

## In case of recurrent wheezing and bronchiolitis: Think again, it may be a primary immunodeficiency

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### Abstract

**Background:** Wheezing, starting early in life, is a heterogeneous medical condition caused by airway obstruction due to different underlying mechanisms. Primary immunodeficiencies are also among the risk factors that cause wheezing and recurrent bronchiolitis. ADA deficiency is a primary immunodeficiency, also a rare metabolic disease associated with multisystemic clinical findings.

**Objective:** This report will be helpful for addressing the importance of thinking primary immunodeficiency in case of wheezing and recurrent bronchiolitis.

**Methods:** The patient was diagnosed by using a targeted next generation sequencing PID panel. Lymphocyte subsets were measured by flow-cytometry.

**Results:** Here we present an infant with ADA deficiency who admitted with wheezing and recurrent bronchiolitis as the first presentation. He was found to have wheezing, relative CD4+ T cell deficiency, and prolonged neutropenia.

**Conclusion:** Primary immunodeficiencies including ADA deficiency should be considered in infants with wheezing, recurrent bronchiolitis, lymphopenia and neutropenia.

**Key words:** Wheezy infant, Lymphopenia, relative CD4 deficiency, Primary Immunodeficiency, ADA deficiency

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### Introduction

Wheezing is the most important symptom and clinical manifestation of partial obstruction of the airways.<sup>1</sup> Its most common causes are asthma, gastroesophageal reflux and infections.<sup>2</sup> Wheezing can be seen at any age but infants are more susceptible to wheezing since their airways are small and require less obstruction to produce an audible wheezing. Approximately 37-56% of infants have at least one wheezing episode in their lives.<sup>1</sup> Wheezy infant or recurrent bronchiolitis can be defined as the occurrence of at least three lower respiratory diseases with wheezing in the first two years of life (first episode before the age of 1). Some of the risk factors for wheezing in infants are pneumonia, upper respiratory tract infection, allergy, asthma, use of oral corticosteroids, cigarette smoking, male sex, lack of breastfeeding, the presence of mold at home and primary immunodeficiency (PID). According to the report published by the European Respiratory Society (ERS), viral respiratory infections (rhinovirus, respiratory syncytial virus, coronavirus, metapneumovirus) are the most common cause of wheezing or recurrent bronchiolitis in children in preschool age.<sup>3</sup>

As one of the most common findings in severe combined immunodeficiency (SCID), wheezing is respiratory tract manifestations in the earlier months of life. Among the genetic subtypes of SCID, certain pulmonary complications are more common in adenosine deaminase (ADA) deficiency.

ADA deficiency is the first PID in which a specific molecular defect is identified.<sup>4</sup> On the whole it accounts for 10-15% of all SCIDs and one-third of the autosomal recessive SCIDs in western countries.<sup>5</sup> ADA is an enzyme involved in the purine salvage pathway, responsible for the conversion of adenosine to inosine and deoxyadenosine to deoxyinosine.<sup>6</sup> ADA enzyme is located in all cells and the deficiency causes accumulation of toxic metabolites which inhibits many important functions such as cell signaling, energy generation, DNA repair, de novo nucleotide and deoxynucleotide synthesis, leading to pathological findings affecting various organ systems. Therefore, ADA deficiency is classified not only as an immunodeficiency but also as a metabolic disease.

Although ADA deficiency typically leads to T-B-NK- SCID, delayed and late onset cases are also present. Different from other SCID forms, patients may present with growth retardation in the early infancy non-infectious respiratory distress, skeletal and thoracic abnormalities, sensorineural hearing loss, and neutropenia.<sup>7,8</sup> Delayed and late onset cases may present with mild clinical findings such as recurrent sinopulmonary infections, viral infections with HPV, autoimmunity, allergic findings and increased IgE levels. Patients with ADA deficiency may be treated with PEG-ADA enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) or gene therapy.<sup>9</sup>

Here, we report a patient with ADA deficiency presented with recurrent wheezing and sinopulmonary infections, neutropenia, relatively low number of CD4+ T cells and low CD40 expression on B cells.

## Case

An 18 month-old boy was admitted to Hacettepe University Ihsan Dogramacı Children's Hospital with the history of recurrent wheezing and sinopulmonary infections. His medical history revealed an upper respiratory tract infection for the first time at 6 months of age. Then, he was treated with inhaled steroids and salbutamol intermittently because of recurrent wheezing attacks and pulmonary infections. He was suffered from fever, cough and respiratory distress and was hospitalized in an another hospital for pneumonia at the age of 9 and 15 months and was given intravenous antibiotics. He was the first child of first-degree consanguineous parents. The physical examination revealed normal growth [his height was 79 cm (25 p), weight was 9.5 kg (10-25 p) and head circumference was 48 cm (75 p)]; diffuse rales, rhonchi on lung auscultation and hepatosplenomegaly.

Laboratory results are given in **Table 1**. A transient eosinophilia which was thought to be secondary to drug allergy or an infection was detected. Immunological evaluation revealed prolonged neutropenia (300-1000/mm<sup>3</sup>), lymphopenia, slightly low IgG and IgM levels, reduced CD4+ and CD19+ cells percentages and CD3+ T cell absolute counts. Sweat chloride test and echocardiography were normal. Rhinovirus antigen was found to be positive in respiratory viral panel.

**Table 1. Patient's Immunological Findings.**

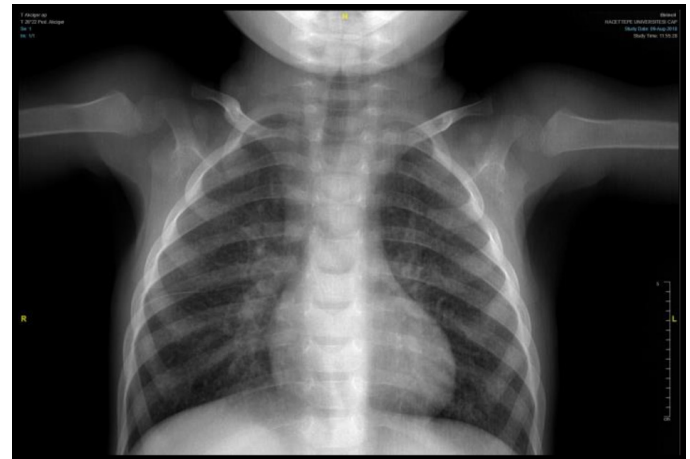
	First admission (18 months old)	References
Complete blood count		
Hemoglobin (g/dL)	<b>9.2</b>	10.9-15.0
Leucocytes (/mm <sup>3</sup> )	<b>3400</b>	6400-12.000
Absolute lymphocyte count (/mm <sup>3</sup> )	<b>700</b>	3600-8900
Absolute neutrophile count (/mm <sup>3</sup> )	<b>300</b>	1000-1700
Absolute eosinophile count (/mm <sup>3</sup> )	400	0-500
Trombocytes (/mm <sup>3</sup> )	290.000	150.000-450.000
Immunoglobulins (mg/dL)		
IgA	57	30-107
IgG	<b>553</b>	605-1430
IgM	<b>43.1</b>	66-228
Total IgE (IU/mL)	53.6	
Lymphocyte subsets % and absolute values		
CD3	83% <b>581</b>	53-75% 2100-6200
CD4	5% <b>35</b>	32-51% 1300-3400
CD8	77% <b>539</b>	14-30% 620-2000
CD16+56	14% <b>98</b>	3-15% 180-920
CD19	1.5% <b>10.5</b>	16-35% 720-2600

**Table 1. (Continued)**

	First admission (18 months old)	References
<b>T lymphocyte subsets</b>		
CD4	4%	29-59%
Naïve (CD4+CCR7+CD45RA+)	<b>0.3%</b>	57.1-84.9%
Central memory (CD4+CCR7+CD45RA-)	5%	11.3-26.7%
Effector memory (CD4+CCR7-CD45RA+)	<b>94.1%</b>	3.3-15.2%
Temra (CD4+CCR7+CD45RA+)	0.3%	0.4-2.6%
TREC (CD4+CCR7-CD45RA+)	3%	40-100%
CD8	64%	19-29%
Naïve (CD8+CCR7+CD45RA+)	0.7%	28.4-80.6%
Central memory (CD4+CCR7+CD45RA-)	<b>69.5%</b>	<b>1.0-4.5%</b>
Effector memory (CD4+CCR7-CD45RA-)	<b>25.4%</b>	<b>6.2-29.3%</b>
Temra (CD4+CCR7+CD45RA+)	<b>4.2%</b>	<b>9.1-49.1%</b>
<b>Lymphocyte activation test</b>		
CD3	1%	<b>59.1-80.7</b>
CD4	3%	
CD25	1%	<b>86.9-99.8</b>
CD69	1%	<b>61.2-91.8</b>
CD3+CD25+	0	<b>52.4-93.7</b>
CD3+CD69+	0	<b>47.9-84.8</b>
CD4+CD25+	0	
CD4+CD69+	0	
ADA enzyme level (nmol/h/mg)	<b>0, 4 (26, 4 ± 10, 0)</b>	
% dAXP	<b>38, 1 (50, 3 ± 18)</b>	

Findings in **bold** are abnormal.

T cell subtypes and lymphocyte activation tests revealed immunodeficiency compatible with combined form. Serum CMV PCR was 12982 copies/mL and therapy was started. Trimethoprim-sulfamethoxazole and fluconazole prophylaxis and intravenous immunoglobulin replacement (IVIG) were initiated with the diagnosis of combined immunodeficiency. HLA-matched family donor could not be found so matched unrelated donor screening was started for HSCT. Since he was vaccinated according to Turkey's schedule (BCG vaccination at 2 months of age), prophylactic isoniazid and rifampicin were initiated. In his follow-up, there was vesicular rash due to herpes infection which was treated with acyclovir. Chest X-Ray and thorax computed tomography showed mediastinal lymphadenopathies, patchy diffuse ground glass areas in both lungs, peribronchial thickening and micronodules in both lower lobes of lungs (**Figure 1**).



**Figure 1. Posteroanterior view of the lung of the patient**

Although the patient had slightly low IgM levels, we firstly investigated about CD40-CD40L deficiency due to male sex, recurrent sinopulmonary infections, neutropenia and immunological test results indicating combined immunodeficiency. Although these disorders are collectively known as Hyper IgM syndrome, serum levels of IgM are normal or even decreased in approximately 50% of patients with CD40L deficiency at diagnosis.<sup>10</sup> So we performed flow cytometric analysis of CD40 and CD40 ligand expression and we found that the CD40 expression on B cells was about 25% of healthy control. Further study, targeted next generation sequencing (NGS) PID panel analysis showed a missense homozygous mutation c.302G>A in exon 3 of ADA gene. ADA enzyme activity [0.4 nmol/h/mg (16.4-36.4)] and deoxyadenosine [38.1% (< 1%)] metabolite levels were consistent with ADA deficiency. PEG-ADA enzyme treatment was started. He underwent HSCT of bone marrow from HLA-matched unrelated donor at the age of 2 years 6 month.

## Discussion

The most significant presentation of our patient was wheezing and respiratory manifestations. Wheezing is the most important clinical finding of partial obstruction of the lower respiratory tract. Approximately 37-56% of infants develop at least one wheezing attack in their life.<sup>1</sup> One of the most common findings in SCID is respiratory tract manifestations in the earlier months of life. Recurrent and severe pulmonary findings are also seen in the patients with ADA deficiency.<sup>11</sup> These patients may experience severe respiratory tract infections because of direct toxicity of adenosine metabolites on the lung. These pulmonary findings such as non-infectious lung disease, pneumonitis, diffuse progressive interstitial lung disease and pulmonary alveolar proteinosis (PAP) are more common in patients with ADA deficiency than other genetic forms of SCID.<sup>12,13</sup> The differential diagnosis of PID should be performed in pediatric patients with recurrent wheezing episodes.

ADA deficiency has a life-threatening heterogeneous clinical presentation with repetitive and opportunistic infections, developmental delay, neurological disorders, abnormal findings in the lung and liver, sensorineural hearing loss and skeletal dysplasias.<sup>7,14</sup> Due to the broad mutation spectrum and diversity of clinical findings, the age of onset, severity of symptoms and cases with variable prognosis have been reported. Therefore, it exhibits a variety of presentations including early-onset and late-onset forms.

Neutropenia and lymphopenia were present in our patient (Table 1). Neutropenia recovered with G-CSF treatment and lymphopenia was normalized after PEG-ADA enzyme replacement. Prolonged neutropenia in patients with ADA deficiency is a rare and interesting finding and reported before in our unit in another patient with the same molecular defect.<sup>15</sup> Myeloid dysplasia and bone marrow hypocellularity have been previously reported in ADA-deficient SCID patients. In these patients, neutropenia may be due to accumulating toxic metabolites, not just autoantibodies against neutrophils. In patients with ADA deficiency, absolute neutrophil counts were found to be inversely proportional to deoxynucleotide accumulation. Myeloid dysplasia and bone marrow hypocellularity may make ADA-deficient patients more susceptible to myelotoxicity due to chemotherapy and antibiotics.<sup>16,17</sup> The patient's IgM and IgG levels were slightly low and IgA level was normal before receiving IVIG. Relatively low number and percentage of CD4+ T cells was detected. Additionally, there was no activation of T cells with the stimulation of phytohemagglutinin (PHA).

Since we considered the possibility of CD40 deficiency in the differential diagnosis due to history of male sex, combined immunodeficiency and neutropenia, we measured CD40 and CD40 ligand expression and found that the CD40 expression was about 25% of healthy control. Interestingly, there was no defect in CD40 gene. Instead, we found c.302G>A mutation in ADA gene in targeted NGS PID panel analysis. Casanova V. et al.<sup>18</sup> showed that ADA enzyme regulates CD40 levels on dendritic cells. Following treatment with ADA enzyme, dendritic cells increased CD40 expression on cell surface.

ADA gene is localized at 20q12-q13.11. There are more than 70 mutations identified in the ADA gene, inherited with autosomal recessive patterns.<sup>19</sup> A targeted PID NGS panel revealed a missense homozygous mutation (c.302G>A) in exon 3, previously reported. ADA-enzyme and metabolite levels were compatible seen in SCID form of ADA deficiency (Duke University).<sup>20</sup> In a series of 13 patients reported from our center, another patient with the same mutation with our patient had also marked neutropenia,<sup>15</sup> since he had underwent HSCT, we could not evaluate CD40/CD40L expression of him.

PEG-ADA ERT was initiated immediately after diagnosis. ERT containing PEG-ADA, is a therapeutic option that provides systemic clearance or "detoxification" of toxic metabolic substrates, although it is not the definitive treatment of the disease. ERT is associated with a long-term suboptimal immune reconstitution in the absence of a compatible HSCT donor.<sup>21</sup> In the presented patient, HSCT was successfully performed following ERT which provided the patient time for achieving the curative treatment.

In conclusion, primary immunodeficiencies including ADA deficiency should be considered in infants with wheezing. Lymphopenia is most prominent laboratory finding in infancy to suggest the diagnosis of SCID including ADA deficiency. ADA-deficient patient should also be evaluated with bone marrow aspiration for myeloid dysplasia in case of neutropenia since it may contribute to infections and may make patients more vulnerable to antibiotic and chemotherapy toxicities.

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