systematic part of the variation across the biomarkers using centered and scaled (equal variance)
192 mediator levels. Scree plots of the Eigen values were used to select the number of components for
193 subsequent association analyses.

194 All results are presented as raw estimates with 95% CI and as estimates adjusted for covariates
195 associated with levels of hs-CRP using a cut-off at $p \leq 0.10$. Birth BMI and maternal smoking during
196 3rd trimester were retained in the multivariable models with infant lung function independently of
197 their association with hs-CRP as these are important determinants of infant lung function²¹. A p-
198 value ≤ 0.05 was considered significant. All analyses were done using SAS version 9.3 (SAS
199 Institute, Cary, NC).

200 RESULTS

201 *Inflammatory Biomarker Assessments*

202 Measurements of IL-1 β , IL-6, TNF- α and CXCL8 were performed on 309 and hs-CRP on 301
203 serum samples collected at age 6 months. One sample was lost for technical reasons while
204 performing the 4-plex assay, resulting in 300 children (73% of the original 411 cohort children)
205 with available measurements for all five biomarkers. We found no significant differences in
206 baseline characteristics between children with and without available biomarker assessments (Table
207 E1).

208 The median hs-CRP level was 1.39 mg/L (inter-quartile range [IQR], 0.46-4.61), IL-1 β was 0.01
209 ng/L (0.001-0.04), IL-6 was 0.20 ng/L (0.11-0.31), TNF- α was 2.34 ng/L (1.92-2.88), and CXCL8
210 was 3.04 ng/L (2.19-4.37). The IL-6 and TNF- α levels were strongly positively correlated with hs-
211 CRP levels ($p < 0.001$ for both) whereas IL-1 β and CXCL8 levels were not correlated with hs-CRP
212 ($p \geq 0.62$). The measured values of hs-CRP, IL-6, TNF- α and CXCL8 were within the expected
213 range²² with very few null values, whilst IL-1 β levels were much lower than expected²² with null
214 values for 72 of 308 children (23%). Due to that and the fact that IL-1 β has been shown to
215 significantly degrade over time even at -80° C²³, IL-1 β was not included in further analyses.

216 *Determinants of hs-CRP*

217 Children with older children in the home at birth had significantly higher hs-CRP level at age 6
218 months compared to children without older children in the home: median hs-CRP level 2.20 mg/L
219 (IQR, 0.63-5.05) vs. 1.16 mg/L (0.41-3.40), $p = 0.005$. In addition, hs-CRP was elevated in children
220 who had suffered an infectious episode within 14 days prior to biomarker assessment compared to
221 children without apparent infections: 4.29 mg/L (1.71-5.34) vs. 0.84 mg/L (0.36-2.67), $p < 0.0001$.
222 We did not detect associations between hs-CRP level and paternal history of asthma, eczema or
223 allergy, child gender, birth BMI, household income, maternal smoking during 3rd pregnancy

224 trimester, birth by caesarean section, breastfeeding, daycare attendance or pets in the home (Table
225 1).

226 *Lung Function and Systemic Low-grade Inflammation*

227 The conventional statistical approach showed a strong linear inverse association between FEV_{0.5} at
228 age 1 month and hs-CRP level at age 6 months (β -coefficient, -0.12; 95% CI, -0.21 to -0.04;
229 $p=0.004$) suggesting increasing grade of inflammation by diminished neonatal lung volume (Figure
230 1). The association was unaffected by adjustment for older children in the home, infections 14 days
231 prior to biomarker assessment, birth BMI and maternal smoking in 3rd trimester: β -coefficient, -
232 0.13; 95% CI, -0.22 to -0.04; $p=0.005$. FEF₅₀ also seemed inversely associated with hs-CRP, but
233 was not significant: β -coefficient, -0.06; 95% CI, -0.15 to 0.02; $p=0.14$.

234 Increasing FEV_{0.5} was also significantly associated with decreasing levels of IL-6 (β -coefficient, -
235 0.10; 95% CI, -0.18 to -0.01; $p=0.03$) (Figure 2). Confounder adjustment did not modify the
236 association: β -coefficient, -0.09; 95% CI, -0.18 to 0.00; $p=0.04$. We did not detect a significant
237 association between FEF₅₀ and IL-6 levels.

238 FEV_{0.5} and FEF₅₀ measurements were not associated with CXCL8 or TNF- α levels although the β -
239 coefficients suggested an inverse association between lung function indices and TNF- α (Table 2).

240 The unsupervised PCA showed that hs-CRP, IL-6, TNF- α and CXCL8 were positively correlated in
241 the first principal component (PC₁) which explained 41% of the total variation in the data. The PCA
242 approach is illustrated in the biplot (Figure 3) showing scores for PC₁ and PC₂ and loadings for the
243 biomarkers. Because of the univocal pattern in PC₁, we focused on PC₁ in the further analyses.

244 Confirming the findings from the conventional statistics, we found that FEV_{0.5} was inversely
245 associated with PC₁ ($p=0.02$) and remained significant after confounder adjustments ($p=0.03$). The

246 β -coefficients also suggested an inverse association between FEF₅₀ and PC₁, but the model was not
247 significant (Table 2).

248 We did not detect any association between inflammatory biomarkers at age 6 months and lung
249 function at age 7 years neither by conventional statistics nor by PCA approach (Table E2).

250 *Bronchial Responsiveness and Systemic Low-grade Inflammation*

251 Bronchial responsiveness to methacholine in neonatal life and at age 7 years was not associated
252 with biomarkers of low-grade inflammation at age 6 months (Tables 2 and E2).

253 *Lung Symptoms, Asthma and Systemic Low-grade Inflammation*

254 Children experiencing TROLS at any time-point from birth till biomarker assessment (0-6mo)
255 compared to children without TROLS had significantly elevated levels of hs-CRP: median 1.79
256 mg/L (IQR,0.50-4.72) vs. 1.19 mg/L (0.46-4.14), p=0.05; IL-6: 0.21 ng/L (0.13-0.42) vs. 0.19 ng/L
257 (0.11-0.29), p=0.05; and CXCL8: 3.37 ng/L (2.18-5.31) vs. 2.90 ng/L (2.22-3.85), p=0.04. The
258 PCA approach confirmed an up-regulated blood inflammatory profile in children experiencing
259 TROLS at age 0-6mo (p=0.01). The findings were unaffected by adjustment for older children in
260 the home and infectious episodes within 14 days prior to biomarker assessment (Table 3).

261 Elevated hs-CRP showed a trend of a 1.5-fold increased risk of recurrent TROLS till age 1yr
262 (hazard ratio, 1.5; 95% CI, 0.9-2.5, p=0.10), but was not associated with recurrent TROLS after age
263 1yr or asthma at age 7yrs. Similar associations were detected with IL-6 and PC₁ (Table 3).

264 **DISCUSSION**265 *Key Findings*

266 This study shows that children with reduced pulmonary capacity as neonates are characterized by
267 elevated levels of hs-CRP and a generally up-regulated blood inflammatory response suggesting
268 presence of systemic low-grade inflammation in early childhood. These findings indicate that
269 reduced infant lung function reflects an ongoing asymptomatic airway inflammation with a
270 measurable systemic component early in life.

271 *Strengths and Limitations of the Study*

272 A major strength of the study is the unique assessment of neonatal lung function with the state-of-
273 the-art raised volume rapid thoraco-abdominal compression technique performed strictly in
274 coherence with recognized guidelines¹⁴. The infant spirometry measurements were obtained in a
275 large sample of asymptomatic children prior to presence of any respiratory symptoms and are thus
276 unbiased from preexisting or concurrent airway disease. Another significant strength of the study is
277 the availability of a range of environmental exposure assessments enabling robust confounder
278 adjustment for factors with possible influence on infant lung function and low-grade inflammation.

279 There were strong linear correlations between IL-6 and TNF- α and hs-CRP levels. As IL-6 and
280 TNF- α are main triggers of CRP release from the liver², these expected correlations serve as a
281 biological validation of the data. The lack of correlation between CXCL8 and hs-CRP levels was
282 not surprising because CXCL8 primarily has a neutrophilic chemotactic function in the innate
283 immune system and does not directly induce CRP release²⁴. The finding of significantly elevated
284 hs-CRP levels in children experiencing an infectious episode within 14 days prior to biomarker
285 assessment further assures a high signal-to-noise ratio as CRP is a reliable biomarker of ongoing
286 infection¹. Even after adjusting for this potentially strong confounder, the association between
287 infant lung function and hs-CRP persisted with largely unchanged effect estimates. Furthermore,

288 both the standard statistical approach and the unsupervised data driven approach revealed identical
289 associations enhancing our confidence in the findings of the study.

290 It is a limitation of the study that we were unable to detect a biologically meaningful signal from IL-
291 1β which is presumably partly due to the sample storage time of up to 13 years. It is well known
292 that circulating IL- 1β levels are approximately x5 lower than TNF- α in healthy adults²², but in our
293 case the median IL- 1β level was x200 lower than the median TNF- α level (0.01 vs. 2.34ng/L) and
294 we were unable to detect association between IL- 1β and hs-CRP. This was not unexpected as IL- 1β
295 is particularly sensitive to freeze-thaw cycles and degrades >50% over time, even when samples are
296 stored at -80 degree C²³.

297 Another limitation of the study is the at-risk nature of the cohort, as all children are born to mothers
298 with a history of asthma. We recently demonstrated that the offspring of mothers with a history of
299 asthma, allergy or eczema in an unselected mother-child cohort has a topical down-regulated
300 immune signature in the airway mucosa compared to children of mothers without such disorders²⁵.
301 The at-risk nature of the studied cohort may have impacted the measured biomarker levels but
302 should not hamper our ability to explore the association between infant spirometry incentives and
303 evident markers of systemic low-grade inflammation within the cohort.

304 *Meaning of the Study*

305 The strong linear inverse association between infant lung function and hs-CRP proposes that
306 neonates with diminished lung function are characterized by manifest systemic low-grade
307 inflammation very early in life. This suggests that airway inflammation accompanies reduced lung
308 function even in asymptomatic neonates and that such airway inflammation is not a local
309 phenomenon but has a measurable systemic component. To our knowledge, no other previous study
310 has investigated the relationship between infant lung function and low-grade inflammation in early
311 life.

312 Hitherto, only very few childhood studies have investigated hs-CRP level in relation to pulmonary
313 function outcomes^{9,26,27}. In line with our findings, a study of 63 asthmatic children aged 2-12 years
314 with and without acute exacerbations²⁷ and a study of 60 school-aged children treated with inhaled
315 corticosteroids as well as steroid-naïve children⁹ showed a reciprocal relationship between FEV₁
316 and hs-CRP. In contrast, another similar study of 62 school-aged children with controlled and
317 uncontrolled asthma²⁶ did not detect association between hs-CRP and FEV₁, but found that hs-CRP
318 was higher in uncontrolled vs. controlled asthma which may reflect degree of airway inflammation.
319 All these studies are significantly hampered by low numbers and wide age-ranges and solely
320 investigate children with manifest asthma. Our study extends the current knowledge by
321 demonstrating an association between hs-CRP and infant lung function measured at age 1 month in
322 asymptomatic neonates prior to onset of any respiratory symptoms.

323 In support of our findings, a number of recent larger cross-sectional analyses in adult and adolescent
324 studies have shown that increased hs-CRP is associated with respiratory impairment in both
325 population-based settings and in asthmatic and non-asthmatic strata^{10,28,29}. Longitudinal lung
326 function follow-up performed 6-9 years after baseline in these studies and in another similar study
327 showed no association between baseline hs-CRP and follow-up FEV₁²⁸⁻³⁰. In line with those
328 findings, we found no association between hs-CRP in early life and lung function at age 7 years
329 suggesting that low-grade systemic inflammation mainly reflects current airway inflammation and
330 does not predict subsequent decline in lung function. This hypothesis aligns with our finding of
331 elevated hs-CRP being associated with a recent history of asthma-like symptoms and an increased
332 risk of developing recurrent asthma-like symptoms in the first year of life but not thereafter.

333 A possible explanation of the identified association between reduced infant lung function and
334 elevated hs-CRP is that diminished forced volume is accompanied by airway inflammation with a

335 systemic component. Thus, in vitro murine and human lung cell studies have established a possible
336 role of the pro-inflammatory cytokines stimulating CRP release such as IL-6, TNF- α and IL-1 β in
337 the pathophysiology of obstructive airway inflammation^{31,32}. Persistently elevated CRP may induce
338 an increased vulnerability to changes in the early life environment through its actions as a general
339 scavenger protein with important innate immune functions in the recognition and elimination of
340 bacteria and damaged human cells via opsonization, phagocytosis, and cell-mediated cytotoxicity¹.

341 Alternatively, reduced neonatal lung function does not per se trigger systemic inflammation, but is
342 rather an independent characteristic of infants with a less efficient inflammatory regulation leading
343 to a cycle of sustained low-grade inflammation in early life. Such inefficient immune-regulation
344 might be driven by the infant's genotype interacting with the intra uterine and early-in-life
345 environment, thereby affecting the plasticity of the developing immune system. In support of the
346 latter theory, higher baseline CRP levels has been demonstrated in westernized populations where
347 obstructive airway disorders are more prevalent compared to rural societies³³.

348 *Conclusion*

349 Children of the Danish COPSAC₂₀₀₀ at-risk cohort with reduced infant lung function are
350 characterized by elevated hs-CRP level and an up-regulated blood inflammatory response
351 suggesting that reduced lung function reflects an ongoing asymptomatic airway inflammation with a
352 measurable systemic component early in life.

353 **TABLES**

354 **Table 1:** Heredity, anthropometrics, demographics, pre-, peri- and postnatal exposures, and
 355 infectious episodes prior to assessment of low-grade inflammation in relation to hs-CRP level at age
 356 6 months.

			hs-CRP (mg/L) at age 6 months	
Characteristic		N	Median (IQR)	P-value
Paternal asthma, allergy or eczema	Yes	135	1.52 (0.46-4.61)	0.54
	No	153	1.31 (0.46-4.44)	
Gender	Male	155	1.37 (0.37-4.44)	0.62
	Female	146	1.51 (0.49-4.81)	
Body mass index (BMI) at birth	7-12m/kg ²	78	1.06 (0.45-4.46)	0.56
	12-13m/kg ²	73	1.80 (0.56-4.69)	
	13-14m/kg ²	74	1.41 (0.48-4.98)	
	14-17m/kg ²	76	1.25 (0.39-3.75)	
Older children in the home at birth	Yes	114	2.20 (0.63-5.05)	0.005
	No	177	1.16 (0.41-3.40)	
Household income at birth (yearly)*	Low	77	0.83 (0.38-3.57)	0.17
	Average	144	1.31 (0.46-4.63)	
	High	70	2.27 (0.67-4.92)	
Maternal smoking during 3 rd trimester	Yes	51	1.40 (0.41-4.46)	0.33
	No	250	1.22 (0.64-5.02)	
Cesarean section	Yes	60	1.81 (0.52-5.13)	0.20
	No	205	1.31 (0.46-3-93)	
Solely breastfeeding period	0-3mo	64	2.16 (0.49-5.35)	0.43

	3-6mo	160	1.51 (0.46-4.31)	
	>6mo	40	1.02 (0.55-3.87)	
Age at start in daycare	0-9mo	89	1.80 (0.50-5.13)	0.26
	9-12mo	77	1.13 (0.36-3.40)	
	>12mo	123	1.59 (0.50-4.82)	
Cat in the home in 1 st year of life	Yes	46	1.74 (0.67-3.87)	0.42
	No	248	1.41 (0.44-4.67)	
Dog in the home in 1 st year of life	Yes	44	1.15 (0.50-3.65)	0.56
	No	249	1.52 (0.49-4.69)	
Infection 14 d before hs-CRP assessment**	Yes	95	4.29 (1.71-5.34)	<0.0001
	No	206	0.84 (0.36-2.67)	

357 *Yearly household income at birth of infant: low (<53.000 €), medium (53.000-80.000 €), high

358 (>80.000 €).

359 **Infections include any upper or lower respiratory tract infection, gastroenteritis or fever with

360 unknown cause within 14 days before the blood sampling for hs-CRP measurement.

361 **Table 2:** Association between infant lung function and inflammatory biomarkers at age 6 months: conventional and principal component
 362 analysis approach.

	Log-hs-CRP		Log-IL-6		Log-TNF- α		Log-CXCL8		PC1	
	β -coefficient (95% CI)	p	β -coefficient (95% CI)	p	β -coefficient (95% CI)	p	β -coefficient (95% CI)	p	β -coefficient (95% CI)	p
UNADJUSTED ANALYSIS										
z-FEV _{0.5}	-0.12 (-0.21 to - 0.04)	0.004	-0.10 (-0.18 to - 0.01)	0.03	-0.11 (-0.38 to 0.17)	0.44	0.02 (-0.15 to 0.19)	0.83	-0.10 (-0.19 to - 0.01)	0.02
z-FEF ₅₀	-0.06 (-0.15 to 0.02)	0.14	-0.02 (-0.11 to 0.06)	0.61	-0.09 (-0.37 to 0.18)	0.52	-0.06 (-0.22 to 0.11)	0.49	-0.06 (-0.14 to 0.03)	0.17
Log-PD ₁₅	0.04 (-0.12 to 0.21)	0.60	-0.03 (-0.21 to 0.15)	0.75	-0.02 (-0.56 to 0.52)	0.94	0.15 (-0.17 to 0.46)	0.36	0.03 (-0.14 to 0.19)	0.76
ADJUSTED ANALYSIS*										
z-FEV _{0.5}	-0.13 (-0.22 to - 0.04)	0.005	-0.09 (-0.18 to 0.00)	0.04	-0.13 (-0.43 to 0.18)	0.41	0.02 (-0.15 to 0.20)	0.79	-0.10 (-0.20 to - 0.01)	0.03
z-FEF ₅₀	-0.06 (-0.16 to 0.03)	0.18	-0.03 (-0.12 to 0.06)	0.49	-0.11 (-0.42 to 0.19)	0.46	-0.06 (-0.23 to 0.12)	0.50	-0.06 (-0.15 to 0.03)	0.19
Log-PD ₁₅	0.02 (-0.16 to 0.20)	0.83	-0.03 (-0.22 to 0.15)	0.72	-0.06 (-0.61 to 0.50)	0.84	0.15 (-0.18 to 0.48)	0.35	-0.02 (-0.19 to 0.16)	0.85

363 PC1 = Principal Component 1; FEV_{0.5} = Forced Expiratory Volume at 0.5 seconds; FEF₅₀ = Forced Expiratory Flow at 50% of the forced
 364 vital capacity; PD₁₅ = Provocative Dose of methacholine causing a 15% drop in transcutaneous oxygen saturation.

365 * Adjusted for birth BMI, maternal smoking during 3rd pregnancy trimester, older children in the home at birth and infectious episodes
 366 within 14days prior to blood sampling for inflammatory biomarkers assessment.

367 **Table 3:** Association between inflammatory biomarkers at age 6 months and asthma-related outcomes at 0-7 years: conventional and
 368 principal component analysis approach.

	Log-hs-CRP		Log-IL-6		Log-TNF- α		Log-CXCL8		PC ₁	
	Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p
	UNADJUSTED ANALYSIS									
Any TROLS, 0-6mo ¹	0.04 (0.00-0.08)	0.05	0.04 (0.00-0.09)	0.05	0.09 (-0.05-0.23)	0.18	0.09 (0.00-0.17)	0.04	0.06 (0.01-0.10)	0.01
Recurrent TROLS, 0-1yr ²	1.5 (0.9-2.5)	0.10	1.5 (0.9-2.4)	0.11	1.5 (0.4-6.6)	0.56	1.2 (0.6-2.3)	0.63	1.4 (0.9-2.0)	0.12
Recurrent TROLS, 0-yrs ²	1.0 (0.8-1.2)	0.95	1.0 (0.8-1.2)	0.79	1.0 (0.6-1.9)	0.88	0.9 (0.6-1.3)	0.43	1.0 (0.8-1.2)	0.97
Asthma, 7yrs ³	1.0 (0.8-1.3)	0.99	1.0 (0.7-1.2)	0.73	0.7 (0.3-1.6)	0.36	0.6 (0.3-1.3)	0.17	0.9 (0.7-1.2)	0.62
	ADJUSTED ANALYSIS*									

Any TROLS, 0-6mo ¹	0.05 (0.00-0.09)	0.04	0.04 (-0.01-0.08)	0.08	0.06 (-0.08-0.21)	0.40	0.07 (-0.01-0.15)	0.1 1	0.05 (0.01-0.10)	0.03
Recurrent TROLS, 0-1yr ²	1.6 (0.9-2.7)	0.09	1.4 (0.8-2.3)	0.20	1.3 (0.3-5.4)	0.76	1.1 (0.6-2.0)	0.7 9	1.3 (0.9-1.9)	0.21
Recurrent TROLS, 0-yrs ²	1.0 (0.8-1.2)	0.97	1.0 (0.8-1.1)	0.62	0.9 (0.5-1.6)	0.66	0.8 (0.5-1.2)	0.3 0	1.0 (0.8-1.2)	0.68
Asthma, 7yrs ³	1.0 (0.8-1.3)	0.97	0.9 (0.7-1.2)	0.66	0.6 (0.3-1.4)	0.26	0.6 (0.3-1.2)	0.1 5	0.9 (0.7-1.2)	0.43

369 PC₁ = Principal Component 1; TROLS = TRoublesome Lung Symptoms.

370 * Adjusted for older children in the home at birth and infectious episodes within 14days prior to blood sampling for inflammatory
371 biomarkers assessment.

372 ¹Occurence of any TROLS from birth till age 6 months: general linear model (estimate= β -coefficient).

373 ²Time to onset of recurrent TROLS: Cox regression (estimate=hazard ratio).

374 ³Asthma at age 7 years (yes/no): logistic regression (estimate=odds ratio).

375 **Table E1 Online:** Comparison of baseline characteristics between children with and without
 376 complete assessment of early-life low-grade inflammation.

Baseline characteristic	Children with biomarker assessment N=300	Children without biomarker assessment N=111	p
Paternal asthma, allergy or eczema, % (N)	47% (135)	46% (50)	0.84 ^c
Male gender, % (N)	51% (154)	44% (49)	0.20 ^c
BMI at birth, mean (SD)	12.79m/kg ² (1.34)	12.84m/kg ² (1.22)	0.63 ^t
Older children in the home at birth, % (N)	39% (114)	40% (38)	0.91 ^c
Household income at birth*, % (N)			0.12 ^c
Low	27% (77)	38% (35)	
Average	49% (143)	41% (39)	
High	24% (70)	21% (20)	
Maternal smoking during 3 rd trimester, % (N)	17% (51)	11% (12)	0.12 ^c
Cesarean section, % (N)	23% (60)	27% (25)	0.45 ^c
Solely breastfeeding length, median (IQR)	122days (90-155)	122days (74-164)	0.90 ^w
Age at start in daycare, median (IQR)	345days (240-415)	307days (216-412)	0.27 ^w
Cat in the home in 1 st year of life, % (N)	16% (46)	14% (14)	0.61 ^c

Dog in the home in 1 st year of life, % (N)	15% (44)	10% (10)	0.16 ^c
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377 *Yearly household income at birth of infant: low (<53.000 €), medium (53.000-80.000 €), high

378 (>80.000 €), ^cChi-square test, ^tt-test, ^wWilcoxon rank sum test

379 **Table E2:** Association between inflammatory biomarkers at age 6 months and lung function at age 7 years: conventional and principal
 380 component analysis approach.

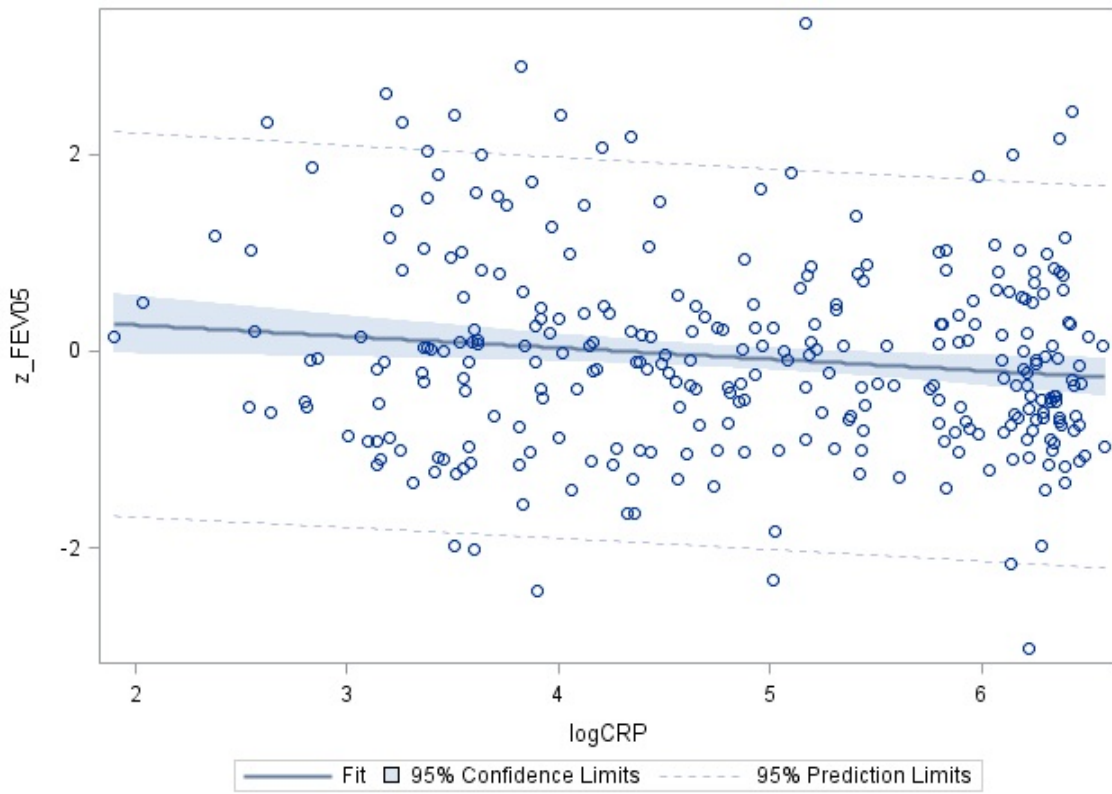
	Log-hs-CRP		Log-IL-6		Log-TNF-α		Log-CXCL8		PC1	
	β -coefficient (95% CI)	p	β -coefficient (95% CI)	p	β -coefficient (95% CI)	p	β -coefficient (95% CI)	p	β -coefficient (95% CI)	p
z-FEV ₁	0.04 (-0.06-0.15)	0.45	0.02 (-0.08-0.12)	0.68	0.20 (-0.12-0.52)	0.21	0.18 (-0.03-0.40)	0.09	0.05 (-0.05-0.15)	0.32
z-MMEF	0.01 (-0.10-0.11)	0.92	-0.01 (-0.11-0.10)	0.91	0.11 (-0.23-0.44)	0.53	0.14 (-0.09-0.36)	0.23	0.03 (-0.08-0.14)	0.57
Log-PD ₂₀	0.13 (-0.15-0.29)	0.08	0.09 (-0.06-0.25)	0.24	0.30 (-0.15-0.75)	0.19	0.02 (-0.30-0.34)	0.90	0.07 (-0.07-0.21)	0.34

381 PC1 = Principal Component 1; FEV₁ = Forced Expiratory Volume at 0.5 seconds; MMEF = Maximal Mid-Expiratory Flow; PD₂₀ =

382 Provocative Dose of methacholine causing a 20% drop in FEV₁ from baseline.

383 **FIGURES**

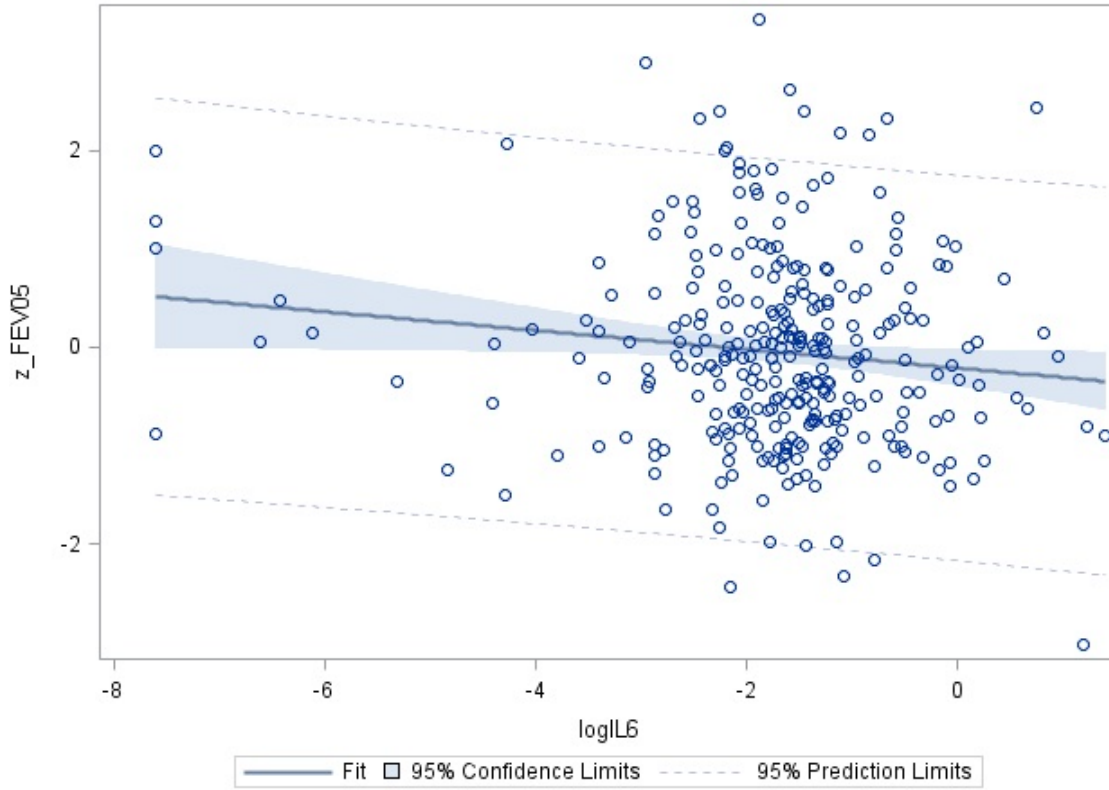
384 **Figure 1:** Scatter plot illustrating the relationship between neonatal lung function (z-score of
385 FEV_{0.5}) and hs-CRP at age 6 months (log-transformed values).



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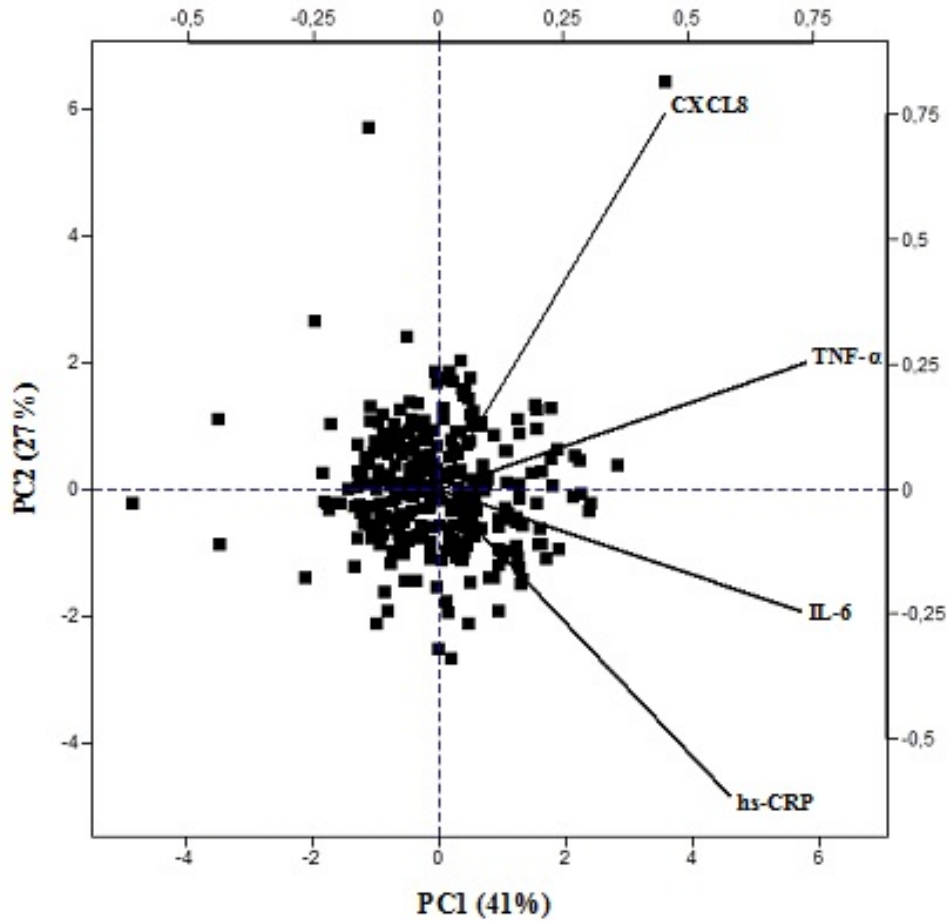
388 **Figure 2:** Scatter plot illustrating the relationship between neonatal lung function (z-score of
389 FEV_{0.5}) and IL-6 at age 6 months (log-transformed values).



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392 **Figure 3:** Principal component analysis biplot showing scores and loadings for hs-CRP, IL-6, TNF-
393 α and CXCL8 in the first principal component (PC1) and second principal component (PC2).
394 Percentages in parenthesis are the part of the total variation in the data set explained by the
395 components.



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