

Correlations of serum 25-hydroxy-vitamin D, CD4⁺CD25⁺CD127⁻ regulatory T cells and total immunoglobulin E in children with recurrent respiratory tract infections.

Yanfei Yang, Na Fan, Xingshu Liu, Lin Wang, Zhihai Wang, Yao Hou, Ting Wang and Yuqin Wu

Ward of Special Needs, Kunming Children's Hospital. Yunnan Province, China.

Keywords: cluster of differentiation; correlation; 25-hydroxy-vitamin D; immunoglobulin; recurrent respiratory tract infection; regulatory T cell.

Abstract. Recurrent respiratory tract infections (RRTIs) have a complex pathogenic mechanism. There may be correlations between expression levels of serum 25-hydroxy-vitamin D (25-OH-D), regulatory T cells (Tregs), total immunoglobulin E (IgE) and RRTIs. We aimed to explore the correlations of serum 25-OH-D, cluster of differentiation 4 (CD4)⁺CD25⁺CD127⁻ Tregs and total IgE in children with RRTIs. A total of 150 children with pneumonia were enrolled as the subjects and randomly assigned into a control group (97 children with non-RRTIs) and an observation group (53 children with RRTIs). The expression levels of serum 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE were compared in RRTI children with different genders, stages, sites of lesions and severities of illness. The correlation was tested by Pearson analysis. The expression level of serum 25-OH-D was significantly higher in children with RRTIs in the upper respiratory tract than that in the lower respiratory tract, whereas the expression level of total IgE was significantly lower in children with RRTIs in the upper respiratory tract ($p < 0.05$). The expression level of 25-OH-D was negatively correlated with expression levels of CD4⁺CD25⁺CD127⁻ Tregs and total IgE ($r = -0.355$, -0.314 , $p < 0.05$), while a positive correlation was found between expression levels of CD4⁺CD25⁺CD127⁻ Treg and total IgE ($r = 0.237$, $p < 0.05$). The area under the curve of combination of 25-OH-D, CD4⁺CD25⁺CD127⁻ Treg and total IgE expression levels for predicting RRTIs was 0.852. The combined detection of the three indicators is conducive to enhancing the detection rate of RRTIs.

Las correlaciones séricas de 25-hidroxivitamina D, CD4⁺CD25⁺CD127⁻ linfocitos T reguladores e inmunoglobulina E total en niños con infecciones recurrentes del tracto respiratorio.

Invest Clin 2024; 65 (4): 426 – 435

Palabras clave: cluster of differentiation; correlacion; 25-hidroxi-vitamina D; inmunoglobulina; infeccion recurrente del tracto respiratorio, T cell reguladora.

Resumen. Las infecciones recurrentes del tracto respiratorio (ITRR) tienen un mecanismo patogénico complejo. Puede haber correlaciones entre los niveles de expresión de 25-hidroxivitamina D (25-OH-D) sérica, células T reguladoras (Treg), inmunoglobulina E total (IgE) y RRTI. Nuestro objetivo fue explorar las correlaciones de la 25-OH-D sérica, el grupo de diferenciación 4 (CD4)⁺CD25⁺CD127⁻ Tregs y la IgE total en niños con IRRR. Se inscribió un total de 150 niños con neumonía como sujetos y se los asignó aleatoriamente a un grupo de control (97 niños sin RRTI) y un grupo de observación (53 niños con RRTI). Se compararon los niveles de expresión de 25-OH-D, (CD4)⁺CD25⁺CD127⁻ Tregs en suero y de IgE total en niños con RRTI con diferentes géneros, estadios, sitios de lesiones y gravedad de la enfermedad. La correlación fue probada mediante análisis de Pearson. El nivel de expresión de 25-OH-D sérico fue significativamente mayor en niños con IRRR en el tracto respiratorio superior que en el tracto respiratorio inferior, mientras que el nivel de expresión de IgE total fue significativamente menor en niños con IRRR en el tracto respiratorio superior ($p < 0,05$). El nivel de expresión de 25-OH-D se correlacionó negativamente con los niveles de expresión de (CD4)⁺CD25⁺CD127⁻ Tregs y la IgE total ($r = -0,355$, $-0,314$, $p < 0,05$), mientras que se encontró una correlación positiva entre los niveles de expresión de (CD4)⁺CD25⁺CD127⁻ Treg e IgE total ($r = 0,237$, $p < 0,05$). El área bajo la curva de combinación de los niveles de expresión de 25-OH-D, (CD4)⁺CD25⁺CD127⁻ Treg y de IgE total para predecir IRRR fue de 0,852. La detección combinada de los tres indicadores conduce a mejorar la tasa de detección de IRRR.

Received: 09-04-2024

Accepted: 25-08-2024

INTRODUCTION

Recurrent respiratory tract infections (RRTIs) possess a relatively complex pathogenic mechanism, which involves various pathogenic factors. Children are susceptible to RRTIs, with a major age of onset of 2-6 years old, and in terms of the prevalence rate, children with RRTIs account for ap-

proximately 20.00% of children with respiratory infections ^{1,2}. The incidence rate of RRTIs is about 16.80-18.70% in children in China, and 80.00% of children with RRTIs are aged below 5 years old ³. 25-hydroxy-vitamin D (25-OH-D) is mainly metabolized by vitamin D-25-hydroxylase. Studies have manifested that 25-OH-D is implicated in the development and progression of RRTIs,

exerting a profound impact on the body immunity of developing children^{4,5}. Regulatory T cells (Tregs), a common subset of T cells, control the autoimmune response of the body. Animal experiments have revealed that the content of Tregs [cluster of differentiation 4 (CD4)⁺CD25⁺ Forkhead box P3 (Foxp3) Tregs] is higher in RRTI mice than that in normal mice, but it declines significantly after drug intervention^{6,7}. Generally, immunoglobulin E (IgE) is mainly produced in plasma cells of the respiratory and digestive tracts, which has an extremely low expression level in blood of normal people (about 0.002% of total Ig in serum), and its abnormal expression is related to the development and progression of some diseases, including RRTIs.⁸ Based on the above analysis, it can be concluded that there are certain correlations between expression levels of serum 25-OH-D, Tregs and total IgE and RRTIs. Kunming is situated in the Yunnan-Guizhou Plateau, China, where the incidence of RRTIs may be of specific characteristics. In addition, research on the relationship between RRTIs and expressions of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE is lacking (or rare) at present, so it is necessary to carry out such research. In this study, therefore, the expression levels and relationships of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE were explored with 150 children with pneumonia (including 53 children with RRTIs) as the subjects, aiming to provide a basis for clinical diagnosis and treatment of children with RRTIs in the plateau section.

PATIENTS AND METHODS

General data

According to the guidelines of the Declaration of Helsinki, the study was performed with approvals from the medical ethics committee of our hospital. One hundred and fifty children with pneumonia admitted to and treated in Kunming Children's Hospital, China from January 2020 to January 2023

were selected as the subjects. The children were assigned into a control group (97 children with non-RRTIs) and an observation group (53 children with RRTIs) using a random number table.

The inclusion criteria for the observation group were set as follows: 1) children aged 0-12 years old, without gender requirement, 2) those who were diagnosed with pneumonia and met the diagnostic criteria for RRTIs, 3) those who lived in the jurisdiction of Kunming, China and 4) those whose family members and caregivers had signed the informed consent and who were willing to participate in this study. The inclusion criteria for the control group were as follows: 1) children aged 0-12 years old, without gender requirement, 2) those who were diagnosed with pneumonia and did not meet the diagnostic criteria for RRTIs, 3) those who lived in the jurisdiction of Kunming, China, and 4) those whose family members and caregivers had signed the informed consent and who were willing to participate in this study.

The exclusion criteria involved: 1) children with all kinds of congenital diseases, cancers (liver, lung, brain, pancreas, thyroid, *etc.*) or severe deformities, 2) those with various hematological, immunological or motor disorders, 3) those with airway obstruction, external tracheal compression, asthma, bronchiectasis or foreign bodies in the bronchus, 4) those with abnormal diet (including those with various nutritional diseases), 5) those who had taken immunosuppressants within 3 months before enrollment, 6) those with hereditary or metabolic diseases, 7) those with anemia or obesity ($BMI > P_{95}$), or 8) those whose family members and caregivers were not informed or had not signed the informed consent or who had incomplete clinical data.

The control group was composed of 97 patients, including 61 (62.89%) boys and 36 (37.11%) girls, aged 0-12 (4.12 ± 1.25) years old. As to severity of illness, there were 51 mild cases, 24 moderate cases and 22 se-

vere cases. The observation group consisted of 53 patients, including 33 (62.26%) boys and 20 (37.74%) girls, with an age of 0-12 (4.23 ± 1.27) years old. In terms of severity of illness, there were 27 mild cases, 16 moderate cases and 10 severe cases, respectively. As to the site of lesions, there were 34 and 19 cases of upper and lower respiratory tract infections, respectively. The general data such as age, gender and severity of illness were of no significant differences between the two groups ($p > 0.05$), which were comparable.

Detection methods

After admission, the demographic data (age, gender, etc.) of patients were collected in both groups. The next morning, an appropriate amount (5 mL) of venous blood was collected from the elbow of each child in the company of their family members and caregivers. Then, the blood was centrifuged with a centrifuge (Beckman Coulter, USA, Optima MAX -TL) under the following conditions: centrifugal radius of 15 cm, centrifugal rate of 2000 rpm and centrifugal time of 5 min. Afterwards, the serum was harvested for measurement of 25-OH-D, CD4⁺CD25⁺CD127⁻ Treg and total IgE expression levels. The expression level of 25-OH-D was detected by magnetic microparticle chemiluminescence assay, while the expression levels of CD4⁺CD25⁺CD127⁻ Tregs and total IgE were measured by a flow cytometer and an automatic protein analyzer, respectively. The measurement was conducted strictly according to the specific standard operating procedures for detecting 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE⁹.

The expressions of serum 25-OH-D, Tregs and total IgE in observation and control groups were measured and compared. Additionally, the expressions of serum 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in the observation group at different stages (remission stage and attack stage), genders (boy and girl), sites of lesions (upper and lower respiratory tracts) and severi-

ties of illness (mild, moderate and severe) were measured and compared.

Statistical analysis

An Excel database was created, in which the baseline data and research data of all subjects were classified, numbered and counted. Next, such data were included in SPSS 23.0 software for processing. Count data were subjected to the χ^2 test and expressed as rate or percentage (%). Measurement data were expressed by mean \pm standard deviation and subjected to the t test, and analysis of variance was employed for pairwise comparison of means of three samples. The correlation was tested by Pearson analysis. Receiver operating characteristic (ROC) curves were plotted, based on which the areas under ROC curves (AUCs) were calculated to analyze the predictive values of expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE for the incidence of RRTIs. The value of AUC was between 0 and 1, where 1 represented a perfect classification and 0.5 represented random guessing. The best classification threshold was selected based on the ROC curve. At the selected optimal cut-off value, the corresponding sensitivity (true positive rate) and specificity (true negative rate) were calculated. At the selected cutoff value, the false positive rate (1-specificity) and false negative rate (1-sensitivity) were calculated. The significant level was set as $\alpha = 0.05$ (bilateral), and $p < 0.05$ denoted that the difference was statistically significant.

RESULTS

Expression levels of serum 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in the two groups

The expression level of serum 25-OH-D in the observation group was significantly lower than that in the control group, whereas the expression levels of CD4⁺CD25⁺CD127⁻ Tregs and total IgE were significantly higher than those in the control group ($p < 0.05$) (Table 1).

Expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in children with RRTIs at different stages in the observation group

Children with RRTIs at remission stage in the observation group had a significantly lower expression level of serum 25-OH-D and significantly higher expression levels of CD4⁺CD25⁺CD127⁻ Tregs and total IgE than those of the control group ($p<0.05$) (Table 2).

Expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in RRTI boys and girls in the observation group

In comparison with RRTI girls, RRTI boys exhibited comparable expression levels of serum 25-OH-D and CD4⁺CD25⁺CD127⁻ Tregs ($p>0.05$), but a slightly elevated expression level of total IgE ($p<0.05$) (Table 3).

Expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in children with different severities of RRTIs in the observation group

A lower expression level of serum 25-OH-D and higher expression levels of CD4⁺CD25⁺CD127⁻ Tregs and total IgE were detected in children with severe RRTIs compared to those in children with mild and moderate RRTIs ($p<0.05$) (Table 4).

Expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in children with RRTIs in the upper and lower respiratory tracts in the observation group

The expression level of serum 25-OH-D in children with RRTIs in the upper respiratory tract was significantly higher than that in the lower respiratory tract, and the ex-

Table 1
Expression levels of serum 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in the two groups

Group	<i>n</i>	25-OH-D (ng/mL)	CD4+CD25+CD127-Treg (%)	Total IgE (U/mL)
Control	97	25.34±3.68	4.87±1.32	81.62±10.28
Observation	53	15.58±2.27	7.15±1.84	233.17±18.62
<i>t</i>		17.554	8.864	64.305
<i>p</i>		<0.05	<0.05	<0.05

Table 2
Expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in children with RRTIs at different stages in the observation group.

Stage	<i>n</i>	25-OH-D (ng/mL)	CD4+CD25+CD127-Treg (%)	Total IgE (U/mL)
Remission	53	22.31±2.25	4.48±1.34	98.67±12.33
Attack	53	15.64±2.17	6.81±1.55	233.61±19.55
<i>t</i>		15.534	8.279	42.502
<i>p</i>		<0.05	<0.05	<0.05

Table 3
Expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE in RRTI boys and girls in the observation group.

Gender	<i>n</i>	25-OH-D (ng/mL)	CD4+CD25+CD127-Treg (%)	Total IgE (U/mL)
Girl	20	15.57±2.26	6.25±1.37	232.51±19.82
Boy	33	15.82±2.23	6.72±1.43	234.57±20.59
<i>t</i>		0.573	1.728	2.045
<i>p</i>		0.568	0.087	0.043

Table 4
Expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁺ Tregs and total IgE in children with different severities of RRTIs in the observation group.

Severity	<i>n</i>	25-OH-D (ng/mL)	CD4 ⁺ CD25 ⁺ CD127 ⁺ Treg (%)	Total IgE (U/mL)
Mild	27	15.57±2.26	4.15±1.02	201.33±16.55
Moderate	16	15.82±2.23	5.84±1.23a**	239.75±20.58a**
Severe	10	13.08±1.85b**c*	6.89±1.52b**c	292.61±23.57b**c**
<i>F</i>		5.75	22.99	84.11
<i>p</i>		0.057	0.0000	0.0000

q test (Newman-Keuls method) for pairwise comparison of sample means (3), a: the first vs. the second, b: the first vs. the third, c: the second vs. the third. **p*<0.05, ***p*<0.01.

Table 5
Expression levels of 25-OH-D, CD4⁺CD25⁺CD127⁺ Tregs and total IgE in children with RRTIs in the upper and lower respiratory tracts in the observation group.

Site	<i>n</i>	25-OH-D (ng/mL)	CD4 ⁺ CD25 ⁺ CD127 ⁺ Treg (%)	Total IgE (U/mL)
Upper respiratory tract	34	16.23±2.58	6.12±1.25	226.87±17.88
Lower respiratory tract	19	13.34±2.27	6.58±1.33	250.64±23.58
<i>t</i>		6.122	1.835	5.848
<i>p</i>		<0.05	0.069	<0.05

pression level of total IgE was significantly lower than that in the lower respiratory tract (*p*<0.05) (Table 5).

Results of correlation analysis

The results of Pearson correlation analysis revealed negative correlations of the expression level of serum 25-OH-D with the expression levels of CD4⁺CD25⁺CD127⁺ Tregs and total IgE (*r*=-0.355, -0.314, *p*<0.05) and a positive correlation between expression levels of CD4⁺CD25⁺CD127⁺ Tregs and total IgE (*r*=0.233, *p*<0.05) (Fig. 1).

Analysis results of predictive value

The AUC value of combination of 25-OH-D, CD4⁺CD25⁺CD127⁺ Tregs and total IgE for predicting the incidence of RRTIs was 0.852, which was significantly larger than that of any of the three indicators alone (0.752, 0.654 and 0.643, respectively; *p*<0.05) (Fig. 2).

DISCUSSION

The expression level of 25-OH-D in children with RRTIs (0.5 months to 14 years old) is lower than that in healthy children (control group), and the insufficiency or deficiency of vitamin D is related to the occurrence of RRTIs ⁴. The lack of serum 25-(OH)-D3 in children with RRTIs is an important reason for the decreased expression level of plasma LL-37 (one of the Cathelicidin antimicrobial peptides) ¹⁰. This suggests that RRTI children have obvious vitamin D deficiency or deficit. In this study, the expression level of 25-OH-D in children with RRTIs was abnormal, which was significantly decreased compared with that in the control group (children with non-RRTIs). Such a decrease in the expression level of serum 25-OH-D may be ascribed to the following factors. Firstly, the immunomodulatory function of vitamin D is related to the expression of vitamin D

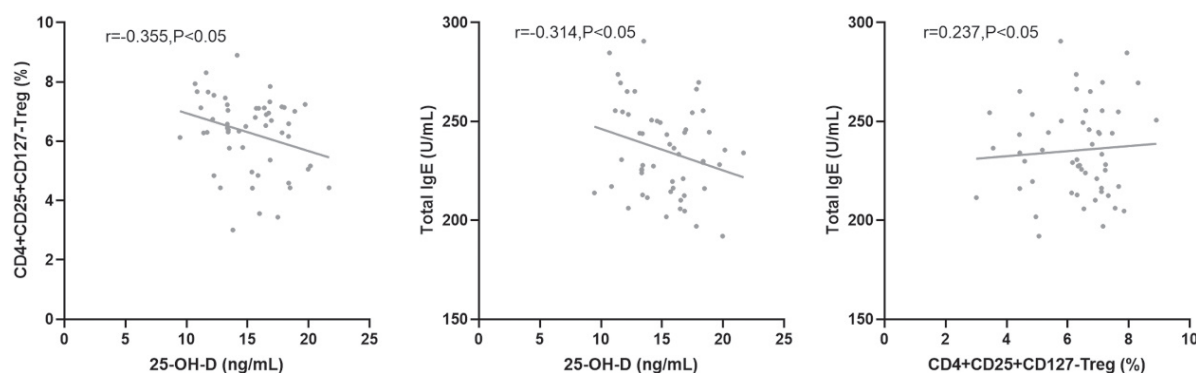


Fig. 1. Correlation analysis results of 25-OH-D, CD4⁺CD25⁺CD127⁺ Tregs and total IgE levels.

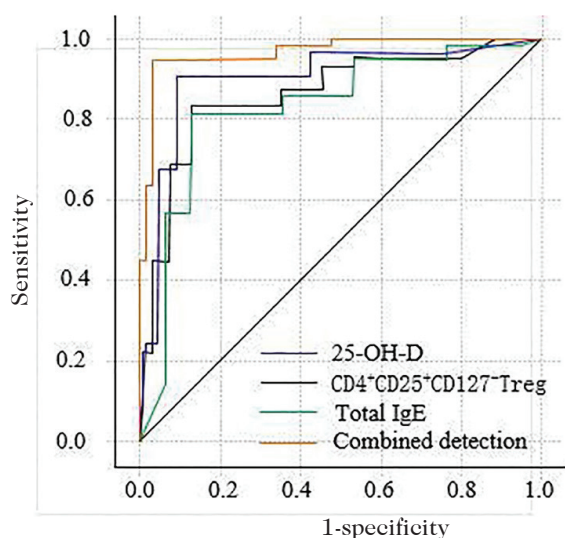


Fig. 2. ROC curves of predictive values of CD4⁺CD25⁺CD127⁺ Tregs and total IgE for the incidence of RRTIs.

receptors (in vascular endothelium, myocardium, skeletal muscle, immune cells, etc.). Vitamin D receptors have been confirmed to have no expression in resting T lymphocytes. However, when the body is infected, T lymphocytes are activated and can participate in the mediation of 25-OH-D by expressing cytochrome P450 family member 27B1, and make it transform into 1,25(OH)₂D. On this condition, the endogenous nature of vitamin D receptors is activated by stimuli, followed by combination (vitamin D and its receptors) and joint action to promote immune cells to produce corresponding cytokines,

playing the role of immune regulation. As a result, body immunity is enhanced^{11,12}. Secondly, 25-OH-D can interact with macrophages and monocytes, producing tubulins and defensins with antibacterial and antiviral activities in the induction period, thus affecting the phagocytic ability and chemotaxis of macrophages and monocytes, and enhancing the differentiation of monocytes to macrophages¹³. It follows that the decrease in the expression level of serum 25-OH-D in children with RRTIs is related to insufficient intake of vitamin D and repressed expression of vitamin D receptors.

Tregs impede the activation and proliferation of target cells inhibiting the expression of CD25 (interleukin-2 receptor subunit alpha, IL-2Ra) chain on the surface of target cells. Second, Tregs suppress the proliferation of effector cells and immunocompetence¹⁴, i.e. Tregs secrete such inhibitory cytokines as IL-10 and transforming growth factor- β to participate in immune suppression of the body. In this study, the expression level of CD4⁺CD25⁺CD127⁺ Tregs in children with RRTIs was subjected to a control study from the perspectives of disease stage, gender, site of lesions and severity of illness. It was found that the expression level of CD4⁺CD25⁺CD127⁺ Tregs in children with RRTIs was abnormal, which was significantly higher than that in the control group (children with non-RRTIs), implying that the development and progression of RRTIs in children

are influenced by CD4⁺CD25⁺CD127⁻ Tregs. Schenck *et al.* found that the expression level of lymphocytes (CD3, CD4) in RRTI children was lower than that in the control group (healthy children) ¹⁵. Moreover, CD4⁺CD25⁺CD127⁻ Tregs can effectively inhibit the immune response mediated by CD4⁺ and CD8⁺ effector cells^{16,17}. This shows that CD4⁺CD25⁺CD127⁻ Tregs exhibit abnormal proliferation and activation in RRTI children, giving rise to enhanced response advantage of Tregs, breaking the balance between T helper type 1 (Th1)/Th2/Th17 cell system and Treg cell system, and leading to the disorder of immune regulation. Eventually, RRTIs develop and progress.

In this study, the expression level of total IgE in children with RRTIs was compared from disease stage, gender, site of lesions and severity of illness. The results showed that the expression level of total IgE in children with RRTIs was abnormal and higher than that in the control group (children with non-RRTIs), signifying that the abnormal expression of total IgE is related to the development and progression of RRTIs. This is attributed to the fact that repeated respiratory infections affect the antigens, leading to the exhaustion of the immune system and increasing the expression level of total IgE in serum.

The results of Pearson correlation analysis uncovered that the expression level of serum 25-OH-D was negatively correlated with the expression levels of CD4⁺CD25⁺CD127⁻ Tregs and total IgE ($r=-0.355$, -0.314), while the expression level of CD4⁺CD25⁺CD127⁻ Tregs was positively correlated with that of total IgE ($r=0.237$). This demonstrates that the development and progression of RRTIs is a comprehensive pathological process, which may be caused by the interaction of serum 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE. It has previously been reported that the children with RRTIs (196 children aged 1 to 18 years old) had reduced serum 25-OH-D expression levels (61.00%), and

vitamin D expression level was negatively correlated with age ¹⁸. In this state, the expression levels of immunoglobulins (IgA, IgG) in children with RRTIs also decrease ¹⁹. In this study, when the expression level of serum 25-OH-D in children with RRTIs dropped, that of total IgE rose, *i.e.*, there was a negative correlation between the two. It suggests that serum 25-OH-D may be involved in mediation of humoral immunity. Beale *et al.* proved that the severity of RRTIs was correlated with the level of vitamin D, and in RRTI children with a low immune level, the serum 25-OH-D level showed a positive relationship with the severity of RRTIs ²⁰. Hatam *et al.* confirmed that CD4⁺CD25⁺CD127⁻ Tregs were abnormally expressed in children with RRTIs, which was an important pathogenesis of RRTIs.²¹ It can be concluded that serum 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE may participate in the development and progression of RRTIs through interaction. Moreover, it was also found in this study that the AUC value of combination of 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE for predicting the incidence of RRTIs was 0.852, which was larger than that of any of the three indicators alone, with significant differences. Collectively, the combined detection of the three indicators is beneficial to improving the detection rate of RRTIs.

Nevertheless, this study is limited. The obtained predictive values are focused on the analysis of global RRTIs (upper and lower respiratory tract) compared to non-RRTIs. Further studies are ongoing in our group.

In summary, serum 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE are abnormally expressed in children with RRTIs, and their expressions are of great differences among children at different stages, sites of lesions and severities of illness. The expression of 25-OH-D has negative correlations with the expressions of CD4⁺CD25⁺CD127⁻ Tregs and total IgE, and a positive correlation is observed between

the expressions of CD4⁺CD25⁺CD127⁻ Tregs and total IgE. The combined detection of serum 25-OH-D, CD4⁺CD25⁺CD127⁻ Tregs and total IgE is conducive to enhancing the detection rate of RRTIs.

ACKNOWLEDGMENTS

None.

Funding

This study was financially supported by the Health Science and Research Project of Kunming Municipal Health Commission (No. 2022-06-01-005).

Conflict of interest

The authors declare no conflict of interest.

ORCID's number authors

- Yanfei Yang (YY):
0009-0006-6324-5357
- Na Fan (NF):
0009-0000-9635-0360
- Xingzhu Liu (XL):
0009-0001-6107-5677
- Lin Wang (LW):
0009-0004-4028-0577
- Zhihai Wang (ZW):
0009-0007-0753-7782
- Yao Hou (YH):
0009-0003-6697-7391
- Ting Wang (TW):
0009-0007-9197-7499
- Yuqin Wu (YW):
0009-0008-1137-5250

Contribution of the authors

YY, TW and YW designed this study and significantly revised this paper; NF, XL, LW, ZW and YH performed this study and drafted this paper. All authors have approved the submission and publication of this paper.

REFERENCES

1. Paśnik J. Vaccines nonspecific - immunostimulation in patients with recurrent respiratory infections. *Otolaryngol Pol* 2016; 70(6): 31-39. <https://doi.org/10.5604/01.3001.0009.4819>
2. Dong F, Yu H, Ma J, Wu L, Liu T, Lv G, Zhen J, Li X, Lewith G, Gu X. Exploring association between gastrointestinal heat retention syndrome and recurrent respiratory tract infections in children: a prospective cohort study. *BMC Complement Altern Med* 2016; 16: 82. <https://doi.org/10.1186/s12906-016-1062-8>
3. Wang X, Li X, Jin C, Bai X, Qi X, Wang J, Zhang L, Li N, Jin N, Song W, Gao H, Gao B, Zhang Y, Wang L. Association between serum Vitamin A levels and recurrent respiratory tract infections in children. *Front Pediatr* 2021; 9: 756217. <https://doi.org/10.3389/fped.2021.756217>
4. Zhang X, Ding F, Li H, Zhao W, Jing H, Yan Y, Chen Y. Low serum levels of vitamins A, D, and E are associated with recurrent respiratory tract infections in children living in Northern China: A case control study. *PLoS One* 2016; 11(12): e0167689. <https://doi.org/10.1371/journal.pone.0167689>
5. Magnus MC, Stene LC, Håberg SE, Nafstad P, Stigum H, London SJ, Nystad W. Prospective study of maternal mid-pregnancy 25-hydroxyvitamin D level and early childhood respiratory disorders. *Paediatr Perinat Epidemiol* 2013; 27(6): 532-541. <https://doi.org/10.1111/ppe.12080>
6. Chen X, Murakami T, Oppenheim JJ, Howard OMZ. Differential response of murine CD4⁺CD25⁺ and CD4⁺CD25⁻ T cells to dexamethasone-induced cell death. *Eur J Immunol* 2004; 34(3): 859-869. <https://doi.org/10.1002/eji.200324506>
7. Chen M, Deng H, Zhao Y, Miao X, Gu H, Bi Y, Zhu Y, Guo Y, Shi S, Xu J, Zhao D, Liu F. Toll-Like Receptor 2 modulates pulmonary inflammation and TNF- α release mediated by *Mycoplasma pneumoniae*. *Front Cell Infect Microbiol* 2022; 12: 824027. <https://doi.org/10.3389/fcimb.2022.824027>

8. Ruskamp J, Smit H, Rovers M, Hoekstra M, Schilder A, Brunekreef B, Wijga A, Kerkhof M, de Jongste J, Sanders E. Neonatal total IgE and respiratory tract infections in children with intrauterine smoke exposure. *Arch Dis Child* 2010; 95(6): 427-431. <https://doi.org/10.1136/adc.2009.162685>
9. Fu Y, Lou H, Wang C, Lou W, Wang Y, Zheng T, Zhang L. T cell subsets in cord blood are influenced by maternal allergy and associated with atopic dermatitis. *Pediatr Allergy Immunol* 2013; 24(2): 178-186. <https://doi.org/10.1111/pai.12050>
10. Eroglu C, Demir F, Erge D, Uysal P, Kirdar S, Yilmaz M, Kurt Omurlu I. The relation between serum vitamin D levels, viral infections and severity of attacks in children with recurrent wheezing. *Allergol Immunopathol* 2019; 47(6): 591-597. <https://doi.org/10.1016/j.aller.2019.05.002>
11. Ginde AA, Mansbach JM, Camargo CA Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2009; 169(4): 384-390. <https://doi.org/10.1001/archinternmed.2008.560>
12. Korf H, Decallonne B, Mathieu C. Vitamin D for infections. *Curr Opin Endocrinol Diabetes Obes* 2014; 21(6): 431-436. <https://doi.org/10.1097/MED.000000000000108>
13. Watkins RR, Lemonovich TL, Salata RA. An update on the association of vitamin D deficiency with common infectious diseases. *Can J Physiol Pharmacol* 2015; 93(5): 363-368. <https://doi.org/10.1139/cjpp-2014-0352>
14. Qin S, Li L, Liu J, Zhang J, Xiao Q, Fan Y, Wei X. CD4+CD25+Foxp3+ regulatory T cells regulate immune balance in unexplained recurrent spontaneous abortion via the Toll-like receptor 4/nuclear factor- κ B pathway. *J Int Med Res* 2020; 48(12): 300060520980940. <https://doi.org/10.1177/0300060520980940>
15. Schenck LP, Surette MG, Bowdish DM. Composition and immunological significance of the upper respiratory tract microbiota. *FEBS Lett* 2016; 590(21): 3705-3720. <https://doi.org/10.1002/1873-3468.12455>
16. Brusko TM, Hulme MA, Myhr CB, Haller MJ, Atkinson MA. Assessing the in vitro suppressive capacity of regulatory T cells. *Immunol Invest* 2007; 36(5-6): 607-628. <https://doi.org/10.1080/08820130701790368>
17. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. *Nat Immunol* 2008; 9(3): 239-244. <https://doi.org/10.1038/ni1572>
18. Ramasamy I. Vitamin D metabolism and guidelines for Vitamin D supplementation. *Clin Biochem Rev* 2020; 41(3): 103-126. <https://doi.org/10.33176/AACB-20-00006>
19. Dąbrowska-Leonik N, Bernatowska E, Pac M, Filipiuk W, Mulawka J, Pietrucha B, Heropolitańska-Pliszka E, Bernat-Sitarz K, Wolska-Kuśnierz B, Mikołuc B. Vitamin D deficiency in children with recurrent respiratory infections, with or without immunoglobulin deficiency. *Adv Med Sci* 2018; 63(1): 173-178. <https://doi.org/10.1016/j.advms.2017.08.001>
20. Beale DJ. Analysis leads to unreliable results in study of Vitamin D and acute respiratory infection. *J Am Geriatr Soc* 2017; 65(5): 1103. <https://doi.org/10.1111/jgs.14797>
21. Hatam LJ, Devoti JA, Rosenthal DW, Lam F, Abramson AL, Steinberg BM, Bonagura VR. Immune suppression in premalignant respiratory papillomas: enriched functional CD4+Foxp3+ regulatory T cells and PD-1/PD-L1/L2 expression. *Clin Cancer Res* 2012; 18(7): 1925-1935. <https://doi.org/10.1158/1078-0432.CCR-11-2941>