Background: Oral immunotherapy (OIT) for cow’s milk allergy (CMA) is an effective treatment option on the basis of its ability to increase the threshold for clinical reactions.

Aim: We aimed to present our experience of OIT with CM at a pediatric allergy outpatient clinic and to evaluate risk factors for the development of adverse reactions during OIT and to show the long-term effectiveness of OIT.

Study Design: Single-center retrospective cohort study.

Methods: We evaluated 42 patients with IgE-mediated CMA who complied with the OIT protocol. The treatment consisted of a rapid escalation phase in which milk doses were part of an oral food challenge (OFC) step. During the buildup phase, increasing quantities of cow’s milk (CM) were administered until the patient was able to consume 200 mL CM intake daily.

Results: The mean age of starting OIT was 40.2±3.2 (range,36-156) months and and 54.8% (n=23) of the patients were male. The mean duration of the build-up phase was 18.1±5.6 (range,9-41) weeks, and the mean maintenance phase was 29.1±11.6 (range,12-63) months. During OIT, 36 adverse reactions (78% mild, 22% moderate) occurred in 16 (38%) patients. Between the two groups of patients with and without adverse reactions, there were no differences in the age of OIT (p=0.19), CM-specific IgE levels (p=0.17) and cumulative provocative doses of OFCs (p=0.78). The wheal diameters to CM were higher in the group with adverse reactions (p=0.03). We successfully administered OIT protocols to children with a history of anaphylaxis (n=7). The adverse reactions and number of reactions during OIT were seen higher in patients with history of anaphylaxis. There was no difference in OIT onset age between patients with and without a history of anaphylaxis (p=0.38). The patients with history of anaphylaxis were higher adverse reactions (p=0.04) and a higher number of reactions during the OIT (p=0.01), and higher mean duration of the updosing phase (p=0.04) compared with patients without anaphylaxis.

Conclusion: OIT is a treatment option in patients with CMA because of its high efficacy and usually mild adverse effects. Due to adverse reactions, it should be applied carefully to patients with higher wheal diameters of CM in SPT. We administered the OIT protocol successfully. Therefore, in experienced centers, with care and the relevant expertise competencies to safely deliver this therapy and manage any adverse effects, OIT can be a safe treatment option.

Keywords: Children, cow’s milk allergy, oral immunotherapy

INTRODUCTION
Food allergy that can cause serious reactions such as anaphylaxis or severe allergic reactions can be managed by allergen avoidance and treatment of symptoms (1). Children with food allergy (FA) and their families have reduced quality of life, especially if the allergy is severe (2). Cow’s milk allergy (CMA) is the most common food allergy in infants and young children affecting 2-3% of the latter population (3). The prevalence of food-challenge-defined allergy to cow’s milk (CM) was 0.6-3% for all age groups (4). Eighty-seven percent of these patients will develop tolerance by the age of 3 years (5). Nonetheless, more recent studies have reported low
rates of CMA resolution (6,7). Skripak et al. reported recovery rates of 19% by age 4 years, and 79% by age 16 years (6).

Immunoglobulin-E (IgE)-mediated reactions due to CM intake may present as cutaneous reactions (e.g. urticaria, angioedema, atopic dermatitis), respiratory reactions (asthma and rhinitis), gastrointestinal reactions (e.g. oral allergy syndrome, vomiting) or systemic reactions (anaphylaxis) (8). The current management of CMA continues to be the avoidance of foods containing CM proteins until tolerance develops and the rescue treatment of acute reactions after accidental ingestions (9,10). However, CM can be present in a wide variety of foods, and strict avoidance is difficult, particularly in patients who react even with small amounts of CM. Avoidance of allergen food leads to a poor quality of life for patients and their families because of the potential for unexpected sudden and life-threatening reactions (11). In various series of anaphylaxis, CMA accounted for 11-28% of reactions, including up to 11% of fatal reactions (8).

As yet, there is no effective pharmacologic agent that offers definitive treatment. Specific oral immunotherapy (OIT) is a treatment option that has been introduced at several referral centers (12,13). Oral immunotherapy increases the threshold for clinical reactions if food tolerance is not achieved with age. The purpose of OIT is to protect against symptoms upon accidental ingestion, and to allow for full reintroduction of the food to the diet. OIT protocols generally start with a rapid escalation phase. In this phase, CM is introduced at a low amount and then rapidly increased to identify the maximum tolerated dose. Then follows the buildup phase, in which the daily dose is increased at weekly intervals until the target dose of 200 mL CM is reached. At the end of the buildup phase, the patient achieves desensitization, and continues to ingest 200 mL CM regularly. The maintenance phase may continue for years. If the sustained unresponsiveness is aimed to be evaluated, the patient avoids CM for a time period (generally 2-8 weeks) and then a new oral food challenge (OFC) is performed (14). Recently, the safety and efficacy of long-term milk oral immunotherapy (OIT) results were investigated (15,16). In this study, we aimed to present our experience of OIT with CM at a pediatric allergy outpatient clinic and to evaluate risk factors for the development of adverse reactions during OIT and to show the long-term effectiveness of OIT.

MATERIALS AND METHODS
This study is a single-center retrospective cohort study. We performed power analysis to calculate minimum number of patient for the study. Approximately 3840 patients is examined the child allergy outpatient clinic, annually. The frequency of developing milk allergy in these patients is p = 0.01. We calculated as 24 people as minimum numbers to the study with d = 0.04 sampling error at 95% (α = 0.05) confidence interval limits for power as 0.8.

Study population
Patient Selection
An oral immunotherapy protocol was administered to 47 patients with only IgE-mediated CMA aged 3 to 13 years between January 2009 to June 2014. The exclusion criteria of the OIT were the unreliability of parents for the management of OIT or concomitant non-controlled asthma.

Study Protocol
Of the 47 subjects, 42 patients (89.3%) successfully complied with the protocol and one patient achieved partial tolerance, 4 (8.5%) patients failed. A 6-year-old female patient achieved only partial tolerance, tolerating the consumption of 45 mL CM once daily rather than the 200 mL. They should be considered as fully desensitized to a dose of 200 mL CM intake daily. Three patients withdrew from the protocol due to mild or moderate adverse effects and their families were non-compliant with the treatment by not giving the doses at home as prescribed. Another patient was forced to withdraw because of the development of moderate and severe reactions. She was a 3-year-old with high CM-specific IgE (>100) levels and wheal diameter in the skin prick test (SPT) of 16 mm. She developed generalized urticaria with 3 mL and moderate bronchospasm with 6 mL in the OFC.

In this study, we evaluated 42 patients who could tolerate 200 mL milk at the end of the OIT. All patients were evaluated with an SPT and serum CM-specific IgE(sIgE) antibodies for diagnosis of CMA. Additionally, an open OFC was performed in all patients except those who had recent anaphylaxis with CM.

Skin prick test
The skin prick tests were performed using ALK-Abello A/S, Horsholm, Denmark standard prick test solutions (cow milk). The positive control was histamine and the negative control was 0.9% sodium chloride. The wheal diameters which were 3 mm and above according to the negative control were considered as positive.

Laboratory evaluation
The total serum IgE and milk-specific IgE levels were measured using the CAP system-FEIA (Pharmacia Upjohn). The detection limit was 0.35 kUA/l for sIgE.

Oral Food Challenge (OFC) Test
Oral food challenge test was an open protocol. OFCs were started using 0.1 mL diluted pasteurized CM with 3.3% protein content (1:10, milk: water), and were continued with increasing amounts of milk as follows: 0.1 mL, 1 mL, 2 mL, 3 mL diluted CM; and 1 mL, 1.5 mL, 3 mL, 6 mL, 12 mL, 24 mL, 50 mL, and 100 mL undiluted CM until a reaction was noted. Oral challenge results were considered positive when objective symptoms occurred. Early and late objective reactions were assessed by such as urticaria, angioedema, airway obstruction signs (e.g. dyspnea, rales, rhonchi), vomiting, and anaphylaxis. We did not stop OFCs for subjective reactions reported such as pruritus, not feeling well, and abdominal pain, which would increase the risk of false-positive test results where the reaction improved spontaneously in 10-20 minutes. We identified the individual tolerated dose of CM during increased-dose OFCs. We performed the OFC test with 6-month or one-year intervals for to evaluate the development of tolerance.

Milk oral immunotherapy protocol
Persistent cow’s milk allergy was evaluated according to the following criteria 4 weeks before oral immunotherapy: The wheal diameter CM in SPT ≥3 mm and CM-sIgE >0.35 kUA/l for whole cows milk. After SPT and specific IgE measurement, we performed OFC tests. If CMA in patients were persistent, we informed the patients and their families about OIT and allowed them to make an informed decision about the therapy. If the patient would be treated with OIT, we accepted the OFC steps as part of an initial escalation phase on the first day. We evaluated the dose in the previous step before developed reaction in OFC as the tolerated dose (the dose steps in OFC as follows: 0.1 mL, 1 mL, 2 mL, 3 mL diluted CM; and 1 mL, 1.5 mL, 3 mL, 6 mL, 12 mL, 24 mL, 50 mL, and 100 mL undiluted CM). On the second day, we continued with the OIT with a dose three steps behind the tolerated dose of the OFC on the first day, different from the other protocols. The patient was instructed to consume the same dose daily at home next week. During the build-up phase, increasing quantities of CM were administered initially at the hospital. If no reaction occurred, the same dose was continued at home weekly until the patient was able to consume the target dose of 200 mL (6540 mg protein) of CM daily at the end of 16 weeks (Figure 1). During home administrations, patients were able to contact the physician on their mobile phones 24 hours per day. The CM dose was modified during the follow-up period, according to the patient’s adverse events. When a dose in the build-up phase was not tolerated, the patient received the previous tolerated dose for one week at home. Patients were treated with antihistamines (1 mg ketotifen) once a day throughout the build-up phase. This phase was longer in patients who experienced adverse reactions. At the end of the build-up phase, the patient had achieved desensitization, and continued daily milk consumption during a maintenance phase. During the maintenance phase of the OIT, our patients’ treatment with antihistamines was discontinued. Family members were instructed in the recognition of the adverse effects. Carrying an epinephrine autoinjector was advised in case of severe adverse effects and both the patients and their families were instructed in their use. Adverse events were recorded as standard at each weekly visit. Adverse reactions were assessed and classified according to severity as mild (oral allergy syndrome, localized erythema or urticaria, vomiting, rhinitis, conjunctivitis, local urticarial, vomiting), moderate (generalized urticaria and angioedema, mild bronchospasm), and severe (moderate/severe bronchospasm, shortness of breath, breathing difficulties with inspiratory stridor, anaphylactic shock) (17). The patients were examined for 1-3 years to measure milk-specific IgE. The study was approved by the local ethics committee (IRB No. 14-4.1/16) and informed consent was obtained from all parents/guardians.

Statistical Analysis
Statistical analyses were performed using IBM SPSS Statistics V25. Descriptive statistics were presented with mean, standard deviation, median, minimum, and maximum values, frequencies, and percentages. Whether the distribution of each variable in the dataset fits the normal distribution was tested and variables that were not suitable for normal distribution were evaluated by nonparametric tests. A chi-square test was used in the analysis of categorical data. Mann Whitney U was used in binary independent group comparison. The Spearman Rho correlation analysis was performed to assess the correlation between the scale scores. Friedman analysis was used to compare repeated measurements and Dunn’s post-hoc test was used for binary comparisons when the significance was achieved. p<0.05 was considered statistically significant.

RESULTS
The mean age of the patients was 40.2±3.2 (range, 36-156) months at the start of the OIT, and 54.8% (n=23) of the patients were male. Seven percent (n=3) of patients were older than 5 years of age. The symptoms at presentation were skin eruption (urticaria and/or angioedema) (83.3%, n=35), respiratory distress (23.8%, n=10), and vomiting (21.4%, n=9) within the first 2 hours of ingesting milk. Concomitant diseases were atopic dermatitis (45%, n=19), asthma (12%, n=5), and allergic rhinitis (10.5%, n=4). Seven (16.7%) patients had a history of anaphylaxis after exposure to CM. Eighty five percent of the patients that presented with only cutaneous symptoms. Ten of 42 patients had CM-sIgE over than 50 kUA/l. Table 1 shows the demographic characteristics and laboratory findings of the patients.
While there was no correlation between laboratory findings and duration of buildup phase of treatment and OIT onset age of the patients; there was a correlation between CM-sIgE and IgE levels and CM-sIgE levels were also high in patients with large wheal diameters of CM in SPTs (Table 2).

All of the patients could not tolerated baked milk. In the OFC, the reactions were generally observed in response to the non-diluted milk dose. The median cumulative provocative dose in the OFC was 6 (0.2-48) mL. The provocation reactions consisted of urticaria (83.3%), respiratory distress findings (including bronchospasm, wheezing and/or sibilant rhonchi) (7.1%), angioedema (12%), and vomiting (2.3%).

The mean duration of the buildup phase was 18.1±5.6 (range, 9-41) weeks. The mean duration of the maintenance phase was 29.1±11.6 (range, 12-63) months. During the OIT, 36 adverse reactions occurred in 16 (38%) patients; 28 adverse reactions were mild and eight were moderate. These adverse reactions consisted of localized urticaria (47%, n=17), respiratory distress (cough and bronchospasm; n=4 or cough and wheezing; n=3) (19.4%, n=7), gastrointestinal symptoms (vomiting, 11%, n=4), exacerbation of atopic dermatitis (8%, n=3), localized erythema and itching of the throat (8%, n=3), urticaria and angioedema (2.7%, n=1), and generalized urticaria (2.7%, n=1) (Table 3). All reactions occurred in the hospital. The reactions were seen during the first 4 weeks in 50% of patients during the build-up phase. The mean reaction time was 5.2±3.5 (range, 1-14) weeks. Urticaria was controlled using oral antihistamines, and respiratory distress using oral antihistamines, steroids, and inhaled β2-agonists. No medications were administered for vomiting. We admitted patients’ reactions as single system involvement (only cutaneous or respiratory or gastrointestinal system signs) and after the treatment of the reactions, patients didn’t develop any more symptom to require use of epinephrine.

Between the two groups of patients with and without adverse reactions, there were no differences in OIT onset age of the patients (p=0.19), CM-specific IgE levels (p=0.17), and cumulative provocative doses of OFCs (p=0.78). The wheal diameters to CM were smaller (p=0.03) and the mean duration of the buildup phase was shorter (p=0.01) in the group without adverse reactions (Table 4). Sex distribution (47%, 52.6% of the patients was female, respectively, p=0.26) and presence of additional atopic disease (40%, 60% of the patients, respectively, p=0.75) both were similar in patients with and without OIT adverse reactions.

There was no difference in OIT onset age between patients with and without a history of anaphylaxis (p=0.38). Also, they had higher median IgE (p=0.02) and CM-sIgE (p=0.004) larger wheal diameters to CM in SPTs (p=0.001), higher cumulative provocative doses of OFCs (p=0.06, NS), higher adverse reactions (p=0.04) and a higher number of reactions during the OIT (p=0.01), and longer mean duration of the build-up phase (p=0.03) compared with patients without anaphylaxis (Table 5). Sex distribution was similar in patients with and without history of anaphylaxis (71.4%, 51.4% of the patients was male, respectively, p=0.42).

After one year of the maintenance phase, wheal diameters with CM in SPTs were significantly decreased (Figure 2). The median CM-sIgE levels decreased significantly step-by-step after the 6th month, and one, two, and three years of maintenance phase (Figure 3).

After completions of OIT, the nutritional status of the patient were normal and none of the patients were developed eosinophilic esophagitis.

DISCUSSION

The treatment of CMA is the dietary elimination of CM protein. The elimination diet is a difficult approach due to the possible risk of reaction including anaphylactic reaction following the accidental ingestion of CM. OIT is the active treatment for IgE-mediated CM allergic children who suitable for treatment instead of avoidance. Successful CM-OIT protocols in different age groups have been reported. In some desensitization protocols, because of the probability that natural tolerance will develop in children by the age of 4 years, children older than 4 years have been treated with OIT (12,13,18,19). However, more recent studies reported lower rates of natural tolerance development. In the studies by Garcia-Ara et al., 68% of patients with CMA developed tolerance by age 4 years (7). Other studies on OIT at an earlier age have been published, such as those by Martorell et al. (median age 2 years) and Staden et al. (median age 2.5 years) (20,21). The mean age of the patients receiving OIT was 40.2±3.2 months in the present study.

The majority of patients with CMA are successfully desensitized, and many effective protocols have been described (13,20,21). In Zapetero et al.’s protocol, treatment began on day 1 with diluted doses, on day 2, patients received a single dose of 1 mL non-diluted milk and then a 2-mL dose 30 min later at the hospital, continuing with the same dose at home for a week. The dose of milk was increased once a week, continuing until the patient was able to tolerate 200-250 mL non-diluted milk without reaction (13). Meglio et al. successfully desensitized 71.4% (n=15) of their patients and partially desensitized 14.3% (n=3) using a 6-month OIT protocol. Their protocol started with one drop of diluted CM, increasing to 200 mL over a period of several months (19). In the study by Patriarca et al., desensitization was started with one drop of CM and continued over a period of 136 days until the patient tolerated 120 mL CM (22).
In our protocol, the initial dose was dependent on the last tolerated dose in the OFC, differently. Due to variable initial doses and development of reactions, the duration of the build-up phase varied from 9 to 41 weeks. In the group of children with CM-specific IgE antibody levels ≥50 kUA/L evaluated by Skripak et al., some children outgrew their allergy during adolescence (6). In our study, 10 of 42 patients had CM-specific IgE levels ≥50 kUA/L (74.7±21 (51.7-100)). In some studies, immunotherapy was associated with a decrease in CM-specific IgE levels (8,9,20). Whereas in some studies, there was no change in the levels of these antibodies (12,23). In our patients, CM-sIgE levels steadily decreased during the 3 years of follow-up after the OIT. As in other studies, wheal diameters to CM decreased after 1 year (18).

The reported rates of adverse reactions in patients undergoing OIT are in the range of 47-100% of patients (12,20,21,24,25). Lower ratios were reported by Meglio et al. (61%) and Patriarca et al. (51.5%) (19,22). Adverse reactions are mainly mild or moderate and develop independently of desensitization regimens (21,23,25), even though the OIT was associated with an increased risk of severe adverse reactions requiring adrenaline injection or systemic corticosteroids compared with those on an elimination diet (26). Some studies reported higher rates for requiring adrenaline injections because of severe adverse reactions (12,20-23,24). In the present study, 38% (n=16) of the patients developed adverse events on 36 occasions; 28 of the adverse reactions were mild and 8 were moderate. No severe reactions developed and there was no need to use adrenaline. In the study by Staden et al, all patients had mild reactions and four patients had moderate reactions (21). Adverse reactions were more frequent in the initial phases, especially during the first 4 weeks (50% of patients), as reported by Martorell et al (20).

Before to the OIT, our patients were premedicated with ketotifen during the buildup phase, whereas cetirizine or sodium cromoglycate was used in other protocols (19,22,27). Jagdis et al. showed that ketotifen premedication reduced the frequency and severity of gastrointestinal adverse reactions during peanut OIT (28). In our study, gastrointestinal adverse reactions were seen in 11% (n=4) of the patients, although they were seen in 2.3% (n=1) of patients in the OFC before the OIT. In our experience, antihistamines did not mask any symptoms except oral allergy syndrome or rash, and facilitated patient compliance as reported by Garcia et al (29). Ketotifen was stopped after the updosing phase of treatment, and CM desensitization persisted.

Rates of successful desensitization to CM-OIT are reported as 50.6-70% (13,19,20,23), although Staden et al. (21) reported lower permanent tolerance rates (36%) after OIT.

Our OIT protocol had a 91.3% (n=42) success rate in achieving the target dose of 200 mL of CM daily. Additionally, 2% (n=1) of patients achieved partial desensitization with 45 mL of CM per day. The success of the treatment may be related to the patient characteristics. However, some protocols excluded patients with a history of anaphylaxis before OIT (20). Alvaro et al.’s study showed that oral desensitization to cow’s milk is efficient even in patients with anaphylactic reactions to CM in one-year assessment (30). In this study, successful completion of treatment by patients with a history of anaphylaxis shows the effectiveness of the OIT protocol in our patients in three years follow-up OIT. OIT trials with patients with anaphylaxis are needed to investigate the safety of OIT. This study showed that adverse reactions and number of reactions during OIT were seen higher in patients with history of anaphylaxis. There is insufficient evidence as to whether clinical tolerance is transient or persistent (31). After stopping OIT, patients may fail desensitization (32,33). In Sato et al.’s study, the clinical tolerance ratio was 27.1% at the end of the milk OIT (31). Therefore, we cannot estimate the loss of tolerance when ingestion is discontinued, which some studies have reported (33). We could not evaluate sustained unresponsiveness rates.

Study limitations
An open OFC protocol was chosen despite the patients were older than 3 years of age. We didn’t evaluate isolated cow’s milk proteins (casein, a-lactoalbumin, b-lactoglobulin) to assess persistence of CM allergy in patients and any parameter except SPT and CM-sIgE was not evaluated after OIT either. We also didn’t perform any implementation for the evaluation of sustained unresponsiveness and permanent tolerance.

CONCLUSION
Oral immunotherapy is a promising treatment option in patients with CMA because of its high efficacy. However, it is a time-consuming treatment and carries risk of adverse reactions. Due to adverse reactions, it should be applied carefully to patients with higher wheal diameters of CM in SPT. It can only be performed by experienced allergy departments and requires patient and family adaptation. However, there are limited data on their safety and long-term clinical follow-up. We successfully administered OIT to children with or without a history of anaphylaxis due to CM. In this study, we demonstrate our OIT experience and the long-term results show that it is an effective treatment option for children with CM allergy. Despite the difficulties of OIT, it can provide a better quality of life for patients and their families. Therefore, in experienced centers, with careful monitoring of patients, OIT can be a safe treatment option, even in patients with a history of anaphylaxis.

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Disclosure Statement
The authors have no conflicts of interest to declare.
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References
### TABLE 1. Demographic Characteristics and Laboratory Findings

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients onset age of the symptoms (month)</td>
<td>5.4±4.6 (1-30)</td>
</tr>
<tr>
<td>OIT onset age of the patients (month)</td>
<td>40.2±3.2 (36-156)</td>
</tr>
<tr>
<td>Median Total IgE (kU/l)</td>
<td>125 (4-1731)</td>
</tr>
<tr>
<td>Median CM-sIgE (kUA/l) (at the start of OIT)</td>
<td>5.8 (0.5-100)</td>
</tr>
<tr>
<td>Median wheal diameter to CM in SPTs (mm)</td>
<td>8.5 (3-35)</td>
</tr>
<tr>
<td>Median cumulative provocative dose of OFC (mL)</td>
<td>6 (0.2-48)</td>
</tr>
<tr>
<td>Median peripheral blood eosinophil (%)</td>
<td>3.4 (1-25.7)</td>
</tr>
</tbody>
</table>

### TABLE 2. Evaluation of the correlation between laboratory findings, the mean duration of the updosing phase and OIT onset age of the patients

<table>
<thead>
<tr>
<th></th>
<th>OIT onset age of the patients</th>
<th>IgE</th>
<th>CM-sIgE</th>
<th>Wheal diameters CM in SPTs</th>
<th>Cumulative provocative dose in the OFC</th>
<th>The mean duration of the buildup phase (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OIT onset age of the patients (month)</td>
<td>rs</td>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgE (kU/l)</td>
<td>0.302</td>
<td>0.070</td>
<td>0.677**</td>
<td>0.265</td>
<td>-0.221</td>
<td>-0.030</td>
</tr>
<tr>
<td>CM-sIgE (kUA/l)</td>
<td>0.076</td>
<td>0.631</td>
<td>0.677**</td>
<td>0.000</td>
<td>0.004</td>
<td>0.093</td>
</tr>
<tr>
<td>Wheal diameters CM in SPTs (mm)</td>
<td>0.053</td>
<td>0.739</td>
<td>0.436**</td>
<td>1.000</td>
<td>0.004</td>
<td>0.093</td>
</tr>
<tr>
<td>Cumulative provocative dose in the OFC (mL)</td>
<td>0.086</td>
<td>0.614</td>
<td>-0.221</td>
<td>-0.091</td>
<td>0.003</td>
<td>0.232</td>
</tr>
<tr>
<td>Patient ages, sex distribution (F/M)</td>
<td>The reactive dose of OFC</td>
<td>Reaction doses of adverse reactions</td>
<td>The mean duration of buildup phase (weeks)</td>
<td>Symptoms</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>37 months, M</td>
<td>0.5 mL</td>
<td>140 mL</td>
<td>25</td>
<td>Lokalized urticaria</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 36 months, M                       | 48 mL                    | 50 mL 55 mL                       | 12                                     | Lokalized urticaria  
Urticaria and angioedema |
| 36 months, M                       | 19 mL                    | 25 mL 30 mL 38 mL                 | 15                                     | Lokalized erythema, itching of the throat  
Cough, wheezing,  
Exacerbation of atopic dermatitis |
| 40 months, M                       | 0.3 mL                   | 0.6 mL 1.5 mL 12 mL               | 20                                     | Lokalized urticaria  
Cough, wheezing,  
Lokalized urticaria |
| 36 months, F                       | 10 mL                    | 15 mL                             | 19                                     | Lokalized urticaria |
| 38 months, F                       | 6 mL 16 mL               |                                    | 14                                     | Vomiting  
Localizer urticaria |
| 37 months, F                       | 6 mL                     | 5 mL 100 mL                       | 20                                     | Vomiting, refuse to take milk  
Localizer urticaria |
| 38 months, M                       | 3 mL                     | 15 mL 30 mL                       | 20                                     | Exacerbation of atopic dermatitis  
Localizer urticaria |
| 36 months, F                       | 2.5 mL                   | 2 mL 6 mL                         | 11                                     | Cough, respiratory distres |
| 50 months, F                       | 1.4 mL                   | 5 mL                              | 20                                     | Localizer urticaria,  
Cough, wheezing,  
Localizer urticaria  
Vomiting |
| 36 months, F                       |                          | 50 mL                             | 19                                     | Sistemic urticaria  
Localizer urticaria |
| 42 months, F                       | 6 mL                     | 12 mL 36 mL                       | 21                                     | Cough, respiratory distres  
Erythema, itching in the mouth and throat |
| 68 months, F                       | 6 mL                     | 10 mL 25 mL                       | 19                                     | Cough, respiratory distres  
Erythema, itching in the mouth and throat |
| 36 months, M                       | 6 mL                     | 12 mL 45 mL                       | 18                                     | Localizer urticaria  
Exacerbation of atopic dermatitis |
| 37 months, M                       | 5 mL                     | 19                                 | Localizer urticaria                     |
### TABLE 4. OIT duration and laboratory findings between the two groups of patients with and without adverse reactions

<table>
<thead>
<tr>
<th>Adverse reactions</th>
<th>No (n=26)</th>
<th>Yes (n=16)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>OIT onset age of the patients (month)</td>
<td>40.9±5.6 (36-156)</td>
<td>40.3±8 (36-68)</td>
<td>0.26</td>
</tr>
<tr>
<td>The mean duration of buildup phase (weeks)</td>
<td>15.5±3.3 (9-24)</td>
<td>21.4±8.6 (12-43)</td>
<td>0.001</td>
</tr>
<tr>
<td>The mean duration of the maintenance phase (weeks)</td>
<td>25.8±5.1 (12-39)</td>
<td>26.8±6.7 (13-37)</td>
<td>0.82</td>
</tr>
<tr>
<td>Median Total IgE (kU/l) (at the beginning of OIT)</td>
<td>125 (4-1731)</td>
<td>118 (9.9-2142)</td>
<td>0.83</td>
</tr>
<tr>
<td>Median CM-sIgE (kUA/l) (at the beginning of OIT)</td>
<td>4.2 (0.5-75.7)</td>
<td>11.9 (1.9-100)</td>
<td>0.17</td>
</tr>
<tr>
<td>Median CM-sIgE (kUA/l) (at the end of the updosing phase)</td>
<td>3.8 (0-63.7)</td>
<td>5.6 (0.2-72.2)</td>
<td>0.288</td>
</tr>
<tr>
<td>Median CM-sIgE (kUA/l) (6 months of maintenance phase)</td>
<td>2 (0-43.2)</td>
<td>2.1 (0.1-72.2)</td>
<td>0.445</td>
</tr>
<tr>
<td>Median CM-sIgE (kUA/l) (1 year of maintenance phase)</td>
<td>0.8 (0-16.5)</td>
<td>13 (0-75.7)</td>
<td>0.487</td>
</tr>
<tr>
<td>Median CM-sIgE (kUA/l) (2 years of maintenance phase)</td>
<td>0.4 (0-9.8)</td>
<td>0.8 (0-9.8)</td>
<td>0.168</td>
</tr>
<tr>
<td>Median CM-sIgE (kUA/l) (3 years of maintenance phase)</td>
<td>0.4 (0-0.4)</td>
<td>0.1 (0-0.2)</td>
<td>***</td>
</tr>
<tr>
<td>Wheal diameters to CM in SPT (mm)</td>
<td>6.5 (0-20)</td>
<td>9.5 (0-35)</td>
<td>0.035</td>
</tr>
<tr>
<td>Median peripheral blood eosinophil (%) (at the beginning of OIT)</td>
<td>4.3 (0.9-25.7)</td>
<td>1.9 (0-8.5)</td>
<td>0.082</td>
</tr>
<tr>
<td>Median peripheral blood eosinophil (%) (at the end of the updosing phase)</td>
<td>3.7 (0.9-15.1)</td>
<td>3.7 (1.4-10.6)</td>
<td>0.841</td>
</tr>
<tr>
<td>Median cumulative provocative dose of OFC (mL)</td>
<td>4.5 (0.2-48)</td>
<td>6 (0.3-48)</td>
<td>0.783</td>
</tr>
</tbody>
</table>

### TABLE 5. Patient characteristics according to the history of anaphylaxis

<table>
<thead>
<tr>
<th>History of anaphylaxis</th>
<th>History of anaphylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(no) (n:35)</td>
<td>(yes) (n:7)</td>
</tr>
<tr>
<td>Median Total IgE kU/l) (at the beginning of OIT)</td>
<td>76 (4-1461)</td>
</tr>
<tr>
<td>Median CM-sIgE values (kUA/l) (at the beginning of OIT)</td>
<td>4.3 (2-75)</td>
</tr>
<tr>
<td>Median wheal diameters to CM in SPT (mm) (at the beginning of OIT)</td>
<td>7 (3-20)</td>
</tr>
<tr>
<td>The mean duration of buildup phase (weeks)</td>
<td>16.8±3.2 (10-25)</td>
</tr>
<tr>
<td>Median cumulative provocative doses of OFC (mL)</td>
<td>0.85 (0.3-1.4)</td>
</tr>
<tr>
<td>Adverse reactions during OIT (yes)</td>
<td>31.4% (n=11)</td>
</tr>
<tr>
<td>Number of reactions during OIT</td>
<td>0 (0-3)</td>
</tr>
</tbody>
</table>
FIG. 1. Oral immunotherapy protocol for cow’s milk.

FIG. 2. Wheal diameters (mm) to cow’s milk (commercial extract) in skin prick test.
FIG. 3. The median CM-sIgE level (kUA/l) results in three years follow-up OIT.