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Long-lasting non-IgE-mediated gastrointestinal cow's milk allergy in infants with Down syndrome

To the Editor,

Non-IgE-mediated gastrointestinal food allergy is characterized by massive gastrointestinal symptoms that reportedly induced by cell-mediated reactions to a specific food in infancy (1). The causative foods of non-IgE-mediated gastrointestinal food allergy have reportedly included cow's milk (67%), fish (12%), egg (6%), rice (4%), soy (4%), poultry (3%), goat's milk (2%), and corn (2%) (1). Patients with Down syndrome occasionally suffer from food allergy (2). The morbidity of food allergy in patients with Down syndrome is reported to be higher than seen in subjects without Down syndrome (2). On the other hand, there are no reports of the association between non-IgE-mediated gastrointestinal food allergy and Down syndrome.

Two Japanese children with Down syndrome were diagnosed as having non-IgE-mediated gastrointestinal food allergy by fulfilling all of Miceli Sopo's five criteria (3). Antigen-specific lymphocyte stimulation test (Bio Medical Laboratories (BML) Inc., Tokyo, Japan) revealed that a significant ³H-thymidine incorporation was observed when patients' PBMCs were cultured with κ -casein and lactoferrin, when compared with those stimulated with a vehicle control (Table 1) (4). Neither serum non-specific IgE antibody nor cow's milk-specific IgE antibody was detected by ImmunoCAP (Phadia KK, Tokyo, Japan).

Patient 1 was a 7-day-old Japanese boy who was born by normal vaginal delivery weighing 3,224 g at 38 weeks of gestation, and was diagnosed as having Down syndrome assessed by the cytogenetic study. He started bottle-feeding with cow's milk one day after birth and showed watery diarrhea 7 days after birth. He led to a clinical remission after the avoidance of cow's milk, but oral challenge tests performed at ages of 3, 7, and 10 showed that he attained no tolerance until 7 years of age.

Patient 2 was a 1-month-old Japanese girl who was born by normal vaginal delivery weighing 2,574 g at 37 weeks of gestation, and was diagnosed as having Down syndrome. She was hospitalized because of fever and the red and swollen vulva. Hematological examinations revealed moderate leukocytosis ($11.83 \times 10^9/l$) with elevated C-reactive protein (0.99 mg/dl). Abdominal computed tomography (CT) showed contrast enhancements around the vulva and the bilateral

Table 1 Antigen-specific immune responses

Patient	No. 1	No. 2
Total IgE (IU/ml)*	<5	<5
Antigen-specific IgE (CAP RAST: IU/ml)		
cow's milk	<0.10	<0.10
α -lactalbumin	<0.10	<0.10
β -lactalbumin	<0.10	<0.10
casein	<0.10	<0.10
Antigen-specific lymphocyte stimulation test (cpm)†		
control (no stimulation)	288	206
lactoferrin	12,269	1,281
κ -casein	4,942	1,720

*The levels of total IgE and antigen-specific IgE were measured by ImmunoCAP (Phadia KK, Tokyo, Japan).

†Antigen-specific lymphocyte stimulation test was performed in Bio Medical Laboratories (BML) Inc., Tokyo, Japan, as described in reference 4 with a slight modification. In brief, PBMCs were resuspended at 1×10^6 cells/ml in RPMI1640 medium supplemented with 10% human AB serum, 100 IU/ml penicillin, and 100 μ g/ml streptomycin. Aliquots (0.5 ml) of the cell suspension were placed in a 48-well culture plate, and cell proliferation was assessed by ³H-incorporation after 5 days of culture.

inguinal lymph nodes, but not indicated abscess formation (*data not shown*). Under the diagnosis of vulvar cellulitis with rectovaginal fistula, antibiotic therapy was started. Colostomy was performed on day 30 after admission. The feeding with cow's milk was resumed on postoperative day 1. She started to have watery but not bloody stools ten times per day right after resuming of cow's milk. The body weight decreased gradually. Severe diarrhea led to dehydration, renal failure, and metabolic acidosis on postoperative day 11. Abdominal CT revealed the remarkable dilatation of the stomach, and the dilation and retention of intestinal juice in whole bowel (Fig. 1). The symptoms were improved after the avoidance of cow's milk. However, oral challenge tests performed at ages of 2 showed that she attained no tolerance to cow's milk.

The clinical phenotype of the gastrointestinal food allergy including cow's milk allergy has been established by Japanese study group (5). The patients were classified into 4 clusters

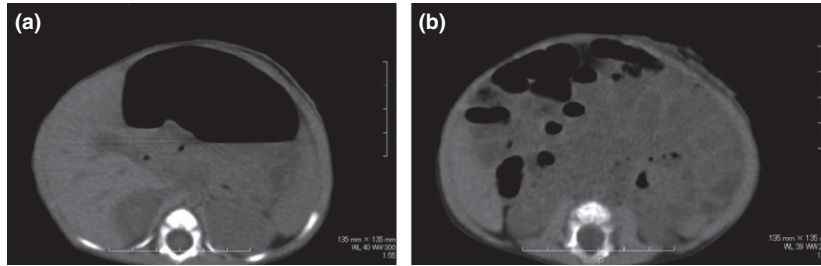


Figure 1 Abdominal CT findings showed the remarkable dilatation of the stomach in patient 2 (a), and the dilation and retention of intestinal juice in whole bowel after restart of cow's milk feeding on the day 11 of post-operation (b).

according to the presence or absence of vomiting and/or bloody stool (5). We concluded that the present patients were diagnosed as having cluster 2 (patient 1: vomiting without bloody stool, food protein-induced enterocolitis syndrome) and cluster 3 (patient 2: neither vomiting nor bloody stool, enteropathy), respectively. The main targeted organ of cluster 2 is an upper gastrointestinal tract, and symptoms appear after 2–24 hours from the oral ingestion of offending food. On the other hand, the affected organ of cluster 3 is chiefly small intestine, and symptoms appear after 24–60 h.

Patient 2 developed non-IgE-mediated gastrointestinal food allergy after the infection and surgical intervention. Several studies have reported the relationships between surgical intervention and the gastrointestinal food allergy (6). Surgical stress or anesthesia also reportedly increases permeability of the gut (7), and these may play some roles in the development of non-IgE-mediated gastrointestinal food allergy in these patients.

Previous reports showed that 52% of the patients acquired the tolerance until 1 year of age, 88% until 2 years, and 94% until 3 years (5). Although patient 1 acquired tolerance at 10 years of age, he remained to be intolerant to cow's milk at 7 years of age. Patient 2 still remained to be intolerant at 2 years of age, suggesting that the acquisition of tolerance in non-IgE-mediated gastrointestinal food allergy might be delayed in Down syndrome.

Recently, Morita et al. have reported that antigen-specific cytokine production profiles in the patients with non-IgE-mediated gastrointestinal food allergy were skewed to Th2 in addition to the production of TNF- α (8). Notably T cells from patients produce a significant amount of IL-10, a regulatory cytokine, which presumably leads to an early outgrowth of this allergy. As a matter of fact, CD4⁺CD25⁺ regulatory T cells (9) or CD4⁺IL-10⁺ T cells (10) were

reportedly associated with the acquisition of tolerance in patients with non-IgE-mediated gastrointestinal food allergy. However, the patients with Down syndrome reportedly have impaired IL-10 production (11), suggesting that such immunological impairment might reflect to the severe and long-lasting symptoms in our patients. Four cytokine receptor genes are located on chromosome 21q22.11, encoding the α and β subunits of the interferon- α receptor, the β subunit of the IL-10 receptor, and the second subunit of the interferon- γ receptor (12). We speculate that serum levels of higher IFN- γ and lower IL-10 may be involved in the pathophysiology of non-IgE-mediated gastrointestinal food allergy in patients with Down syndrome (11).

The rare association of non-IgE-mediated gastrointestinal food allergy with two patients with Down syndrome suggested that the condition might be more severe and need longer duration to establish the tolerance than seen in patients without Down syndrome. Further studies are required to characterize the distinct process of non-IgE-mediated gastrointestinal food allergy in patients with Down syndrome.

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Mean platelet volume increased in children with asthma

To the Editor,

Asthma is a chronic respiratory disease which is characterized by recurrent attacks of cough, wheezing, dyspnea, and variable expiratory airflow limitation. Asthma is a worldwide problem with an estimated 300 million affected individuals. Inflammation in asthma involves several inflammatory cells. Inflammatory cells such as eosinophils and/or neutrophils are defined well in the asthmatic airway (1). Platelets primarily involve in prevention of bleeding. Besides, they may also involve in various processes as secretion of proinflammatory, anti-inflammatory, and angiogenic factors, and collection of leukocytes and progenitor cells at areas of vascular damage and inflammation. Platelets play a role in allergic reactions that are inflammatory processes. Both intact platelets and platelet-released inflammatory mediators play crucial roles in the sustained inflammatory response of many allergic diseases. The platelet counts and platelet-activating markers in the plasma were significantly altered after exposure to an allergen in patients suffering from allergic inflammatory reactions. Platelet activation is necessary to transform arachidonic acid into lipid mediators which exacerbates the allergic response such as thromboxane A2 (TXA2), cysteinyl leukotrienes, and lipoxins (2). Platelet activation was demonstrated in asthmatic patients (3, 4). Experimental evidence demonstrated that platelets had a role in each stage of asthma pathogenesis in development of bronchoconstriction, airway inflammation, airway remodeling, and bronchial hyper-responsiveness (5). Platelets' volume increase when platelets become activated. Mean platelet volume (MPV) is one of the parameters in routine complete blood count test and shows the average volume of platelets in the circulation. MPV is one marker of platelet activation. In previous studies; relation between mean platelet volume changes and different diseases such as acute pyelonephritis had been shown (6). We have limited information about MPV in asthmatic patients (7, 8). In our country, in the study of Tuncel et al., they found no difference in terms of MPV among asthmatic patients during asymptomatic periods and exacerbations and control group (7). A new study on adults showed that decreased mean platelet volume was associated with stable and exacerbated asthma (8). In our study, we aimed to evaluate MPV and to assess its relationship with clinical parameters in children with stable and exacerbated asthma.

Six hundred and thirteen children with asthma, followed up in the pediatric allergy and immunology department of a public hospital of obstetrics and pediatrics, were included in the study.

Patients were evaluated retrospectively between September 2012 and August 2014. Two hundred and nineteen children with no evidence of allergic disease (i.e., asthma, allergic rhinitis, and eczema) were included in the control group. 162 patients in the patient group were evaluated during exacerbation and classified as exacerbation group separately. Patients who have had an acute/chronic infection (except for exacerbation) and patients with any other systemic disease such as hepatic, renal, cardiovascular diseases, diabetes mellitus, cancer, and systemic inflammatory disorders were excluded. In addition, stable asthmatic patients with anemia/polycythemia, leukopenia/leukocytosis, and thrombocytopenia/thrombocytosis in complete blood count analysis were excluded from the study. A detailed allergic history, including the age, a familial atopy history, and exposure to cigarette smoke were recorded. Familial atopy was accepted as positive when having allergic disease in first degree relatives (parents and siblings). The diagnosis of asthma was evaluated according to the GINA guidelines (1). Skin prick tests were performed using the same antigens ((*Dermatophagoides pteronyssinus*, *Dermatophagoides farinea*, grasses mix, cereals pollen mix, trees mix, weed mix, *Alternaria alternaria*, cockroaches, cat dander, and dog dander (Stallergenes SA, 92160 Antony, France) for all patients. The immunoglobulin (Ig) E levels and complete blood count were measured. The study was approved by the Zeynep Kamil Woman's and Children's Diseases Training and Research Hospital Ethics committee, and an oral consent was obtained from all subjects and/or their parents.

Results were given as either mean $\pm$ standard deviation (SD) or as mean $\pm$ standard deviation (median) according to the distribution. Student's t-test was used for the comparison of normally distributed variables. Chi-square, Mann–Whitney U-test, and Kruskal–Wallis tests were used for non-normally distributed variables. Pearson's correlation test was used for the correlation analyses of continuous variables. Paired samples t-test was used to compare the change of MPV during exacerbation and stable period. $p < 0.05$ was considered as significant.

There was no difference between the groups with regard to gender and age. Mean MPV was 8.21 ± 0.81 fL (5.3–11.3) in the study group and 7.95 ± 1.05 (5.6–12.6) in the control group. The difference was statistically significant ($p = 0.004$). Platelet count was significantly higher in asthmatic children compared to controls ($p = 0.008$). The demographic features, MPV, and platelet counts of the study population are shown in Table 1.