

Association between neopterin in cord blood, urinary neopterin in early childhood and the development of atopic dermatitis, asthma and hay fever

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It is generally accepted that the increased prevalence of atopic disease is due to a disturbed balance of T-helper (Th)1/Th2-type immunity. Upon stimulation by the Th1-type cytokine interferon (IFN)- γ , human monocytes/macrophages release large amounts of neopterin. Thus, the determination of neopterin concentrations is an indirect measure of the levels of IFN- γ and allows us to monitor Th1-type immune response. We evaluated whether neopterin concentrations in the neonatal cord blood could be a valuable marker predicting atopic disease in early childhood and whether there is a difference in actually determined urinary neopterin concentrations in children with and without atopic disease. Five hundred and five children born during 1997–1999 were enrolled, with cord blood neopterin data available at birth. The International study of asthma and allergies in childhood (ISAAC) questionnaire was used to assess the prevalence of wheezy bronchitis (asthma), atopic dermatitis and allergic rhinitis. Morning urinary samples were collected and urinary neopterin concentration was measured by high-pressure liquid chromatography. By the average age of 6 yr, the prevalence of atopic disease in the last 12 months was 31%. There was no significant correlation between cord blood and urinary neopterin concentrations at age 6 yr, and between cord blood neopterin and later atopic disease. Urinary neopterin concentrations were significantly lower in children with a family history of atopic disease ($p = 0.02$). In this study, cord blood neopterin concentration was not a predictor for atopic disease in early childhood. Family history of atopic disease was associated with lower urinary neopterin levels at age 6 yr, which might mirror a Th1/Th2 imbalance.

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Atopic diseases such as asthma, hay fever and atopic dermatitis have multifactorial origins, arising from complex interactions between genes and environment, and their prevalence has increased dramatically during the last decade (1). Early recognition of high-risk children and the possibility to initiate primary prevention is therefore important, and identification of early markers of allergy, shortly after birth or during infancy, is a major challenge.

Despite environmental factors (early allergen contact and pollution exposure) a family history of atopic disease is consistently identified as a risk factor for atopic disease in children, but the predictive value is low and most atopic children have non-atopic parents (2, 3). Markers like immunoglobulin (Ig)E in cord blood, skin prick test or specific IgE have either low sensitivity or are not useful for general screening. Other early markers are

under research and cannot yet be used in daily practice (4).

It is generally accepted that the increased prevalence of atopic disease is due to the disturbed balance of T-helper (Th)1- and Th2-type immunity. During early childhood, there is a transition from the fetal Th2-type cytokine phenotype to the adult Th1-type phenotype. Factors that determine the degree of immune competence during early infancy are only partially understood. In subjects with a high risk of atopy, a deficiency in interferon- γ (IFN- γ) production early in life supports immune deviation toward an atopic Th2-type phenotype (5, 6). Upon stimulation by the Th1-type cytokine IFN- γ human monocytes/macrophages produce and release large amounts of neopterin (7). Thus, the determination of neopterin concentrations is an indirect measure of both, the levels of IFN- γ and of IFN- γ -induced macrophage activity, and allows us to monitor Th1-type immune response. In contrast, IFN- γ and consequently neopterin formation is suppressed by the Th2-type cytokines IL-4 or IL-10 *in vitro* (8), and therefore, a defect in IFN- γ production as well as suppression of IFN- γ formation may result in decreased release of neopterin in patients also.

Although the premise that neopterin may be an indicator of a Th1/Th2 imbalance seems somewhat indirect, and a number of studies have examined IFN- γ production in atopic and non-atopic individuals directly, we postulated that neopterin – a useful marker of Th1-type immunity – might be a valuable marker predicting atopic disease in early childhood. In this study, we evaluated whether neopterin concentrations in the neonatal cord blood can predict atopic disease in early childhood, and whether there is a difference in actually determined urinary neopterin concentrations in children with and without atopic disease.

Materials and methods

Study population

A total of 505 children born between 1997 and 1999 at the University Hospital of Innsbruck with cord blood neopterin data available at birth from a previous study (9) were enrolled. A questionnaire and a tube for collecting morning urinary sample were sent to their parents during the summer of 2004. The study was approved by the local ethics committee and consent was obtained from all parents.

Neopterin measurements

Cord blood samples were taken from newborns with uncomplicated pregnancies and without any signs of perinatal infection. During pregnancy, all participating mothers took part in the Austrian healthcare program called 'Mutter-Kind-Pass' and they underwent all recommended examinations at the same institution. This program includes at least five gynaecologic examinations and one internal medical investigation during pregnancy. In addition, all pregnant women are tested for antibodies against *Treponema pallidum*, *Toxoplasma gondii* and hepatitis B surface antigen. None of them had medical or obstetric complications. In keeping with the healthcare practice in Austria, the children were checked by medical investigations after birth, on day 1, on day 5 and if there were any problems, reported by the nurse. None of the participating newborns had any postnatal problems in the sense of adaptation problems, feeding difficulties, fever, irritability or other clinical signs indicative of possible perinatal infections.

For urinary neopterin measurements, the sample was collected from the first morning urine, and the parents were instructed to collect the sample only if the child was without any signs of infection for at least 1 week.

Serum neopterin concentrations were determined by a commercially available enzyme-immunoassay (BRAHMS Diagnostica, Berlin, Germany) with a detection limit of 2 nmol/L neopterin. Urinary neopterin concentrations were measured by high-pressure liquid chromatography on reversed phase (10). Neopterin concentrations were related to concomitantly measured creatinine levels and expressed as μmol neopterin per mol creatinine.

Usually, urinary neopterin concentrations compare favorably with serum levels, thus neopterin concentrations in serum or urine seem to be of equal value to monitor cellular immune activation (11). For this reason, we favored measuring urinary neopterin levels rather than serum levels at age 6 yr, which seemed too invasive for this pilot study.

Questionnaire

The ISAAC questionnaire was used to assess prevalence of wheezy bronchitis (asthma), atopic dermatitis and allergic rhinitis. Further questions about asthma medication, diagnosis by a doctor and the family history regarding atopic disease were added.

Statistical analysis

Frequencies within subgroups were compared by Fisher's exact test. Correlation between variables was assessed by the non-parametric Spearman's rank correlation test, as the distributions of observed values were generally non-Gaussian. Differences of mean neopterin values among patient groups with different clinical symptoms were tested for significance by the Welch's t-test, which does not assume equal variances. Calculations were performed with the programs Graph Pad Prism (Graph Pad Software, Inc., San Diego, CA, USA) and SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Of 505 children, data of 408 children (189 girls, 219 boys) were eligible for analysis. The mean age of the cohort was 6 yr (s.d. ± 0.8 , range 4.7–7.4). Seventy-eight parents did not want to participate, and 19 children either did not sent an urinary sample for neopterin measurement or the questionnaire was missing.

The prevalence of wheezing, sneezing or itchy rash *ever* was 45%. By the average age of 6 yr, 126 of 408 (31%) children were reported to have one or more symptoms of atopic disease in the *last 12 months*. In contrast, only 4% had doctor-diagnosed asthma, 6% doctor-diagnosed allergic rhinitis, 14% doctor-diagnosed atopic dermatitis and 20% had doctor-diagnosed wheezy bronchitis. The prevalence of atopic complaints in association with family history is shown in Table 1.

Median neopterin concentration in cord blood was 13.0 nmol/l (interquartile range: 10.6–15.5), median urinary neopterin concentration was 358 $\mu\text{mol/mol}$ creatinine (interquartile range: 288–450). There was no significant correlation between cord blood and urinary neopterin concentrations (Spearman's rank correlation: $r_s = 0.007$; $p = \text{n.s.}$; Fig. 1).

Table 1. Prevalence of reported symptoms of atopic disease in association with family history

Family history	Positive		Negative		p-value
	Yes (%)	No (%)	Yes (%)	No (%)	
Wheezing ever (n)	56 (33)	116 (77)	55 (24)	172 (76)	n.s.
Wheezing last 12 months	34 (20)	138 (80)	23 (10)	206 (90)	0.009
Sneezing ever	47 (28)	123 (72)	38 (17)	187 (83)	0.013
Sneezing last 12 months	41 (24)	130 (76)	29 (13)	196 (87)	0.005
Itchy rash ever	33 (20)	135 (80)	28 (12)	199 (88)	0.05
Itchy rash last 12 months	24 (14)	146 (86)	15 (7)	211 (93)	0.017

n.s. denotes not significant or $p > 0.05$ as calculated by Fisher's exact test.

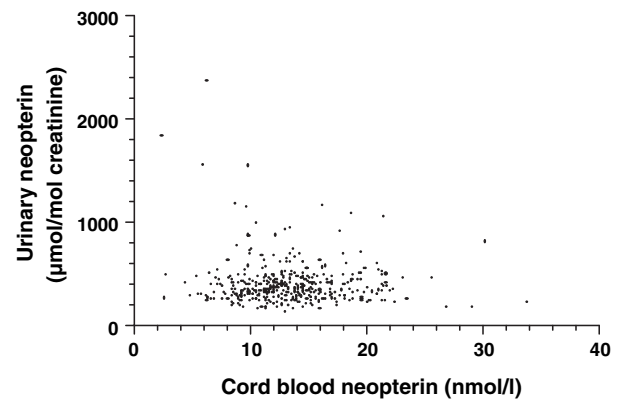


Fig. 1. Scatter plot of cord blood neopterin concentrations vs. urinary neopterin per creatinine ratios.

Cord blood neopterin concentrations and urinary neopterin per creatinine ratios were tested for differences for the variables: *with or without* – wheezing during the last 12 months ($n = 58$ vs. 348), asthma ever ($n = 15$ vs. 386), sneezing during the last 12 months ($n = 71$ vs. 330), hay fever ever ($n = 37$ vs. 355), itchy rash during the last 12 months ($n = 39$ vs. 362), atopic dermatitis ever ($n = 66$ vs. 321), doctor-diagnosed asthma ($n = 15$ vs. 385), doctor-diagnosed wheezy bronchitis ($n = 80$ vs. 312), doctor-diagnosed hay fever ($n = 26$ vs. 375), doctor-diagnosed atopic dermatitis ($n = 59$ vs. 332), use of antibiotics during the first year of life ($n = 176$ vs. 194), living with domestic animals ($n = 170$ vs. 236), living on a farm ($n = 41$ vs. 364), siblings ($n = 329$ vs. 77), older siblings ($n = 231$ vs. 175), younger siblings ($n = 142$ vs. 263), family history of allergic disease ($n = 173$ vs. 230) and nursery during the first year ($n = 20$ vs. 386).

Cord blood neopterin analysis revealed that those with siblings had lower cord blood neopterin concentrations (mean $\pm$ s.d.: 13.2 ± 4.2 nmol/l) than those without (14.5 ± 4.4 nmol/l; $t = -2.276$, $p = 0.025$). Thereby, those with older siblings had lower cord blood neopterin concentrations (13.1 ± 4.1 nmol/l) than those without older siblings (13.9 ± 4.4 nmol/l; $t = -1.762$, $p = 0.079$), but the difference was not significant.

Symptoms or atopic disease diagnosed by a doctor, in general, showed a tendency for decreased urinary neopterin concentrations, but the data did not reach significance (Fig. 2). Children with a family history of atopic disease had significant lower urinary neopterin concentrations than those without (372 ± 133 vs. 414 ± 228 $\mu\text{mol/mol}$ creatinine; $t = -2.332$, $p = 0.02$). Thereby, having a sibling with atopic disease was associated with significant lower urinary neopterin concentrations than

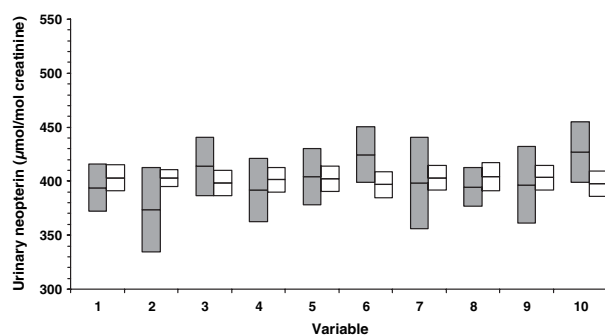


Fig. 2. Box and Whiskers plots of urinary neopterin per creatinine ratios in relationship to symptoms of atopic disease. The box extends from the mean (horizontal line) $\pm$ 1 s.e. Dark boxes indicate with, light boxes without the indicated variable. Investigated variables are indicated by numbers: 1, wheezing last 12 months; 2, asthma ever; 3, sneezing last 12 months; 4, hay fever ever; 5, itchy rash last 12 months; 6, atopic dermatitis ever; 7, doctor-diagnosed asthma; 8, doctor-diagnosed wheezy bronchitis; 9, doctor diagnosed hay fever; 10, doctor-diagnosed atopic dermatitis; p-values not significant for all comparisons.

having a non-atopic sibling (348 ± 128 vs. 416 ± 214 $\mu\text{mol/mol}$ creatinine; $t = -3.497$, $p = 0.001$). Urinary neopterin was, in particular, lower in those who had siblings with hay fever than those who had non-atopic siblings (331 ± 122 vs. 416 ± 214 $\mu\text{mol/mol}$ creatinine; $t = -3.635$, $p = 0.001$). No other variables showed a significant influence on cord blood or urinary neopterin concentrations.

Discussion

Our data regarding prevalence of atopic diseases in early childhood are in agreement with those in recent studies (2, 12–15). Approximately one-third of the cohort showed signs of atopic disease by the age of 6 yr in the past 12 months. Children with a positive family history of atopic disease were at higher risk.

In the present study, neopterin concentrations in cord blood were not found to be correlated with symptoms of atopic disease at the age of 6 yr. Therefore, neopterin in cord blood does not have a predictive value for the development of later atopic disease, which would allow us to consider preventive measures. Neopterin is produced upon stimulation with the Th1-type cytokine IFN- γ and thus the determination of neopterin allows monitoring Th1-type immune responses. Neopterin concentrations measured in urine or blood reflect the activation of cellular immunity and endogenous release of IFN- γ (16, 17). Low IFN- γ has been found in individuals with atopic disease and reduced levels of secreted IFN- γ in cord blood have been found in neonates

who developed atopic symptoms during their first year of life (18–20).

Previous data indicated that the first 6 months of life represent a critical time window for the initiation of immunological changes resulting in the development of atopy. The selective development of a Th2-type cytokine profile in high-risk children who develop atopy is possibly caused by impaired mitogen-induced IFN- γ production in the neonatal period thus producing increased amounts of Th2-type cytokines. Rinas et al. (21) found, in newborns with a familiar risk, a significant decrease in phytohemagglutinine (PHA)-induced IFN- γ production, but this was not related to cord blood IgE. They concluded that immunoregulatory abnormalities in newborns of families with a history of atopic disease are detectable already at birth and are unrelated to cord blood IgE. Tang et al. (18) found that infants who developed either symptoms of atopic disease or a positive IgE-mediated skin prick test at 12 months of age produced significantly less IFN- γ at birth and concluded that the defect in interferon secretion is a primary component of the atopic state rather than an effect of the disease process. If IFN- γ is low, as a consequence, neopterin levels would also be expected to be low as well in such individuals, but with the methods chosen we were not able to detect any differences in cord blood neopterin in children with later atopic disease.

The second finding of this study is that children with a family history of allergic disease had significant lower urinary neopterin levels than those with no family history, and this association was strongest if siblings rather than parents were affected.

A family history of atopic disease was consistently identified as a risk factor for atopic disease in children in epidemiological studies (2, 3, 22). Tariq et al. (13) followed up a birth cohort up to 4 yr of age to record the development of allergic disorders and to study the influence of genetic and environmental factors. A family history of atopic disease was the single most important risk factors for atopy in children. In agreement with our results, sibling atopic disease was a stronger predictor of clinical disease than parental atopic disease.

Symptoms or the diagnosis of atopic disease by a doctor, in general, showed a tendency for decreased urinary neopterin concentrations, but the data did not reach significance. The inability to detect significant differences despite the family history might have several reasons. First, neopterin as a surrogate marker of Th1-type immune response, might not be sensitive enough

to represent the differences between children with and without atopy. Publications regarding atopy and neopterin are rare. In healthy adults, an inverse relationship between serum neopterin and IgE was found. This result agrees well with the cross-regulatory interplay between the activation of Th1- and Th2-type immune response (23). Recent data have shed some light on the cross-regulatory impact of Th1- and Th2-type immune response on the production of neopterin, i.e. neopterin production by human peripheral blood mononuclear cells upon stimulation with the Th1-type cytokine IFN- γ could be suppressed by typical Th2-type cytokines like IL-4 or IL-10, and histamine – a mediator of allergic response mainly released from stimulated mast cells – was also found to down-regulate neopterin production in peripheral blood mononuclear cells (24, 25). Neale et al. (26) measured serum and urine concentrations of neopterin in groups of patients with acute severe as well as stable asthma and neopterin values tended to be lower in patients with asthma compared with those in healthy controls.

Secondly, classification for atopy was made exclusively by parents' reports. Symptoms might be misinterpreted or ignored, and a definitive diagnosis of atopic disease during the first years of life might be difficult and avoided for a long time. About 50% of children with wheezing grow out of their 'asthma' by the first years of their life and do not develop atopic asthma (14). A runny nose is very common in early childhood and sometimes difficult to differentiate from allergic rhinitis, therefore it frequently goes unrecognized and untreated (27). Although the children examined in this cohort are already aged around 6 yr, the reported symptoms might still include a substantial number of symptom reports not related to atopic disease. The main limitation of this study is, therefore, that objective measures of atopic status, like IgE, skin prick testing or specific IgE measurements, are not included.

In summary, in this study, neopterin in cord blood was not a predictor for atopic disease in early childhood. At the age of 6 yr, only the family history, but not symptoms of atopic disease were associated with lower urinary neopterin levels. Further investigations, taking in account objective markers of atopic disease (i.e. specific IgE and/or skin prick testing) in addition to symptoms might clarify some of those issues.

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