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

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Background: Children and adults with asthma and impaired lung function have been reported to have low-grade systemic inflammation, but it is unknown whether this inflammation starts before symptoms and in particular whether low-grade inflammation is present in asymptomatic neonates with reduced lung function.

Objective: We sought to investigate the possible association between neonatal lung function and biomarkers of systemic inflammation.

Methods: Plasma levels of high-sensitivity C-reactive protein (hs-CRP), IL-1β, IL-6, TNF-α, and CXCL8 (IL-8) were measured at age 6 months in 300 children of the Copenhagen Prospective Study on Asthma in Childhood$_{2000}$ birth cohort who had completed neonatal lung function testing at age 4 weeks. Associations between neonatal lung function indices and inflammatory biomarkers were investigated by conventional statistics and unsupervised principal component analysis.

Results: The neonatal forced expiratory volume at 0.5 seconds was inversely associated with hs-CRP (β-coefficient, −0.12; 95% CI, −0.21 to −0.04; $P < .01$) and IL-6 (β-coefficient, −0.10; 95% CI, −0.18 to −0.01; $P = .03$) levels. The multivariate principal component analysis approach, including hs-CRP, IL-6, TNF-α, and CXCL8, confirmed a uniform upregulated inflammatory profile in children with reduced forced expiratory volume at 0.5 seconds ($P = .02$). Adjusting for body mass index at birth, maternal smoking, older children in the home, neonatal bacterial airway colonization, infections 14 days before, and asthmatic symptoms, as well as virus-induced wheezing, at any time before biomarker assessment at age 6 months did not affect the associations.

Conclusion: Diminished neonatal lung function is associated with upregulated systemic inflammatory markers, such as hs-CRP. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Asthma, children, high-sensitivity C-reactive protein, proinflammatory cytokines, spirometry*

C-reactive protein (CRP) is an acute-phase reactant found in the blood in response to acute and chronic inflammatory conditions and has a broad clinical application in screening for infectious and immune-mediated diseases.[1] CRP has important innate immunity properties and is released from the liver after triggering by proinflammatory cytokines, such as IL-6, IL-1β, and TNF-α.[2]

CRP assays[3] with increased sensitivity (high-sensitivity C-reactive protein [hs-CRP]) have demonstrated low-grade inflammation in patients with disorders such as cardiovascular disease,[4] obesity,[5] and diabetes mellitus.[6] Increased hs-CRP levels have also been demonstrated during and shortly after viral respiratory tract infections[7] and in patients with symptomatic airway diseases, such as asthma[8] and chronic obstructive pulmonary disease.[9] In addition, impaired lung function in asthmatic children and adults has been associated with the presence of systemic low-grade inflammation.[10,11]

We hypothesized that impaired lung function would be associated with the systemic inflammatory process, even before development of any respiratory symptoms. Therefore we measured plasma hs-CRP, IL-1β, IL-6, TNF-α, and CXCL8 (formerly IL-8) levels at the early age of 6 months and related these to neonatal lung function assessed at age 4 weeks in the Copenhagen Prospective Study on Asthma in Childhood$_{2000}$ (COPSAC$_{2000}$) birth cohort.

METHODS

Study cohort

The study participants were 411 neonates born of mothers with a history of asthma and enrolled at 4 weeks of age in the COPSAC$_{2000}$ prospective birth cohort study.[12-14] Exclusion criteria were any respiratory symptoms or respiratory support before inclusion, gestational age of less than 36 weeks, and any congenital abnormality or systemic illness, such as severe neonatal sepsis. The children attended the COPSAC research clinic at age 4 weeks for assessment of neonatal lung function and subsequently at 6-month intervals, as previously detailed.[12-14]

Ethics

The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the local ethics committee (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754). Both parents provided oral and written informed consent before enrollment.

Inflammatory biomarkers

Blood was drawn in an EDTA tube from a cubital vein at the age of 6 months, centrifuged to separate plasma and plasma cells, and immediately stored at −80°C

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Abbreviations used
BMI: Body mass index
$COPSAC_{2000}$: Copenhagen Prospective Study on Asthma in Childhood
CRP: C-reactive protein
FEF_{50}: Forced expiratory flow at 50% of the forced vital capacity
$FEV_{0.5}$: Forced expiratory volume at 0.5 seconds
FVC: Forced vital capacity
hs-CRP: High-sensitivity C-reactive protein
IQR: Interquartile range
PC1: First principal component
PCA: Principal component analysis
TROLS: Troublesome lung symptoms

until analysis. The samples were transported on dry ice to the laboratory, where levels of the *a priori* selected biomarkers were determined by using high-sensitivity ELISAs based on electrochemiluminescence in a 4-plex setting for IL-1β, IL-6, CXCL8, and TNF-α and as a single assay for hs-CRP. Samples were read in duplicates by using the Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, Md). The limit of detection (mean signal from blanks + 3SD) was 9.54 pg/mL for hs-CRP, 0.15 pg/mL for IL-1β, 0.17 pg/mL for IL-6, 0.09 pg/mL for CXCL8, and 0.08 pg/mL for TNF-α.

Neonatal lung function

Neonatal spirometric results were measured at age 4 weeks, applying the raised-volume rapid thoracoabdominal "squeeze" jacket compression technique.[15] Repeated ventilations to predefined mouth pressures ensured expansion of the lung volume before an instant inflation of the jacket caused a full exhalation during which the flow was measured by using a pneumotachograph with an air-cushion facemask.[16,17] The software identified forced vital capacity (FVC) as the first plateau on the volume-time curve, and measurements with FVC appearing after 0.5 seconds and the forced expiratory volume at 0.5 seconds ($FEV_{0.5}$) being less than or equal to FVC were accepted. Three to 5 acceptable curves were obtained for each measurement, and the curve containing the median value of $FEV_{0.5}$ was used for analysis of $FEV_{0.5}$ and forced expiratory flow at 50% of forced vital capacity (FEF_{50}).

For neonatal bronchial responsiveness, after an initial saline inhalation, methacholine was administered in quadrupling dose steps with a dosimeter attached to a nebulizer (SPIRA 08 TSM 133; Respiratory Care Center, Hämeenlinna, Finland).[17] Bronchial responsiveness was determined by means of continuous assessment of transcutaneous oxygen saturation (TCM3; Radiometer, Copenhagen, Denmark). The provocative dose of methacholine causing a 15% decrease in transcutaneous oxygen saturation was estimated from the dose-response curves fitted with a logistic function.

Troublesome lung symptoms

Troublesome lung symptoms (TROLS) were defined as significant cough or wheeze or dyspnea severely affecting the well-being of the child and recorded by the parents in a daily diary chart as a dichotomized score (yes/no) from birth.[18-20] At acute episodes of TROLS (≥3 consecutive days with TROLS), the children were seen at the COPSAC clinic for a clinical examination, including a rhinopharyngeal aspirate for viral detection (picornaviruses, respiratory syncytial virus, coronaviruses, parainfluenza viruses, influenza viruses, human metapneumoviruses, adenoviruses, and bocavirus).[21]

Covariates

Covariates included heredity (father's history of asthma, eczema, or allergy [yes/no]); anthropometrics (birth body mass index [BMI; 7-12, 12-13, 13-14, and 14-17 m/kg^2]); demographics (sex, older children in the home at birth [yes/no], and yearly household income [low at <€53,000, medium at €53,000-€80,000, and high at >€80,000]); prenatal and antenatal exposures (maternal smoking during the third trimester of pregnancy [yes/no] and cesarean section [yes/no]); postnatal exposures (bacterial airway colonization with *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* at age 4 weeks [yes/no],[14] length of sole breast-feeding [0-3, 3-6, and >6 mo], age at start in day care [0-9, 9-12, and >12 mo], and pets in the home in the first year of life: cat [yes/no] or dog [yes/no]); any TROLS (yes/no) and any episodes of TROLS with virus detected before biomarker assessment (yes/no); and any infection 14 days before biomarker assessment (upper and lower respiratory tract infections, gastroenteritis, or fever with unknown cause [yes/no]).

Statistics

Biomarker null values were set to half of the lowest detected value for the specific biomarker, values were log-transformed, and the mean of the duplicate measurements were used for association analyses. z Scores were calculated for $FEV_{0.5}$ and FEF_{50}, and the provocative dose of methacholine causing a 15% decrease in transcutaneous oxygen saturation was log-transformed to obtain normality.

The associations between neonatal lung function indices and inflammatory biomarkers were tested by using conventional statistics with general linear models and by using unsupervised pattern recognition with principal component analysis (PCA). In the PCA analyses we extracted underlying orthogonal components that described the systematic part of the variation across the biomarkers using log-transformed and z score mediator levels.

All results are presented as raw estimates with 95% CIs and as estimates obtained from partial regression analyses, adjusting for covariates associated with levels of hs-CRP by using a cutoff P value of .10 or less. Birth BMI and maternal smoking during the third trimester were retained in the multivariable models independently of their association with hs-CRP because these are important determinants of neonatal lung function.[22] Interaction with bacterial airway colonization, any TROLS, and acute episodes of TROLS with virus detected was tested by adding cross-products to the models. A P value of .05 or less was considered significant. All analyses were done with SAS software, version 9.3 (SAS Institute, Cary, NC).

RESULTS

Inflammatory biomarker assessments

Measurements of IL-1β, IL-6, TNF-α, and CXCL8 levels were performed on 309 plasma samples collected at age 6 months, and measurements of hs-CRP levels were performed on 301 plasma samples collected at age 6 months. One sample was lost for technical reasons while performing the 4-plex assay, resulting in 300 children (73% of the original 411 cohort children) with available measurements for all 5 biomarkers. We found no significant differences in baseline characteristics between children with and without available biomarker assessments (see Table E1 in this article's Online Repository at www.jacionline.org).

Median levels were as follows: hs-CRP, 1.39 mg/L (interquartile range [IQR], 0.46-4.61 mg/L); IL-1β, 0.01 ng/L (IQR, 0.001-0.04 ng/L); IL-6, 0.20 ng/L (IQR, 0.11-0.31 ng/L); TNF-α, 2.34 ng/L (IQR, 1.92-2.88 ng/L); and CXCL8, 3.04 ng/L (IQR, 2.19-4.37 ng/L). IL-6 and TNF-α levels were strongly positively correlated with hs-CRP levels ($P < .001$ for both), whereas IL-1β and CXCL8 levels were not correlated with hs-CRP levels ($P \geq .62$). The measured values of hs-CRP, IL-6, TNF-α, and CXCL8 were within the expected range,[23] with very few null values, whereas IL-1β levels were much lower than expected,[23] with null values for 72 (23%) of 308 children. Because of this and the fact that IL-1β has been shown to significantly degrade over time, even at −80°C,[24] IL-1β was not included in further analyses.