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ORIGINAL ARTICLE

Food Allergy and Gastrointestinal Disease

# Long-term changes in milk component immunoglobulins reflect milk oral immunotherapy outcomes in Finnish children

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## Abstract

**Background:** Milk oral immunotherapy (OIT) may increase the amount of milk protein that can be ingested without triggering an allergic reaction. It is important to understand why some patients benefit from the treatment while others do not.

**Objective:** The aim was to define the differences in the milk allergen component-specific (casein, α-lactalbumin, ß-lactoglobulin) immunoglobulin (sIg [sIgE, sIgG4, and sIgA]) levels relative to the long-term outcomes of milk OIT.

**Methods:** In this long-term, open-label follow-up study, 286 children started milk OIT between 2005 and 2015. Follow-up data were collected at two points: the post-buildup phase and long term (range 1–11 years, median 6 years). Comparisons of sIg levels were made among three outcome groups of self-reported long-term milk consumption (high-milk dose, low-milk dose, and avoidance).

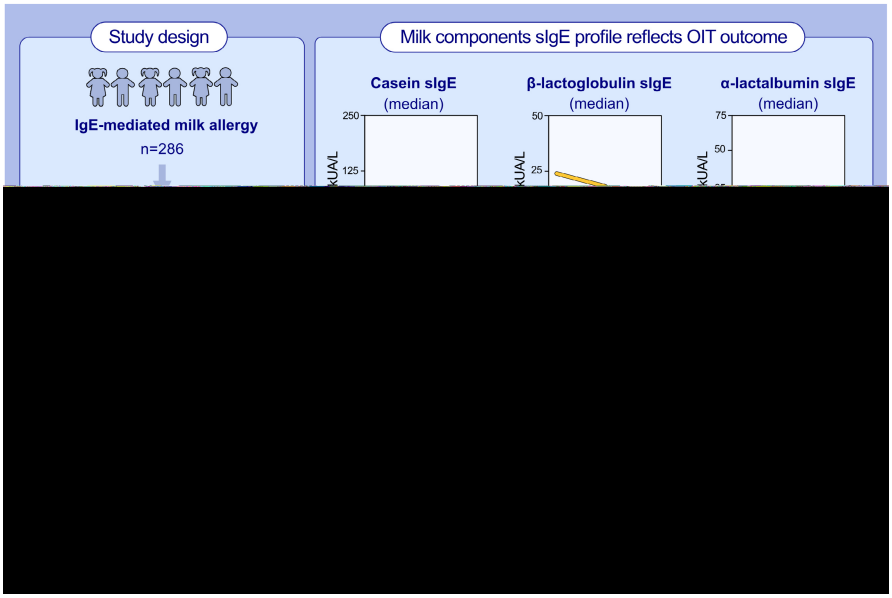
**Results:** A total of 168 (59%) of the 286 patients on OIT participated. Most patients (57%) were in the high-dose group; here, 80% of these patients had a baseline casein sIgE value less than 28 kUA/L, they had the lowest casein sIgE levels at all time ($p < .001$), their casein sIgG4/IgE levels increased, and long-term casein sIgA was highest compared with the low-dose and avoidance groups ($p = .02$). Low-milk dose group had the highest casein sIgG4/IgE levels in long term ($p = .002$).

**Conclusion:** The baseline Ig profiles and responses to milk OIT differed depending on long-term milk consumption. Lower casein sIgE levels were associated with better outcome. Milk casein sIgA differed in the long term among high-milk consumers.

KEYWORDS
cow's milk allergy, immunoglobulins, long-term follow-up, milk components, oral immunotherapy

**Abbreviations:** AUC, Area under the curve; kUA/L, kilounits of allergen-specific IgE per liter; mL, milliliter; NNT, number needed to treat.; OIT, oral immunotherapy; ROC curves, receiver operating characteristics curves; sIgE, sIgA, sIgG4, specific immunoglobulin E, A G4.

Kauppila and Hinkkanen equal contribution.




**GRAPHICAL ABSTRACT**

This long-term open-label follow-up study evaluates the differences in the milk allergen component-specific Ig levels relative to the long-term outcomes of milk OIT. The baseline Ig profiles and responses to milk OIT differ depending on long-term milk consumption; lower casein sIgE levels are associated with better outcome. sIgG4/IgE ratio distinguishes the long-term OIT outcome at early timepoints. Higher casein IgA is associated with high-milk dose in the long-term phase.

Abbreviations: OIT, oral immunotherapy; Ig, immunoglobulin; sIg, specific immunoglobulin; kUA/L, kilounits of allergen-specific IgE per liter; T, time point

## 1 | INTRODUCTION

Among young children, a milk allergy is the most common food allergy in Finland.[1] Usually, a milk allergy is outgrown by school age, but a severe milk allergy tends to be more persistent.[2] The current guidelines for treating a milk allergy include dietary avoidance of milk and preparations for the accidental ingestion of milk protein by ensuring that allergy medicine is available.[1,3] In experimental settings, oral immunotherapy (OIT) for a milk allergy has shown promise in promoting desensitization. In milk OIT, patients' immune systems are trained to manage increasing amounts of milk protein.[4–10] However, milk OIT is associated with adverse effects, and the long-term success rate in our previous study was 56%.[11]

The search to better understand the immunological mechanism of OIT is ongoing, and one aim is to identify the patients who will benefit from milk OIT.[12,13] Specific immunoglobulin E (sIgE) levels toward milk proteins are important in defining the outcome of milk OIT. Higher milk or milk component sIgE concentrations at baseline are associated with adverse effects to milk OIT and with treatment failure.[7–11,14,15]

Little by little, there are more studies available on long-term outcomes of milk OIT.[10,11,15,16] Patients might react to milk occasionally, even after years on maintenance dose.[11,16] The sIgG4 has shown to increase during OIT.[12,14] Further, the role of sIgA in OIT is unknown. In the current study, we sought to longitudinally determine sIgE, sIgG4, sIgG4/IgE ratio, and sIgA responses to milk-specific components in relation to the long-term outcomes of milk OIT in a real-life setting.

## 2 | METHODS

### 2.1 | Study design

The current study was a long-term, open-label follow-up study of patients undergoing milk OIT. Previously, we reported the clinical outcomes, and here, we focus on immunological changes related to milk OIT outcomes of the same study group.[11] Data were collected at three time points: before treatment (T1), after buildup (T2, 3 months after reaching the maintenance phase), and in the long-term follow-up, which ranged between 1 and 11 years (T3). The first two time points were studied longitudinally, and the long-term data from the third time point were collected as a cross-sectional study. All patients started the same OIT protocol (Table S1) and were divided into groups according to their self-reported long-term milk consumption, which reflected the achieved milk desensitization level. Comparisons were made among the following groups: a high-dose group (consuming at least 200 ml of milk daily, >200 ml), a low-dose group (consuming 10–199 ml of milk), and an avoidance group (discontinued OIT at some point during the treatment, 0 ml of milk). Figure 1 presents the study flow chart. To obtain the results, we compared the differences between the groups based on their milk consumption levels in the long term (T3).

### 2.2 | Study setting

The study was performed at two university hospitals in Finland (Skin and Allergy Hospital, Helsinki and Tampere University Hospital,

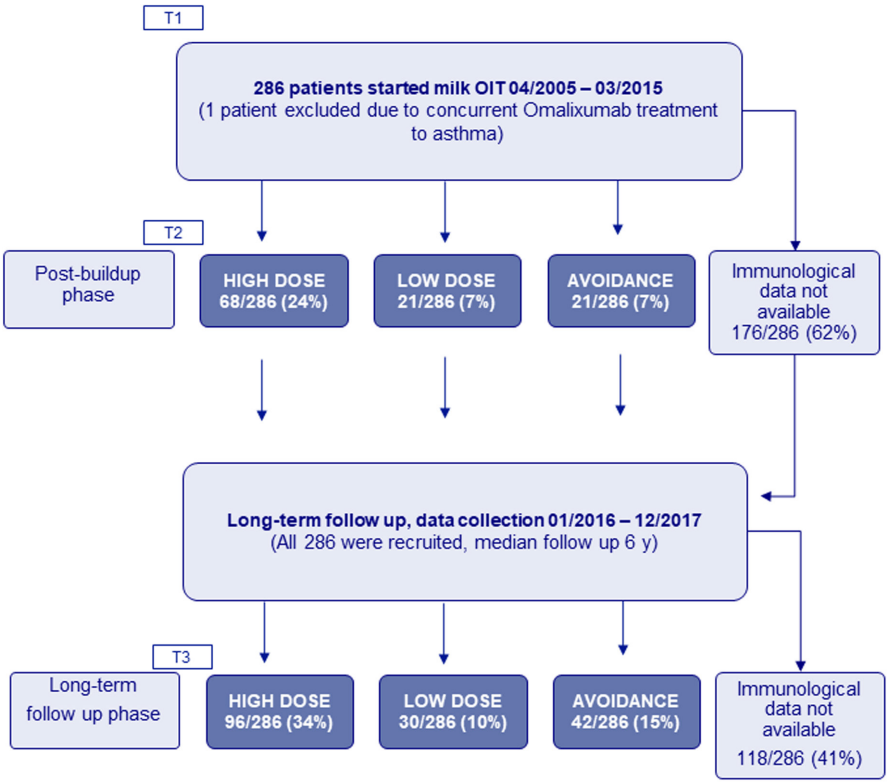



**FIGURE 1** Study flowchart represents study design and patient's disposition in three different time points: baseline (T1), post-buildup (T2), and at the long term (T3). Groups according to use of milk in long term: high dose ≥200 ml, low dose 10–199 ml, and avoidance 0 ml of milk daily.

Tampere). A nonrandomized milk OIT program started in 2005 with a protocol adapted from Meglio et al.[5] Increasing doses of milk protein were administered daily at home, from 0.5 μg at the beginning to a maintenance dose of 200 ml of milk (6.4 g of milk protein) at the end of a four-month buildup phase. After the patients reached the maintenance dose, they were advised to continue daily milk consumption (Table S1).

Follow-up data were collected 3 months after the patients reached the maintenance dose, and cross-sectional long-term data were gathered between January 2016 and December 2017 (Figure 1). Blood serum samples were collected at all time points and frozen for later analysis. An open milk challenge was offered to the patients in the low-dose or avoidance group during the long-term follow-up. At the long-term time point, 18 patients with a milk allergy who did not undergo milk OIT were included for comparison.

## 2.3 | Participants

Patients were at least 5 years old and had a challenge-confirmed IgE-mediated milk allergy. The patients had a positive reaction to rechallenge or a recent severe reaction from milk protein before starting the milk OIT protocol. Uncontrolled asthma or any other severe medical condition was a criterion for exclusion. We recruited all patients who had started milk OIT between April 2005 and March 2015, regardless of the primary outcome of the OIT (Figure 1). This time frame varied from the previous report[11] related to a more detailed serum sample collection.

We also enrolled patients from Helsinki who did not participate in the milk OIT; these patients had been offered the OIT protocol during the study period (i.e., they fulfilled the inclusion criteria for milk OIT) but had decided not to start the treatment. This recruitment was done by phone, mail, or during the routine follow-up visit. These patients were asked to participate in an open milk challenge and to give a blood sample.

The Institutional Ethics Committees of the Helsinki University Hospitals and the University Hospitals of Helsinki and Tampere approved the study protocol and the follow-up. The study was registered at ClinicalTrials.gov (Identifier: NCT02640014). Informed consent was obtained from the participants and from parents of children under 18 years of age.

## 2.4 | Variables

The primary objective was to determine the differences in milk component sIg concentrations in patients who underwent milk OIT but achieved different consumption abilities in the long term. The study aimed to define why some patients benefited from the treatment while others had difficulties or discontinued the treatment.

## 2.5 | Data sources

The primary data were collected during patient visits to the clinic before the milk OIT began and 3 months after reaching the maintenance dose. Long-term data were collected by mail or phone, and

patients were asked to provide a serum sample. We used a detailed questionnaire to define milk consumption and collected data from medical records.

An open milk challenge was offered to the patients in the low-dose and avoidance groups, and it was performed according the PRACTALL guidelines.[17]

The sIgE, sIgG4, and sIgA concentrations for casein, β-lactoglobulin, and α-lactalbumin were measured by ImmunoCAP (ThermoFisher Scientific; Phadia, Uppsala, Sweden).

### 2.6 | Statistics (bias, study size, quantitative variables, methods)

Our study results have the possibility of bias because the patients with successful milk OIT results were more likely to be eager to participate in the study (see article's Online Repository for lost to follow-up analysis, Table S2). We included all participants who had a long-term milk casein sIgE value and milk consumption information available. We enrolled some patients not undergoing milk OIT as a comparison group to empower the results (see Appendix S1).

Descriptive statistics were used to represent demographics, clinical characteristics, and comorbidities. Distributional differences in the variables were compared with the chi-square test for nominal data and with the nonparametric Mann–Whitney U-test or Kruskal–Wallis test for continuous data. For the IgG4/IgE ratios, IgE was converted into mg/L with a conversation factor of 1 kU/L = 0.0024 mg/L.[18]

The reported *P* values were two-tailed when applicable, and values less than 0.05 were considered statistically significant. SPSS statistics software version 27.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 8.4.2 (GraphPad Software Inc., La Jolla, CA) were used for the analyses. Figures and heatmaps were created using GraphPad Prism and the Perseus data analysis platform.[19] For heatmaps, the data were Z-score normalized, and the Euclidean method and the k-means algorithm were used for clustering. We drafted receiver operating characteristics (ROC) curves to illustrate the utility of the different immunoglobulins; cutoff values were evaluated based on clinical importance.[20]

For the heatmap and box and whisker plot, the data were log2 transformed. Prior to the log transformation and IgG4/IgE ratios, laboratory results that had a value of 0.00 were replaced with a value that was just under the detection limit of each antibody (see Appendix S1).

## 3 | RESULTS

A total of 286 milk OIT participants were recruited, and 168 patients (59%) participated. Here, 57% (96/168) of the study participants were in the high-dose group, 18% (30/168) of the patients were in the low-dose group, and 25% (42/168) of the patients discontinued treatment. Demographic and clinical characteristics are outlined in Table S3. Some plasma samples were lacking in relation to the challenges of the real-life, long-term study (Table 1A–C). No demographic variables or baseline open food challenge result differences were observed between the three long-term outcome groups (Table 1A). In the post-buildup phase, most (94%) of the high-dose group was consuming a high-milk dose, and a minority (32%) of the long-term avoidance group was able to achieve a high-milk dose (with these patients discontinuing milk consumption later; Table 1B). The median follow-up time (6 years) did not differ between the outcome groups ($p = .66$, Table 1C).

### 3.1 | Milk component sIgE and sIgG4/sIgE profiles differ depending on milk OIT outcome

Milk component sIgE concentrations differ in each long-term milk consumption group with all tested allergens (casein, ß-lactoglobulin, and α-lactalbumin) at each time point. The avoidance group (OIT discontinuation) had the highest sIgE levels (Figure 2, Table 1A-C, and Figure S1). In the avoidance group, 76% (22/29) of the patients had a baseline casein sIgE value of 17 kUA/L or more. In the high-dose group, 73% (52/71) of the patients had a baseline casein sIgE level <17 kUA/L, and 80% (57/71) had a value <28 kUA/L. Likewise, the ROC analysis of the baseline milk and milk component sIgE levels differed when comparing the values of the long-term high-dose and avoidance groups (Figure 3A). The cutoff for a long-term high-dose outcome with 35% sensitivity and 91% specificity was a baseline casein sIgE <3.6 kUA/L (Figure 3A).

The milk component ratios of sIgG4 to sIgE (sIgG4/IgE) in the baseline were highest among the high-dose group compared with the other groups ($p < .001$ to $p = .04$, respectively, Table 1A). In contrast, casein sIgG4 was lowest at baseline among the high-dose group compared to the other groups ($p = .01$ Table 1A). ROC curves comparing the baseline and post-buildup casein sIgG4 and sIgG4/IgE ratio values among the long-term high-dose or avoidance groups showed that the sIgG4/IgE ratio distinguished the long-term outcome at early timepoints (Figure 3B). The cutoff for a long-term high-dose outcome with 43% sensitivity, 95% specificity, and a likelihood ratio of nine was a baseline casein sIgG4/IgE > 87 kUA/L.

The milk component sIgG4 increased during the post-buildup phase (Figure S2). The high-dose group had the highest milk component sIgG4/IgE ratio in the post-buildup phase compared with the other groups ($p < .001$ to $p = .003$; Table 1B).

In the long term, the participants in the high-dose group had the lowest milk protein and milk component sIgE ($p < .001$ in all, Table 1C). The casein IgG4/IgE ratio was the highest in the low-dose group ($p = .002$) compared with the other groups (Table 1C).

### 3.2 | Higher casein sIgA was associated with high-milk dose in the long-term phase compared to the other groups

All three outcome groups had an sIgA toward milk components at baseline and post-buildup phases; furthermore, those

**TABLE 1** Characteristics, and results from the milk OIT follow-up, depending on the long-term milk consumption and the comparison between the three groups; (A) baseline (T1), (B) post-buildup (T2, 3 months after reaching the maintenance phase), (C) long term of up to 11 years of follow-up (T3).

| Characteristics | High-dose group ($n = 96$)[a] | Low-dose group ($n = 30$)[a] | Avoidance ($n = 42$)[a] | *p* value |
|---|---|---|---|---|
| (A) Baseline (T1) | | | | |
| Male sex | 55 (57) | 16 (53) | 23 (55) | .92 |
| Age (y) when OIT started | 7 (6.0–10) | 6.5 (6.0–8.0) | 7 (6.0–11) | .12 |
| Baseline OFC cumulative threshold, milk protein (mg)[b] | 320 (32–400) | 112 (56–416) | 96 (24–400) | .97 |
| Adrenalin used at the baseline milk challenge | 5/88 (5.7) | 4 (13) | 7 (17) | .09 |
| Milk sIgE (kUA/L) | 11 (5.0–26) | 39 (8.8–90) | 86 (18–335) | **<.001** |
| Casein sIgE (kUA/L) | 7.3 (1.9–22) $n = 71$ | 35 (2.4–121) $n = 22$ | 83 (14–222) $n = 29$ | **<.001** |
| α-lactalbumin sIgE (kUA/L) | 3.7 (1.6–14) $n = 72$ | 10 (3.8–24) $n = 22$ | 19 (2.8–48) $n = 28$ | **.003** |
| ß-lactoglobulin sIgE (kUA/L) | 3.5 (0.9–11) $n = 72$ | 11 (1.2–26) $n = 22$ | 23 (4.4–49) $n = 29$ | **<.001** |
| Casein sIgG4 (mg/L) | 0.9 (0.5–1.9) $n = 71$ | 1.8 (0.7–3.7) $n = 22$ | 2.5 (0.8–4.3) $n = 29$ | **.01** |
| α-lactalbumin sIgG4 (mg/L) | 0.6 (0.3–1.4) $n = 71$ | 0.8 (0.4–1.6) $n = 22$ | 1.5 (0.4–2.6) $n = 29$ | .051 |
| ß-lactoglobulin sIgG4 (mg/L) | 0.5 (0.2–1.3) $n = 71$ | 1.0 (0.4–2.4) $n = 22$ | 1.1 (0.4–2.6) $n = 29$ | **.03** |
| Casein sIgA (mg/L) | 0.3 (0.2–0.4) $n = 68$ | 0.3 (0.2–0.6) $n = 21$ | 0.4 (0.2–0.6) $n = 28$ | .20 |
| α-lactalbumin sIgA (mg/L) | 0.2 (0.1–0.3) $n = 68$ | 0.2 (0.2–0.2) $n = 21$ | 0.2 (0.2–0.4) $n = 28$ | .51 |
| ß-lactoglobulin sIgA (mg/L) | 0.2 (0.2–0.3) $n = 68$ | 0.2 (0.2–0.3) $n = 21$ | 0.3 (0.2–0.4) $n = 28$ | .30 |
| Casein sIgG4/sIgE (mg/L) | 55 (23–164) $n = 70$ | 36 (8.6–128) $n = 22$ | 15 (6.6–31) $n = 29$ | **<.001** |
| α-lactalbumin sIgG4/sIgE (mg/L) | 77 (25–146) $n = 71$ | 48 (17–109) $n = 22$ | 29 (9.6–52) $n = 28$ | **.04** |
| ß-lactoglobulin sIgG4/sIgE (mg/L) | 99 (26–219) $n = 71$ | 65 (27–210) $n = 22$ | 20 (11–63) $n = 29$ | **.001** |
| (B) Post-buildup results (T2) | | | | |
| Able to reach 200 ml milk consumption | 87/93 (94) | 21 (70) | 13/41 (32) | **<.001** |
| Milk sIgE (kUA/L) | 18 (5.3–34) $n = 22$ | 35 (3.6–144) $n = 8$ | 43 (11–221) $n = 6$ | .30 |
| Casein sIgE (kUA/L) | 5.5 (1.8–16) $n = 68$ | 21 (1.6–72) $n = 21$ | 53 (12–193) $n = 21$ | **<.001** |
| α-lactalbumin sIgE (kUA/L) | 5.5 (1.8–14) $n = 68$ | 11 (2.3–25) $n = 21$ | 21 (9.5–64) $n = 21$ | **.001** |
| ß-lactoglobulin sIgE (kUA/L) | 3.7. (1.0–9.3) $n = 68$ | 7.6 (0.7–20) $n = 21$ | 17 (4.6–31) $n = 21$ | **.003** |
| Casein sIgG4 (mg/L) | 2.8 (0.6–8.2) $n = 67$ | 3.6 (0.7–10) $n = 21$ | 7.4 (0.6–11) $n = 21$ | .68 |
| α-lactalbumin sIgG4 (mg/L) | 6.0 (1.3–13) $n = 67$ | 6.2 (1.6–15) $n = 21$ | 5.3 (0.9–16) $n = 21$ | .94 |
| ß-lactoglobulin sIgG4 (mg/L) | 4.5 (0.4–12) $n = 67$ | 4.7 (1.2–15) $n = 21$ | 4.2 (0.3–8.2) $n = 21$ | .60 |
| Casein sIgA (mg/L) | 0.5 (0.3–0.7) $n = 64$ | 0.4 (0.3–0.7) $n = 20$ | 0.7 (0.3–0.9) $n = 20$ | .50 |
| α-lactalbumin sIgA (mg/L) | 0.2 (0.2–0.4) $n = 64$ | 0.3 (0.2–0.4) $n = 20$ | 0.3 (0.2–0.6) $n = 20$ | .80 |
| ß-lactoglobulin sIgA (mg/L) | 0.3 (0.2–0.4) $n = 64$ | 0.3 (0.2–0.3) $n = 20$ | 0.3 (0.2–0.6) $n = 20$ | .53 |
| Casein sIgG4/sIgE (mg/L) | 163 (87669) $n = 67$ | 111 (16–664) $n = 21$ | 45 (14–103) $n = 21$ | **<.001** |
| α-lactalbumin sIgG4/sIgE (mg/L) | 454 (130–1199) $n = 67$ | 275 (47–595) $n = 21$ | 146 (32–224) $n = 21$ | **.001** |
| ß-lactoglobulin sIgG4/sIgE (mg/L) | 686 (227–1362) $n = 67$ | 428 (73–2048) $n = 21$ | 118 (32–387) $n = 21$ | **.003** |
| (C) Long-term results (T3) | | | | |
| Age (year), median | 15 (12–18) | 13 (12–16) | 14 (12–18) | .28 |
| Update follow-up time (year) | 6.0 (4.0–8.0) | 6.0 (4.0–7.3) | 6.0 (4.0–7.0) | .66 |
| Asthma | 62/91 (68) | 22 (73) | 30/41 (73) | .78 |
| Atopic skin | 68/90 (76) | 23/28 (82) | 36/41 (88) | .25 |
| Allergic rhinitis | 64/90 (67) | 21 (70) | 30/41 (71) | .95 |
| Milk sIgE (kUA/L) | 1.8 (0.7–5.0) | 5.9 (0.6–12) $n = 29$ | 21 (7.4–93) $n = 41$ | **<.001** |
| Casein sIgE (kUA/L) | 1.0 (0.3–3.1) | 3.4 (0.5–12) | 20 (1.8–75) | **<.001** |

TABLE 1 (Continued)

| Characteristics | High-dose group ($n = 96$)[a] | Low-dose group ($n = 30$)[a] | Avoidance ($n = 42$)[a] | $p$ value |
|---|---|---|---|---|
| α-lactalbumin sIgE (kUA/L) | 0.9 (0.2–3.3) $n = 83$ | 2.5 (0.4–5.9) $n = 27$ | 6.9 (2.2–21) $n = 39$ | **<.001** |
| ß-lactoglobulin sIgE (kUA/L) | 0.7 (0.2–1.5) $n = 83$ | 0.8 (0.1–4.4) $n = 27$ | 4.6 (1.4–17) $n = 38$ | **<.001** |
| Casein sIgG4 (mg/L) | 2.3 (0.0–10) $n = 67$ | 2.0 (1.3–16) $n = 21$ | 2.1 (1.0–3.9) $n = 28$ | .33 |
| α-lactalbumin sIgG4 (mg/L) | 1.7 (0.0–5.6) $n = 67$ | 1.7 (0.2–5.0) $n = 21$ | 1.1 (0.5–2.3) $n = 28$ | .87 |
| ß-lactoglobulin sIgG4 (mg/L) | 1.5 (0.0–8.3) $n = 67$ | 1.9 (0.3–4.4) $n = 21$ | 1.2 (0.5–1.8) $n = 28$ | .62 |
| Casein sIgA (mg/L) | 0.4 (0.3–1.2) $n = 67$ | 0.3 (0.2–0.4) $n = 22$ | 0.3 (0.2–0.4) $n = 28$ | **.002** |
| α-lactalbumin sIgA (mg/L) | 0.2 (0.2–0.3) $n = 67$ | 0.2 (0.1–0.3) $n = 22$ | 0.2 (0.1–0.3) $n = 28$ | .15 |
| ß-lactoglobulin sIgA (mg/L) | 0.2 (0.2–0.4) $n = 67$ | 0.2 (0.2–0.3) $n = 22$ | 0.3 (0.2–0.3) $n = 28$ | .35 |
| Casein sIgG4/sIgE (mg/L) | 1130 (2.4–4658) $n = 69$ | 1313 (352–5241) $n = 21$ | 37 (16–104) $n = 41$ | **.002** |
| α-lactalbumin sIgG4/sIgE (mg/L) | 898 (1.94–5500) $n = 69$ | 855 (89–1347) $n = 21$ | 62 (20–154) $n = 41$ | **.02** |
| ß-lactoglobulin sIgG4/sIgE (mg/L) | 1649 (77–7983) $n = 69$ | 1473 (275–9550) $n = 21$ | 66 (24–207) $n = 41$ | **<.001** |

*Note*: Statistical tests; chi-square test for groups of nominal data and Kruskal–Wallis test for nonparametric continuous data. The high-dose (consuming at least 200 ml of milk daily), low-dose (consuming less than 200 ml of milk daily), and avoidance (maintaining a milk-avoidance diet) groups. Bold values are $p < .05$.

Abbreviations: OIT, Oral immunotherapy; OFC, open food challenge; sIgE, specific IgE; sIgG4, specific IgG4; sIgA, specific IgA.

[a]Estimates for all variables are presented in percentage, unless otherwise indicated or with continuous data the sample median and the sample first and third quartiles.

[b]Data are available only from study unit 1, $n = 102$.

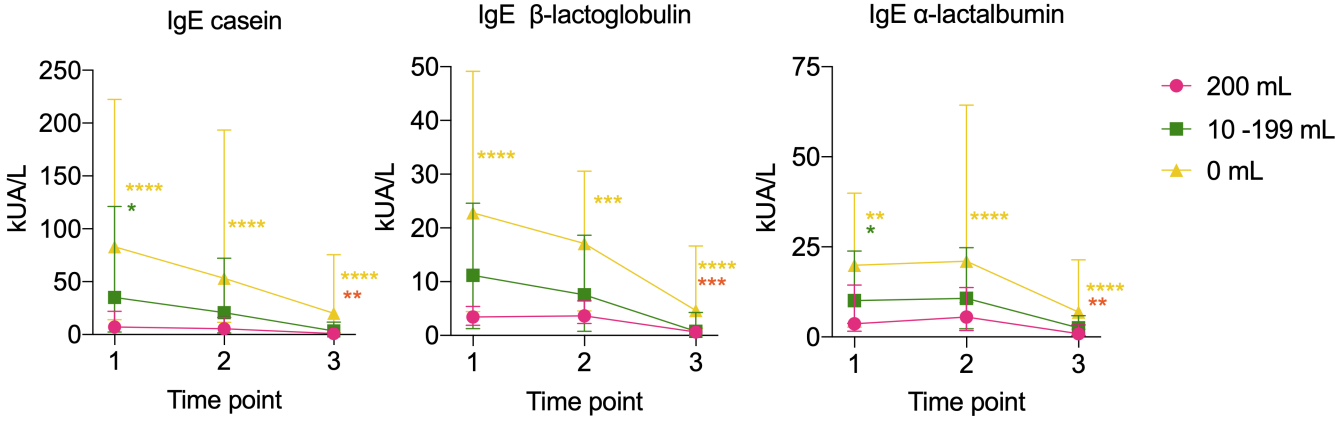


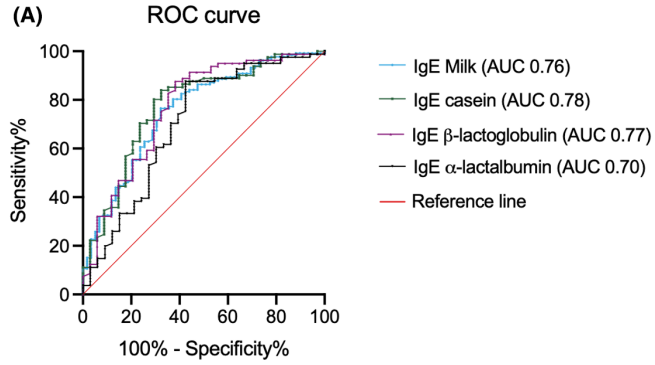

FIGURE 2 Milk sIgE (median, IR) at the baseline (T1), post-buildup (T2), and at the long term (T3). Groups according to use of milk in long term: 0 ml, 10–199 ml, and ≥ 200 ml of milk daily. Yellow 0 vs. >200 ml, green 10–199 vs. >200 ml, and orange 10–199 vs. 0 ml (Mann–Whitney U).

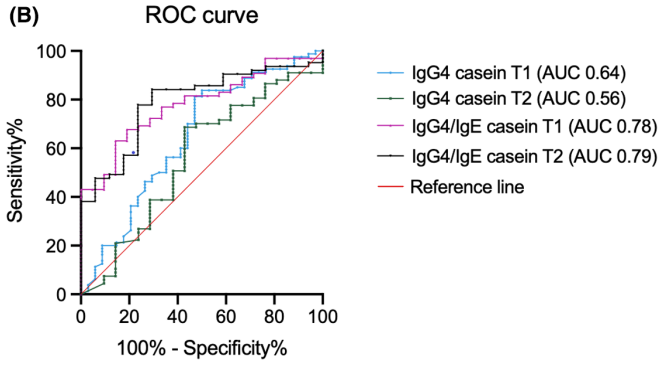
concentrations did not differ significantly between the groups (Table 1A, B, Figure S3). Median casein sIgA increased during the treatment (T1–T2), and the high-dose group had a significantly higher concentration ($p = .002$; Table 1C, Figure S3) compared with the low-dose and avoidance groups at the long-term time point. In hierarchical clustering, the patients with the highest concentrations of casein sIgA were mostly located in the high-dose group (Figure 4).

### 3.3 | Patients not undergoing milk OIT were less likely to consume milk

In addition, we enrolled milk allergy patients who had been offered milk OIT ($n = 18$) but who declined to highlight the results (Table S4). There were no statistically significant differences in sex, age, or atopic comorbidities between the two groups. Among the patients not undergoing milk OIT, 2 (11%) of the 18 patients had outgrown their milk allergy; in the milk OIT group, 56% (96 out of 168 patients) consumed at least 200 ml of daily milk ($p < .001$). To evaluate the effectiveness of this noncontrolled, real-life setup, the number needed to treat with successful milk OIT (consumed at least 200 ml of daily milk) was 2.2 patients (Figure S4).

Milk protein, casein, and ß-lactoglobulin sIgE were lower among participants in the milk OIT group compared with the nonmilk OIT group ($p < .001$, $p < .001$, and $p = .004$, respectively). Furthermore, the casein and ß-lactoglobulin IgG4/IgE ratios were higher among the participants of the milk OIT group ($p = .002$ and $p = .003$, respectively).




| 3A. 200 mL of milk at long-term | AUC (95% CI) | Cutoff (kUA/L) | Specificity % | Sensitivity % | Likelihood ratio |
|---|---|---|---|---|---|
| **Baseline** | | | | | |
| Whole milk sIgE | 0.76 (0.68-0.83) | <5.8 | 90 | 33 | 3,2 |
| Casein sIgE | 0.78 (0.67-0.87) | <3.6 | 91 | 35 | 3,9 |
| β-lactoglobulin sIgE | 0.76 (0.65-0.87) | <1.4 | 91 | 32 | 3,6 |
| α-lactalbumin sIgE | 0.70 (0.59-0.82) | <1.1 | 91 | 20 | 2,2 |

| 3B. 200 mL of milk at long-term | AUC (95% CI) | Cutoff (kUA/L) | Specificity % | Sensitivity % | Likelihood ratio |
|---|---|---|---|---|---|
| **Baseline** | | | | | |
| Casein sIgG4 | 0.64 (0.53-0.76) | <0.38 | 91 | 20 | 2,3 |
| Casein sIgG4/IgE | 0.78 (0.68-0.88) | >87 | 95 | 43 | 9,0 |
| **Post buildup** | | | | | |
| Casein sIgG4 | 0.56 (0.41-0.71) | <0.42 | 86 | 19 | 1,4 |
| Casein sIgG4/IgE | 0.79 (0.68-0.88) | >179 | 94 | 48 | 8,1 |

**FIGURE 3** Receiver operating characteristics (ROCs) depending on the long-term outcome (high-milk dose or avoidance) and cutoff levels, sensitivity, specificity, and likelihood ratios. (A) Baseline milk and milk component sIgE levels. (B) Baseline and post-buildup casein sIgG4 and sIgG4/IgE levels.

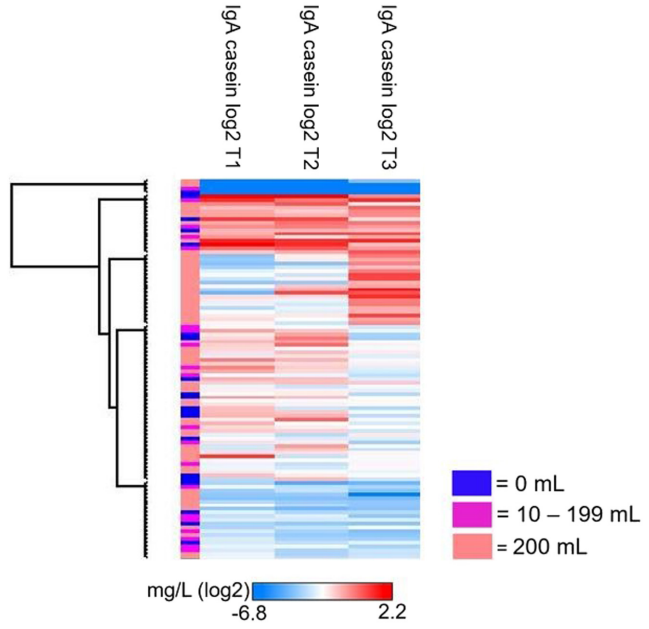



**FIGURE 4** Hierarchical Euclidian clustering of casein sIgA (T1 = baseline, T2 = post-buildup, and T3 = long term) in relation to other patients at same time point. Each row represents one patient and each column log2 transformed IgA at a different time point. High concentration is shown as red, and low concentration is shown as blue.

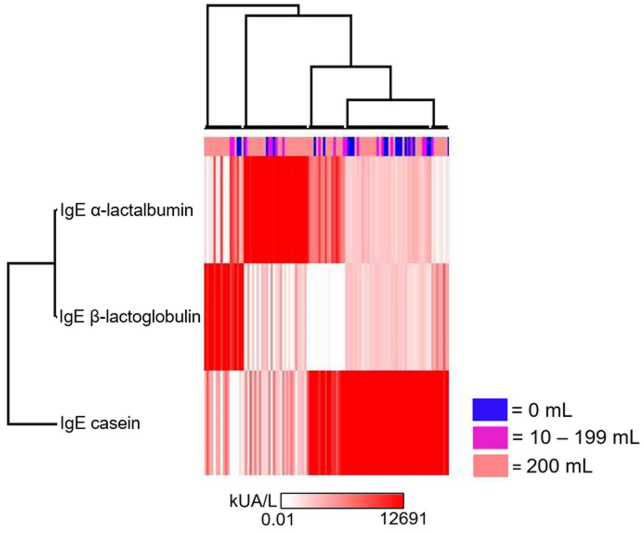



**FIGURE 5** Hierarchical Euclidian clustering of combined sIgE from three time points ($n = 108$). Individual patients' casein sIgE are compared to the ß-lactoglobulin and α-lactalbumin. Each column represents one patient and each row the concentration of allergen-specific IgE. High concentration is shown as red and low is white.

## 3.4 | Reactivity to milk in open food challenges was associated with higher sIgE

To test the reactivity to milk at the long-term time point among the low-dose group and the avoidance group, we performed 25 open milk challenges (35%, 25/72 participated) (Table S5). The baseline milk, casein, and ß-lactoglobulin sIgE concentrations were statistically higher in the group that had a positive challenge ($n = 13$) than in the negative group ($n = 12$; $p = .02$, $p = .001$, and $p = .007$, respectively; Table S5). Long-term milk component sIgE concentrations were statistically higher in the positive challenge group. In the group without a reaction to milk after an oral challenge, the casein and ß-lactoglobulin sIgG4/IgE ratios were higher than the positive challenge group at the baseline ($p = .002$, $p = .01$) and in the long term ($p = .007$, $p = .01$; Table S5).

## 3.5 | Patients with sensitization to casein sIgE were more likely to discontinue treatment

We compared the combined sIgE levels to different milk protein components, as shown in Figure 5. Related to the limitations of a real-life, long-term study, complete data (all samples available from

three different time points from same patient) were available from 108 patients. The casein sIgE of single patients was compared with the ß-lactoglobulin and α-lactalbumin, showing that sensitization to casein sIgE was clustered in the avoidance group. Vice versa, sensitization to ß-lactoglobulin and α-lactalbumin was more prominent in the high-dose group. In addition, our ROC curves showed that the baseline casein sIgE had the largest area under the curve (AUC 0.78), when compared to the long-term high-dose and avoidance groups (Figure 3A).

## 4 | DISCUSSION

The current study showed that patients who achieve different outcomes with milk OIT present with variable immunological profiles at three time points—baseline, after OIT completion, and at a median of 6 years of follow-up. Casein, ß-lactoglobulin, and α-lactalbumin sIgE concentrations were lower in the group consuming a high-milk dose than in the low-dose group or avoidance group at all time points. The milk component of sIgE decreased over time, but reactivity toward the milk protein remained among those who had high-milk or casein sIgE before milk OIT and at the long-term follow-up time point, as our open milk challenges showed. This indicated that there were similarities in milk sIgE patterns among patients with persistent milk allergy and those in other milk OIT long-term study.[15,21]The baseline milk IgE and the casein sIgE are major players in defining the outcome of milk OIT shown in many studies; higher milk or casein sIgE are associated with less successful OIT outcomes.[6–11,14,15,22,23] In addition to the current knowledge, we showed that the immunological responses related to milk OIT varied between the outcome groups and that the sIgG4 levels should be evaluated with sIgG4/IgE ratio.

Our results related to casein sIgE are in line with the current understanding of the role of sIgE in assessing OIT outcomes.[23] The role of sIgG4 is presumed to be opposite that of sIgE; sIgG4 might have a protective effect against allergies. Allergen-specific IgG4 production is induced by exposure to protein antigens, so IgG4 increases during the OIT buildup phase.[14,15,22,23] The sIgG4/IgE ratio has been presented as perhaps an even more important value than the absolute quantity of sIgG4 in relation to the clinical outcomes of OIT, and a high baseline sIgG4/IgE ratio may be predictive of OIT-induced sustained unresponsiveness to an allergen.[23] We show here that the casein sIgG4/IgE ratio was the highest at baseline in the high-dose group, but the absolute casein sIgG4 level was the lowest. In terms, this highlights the importance of evaluating the ratio, not the absolute number of sIgG4, as demonstrated with ROC curves. In the post-buildup phase, the high-dose group had the highest milk components sIgG4/IgE. Interestingly, at the long-term time point, the low-dose group had the highest casein sIgG4/IgE concentration.

IgA is usually presented as an antibody of mucous membranes, and little is known about its role in the outcome of OIT.[23] Higher milk sIgA concentrations in the serum have been associated with the development of a natural tolerance to a milk allergy.[24] We were able to detect milk component sIgA among all outcome groups at all three time points. At the long-term time point, the high-dose group had the highest casein sIgA, and this difference was statistically significant compared to the other dose groups. It is unclear whether this was related to the positive response to the milk OIT or whether this group included many patients who would have outgrown their milk allergy anyway. Casein sIgA level might not be able to predict the outcome of milk OIT but higher level was associated with better outcome in long term.

In the literature, casein has been shown to be a major allergenic component of cow's milk.[25,26] When the allergen sIgE concentrations were compared within each patient, a high sIgE concentration to casein was indicated as a major allergen component among milk OIT patients with less successful outcomes, and casein had the largest AUC in the ROC analysis. Casein is very resistant to heating compared with whey proteins (ß-lactoglobulin and α-lactalbumin).[26,27] In line with understanding the role of casein in a milk allergy, children with a persistent milk allergy have been reported to largely produce IgE antibodies directed against certain casein epitopes, and serum IgE to casein has been predictive of the outcome of an oral milk challenge.[28,29]

Our study has several limitations. We did not have a control group from the beginning of the study to ensure that the measured changes were caused by the milk OIT and not simply a reflection of the natural course of the individual's milk allergy. However, we obtained some immunological data from nonmilk OIT patients to strengthen our results, but it should be noted that we only had data from these patients for the long term, and the small sample size limits the usability of these results. We had some gaps in the follow-up data collection (e.g., some serum samples were missing in some patients, especially at T2), and we did not have immunological data available for 44% of the participants in the original milk OIT study.[11] We did not discontinue treatment to define sustained unresponsiveness, nor did we test the reactivity to baked milk, though those results would be of interest. The generalizability of our results is limited to milk OIT. Differences may exist in OIT responses among different food allergies; for example, a milk allergy is usually outgrown by age, but a peanut allergy is more persistent.[3] Moreover, our immunological results are restricted to current understanding of immunoglobulin values, and no cellular analyses were performed. The usability of cutoff values to predict the long-term outcome of milk OIT was limited with low sensitivity.

The strengths of this article include its large sample size of patients undergoing milk OIT ($n$ = 168) and its long follow-up time (up to 11 years). Our study offers clinical value in terms of supporting the idea of personalized medicine approaches to milk OIT,[13] because our results highlight the differences between the patient's immunoglobulin values and outcomes. Patients with a persistent milk allergy might benefit from different types of OIT protocols, depending on their baseline immunological profiles (e.g., a patient with low casein sIgE and higher sIgG4/IgE ration could aim for larger maintenance doses of milk or a faster buildup phase and vice versa). Our calculation for the number needed to

treat (2.2) for milk OIT was at the same level as in peanut OIT (2).[30] We reinforced our results with data from open milk challenges and nonmilk OIT patients. Among those groups, the casein sIgE level was lower, and casein sIgG4/IgE was higher within non-reactive group or milk OIT participants.

## 5 | CONCLUSIONS

In this study, we showed that milk OIT patients have variable immunological paths at the start of the treatment and these paths may define the outcome. Casein sIgE was higher at all time points among the avoidance group compared to the other groups, but casein sIgE decreased over time. The reactivity toward milk remained especially among patients with a higher casein sIgE. Casein sIgG4/IgE ratio is more important than casein sIgG4 level itself to define the outcome of milk OIT. Casein sIgG4/IgE increased among milk consumers. There were some levels of casein sIgA detectable in all groups at all time points, and a high-milk dose was associated with higher casein sIgA in the long term compared with the other groups.

### AUTHOR CONTRIBUTIONS

AKK, AP, and MJM designed the study. TK and MP gathered the clinical data. TK, AKK, and MP performed the open food challenges. VH and TS analyzed immunological data. TK, VH, TS, and PK performed data analysis. TK wrote the manuscript. All authors participated in editing the manuscript.

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### CONFLICT OF INTEREST

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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