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Factors influencing the natural history of non-IgE-mediated gastrointestinal food allergies in paediatric age: a prospective multicentre cohort study

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ABSTRACT

Background We aimed at identifying the factors influencing the natural history of non-IgE-mediated gastrointestinal food allergies (non-IgE-GIFA), a group of common paediatric conditions including food protein–induced: enteropathy (FPE), allergic proctocolitis (FPIAP), enterocolitis syndrome (FPIES), and motility disorders (FPIMD).

Methods Prospective multicentre cohort study involving paediatric patients (both sexes, aged ≤14 y) with non-IgE-GIFA diagnosed and followed for 24 months at a Tertiary Centre for Paediatric Allergy, Gastroenterology and Nutrition. Anamnestic and clinical data were collected from all enrolled patients.

Results 123 non-IgE-GIFA patients were enrolled (56% male, median age (IQR) 150 (60–300) days): FPE (39%), FPIES (17%), FPIAP (16%) and FPIMD (28%). 42% of patients had multiple food allergies (FAs) at baseline, and 64% had a positive family history of allergy. Male sex (OR = 2.24, 95% CI 1.07 to 4.71) and every 1 month of diagnostic delay (OR=1.09, 95% CI 1.01 to 1.18) were positively associated with the occurrence of multiple FAs. At 24-month follow-up, 54% of patients acquired immune tolerance. This rate was higher in FPIAP (75%), when compared with FPIMD (62%), FPE (54%) and FPIES (24%). The odds of 24-month immune tolerance acquisition rate was lower in children with family history of allergy (OR=0.41, 95% CI 0.19 to 0.89) and in those with multiple FAs at baseline (OR=0.24, 95% CI 0.11 to 0.51). At 24-month follow-up, the rate of patients with allergic march was 0.46 (95% CI 0.38 to 0.55, n=57/123), without differences comparing the four phenotypes. The presence of multiple FAs at baseline was associated with an increased risk of developing allergic march (OR=2.22, 95% CI 1.07 to 4.61) at 24-month follow-up.

Conclusions The results of the study suggest the potential role of modifiable and non-modifiable risk factors influencing the natural history of paediatric patients affected by non-IgE-GIFA.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Non-IgE-mediated gastrointestinal food allergies (non-IgE-GIFA) are a group of common conditions in the paediatric age characterised by subacute/chronic gastrointestinal symptoms. The paucity of data on factors influencing natural history contributes to the actual difficulties in the management of these conditions.

WHAT THIS STUDY ADDS

⇒ This prospective multicentre cohort study highlighted the presence of non-modifiable (i.e., male sex, allergy family risk, non-IgE-GIFA phenotype) and modifiable factors (e.g., diagnostic delay, presence of multiple FAs, formula choice in children with cow's milk allergy) influencing the immune tolerance acquisition and occurrence of allergic march.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ A better knowledge of the main non-IgE-GIFA features could facilitate an earlier recognition of these conditions with a potential impact on disease course. The early diagnosis could limit the occurrence of multiple FAs facilitating the acquisition of immune tolerance and limiting the development of allergic march in these patients.

INTRODUCTION

Food allergy (FA) is one of the most common chronic conditions in paediatric age, affecting up to 10% of children in westernised countries.[1] It has been estimated that up to 50% of paediatric FA patients present a predominant gastrointestinal involvement deriving from a non-IgE-mediated immune mechanism.[1] Non-IgE-mediated gastrointestinal food allergies (non-IgE-GIFA) are an evolving web of conditions including food protein–induced enteropathy (FPE), food protein–induced allergic proctocolitis (FPIAP), food protein–induced enterocolitis syndrome (FPIES) and food protein–induced motility disorders (FPIMD).[2 3] Data on clinical features of these conditions mainly derived from retrospective studies and single-centre experience, focusing on single non-IgE-GIFA phenotypes.[4–19]

Retrospective studies suggested the potential role of selected factors influencing the natural history of paediatric patients with FPIES and FPIAP.[4 20–22] Prospective comparative studies investigating the potential role of prognostic factors in modulating the natural history of the four different non-IgE-GIFA phenotypes are lacking.

The non-IgE-mediated gastrointestinal food allergy (NIGEFA) prospective multicentre cohort study was designed to address current knowledge gaps by comparatively investigating several aspects of the four non-IgE-GIFA phenotypes. Here, we reported data on factors potentially influencing the disease course of a cohort of children affected by these conditions followed for 24 months at a Tertiary Centre for Paediatric Allergy, Gastroenterology and Nutrition. The paper follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Supplementary Appendix 1).

METHODS

Study design

Prospective multicentre comparative cohort study, conducted from January 2017 to January 2022. The study was carried out at the coordinating Tertiary Centre for Paediatric Allergy, Gastroenterology and Nutrition in collaboration with 10 affiliated Italian paediatric hospitals, each evaluating at least 20 patients for suspected non-IgE-GIFA. The aims and the design of the study were presented and discussed during one meeting with all investigators. Once evaluated at one of the affiliated paediatric hospitals, the subjects with suspected non-IgE-GIFA were referred to the coordinating centre for the diagnostic work-up and follow-up.

The inclusion criteria were age ≤14 years, with anamnestic and clinical features suggestive for non-IgE-GIFA, as previously described.[2]

The exclusion criteria were the following: age >14 years; concomitant presence at the baseline of infectious diseases; chronic systemic diseases; malignancies; immunodeficiencies; autoimmune diseases; coeliac disease; metabolic and genetic diseases; cystic fibrosis or other forms of primary pancreatic insufficiency; malformations or previous major surgery procedures of gastrointestinal, cardiovascular, urinary or respiratory tract; psychiatric and neurological diseases; and eosinophilic gastrointestinal disorders.

Study outcome

The study outcome was the evaluation of the predictors (i.e., sex, mode of delivery, breastfeeding, age at FA onset, age at diagnosis, the presence of atopic dermatitis, type of food antigens, non-IgE-GIFA phenotype, family allergy risk, diagnostic delay, multiple FAs) potentially influencing the natural history (i.e, time of immune tolerance acquisition and of occurrence of other allergic manifestations (AM)).

Bias

The prospective design of the study was expected to substantially reduce the risk of bias associated with the assessment of the outcome and of the predictors. As outlined in detail below under *Study Procedures*, all data were prospectively collected and thoroughly re-evaluated by three members of the paediatric team responsible for the study.

Study size

No formal sample size calculation was performed.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Study procedures

All subjects with suspected non-IgE-GIFA were evaluated for the study by a research team (RT) composed by three paediatricians highly experienced in FA operating at the coordinating centre. After the evaluation of inclusion and exclusion criteria, written informed consent was collected, and enrolled subjects underwent to the diagnostic work-up for FA diagnosis according to the actual guidelines.[2 18 19] For all study subjects with a definitive non-IgE-GIFA diagnosis, a 24-month follow-up was planned with a visit every 6 months. At each visit, the patients underwent a full anamnestic and clinical evaluation at the coordinating centre. The occurrence of other AM was monitored and diagnosed according to the current guidelines.[23–26] The oral food challenge (OFC) was conducted every 6 months to monitor the potential development of immune tolerance. Only in patients affected by FPIES the OFC was performed every 12 months, according to the actual guidelines.[18]

Definitive diagnoses of non-IgE-mediated gastrointestinal food allergies (non-IgE-GIFA) phenotypes

The RT evaluated the anamnestic and clinical data of all patients, including the result of the diagnostic OFC, and made a final assignment to one of non-IgE-GIFA phenotypes only when ≥2 out of three RT members agreed. The final assignment to one of non-IgE-GIFA phenotypes was based on the presence of the following signs/symptoms, as previously reported.[2 3 18 27]

- FPE, if the patient presented the cardinal symptom (diarrhoea) with or without the additional symptoms (mucus and bloating, abdominal pain, hypoalbuminemia, faltering on growth, malabsorption, or vomiting).
- FPIAP, if the patient presented the cardinal symptom (visible blood in the stools) with or without the additional symptoms (mucus, loose stools, painful flatus, anal excoriations).
- FPIMD, constipation if the patient presented the cardinal symptom (straining with hard/soft stools) with or without the additional symptoms (faecal impaction, bloating, abdominal pain, crying);

gastro-oesophageal reflux disease if the patient presented the cardinal symptom (intermitted painful vomiting/regurgitation, feeding difficulties) with or without the additional symptoms (cough, back-arching with pain, crying); colic if the patient presented recurrent and prolonged periods of crying, fussing or irritability without evidence of failure to thrive or other symptoms illness.

- FPIES, if the patient presented vomiting in the 1–4-hours period after the ingestion of the suspected food, in the absence of classic IgE-mediated allergic skin or respiratory symptoms, in combination with ≥2 minor criteria (lethargy, pallor, diarrhoea 5–10 hours after food ingestion, hypotension, hypothermia, increased neutrophil count of ≥500 neutrophils above the baseline count).

Data management and analysis

Data of all study subjects were recorded into a dedicated clinical chart. Then, using a single data entry method, all data were entered anonymously in the study database. Then, the statistical team reviewed the study database and performed data cleaning and verification according to standard procedures.

Statistical analysis

Most continuous variables were not Gaussian-distributed, and all are reported as median (50th percentile) and IQR (25th and 75th percentiles). Discrete variables are reported as the number and proportion of subjects with the characteristic of interest. Univariable logistic regression was used to identify potential baseline predictors of multiple FA at baseline, overall immune tolerance at 24 months, tolerance to cow's milk proteins (CMP) at 24 months and the occurrence of allergic march at 24 months. The rates of immune tolerance, tolerance to CMP and allergic march at 24 months were calculated as marginal probabilities from an univariable logistic model using the occurrence of the given outcome at 24 months (discrete, 0=no, 1=yes) as response variable. A similar model employing non-IgE-GIFA (discrete, 0=FPE, 1=FPIES, 2=FPIAP, 3=FPMID) as response variable was used to calculate the corresponding rates among the non-IgE-GIFA phenotypes. Time-related changes of overall immune tolerance, CMP tolerance and AM were calculated as marginal estimates from logistic regression models including the outcome of interest (discrete, 0=no; 1=yes) as response variable and time (discrete, 0=6 months, 1=12 months, 2=18 months, 3=24 months), multiple FA at baseline (discrete; 0=no; 1=yes) and a multiple FA at baseline X time interaction (discreteXdiscrete) as predictors and cluster CIs to take into account repeated measures.[28] Differences in incidence rates between single and multiple baseline FA groups were obtained from the same logistic regression models with Bonferroni correction applied at each follow-up interval (6, 12, 18 and 24 months). There were no missing data. Statistical analysis was performed using Stata 17.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Study population

From January 2017 to January 2020, a total of 200 subjects were evaluated for suspected non-IgE-GIFA at the 10 affiliated Italian paediatric hospitals. Twenty-five subjects were excluded because of the presence of ≥1 exclusion criteria. In 123 out of 175 children, the OFC resulted positive with typical timing and symptoms of non-IgE-GIFA, whereas five presented with immediate allergic reaction and received a final diagnosis of IgE-mediated FA and were excluded from the study.

Baseline clinical features

The main anamnestic, demographic and clinical features of the study population are reported in table 1. At baseline, 52 (42%) subjects presented multiple FA, 24 (50%) among FPE, 12 (57%) among FPIES, three (15%) among FPIAP and 13 (38%) among FPMID.

Diagnostic delay was associated with a higher odds of multiple FAs, with each additional month of delay increasing the odds by 9% (OR 1.09, 95% CI 1.10 to 1.18). In addition, also male sex was identified as risk factor for multiple FAs occurrence (OR 2.24, 95% CI 1.07 to 4.71) (table 2). Because of the large 95% CI and the relatively low sample size, multivariable modelling of potential predictors was not performed.

The food allergens were CMP in 99 children (80.5%), followed by hen's egg (23.6%), soy (14.6%), wheat (13.8%), rice (9.8%), meat (9.8%), legumes (8.9%), fish (7.3%) and other foods (8.9%). The rates did not sum to 100 because of multiple FAs. The food antigens distribution among FPE, FPIES, FPIAP and FPMID patients is reported in online supplemental table 1. Among the 99 children with cow's milk allergy (CMA), 40 had FPE, 12 FPIES, 19 FPIAP and 28 FPIMD. Nine CMA children were being breastfed at the first visit, while 90 were already receiving a special formula previously prescribed by the physicians operating at the paediatric hospitals involved in the study. Seven children (7.8%) were fed with soy proteins–based formula (SF), nine (10.0%) with extensively hydrolysed casein formula (EHCF), 10 (11.1%) with amino acid–based formula (AAF), 10 (11.1%) with hydrolysed rice formula (RF), 14 (15.6%) with extensively hydrolysed whey formula (EHWF) and 40 (44.4%) with EHCF supplemented with the probiotic *L.rhamnosus* GG (EHCF+LGG). At the end of diagnostic work-up in children with positive OFC, the formula previously prescribed by the physicians operating at the paediatric hospitals involved in the study was continued.

Table 1 Demographics and clinical features of the study population

	Total	FPE	FPIES	FPIAP	FPMID
	n=123	n=48	n=21	n=20	n=34
Male sex, n (%)	69 (56.1)	32 (66.7)	9 (42.9)	9 (45.0)	19 (55.9)
Family history of allergy, n (%)	79 (64.2)	34 (70.8)	15 (71.4)	11 (55.0)	19 (55.9)
Caesarean delivery, n (%)	75 (61.0)	29 (60.4)	15 (71.4)	12 (60.0)	19 (55.9)
Preterm birth, n (%)	8 (6.5)	5 (10.4)	0 (0)	1 (5.0)	2 (5.9)
Breastfed for at least 1 month, n (%)	67 (54.5)	24 (50)	13 (61.9)	14 (70)	16 (47.1)
Breastfeeding duration (months), median (IQR)	0 (0; 6)	0 (0; 6)	6 (0; 7)	0 (0; 6)	0 (0; 0)
Breastfed at diagnosis, n (%)	9 (7.3)	1 (2.0)	2 (9.5)	1 (5.0)	5 (10.3)
Age at onset (days), median (IQR)	90 (30; 180)	105 (30; 330)	120 (30; 180)	60 (30; 90)	105 (30; 180)
Age at diagnosis (days), median (IQR)	150 (60; 300)	195 (90; 495)	150 (60; 180)	60 (60; 105)	180 (60; 360)
Time from onset to diagnosis (days), median (IQR)	30 (0; 90)	60 (26; 195)	5 (0; 30)	0 (0; 30)	30 (0; 150)
Atopic dermatitis before food allergy, n (%)	45 (36.6)	20 (41.7)	7 (33.3)	9 (45.0)	9 (26.5)
Multiple food allergies, n (%)	52 (42.3)	24 (50.0)	12 (57.1)	3 (15.0)	13 (38.2)
Symptoms					
Diarrhoea	48 (39)	48 (100)	–	–	–
Irritability	38 (30.9)	13 (27.1)	–	–	25 (73.5)
Abdominal pain	7 (5.7)	4 (8.3)	–	–	3 (8.8)
Mucus in the stool	17 (13.8)	4 (8.3)	–	13 (65)	–
Blood in the stool	22 (17.9)	2 (4.2)	–	20 (100)	–
Bloating	28 (22.8)	26 (54.2)	–	–	2 (5.9)
Vomiting	53 (43.1)	12 (25)	21 (100)	–	20 (58.8)
Anal excoriation	5 (4.1)	–	1 (4.8)	2 (10)	2 (5.9)
Hard stool	7 (5.7)	–	–	–	7 (20.6)
Regurgitation	30 (24.4)	2 (4.2)	–	–	28 (82.4)
Crying	26 (21.1)	4 (8.3)	–	–	22 (64.7)
Feeding difficulties/adversion	17 (13.8)	7 (14.6)	–	–	10 (29.4)
Back-arching	16 (13)	–	–	–	16 (47.1)
Lethargy	20 (16.3)	–	20 (95.2)	–	–
Pallor	19 (15.5)	–	19 (90.5)	–	–
Hypotension	8 (6.5)	–	8 (38.1)	–	–
Hypotermia	3 (2.4)	–	3 (14.3)	–	–
Faltering growth	48 (39)	41 (85.4)	–	–	7 (20.6)

Diagnostic delay was considered if the diagnosis occurred more than 30 days after symptoms onset.
FPE, food protein–induced enteropathy; FPIAP, food protein–induced allergic proctocolitis; FPIES, food protein–induced enterocolitis syndrome; FPIMD, food protein–induced motility disorders.

Factors influencing the natural history of non-IgE-mediated gastrointestinal food allergies (non-IgE-GIFA)

Immune tolerance acquisition

At the 24-month follow-up, 54% of patients (67 out of 123) achieved immune tolerance (95% CI 46% to 63% (marginal estimate from logistic regression). Such rate was 54% (40% to 68%) for FPE, 24% (6% to 42%) for FPIES, 75% (56% to 94%) for FPIAP and 62% (45% to 78%) for FPMID children (marginal estimates from logistic regression) (figure 1). The rate of immune tolerance acquisition for each non-IgE-GIFA phenotype is reported in online supplemental table 2. Comparing patients with single versus multiple FA, the former had a higher rate of immune tolerance acquisition at 24-month follow-up (figure 2a,b).

To evaluate the role of multiple FAs and other potential predictors on the immune tolerance acquisition rate at 24-month follow-up, a univariable analysis was performed. The odds of acquiring immune tolerance at 24 months was inversely associated with the presence of multiple FAs, the presence of family allergy risk and the FPIES phenotype (table 3). Lastly, in children with multiple FAs including CMA, and receiving special formulas, the overall immune tolerance rate at 24-month

Table 2 Potential predictors on the occurrence of multiple FAs at baseline

Male sex	2.24* (1.07, 4.71)						
Age at onset		1.00 (1.00, 1.00)					
Age at diagnosis			1.00 (1.00, 1.00)				
Diagnostic delay				1.09* (1.01, 1.18)			
Caesarean delivery					1.04 (0.50, 2.17)		
Breastfed						0.90 (0.33, 2.48)	
Family history of allergy							0.82 (0.39, 1.72)
Observations	123	123	123	123	123	123	123

Values are ORs and 95% CIs from univariable logistic regression.
*p<0.05

follow-up was 75% (95% CI 62% to 88%) for subjects receiving EHCF+LGG versus 46% (32% to 60%) for patients treated with other formulas (p=0.003) (marginal estimate from logistic regression).

Cow's milk protein tolerance acquisition

A total of 62 patients (63%, 95% CI 53% to 72%) achieved immune tolerance to CMP at the end of a 24-month follow-up: 25 FPE (62.5%); two FPIES (16.7%); 15 FPIAP (79.0%) and 20 FPIMD (7.4%) (figure 3). The acquisition of CMP tolerance by the distinct non-IgE-GIFA phenotypes is reported in online supplemental table 3. At the 24-month follow-up, CMP immune tolerance acquisition rate was lower in children with multiple FAs (figure 4a,b). The univariable analysis of potential predictors of CMP immune tolerance acquisition at 24 months showed that the presence of multiple FAs and the FPIES phenotype were associated with a lower odds to achieve immune tolerance to CMP (online supplemental table 4). Lastly, in formula-fed patients, the CMP tolerance rate at 24 months was 83% (95% CI 71% to 94%) for subjects receiving EHCF+LGG versus 48% (34% to 62%) for those treated with other formulas (p=0.0002) (marginal estimates from logistic regression).

Allergic march occurrence

The occurrence of allergic march was defined as the presence of ≥1 additional AM during the follow-up. At the end of the 24-month follow-up, the allergic march rate was 46% (95% CI 38% to 55%, n=57/123) (marginal estimate from logistic regression for repeated measures). Fifty children developed at least one AM, whereas seven patients developed >2 AM. The allergic march occurrence rate was similar in the four phenotypes: 48% (95% CI 34% to 62%) for FPE, 48% (26% to 69%) for FPIES, 40% (19% to 61%) for FPIAP and

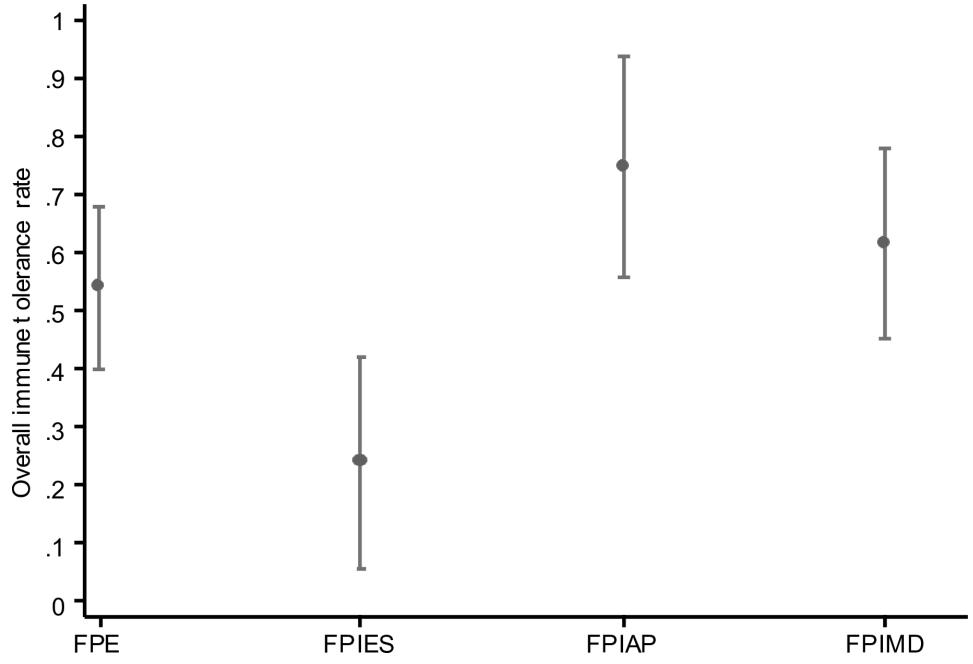

Figure 1 Overall immune tolerance rate at 24 months follow-up among non-IgE-mediated gastrointestinal food allergies (non-IgE-GIFA) phenotypes food protein–induced enteropathy (FPE), food protein–induced enterocolitis syndrome (FPIES), food protein–induced allergic proctocolitis (FPIAP) and food protein–induced motility disorders (FPIMD).

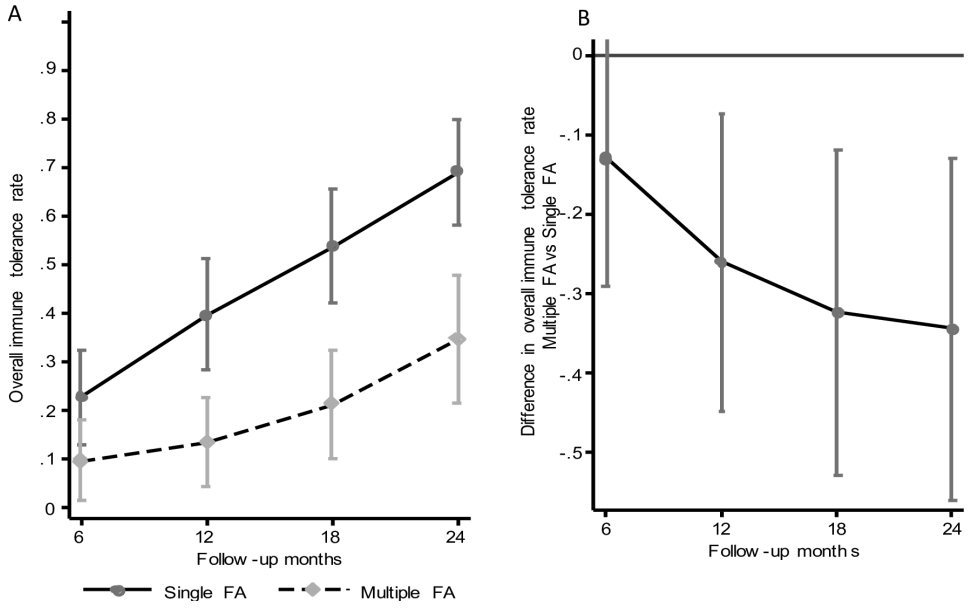

Figure 2 Overall immune tolerance acquisition rate during 24-month follow-up in non-IgE-mediated gastrointestinal food allergies (non-IgE-GIFA) paediatric patients with single versus multiple food allergies. (**A**) Values are marginal probabilities with 95% CI obtained from logistic regression for repeated measurements. (**B**) Differences in marginal probabilities with 95% CI calculated from logistic regression for repeated measurements, with Bonferroni correction for four contrasts.

47% (30% to 64%) for FPMID (online supplemental figure 1) (marginal estimates from logistic regression for repeated measures).

At univariable logistic regression, the OR of allergic march at 24 months for multiple versus single FA at baseline was 2.22 (95% CI 1.07 to 4.61) (table 4). The distribution of AM among non-IgE-GIFA phenotypes at 24-month follow-up is reported in online supplemental table 5.

In formula-fed CMA children, the allergic march rate at 24 months was 37% (95% CI 23% to 49%) for subjects receiving EHCF+LGG versus 53% (95% CI 37% to 68%) for patients treated with other formulas (p=0.113).

DISCUSSION

This multicentre prospective study comparatively evaluated a cohort of paediatric patients affected by the four phenotypes of non-IgE-GIFA for 24 months.

Overall, the non-IgE-GIFA patients were mainly male (56%), born by caesarean section (61%), with family allergy risk (64%) and formula fed at the diagnosis (93%). The median age at symptoms onset was 90 days and 150 days at diagnosis, with a median diagnostic delay of 60 days. Data regarding diagnostic delay in non-IgE-GIFA are conflicting and ranging from 2 to 6 months, depending on food antigens and the clinical phenotypes.[29 30] The prognostic value of diagnostic delay was

Table 3 Potential predictors on the occurrence of the overall immune tolerance at 24 months

Age at onset	0.97 (0.91, 1.03)						
Age at diagnosis		0.96 (0.92, 1.01)					
Diagnostic delay			0.96 (0.90, 1.02)				
Male sex				0.81 (0.40, 1.66)			
Family history of allergy					0.41* (0.19, 0.89)		
Multiple food allergies						0.24† (0.11, 0.51)	
FPE							1.00 (1.00, 1.00)
FPIES							0.26* (0.08, 0.84)
FPIAP							2.54 (0.80, 8.10)
FPMID							1.37 (0.56, 3.34)
Observations	123	123	123	123	123	123	123

Values are ORs and 95% CIs from univariable logistic regression.
*p<0.05
†p<0.001
FPE, food protein–induced enteropathy; FPIAP, food protein–induced allergic proctocolitis; FPIES, food protein–induced enterocolitis syndrome; FPIMD, food protein–induced motility disorders.

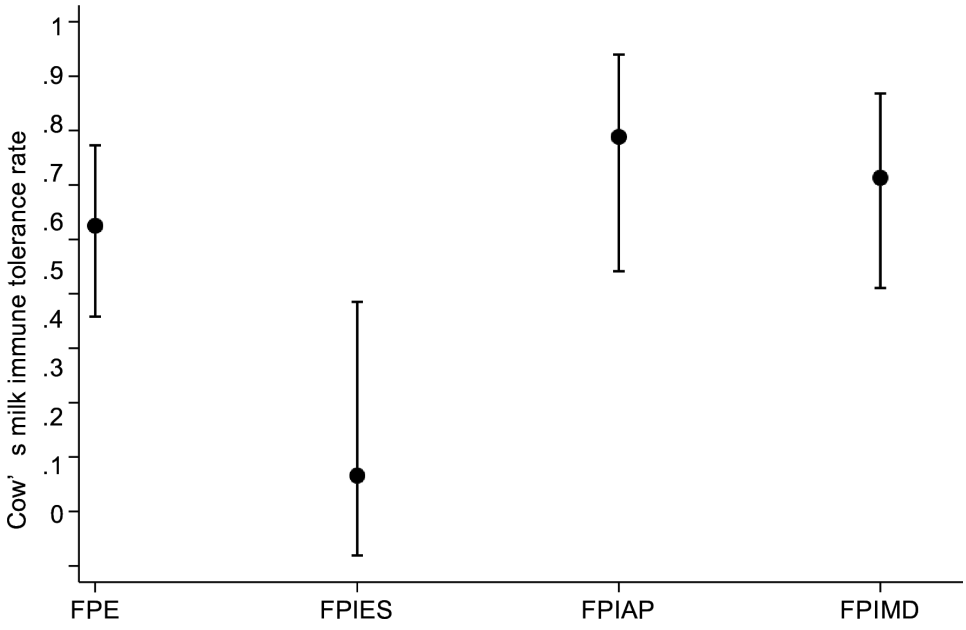

Figure 3 Cow's milk protein immune tolerance acquisition rate at 24-month follow-up among non-IgE-mediated gastrointestinal food allergies (non-IgE-GIFA) phenotypes food protein–induced enteropathy (FPE), food protein–induced enterocolitis syndrome (FPIES), food protein–induced allergic proctocolitis (FPIAP) and food protein–induced motility disorders (FPIMD).

not evaluated in these studies. We found that diagnostic delay increased the likelihood of multiple FAs, with each additional month of delay raising the odds of 9%. In addition to diagnostic delay, also male sex was a risk factor for the occurrence of multiple FAs. These data suggest the importance of an early diagnosis to limit the occurrence of multiple FAs.

In our population, more than 40% of patients showed multiple FAs, with the lower rate among FPIAP and the higher rate among FPIES children. Accordingly, other authors reported that FPIAP patients were most frequently affected by single FA and that in a European FPIES cohort, including Italian patients, more than half had multiple FAs.[17 31 32]

CMA was the main cause of non-IgE-GIFA independently of the phenotype in our study population, and this could be related at least in part to the age of patients at symptoms onset. This data aligns well with

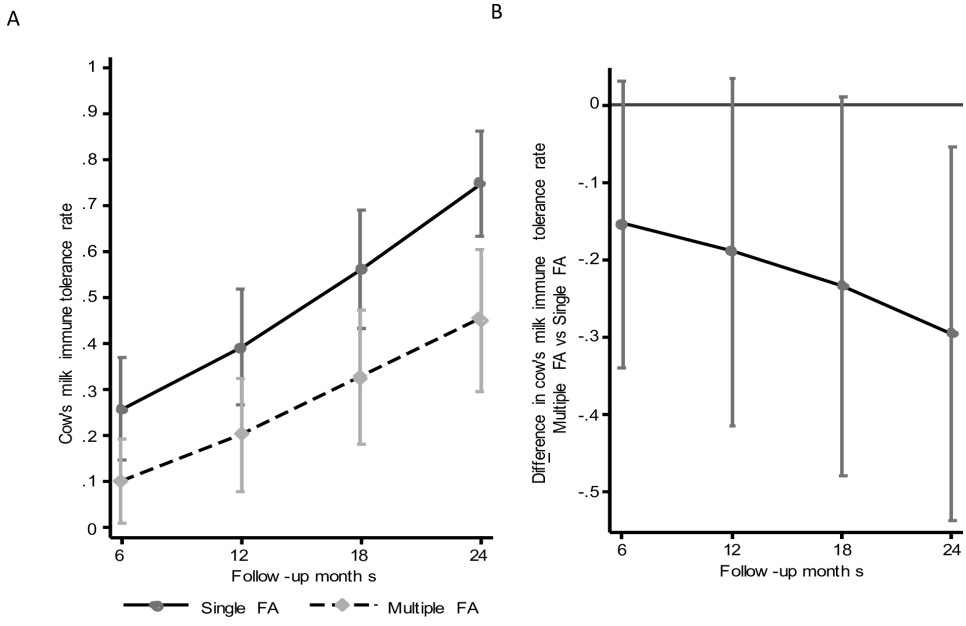

Figure 4 Cow's milk protein immune tolerance acquisition rate during 24-month follow-up in patients with single versus multiple food allergies (FA). (**A**) Values are marginal probabilities with 95% CI obtained from logistic regression for repeated measurements. (**B**) Differences in marginal probabilities with 95% CI obtained from logistic regression for repeated measurements, with Bonferroni's correction for four contrasts.

Table 4 Potential predictors on the occurrence of atopic march at 24 months

Age at onset	1.04 (0.98, 1.10)						
Age at diagnosis		1.04 (1.00, 1.09)					
Diagnostic delay			1.05 (0.99,1.13)				
Male sex				1.50 (0.73, 3.07)			
Family history of allergy					0.80 (0.38, 1.67)		
Multiple food allergies						2.22* (1.07, 4.61)	
FPE							1.00 (1.00, 1.00)
FPIES							0.99 (0.35, 2.76)
FPIAP							0.72 (0.25, 2.09)
FPMID							0.97 (0.40, 2.33)
Observations	123	123	123	123	123	123	123

Values are ORs and 95% CIs from univariable logistic regression.
*p<0.05.
FPE, food protein–induced enteropathy; FPIAP, food protein–induced allergic proctocolitis; FPIES, food protein–induced enterocolitis syndrome; FPIMD, food protein–induced motility disorders.

previous results, including systematic reviews on FPIES, and with a recent nationwide cross-sectional, retrospective medical-record survey on non-IgE-GIFA patients, in which CMA was diagnosed in almost 90% of patients with OFC confirmed non-IgE-GIFA.[4 5 31–33]

In our study, the time of immune tolerance acquisition was accurately evaluated in all patients. The immune tolerance to all foods in patients with multiple FA (i.e., overall immune tolerance rate) was achieved by 54% of patients at 24 months. The rate of overall immune tolerance acquisition seems to be lower when compared with previous studies.[27 29 32] It is crucial to emphasise that we considered a patient overall tolerant only if he had overcome all FAs, regardless of the number of FAs. In our population, nearly 50% of patients presented multiple FAs. Having multiple FAs or a family risk for allergy, negatively influenced the odds to acquire immune tolerance in our population, as previously reported in FPIAP patients.[20] Furthermore, the analysis of the different phenotypes of non-IgE-GIFA suggests that having FPIES negatively influence the rate of immune tolerance acquisition at 24 months. Other authors indicated patient age as potential factor influencing the immune tolerance acquisition, reporting a greater chance of acquiring immune tolerance in patients aged from 3 to 5 years.[4 21 22] The lower patients' age range in our cohort made impossible the confirmation of this data.

Previous studies involving paediatric patients with non-IgE mediated CMA suggested a potential role of formula selection in facilitating the acquisition of immune tolerance.[34 35] Analysing CMA formula-fed patients in our study cohort, we confirmed a higher rate of immune tolerance acquisition in patients treated with EHCF+LGG. Similar results have been recently reported also by others in FPIAP patients.[36] Interestingly, CMA patients with multiple FAs treated with EHCF+LGG showed a higher immune tolerance rate also for other food antigens, supporting the hypothesis of a potential non-specific immunomodulatory action elicited by this dietary intervention.[37]

During the 24-month follow-up, 46% of patients developed at least one AM. Similar rates have been reported by others for patients with other forms of FA.[38 39] The occurrence of AD after the diagnosis of non-IgE-GIFA was the most common AM, reported in 22% of patients. Previous studies viewed AD as a comorbidity of non-IgE-GIFA rather than a step in the allergic march, which makes it challenging to compare our findings.[8 39] The occurrence of AR was reported in 19% of the study subjects. A similar rate was previously reported in IgE-mediated FA children.[38] Retrospective studies with longer follow-up and different patient ages reported a higher occurrence of AR (up to 45%) in subjects with non-IgE-GIFA.[8 40] Also, the 6% rate of asthma in our cohort was lower if compared with previous observations (27% to 32%), again probably due to the shorter follow-up and the lower age of patients in our study.[8 40] Lastly, 6% of patients (four FPE, two FPIES, one FPIAP) developed IgE-mediated FA during the follow-up. Previous observations were mainly focused on concomitant IgE sensitisation in non-IgE-GIFA (e.g., atypical FPIES) or in transient forms of FPIAP.[20 40–42]

Data regarding potential predictors of allergic march development in non-IgE-GIFA are lacking. The results of this study suggest the potential role of multiple FA as a facilitating factor for the occurrence of other AM.

The present study has several strengths. First, our sample size including all the four non-IgE-GIFA phenotypes is larger than that available in most published studies. Second, the prospective, comparative and highly standardised design employed minimised bias in the assessment of both outcomes and predictors. The study has nonetheless some limitations. First, evaluating immune tolerance at 24 months is a clinically relevant outcome but clearly less informative compared with a longer time frame. Second, our findings are based on children consecutively observed at 10 Italian hospitals and sent to a tertiary care centre. Consequently, these findings cannot be generalised to the general population of Italian children with non-IgE-GIFA, even though

these children are usually managed in secondary and/or tertiary care centres, which are the most suitable settings to track the natural history of the disease.

In conclusion, the NIGEFA study highlights the importance of non-modifiable (i.e., male sex, family allergy risk, different phenotypes of non-IgE-GIFA) and modifiable factors (i.e., diagnostic delay, presence of multiple FAs, formula choice in children with CMA) influencing the time of immune tolerance acquisition and the occurrence of allergic march in non-IgE mediated FA. These data, if confirmed by future studies, may have a relevant impact on clinical practice and could help an effective management of these patients.

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