

Cord Blood Soluble CD14 Predicts Wheeze and Prolonged Cough in Young Children: The PATCH Study

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Key Words

Cord blood · Soluble CD14 · Wheezing infants · Birth cohort · Prolonged cough

Abstract

Background: Soluble cluster of differentiation 14 (sCD14) plays a role in the development and manifestation of atopic symptoms, although the results of previous studies have been inconclusive. The aim of this study is to evaluate the practical use of sCD14 as a predictive biomarker of allergy in young children. **Methods:** Children aged 0–1 year from a birth cohort in the Prediction of Allergies in Taiwanese Children (PATCH) study were enrolled. Cord blood sCD14 concentrations were measured. Pediatrician evaluation and questionnaire interviews were performed periodically until 1 year of age to determine the children's allergic and respiratory symptoms. **Results:** Two hundred and six 1-year-old subjects were enrolled. Wheeze was positively associated with cord blood sCD14, a family member with asthma and parental smoking. Prolonged cough was associated with

cord blood sCD14, older maternal age and more siblings. In the multivariate logistic regression analysis, cord blood sCD14 was the only independent predictive biomarker for wheeze and prolonged cough by 1 year of age. Every 100-ng/ml increase in cord blood sCD14 resulted in a 1.56-fold higher risk of developing wheeze and a 1.62-fold higher risk of prolonged cough in children by 1 year of age. **Conclusions:** Cord blood sCD14 may be a useful biomarker for predicting infant wheeze and prolonged cough by 1 year of age.

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Introduction

The prevalence of atopic diseases, including asthma, allergic rhinitis and atopic dermatitis, is increasing gradually in most industrialized countries [1]. In the past decades, several studies have investigated the risk factors for early development of atopy or allergic symptoms in young children [2–4]. Various mechanisms, such as viral infection, cytokine dysregulation, genetic variation and im-

mune dysfunction, have been identified as possible explanations for the development of allergic diseases in early life [5–7]. Recently, the interest has shifted towards identifying reliable predictors of allergic diseases in young infants [8, 9], as early identification of those at risk allows prevention and early intervention [10].

Cluster of differentiation 14 (CD14) is a glycoprotein that mediates the interaction of lipopolysaccharide (LPS) and toll-like receptor 4 (TLR4), acting as a coreceptor. When LPS binds to TLR4 through CD14, the cells produce proinflammatory cytokines such as interleukin (IL)-6, IL-8, and IL-12, and tumor necrosis factor (TNF)- α [11, 12]. CD14 can exist in either a soluble or membrane-bound form; cells that do not express membrane-bound CD14, such as the epithelial, endothelial and smooth-muscle cells, depend upon soluble CD14 (sCD14) to be activated by LPS. However, sCD14 has also been reported to have anti-inflammatory effects mediated by transferring LPS to plasma lipoproteins, which neutralize its activity [13]. Moreover, sCD14 also diverts LPS from membrane-bound CD14, and promotes LPS efflux from cells [14], suggesting that it has both pro- and anti-inflammatory effects.

The *CD14* gene is located on chromosome 5q31.1 and has been linked to childhood asthma in genetic association studies [15, 16]. Exposure to environmental factors, including pets and endotoxins, has been demonstrated to be involved in the interaction between CD14 and allergic diseases [17]. However, the available data are conflicting, as some studies showed that decreased sCD14 levels were related to atopy [18], while others found that increased sCD14 levels were related to allergic disease [19, 20]. Moreover, most studies performed to date have focused on populations of Caucasian or African descent, while studies in Asian populations are lacking.

Many different types of cord blood soluble mediators have been studied to establish their predictive value for atopic disorders. Cord blood immunoglobulin E (IgE) has received much attention over the years. However, because of its low sensitivity, it is not a reliable predictive marker of atopic diseases [21–23]. Various soluble cytokines and cytokine receptors in cord blood, such as sCD30, sCD23 and the IL-4 receptor, have also been studied, but none has been found to be a significant predictive factor for atopic disorders [24, 25]. However, the presence of sCD14 in early life may be related to further atopy [26], although its predictive value for atopic disease and the corresponding optimal cut-off point require further research.

This study is part of the Prediction of Allergies in Taiwanese Children (PATCH) study. Cord blood sCD14 is a

potential candidate for a reliable predictor of early atopy as it can be measured with simple, noninvasive procedures that are well tolerated by the infants and their parents. Thus, we aimed to examine whether cord blood sCD14 can predict atopic symptoms in children within the first year of life and also to identify a suitable cut-off level in this prospective Asian birth cohort.

Material and Methods

Study Design and Study Population

The PATCH study is an integrated-study project, which includes several birth and schoolchildren cohorts [27, 28]. The study focuses on the epidemiology and predictive factors of allergies and asthma in Asian children.

In this prospective birth cohort study, we recruited newborns from a general population rather than from a particular high-risk group. Newborns delivered at the Chang Gung Memorial Hospital in Keelung between 1 October 2007 and 30 September 2009 were invited to participate in this study. Initial recruitment was carried out at the Department of Gynecology and Obstetrics of the Chang Gung Memorial Hospital, where the investigators explained this study to pregnant women with >34 weeks of gestation. The Hospital's institutional review board approved the study and all participants' parents provided written informed consent. The study was approved by the Ethics Committee of Chang Gung Memorial Hospital.

Inclusion and Exclusion Criteria

Newborns born after 34 weeks of gestation and with a birth weight >2,500 g were eligible for enrolment in the study. Infants with perinatal insult, hypoxic ischemic encephalopathy, chronic lung diseases or congenital anomalies were excluded. Since participation was voluntary, cases of dropouts during the follow-up period were likewise excluded.

Data Collection

At baseline, all mothers underwent standardized interviews conducted by trained investigators soon after delivery. The interviews included detailed questions about family demographics, socioeconomic status, housing and living conditions (incense-burning, pets, carpets, water damage, fungi on walls and pesticide use) and family history of atopic diseases (i.e., asthma, allergic rhinitis and atopic dermatitis). Each neonate's birth data (sex, weight, height, head circumference and gestational week) were collected from the hospital records.

Evaluation of Atopic Diseases in Children

All children were followed up at 1 month, 6 months and 1 year of age, using a questionnaire that included specific questions related to the development of allergic diseases and symptoms. All of them were evaluated by a pediatric allergist at our outpatient clinic at each visit. We collected information about the children's lifestyles within the first year.

The following definitions of the various respiratory symptoms and allergic diseases were used: wheeze was defined based on a physical examination by a pediatric allergist and the response to

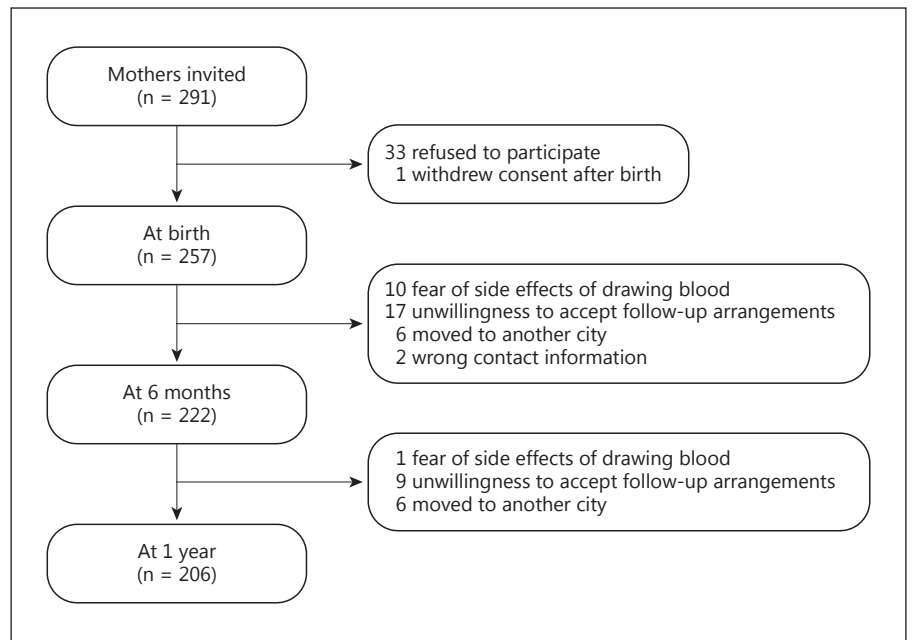


Fig. 1. Schematic presentation of the study recruitment process.

the question ‘Has your child ever had wheeze or whistling in the chest at any time since he or she was born?’ It was reported as a single or multiple episodes during the study period. Prolonged cough was defined as persistent cough lasting >3 weeks that occurred in a single or multiple episodes during the study period. Allergic rhinoconjunctivitis was diagnosed if rhinitis and/or conjunctivitis appeared at least twice, with symptoms of rhinorrhea, sneezing, nasal congestion, eye itching or tearing not associated with a viral infection [29]. Eczema was defined as a pruritic rash over the face and/or extensors with a chronic relapsing course, as described by Hanifin [30]. We used the pediatric allergist’s diagnosis and answers from questionnaires at 6 months and 1 year of age to define allergic symptoms and diseases.

The total IgE levels in the cord blood serum were measured using a commercial, ultrasensitive CAP IgE fluorescence enzyme immunoassay (ImmunoCAP®; detection limit 0.1 kU/l; Phadia, Uppsala, Sweden). Serum was collected at 6 and 12 months of age for the quantification of total IgE and specific IgE against food allergens (egg white, cow’s milk and wheat) and inhalant allergens (*Dermatophagoides pteronyssinus*, *D. farinae* and *Cladosporium herbarum*). Atopy was defined as a positive test (≥ 0.35 kU/l) to any of the allergens tested.

Measurement of sCD14 in Cord Blood

The sCD14 levels were determined in the cord blood samples using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn., USA). The detection limit of this assay is 125 pg/ml.

Statistical Analysis

SPSS v13.0 for Windows (SPSS, Chicago, Ill., USA) was used for data analysis. Differences between infants with and without specific atopic or allergic diseases were assessed by Student’s *t* test or nonparametric tests for continuous variables, and the χ^2 test or

Fisher’s exact test for categorical variables. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to examine the effects of potential risk factors. It was considered statistically significant when *p* values were <0.05.

Forward step-wise regression was applied to select the predictive factors in the multiple regression analysis. If specific atopic or allergic diseases were found to correlate with 1 of the examined biomarkers in the univariate regression analyses (*p* < 0.1), these were included in the predictive model for identifying independent factors. Variables included in the univariate analyses were parental asthma, atopy or other allergic diseases, parental smoking during pregnancy and the child’s first year of life, maternal age, the child’s demographics, breastfeeding, the presence of siblings at home, daycare center attendance and infant probiotics use as well as IgE levels in the cord blood and at 6 months old.

Receiver operating characteristic (ROC) curves were generated to assess the cord blood sCD14 predicting capability and to determine the cut-off level that would provide the highest positive predictive value. The Kaplan-Meier survival analysis and the log-rank test were used to evaluate the association between the cord blood sCD14 level and patient disease-free survival (wheeze and prolonged cough for >3 weeks).

Results

During the study period, approximately 70% of the parents of newborns were contacted at the Department of Gynecology and Obstetrics, and the initial response rate was 41%. Parents of 291 eligible newborns who fulfilled the inclusion criteria were contacted. The initial participation rate was 88.3% (257 families agreed to participate,

Table 1. Demographic characteristics and parental history of atopic disease and smoking of the study population of 206 children

Characteristics		
Gender, M:F	105:101	
Delivery by Cesarean section	79 (38.3)	
Gestational age, weeks	38±1.8	
Nationality of mothers		
Chinese	200 (97.1)	
Vietnamese	4 (1.9)	
Indonesian	2 (1.0)	
Family members, n	4.3±1.8	
Annual family income, USD		
<17,000	40 (19.4)	
17,000–34,000	119 (57.7)	
>34,000	47 (22.8)	
Parental history of atopic disease and smoking		
	Mother	Father
Asthma	12 (5.8)	8 (3.9)
Allergic rhinitis	65 (31.5)	91 (44.2)
Atopic dermatitis	11 (5.3)	14 (6.8)
Smoking	13 (6.3)	93 (45.1)
Values are expressed as n (%) or means ± SD.		

Table 2. Cord blood sCD14 level and respiratory symptoms during the first year of life

	Yes/no	Cord blood sCD14, ng/ml	p value
Wheeze	yes, n = 29 (14.1%) no, n = 177	546.7 (519.9–652.2) 493.8 (417.4–569.2)	0.001
Prolonged cough	yes, n = 15 (7.2%) no, n = 191	584.8 (512.0–671.6) 501.3 (419.8–557.3)	0.003
Allergic rhinitis	yes, n = 41 (19.9%) no, n = 165	506.1 (424.3–575.9) 521.3 (432.0–577.0)	0.678
Allergic conjunctivitis	yes, n = 42 (20.4%) no, n = 164	535.3 (433.4–589.6) 509.1 (424.0–561.4)	0.170
Eczema	yes, n = 58 (28.2%) no, n = 148	527.0 (435.6–570.9) 497.5 (420.8–581.0)	0.332
Atopy	yes, n = 86 (41.7%) no, n = 120	503.0 (429.8–573.5) 523.1 (435.2–563.7)	0.658

The cord blood sCD14 values are expressed as median (interquartile range).

including 130 boys and 127 girls) and 80.2% (206/257) of the children included in the baseline examination were evaluated at the 1-year follow-up. The recruitment process is summarized in figure 1.

The major characteristics of the study population are summarized in table 1. As recorded by the questionnaire, parents reported a history of wheezy breathing, prolonged cough, allergic rhinitis, allergic conjunctivitis and eczema during the first year of life in 29 (14.1%), 15 (7.2%), 41 (19.9%), 42 (20.4%) and 58 (28.2%) children, respectively. Of the 206 children, 86 (41.7%) had a positive specific-IgE value at 1 year of age.

The median values and interquartile ranges for cord blood sCD14 levels in children with or without specific allergic symptoms or atopy are shown in table 2. Children with wheeze or prolonged cough at 1 year of age had significantly higher levels of cord blood sCD14 than those without these symptoms ($p = 0.001$ and 0.003 , respectively). The cord blood sCD14 levels did not correlate with the serum IgE levels, socioeconomic status, parental atopy, paternal education levels or housing conditions.

For 1-year-old children with wheeze and prolonged cough, all the above-mentioned variables were analyzed (table 3). In the univariate analyses, the cord blood sCD14 level, family members with asthma and parental smoking were identified as significant risk factors for developing wheeze ($p = 0.002$, 0.02 and 0.045 , respectively). Moreover, the cord blood sCD14 level, older maternal age and a greater number of elder siblings were associated with higher rates of prolonged cough ($p = 0.001$, 0.016 and 0.041 , respectively). Conversely, exposure to pets in the home was not associated with infant wheeze, prolonged cough, eczema, allergic rhinoconjunctivitis, the total serum IgE levels or atopy. IgE levels in the cord blood and at the age of 6 months were not associated with infant wheeze or prolonged cough.

After adjusting for covariates in the multivariate model, the cord blood sCD14 level was the only significant predictor of wheeze and prolonged cough. Every 100-ng/ml increase in the cord blood sCD14 level resulted in a 1.56-fold higher risk of developing wheeze ($p = 0.001$) and a 1.62-fold higher risk of having prolonged cough by 1 year of age ($p = 0.003$).

For additional delineation of the role of sCD14 in predicting wheeze and prolonged cough, we generated ROC curves. For wheeze at 1 year of age, the area under the ROC curve (AUC) was 0.700. The highest positive predictive value occurred at a cut-off point of 509.0 ng/ml with sensitivity of 86.2% and specificity of 52.8% (fig. 2a). For prolonged cough, the AUC was 0.733. The highest positive predictive value occurred at a cut-off point of 570.9 ng/ml with sensitivity of 66.7% and specificity of 77.0% (fig. 2b).

Table 3. Predictors for allergic symptoms at 1 year of age by univariate and multivariate logistic regression analysis

Variable	Univariate analysis, OR (95% CI)	p value	Multivariate analysis, OR (95% CI)	p value
<i>Predictors of wheeze</i>				
Cord blood sCD14 level, ng/ml (every 100-increment)	1.459 (1.149–1.853)	0.002	1.556 (1.205–2.009)	0.001
Parent or sibling asthma	3.01 (1.184–7.603)	0.020	1.825 (0.582–5.723)	0.302
Paternal smoking amount (packs/day)	1.75 (1.01–3.033)	0.045	1.872 (0.993–3.528)	0.053
<i>Predictors of prolonged cough</i>				
Cord blood sCD14 level, ng/ml (every 100-increment)	1.602 (1.201–2.139)	0.001	1.617 (1.180–2.215)	0.003
Mother's age (years)	1.167 (1.029–1.324)	0.016	1.121 (0.967–1.300)	0.130
Number of elder siblings	1.919 (1.026–3.59)	0.041	1.847 (0.908–3.757)	0.090

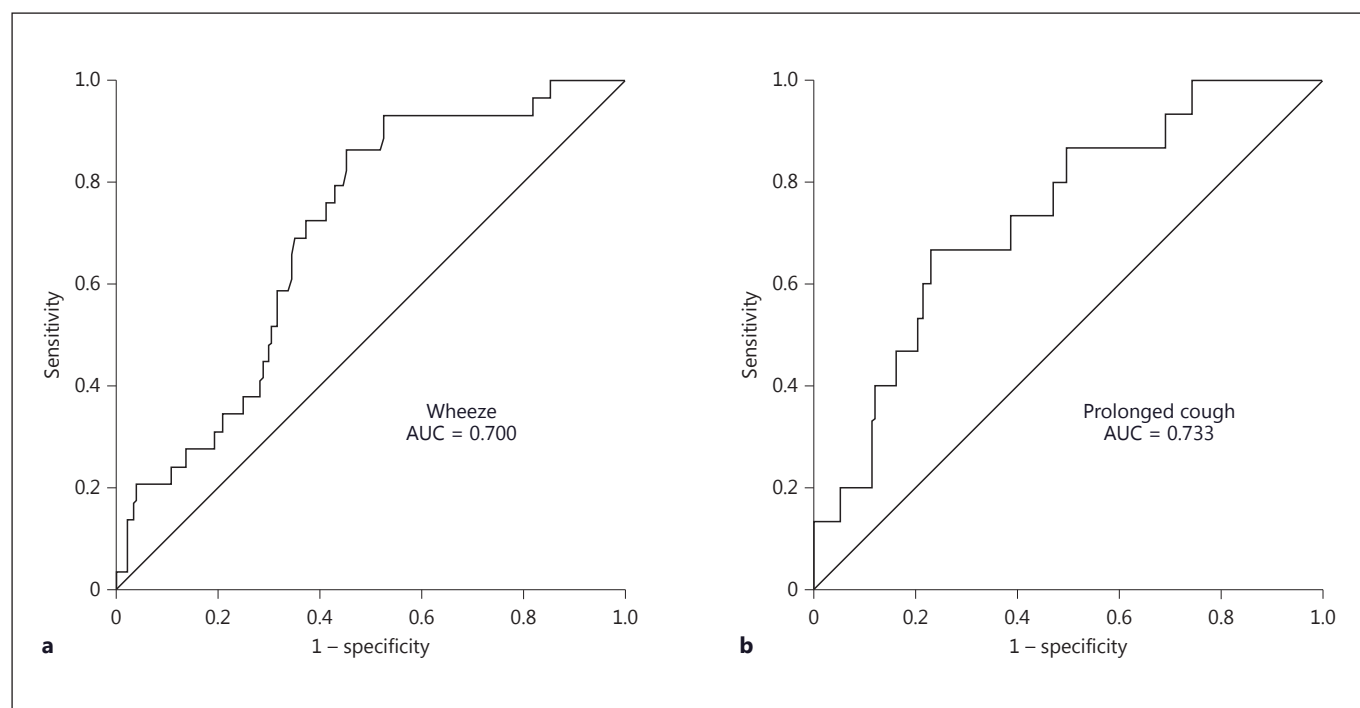


Fig. 2. The ROC curve was used to determine the discriminating capability of cord blood sCD14 for predicting wheeze (**a**; AUC = 0.700) and prolonged cough (**b**; AUC = 0.733) by 1 year of age.

Using the cut-off points revealed by ROC analysis, the Kaplan-Meier survival analysis showed that patients with lower levels of cord blood sCD14 had significantly longer disease-free survival, with respect to both wheeze and prolonged cough (both $p < 0.0001$; fig. 3).

Discussion

Although it was not preregistered on the public web-site, the measurement of sCD14 was included in the central hypothesis, trying to find predictive factors of asthma and allergies, especially in Asian children. Other predic-

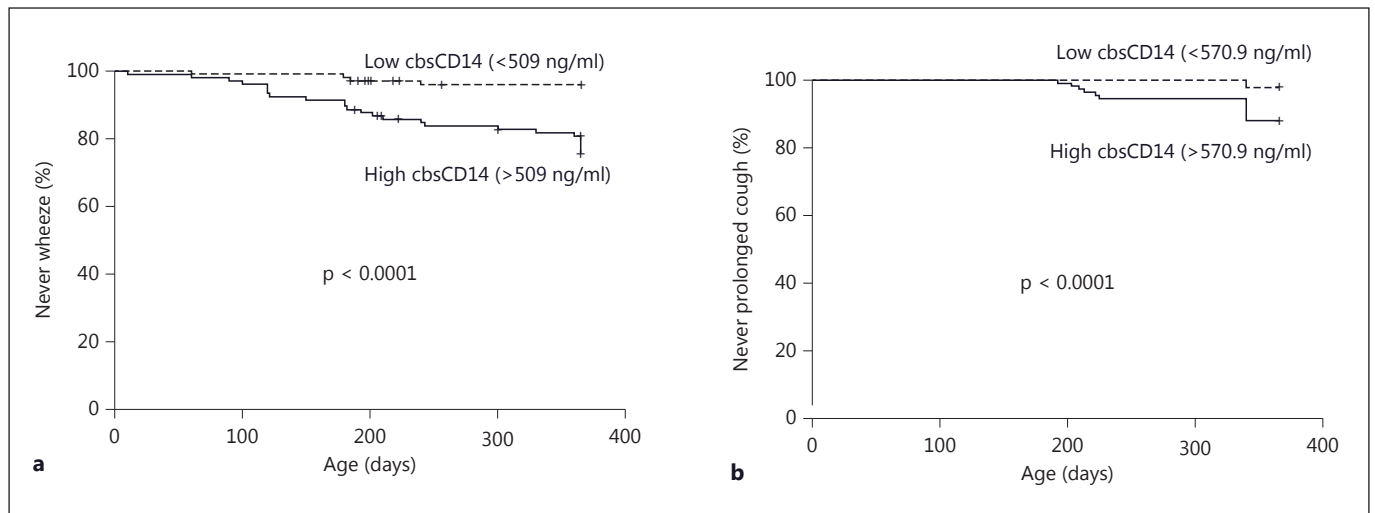


Fig. 3. Kaplan-Meier survival curves showing the proportions of children who remained symptom free during the first year of life in the low and high cord blood (cb)sCD14 groups. **a** Wheeze-free survival, $p < 0.0001$. **b** Prolonged cough-free survival, $p < 0.0001$.

tive factors in the central hypothesis were serum IgE, urinary leukotriene E4 and eosinophil protein X. During the first year of life, a higher cord blood sCD14 level was independently associated with infant wheeze and prolonged cough.

It is difficult to diagnose asthma before 1 year of age, although wheeze and prolonged cough are frequently its initial presentations. In a previous study, wheeze in the first year of life was associated with an increased risk of asthma at 6 years of age in both nonatopic and atopic children, especially if there had been frequent episodes of wheeze [31]. Hence, the cord blood sCD14 level may be a predictor of subsequent asthma. We will follow up these participants to assess the relationship between the cord blood sCD14 levels and the development of asthma in the future.

The sCD14 level has been previously shown to be elevated in patients with allergic diseases, including in children with an acute exacerbation of asthma [20]. In other studies, the sCD14 levels were found to be increased 18 h after segmental allergen provocation in asthmatic patients [32], while in atopic dermatitis patients, the sCD14 levels were increased at the acute stage during admission and were found to correlate with the skin intensity score [19].

CD14 gene polymorphisms have also been linked to atopic disease, although this association shows ethnic differences. In Caucasian children, the CC genome at position -159 of the *CD14* promoter was shown to be associ-

ated with a higher mean number of positive skin tests to aeroallergens [33], whereas in Hutterites, the T allele instead of the C allele in *CD14*-159 was found to be associated with atopy [34]. These inconsistencies may be explained by a gene-environment interaction, as, for example, different degrees of environmental endotoxins or pet exposure interacting with gene expression have been demonstrated to result in different atopic manifestations [17, 35]. In this study, sCD14 was found to be an independent predictor for wheeze or prolonged cough by 1 year of age, but pet exposure was not associated with infant wheeze, prolonged cough or other atopic manifestations, indicating that the gene-environment interaction is more complicated than we originally thought.

In 1999, Baldini et al. [33] reported that *CD14*-159 CC homozygous children had significantly higher serum IgE levels and lower sCD14 levels than those with the TT or TC genotype. This relationship between *CD14*-159 gene polymorphisms and serum IgE was also confirmed in a British population [36]. However, this relationship did not exist in studies performed on populations of Japanese, German or African descent [36–38]. Similarly, in our study, the cord blood sCD14 levels did not correlate with the serum plasma IgE levels; however, we did not analyze the *CD14* gene polymorphisms.

To our knowledge, to date, only one single Asian study, performed in Hong Kong, has analyzed the relationship between *CD14* gene polymorphisms and atopy [39]. This study found that atopic subjects with the CC genotype in

CD14/-159 had higher total serum IgE levels. However, it should be noted that all subjects with the CC genotype had lower sCD14 levels, although this polymorphism was not associated with asthma or aeroallergens. The above data differed from those of similar studies on different ethnic groups, indicating that Asian populations show unique characteristics in their gene expression and atopic presentation.

The influence of the maternal immunity has been reported to have long-term effects on the immunity of the offspring. Maternal atopy is an important risk factor for the development of allergic diseases in children [40]. The cord blood provides a unique opportunity to investigate the immunity of newborns prior to disease onset as well as representing a bond between mother and child. Only in atopic mothers have sCD14 levels at the time of delivery been found to be related to the cord blood sCD14 of their children [41]. In our birth cohort, one limitation was that maternal sCD14 levels were not checked at the time of delivery. However, cord blood sCD14 levels were not correlated with parental atopy or IgE levels after analysis. The relationship of sCD14 levels between mothers and their children requires further investigation.

The predictive role of cord blood IgE for childhood atopy and allergic disease has been discussed for a long time. However, the sensitivity of cord blood IgE was quite low at 8.5–33% [21–23]. A family history of atopic disease or maternal total IgE levels was more sensitive than the level of cord blood IgE to detect children at risk of atopy [22, 40]. In addition, gene-gene and gene-environment interactions interfered with this level [42]. At the different ages of the children, the genes that determined the IgE production had also changed over time [43]. Our study also demonstrated that IgE levels in cord blood and at 6 months were not associated with chronic cough or wheeze by 1 year of age.

Traditionally, male gender has been thought to be a risk factor for allergic diseases, especially in school-aged children [44, 45]. However, surveys rarely include preschool children. In our study, male sex was not a risk factor for the development of wheeze and prolonged cough by 1 year of age. In the preschool- and school-aged children cohort of the PATCH study, male predominance was only noted at 6–15 years of age and a slightly female predominance at 4–5 years [46]. Another study, by Dodge and Burrows [47], demonstrated that, at the age of 0–4 years, asthma prevalence was equal in both sexes. Therefore, maybe in the very young children, male predominance was not as obvious as in the schoolchildren. Ethnic difference also needs to be considered.

This study had a number of limitations. Central working hypotheses are important for a prospective cohort study. One of the limitations of this prospective birth cohort study is that we do not have a public documentation to show that sCD14 is preregistered as a central hypothesis. For a better study design, such central hypotheses should be published before the study is initiated, by using a website or a protocol paper. The AUC was between 0.7 and 0.8, which means that sCD14 only had acceptable discrimination for infant wheeze and prolonged cough. The sensitivity and specificity were not very good. Our outcomes, i.e. infant wheeze and prolonged cough, were mainly based on questionnaires, but not confirmed each time by pediatric allergists. The difficulty of drawing blood from children of different ages resulted in the study cohort being rather small, thus preventing subdivisions of the parental risk groups and analysis of the many potential confounding and effect-modifying variables. Therefore, our findings will need to be further validated in larger cohorts. Given the natural course of allergic diseases and symptoms, which may appear gradually after 1 year of age [48], some subjects without allergy or atopy may experience prolonged cough or wheeze that is later diagnosed as asthma. Thus, our study provided only preliminary data regarding the clinical application of cord blood sCD14 as a predictor in Asian children. Further, long-term, longitudinal studies are warranted to delineate the relationship between sCD14 and allergies, especially when facing ethnic genetic variations and environmental differences.

In summary, the cord blood sCD14 level may be a predictor of infant wheeze and prolonged cough by 1 year of age. Further follow-up and large-scale studies on different ethnic groups are needed to confirm the usefulness of this simple, noninvasive marker.

Acknowledgements

This study was sponsored by a research grant (CMRPG-G3E1191) from the Chang Gung Memorial Hospital.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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