Aus der Klinik für Pädiatrie mit Schwerpunkt Pneumologie, Immunologie und Intensivmedizin
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Klinische Merkmale des Pollen Food Allergy Syndrome in südeuropäischen Ländern

Clinical Characteristics of Pollen Food Allergy Syndrome in Southern European Countries

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Theresa Lipp

aus Berlin-Charlottenburg

Datum der Promotion: 25.06.2023

Table of Contents

List of Abbreviations

AIT – allergen immunotherapy

ARIA – Allergic Rhinitis and its Impact on Asthma

ATH – Athens

BER – Berlin

CARAT – Control of Allergic Rhinitis and Asthma Test

CDSS – clinical decision support system

CRD – component resolved diagnostics

ESEP – EUROLINE Southern European Pollen Profile

IgE – immunoglobulin E

IQR – interquartile range

IST – Istanbul

IZM – Izmir

MAR – Marseille

MES – Messina

nsLTP – non-specific lipid transfer protein

OR – odds ratio

PFAS – pollen food allergy syndrome

POR – Porto

PR-10 – pathogenesis-related class 10 protein

ROM – Rome

SD – standard deviation

SAR – seasonal allergic rhinitis

SPT – skin prick test

TIR – Tirana

VAL – Valencia

Abstract

Background

Worldwide, allergic rhinitis is a disease affecting an estimated 10-30% of the world population and thus having a high socioeconomic impact. Seasonal allergic rhinitis (SAR) can be difficult to diagnose, especially in Southern Europe, where pollen seasons are long and often overlap. Amongst patients with SAR, pollen food allergy syndrome (PFAS) is a frequent comorbidity. Yet, due to its heterogeneity in symptoms, triggers, and laboratory results, it is underdiagnosed. As of now, no comprehensive study examining the syndrome in the Mediterranean region has been published.

Objectives

To identify the clinical characteristics of PFAS in Southern Europe as well as possible similarities, differences, and unique features within the region.

Methods

The @IT.2020 Multicenter Study recruited 815 patients suffering from SAR aged 10 to 60 years from nine Southern European centers. They were included in an initial study visit, during which questionnaires on SAR, comorbidities, family history, and PFAS as well as skin prick and immunoglobulin E (IgE) testing were performed. Afterwards, they participated in a digital monitoring period as well as a second study visit with follow-up questionnaires. The resulting data regarding PFAS was analyzed for this dissertation.

Results

167 out of the 815 patients (20.5%) reported PFAS reactions. Kiwi (58, 34.7%), peach (43, 25.7%), and melon (26, 15.6%) were the most frequently named elicitors. Most of the reported reactions were localized (216/319, 67.7%) and occurred within five minutes after contact with the eliciting food (209/319, 65.5%). The characteristics associated with PFAS included positive IgE results to one or more of the tested panallergen groups (profilin, pathogenesis-related class 10 protein (PR-10), or non-specific lipid transfer protein (nsLTP)), positive maternal history of PFAS, and positive history of asthma. The included centers showed a vast heterogeneity in prevalence of PFAS and its associated clinical characteristics.

Conclusions

The current findings portray the clinical relevance of PFAS in Southern Europe. Vast differences within the region were found, which may be due to differing pollen sensitization patterns. The new insights on associated clinical characteristics and

common elicitors can aid physicians and patients in diagnosis and therefore food allergen avoidance. The data will also become relevant to clinical life in Germany, as plants currently only found in Southern Europe are predicted to spread north and the length of pollen seasons is predicted to increase due to climate change.

Zusammenfassung

Hintergrund

Weltweit ist allergische Rhinitis eine Erkrankung, die geschätzte 10-30% der weltweiten Bevölkerung beeinträchtigt und daher eine hohe sozioökonomische Auswirkung hat. Saisonale allergische Rhinitis (SAR) kann schwer diagnostizierbar sein, vor allem in Südeuropa, da hier die Pollensaisons lange anhalten und häufig überlappen.

Bei Patienten mit SAR ist das Pollen Food Allergy Syndrome (PFAS) eine häufige Komorbidität. Die Symptome, Auslöser, und laborchemischen Erkennungsmerkmale fallen sehr heterogen aus, weshalb PFAS häufig unterdiagnostiziert wird. Bislang wurde noch keine umfassende Studie zu PFAS in der mediterranen Region publiziert.

Zielsetzung

Die klinischen Charakteristika von PFAS in Südeuropa im Hinblick auf Ähnlichkeiten, Unterschiede und phänotypische Besonderheiten innerhalb der Region zu identifizieren.

Methodik

Die @IT.2020 Multicenter Study rekrutierte 815 PatientInnen mit SAR im Alter von 10 bis 60 Jahren in neun südeuropäischen Zentren. Im ersten Schritt wurden PatientInnendaten hinsichtlich SAR, Komorbiditäten, Familienanamnese und PFAS erhoben. Zudem wurden Hautpricktest und Immunglobulin E (IgE)-Messungen durchgeführt. Es schloss sich ein digitaler Überwachungszeitraum, gefolgt von einer zweiten, abschließenden Datenerhebung per Fragebogen an. Die Daten zu PFAS wurden für diese Dissertation analysiert.

Ergebnisse

167 von 815 PatientInnen (20,5%) berichteten über PFAS-Reaktionen. Kiwi (58, 34,7%), Pfirsich (43, 25,7%) und Melone (26, 15,6%) waren die meist genannten Auslöser. Die häufigsten Reaktionen waren lokal (216/319, 67,7%) und traten innerhalb von fünf Minuten nach Kontakt mit dem auslösenden Lebensmittel auf (209/319, 65,5%). Die mit PFAS assoziierten Charakteristika beinhalteten eine positive IgE-Testung auf mindestens

eine der eingeschlossenen Panallergengruppen (Profilin, Pathogenesis-Related Class 10 Protein (PR-10) oder Non-Specific Lipid Transfer Protein (nsLTP)), eine positive maternale Familienanamnese für PFAS und die positive Eigenanamnese für Asthma. Zwischen den beteiligten Zentren zeigte sich eine große Divergenz hinsichtlich der Prävalenz sowie den assoziierten klinischen Charakteristika.

Schlussfolgerung

Die aktuellen Ergebnisse zeigen die klinische Relevanz sowie geographisch verteilte Heterogenität von PFAS in Südeuropa. Letztere könnte durch Unterschiede der Pollensensibilisierungsmuster erklärt werden. Die Einblicke in die klinischen Charakteristika und häufigen Auslöser können sowohl ÄrztInnen als auch PatientInnen in der Diagnose sowie im Management von PFAS unterstützen. Die Daten werden auch im klinischen Alltag in Deutschland relevant werden, da vorhergesagt wird, dass aufgrund des Klimawandels Pflanzen, die aktuell primär in südlichen Breitengraden auftreten, sich ausbreiten und die Pollenflugzeiten sich ausdehnen werden.

Manteltext

1. Introduction

Seasonal allergic rhinitis (SAR) is an inflammatory, antibody facilitated disease caused by exposure to seasonal allergens such as trees, grasses, and weeds (Passali et al., 2018; Skoner, 2001). Patients with SAR suffer from one or more of these symptoms: sneezing, itching, nasal congestion, and rhinorrhea, due to exposure to seasonal allergens. These can be accompanied by symptoms of the eyes, ears, and throat. The patients show a rapid onset of symptoms upon exposure to the allergen and improving symptoms when the season of the causative allergen is over (Skoner, 2001). Additionally, allergic rhinitis can cause mental symptoms including fatigue, decreased concentration, and depression, which is reflected in its impact on school or work performance and quality of life (Bachert and Andreasen, 2015).

1.1 Pathogenesis

SAR is caused by an early- and a late-phase allergic response. In previously sensitized patients, allergens are recognized upon re-encounter by immunoglobulin E (IgE) bound to the surface of mast cells and basophils (Bjermer et al., 2019). In the early-phase, mast cell degranulation occurs predominantly in the nasal mucosa, eliciting histamine release (Figure 1A). This causes the onset of nasal and ocular symptoms such as sneezing, rhinorrhea, and itching within minutes of exposure. Due to the release of histamine and pro-inflammatory cytokines, vascular permeability increases and edema form (Bjermer et al., 2019). The ensuing late-phase reaction occurs hours after exposure due to the recruitment of basophils, neutrophils, T-lymphocytes, monocytes, and eosinophils, as well as the release of cytokines, prostaglandins, and leukotrienes (Figure 1B). It causes mucosal inflammation including edema and nasal congestion and leads to an increased susceptibility of the patient for further symptom development due to allergen exposure (Bjermer et al., 2019).

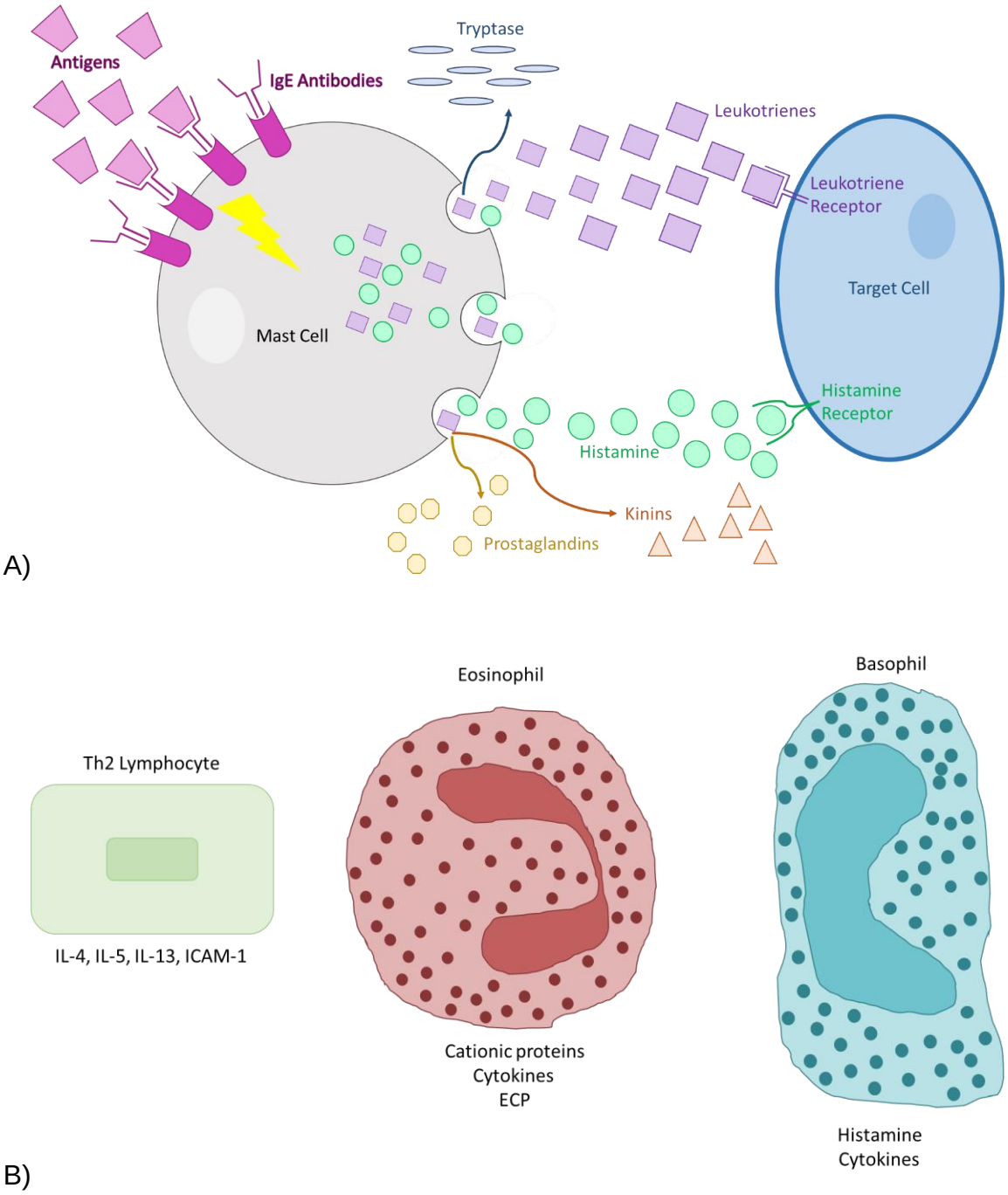


Figure 1: *A) Early phase allergic reaction, in which the antigens and IgE (immunoglobulin E) antibodies activate mast cells to release leukotrienes, and other cytokines (tryptase, kinins, prostaglandins).*

B) Late phase allergic reaction, in which Th2 lymphocytes, eosinophils, and basophils are activated and release mediators, which prolong the inflammatory response. (IL: interleukin, ICAM: intercellular adhesion molecule, ECP: eosinophil cationic protein) Modified from Bjermer et al., 2019.

1.2 Epidemiology

It is estimated that 10-30% of the world population suffer from allergic rhinitis (Pawanker et al., 2013). Worldwide, 0.1-25.8% of children aged 8-12 years are affected by SAR (Weinmayr et al., 2008). The prevalence is steadily on the rise in most countries (Asher et al., 2006). Due to its high prevalence and impact on quality of life, SAR has a high socioeconomic cost. According to an analysis by Zuberbier et al., untreated patients with allergic rhinitis in Europe produce unavoidable indirect costs of EUR 2405 per patient annually due to absence from work and reduced productivity at work (Zuberbier et al., 2014).

1.3 Diagnostics

The diagnosis off SAR greatly depends on accurate history taking to identify the associated symptoms and possible elicitors for the patients. Additionally, diagnostic scores can be helpful in assessing the severity and quality of SAR and thus its impact on quality of life, such as the Allergic Rhinitis and its Impact on Asthma (ARIA) score (Greiner et al., 2011). Furthermore, diagnostic tools are used to identify the eliciting pollens or molecules of SAR: skin prick testing (SPT) and IgE testing (Skoner, 2001).

1.4 Therapy

As pollen allergen avoidance is not possible for patients suffering from SAR due to their ubiquitous nature, pharmacological therapy aims at symptom reduction (Dykewicz et al., 2017). Treatment options include antihistamines, decongestants, corticosteroids, cromolyn, anticholinergics, and leukotriene receptor antagonists (Bjermer et al., 2019; Dykewicz et al., 2017). These therapies solely intend symptom relief and do not impact the natural history of SAR and may also cause side effects (Roberts et al., 2018).

Currently, the sole viable option for disease-modifying treatment is allergen immunotherapy (AIT), which is used to reach allergen tolerance through increasing doses of allergen extracts (Fujita et al., 2012; Roberts et al., 2018). This is achieved through the inducement of T-cell tolerance, modulation of the mast cell and basophil activation threshold, and a decreased histamine release (Fujita et al., 2012). It can be administered either subcutaneously or sublingually (Roberts et al., 2018). AIT can optimally lead to desensitization of the patient, leading to a decrease in symptoms. In addition to symptomatic improvement, AIT can lead to long-term clinical benefits, which may persist

for years after cessation of treatment (Roberts et al., 2018). The efficacy of AIT is dependent on the exact identification of the causative allergen and targeted treatment, which can be achieved via SPT or IgE testing (Roberts et al., 2018). Thus, component resolved diagnostics (CRD) plays an important role in correctly identifying the elicitor and targeting the AIT appropriately (Roberts et al., 2018). CRD uses IgE testing to identify specific allergenic molecules that the patient reacts to, rather than testing reactions to allergenic extracts, where different types of molecules are tested simultaneously. This leads to a more precise identification of the causative allergic agent and aids in the management of allergic patients. Therefore, AIT can be tailored to the patient and its outcome can be improved (Barber et al., 2021; Treudler and Simon, 2013). The interpretation of CRD results is a complicated matter (Matricardi et al., 2016), as allergologists are required to interpret more complex information, such as allergenic properties and cross-reactivity of allergic molecules (Treudler and Simon, 2013).

1.5 Pollen Food Allergy Syndrome

SAR is associated with many allergic comorbidities (Greiner et al., 2011). One of these is pollen food allergy syndrome (PFAS) (Werfel et al., 2015). Around 9.6-55 % of patients with SAR are reported to suffer from PFAS worldwide (Bedolla-Barajas et al., 2017; Bircher et al., 1994). PFAS is a hypersensitivity reaction based on cross-reactivity between food allergens and pollen allergens (Werfel et al., 2015). Reactions include symptoms of the oropharynx such as itching, stinging, pain, and edema that typically appear minutes after contact with the eliciting food (Kondo and Urisu, 2009; Price et al., 2015). Additionally, more severe symptoms have been reported in around 5% of the cases, affecting the skin, gastrointestinal, cardiovascular, and respiratory system and in rare cases may even lead to anaphylaxis (Price et al., 2015; Webber and England, 2010). The causative cross-reactive allergens share sequence-, structure-, and function-similarities. Since they are wide-spread, they are called panallergens. Major panallergen groups involved in PFAS reactions include profilins (Figure 2), pathogenesis-related class 10 proteins (PR-10) (Figure 3), and non-specific lipid transfer proteins (nsLTP) (Figure 4) (Hauser et al., 2010; Matricardi et al., 2016).

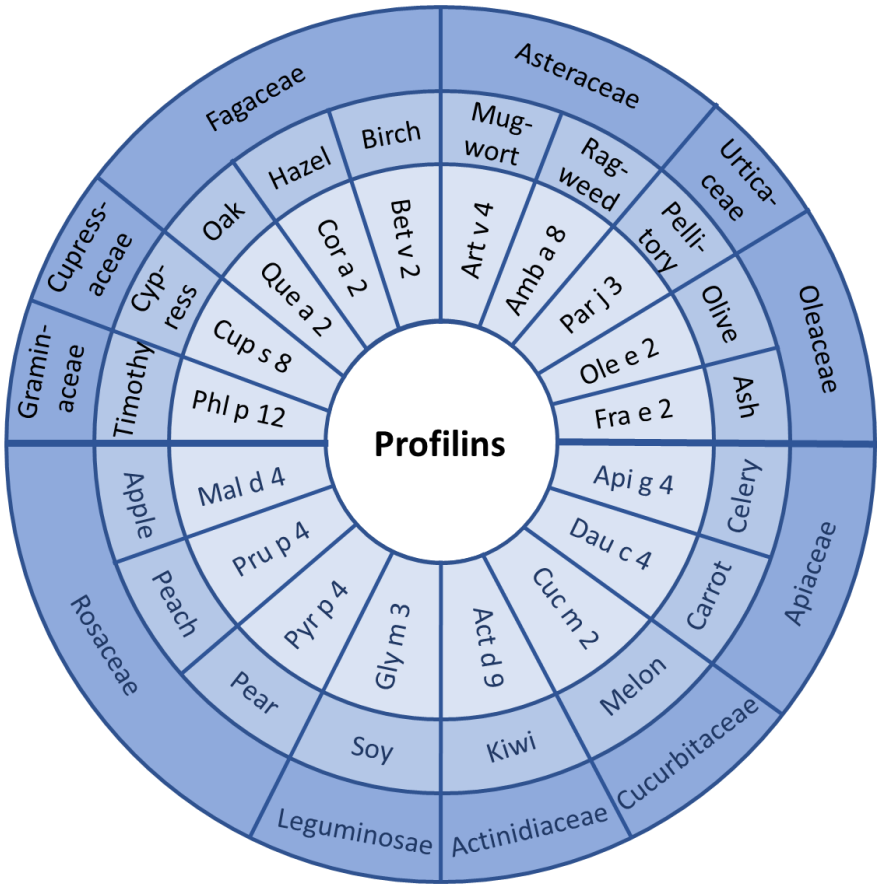


Figure 2: *Profilins: examples of allergen molecules (central), their corresponding allergen source (middle), and botanical family (peripheral). Black indicates pollen sources while blue indicates plant food sources* (Matricardi et al., 2016).
Created by the author for this dissertation.

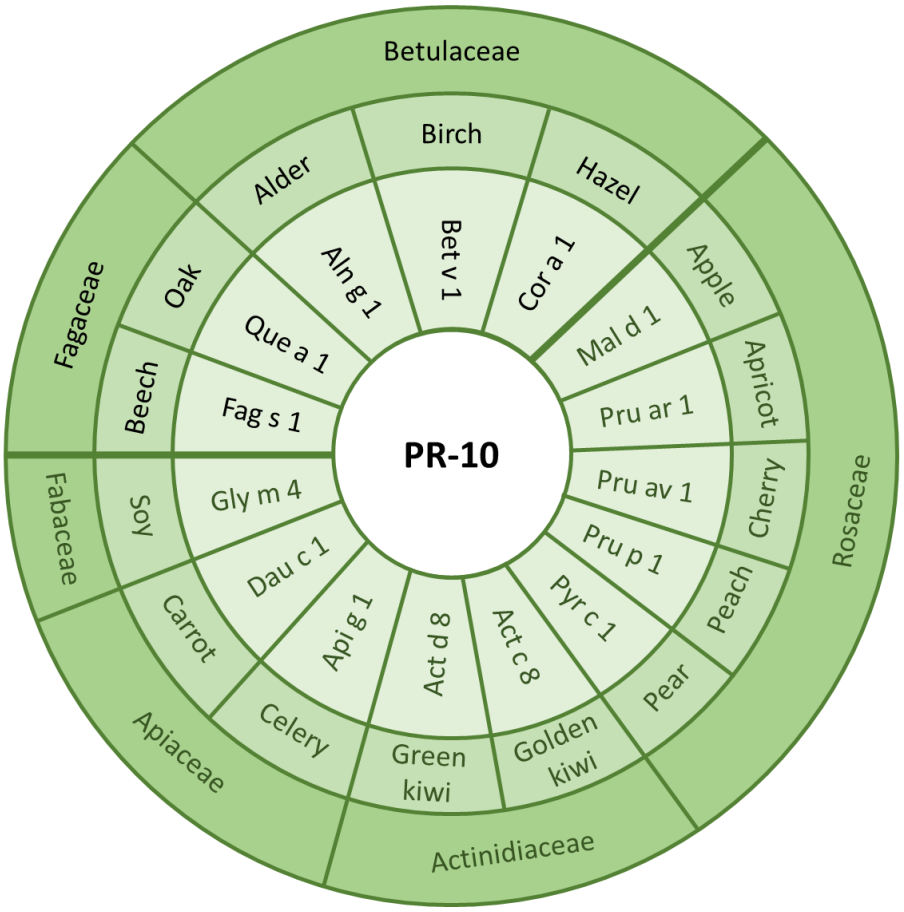


Figure 3: *Pathogenesis related class-10 proteins (PR-10): examples of allergen molecules (central), their corresponding allergen source (middle), and botanical family (peripheral). Black indicates pollen sources while green indicates plant food sources* (Matricardi et al., 2016).
Created by the author for this dissertation.

While the profilins and PR-10 cause PFAS reactions due to initial sensitization to an aeroallergen, nsLTPs are currently classified as class I food allergens, with recent evidence showing that, in some cases, the initial sensitization may occur to pollen such as Ole e 7 from the olive tree (Bogas et al., 2020; Oeo-Santos et al., 2020; Werfel et al., 2015). Additionally, Profilins and PR-10 molecules are heat- and acid-labile, therefore symptoms are rarely caused by cooked elicitors and typically only local symptoms appear after contact with raw foods (Hauser et al., 2010; Price et al., 2015). Yet, nsLTPs are resistant to heat and acid and are therefore frequently associated with systemic PFAS reactions (Hauser et al., 2010; Matricardi et al., 2016).

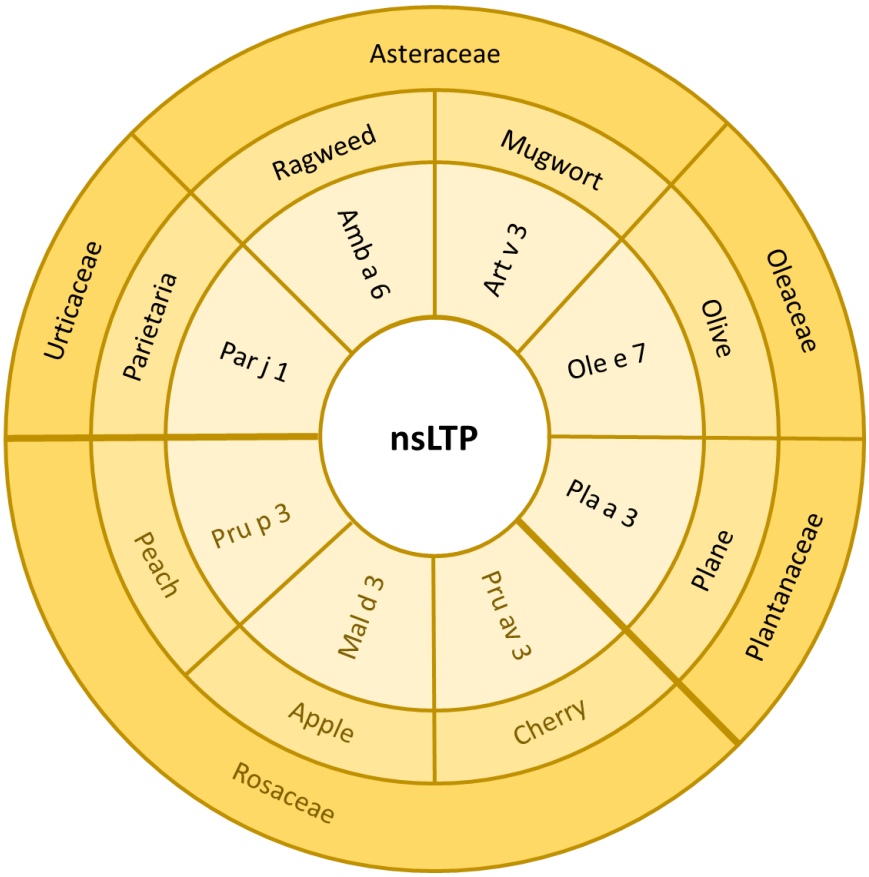


Figure 4: *Non-specific lipid proteins (nsLTP): examples of allergen molecules (central), their corresponding allergen source (middle), and botanical family (peripheral). Black indicates pollen sources while yellow indicates plant food sources* (Matricardi et al., 2016)*. Created by the author for this dissertation.*

1.6 SAR and PFAS in Southern Europe

Due to its milder climates and diversity of vegetation, pollen seasons in Southern Europe differ from those in other European regions and frequently overlap (Hoffmann et al., 2020). Since clinically identifying the causative allergen is more complicated under these circumstances, the diagnosis is also dependent on further testing. Tools aiding physicians in the correct diagnosis of SAR include SPT, IgE testing, and the complex CRD (Matricardi et al., 2016). Without adequate prior data and proper instruction on diagnostic tools, the correct identification of the causative agent and therefore treatment of SAR and its associated comorbidities including PFAS can be challenging. In order to expand the body of knowledge of SAR in Southern Europe and aid clinicians in its diagnosis and proper treatment, a cohesive study with unified methodology including nine study centers

in seven Southern European countries was established: @IT.2020 Observational Longitudinal Multicenter Clinical Study (Matricardi, 2017). Its aim was to develop a clinical decision support system (CDSS) integrating CRD and to assess the efficacy of this system. Included in this study was the analysis of pollen seasons in Southern Europe, a questionnaire on SAR, comorbidities, personal and family history of recruited patients, a monitoring period of one year with a digital symptom diary, Allergymonitor®, a second questionnaire at the end of the year for each of the patients reflecting upon their allergic symptoms during the past year and their digital literacy, and finally a workshop for physicians assessing the usefulness of the CDSS.

The main objectives were describing the molecular sensitization profile and clinical phenotype of SAR in Southern Europe and testing the use of CRD and Allergymonitor® and the impact on diagnosis and AIT prescription. Additional research questions included the frequency and clinical characteristics of PFAS as a comorbidity of SAR in Southern Europe (Matricardi, 2017). The author of this dissertation focused on that particular aspect. The related work included the establishment and upkeep of all databases, the statistical analyses, relevant research in connection to this topic, and the interpretation of gathered data embedded in that context.

1.7 Impact on Middle and Northern Europe

While overlapping pollen seasons and certain plants currently do not play a relevant role in SAR in Middle and Northern Europe, this is predicted to alter due to climate change. For example, Lake et al. predicted that by 2041-2060, ragweed will spread across Europe, excluding Scandinavia, Baltic States, Spain, and Portugal. Additionally, they projected that ragweed sensitization will increase to 77 million people by then, more than double its current prevalence. This change will especially affect countries where the incidence is currently low, including Germany (Lake et al., 2017). Therefore, the gathered data and conclusions will not only be able to support clinicians and patients in Southern Europe, but the rest of the continent as well.

2. Methods

The @IT.2020 Multicenter Study included nine centers in seven Southern European countries: Porto (POR), Portugal; Valencia (VAL), Spain; Marseille (MAR), France; Rome (ROM) and Messina (MES), Italy; Tirana (TIR), Albania; Athens (ATH), Greece; Istanbul (IST) and Izmir (IZM), Turkey. Berlin (BER), Germany served as the coordinating center. Within the study, patient data from 815 patients attending allergy clinics at all nine centers as well as aerobiological data for the calendar year 2018 were collected. The study consisted of four components: an initial study visit (T0), a monitoring period, a second study visit (T1), and a workshop (Matricardi, 2017).
The study was approved by the ethics committee in BER and all local ethics committees (Matricardi, 2017).

2.1 Study Population

Patients with SAR were recruited between November 2017 and May 2018. They were included based on following criteria: 1) age 10-18 years for children or 19-60 years for adults; 2) a good understanding of the national language or one of the languages offered in the Allergymonitor® application (TPS software production, Rome, Italy); 3) availability of a smart phone; 4) written informed consent. Exclusion criteria consisted of: 1) prior pollen allergen immunotherapy; 2) any severe chronic disease; 3) living further than 30 km away from the local aerobiological center used for pollen counts (Matricardi, 2017). The diagnosis of SAR was based on clinical history and previous positive SPT or IgE assay, with local study physicians under the supervision of the local coordinators determining the diagnosis (Matricardi, 2017).

2.2 Study Design

The following information is based on the standard operating procedure of the @IT.2020 Multicenter Study (Matricardi, 2017).

2.2.1 *T0 Visit*

At the initial study visit, patients were recruited to the study, and the diagnosis of SAR as well as fulfillment of the inclusion and exclusion criteria were verified (Figure 5). A questionnaire consisting of eight sections was completed by each patient with the aid of

the local study physicians. The initial section was comprised of sociodemographic questions such as sex, date of birth, height, and weight. Section two regarded the family history of allergic diseases, including both parents and any siblings. Allergic comorbidities (oral allergy syndrome, anaphylaxis, urticaria, atopic eczema, food allergy, asthma not related to pollen, and other allergic diseases) of the patients were assessed in section three. Pollen food allergy syndrome was assessed in the fourth section. This included fifteen possible elicitors (peach, apple, almond, apricot, soybean, cherry, pear, watermelon, melon, sesame, banana, carrot, fennel, kiwi, celery) and the category “others”. Patients were initially asked whether they had ever ingested the food and if they had, whether they suffered from an allergic reaction. For positive responses to the initial two questions, the type and timing of the reactions were assessed. Options for symptoms included local symptoms: oral pruritus, swelling of tongue/face, difficulty talking/swallowing, oral vesicles; as well as systemic symptoms: urticaria, skin redness, cough/wheezing/respiratory difficulties, swelling of eyes/eyelids, nose closed/running, vomiting, diarrhea, pallor/hypotension, palpitations/tachycardia, loss of consciousness. Five possible categories for times to onset of symptoms were provided: 1) ≤ 5 minutes; 2) 6-20 minutes; 3) 21-60 minutes; 4) 61-120 minutes; 5) ≥ 121 minutes (Figure 6). Positive responses to multiple PFAS elicitors were possible. The fifth section of the questionnaire focused on allergic rhinoconjunctivitis, assessing the age at onset, severity and frequency of symptoms. In section six, frequency and efficacy of administered allergic rhinoconjunctivitis drug therapy during the previous pollen season was reported by patients. The Control of Allergic Rhinitis and Asthma Test (CARAT) comprised section seven (Fonseca et al., 2010). Lastly, section eight regarded allergic asthma, including severity, control, and therapy.

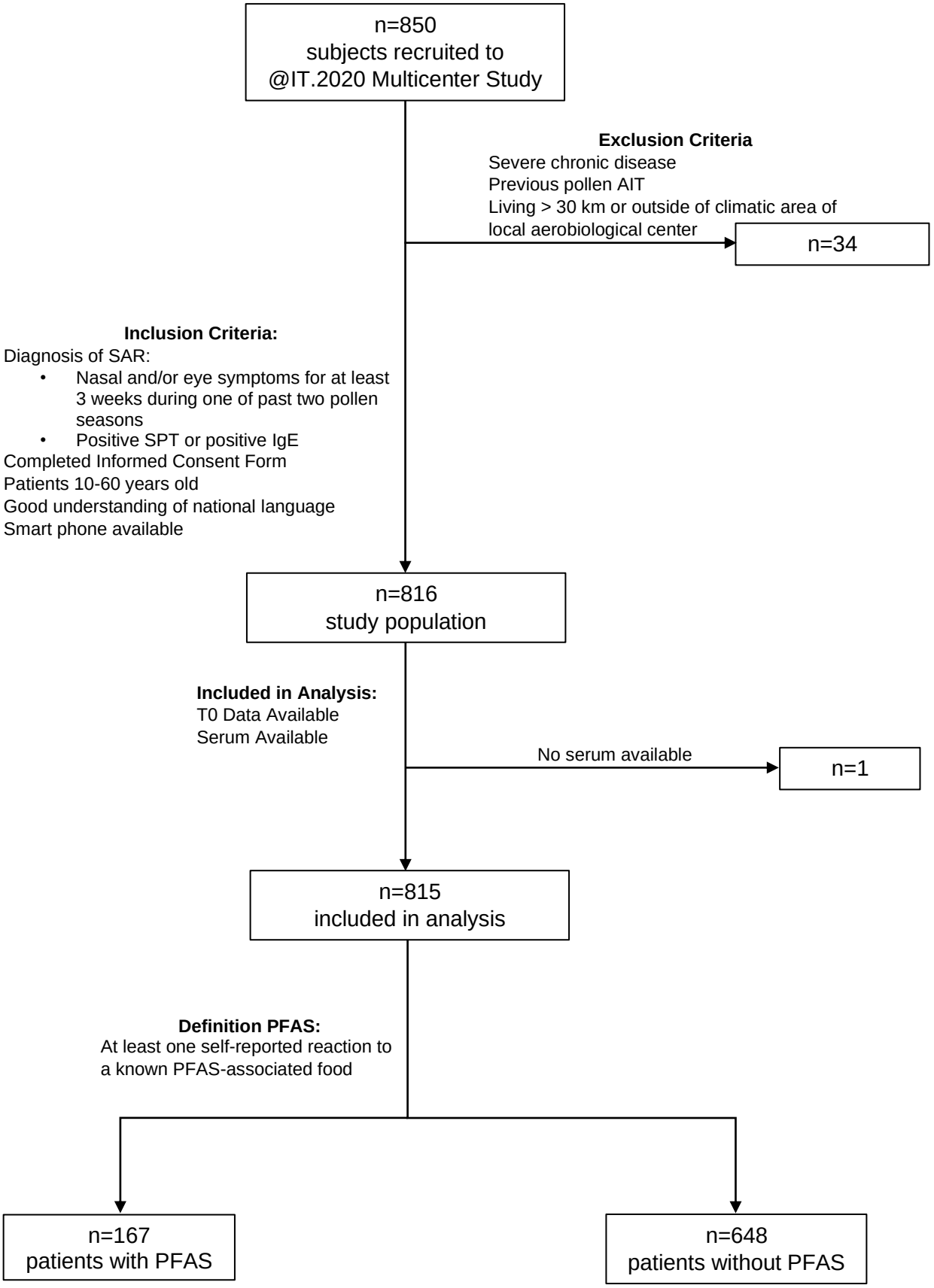


Figure 5: *Flow chart depicting the patient selection and final population used in the PFAS analysis* (Matricardi, 2017)*. Created by the author for this dissertation.*

*Food-induced symptoms (Choose one or more of the following options):	** Time lapse between ingestion of the offending food and symptoms onset:
1) pruritus throat / mouth / tongue	1) <= 5 minutes
2) vesicules to the oral cavity	2) 6-20 minutes
3) skin redness	3) 21-60 minutes
4) urticaria	4) 61-120 minutes
5) swelling of eyes / eyelids	5) >121minutes
6) swelling tongue / face	
7) difficulty talk / swallow	
8) nose closed / running	
9) cough / wheezing / respiratory difficulties	
10) vomiting	
11) diarrhea	
12) palpitations / tachycardia	
13) pallor / hypotension	
14) loss of consciousness	

Figure 6: *AllergyCard Portal for digital input of patient responses by the study physicians, using the PFAS questionnaire as an example. Reproduced with written consent by Salvatore Tripodi, co-founder of TPS software production.*

Additionally, SPT was performed by the study physicians in accordance with international guidelines (Bousquet et al., 2012; Heinzerling et al., 2013) using reagents from two

providers due to limitations of availability: mugwort, wall pellitory, olive tree, hazel tree, birch, bermuda grass, juniper ash, ragweed, *D. pteronyssinus*, cat, dog, histamine control, saline control (Stallergenes Greer, London, UK), timothy grass, *Alternaria alternata*, plane tree, *Salsola kali* (Russian thistle), and mixed grasses (ALK Abelló, Hørsholm, Denmark). 15 minutes after application of the extracts to the volar surface of the forearms, the results were noted. If the wheal diameters were ≥ 3 mm after subtracting the negative control, the results were counted as positive (Matricardi, 2017).

At the initial study visit, a blood draw was performed. Sera were obtained and sent to BER, where they were analyzed using the EUROLINE Southern European Pollen Profile (ESEP) (EUROIMMUN Medizinische Diagnostika AG, Lübeck, Germany). This semiquantitative, customized multiplex immunoblot assay was previously validated by Di Fraia et al., 2019. It tests for IgE antibodies to a multitude of pollen extracts and molecules (Di Fraia et al., 2019). Included in the assay are the following extracts: cypress, birch, oak, olive tree, Bermuda grass, timothy grass, wall pellitory, common ragweed, mugwort, *Alternaria alternata*, Russian thistle, English plantain, plane tree; and the following molecules: Cup a 1, Bet v 1, Bet v 2, Bet v 4, Que a 1, Cor a 1, Ole e 1, Ole e 7, Cyn d 1, Phl p 1, Phl p 4, Phl p 5, Phl p 7, Phl p 12, Par j 2, Amb a 1, Art v 1, Art v 3, Alt a 1, Sal k 1, Pla l 1, Pla a 1, Pla a 2 (Matricardi, 2017). All results were expressed in kU/l and considered as positive at ≥ 0.35 kU/l. For more details on the experimental procedures, please refer to Di Fraia et al. (Di Fraia et al., 2019).

Finally, as part of the T0 visit, the study physicians were asked to assess which pollen they consider clinically relevant for each patient and whether they would prescribe AIT based on the information gathered through the questionnaire and the SPT.

2.2.2 *Monitoring Period*

Via the Allergymonitor® application for mobile phones, daily health data was registered by patients in 2018. This included clinical symptoms and medication use (Figure 7). Based on the information obtained in the initial study visit, local study physicians established pollen seasons during which each patient was asked to document their disease. Patients with a low response rate were contacted by the study physicians in order to improve the volume of data generation.

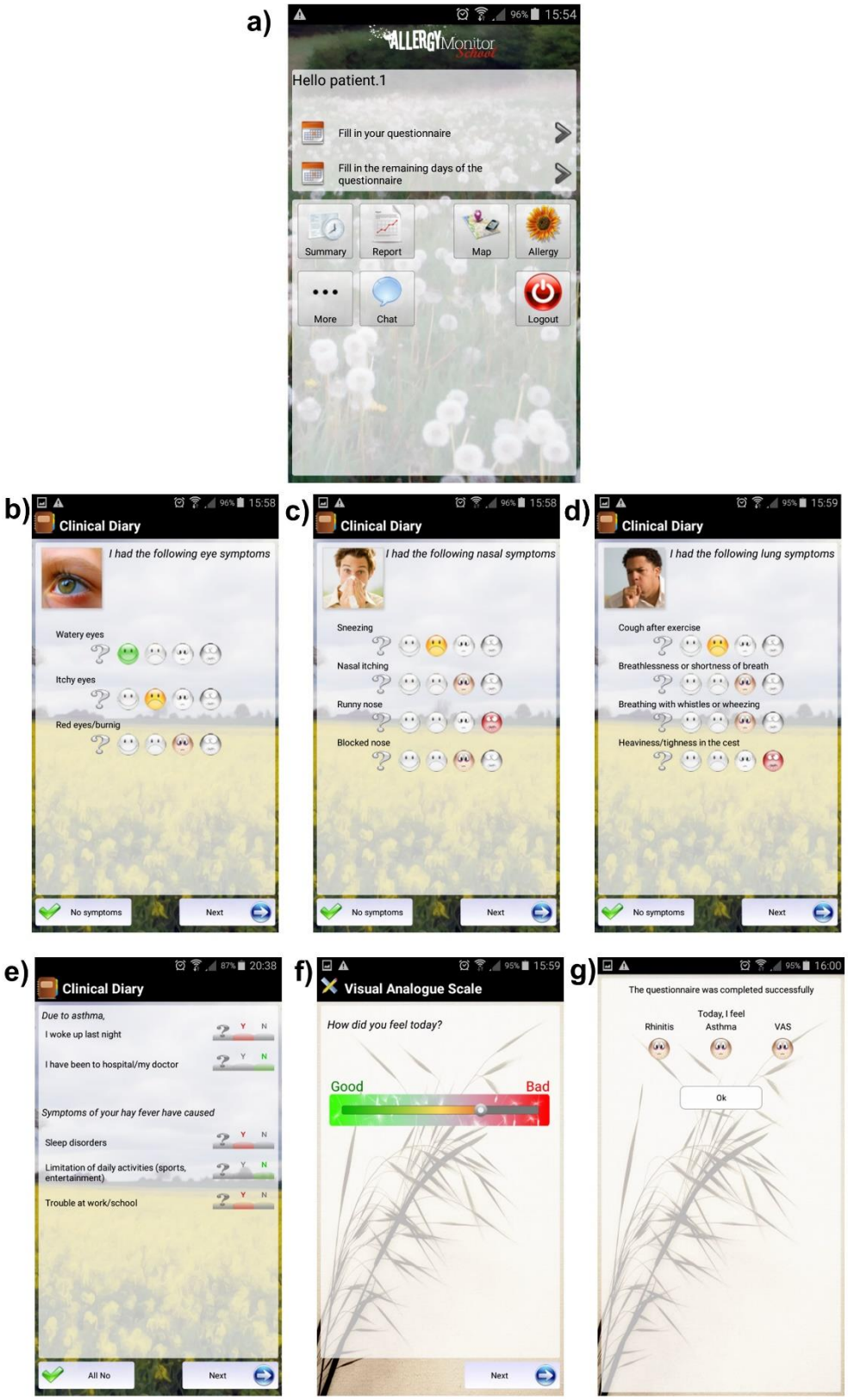


Figure 7: *Screenshots from Allergymonitor®, reproduced with written consent by Salvatore Tripodi, co-founder of TPS software production.*
a) Welcome screen; b) ocular symptoms; c) nasal symptoms; d) pulmonary symptoms; e) asthma; f) visual analogue scale; g) questionnaire completed.

2.2.3 *T1 Visit*

At the end of the monitoring period, a follow-up visit (T1 visit) was scheduled with the patients. Once more, a questionnaire was completed under the supervision of the study physicians. This questionnaire included sections on general personal information, rhino-conjunctivitis, drug therapy during the 2018 pollen season, asthma, and the patients' opinion on which pollens were causative for their symptoms. Additionally, questions regarding Allergymonitor® were included to assess the usability of the application and the digital literacy of the patient.

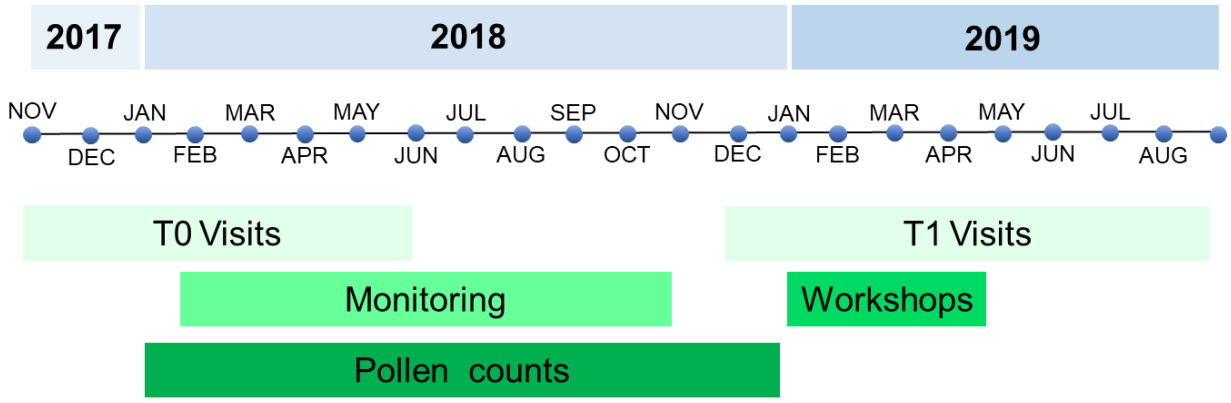


Figure 8: *Timeline for the @IT.2020 Multicenter Study*
T0 visit: patient recruitment, initial questionnaire, SPT, serum collection for IgE testing, installation of Allergymonitor® App.
Monitoring: daily symptom recording by the patients through Allergymonitor® App, pollen collection.
T1 visit: IgE result discussion, second questionnaire.
Workshops: organized for physicians to assess the CDSS.
Modified from Matricardi, 2017 by the author for this dissertation (Matricardi et al., 2016).

2.2.4 *Workshops*

Workshops were organized for each of the centers in early 2019. Physicians were instructed on general concepts and methodologies of the diagnostic tools used during the @IT.2020 Multicenter Study. They assessed which pollens were causative for the seasonal allergic rhinitis of the patients and whether they would prescribe AIT to the patients 1) based on the clinical history and SPT results, 2) also using the ESEP results, and 3) including the information from the Allergymonitor® application. Additionally, physicians evaluated all diagnostic tools used within the study.

2.3 PFAS Analysis

For the study on PFAS as a comorbidity of SAR, data from the T0 visit was analyzed (Figure 5). The focus was placed on sociodemographic data, family history, additional allergic comorbidities, and the reported PFAS reactions from the questionnaire. Furthermore, SPT results for seasonal aeroallergens (mugwort, wall pellitory, olive tree, hazel tree, birch, Bermuda grass, juniper ash, ragweed, timothy grass, *Alternaria alternata*, plane tree, Russian thistle, and mixed grasses) were included. Regarding the IgE results, focus was placed on allergenic molecules from three panallergen groups: profilins (Bet v 2, Phl p 12), PR-10 (Bet v 1, Cor a 1, Que a 1), and nsLTP (Art v 3, Ole e 7) (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation).

All statistical analyses were performed using IBM SPSS Statistics 25, Armonk, NY, USA. For a detailed description on the performed analyses please refer to Lipp et al. (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation).

3. Results

The focus of the analyses was placed on the clinical characteristics of patients suffering from the comorbidity PFAS within the cohort of recruited SAR patients in Southern Europe. Of the SAR patients, 20.5% reported PFAS reactions in their clinical history. These patients had significantly more maternal history of PFAS and reported further comorbidities (asthma, anaphylaxis, urticaria, and atopic dermatitis) than the SAR patients without PFAS. Additionally, the number of positive SPT results and the IgE reactions to panallergens showed a significant difference (Table 1). Sociodemographic data, SAR history, and ARIA severity and quality did not significantly differ between the patients with and without PFAS (Table 1).

As shown by Lipp et al. in Table e2 and Figure e1 (reproduced on pages 59 and 63, respectively, of this dissertation), hierarchical regression analysis demonstrates that associated characteristics of PFAS in Southern Europe include positive IgE response to one or more panallergens (profilin, PR-10, or nsLTP), reported history of maternal PFAS, and personal history of asthma (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation).

The SPT results showed significant differences between patients with and without PFAS, both regarding total number of positive results ($p < 0.001$) and for almost all tested extracts (Figure 9, Table 2). Especially Timothy grass, mugwort, *Alternaria alternata*, plane tree, birch, and hazel tree were more frequently positive in patients with PFAS. No significant difference in positive SPT results for juniper ash was found (Table 2).

Table 1. Clinical characteristics of SAR patients with and without PFAS in Southern Europe

	With PFAS (n=167)		Without PFAS (n=648)		Odds Ratio	*P*-Value
Male [n (%)]	82	*49.1*	359	*55.4*	1.288	.146
Age (y) [mean (*SD*)]	25.2	*13.1*	26.3	*13.7*	0.994	.318
Family history						
Atopic relative in immediate family [n (%)]	126	*75.5*	449	*69.3*	1.362	.120
Sibling(s) with PFAS [n (%)]	5	*3.0*	16	*2.5*	1.219	.703
Father with PFAS [n (%)]	1	*0.6*	6	*0.9*	0.645	.685
Mother with PFAS [n (%)]	13	*7.8*	12	*1.9*	4.474	<.001***
Allergic rhinitis						
Age at onset (y) [median (*IQR*)]	9	*12*	12	*14*	0.973	.003**
Disease duration (y) [median (*IQR*)]	9	*13.5*	8	*12*	1.013	.097
Months/year with symptoms [mean (*SD*)]	4.8	*2.4*	4.7	*2.4*	1.016	.659
ARIA severity						
Mild intermittent [n (%)]	6	*3.6*	35	*5.4*	-	.297
Mild persistent (ref.: mild intermittent) [n (%)]	9	*5.4*	51	*7.9*	1.029	.960
Mod./sev. intermittent (ref.: mild intermittent) [n (%)]	27	*16.2*	125	*19.3*	1.260	.637
Mod./sev. persistent (ref.: mild intermittent) [n (%)]	125	*74.9*	437	*67.4*	1.669	.259
ARIA quality						
Unclassified [n (%)]	19	*11.7*	108	*16.7*	-	.073
Rhinitis sneezer/runner (ref.: unclassified) [n (%)]	123	*73.7*	417	*64.4*	1.677	.055
Rhinitis blocker (ref.: unclassified) [n (%)]	25	*15.0*	123	*19.0*	1.155	.663
Other allergy comorbidities						
Number of patients with comorbidities [n (%)]	111	*66.5*	298	*46.0*	2.328	<.001***
Number of comorbidities [mean (*SD*)]	1.2	*1.0*	0.7	*0.8*	1.748	<.001***
Asthma [n (%)]	51	*30.5*	123	*19.0*	1.877	.001**
Anaphylaxis [n (%)]	26	*15.6*	23	*3.6*	5.001	<.001***
Urticaria [n (%)]	63	*37.7*	131	*20.2*	2.391	<.001***
Atopic dermatitis [n (%)]	50	*29.9*	129	*19.9*	1.719	.006**
Other [n (%)]	4	*2.4*	22	*3.4*	0.698	.514
IgE results						
No panallergen [n (%)]‡	102	*61.1*	559	*86.3*	-	<.001***
Mono-panallergen (ref.: no panallergen) [n (%)]‡	53	*31.7*	79	*12.2*	3.677	<.001***
Multi-panallergen (ref.: no panallergen) [n (%)]‡	12	*7.2*	10	*1.5*	6.576	<.001***
Profilins [n (%)]‡	26	*15.6*	42	*6.5*	2.661	<.001***
PR-10-like allergenic proteins [n (%)]‡	26	*15.6*	26	*4.0*	4.411	<.001***
nsLTPs [n (%)]‡	26	*15.6*	33	*5.1*	3.436	<.001***

PFAS: pollen food allergy syndrome; n: number; y: years; SD: standard deviation; IQR: interquartile range; mod.: moderate; sev.: severe; ref.: reference; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; ‡ Testpanel included: profilins (Bet v 2, Phl p 12), PR-10-like allergenic proteins (Bet v 1, Cor a 1, Que a 1), and nsLTPs (Art v 3, Ole e 7). Adapted from Lipp et al., 2021 (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation)..

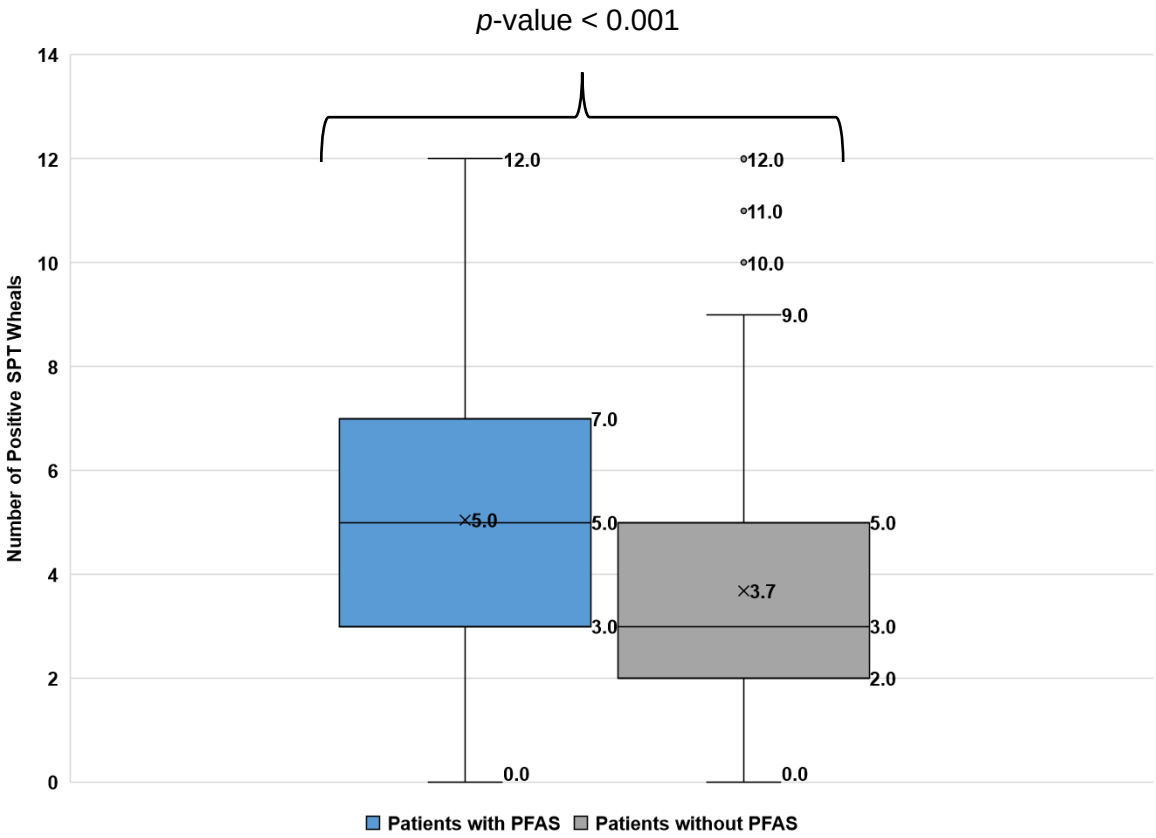


Figure 9: *Box-and-whiskers plot of number of positive SPT wheals for SAR patients with and without PFAS. The range of positive SPT wheals is displayed via the whiskers outside of the box, with outliers being marked as points above the upper end of the whisker. The lower and upper parts of the box portray the second and third quartile, respectively. The IQR is shown by the entire box, with the horizontal line displaying the median. The x marks the mean. Created by the author for this dissertation.*

Table 2. SPT results

	With PFAS (n=167)		**Without PFAS (n=648)**		***P*-Value**
Timothy grass	134	*80.2*	440	*67.9*	<0.001***
Olive tree	102	*61.1*	312	*48.2*	0.001**
Bermuda grass	94	*56.3*	321	*49.5*	0.04*
Mugwort	70	*41.9*	143	*22.1*	<0.001***
Pellitory	69	*41.3*	193	*29.8*	0.002**
Plane tree	59	*35.3*	139	*21.5*	<0.001***
Juniper Ash	59	*35.3*	252	*38.9*	0.132
Birch	54	*32.3*	113	*17.4*	<0.001***
Hazel tree	54	*32.3*	107	*16.5*	<0.001***
Salsola kali	51	*30.5*	132	*20.4*	0.002**
Ragweed	49	*29.3*	129	*19.9*	0.003**
Alternaria alternata	48	*28.7*	108	*16.7*	<0.001***

* $p<0.05$; ** $p<0.01$; *** $p<0.001$
Created by the author for this dissertation.

While patients in Southern Europe reported an overall high prevalence of PFAS amongst the SAR cohort, a significant variance of prevalence was reported between the centers. Few patients in Marseille (6/80, 7.5%) reported reactions to known PFAS-eliciting foods, while patients from Rome showed a high rate of PFAS (41/99, 41.4%) (Table 3). The heterogeneity between the included centers was not only apparent in regards to prevalence of PFAS but also regarding their clinical characteristics.

Table 3. Patients with PFAS per center

Total (n=815)	167	*20.5%*
POR (n=102)	24	*23.5%*
VAL (n=71)	10	*14.1%*
MAR (n=80)	6	*7.5%*
ROM (n=99)	41	*41.4%*
MES (n=82)	24	*29.3%*
TIR (n=93)	13	*14.0%*
ATH (n=97)	22	*22.7%*
IST (n=96)	13	*13.5%*
IZM (n=95)	14	*14.7%*

All percentages are calculated from the total number of patients from each center.
Created by the author for this dissertation.

The age at onset of SAR, months per year with SAR symptoms, ARIA severity and quality showed a significant divergence between the centers. Additionally, the number of patients with comorbidities and the number of additional comorbidities per patient also differed significantly between the centers, especially regarding urticaria and atopic dermatitis. The recorded SPT reactions showed significant heterogeneity both in number and in average wheal size, while for the IgE test results, especially PR-10 showed heterogeneity (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation).

Overall, kiwi, peach, and melon were found to be the three most common elicitors of PFAS in Southern Europe. A large number of patients also reported reactions to foods not included in the questionnaire (Figure 10).

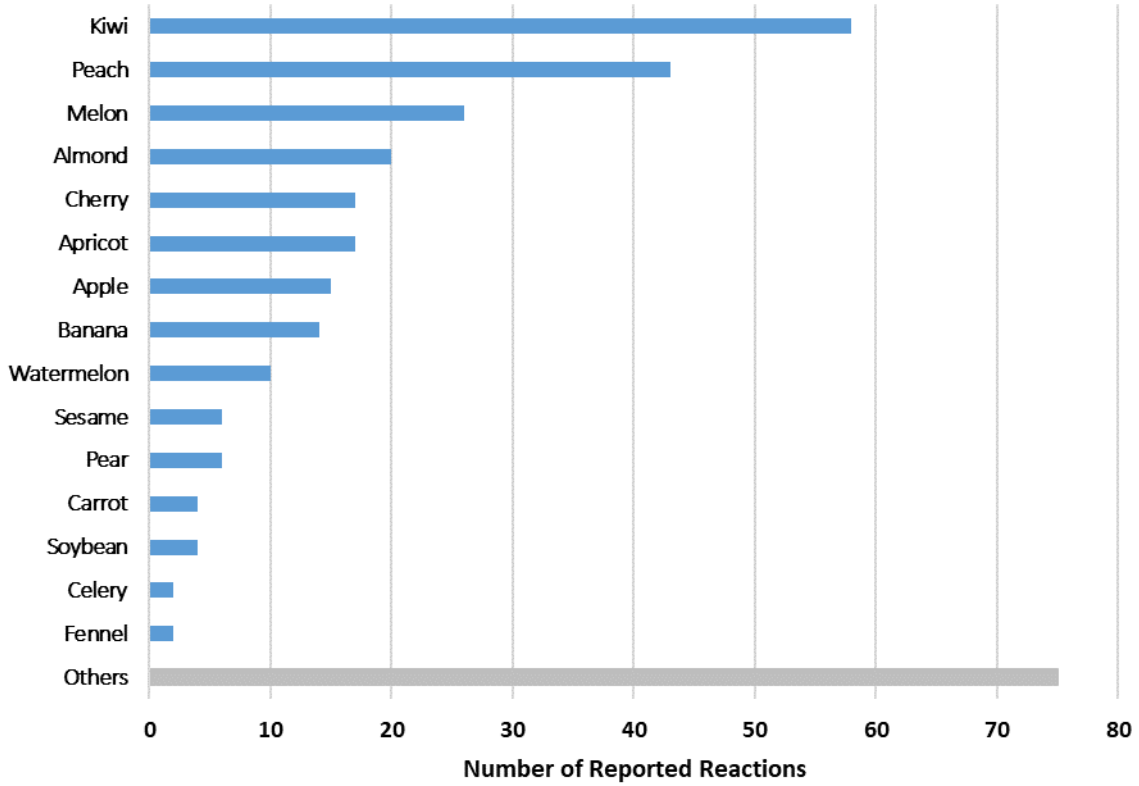


Figure 10: *Number of reported PFAS reactions to the different possible symptom-eliciting foods.*
Created by the author for this dissertation.

The frequency of reactions for the differing elicitors included in the questionnaire varied greatly between the centers. While kiwi was the most frequently reported PFAS-eliciting food in POR, MAR, ROM, IST, and IZM, peach played an equally large role in patients from ATH. It was the most reported elicitor in MES and in VAL it was the most prominent together with almond. TIR named almond alone as the most common elicitor. ROM was the only center where carrot, fennel, and celery were reported as elicitors and both Italian centers (ROM and MES) reported reactions to pear (Table 4).

Table 4. Reported reactions to different foods in patients with PFAS per center

	POR (n=24)		VAL (n=10)		MAR (n=6)		ROM (n=41)		MES (n=24)		TIR (n=13)		ATH (n=22)		IST (n=13)		IZM (n=14)	
Kiwi [n (%)]	11	*45.8*	2	*20.0*	2	*33.3*	16	*39.0*	9	*37.5*	2	*15.4*	5	*22.7*	3	*23.1*	8	*57.1*
Peach [n (%)]	5	*20.8*	5	*50.0*	1	*16.7*	9	*22.0*	13	*54.2*	3	*23.1*	5	*22.7*	1	*7.7*	1	*7.1*
Melon [n (%)]	8	*33.3*	1	*10.0*	0	*0.0*	9	*22.0*	0	*0.0*	2	*15.4*	4	*18.2*	0	*0.0*	2	*14.3*
Almond [n (%)]	2	*8.3*	5	*50.0*	1	*16.7*	2	*4.9*	2	*8.3*	4	*30.8*	2	*9.1*	2	*15.4*	0	*0.0*
Apricot [n (%)]	5	*20.8*	0	*0.0*	0	*0.0*	4	*9.8*	5	*20.8*	0	*0.0*	1	*4.6*	1	*7.7*	1	*7.1*
Cherry [n (%)]	2	*8.3*	1	*10.0*	1	*16.7*	4	*9.8*	4	*16.7*	3	*23.1*	1	*4.6*	0	*0.0*	1	*7.1*
Apple [n (%)]	4	*16.7*	0	*0.0*	1	*16.7*	3	*7.3*	2	*8.3*	3	*23.1*	0	*0.0*	0	*0.0*	2	*14.3*
Banana [n (%)]	3	*12.5*	0	*0.0*	1	*16.7*	3	*7.3*	0	*0.0*	1	*7.7*	3	*13.6*	1	*7.7*	2	*14.3*
Watermelon [n (%)]	3	*12.5*	0	*0.0*	1	*16.7*	2	*4.9*	0	*0.0*	0	*0.0*	1	*4.6*	1	*7.7*	2	*14.3*
Pear [n (%)]	0	*0.0*	0	*0.0*	0	*0.0*	4	*9.8*	2	*8.3*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Sesame [n (%)]	0	*0.0*	0	*0.0*	1	*16.7*	1	*2.4*	1	*4.2*	1	*7.7*	1	*4.6*	1	*7.7*	0	*0.0*
Soybean [n (%)]	0	*0.0*	0	*0.0*	0	*0.0*	2	*4.9*	1	*4.2*	1	*7.7*	0	*0.0*	0	*0.0*	0	*0.0*
Carrot [n (%)]	0	*0.0*	0	*0.0*	0	*0.0*	4	*9.8*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Fennel [n (%)]	0	*0.0*	0	*0.0*	0	*0.0*	2	*4.9*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Celery [n (%)]	0	*0.0*	0	*0.0*	0	*0.0*	2	*4.9*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Others [n (%)]	14	*58.3*	1	*10.0*	1	*16.7*	20	*48.8*	12	*50.0*	7	*53.9*	12	*54.6*	7	*53.9*	1	*7.1*

All percentages are calculated from the total number of PFAS patients in each center. Reactions to multiple foods may have been reported.
Created by the author for this dissertation.

Overall, most patients experienced local symptoms, especially oral pruritus. The most common systemic symptom was urticaria, which was especially frequent in patients consuming peach, soy, and apricot. Kiwi caused most of the reported gastrointestinal symptoms, while more severe systemic reactions (pallor/hypotension, palpitations/tachycardia/loss of consciousness) were reported by patients consuming peach, almond, soy, and melon. While patients reporting reactions to “others” also mostly reported oral pruritus as a symptom, the rate of systemic symptoms was high, including the more severe symptoms listed above. 85% of all patients experienced symptoms within the first twenty minutes of contact with the elicitor. Reactions occurring after two hours were rare. Patients reacting to cherry had the highest frequency of symptom onset over 60 minutes after contact with the symptom-eliciting food (Table 5).

Additional analyses regarding cypress pollen, an important pollen causing SAR in Southern Europe, were performed to gain further insight into its potential role regarding PFAS. As a result, it was found that SPT mono-sensitized patients to juniper ash, an indicator for cypress sensitization (André et al., 2000), only had a frequency of 1/22 of reporting PFAS. None of the patients with IgE mono-sensitization to cypress reported PFAS reactions. For further details, please refer to Lipp et al. (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation). Additionally, as seen in Table 3, no significant difference could be found between patients with and without PFAS regarding SPT sensitization when testing for juniper ash.

Due to its unique quality as a heat- and acid-stable panallergen, nsLTP was examined in more depth and two things stood out. First, in nsLTP IgE positive patients, the most common elicitors were peach, kiwi, and almond. Second, these patients also reported a high rate of systemic symptoms. For further details, please see Lipp et al. (Lipp et al., 2021 (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation).

Table 5. Reported PFAS symptoms and times to onset of reaction for different possible eliciting foods

	Total (n=319)		Kiwi (n=58)		Peach (n=43)		Melon (n=26)		Almond (n=20)		Cherry (n=17)		Apricot (n=17)		Apple (n=15)		Banana (n=14)	
Local symptoms																		
Oral pruritus [n (%)]	252	*79.0*	49	*84.5*	35	*81.4*	21	*80.8*	15	*75.0*	13	*76.5*	13	*76.5*	12	*80.0*	12	*85.7*
Swelling of tongue/face [n (%)]	49	*15.4*	11	*19.0*	11	*25.6*	2	*7.7*	1	*5.0*	2	*11.8*	3	*17.7*	3	*20.0*	1	*7.1*
Difficulty talking/swallowing [n (%)]	13	*4.1*	4	*6.9*	2	*4.7*	0	*0.0*	0	*0.0*	1	*5.9*	1	*5.9*	1	*6.7*	0	*0.0*
Oral vesicles [n (%)]	5	*1.6*	1	*1.7*	3	*7.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Systemic symptoms																		
Urticaria [n (%)]	48	*15.1*	5	*8.6*	10	*23.3*	3	*11.5*	3	*15.0*	4	*23.5*	2	*11.8*	2	*13.3*	1	*7.1*
Skin redness [n (%)]	41	*12.9*	7	*12.1*	9	*20.9*	3	*11.5*	1	*5.0*	3	*17.7*	2	*11.8*	2	*13.3*	0	*0.0*
Cough/wheezing/respiratory difficulties [n (%)]	34	*10.7*	11	*19.0*	5	*11.6*	1	*3.9*	4	*20.0*	1	*5.9*	1	*5.9*	1	*6.7*	1	*7.1*
Swelling of eyes/eyelids [n (%)]	27	*8.5*	5	*8.6*	8	*18.6*	1	*3.9*	1	*5.0*	2	*11.8*	1	*5.9*	1	*6.7*	0	*0.0*
Nose closed/running [n (%)]	16	*5.0*	4	*6.9*	1	*2.3*	1	*3.9*	1	*5.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Vomiting [n (%)]	10	*3.1*	2	*3.5*	0	*0.0*	1	*3.9*	1	*5.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*7.1*
Diarrhea [n (%)]	9	*2.8*	4	*6.9*	0	*0.0*	1	*3.9*	0	*0.0*	0	*0.0*	0	*0.0*	1	*6.7*	0	*0.0*
Pallor/hypotension [n (%)]	6	*1.9*	0	*0.0*	1	*2.3*	1	*3.9*	1	*5.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Palpitations/tachycardia [n (%)]	2	*0.6*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Loss of consciousness [n (%)]	1	*0.3*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Time to onset of reaction																		
≤ 5 min [n (%)]	209	*65.5*	48	*82.8*	24	*55.8*	20	*76.9*	10	*50.0*	10	*58.8*	11	*64.7*	11	*73.3*	9	*64.3*
6-20 min [n (%)]	62	*19.4*	5	*8.6*	12	*27.9*	5	*19.2*	6	*30.0*	2	*11.8*	4	*23.5*	2	*13.3*	3	*21.4*
21-60 min [n (%)]	29	*9.1*	5	*8.6*	3	*7.0*	1	*3.9*	1	*5.0*	2	*11.8*	1	*5.9*	1	*6.7*	2	*14.3*
61-120 min [n (%)]	6	*1.9*	0	*0.0*	2	*4.7*	0	*0.0*	1	*5.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
>120 min [n (%)]	13	*4.1*	0	*0.0*	2	*4.7*	0	*0.0*	2	*10.0*	3	*17.7*	1	*5.9*	1	*6.7*	0	*0.0*

All percentages are calculated from the total number of reactions reported for each PFAS-elicitor. Multiple symptoms may have been reported. Min: minutes.
Created by the author for this dissertation.

Table 5 continued. Reported PFAS symptoms and times to onset of reaction for different possible eliciting foods

	Total (n=319)		Watermelon (n=10)		Sesame (n=6)		Pear (n=6)		Carrot (n=4)		Soybean (n=4)		Celery (n=2)		Fennel (n=2)		Others (n=75)	
Local symptoms																		
Oral pruritus [n (%)]	252	*79.0*	8	*80.0*	4	*66.7*	6	*100.0*	4	*100.0*	2	*50.0*	2	*100.0*	2	*100.0*	54	*72.0*
Swelling of tongue/face [n (%)]	49	*15.4*	1	*10.0*	0	*0.0*	1	*16.7*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	13	*17.3*
Difficulty talking/swallowing [n (%)]	13	*4.1*	0	*0.0*	1	*16.7*	1	*16.7*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	2	*2.7*
Oral vesicles [n (%)]	5	*1.6*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*1.3*
Systemic symptoms																		
Urticaria [n (%)]	48	*15.1*	0	*0.0*	1	*16.7*	1	*16.7*	0	*0.0*	1	*25.0*	0	*0.0*	1	*50.0*	14	*18.7*
Skin redness [n (%)]	41	*12.9*	0	*0.0*	0	*0.0*	1	*16.7*	0	*0.0*	1	*25.0*	0	*0.0*	0	*0.0*	12	*16.0*
Cough/wheezing/respiratory difficulties [n (%)]	34	*10.7*	0	*0.0*	1	*16.7*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	8	*10.7*
Swelling of eyes/eyelids [n (%)]	27	*8.5*	0	*0.0*	0	*0.0*	1	*16.7*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	7	*9.3*
Nose closed/running [n (%)]	16	*5.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	9	*12.0*
Vomiting [n (%)]	10	*3.1*	1	*10.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	4	*5.3*
Diarrhea [n (%)]	9	*2.8*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*25.0*	0	*0.0*	0	*0.0*	2	*2.7*
Pallor/hypotension [n (%)]	6	*1.9*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*25.0*	0	*0.0*	0	*0.0*	2	*2.7*
Palpitations/tachycardia [n (%)]	2	*0.6*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	2	*2.7*
Loss of consciousness [n (%)]	1	*0.3*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*1.3*
Time to onset of reaction																		
≤ 5 min [n (%)]	209	*65.5*	7	*70.0*	2	*33.3*	4	*66.7*	3	*75.0*	2	*50.0*	2	*100.0*	1	*50.0*	45	*60.0*
6-20 min [n (%)]	62	*19.4*	2	*20.0*	3	*50.0*	1	*16.7*	1	*25.0*	1	*25.0*	0	*0.0*	1	*50.0*	14	*18.7*
21-60 min [n (%)]	29	*9.1*	0	*0.0*	1	*16.7*	1	*16.7*	0	*0.0*	1	*25.0*	0	*0.0*	0	*0.0*	10	*13.3*
61-120 min [n (%)]	6	*1.9*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	3	*4.0*
>120 min [n (%)]	13	*4.1*	1	*10.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	3	*4.0*

All percentages are calculated from the total number of reactions reported for each PFAS-elicitor. Multiple symptoms may have been reported. Min: minutes.
Created by the author for this dissertation.

4. Discussion

The analyses of SAR patients with PFAS in Southern Europe showed:
a) a significantly higher rate of further allergic comorbidities, positive maternal history for PFAS, and higher rate of positive IgE to the included panallergens (profilin, PR-10, nsLTP); b) more positive SPT results for mugwort, wall pellitory, olive tree, hazel tree, birch, Bermuda grass, ragweed, timothy grass, *Alternaria alternata*, plane tree, Russian thistle, and mixed grasses, but not for juniper ash; c) a large variance in reported PFAS between the centers; d) kiwi, peach, and melon as the most frequently reported PFAS elicitors overall, with heterogeneity in frequency between the centers; e) mostly local reactions with onset within minutes after contact with the eliciting foods (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation).
The overall prevalence of PFAS amongst patients with SAR worldwide falls between 9.6-55% (Bedolla-Barajas et al., 2017; Bircher et al., 1994), which is supported by our study, since 20.5% of SAR patients overall reported PFAS reactions. There was a breadth of range of 7.5-41.4% in the different centers, showing similar results to previous data collected in Italy and Turkey, respectively (Mastrorilli et al., 2016; Özdemir and Özgüçlü, 2018).
The study corroborates findings to those by Mastrorilli et al. by showing the significant link between PFAS and positive maternal history of PFAS, a positive correlation between PFAS as a comorbidity of SAR and further allergic comorbidities, and IgE to panallergens (Mastrorilli et al., 2016). Especially the stepwise regression analysis was able to demonstrate this link, as maternal history, asthma, and IgE to panallergens were shown to be associated factors of PFAS for the @IT.2020 cohort (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation).
While a higher rate of overall SPT responses has been described before (Mastrorilli et al., 2016), the current data was able to expand on that knowledge and show that patients with PFAS had a higher rate of positive SPT responses to each individual seasonal aeroallergen included in the present study, except juniper ash.
In IgE testing, the following extracts showed an especially significant difference in patients with and without PFAS, possibly based on the prominence of panallergenic molecules associated with these elicitors: birch (with Bet v 1 as PR-10 and Bet v 2 as profilin panallergen), timothy grass (with Phl p 12 as profilin panallergen), mugwort (with Art v 4 as profilin and Art v 3 as nsLTP panallergen), plane tree (with Pla a 3 as nsLTP

panallergen), and hazel tree (with Cor a 1 as PR-10 and Cor a 2 as profilin panallergen) (Matricardi et al., 2016). Additionally, SPT reactions to *Alternaria alternata* showed a highly significant difference between patients with and without PFAS. To date, no panallergens from the included groups have been described for *Alternaria alternata* ("WHO/IUIS Allergen Nomenclature Home Page," 2021). This could be due to the fact that PFAS patients are frequently polysensitized, as shown above, and the sensitization to *Alternaria alternata* could be a reflection of that polysensitization.

Previously, a correlation between peach allergy and cypress sensitization has been described (Caimmi et al., 2013; Hugues et al., 2006). Yet this cohort, as demonstrated by Lipp et al., showed that no cypress-monosensitized patient reported PFAS reactions to peach (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation). Interestingly, the result for the juniper ash SPT lends further support that cypress pollen allergies play a lesser role in PFAS in the studied cohort than expected, as juniper ash is used as an indicator of cypress pollen allergy (André et al., 2000).

Typical food reactions from previous studies performed in Southern Europe included kiwi, peach, tomato, melon, and watermelon in Turkey (Özdemir and Özgüçlü, 2018) and kiwi, peach, and apple in Italy (Mastrorilli et al., 2016). In the nine study centers, kiwi, peach, and melon were found to be the most frequent elicitors overall. Yet some differences were noted in the frequency of named elicitors, which could be explained by the differing pattern in pollen exposure due to the various levels of pollens and variations in seasons, resulting in differing sensitization (Hoffmann et al., 2020).

According to current literature, patients with PFAS mostly report localized reactions occurring in the oropharynx with a rapid onset (Price et al., 2015). The reported reactions by patients from the nine included Southern European centers support this assertion. Yet, while previous studies showed more generalized reactions at a rate of around 5%, a higher rate of systemic symptoms was reported by the present cohort, especially in patients that were nsLTP positive (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation; Price et al., 2015). This phenomenon is likely due to the fact that nsLTPs are heat and acid stable (Matricardi et al., 2016).

The present study showed certain limitations, mainly, that the diagnosis of PFAS was based on clinical history of PFAS only, not on the basis of prick-by-prick testing or oral food challenges. Furthermore, the patient recruitment occurred in allergy clinics in the different centers, therefore, the present results are not representative of the entire

population of the included countries (Lipp et al., 2021, reproduced on pages 43-63 of this dissertation).

5. Conclusions

The data and information from the PFAS analyses from the @IT.2020 Multicenter Study reported here provide important first insights into the clinical make-up of SAR patients in Southern Europe. As such, they will fundamentally serve physicians practicing in the region both in the diagnostic work-up of allergic patients and making the correct recommendations to their patients regarding food elicitor avoidance and therapy. Additionally, due to the uniform methodology applied throughout the @IT.2020 Multicenter Study, the data and information obtained affords a sound basis for future research in the domain of PFAS and SAR.

These results have shown that, due to its vast reach and unified methodology, the @IT.2020 Multicenter Study can add much relevant knowledge to the field of allergology and thus aid physicians treating and patients suffering from SAR and PFAS. As this is only one part of the comprehensive data sets collected through the @IT.2020 Multicenter Study and given that SAR is on the rise globally (Asher et al., 2006), the additional information that will be gleaned by further analyzing the data obtained from this study is expected to serve as a great tool for physicians and researchers not only in Southern Europe but other parts of the world as well.

Bibliography

André, C., Dumur, J.P., Hrabina, M., Lefebvre, E., Sicard, H., 2000. Juniperus ashei: the gold standard of the Cuppressaceae. Allerg. Immunol. (Leipz.) 32, 104–106.

Asher, M.I., Montefort, S., Björkstén, B., Lai, C.K., Strachan, D.P., Weiland, S.K., Williams, H., 2006. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. The Lancet 368, 733–743. https://doi.org/10.1016/S0140-6736(06)69283-0

Bachert, C., Andreasen, J.N., 2015. Cost-Effectiveness of Immunotherapy in the Treatment of Seasonal Allergic Rhinitis: Identifying Product-Specific Parameters of Relevance for Health Care Decision-Makers and Clinicians. Int. Arch. Allergy Immunol. 168, 213–217. https://doi.org/10.1159/000443272

Barber, D., Diaz-Perales, A., Escribese, M.M., Kleine-Tebbe, J., Matricardi, P.M., Ollert, M., Santos, A.F., Sastre, J., 2021. Molecular allergology and its impact in specific allergy diagnosis and therapy. Allergy n/a. https://doi.org/10.1111/all.14969

Bedolla-Barajas, M., Kestler-Gramajo, A., Alcalá-Padilla, G., Morales-Romero, J., 2017. Prevalence of oral allergy syndrome in children with allergic diseases. Allergol. Immunopathol. (Madr.) 45, 127–133. https://doi.org/10.1016/j.aller.2016.04.017

Bircher, A.J., Van Melle, G., Haller, E., Curty, B., Frei, P.C., 1994. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol. 24, 367–374. https://doi.org/10.1111/j.1365-2222.1994.tb00248.x

Bjermer, L., Westman, M., Holmström, M., Wickman, M.C., 2019. The complex pathophysiology of allergic rhinitis: scientific rationale for the development of an alternative treatment option. Allergy Asthma Clin. Immunol. 15, 24. https://doi.org/10.1186/s13223-018-0314-1

Bogas, G., Muñoz-Cano, R., Mayorga, C., Casas, R., Bartra, J., Pérez, N., Pascal, M., Palomares, F., Torres, M.J., Gómez, F., 2020. Phenotyping peach-allergic patients sensitized to lipid transfer protein and analysing severity biomarkers. Allergy 75, 3228–3236. https://doi.org/10.1111/all.14447

Bousquet, J., Heinzerling, L., Bachert, C., Papadopoulos, N.G., Bousquet, P.J., Burney, P.G., Canonica, G.W., Carlsen, K.H., Cox, L., Haahtela, T., Lodrup Carlsen, K.C., Price, D., Samolinski, B., Simons, F.E.R., Wickman, M., Annesi-Maesano, I., Baena-Cagnani, C.E., Bergmann, K.C., Bindslev-Jensen, C., Casale, T.B., Chiriac, A., Cruz, A.A., Dubakiene, R., Durham, S.R., Fokkens, W.J., Gerth-van-Wijk, R., Kalayci, O., Kowalski, M.L., Mari, A., Mullol, J., Nazamova-Baranova, L., O'Hehir, R.E., Ohta, K., Panzner, P., Passalacqua, G., Ring, J., Rogala, B., Romano, A., Ryan, D., Schmid-Grendelmeier, P., Todo-Bom, A., Valenta, R., Woehrl, S., Yusuf, O.M., Zuberbier, T., Demoly, P., 2012. Practical guide to skin prick tests in allergy to aeroallergens: Practical use of skin tests. Allergy 67, 18–24. https://doi.org/10.1111/j.1398-9995.2011.02728.x

Caimmi, D., Barber, D., Hoffmann-Sommergruber, K., Amrane, H., Bousquet, P.-J., Dhivert-Donnadieu, H., Demoly, P., 2013. Understanding the molecular sensitization for Cypress pollen and peach in the Languedoc-Roussillon area. Allergy 68, 249–251. https://doi.org/10.1111/all.12073

Di Fraia, M., Arasi, S., Castelli, S., Dramburg, S., Potapova, E., Villalta, D., Tripodi, S., Sfika, I., Zicari, A.M., Villella, V., Perna, S., Travaglini, A., Verardo, P.L., Matricardi, P.M., 2019. A new molecular multiplex IgE assay for the diagnosis of

pollen allergy in Mediterranean countries: A validation study. Clin. Exp. Allergy 49, 341–349. https://doi.org/10.111/cea.13264
Dykewicz, M.S., Wallace, D.V., Baroody, F., Bernstein, J., Craig, T., Finegold, I., Huang, F., Larenas-Linnemann, D., Meltzer, E., Steven, G., Bernstein, D.I., Blessing-Moore, J., Dinakar, C., Greenhawt, M., Horner, C.C., Khan, D.A., Lang, D., Oppenheimer, J., Portnoy, J.M., Randolph, C.R., Rank, M.A., Dykewicz, M.S., Wallace, D.V., 2017. Treatment of seasonal allergic rhinitis. Ann. Allergy. Asthma. Immunol. 119, 489-511.e41. https://doi.org/10.1016/j.anai.2017.08.012
Fonseca, J.A., Nogueira-Silva, L., Morais-Almeida, M., Azevedo, L., Sa-Sousa, A., Branco-Ferreira, M., Fernandes, L., Bousquet, J., 2010. Validation of a questionnaire (CARAT10) to assess rhinitis and asthma in patients with asthma. Allergy 65, 1042–1048. https://doi.org/10.1111/j.1398-9995.2009.02310.x
Fujita, H., Soyka, M.B., Akdis, M., Akdis, C.A., 2012. Mechanisms of allergen-specific immunotherapy. Clin. Transl. Allergy 2, 2. https://doi.org/10.1186/2045-7022-2-2
Greiner, A.N., Hellings, P.W., Rotiroti, G., Scadding, G.K., 2011. Allergic rhinitis. The Lancet 378, 2112–2122. https://doi.org/10.1016/S0140-6736(11)60130-X
Hauser, M., Roulias, A., Ferreira, F., Egger, M., 2010. Panallergens and their impact on the allergic patient. Allergy Asthma Clin. Immunol. Off. J. Can. Soc. Allergy Clin. Immunol. 6, 1. https://doi.org/10.1186/1710-1492-6-1
Heinzerling, L., Mari, A., Bergmann, K.-C., Bresciani, M., Burbach, G., Darsow, U., Durham, S., Fokkens, W., Gjomarkaj, M., Haahtela, T., Bom, A.T., Wöhrl, S., Maibach, H., Lockey, R., 2013. The skin prick test – European standards. Clin. Transl. Allergy 3, 3. https://doi.org/10.1186/2045-7022-3-3
Hoffmann, T.M., Acar Şahin, A., Aggelidis, X., Arasi, S., Barbalace, A., Bourgoin, A., Bregu, B., Brighetti, M.A., Caeiro, E., Caglayan Sozmen, S., Caminiti, L., Charpin, D., Couto, M., Delgado, L., Di Rienzo Businco, A., Dimier, C., Dimou, M.V., Fonseca, J.A., Goksel, O., Guvensen, A., Hernandez, D., Jang, D.T., Kalpaklioglu, F., Lame, B., Llusar, R., Makris, M., Mazon, A., Mesonjesi, E., Nieto, A., Öztürk, A., Pahus, L., Pajno, G., Panasiti, I., Panetta, Papadopoulos, N.G., Pellegrini, E., Pelosi, S., Pereira, A.M., Pereira, M., Pinar, N.M., Pfaar, O., Potapova, E., Priftanji, A., Psarros, F., Sackesen, C., Sfika, I., Suarez, J., Thibaudon, M., Travaglini, A., Tripodi, S., Verdier, V., Villella, V., Xepapadaki, P., Yazici, D., Matricardi, P.M., Dramburg, S., 2020. “Whole” vs. “Fragmented” approach to EAACI Pollen Season Definitions: A Multicenter Study in Six Southern European Cities. Allergy. https://doi.org/10.1111/all.14153
Hugues, B., Didierlaurent, A., Charpin, D., 2006. Cross-reactivity between cypress pollen and peach: a report of seven cases. Allergy 61, 1241–1243. https://doi.org/10.1111/j.1398-9995.2006.01156.x
Kondo, Y., Urisu, A., 2009. Oral Allergy Syndrome. Allergol. Int. 58, 485–491. https://doi.org/10.2332/allergolint.09-RAI-0136
Lake, I.R., Jones, N.R., Agnew, M., Goodess, C.M., Giorgi, F., Hamaoui-Laguel, L., Semenov, M.A., Solomon, F., Storkey, J., Vautard, R., Epstein, M.M., 2017. Climate Change and Future Pollen Allergy in Europe. Environ. Health Perspect. 125, 385–391. https://doi.org/10.1289/EHP173
Lipp, T., Acar Şahin, A., Aggelidis, X., Arasi, S., Barbalace, A., Bourgoin, A., Bregu, B., Brighetti, M.A., Caeiro, E., Caglayan Sozmen, S., Caminiti, L., Charpin, D., Couto, M., Delgado, L., Di Rienzo Businco, A., Dimier, C., Dimou, M.V., Fonseca, J.A., Goksel, O., Guvensen, A., Hernandez, D., Hoffmann, T.M., Jang, D.T., Kalpaklioglu, F., Lame, B., Llusar, R., Makris, M., Mazon, A., Mesonjesi, E., Nieto, A., Öztürk, A., Pahus, L., Pajno, G., Panasiti, I., Papadopoulos, N.G.,

Pellegrini, E., Pelosi, S., Pereira, A.M., Pereira, M., Pinar, N.M., Potapova, E., Priftanji, A., Psarros, F., Sackesen, C., Sfika, I., Suarez, J., Thibaudon, M., Travaglini, A., Tripodi, S., Verdier, V., Villella, V., Xepapadaki, P., Yazici, D., Matricardi, P.M., Dramburg, S., 2021. Heterogeneity of Pollen Food Allergy Syndrome in Seven Southern European Countries: the @IT.2020 Multicenter Study. Allergy. https://doi.org/10.1111/all.14742

Mastrorilli, C., Tripodi, S., Caffarelli, C., Perna, S., Rienzo-Businco, A.D., Sfika, I., Asero, R., Dondi, A., Bianchi, A., Dascola, C.P., Ricci, G., Cipriani, F., Maiello, N., Giudice, M.M.D., Frediani, T., Frediani, S., Macrì, F., Pistoletti, C., Iacono, I.D., Patria, M.F., Varin, E., Peroni, D., Comberiati, P., Chini, L., Moschese, V., Lucarelli, S., Bernardini, R., Pingitore, G., Pelosi, U., Olcese, R., Moretti, M., Cirisano, A., Faggian, D., Travaglini, A., Plebani, M., Verga, M.C., Calvani, M., Giordani, P., Matricardi, P.M., 2016. Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification. Allergy 71, 1181–1191. https://doi.org/10.1111/all.12888

Matricardi, P.M., 2017. Standard Operating Procedures CRD and mHealth for pollen allergy in Southern Europe. A multicenter observationals study @IT.2020.

Matricardi, P.M., Kleine-Tebbe, J., Hoffmann, H.J., Valenta, R., Hilger, C., Hofmaier, S., Aalberse, R.C., Agache, I., Asero, R., Ballmer-Weber, B., Barber, D., Beyer, K., Biedermann, T., Bilò, M.B., Blank, S., Bohle, B., Bosshard, P.P., Breiteneder, H., Brough, H.A., Caraballo, L., Caubet, J.C., Crameri, R., Davies, J.M., Douladiris, N., Ebisawa, M., EIgenmann, P.A., Fernandez-Rivas, M., Ferreira, F., Gadermaier, G., Glatz, M., Hamilton, R.G., Hawranek, T., Hellings, P., Hoffmann-Sommergruber, K., Jakob, T., Jappe, U., Jutel, M., Kamath, S.D., Knol, E.F., Korosec, P., Kuehn, A., Lack, G., Lopata, A.L., Mäkelä, M., Morisset, M., Niederberger, V., Nowak-Węgrzyn, A.H., Papadopoulos, N.G., Pastorello, E.A., Pauli, G., Platts-Mills, T., Posa, D., Poulsen, L.K., Raulf, M., Sastre, J., Scala, E., Schmid, J.M., Schmid-Grendelmeier, P., van Hage, M., van Ree, R., Vieths, S., Weber, R., Wickman, M., Muraro, A., Ollert, M., 2016. EAACI Molecular Allergology User's Guide. Pediatr. Allergy Immunol. 27, 1–250. https://doi.org/10.1111/pai.12563

Oeo-Santos, C., Navas, A., Benedé, S., Ruíz-León, B., Díaz-Perales, A., Vogel, L., Moreno-Aguilar, C., Jurado, A., Villalba, M., Barderas, R., 2020. New insights into the sensitization to nonspecific lipid transfer proteins from pollen and food: New role of allergen Ole e 7. Allergy 75, 798–807. https://doi.org/10.1111/all.14086

Özdemir, S.K., Özgüçlü, S., 2018. Pollen food allergy syndrome in Turkey: Clinical characteristics and evaluation of its association with skin test reactivity to pollens. Asian Pac. J. Allergy Immunol. 36, 77–81. https://doi.org/10.12932/AP0881

Passali, D., Cingi, C., Staffa, P., Passali, F., Muluk, N.B., Bellussi, M.L., 2018. The International Study of the Allergic Rhinitis Survey: outcomes from 4 geographical regions. Asia Pac. Allergy 8. https://doi.org/10.5415/apallergy.2018.8.e7

Pawanker, R., Holgate, S.T., Canonica, G.W., Lockey, R.F., Blaiss, M.S., 2013. WAO White Book on Allergy | World Allergy Organization [WWW Document]. URL https://www.worldallergy.org/wao-white-book-on-allergy (accessed 12.18.20).

Price, A., Ramachandran, S., Smith, G.P., Stevenson, M.L., Pomeranz, M.K., Cohen, D.E., 2015. Oral Allergy Syndrome (Pollen-Food Allergy Syndrome). Dermatitis 26, 78. https://doi.org/10.1097/DER.0000000000000087

Roberts, G., Pfaar, O., Akdis, C.A., Ansotegui, I.J., Durham, S.R., Wijk, R.G. van, Halken, S., Larenas-Linnemann, D., Pawankar, R., Pitsios, C., Sheikh, A., Worm,

M., Arasi, S., Calderon, M.A., Cingi, C., Dhami, S., Fauquert, J.L., Hamelmann, E., Hellings, P., Jacobsen, L., Knol, E.F., Lin, S.Y., Maggina, P., Mösges, R., Elberink, J.N.G.O., Pajno, G.B., Pastorello, E.A., Penagos, M., Rotiroti, G., Schmidt-Weber, C.B., Timmermans, F., Tsilochristou, O., Varga, E.-M., Wilkinson, J.N., Williams, A., Zhang, L., Agache, I., Angier, E., Fernandez-Rivas, M., Jutel, M., Lau, S., Ree, R. van, Ryan, D., Sturm, G.J., Muraro, A., 2018. EAACI Guidelines on Allergen Immunotherapy: Allergic rhinoconjunctivitis. Allergy 73, 765–798. https://doi.org/10.1111/all.13317

Skoner, D.P., 2001. Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. J. Allergy Clin. Immunol. 108, S2-8. https://doi.org/10.1067/mai.2001.115569

Treudler, R., Simon, J.C., 2013. Overview of Component Resolved Diagnostics. Curr. Allergy Asthma Rep. 13, 110–117. https://doi.org/10.1007/s11882-012-0318-8

Webber, C.M., England, R.W., 2010. Oral allergy syndrome: a clinical, diagnostic, and therapeutic challenge. Ann. Allergy. Asthma. Immunol. 104, 101–108. https://doi.org/10.1016/j.anai.2009.11.007

Weinmayr, G., Forastiere, F., Weiland, S.K., Rzehak, P., Abramidze, T., Annesi-Maesano, I., Björkstén, B., Brunekreef, B., Büchele, G., Cookson, W.O.C., Mutius, E. von, Pistelli, R., Strachan, D.P., 2008. International variation in prevalence of rhinitis and its relationship with sensitisation to perennial and seasonal allergens. Eur. Respir. J. 32, 1250–1261. https://doi.org/10.1183/09031936.00157807

Werfel, T., Asero, R., Ballmer-Weber, B.K., Beyer, K., Enrique, E., Knulst, A.C., Mari, A., Muraro, A., Ollert, M., Poulsen, L.K., Vieths, S., Worm, M., Hoffmann-Sommergruber, K., 2015. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy 70, 1079–1090. https://doi.org/10.1111/all.12666

WHO/IUIS Allergen Nomenclature Home Page [WWW Document], 2021. URL http://www.allergen.org/index.php (accessed 11.5.19).

Zuberbier, T., Lötvall, J., Simoens, S., Subramanian, S.V., Church, M.K., 2014. Economic burden of inadequate management of allergic diseases in the European Union: a GA2LEN review. Allergy 69, 1275–1279. https://doi.org/10.1111/all.12470

Eidesstattliche Versicherung

Ich, Theresa Lipp, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema:

„Klinische Merkmale des Pollen Food Allergy Syndrome in südeuropäischen Ländern“

“Clinical Characteristics of Pollen Food Allergy Syndrome in Southern European Countries”

selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem Erstbetreuer, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.

Datum Unterschrift

Statement of Contribution

Lipp, T., Acar Şahin, A., Aggelidis, X., Arasi, S., Barbalace, A., Bourgoin, A., Bregu, B., Brighetti, M.A., Caeiro, E., Caglayan Sozmen, S., Caminiti, L., Charpin, D., Couto, M., Delgado, L., Di Rienzo Businco, A., Dimier, C., Dimou, M.V., Fonseca, J.A., Goksel, O., Guvensen, A., Hernandez, D., Hoffmann, T.M., Jang, D.T., Kalpaklioglu, F., Lame, B., Llusar, R., Makris, M., Mazon, A., Mesonjesi, E., Nieto, A., Öztürk, A., Pahus, L., Pajno, G., Panasiti, I., Papadopoulos, N.G., Pellegrini, E., Pelosi, S., Pereira, A.M., Pereira, M., Pinar, N.M., Potapova, E., Priftanji, A., Psarros, F., Sackesen, C., Sfika, I., Suarez, J., Thibaudon, M., Travaglini, A., Tripodi, S., Verdier, V., Villella, V., Xepapadaki, P., Yazici, D., Matricardi, P.M., Dramburg, S., 2021. **Heterogeneity of Pollen Food Allergy Syndrome in Seven Southern European Countries: the @IT.2020 Multicenter Study.** Allergy. 2021; 00: 1– 12. https://doi.org/10.1111/all.14742

Contribution by Theresa Lipp:

- Establishing and managing the @IT.2020 MC Study databases from raw data provided by study physicians via the AllergyCard platform
- Performing patient selection for pollen food allergy syndrome (PFAS) analysis using inclusion and exclusion criteria from the standard operating procedure and defining PFAS as any reported reaction to any of the included foods in the PFAS section of the questionnaire at the T0 visit
- Designing and executing the statistical analyses using IBM SPSS Statistics 25, Armonk, NY, USA and Microsoft Office Excel 2016, Redmond, Washington, USA
- Creating all tables (tables 1, 2, E1-E4) and figures (figures 1-4, E1) to best illustrate the findings using Microsoft Office Excel, Redmond, Washington, USA
- Interpreting the resulting data and embedding it in current scientific knowledge
- Writing the original manuscript
- Submitting the manuscript to Allergy and completing all following steps until final acceptance by the editors
- Designing the corresponding graphical abstract

_____

Unterschrift, Datum und Stempel des erstbetreuenden Hochschullehrers

_____

Unterschrift des Doktoranden/der Doktorandin

Journal Summary List – Allergy

Journal Data Filtered By: **Selected JCR Year: 2019** Selected Editions: SCIE,SSCI
Selected Categories: **'ALLERGY'** Selected Category Scheme: WoS
Gesamtanzahl: 28 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY	52,417	10.228	0.077410
2	ALLERGY	18,217	8.706	0.024340
3	Journal of Allergy and Clinical Immunology-In Practice	5,946	7.574	0.018100
4	CLINICAL REVIEWS IN ALLERGY & IMMUNOLOGY	3,317	6.437	0.005910
5	Clinical and Translational Allergy	1,163	5.129	0.002820
6	ANNALS OF ALLERGY ASTHMA & IMMUNOLOGY	8,213	4.969	0.011830
7	ALLERGOLOGY INTERNATIONAL	2,335	4.806	0.004120
8	PEDIATRIC ALLERGY AND IMMUNOLOGY	4,456	4.699	0.005920
9	CLINICAL AND EXPERIMENTAL ALLERGY	10,602	4.217	0.011490
10	Allergy Asthma & Immunology Research	1,589	4.157	0.003110
11	CONTACT DERMATITIS	6,326	3.952	0.003550
12	Journal of Asthma and Allergy	577	3.730	0.001450
13	CURRENT ALLERGY AND ASTHMA REPORTS	2,574	3.577	0.005490
14	World Allergy Organization Journal	1,872	3.506	0.002750
15	JOURNAL OF INVESTIGATIONAL ALLERGOLOGY AND CLINICAL IMMUNOLOGY	2,263	3.488	0.002200
16	Current Opinion in Allergy and Clinical Immunology	2,838	3.246	0.003820
17	IMMUNOLOGY AND ALLERGY CLINICS OF NORTH AMERICA	1,709	3.000	0.002840

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
18	INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY	5,058	2.917	0.003550
19	ALLERGY AND ASTHMA PROCEEDINGS	2,039	2.414	0.002620
20	Allergy Asthma and Clinical Immunology	1,226	2.104	0.002250
21	JOURNAL OF ASTHMA	3,738	1.899	0.004950
22	Postepy Dermatologii i Alergologii	815	1.361	0.001550
23	ALLERGOLOGIA ET IMMUNOPATHOLOGIA	1,190	1.276	0.001540
24	ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY	755	1.247	0.000720
25	Iranian Journal of Allergy Asthma and Immunology	761	1.109	0.001010
26	Pediatric Allergy Immunology and Pulmonology	242	0.785	0.000420
27	Revue Francaise d Allergologie	278	0.254	0.000120
28	ALLERGOLOGIE	133	0.078	0.000050

Manuscript as published by Allergy on January 25, 2021

Received: 28 August 2020 | Revised: 17 November 2020 | Accepted: 25 November 2020

DOI: 10.1111/all.14742

Allergy EAACI WILEY

ORIGINAL ARTICLE

Rhinitis, Sinusitis, and Upper Airway Disease

Heterogeneity of pollen food allergy syndrome in seven Southern European countries: The @IT.2020 multicenter study

Theresa Lipp[1] | Aydan Acar Şahin[2] | Xenophon Aggelidis[3] | Stefania Arasi[4] | Andrea Barbalace[5] | Anne Bourgoin[6] | Blerina Bregu[7] | Maria Antonia Brighetti[8] | Elsa Caeiro[9,10] | Sule Caglayan Sozmen[11] | Lucia Caminiti[5] | Denis Charpin[6] | Mariana Couto[12] | Luís Delgado[13,14,15] | Andrea Di Rienzo Businco[16] | Claire Dimier[6] | Maria V. Dimou[17] | João A. Fonseca[14,15,18] | Ozlem Goksel[19] | Aykut Guvensen[20] | Dolores Hernandez[21] | Tara Maria Hoffmann[1] | Dah Tay Jang[22] | Fusun Kalpaklioglu[23] | Blerta Lame[7] | Ruth Llusar[22] | Michael P. Makris[3] | Angel Mazon[22] | Eris Mesonjesi[7] | Antonio Nieto[22] | Ayse Öztürk[24] | Laurie Pahus[25] | Giovanni Battista Pajno[5] | Ilenia Panasiti[5] | Nikolaos G. Papadopoulos[17,26] | Elisabetta Pellegrini[27] | Simone Pelosi[28] | Ana M. Pereira[14,15,18] | Mariana Pereira[14,15] | Munevver Pinar[2] | Ekaterina Potapova[1] | Alfred Priftanji[7] | Fotis Psarros[29] | Cansin Sackesen[30] | Ifigenia Sfika[16] | Javier Suarez[31] | Michel Thibaudon[32] | Alessandro Travaglini[8,33] | Salvatore Tripodi[16,34] | Valentine Verdier[6] | Valeria Villella[16] | Paraskevi Xepapadaki[35] | Duygu Yazici[36] | Paolo M. Matricardi[1] | Stephanie Dramburg[1]

[1]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany

[2]Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey

[3]Allergy Unit, 2nd Department of Dermatology and Venereology, National and Kapodistrian University of Athens, University Hospital "Attikon", Athens, Greece

[4]Pediatric Allergology Unit, Department of Pediatric Medicine, Bambino Gesù Children's Research Hospital (IRCCS), Rome, Italy

[5]Department of Pediatrics-Allergy Unit, University of Messina, Messina, Italy

[6]Department of Pneumonology and Allergy, La Timone Hospital, APHM, Aix-Marseille University, Marseille, France

[7]Department of Allergology and Clinical Immunology, UHC Mother Teresa, Medical University Tirana, Tirana, Albania

[8]Department of Biology, Tor Vergata University, Rome, Italy

[9]MED-Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, University of Évora, Évora, Portugal

[10]Portuguese Society of Allergology and Clinical Immunology, Lisbon, Portugal

[11]Department of Pediatric Allergy and Immunology, Okan University Faculty of Medicine, Istanbul, Turkey

[12]Department of Immunoallergology, CUF Decobertas Hospital, José de Mello Saúde, Porto, Portugal

[13]Basic and Clinical Immunology Unit, Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal

[14]CINTESIS, Center for Health Technology and Services Research, Porto, Portugal

[15]Allergy Unit, Instituto & Hospital CUF Porto, Porto, Portugal

[16]Pediatric Allergy Unit, Sandro Pertini Hospital, Rome, Italy

Abbreviations: ARIA, Allergic Rhinitis and its Impact on Asthma; ATH, Athens; IgE, Immunoglobulin E; IQR, interquartile range; IST, Istanbul; IZM, Izmir; MAR, Marseille; MES, Messina; nsLTP, non-specific lipid transfer protein; OR, odds ratio; PFAS, pollen food allergy syndrome; POR, Porto; PR-10, pathogenesis-related class 10 proteins; ROM, Rome; SAR, seasonal allergic rhinitis; SD, standard deviation; SPT, skin prick test; TIR, Tirana; VAL, Valencia.

[17]Allergy Department, 2nd Pediatric Clinic, Athens General Children's Hospital "P&A Kyriakou", University of Athens, Athens, Greece
[18]MEDCIDS-Department of Community Medicine, Information, and Health Sciences, Faculty of Medicine, University of Porto, Porto, Portugal
[19]Department of Pulmonary Medicine, Division of Immunology, Allergy and Asthma, Faculty of Medicine, Ege University, Izmir, Turkey
[20]Department of Biology, Faculty of Science, Ege University, Izmir, Turkey
[21]Department of Allergy, Health Research Institute Hospital La Fe, Valencia, Spain
[22]Pediatric Allergy and Pneumology Unit, Children's Hospital La Fe, Health Research Institute La Fe, Valencia, Spain
[23]Department of Immunology and Allergic Diseases, Kırıkkale University School of Medicine, Ankara, Turkey
[24]Department of Pulmonary Medicine, Division of Allergy and Immunology, Koç University, School of Medicine, Istanbul, Turkey
[25]Department of Pneumonology and Allergy, North Hospital, APHM, Aix-Marseille University, Marseille, France
[26]Division of Infection, Immunity & Respiratory Medicine, Royal Manchester Children's Hospital, University of Manchester, Manchester, UK
[27]Department of Reggio Calabria, ARPA-Regional Agency for Environmental Protection, Calabria, Italy
[28]TPS Production srl, Rome, Italy
[29]Allergy Department, Athens Naval Hospital, Athens, Greece
[30]Division of Pediatric Allergy, Koç University School of Medicine, Istanbul, Turkey
[31]Department of Biology of Organisms and Systems, Area of Botany, University of Oviedo, Oviedo, Spain
[32]Réseau National de Surveillance Aérobiologique, Brussieu, France
[33]Italian Aerobiology Monitoring Network-Italian Aerobiology Association, Rome, Italy
[34]Allergolology Service, Policlinico Casilino, Rome, Italy
[35]Allergy Department, 2nd Pediatric Clinic, National and Kapodistrian University of Athens, Athens, Greece
[36]Cellular and Molecular Medicine, KUTTAM, Graduate School of Health Sciences, Koç University, Istanbul, Turkey

Correspondence
Paolo M. Matricardi, Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité Medical University, Augustenburgerplatz, 1, 13353 Berlin, Germany.
Email: paolo.matricardi@charite.de

Funding information
SA was supported by the EAACI Fellowship Award of the European Academy of Allergology and Clinical Immunology. The study has been supported by an unrestricted grant from Euroimmun (grant number 118583). The ESEP assay has been provided by Euroimmun. The Informatics Platform "AllergyCARD™" and the app "AllergyMonitor" ® have been provided by TPS Production.

Abstract

Background: Pollen food allergy syndrome (PFAS) is a frequently underdiagnosed disease due to diverse triggers, clinical presentations, and test results. This is especially relevant in geographic areas with a broad spectrum of pollen sensitization, such as Southern Europe.

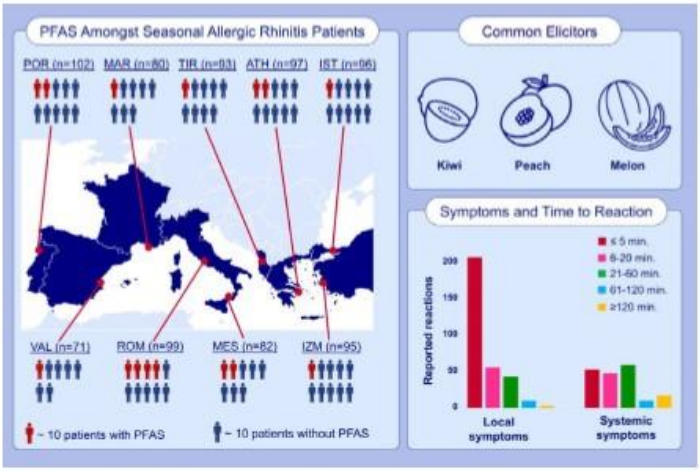
Objectives: To elucidate similarities and differences of PFAS in nine Southern European centers and identify associated characteristics and unique markers of PFAS.

Methods: As part of the @IT.2020 Multicenter Study, 815 patients with seasonal allergic rhinitis (SAR), aged 10–60 years, were recruited in seven countries. They completed questionnaires regarding SAR, comorbidities, family history, and PFAS, and underwent skin prick testing (SPT) and serum IgE testing.

Results: Of the 815 patients, 167 (20.5%) reported PFAS reactions. Most commonly, eliciting foods were kiwi (58, 34.7%), peach (43, 25.7%), and melon (26, 15.6%). Reported reactions were mostly local (216/319, 67.7%), occurring within 5 min of contact with elicitors (209/319, 65.5%). Associated characteristics included positive IgE to at least one panallergen (profilin, PR-10, or nsLTP) ($p = 0.007$), maternal PFAS (OR: 3.716, $p = 0.026$), and asthma (OR: 1.752, $p = 0.073$). Between centers, heterogeneity in prevalence (Marseille: 7.5% vs. Rome: 41.4%, $p < 0.001$) and of clinical characteristics was apparent. Cypress played a limited role, with only 1/22 SPT mono-sensitized patients reporting a food reaction ($p < 0.073$).

Conclusions: PFAS is a frequent comorbidity in Southern European SAR patients. Significant heterogeneity of clinical characteristics in PFAS patients among the centers was observed and may be related to the different pollen sensitization patterns in each geographic area. IgE to panallergen(s), maternal PFAS, and asthma could be PFAS-associated characteristics.

KEYWORDS
oral allergy syndrome, panallergen, pollen food allergy syndrome, seasonal allergic rhinitis, Southern Europe



GRAPHICAL ABSTRACT
In Southern Europe, there is a high heterogeneity in clinical characteristics of pollen food allergy syndrome among patients with seasonal allergic rhinitis. Patients frequently report reactions to kiwi, peach, and melon. Most patients report early localized reactions. The map was created using mapchart.net.

1 | INTRODUCTION

Pollen food allergy syndrome (PFAS) is a hypersensitivity reaction that can occur in patients with seasonal allergic rhinitis (SAR) after contact with certain foods due to sensitization to cross-reactive pollen and/or food allergens.[1,2] Prevalences of PFAS in patients with pollen allergies ranging from 9.6% to 55% have been reported worldwide.[3-5] Typical symptoms affect the oropharynx, including itching, stinging, pain, and edema, appearing within minutes of contact with the offending food[4] and lasting minutes to hours.[4-6] In around five percent of cases, more severe symptoms affecting other organ systems (e.g. skin, gastrointestinal, cardiovascular, and respiratory systems) have been reported.[7-9] Rarely, patients suffered from life-threatening anaphylaxis.[10-13]

Regional differences in pollen sensitization patterns influence the prevalence, elicitors, and typical symptoms of PFAS.[14]

While much is known about the typical sensitization pattern for PFAS in Northern Europe,[15,16] less information is available for Southern Europe. Studies regarding PFAS in Italy, Turkey, and Spain have been published but show little overlap in methodology and are therefore difficult to compare.[17-19] Additionally, different pollens are present in Southern Europe.[20] One of these is cypress pollen, a primary cause of SAR in the Mediterranean.[21] The exact role of cypress pollen in relation to PFAS is yet unknown and subject of current research.[22-25]

Pollen food allergy syndrome cross-reactions are caused by plant-food allergens that share sequence, structure, and function similarities with pollen allergens. Due to their widespread nature, these are known as panallergens.[26,27] In this study, the focus was placed on the following panallergen families: profilins , pathogenesis-related class 10 proteins (PR-10), and non-specific lipid transfer proteins (nsLTPs).[28] While the first two categories are markers of PFAS based on a primary sensitization to aeroallergens, the latter are currently categorized as class I food allergens which, due to their cross-reactivity with airborne allergens, may elicit also respiratory symptoms.[1,29] However, recent evidence suggests that the nsLTP molecule Ole e 7 from olive pollen may play a role as primary sensitizer in peach allergic patients from areas with extensive exposure to olive pollen.[30] Independently from the different perspectives on primary sensitization, nsLTPs play an important role in pollen and food allergies in the Mediterranean region and are therefore being considered in the present analysis.

Currently, no study has been published describing PFAS in Southern Europe with a unified methodology. As greater understanding of this complex syndrome is vital for the proper diagnosis of and care for patients, we have examined the clinical history, characteristics, and diagnostic results of patients in nine study centers from seven Southern European countries using a uniform method. Furthermore, we focused on finding the connections between PFAS and both cypress pollen and nsLTP in our cohort.

2 | MATERIALS AND METHODS

2.1 | Study population

The @IT.2020 Observational Longitudinal Multicenter Clinical Study was conducted to determine the impact of component resolved diagnostics and mobile health on the diagnosis of SAR in Southern Europe. In this context, we recruited patients suffering from SAR in nine study centers in seven Southern European countries between November 2017 and May 2018 (Porto (POR), Portugal; Valencia (VAL), Spain; Marseille (MAR), France; Rome (ROM) and Messina (MES), Italy; Tirana (TIR), Albania; Athens (ATH), Greece; and Istanbul (IST) and Izmir (IZM), Turkey). The patients fulfilled the following inclusion criteria: (1) age 10 to 18 years for children or 19 to 60 years for adults; (2) a good understanding of the national language or one of the languages offered in the AllergyMonitor® application (TPS software production, Rome, Italy); (3) availability of a smartphone; and (4) written informed consent. Exclusion criteria consisted of (1) prior pollen allergen immunotherapy; (2) any severe chronic disease; and (3) living further than 30 km away from the local aerobiological center used for pollen counts. The study was approved by the local ethics committees.

2.2 | Study design

2.2.1 | T0 questionnaire

Under the supervision of an allergy specialist, the patients or legal guardians completed a questionnaire regarding social demographics, clinical history of SAR and asthma, comorbidities, and family history. After indicating whether they had ever ingested one of the 15 selected known PFAS-associated foods (peach, apple, almond, apricot, soybean, cherry, pear, watermelon, melon, sesame, banana, carrot, fennel, kiwi, celery) or "others", patients were asked about the type and timing of potential resulting symptoms. Possible symptoms were (1) pruritus throat/mouth/tongue; (2) vesicles to the oral cavity; (3) skin redness; (4) urticaria; (5) swelling of eyes/eyelids; (6) swelling of tongue/face; (7) difficulty talking/swallowing; (8) nose closed/running; (9) cough/wheezing/respiratory difficulties; (10) vomiting; (11) diarrhea; (12) palpitations/tachycardia; (13) pallor/hypotension; and (14) loss of consciousness. Of these symptoms, (1), (2), (6), and (7) were classified as local reactions, while the rest was categorized as systemic. The possible times to onset of symptoms were divided into five categories: (1) ≤5 min; (2) 6–20 min; (3) 21–60 min; (4) 61–120 min; and (5) ≥120 min. The selection of included foods was based on the experience from previous studies as well as expert opinion.[17,28] Symptom assessment has been adapted from a validated questionnaire.[31]

2.2.2 | Skin prick tests (SPTs)

Skin prick tests were performed by local physicians on the volar surface of both forearms using 1 mm Osterballe type metal lancets and allergen extracts from mugwort, wall pellitory, olive tree, hazel tree, birch, bermuda grass, juniper ash, ragweed, *Dermatophagoides pteronyssinus*, cat, dog, histamine control, saline control (Stallergenes Greer), timothy grass, *Alternaria*, plane tree, *Salsola kali* (Russian thistle), and mixed grasses (ALK Abelló). All results were noted 15 min after application of the extracts. Positive results were defined as wheal diameters ≥3 mm after subtraction of the negative control. For the current analysis regarding PFAS, results obtained from *D. pteronyssinus*, cat, and dog dander SPTs were not included.

2.2.3 | IgE results

Serum was obtained and tested for IgE antibodies to multiple extracts and molecules using the EUROLINE Southern European Pollen Profile (EUROIMMUN Medizinische Diagnostika AG), a semi-quantitative, validated, customized multiplex immunoblot assay method.[32] Results were expressed in kU/L and considered positive at levels ≥0.35 kU/L. This current analysis focused on Bet v 2, Phl p 12 (profilins), Bet v 1, Cor a 1, Que a 1 (PR-10), and Art v 3, Ole e 7 (nsLTP).

2.3 | Statistics

Results were calculated using IBM SPSS Statistics 25. All categorical data were summarized as numbers (*n*) and frequencies (%). Quantitative data were given as mean and standard deviation (*SD*) or median and interquartile range (*IQR*). Further analysis was performed using logistic regression analysis to calculate the influence of select variables on the outcome of PFAS. Hierarchical regression analysis was used to investigate possible associated characteristics for PFAS based on backward stepwise logistic regression using Wald's method. Significance of differences between the centers were calculated using Pearson-chi-square test for frequencies, Kruskal-Wallis test for medians, and ANOVA for means. When comparing two groups, Pearson-chi-square test was used to calculate the significance for frequencies, Mann-Whitney U-test for medians, and t-test for means. Values of $p < 0.05$ were considered significant.

3 | RESULTS

3.1 | Study population

815 patients (mean age 26.1 years (13.6); 441/815, 54.1% male) from nine study centers were included. 167 of them (20.5%) reported reactions to at least one PFAS-associated food. The age and sex distribution among these patients showed no significant difference to those without PFAS (25.2 years and 82/167 male (49.1%) vs. 26.3 years and 359/648 male (55.4%)) (Table 1).

3.2 | PFAS in Southern Europe

- *Clinical characteristics*: Compared to patients without PFAS, patients with PFAS had a lower age at onset of SAR (9 years vs. 12 years, $p < 0.003$), a higher prevalence of maternal PFAS history as well as of additional allergic comorbidities, especially anaphylaxis and urticaria ($p < 0.001$ for all), but also asthma and atopic dermatitis ($p = 0.001$ and $p = 0.006$, respectively). By contrast, no significant differences were observed in disease duration, severity, and quality according to Allergic Rhinitis and its Impact on Asthma (ARIA) classification (Table 1).
- *PFAS-associated foods*: While kiwi (58/167, 34.7%), peach (43/167, 25.7%), and melon (26/167, 15.6%) were most commonly named as elicitors, 44.9% of the patients reported reactions to foods not listed in the questionnaire (Figure 1).
- *PFAS symptoms and time to reaction*: A total of 319 reactions were reported. Frequent symptoms were oral pruritus (252, 79.0%), swelling of the tongue/face (49, 15.4%), and urticaria (48, 15.0%) (Figure 2). Loss of consciousness (1, 0.3%), palpitations/tachycardia (2, 0.6%), oral vesicles (5, 1.6%), and pallor/hypotension (6, 1.9%) were least frequently reported. The majority of reactions occurred within 5 min of contact with the offending food (209, 65.5%) (Figure 2). 216 reported reactions (67.7%) consisted solely of oral symptoms (Figure 3). Systemic reactions were reported by 40.7% (68/167) of the patients (Table e1), most commonly to soy (2/4, 50.0%), peach (17/43, 39.5%), almond (7/20, 35.0%), apple (5/15, 33.3%), sesame (2/6, 33.3%), kiwi (19/58, 32.8%), and cherry (5/17, 29.4%) (Figure 3). Patients suffering from systemic symptoms showed a significantly higher prevalence of anaphylaxis ($p < 0.001$) (Table e1).

TABLE 1 Clinical characteristics of patients with and without PFAS in Southern Europe

	With PFAS ($n = 167$)		Without PFAS ($n = 648$)		Odds ratio	*p*-value
Male [n (%)]	82	49.1	359	55.4	1.288	0.146
Age (y) [mean (SD)]	25.2	13.1	26.3	13.7	0.994	0.318
Family history						
Atopic relative in immediate family [n (%)]	126	75.5	449	69.3	1.362	0.120
Sibling(s) with PFAS [n (%)]	5	3.0	16	2.5	1.219	0.703
Father with PFAS [n (%)]	1	0.6	6	0.9	0.645	0.685
Mother with PFAS [n (%)]	13	7.8	12	1.9	4.474	<0.001***
Allergic rhinitis						
Age at onset (y) [median (IQR)][a]	9	12	12	14	0.973	0.003**
Disease duration (y) [median (IQR)][a]	9	13.5	8	12	1.013	0.097
Months/year with symptoms [mean (SD)]	4.8	2.4	4.7	2.4	1.016	0.659
ARIA severity						
Mild intermittent [n (%)]	6	3.6	35	5.4	–	0.297
Mild persistent (ref.: mild intermittent) [n (%)]	9	5.4	51	7.9	1.029	0.960
Mod./severe intermittent (ref.: mild intermittent) [n (%)]	27	16.2	125	19.3	1.260	0.637
Mod./severe persistent (ref.: mild intermittent) [n (%)]	125	74.9	437	67.4	1.669	0.259
ARIA quality						
Unclassified [n (%)]	19	11.7	108	16.7	–	0.073
Rhinitis sneezer/runner (ref.: unclassified) [n (%)]	123	73.7	417	64.4	1.677	0.055
Rhinitis blocker (ref.: unclassified) [n (%)]	25	15.0	123	19.0	1.155	0.663
Other allergic comorbidities						
Number of patients with comorbidities [n (%)]	111	66.5	298	46.0	2.328	<0.001***
Number of comorbidities [mean (SD)]	1.2	1.0	0.7	0.8	1.748	<0.001***
Asthma [n (%)]	51	30.5	123	19.0	1.877	0.001**
Anaphylaxis [n (%)]	26	15.6	23	3.6	5.001	<0.001***
Urticaria [n (%)]	63	37.7	131	20.2	2.391	<0.001***
Atopic dermatitis [n (%)]	50	29.9	129	19.9	1.719	0.006**
Other [n (%)]	4	2.4	22	3.4	0.698	0.514

Abbreviations: IQR, interquartile range; mod., moderate; n, number; PFAS, pollen food allergy syndrome; ref., reference; SD, standard deviation.

[a]Due to incomplete data sets, 2 patients were excluded.

*$p < .05$; **$p < .01$; ***$p < .001$.

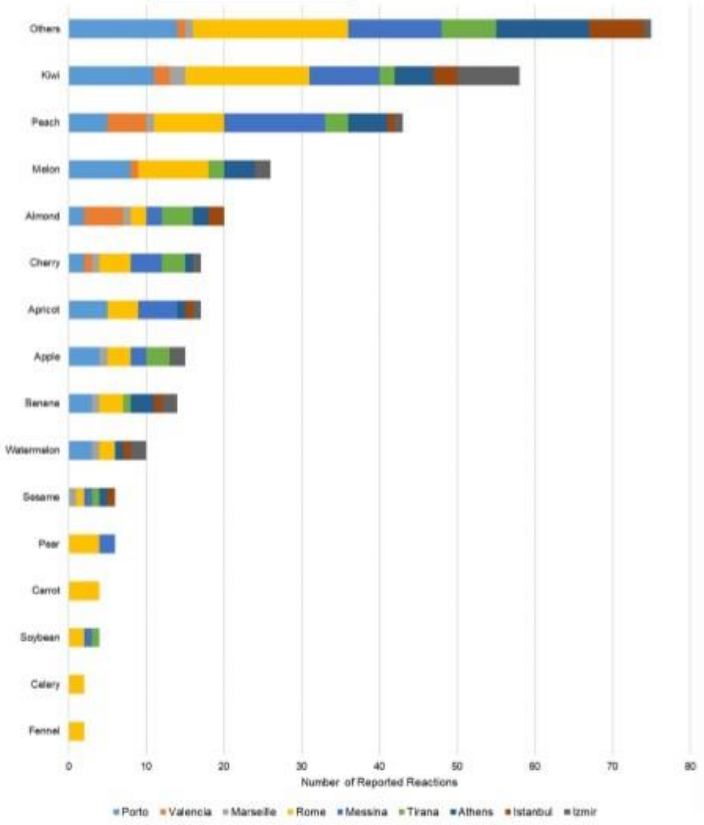


FIGURE 1 Number of reported PFAS reactions to 15 different PFAS-associated foods. The number of reported reactions is shown for the nine different centers: Porto (light blue), Valencia (orange), Marseille (gray), Rome (yellow), Messina (royal blue), Tirana (green), Athens (dark blue), Istanbul (brown), and Izmir (dark gray)

- *Atopic reactivity*: Patients with PFAS tested positive to a higher mean number of allergens in SPTs than those without (5.0 vs. 3.7, $p < 0.001$) but did not show a larger mean wheal diameter (Table 2). In IgE testing, PFAS patients had higher frequency of mono- or multi-panallergen-positive results. The prevalence of positive IgE results for the three analyzed panallergen groups, profilin, PR-10, and nsLTP, was higher in PFAS-positive patients ($p < 0.001$ for all) (Table 2).
- *PFAS-associated characteristics*: The following associated characteristics were identified: (1) positive panallergen IgE results ($p = 0.007$), especially multi-panallergen-positive (*OR*: 6.353, $p = 0.021$) and PR-10-positive results (*OR*: 5.582, $p = 0.004$), (2) anaphylaxis (OR: 6.210, $p < 0.001$), (3) maternal history of PFAS (*OR*: 3.716, $p = 0.026$), and (4) asthma (*OR*: 1.752, $p = 0.073$) (Table e2). The model generated by hierarchical regression analysis shows solid diagnostic ability in a receiver operating characteristics curve with an area under the curve of 0.688 (Figure e1).

3.3 | PFAS in nine different Southern European centers

The prevalence of PFAS differed significantly between the nine centers ($p < 0.001$), ranging from 6/80 (7.5%) in MAR to 41/99 (41.4%) in ROM (Table e4). Heterogeneity was particularly observed regarding age at SAR onset ($p = 0.003$), months per year with SAR symptoms ($p = 0.001$), ARIA severity and frequency (p from <0.001 to 0.080), number of patients with comorbidities ($p = 0.035$), and mean number of comorbidities per patient ($p = 0.016$), especially concerning urticaria and atopic dermatitis ($p = 0.022$ and $p = 0.018$, respectively).

Skin prick test results varied regarding the number of positive tests and average wheal diameter ($p < 0.001$).

Heterogeneous panallergen IgE results were observed for panallergen-negative ($p = 0.030$) and PR-10-positive results (p-value < 0.001).

A focused description of the unique characteristics of patients with PFAS in each center, in order of decreasing PFAS prevalence, is given below (Tables e1 and e3; Figure 1).

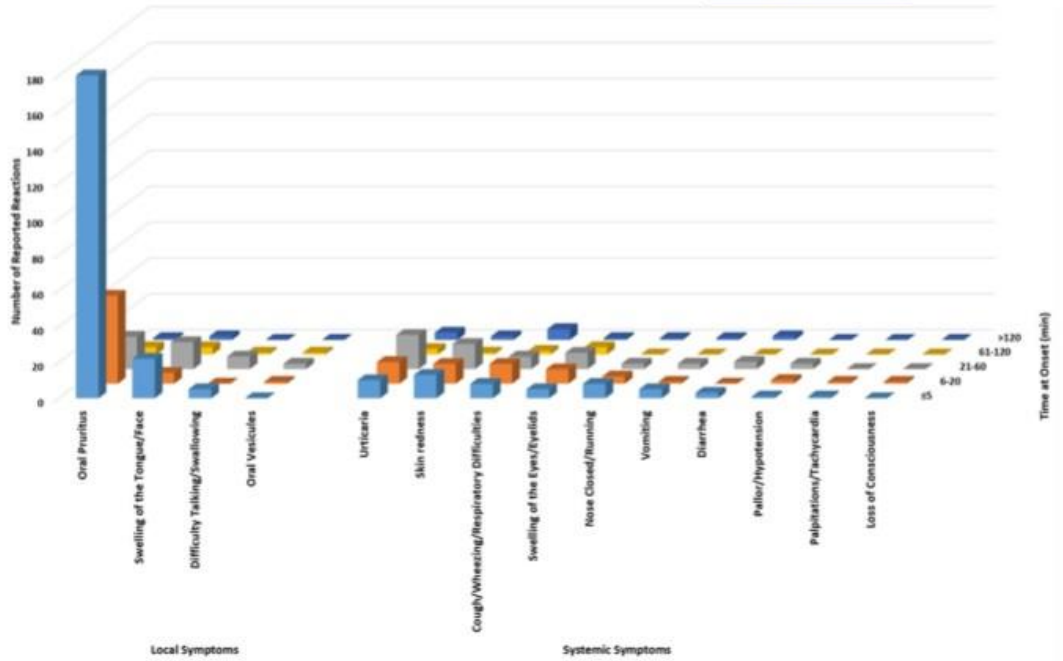


FIGURE 2 Symptoms reported by patients with PFAS after contact with PFAS-eliciting foods and times at onset. Symptoms are split into two categories: local symptoms (left) and systemic symptoms (right). The times at onset are grouped into five categories: ≤5 min (blue), 6–20 min (orange) 21–60 min (gray), 61–120 min (yellow), and >120 min (dark blue)

- ROM had the highest occurrence of PFAS, and 43.9% of these patients also reported urticaria. Reactions to carrot, celery, and fennel were solely reported here. 78% of patients experienced only oral reactions (32/41, $p = 0.005$). Profilin, PR-10, and nsLTP IgE positivity were observed in 9, 13, and 8 out of 41 patients, respectively.
- In MES, patients showed a mean age at onset of SAR of 10 years plus high rates of urticaria and asthma (18/24 and 12/24, respectively). Instead of melon, apricot was the third most frequent elicitor (5/24). Systemic reactions were especially common (14/24). A predominance of nsLTP IgE positivity was shown (4/24).
- POR reported patients with young age at onset at 7 years, and 11/24 patients also reported atopic dermatitis. Patients experiencing at least one systemic reaction were common (13/23). Profilin was the predominant panallergen in IgE results (5/24).
- Patients in TIR had a mean age at onset of SAR of 22 years, high frequency of comorbidities (9/13), especially urticaria (8/13), and solely moderate/severe SAR. Reactions to almond were frequent (4/13). While only 5/13 patients were panallergen-negative in IgE tests, 7/13 were PR-10-positive.
- In ATH, all 22 patients reported severe SAR with a high number of positive SPTs and large mean wheal diameter. Half of the patients reported experiencing at least one systemic symptom. None were PR-10 IgE-positive; instead, IgE to nsLTP and profilin was found (5/22 and 4/22, respectively).
- IZM reported patients with an onset of SAR at 26 years of age and an average of 2.6 months per year with symptoms. 3/14 patients had mild intermittent SAR, and on average, the patients had <1 comorbidity. Kiwi was by far the most common elicitor. 11/14 patients were IgE-negative to all panallergens, and none were PR-10 IgE-positive.
- In VAL, patients typically suffered from SAR during 3.2 months/year on average and reported a high rate of atopic dermatitis (6/10). Moderate/severe intermittent and moderate/severe persistent SAR were equally common at 4/10 each. The most frequently named elicitors included peach (5/10) and almond (5/10). 4/10 patients were IgE-positive to nsLTP.
- IST showed relatively high age at onset and low frequency of comorbidities. While no reactions to melon were recorded, reactions to almond were common (2/13). A predominance of patients had systemic reactions (7/13). No PR-10 IgE-positive patients were found.
- MAR reported the lowest prevalence of PFAS (6/80), showing a relatively high age at onset of SAR at 14.5 years. All PFAS patients had moderate/severe ARIA scores and reported comorbidities, especially urticaria (4/6) and atopic dermatitis (3/6). The patients presented with low average SPT wheal size (4.4 cm) and high rate of positive IgE to PR-10 (3/6).

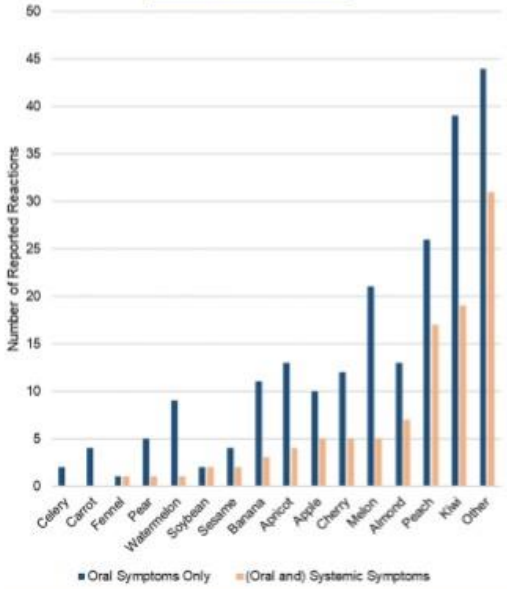


FIGURE 3 Number of reported PFAS reactions to the questioned PFAS-associated foods, categorized by oral symptoms only (blue) and (oral and) systemic symptoms (orange)

3.4 | Specific research questions

- *The role of cypress in PFAS in Southern Europe*: As an indicator of cypress pollen sensitization, juniper ash extract SPT was performed. 311/815 (38.2%) patients tested positive. 22 of these (7.1%) were mono-sensitized. Only one mono-sensitized patient reported a PFAS reaction, compared to 58/289 of multi-sensitized patients ($p = 0.073$). Similarly, out of 275 (33.7%) IgE-sensitized patients to cypress pollen extract and/or Cup a 1, only 16 (5.8%) were mono-sensitized. None of these patients were PFAS-positive, compared to 60/259 of cypress pollen multi-sensitized patients ($p = 0.029$).
- *PFAS and nsLTP in Southern Europe*: 26/167 (15.6%) of the patients reporting symptoms to one or more of the 15 PFAS-associated foods were nsLTP IgE-positive (Table 2). The most frequent elicitors of clinical symptoms among this group were peach (12, 46.2%), kiwi (10, 38.5%), and almond (8, 30.8%). Half of the nsLTP IgE-positive patients reported at least one systemic symptom (Figure 4). No significant differences between patients with and without sensitization to nsLTP were observed with regard to clinical characteristics (Table e4).

4 | DISCUSSION

In our analysis of PFAS based on a cohort of 815 Southern European patients, we discovered (1) an overall prevalence of 20.5% of PFAS in patients suffering from SAR in Southern Europe; (2) substantial heterogeneity in prevalence and clinical characteristics of PFAS among the different centers; (3) a significant lack of PFAS in cypress pollen mono-sensitized patients; and (4) a high frequency of systemic reactions in nsLTP IgE-positive patients.

The overall prevalence of PFAS in our study falls within the range of previous reports, but is much lower than the frequency of PFAS among birch pollen allergic patients in Northern Europe.[15] This can be explained by the decreased role of birch pollinosis in Southern Europe,[15,33] with a lower sensitization to Bet v 1 and a higher sensitization to Bet v 2.[16] This is reflected by our data, showing an equal distribution of sensitization to PR-10, profilin, and nsLTP. Furthermore, the most commonly reported reactions were to foods typically associated with nsLTP or profilin: kiwi, peach, and melon. This reflects similar findings as previous studies performed in Italy and Turkey, where kiwi and peach were also reported as the most common elicitors.[17,18]

In terms of symptoms, our data show a fast onset and a predominance of oral pruritus. This corroborates current literature, where reactions are described as mainly oral and with a rapid onset.[2] Yet, contrary to previous publications on PFAS, where systemic symptoms only comprised 5% of all reactions,[8] 32.3% of the reported reactions in our cohort included at least one systemic symptom. This may be explained by the frequency of nsLTP sensitization in Southern Europe,[34] as these molecules are heat and acid resistant and therefore more likely to cause extraoral symptoms.[29]

Within Southern Europe, a vast heterogeneity of pollen has been reported.[20,35] This heterogeneity can lead to variance in sensitization patterns and therefore in the development of SAR and PFAS, even within the same country as shown by Mastrorilli et al.[17] In our study, a difference in latitude appears to have a bigger impact on the heterogeneity of PFAS than longitudinal differences. This could be due to changes in climatic zones with accordingly differing vegetation. The present analysis aimed at elucidating these potential differences with a uniform methodological approach in several countries and was able to describe a high degree of heterogeneity, certain similarities, and certain unexpected observations.

While a low frequency of birch sensitization has previously been reported in the South of France (1.05%),[33] we found a high rate of PR-10 IgE sensitization in MAR PFAS patients (3/6). This could indicate that patients may have been exposed to birch in a different geographic area.

Surprisingly, PFAS-positive patients in TIR suffered from severe allergic disease and many comorbidities. This is in contrast to previous epidemiological studies from the same geographic region, where low asthma severity has been reported.[36] Additionally, in 1999, Priftanji et al. described that only 2.7% of the tested patients were SPT-positive for *Betula*,[37] yet our cohort of PFAS patients was predominantly PR-10 IgE-positive.

Mastrorilli et al. reported in 2016 a PFAS frequency of 16.9%[17] in Southern Italy, while MES showed a higher rate of PFAS 24/82 (29.3%) in our study. This may be explained by an increased incidence in allergic diseases, since our study recruited patients almost

TABLE 2 Atopic reactivity of patients with and without PFAS in Southern Europe

	With PFAS ($n = 167$)		Without PFAS ($n = 648$)		Odds ratio	p-value
Skin prick test (SPT)						
Positive SPT to seasonal aeroallergen(s)[a] [mean (*SD*)]	5.0	*3.1*	3.7	*2.7*	1.166	<0.001***
Average SPT size of seasonal aeroallergens (mm)[a] [mean (*SD*)]	6.1	*1.6*	6.0	*1.7*	1.028	0.589
IgE results						
No panallergen [n (%)][b]	102	*61.1*	559	*86.3*	-	<0.001***
Mono-panallergen (ref.: no panallergen) [n (%)][b]	53	*31.7*	79	*12.2*	3.677	<0.001***
Multi-panallergen (ref.: no panallergen) [n (%)][b]	12	*7.2*	10	*1.5*	6.576	<0.001***
Profilins [n (%)][b]	26	*15.6*	42	*6.5*	2.661	<0.001***
PR-10-like allergenic proteins [n (%)][b]	26	*15.6*	26	*4.0*	4.411	<0.001***
nsLTPs [n (%)][b]	26	*15.6*	33	*5.1*	3.436	<0.001***

Abbreviations: IQR, interquartile range; n, number; PFAS, pollen food allergy syndrome; ref., reference; SD, standard deviation.

[a]Test panel included mugwort, wall pellitory, olive tree, hazel tree, birch, bermuda grass, juniper ash, and ragweed.

[b]Test panel included profilins (Bet v 2, Phl p 12), PR-10-like allergenic proteins (Bet v 1, Cor a 1, Que a 1), and nsLTPs (Art v 3, Ole e 7).

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

10 years later than Mastrorilli et al. However, both studies showed an early onset of SAR and a predominance of nsLTP IgE positivity.[17]

Among PFAS patients in ATH, our cohort reported a higher rate of IgE to profilin (18.2%) than previously reported (10.9%).[38] As LTP syndrome has been described as a common allergenic syndrome in Greece,[39] it is not surprising that the prevalence of nsLTP IgE-positive patients among our cohort was 22.7%. The absence of sensitization to PR-10 in ATH is noticeable and corroborates current literature.[40]

The high prevalence of IgE to profilins in our PFAS cohort in POR is similar to that found in central Portugal by Tavares et al.[41] and can be explained by the predominance of Urticaceae (including pellitory of the wall) and grass pollen in Portugal.[42]

The frequency of peach and almond as causative foods for PFAS reactions in VAL reported by our study shows some similarity to findings by Flores et al,[19] where peach and nuts were the most common elicitors. Their results showed walnut as the main symptom-causing nut,[19] which was not included in our questionnaire. The high prevalence of nsLTP sensitization found in our cohort corroborates previous reports for the region.[43]

Compared to an earlier study focusing on PFAS in Italian children,[17] our cohort in ROM reported fewer reactions to banana and watermelon. Peach, kiwi, and melon were the three most common elicitors in both central Italian groups. While a higher frequency of urticaria as comorbidity was reported in the present study, the frequency of asthma as a comorbidity was lower than reported by Mastrorilli et al.[17] In addition to a high frequency of IgE to profilins and PR-10, our study found a high rate of positive IgE to nsLTP.

The results from IST and IZM shared some similarities with a previous study. While the overall prevalence of PFAS in Turkey reported by our study was lower than the previously reported 19.3%,[18] kiwi was by far the most common elicitor of PFAS in both studies.[18] Asthma was the most frequent comorbidity of PFAS-positive patients in Turkey both in our cohort and in the previous study.[18]

4.1 | Interesting results regarding the role of cypress in PFAS in Southern Europe

Patients with both cypress pollen allergy and PFAS reactions to peach have been described in literature.[23,24] These two allergic reactions have been linked through molecular similarities between the cypress molecule Cup s 7 and the peach molecule Pru p 7.[22] While such cases have been published, in our analysis no patients with cypress pollen mono-sensitization (based on SPT or IgE results) reported peach PFAS. This result concurs with recent findings by Asero et al.[25] that mono-sensitization to Pru p 7 is rare among cypress pollen hypersensitive patients in Italy. It also supports the authors' conclusion that peach and cypress pollen might share other, currently unknown cross-reactive molecules.

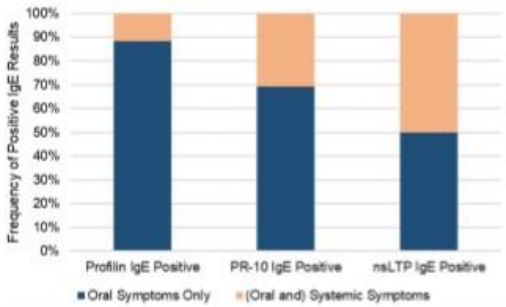


FIGURE 4 Frequency of panallergen-positive IgE results in patients who only reported oral symptoms (blue) versus those who reported (oral and) systemic symptoms (orange) to any of the questioned PFAS-associated foods. Results are shown based on different panallergen groups: profilins (Bet v 2 and Phl p 12), PR-10 (Bet v 1, Cor a 1, and Que a 1), and nsLTPs (Art v 3 and Ole e 7)

4.2 | Limitations

We acknowledge certain limitations of this study. First, the diagnosis of PFAS was based on the clinical history and no objective measurement of reaction, such as prick-by-prick testing or oral food challenges, was performed. Second, the IgE test performed was developed for the diagnosis of seasonal pollen allergies in Southern Europe and no specific panallergen molecules found in PFAS-associated foods were included in the test. Third, the focus of our study was placed on patients attending allergy clinics in different centers. Therefore, the present project is not an epidemiological study representative of the included countries.

4.3 | Conclusion

While some overall similarities within Southern Europe can be seen, the region shows significant heterogeneity in many aspects of its clinical characteristics. These can frequently be explained by the differing pollen types in the area and the differing development of allergic disease. Unlike patients with PFAS in Northern Europe, patients in Southern Europe report more reactions to peach, melon, and kiwi and suffer more frequently from systemic reactions. Cypress pollen mono-sensitized patients were significantly less likely to report PFAS than multi-sensitized patients, and no link to peach was supported by our findings.

4.4 | Outlook

Further insight may be provided by studies focusing on prick-by-prick tests and/or oral challenges and more specific IgE testing with a broader panel of panallergens.

ACKNOWLEDGEMENTS

This study was supported by an unrestricted educational grant from Euroimmun (code 118583), Euroimmun also provided the ESEP strips for the study.

CONFLICT OF INTEREST

Dr. Couto reports personal fees from Roche, outside the submitted work; Dr. Delgado reports personal fees from Laboratórios Vitoria, SA, outside the submitted work; Dr. Makris reports personal fees from Novartis, personal fees from Astra Zeneca, personal fees from Sanofi, personal fees from GSK, and personal fees from Mylan, outside the submitted work; Dr. Pahus reports personal fees from GlaxoSmithKline, personal fees from Astra Zeneca, and personal fees from Chiesi, outside the submitted work; Dr. Papadopoulos reports personal fees from Novartis, personal fees from Nutricia, personal fees from HAL, personal fees from MENARINI/FAES FARMA, personal fees from SANOFI, personal fees from MYLAN/MEDA, personal fees from BIOMAY, personal fees from AstraZeneca, personal fees from GSK, personal fees from MSD, personal fees from ASIT BIOTECH, personal fees from Boehringer Ingelheim, grants from Gerolymatos International SA, and grants from Capricare, outside the submitted work; and Simone Pelosi reports other from TPS Production srl, during the conduct of the study. Dr. Psarros reports personal fees from Novartis Hellas, Takeda, Astra Zeneca, and Sanofi, outside the submitted work; Dr. Sackesen reports grants from MSD to support laboratory tests for the study "Effects of the montelukast therapy on asthma and allergic inflammation in children with food allergy" and from Abbott to support "Metabolomics study in children with food allergy," outside the submitted work; Dr. Tripodi reports other from TPS Production srl, during the conduct of the study; Dr. Xepapadaki reports personal fees from Uriach, personal fees from Novartis, personal fees from Nestle, and personal fees from Nutricia, outside the submitted work; and Dr. Matricardi reports grants from Deutsche Forschungsgemeinschaft, grants and personal fees from Hycor Biomedical, grants and personal fees from Euroimmun, and personal fees and non-financial support from Thermo Fisher Scientific, outside the submitted work.

AUTHOR CONTRIBUTIONS

Patient recruitment and data collection: Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A, Hernandez D, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris M, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno G, Panasiti I, Papadopoulos NG, Pellegrini E, Pereira AM, Pereira M, Pinar NM, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Travaglini A, Verdier V, Villella V, Xepapadaki P, and Yazici D. Aerobiological data analysis: Thibaudon M. App development: Pelosi S and Tripodi S. Study conception and organization, and data analysis and interpretation: Matricardi PM, Dramburg S, Lipp T, Hoffmann TM, and Potapova E. All authors reviewed and approved the final manuscript.

ORCID

Theresa Lipp https://orcid.org/0000-0001-6808-5942
Stefania Arasi https://orcid.org/0000-0002-8135-0568
Mariana Couto https://orcid.org/0000-0003-4987-9346
Nikolaos G. Papadopoulos https://orcid.org/0000-0002-4448-3468
Ekaterina Potapova https://orcid.org/0000-0003-4427-6335
Cansin Sackesen https://orcid.org/0000-0002-1115-9805
Paraskevi Xepapadaki https://orcid.org/0000-0001-9204-1923
Paolo M. Matricardi https://orcid.org/0000-0001-5485-0324
Stephanie Dramburg https://orcid.org/0000-0002-9303-3260

REFERENCES

1. Werfel T, Asero R, Ballmer-Weber BK, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. *Allergy* 2015;70(9):1079–1090. https://doi.org/10.1111/all.12666
2. Poncet P, Sénéchal H, Charpin D. Update on pollen-food allergy syndrome. *Expert Rev Clin Immunol*. 2020;16(6):561–578. https://doi.org/10.1080/1744666X.2020.1774366
3. Bedolla-Barajas M, Kestler-Gramajo A, Alcalá-Padilla G, Morales-Romero J. Prevalence of oral allergy syndrome in children with allergic diseases. *Allergol Immunopathol (Madr)*. 2017;45(2):127–133. https://doi.org/10.1016/j.aller.2016.04.017
4. Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. *Clin Exp Allergy J Br Soc Allergy Clin Immunol*. 1994;24(4):367–374. https://doi.org/10.1111/j.1365-2222.1994.tb00248.x
5. Guvenir H, Dibek Misirlioglu E, Buyuktiryaki B, et al. Frequency and clinical features of pollen-food syndrome in children. *Allergol Immunopathol (Madr)* 2020;48:78–83.
6. Li JD, Du ZR, Liu J, Xu YY, Wang RQ, Yin J. Characteristics of pollen-related food allergy based on individual pollen allergy profiles in the Chinese population. *World Allergy Organ J*. 2020;13(5):100120. https://doi.org/10.1016/j.waojou.2020.100120
7. Osawa Y, Ito Y, Takahashi N, et al. Epidemiological study of oral allergy syndrome in birch pollen dispersal-free regions. *Allergol Int*. 2020;69(2):246–252. https://doi.org/10.1016/j.alit.2019.09.008. Epub 2019 Nov 7.
8. Price A, Ramachandran S, Smith GP, Stevenson ML, Pomeranz MK, Cohen DE. Oral Allergy Syndrome (Pollen-Food Allergy Syndrome). *Dermatitis*. 2015;26(2):78. https://doi.org/10.1097/DER.0000000000000087
9. Asero R, Ariano R, Aruanno A, et al. Systemic allergic reactions induced by labile plant-food allergens: Seeking potential cofactors. A multicenter study. *Allergy*. 2021;76:1473–1479. https://doi.org/10.1111/all.14634
10. Kim M, Ahn Y, Yoo Y, et al. Clinical manifestations and risk factors of anaphylaxis in pollen-food allergy syndrome. *Yonsei Med J* 2019;60:960–968.
11. Skypala IJ. Can patients with oral allergy syndrome be at risk of anaphylaxis? *Curr Opin Allergy Clin Immunol* 2020;20(5):459–464. https://doi.org/10.1097/ACI.0000000000000679. PMID: 32842037.
12. Florido Lopez JF, Quiralte Enriquez J, de Saavedra A, et al. An allergen from Olea europaea pollen (Ole e 7) is associated with plant-derived food anaphylaxis. *Allergy* 2002;57(Suppl 71):53–59. https://doi.org/10.1034/j.1398-9995.2002.057s71053.x
13. Asero R, Celi G, Scala E. Labile plant food allergens: Really so harmless? Case series and literature review. *Allergy*. 2020;75(6):1517–1518. https://doi.org/10.1111/all.14184. Epub 2020 Jan 31.
14. Mastrorilli C, Cardinale F, Giannetti A, Caffarelli C. Pollen-Food Allergy Syndrome: A not so Rare Disease in Childhood. *Medicina (Mex)*. 2019;55(10):641. https://doi.org/10.3390/medicina55100641
15. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. *Allergy* 2019;74(7):1237–1248. https://doi.org/10.1111/all.13758
16. Movérare R, Westritschnig K, Svensson M, et al. Different IgE Reactivity Profiles in Birch Pollen-Sensitive Patients from Six European Populations Revealed by Recombinant Allergens: An Imprint of Local Sensitization. *Int Arch Allergy Immunol* 2002;128(4):325–335.
17. Mastrorilli C, Tripodi S, Caffarelli C, et al. Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification. *Allergy* 2016;71(8):1181–1191. https://doi.org/10.1111/all.12888
18. Özdemir SK, Özgüçlü S. Pollen food allergy syndrome in Turkey: Clinical characteristics and evaluation of its association with skin test reactivity to pollens. *Asian Pac J Allergy Immunol* 2018;36(2):77–81. https://doi.org/10.12932/AP0881
19. Flores E, Cervera L, Sanz ML, Diaz-Perales A, Fernández J. Plant Food Allergy in Patients with Pollinosis from the Mediterranean Area. *Int Arch Allergy Immunol* 2012;159(4):346–354. https://doi.org/10.1159/000338282
20. Hoffmann TM, Acar Şahin A, Aggelidis X, et al. "Whole" vs. "fragmented" approach to EAACI pollen season definitions: A multicenter study in six Southern European cities. *Allergy* 2020;75(7):1659–1671. https://doi.org/10.1111/all.14153
21. Castillo Marchuet MJ, Luengo O, Cardona V. Cypress Pollen Allergy in a Mediterranean Area. *J Investig Allergol Clin Immunol*. 2020;30(1):67. https://doi.org/10.18176/jiaci.0444. Epub 2019 Sep 18.
22. Sénéchal H, Šantrůček J, Melčová M, et al. A new allergen family involved in pollen food-associated syndrome: Snakin/gibberellin-regulated proteins. *J Allergy Clin Immunol* 2018;141(1):411–414.e4. https://doi.org/10.1016/j.jaci.2017.06.041. Epub 2017 Aug 4.
23. Caimmi D, Barber D, Hoffmann-Sommergruber K, et al. Understanding the molecular sensitization for Cypress pollen and peach in the Languedoc-Roussillon area. *Allergy* 2013;68(2):249–251. https://doi.org/10.1111/all.12073
24. Hugues B, Didierlaurent A, Charpin D. Cross-reactivity between cypress pollen and peach: a report of seven cases. *Allergy* 2006;61(10):1241–1243. https://doi.org/10.1111/j.1398-9995.2006.01156.x
25. Asero R, Abbadessa S, Aruanno A, et al. Detection of Gibberellin-Regulated Protein (Peamaclein) Sensitization among Italian Cypress Pollen-Sensitized Patients. *J Investig Allergol Clin Immunol* 2022;32(1). https://doi.org/10.18176/jiaci.0542. [Epub ahead of print]
26. Hauser M, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. *Allergy Asthma Clin Immunol* 2010;6(1):1. https://doi.org/10.1186/1710-1492-6-1
27. Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004;59(3):243–267. https://doi.org/10.1046/j.1398-9995.2003.00407.x
28. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al. EAACI Molecular Allergology User's Guide. *Pediatr Allergy Immunol* 2016;27:1–250. https://doi.org/10.1111/pai.12563
29. Bogas G, Muñoz-Cano R, Mayorga C, et al. Phenotyping peach-allergic patients sensitized to LTP and analysing severity biomarkers. *Allergy* 2020;75:3228–3236. https://doi.org/10.1111/all.14447
30. Oeo-Santos C, Navas A, Benedé S, et al. New insights into the sensitization to nonspecific lipid transfer proteins from pollen and food: New role of allergen Ole e 7. *Allergy* 2020;75(4):798–807. https://doi.org/10.1111/all.14086. Epub 2019 Nov 6.

31. Skypala IJ, Calderon MA, Leeds AR, Emery P, Till SJ, Durham SR. Development and validation of a structured questionnaire for the diagnosis of oral allergy syndrome in subjects with seasonal allergic rhinitis during the UK birch pollen season. *Clin Exp Allergy* 2011;41(7):1001–1011. https://doi.org/10.1111/j.1365-2222.2011.03759.x
32. Di Fraia M, Arasi S, Castelli S, et al. A new molecular multiplex IgE assay for the diagnosis of pollen allergy in Mediterranean countries: A validation study. *Clin Exp Allergy* 2019;49:341–349. https://doi.org/10.111/cea.13264
33. Charpin D, Ramadour M, Lavaud F, et al. Climate and Allergic Sensitization to Airborne Allergens in the General Population: Data from the French Six Cities Study. *Int Arch Allergy Immunol* 2017;172(4):236–241. https://doi.org/10.1159/000471511
34. Rial MJ, Sastre J. Food Allergies Caused by Allergenic Lipid Transfer Proteins: What Is behind the Geographic Restriction? *Curr Allergy Asthma Rep* 2018;18:56. https://doi.org/10.1007/s11882-018-0810-x
35. D'Amato G, Cecchi L, Bonini S, et al. Allergenic pollen and pollen allergy in Europe. *Allergy* 2007;62(9):976–990. https://doi.org/10.1111/j.1398-9995.2007.01393.x
36. Mesonjesi E, Piluri Ziu E, Gupta R, Strachan D, Priftanji A. The prevalence and time trend of asthma in Albanian children in 2011 – Alb ISAAC. *Clin Transl. Allergy* 2015;5(S2):2045–7022. https://doi.org/10.1186/2045-7022-5-S2-P8
37. Priftanji AV, Qirko E, Layzell JCM, Burr ML, Fifield R. Asthma and allergy in Albania. *Allergy* 1999;54(10):1042–1047. https://doi.org/10.1034/j.1398-9995.1999.00108.x
38. Iliopoulou A, Petrodimopoulou M, Konstantakopoulou M, et al. Profilin sensitization and its clinical relevance to a population of atopic adults in Greece. *Rev Fr Allergol* 2018;58(2):72–76. https://doi.org/10.1016/j.reval.2017.11.001
39. Karantoumanis D, Savvatianos S, Konstantinopoulos AP, et al. PD19 - Co-recognition of lipid transfer protein in pollen and foods in a Greek pediatric population. *Clin Transl Allergy*. 2014;4(Suppl 1):P19. https://doi.org/10.1186/2045-7022-4-S1-P19.
40. Lyons SA, Clausen M, Knulst AC, et al. Prevalence of Food Sensitization and Food Allergy in Children Across Europe. *J Allergy Clin Immunol Pract*. 2020;8(8):2736–2746.e9. https://doi.org/10.1016/j.jaip.2020.04.020. Epub 2020 Apr 21 PMID: 32330668.
41. Tavares B, Machado D, Loureiro G, Cemlyn-Jones J, Pereira C. Sensitization to profilin in the Central region of Portugal. *Sci Total Environ* 2008;407(1):273–278. https://doi.org/10.1016/j.scitotenv.2008.08.013.
42. Pereira C, Valero A, Loureiro C, et al. Iberian study of aeroallergens sensitisation in allergic rhinitis. *Eur Ann Allergy Clin Immunol*. 2006;38(6):186–194.
43. Barber D, de la Torre F, Feo F, et al. Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy* 2008;63(11):1550–1558. https://doi.org/10.1111/j.1398-9995.2008.01807.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Lipp T, Acar Şahin A, Aggelidis X, et al. Heterogeneity of pollen food allergy syndrome in seven Southern European countries: The @IT.2020 multicenter study. *Allergy*. 2021;00:1–12. https://doi.org/10.1111/all.14742

Supplementary Material

Clinical Characteristics of Pollen Food Allergy Syndrome amongst Patients with Seasonal Allergic Rhinitis in Seven Southern European Countries: Results from the @IT.2020 Multicenter Study

Lipp T[1], Acar Şahin A[2], Aggelidis X[3], Arasi S[4], Barbalace A[5], Bourgoin A[6], Bregu B[7], Brighetti MA[8], Caeiro E[9,10], Caglayan Sozmen S[11], Caminiti L[5], Charpin D[6], Couto M[12], Delgado L[13,14,15], Di Rienzo Businco A[16], Dimier C[6], Dimou MV[17], Fonseca JA[14,15,18], Goksel O[19], Guvensen A[20], Hernandez D[21], Hoffmann TM[1], Jang DT[22], Kalpaklioglu F[23], Lame B[7], Llusar R[22], Makris M[3], Mazon A[22], Mesonjesi E[7], Nieto A[22], Öztürk A[24], Pahus L[25], Pajno G[5], Panasiti I[5], Papadopoulos NG[17,26], Pellegrini E[27], Pelosi S[28], Pereira AM[14,15,18], Pereira M[14,15], Pinar NM[2], Potapova E[1], Priftanji A[7], Psarros F[29], Sackesen C[30], Sfika I[16], Suarez J[31], Thibaudon M[32], Travaglini A[8,33], Tripodi S[16,34], Verdier V[6], Villella V[16], Xepapadaki P[35], Yazici D[36], Matricardi PM[1*], Dramburg S[1]

[1] Department of Pediatric Pulmonology, Immunology and Intensive Care Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany.
[2] Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey.
[3] Allergy Unit, 2nd Department of Dermatology and Venereology, National and Kapodistrian University of Athens, University Hospital "Attikon", Athens, Greece.
[4] Pediatric Allergology Unit, Department of Pediatric Medicine, Bambino Gesù Children´s Research Hospital (IRCCS), Rome, Italy.
[5] Department of Pediatrics- Allergy Unit, University of Messina, Messina, Italy.
[6] Department of Pneumonology and Allergy, La Timone Hospital, APHM, Aix-Marseille University, Marseille, France.
[7] Department of Allergology and Clinical Immunology. UHC Mother Teresa, Medical University Tirana, Tirana, Albania.
[8] Department of Biology, Tor Vergata University, Rome, Italy.
[9] MED- Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, University of Évora, Évora, Portugal.
[10] Portuguese Society of Allergology and Clinical Immunology, Lisbon, Portugal.
[11] Department of Pediatric Allergy and Immunology, Okan University Faculty of Medicine, Istanbul, Turkey.
[12] Department of Immunoallergology, CUF Decobertas Hospital, José de Mello Saúde, Porto, Portugal.
[13] Basic and Clinical Immunology Unit, Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal.
[14] CINTESIS, Center for Health Technology and Services Research, Porto, Portugal.
[15] Allergy Unit, Instituto & Hospital CUF Porto, Porto, Portugal.
[16] Pediatric Allergy Unit, Sandro Pertini Hospital, Rome, Italy.
[17] Allergy Department, 2nd Pediatric Clinic, Athens General Children's Hospital "P&A Kyriakou," University of Athens, Athens, Greece.
[18] MEDCIDS-Department of Community Medicine, Information, and Health Sciences, Faculty of Medicine, University of Porto, Porto, Portugal.
[19] Department of Pulmonary Medicine, Division of Immunology, Allergy and Asthma. Faculty of Medicine, Ege University, Izmir, Turkey.
[20] Department of Biology, Faculty of Science, Ege University, Izmir, Turkey.
[21] Department of Allergy, Health Research Institute Hospital La Fe, Valencia, Spain.
[22] Pediatric Allergy and Pneumology Unit, Children's Hospital La Fe; Health Research Institute La Fe, Valencia, Spain.
[23] Kırıkkale University School of Medicine, Department of Immunology and Allergic Diseases, Ankara, Turkey.

[24] Department of Pulmonary Medicine, Division of Allergy and Immunology, Koç University, School of Medicine, Istanbul, Turkey.
[25] Department of Pneumonology and Allergy, North Hospital, APHM, Aix-Marseille University, Marseille, France.
[26] Division of Infection, Immunity & Respiratory Medicine, Royal Manchester Children's Hospital, University of Manchester, Manchester, UK.
[27] ARPA- Regional Agency for Environmental Protection, Department of Reggio Calabria, Calabria, Italy
[28] TPS Production srl, Rome, Italy.
[29] Allergy Department, Athens Naval Hospital, Athens, Greece.
[30] Division of Pediatric Allergy, Koç University School of Medicine, Istanbul, Turkey
[31] Department of Biology of Organisms and Systems, Area of Botany, University of Oviedo, Oviedo, Spain.
[32] Réseau National de Surveillance Aérobiologique, Brussieu, France.
[33] Italian Aerobiology Monitoring Network - Italian Aerobiology Association, Rome, Italy.
[34] Allergolology Service, Policlinico Casilino, Rome, Italy
[35] Allergy Department, 2nd Pediatric Clinic, National and Kapodistrian University of Athens, Athens, Greece.
[36] Cellular and Molecular Medicine, KUTTAM, Graduate School of Health Sciences, Koç University, Istanbul, Turkey.

Tables: 4

Figures: 1

*Corresponding author:
Paolo M. Matricardi
Dept. of Paediatric Pneumology, Immunology and Critical Care
Charitè Medical University
Augustenburgerplatz, 1
13353 Berlin, Germany
+49 30 450 566 406
+49 30 450 566 931
paolo.matricardi@charite.de

Legend to figures

Figure E1 - Receiver Operating Characteristics (ROC) Curve for the model generated by the logistic stepwise backward selection (Wald), based on data from mothers with PFAS, anaphylaxis, asthma as comorbidity, and panallergen IgE results (blue line). Compared to reference line (green).

Area under the curve: 0.688

Table e1. Clinical characteristics of patients with PFAS who reported only oral symptoms compared to those who reported (oral and) systemic symptoms

	Oral Symptoms Only (n=99)		(Oral and) Systemic Symptoms (n=68)		*P*-Value
Male [n (%)]	47	*47.5*	35	*51.5*	.612
Age (y) [mean (*SD*)]	24.7	*12.8*	25.9	*13.5*	.558
Centers					
Porto [n (%)]	11	*11.1*	13	*19.1*	.147
Valencia [n (%)]	6	*6.1*	4	*5.9*	.961
Marseille [n (%)]	3	*3.0*	3	*4.4*	.637
Rome [n (%)]	32	*32.3*	9	*13.2*	.005**
Messina [n (%)]	10	*10.1*	14	*20.6*	.058
Tirana [n (%)]	9	*9.1*	4	*5.9*	.447
Athens [n (%)]	11	*11.1*	11	*16.2*	.342
Istanbul [n (%)]	6	*6.1*	7	*10.3*	.316
Izmir [n (%)]	11	*11.1*	3	*4.4*	.125
Allergic rhinitis					
Age at onset (y) [median (*IQR*)]†	8	*11.3*	10	*14*	.337
Months/year with symptoms [mean (*SD*)]	4.9	*2.4*	4.5	*2.4*	.362
Other allergic comorbidities					
Asthma [n (%)]	28	*28.3*	23	*33.8*	.445
Anaphylaxis [n (%)]	7	*7.1*	19	*27.9*	<.001***
Urticaria [n (%)]	34	*34.3*	29	*42.7*	.277
Atopic dermatitis [n (%)]	26	*26.3*	24	*35.3*	.211
Other [n (%)]	1	*1.0*	3	*4.4*	.158
Skin prick test (SPT)					
Positive SPT to seasonal aeroallergen(s) [mean (*SD*)]	5.4	*2.9*	4.6	*3.4*	.209
Average SPT size of seasonal aeroallergens (mm) [mean (*SD*)]	6.2	*1.6*	6.0	*1.7*	.486
IgE results					
Profilins [n (%)]‡	23	*23.2*	3	*4.4*	.001**
PR-10-like allergenic proteins [n (%)]‡	18	*18.2*	8	*11.8*	.261
nsLTPs [n (%)]‡	13	*13.1*	13	*19.1*	.294

PFAS: pollen food allergy syndrome; n: number; SD: standard deviation; IQR: interquartile range

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

†Due to incomplete data sets, 1 patient was excluded

‡ Testpanel included: profilins (Bet v 2, Phl p 12), PR-10-like allergenic proteins (Bet v 1, Cor a 1, Que a 1), and nsLTPs (Art v 3, Ole e 7)

Table e2. Results of hierarchical regression analysis of predictors for PFAS in Southern Europe

	Full model (Step 1)			Late-stage specific model (Step 17)		
	OR	95% CI	*P*-Value	OR	95% CI	*P*-Value
Male	0.926	0.496-1.730	.810	-	-	-
Age	1.028	0.994-1.062	.108	-	-	-
Centers						
Porto	-	-	.338	-	-	-
Valencia (ref.: Porto)	0.592	0.155-2.251	.441	-	-	-
Marseille (ref.: Porto)	0.167	0.037-0.746	.019*	-	-	-
Rome (ref.: Porto)	0.876	0.282-2.720	.819	-	-	-
Messina (ref.: Porto)	0.806	0.253-2.569	.716	-	-	-
Tirana (ref.: Porto)	0.336	0.079-1.421	.138	-	-	-
Athens (ref.: Porto)	1.182	0.371-3.762	.778	-	-	-
Istanbul (ref.: Porto)	0.500	0.117-2.137	.350	-	-	-
Izmir (ref.: Porto)	0.557	0.152-2.036	.376	-	-	-
Family history						
Atopic relative in immediate family	3.410	0.247-47.031	.360	-	-	-
Sibling(s) with PFAS	0.831	0.228-3.028	.779	-	-	-
Father with PFAS	1.192	0.071-20.148	.903	-	-	-
Mother with PFAS	4.030	0.973-16.693	.055	3.716	1.165-11.845	.026*
Allergic rhinitis						
Age at onset	0.984	0.942-1.028	.476	-	-	-
Months/year with symptoms	0.950	0.826-1.092	.469	-	-	-
ARIA severity						
Mild intermittent	-	-	.430	-	-	-
Mild persistent (ref: mild intermittent)	0.139	0.012-1.576	.111	-	-	-
Mod./severe intermittent (ref: mild intermittent)	0.773	0.206-2.905	.704	-	-	-
Mod./severe persistent (ref: mild intermittent)	0.637	0.182-2.230	.480	-	-	-
ARIA quality						
Unclassified	-	-	.351	-	-	-
Rhinitis sneezer/runner (ref. unclassified)	1.253	0.494-3.180	.635	-	-	-
Rhinitis blocker (ref. unclassified)	0.665	0.210-2.107	.488			
Other allergic comorbidities						
Patients with comorbidities	0.881	0.292-2.658	.822	-	-	-
Asthma	1.724	0.704-4.224	.233	1.752	0.949-3.234	.073
Anaphylaxis	6.358	2.209-18.296	.001**	6.210	2.561-15.057	<.001***
Urticaria	1.813	0.734-4.482	.197	-	-	-
Atopic dermatitis	1.160	0.486-2.768	.739	-	-	-
Other	0.217	0.019-2.444	.216	-	-	-
Skin prick test (SPT)						
Positive SPT to seasonal aeroallergen(s)	1.055	0.921-1.208	.440	-	-	-
Average SPT size of seasonal aeroallergens	1.037	0.863-1.246	.700	-	-	-
IgE results						
No panallergen IgE[†]	-	-	.091	-	-	.007**
Profilin IgE only (ref.: no panallergen IgE)[†]	1.550	0.471-5.107	.471	2.113	0.794-5.623	.134
PR-10-like allergenic proteins IgE only (ref.: no panallergen IgE)[†]	5.023	1.214-20.776	.026*	5.582	1.709-18.232	.004**
nsLTPs IgE only (ref.: no panallergen IgE)[†]	1.390	0.447-4.325	.569	1.401	0.483-4.058	.535
Multi-panallergen IgE (ref.: no panallergen IgE)[†]	7.421	1.166-47.241	.034*	6.353	1.323-30.503	.021*

PFAS: pollen food allergy syndrome; n: number; OR: odds ratio; CI: confidence interval; ref.: reference
* $p<0.05$; ** $p<0.01$; *** $p<0.001$
[†] Testpanel included: profilins (Bet v 2, Phl p 12), PR-10-like allergenic proteins (Bet v 1, Cor a 1, Que a 1), and nsLTPs (Art v 3, Ole e 7)

Table e3. Clinical characteristics of patients with PFAS in the nine Southern European centers

	TOTAL (n=167)		POR (n=24)		VAL (n=10)		MAR (n=6)		ROM (n=41)	
Number PFAS positive relative to total number recruited [n (%)]	167/815	20.5	24/102	23.5	10/71	14.1	6/80	7.5	41/99	41.4
Male [n (%)†]	82	*49.1*	14	*58.3*	3	*30.0*	4	*66.7*	19	*46.3*
Age (y) [mean (*SD*)]	25.2	*13.1*	26.5	*13.0*	25.7	*13.7*	33.3	*7.8*	27.3	*16.0*
Family history										
Atopic relative in immediate family [n (%†)]	126	*75.5*	16	*66.7*	7	*70.0*	6	*100.0*	30	*73.2*
Sibling(s) with PFAS [n (%†)]	5	*3.0*	0	*0.0*	0	*0.0*	0	*0.0*	2	*4.9*
Father with PFAS [n (%†)]	1	*0.6*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Mother with PFAS [n (%†)]	13	*7.8*	0	*0.0*	0	*0.0*	0	*0.0*	5	*12.2*
Allergic rhinitis										
Age at onset (y) [median (*IQR*)]‡	9	*12*	7.0	*16.0*	13.5	*17.8*	14.5	*18.8*	7.0	*8.0*
Disease duration (y) [median (*IQR*)]‡	9	*13.5*	11.0	*15.0*	6.5	*16.0*	20.0	*10.5*	12.0	*25.0*
Months/year with symptoms [mean (*SD*)]	4.8	*2.4*	5.0	*2.9*	3.2	*1.4*	4.8	*1.7*	4.4	*1.8*
ARIA severity										
Mild intermittent [n (%†)]	6	*3.6*	1	*4.2*	0	*0.0*	0	*0.0*	0	*0.0*
Mild persistent [n (%†)]	9	*5.4*	1	*4.2*	2	*20.0*	0	*0.0*	0	*0.0*
Mod./severe intermittent [n (%†)]	27	*16.2*	4	*16.7*	4	*40.0*	1	*16.7*	2	*4.9*
Mod./severe persistent [n (%†)]	125	*74.9*	18	*75.0*	4	*40.0*	5	*83.3*	39	*95.1*
ARIA quality										
Unclassified [n (%†)]	19	*11.7*	0	*0.0*	1	*10.0*	0	*0.0*	0	*0.0*
Rhinitis sneezer/runner [n (%†)]	123	*73.7*	19	*79.2*	5	*50.0*	5	*83.3*	39	*95.1*
Rhinitis blocker [n (%†)]	25	*15.0*	5	*20.8*	4	*40.0*	1	*16.7*	2	*4.9*
Other allergic comorbidities										
Number of patients with comorbidities [n (%†)]	111	*66.5*	16	*66.7*	8	*80.0*	6	*100.0*	29	*70.7*
Number of comorbidities [mean (*SD*)]	1.2	*1.0*	1.3	*1.2*	1.7	*1.2*	1.8	*0.8*	1.2	*1.0*
Asthma [n (%†)]	51	*30.5*	7	*29.2*	3	*30.0*	2	*33.3*	12	*29.3*
Anaphylaxis [n (%†)]	26	*15.6*	7	*29.2*	3	*30.0*	2	*33.3*	5	*12.2*
Urticaria [n (%†)]	63	*37.7*	5	*20.8*	5	*50.0*	4	*66.7*	18	*43.9*
Atopic Dermatitis [n (%†)]	50	*29.9*	11	*45.8*	6	*60.0*	3	*50.0*	14	*34.1*
Other [n (%†)]	4	*2.4*	0	*0.0*	0	*0.0*	0	*0.0*	2	*4.9*
Skin prick test (SPT)										
Positive SPT to seasonal aeroallergen(s) [mean (*SD*)]	5.0	*3.1*	4.0	*2.4*	4.3	*2.8*	4.8	*2.6*	7.0	*2.9*
Average SPT size of seasonal aeroallergens (mm) [mean (*SD*)]	6.1	*1.6*	6.5	*1.8*	7.0	*1.7*	4.4	*0.8*	6.1	*1.3*
IgE results										
No panallergen [n (%†)]§	102	*61.1*	16	*66.7*	6	*60.0*	2	*33.3*	19	*46.3*
Mono-panallergen [n (%†)]§	53	*31.7*	7	*29.2*	3	*30.0*	3	*50.0*	15	*36.6*
Multi-panallergen [n (%†)]§	12	*7.2*	1	*4.2*	1	*10.0*	1	*16.7*	7	*17.1*
Profilins [n (%†)]§	26	*15.6*	5	*20.8*	1	*10.0*	1	*16.7*	9	*22.0*
PR-10-like allergenic proteins [n (%†)]§	26	*15.6*	2	*8.3*	0	*0.0*	3	*50.0*	13	*31.7*
nsLTPs [n (%†)]§	26	*15.6*	2	*8.3*	4	*40.0*	1	*16.7*	8	*19.5*

PFAS: pollen food allergy syndrome; POR: Porto; VAL: Valencia; MAR: Marseille; ROM: Rome; MES: Messina; TIR: Tirana; ATH: Athens; IST: Istanbul; IZM: Izmir; n: number; SD: standard deviation; IQR: interquartile range

**p*<0.05; ***p*<0.01; ****p*<0.001

† Percentages calculated from total number of PFAS positive patients overall/in the centers

‡ Due to an incomplete data set, 1 patient was excluded

§ Testpanel included: profilins (Bet v 2, Phl p 12), PR-10-like allergenic proteins (Bet v 1, Cor a 1, Que a 1), and nsLTPs (Art v 3, Ole e 7)

104

105

Table e3 continued. Clinical characteristics of patients with PFAS in the nine Southern European centers

	MES (n=24)		TIR (n=13)		ATH (n=22)		IST (n=13)		IZM (n=14)		P-Value
Number PFAS positive relative to total number recruited [n (%)]	24/82	29.3	13/93	14.0	22/97	22.7	13/96	13.5	14/95	14.7	<.001***
Male [n (%)†]	11	*45.8*	8	*61.5*	12	*54.5*	5	*38.5*	6	*42.9*	.745
Age (y) [mean (*SD*)]	23.2	*12.7*	29.5	*8.9*	21.7	*11.8*	25.8	*12.0*	16.9	*8.1*	.116
Family history											
Atopic relative in immediate family [n (%†)]	21	*87.5*	9	*69.2*	18	*81.8*	9	*69.2*	10	*71.4*	.619
Sibling(s) with PFAS [n (%†)]	3	*12.5*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	.195
Father with PFAS [n (%†)]	0	*0.0*	0	*0.0*	1	*4.5*	0	*0.0*	0	*0.0*	.577
Mother with PFAS [n (%†)]	4	*16.7*	0	*0.0*	4	*18.2*	0	*0.0*	0	*0.0*	.087
Allergic rhinitis											
Age at onset (y) [median (*IQR*)]‡	10.0	*10.8*	22.0	*16.5*	7.5	*5.5*	18.0	*13.0*	6.0	*4.8*	.003**
Disease duration (y) [median (*IQR*)]‡	9.0	*11.8*	8.0	*8.5*	9.0	*11.0*	8.0	*12.0*	8.0	*10.3*	.079
Months/year with symptoms [mean (*SD*)]	5.9	*3.2*	5.9	*2.1*	5.2	*2.0*	4.9	*2.5*	2.6	*0.9*	.001**
ARIA severity											
Mild intermittent [n (%†)]	2	*8.3*	0	*0.0*	0	*0.0*	0	*0.0*	3	*21.4*	.019*
Mild persistent [n (%†)]	2	*8.3*	0	*0.0*	0	*0.0*	2	*15.4*	2	*14.3*	.080
Mod./severe intermittent [n (%†)]	9	*37.5*	1	*7.7*	2	*9.1*	2	*15.4*	2	*14.3*	.024*
Mod./severe persistent [n (%†)]	11	*45.8*	12	*92.3*	20	*90.9*	9	*69.2*	7	*50.0*	<.001***
ARIA quality											
Unclassified [n (%†)]	3	*12.5*	3	*23.1*	2	*9.1*	5	*38.5*	5	*35.7*	<.001***
Rhinitis sneezer/runner [n (%†)]	19	*79.2*	10	*76.9*	15	*68.2*	6	*46.2*	5	*35.7*	<.001***
Rhinitis blocker [n (%†)]	2	*8.3*	0	*0.0*	5	*22.7*	2	*15.4*	4	*28.6*	.058
Other allergic comorbidities											
Number of patients with comorbidities [n (%†)]	18	*75.0*	9	*69.2*	13	*59.1*	3	*23.1*	9	*64.3*	.035*
Number of comorbidities [mean (*SD*)]	1.4	*1.1*	1.2	*0.9*	1.0	*1.0*	0.3	*0.6*	0.8	*0.7*	.016*
Asthma [n (%†)]	10	*41.7*	1	*7.7*	7	*31.8*	2	*15.4*	7	*50.0*	.377
Anaphylaxis [n (%†)]	3	*12.5*	4	*30.8*	2	*9.1*	0	*0.0*	0	*0.0*	.061
Urticaria [n (%†)]	12	*50.0*	8	*61.5*	7	*31.8*	1	*7.7*	3	*21.4*	.022*
Atopic Dermatitis [n (%†)]	8	*33.3*	3	*23.1*	3	*13.6*	1	*7.7*	1	*7.1*	.018*
Other [n (%†)]	0	*0.0*	0	*0.0*	2	*9.1*	0	*0.0*	0	*0.0*	.448
Skin prick test (SPT)											
Positive SPT to seasonal aeroallergen(s) [mean (*SD*)]	2.6	*2.1*	6.3	*3.0*	6.9	*2.7*	2.3	*1.6*	4.3	*3.3*	<.001***
Average SPT size of seasonal aeroallergens (mm) [mean (*SD*)]	5.5	*1.7*	6.6	*1.4*	6.9	*1.7*	5.0	*1.3*	5.7	*1.7*	<.001***
IgE results											
No panallergen [n (%†)]§	20	*83.3*	5	*38.5*	13	*59.1*	10	*76.9*	11	*78.6*	.030*
Mono-panallergen [n (%†)]§	3	*12.5*	7	*53.8*	9	*40.9*	3	*23.1*	3	*21.4*	.233
Multi-panallergen [n (%†)]§	1	*4.2*	1	*7.7*	0	*0.0*	0	*0.0*	0	*0.0*	.180
Profilins [n (%†)]§	0	*0.0*	1	*7.7*	4	*18.2*	2	*15.4*	3	*21.4*	.480
PR-10-like allergenic proteins [n (%†)]§	1	*4.2*	7	*53.8*	0	*0.0*	0	*0.0*	0	*0.0*	<.001***
nsLTPs [n (%†)]§	4	*16.7*	1	*7.7*	5	*22.7*	1	*7.7*	0	*0.0*	.221

PFAS: pollen food allergy syndrome; POR: Porto; VAL: Valencia; MAR: Marseille; ROM: Rome; MES: Messina; TIR: Tirana; ATH: Athens; IST: Istanbul; IZM: Izmir; n: number; SD: standard deviation; IQR: interquartile range

*p<0.05; **p<0.01; ***p<0.001

† Percentages calculated from total number of PFAS positive patients overall/in the centers

‡ Due to an incomplete data set, 1 patient was excluded

§ Testpanel included: profilins (Bet v 2, Phl p 12), PR-10-like allergenic proteins (Bet v 1, Cor a 1, Que a 1), and nsLTPs (Art v 3, Ole e 7)

106

Table e4. Clinical characteristics of PFAS patients with and without IgE to nsLTP

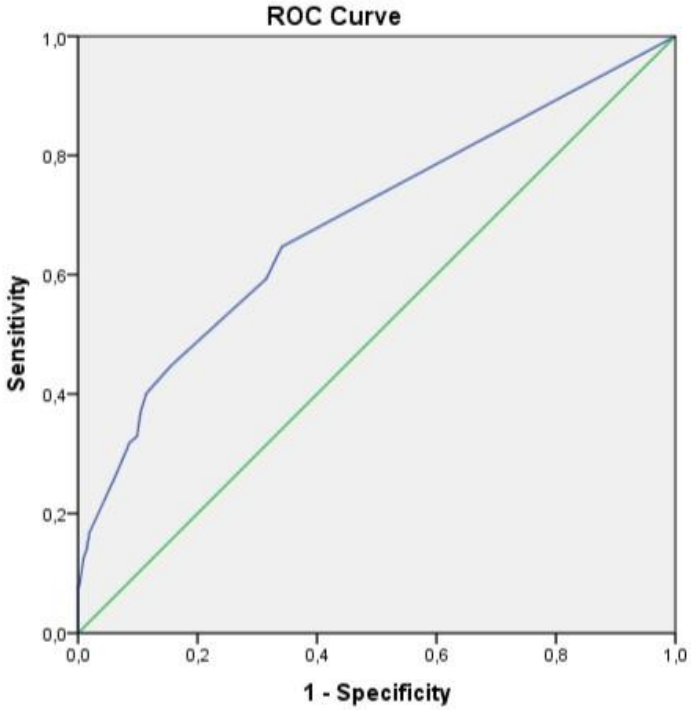
	PFAS - nsLTP Negative Patients (n=141)		PFAS - nsLTP Positive Patients (n=26)		*P*-Value
Male [n (%)]	67	*47.5*	15	*57.7*	.340
Age (y) [mean (*SD*)]	24.9	*12.8*	26.4	*14.8*	.590
Family history					
Atopic relative in immediate family [n (%)]	108	*76.6*	18	*69.2*	.459
Sibling(s) with PFAS [n (%)]	4	*2.8*	1	*3.9*	.576
Father with PFAS [n (%)]	1	*0.7*	0	*0.0*	1.000
Mother with PFAS [n (%)]	10	*7.1*	3	*11.5*	.429
Allergic rhinitis					
Age at onset (y) [median (*IQR*)]†	9	*12.8*	9	*10.5*	.878
Disease duration (y) [median (*IQR*)]†	9	*12*	10.5	*19.5*	.239
Months/year with symptoms [mean (*SD*)]	4.7	*2.3*	5.3	*2.8*	.228
ARIA severity					
Mild intermittent [n (%)]	6	*4.3*	0	*0.0*	.591
Mild persistent (ref.: mild intermittent) [n (%)]	6	*4.3*	3	*11.5*	.148
Mod./severe intermittent (ref.: mild intermittent) [n (%)]	24	*17.0*	3	*11.5*	.772
Mod./severe persistent (ref.: mild intermittent) [n (%)]	105	*74.5*	20	*76.9*	.791
ARIA quality					
Unclassified [n (%)]	17	*12.1*	2	*7.7*	.160
Rhinitis sneezer/runner [n (%)]	100	*70.9*	23	*88.5*	
Rhinitis blocker [n (%)]	24	*17.0*	1	*3.9*	
Other allergic comorbidities					
Number of patients with comorbidities [n (%)]	94	*66.7*	17	*65.4*	.899
Number of comorbidities [mean (*SD*)]	1.1	*1.0*	1.3	*1.2*	.281
Asthma [n (%)]	43	*30.5*	8	*30.8*	.978
Anaphylaxis [n (%)]	22	*15.6*	4	*15.4*	1.000
Urticaria [n (%)]	53	*37.6*	10	*38.5*	.933
Atopic dermatitis [n (%)]	39	*27.7*	11	*42.3*	.163
Other [n (%)]	3	*2.1*	1	*3.9*	.495
Skin prick test (SPT)					
Positive SPT for seasonal aeroallergen(s) [mean (*SD*)]	4.8	*3.1*	6.5	*2.9*	.012
Average SPT size of seasonal aeroallergens (mm) [mean (*SD*)]	6.0	*1.7*	6.4	*1.5*	.261

PFAS: pollen food allergy syndrome; n: number; SD: standard deviation; IQR: interquartile range; ref.: reference
* $p<0.05$; ** $p<0.01$; *** $p<0.001$
†Due to incomplete data sets, 2 patients were excluded

107

8

Figure e1



Curriculum Vitae of Theresa Lipp

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

List of Publications

Manuscripts

Lipp, T., Acar Şahin, A., Aggelidis, X., Arasi, S., Barbalace, A., Bourgoin, A., Bregu, B., Brighetti, M.A., Caeiro, E., Caglayan Sozmen, S., Caminiti, L., Charpin, D., Couto, M., Delgado, L., Di Rienzo Businco, A., Dimier, C., Dimou, M.V., Fonseca, J.A., Goksel, O., Guvensen, A., Hernandez, D., Hoffmann, T.M., Jang, D.T., Kalpaklioglu, F., Lame, B., Llusar, R., Makris, M., Mazon, A., Mesonjesi, E., Nieto, A., Öztürk, A., Pahus, L., Pajno, G., Panasiti, I., Papadopoulos, N.G., Pellegrini, E., Pelosi, S., Pereira, A.M., Pereira, M., Pinar, N.M., Potapova, E., Priftanji, A., Psarros, F., Sackesen, C., Sfika, I., Suarez, J., Thibaudon, M., Travaglini, A., Tripodi, S., Verdier, V., Villella, V., Xepapadaki, P., Yazici, D., Matricardi, P.M., Dramburg, S., 2021. Heterogeneity of Pollen Food Allergy Syndrome in Seven Southern European Countries: the @IT.2020 Multicenter Study. Allergy. https://doi.org/10.1111/all.14742

Poster Presentations

Lipp T., Acar Sahin A., Aggelidis, X., Arasi, S., Barbalace, A., Bourgoin, A., Bregu, B., Brighetti, M.A., Caeiro, E., Caglayan Sozmen, S., Caminiti, L., Charpin, D., Couto, M., Delgado, L., Di Rienzo Businco, A., Dimier, C., Dimou, M.V., Fonseca, J.A., Goksel, O., Guvensen, A., Hernandez, D., Hoffmann, T.M., Jang, D.T., Kalpaklioglu, F., Lame, B., Llusar, R., Makris, M., Mazon, A., Mesonjesi, E., Nieto, A., Öztürk, A., Pahus, L., Pajno, G., Panasiti, I., Papadopoulos, N.G., Pellegrini, E., Pelosi, S., Pereira, A.M., Pereira, M., Pinar, N.M., Potapova, E., Priftanji, A., Psarros, F., Sackesen, C., Sfika, I., Suarez, J., Thibaudon, M., Travaglini, A., Tripodi, S., Verdier, V., Villella, V., Xepapadaki, P., Yazici, D., Matricardi, P.M., Dramburg, S. “Pollen Food Allergy Syndrome in Seven Southern European Countries: Results from the @IT.2020 Multicenter Study”. Poster. European Academy of Allergy and Clinical Immunology Congress 2020. London, GB, 06.-10.06.2020.

Lipp T., Acar Sahin A., Aggelidis, X., Arasi, S., Barbalace, A., Bourgoin, A., Bregu, B., Brighetti, M.A., Caeiro, E., Caglayan Sozmen, S., Caminiti, L., Charpin, D., Couto, M., Delgado, L., Di Rienzo Businco, A., Dimier, C., Dimou, M.V., Fonseca, J.A., Goksel, O., Guvensen, A., Hernandez, D., Hoffmann, T.M., Jang, D.T., Kalpaklioglu, F., Lame, B., Llusar, R., Makris, M., Mazon, A., Mesonjesi, E., Nieto, A., Öztürk, A., Pahus, L., Pajno, G., Panasiti, I., Papadopoulos, N.G., Pellegrini, E., Pelosi, S., Pereira, A.M., Pereira, M., Pinar, N.M., Potapova, E., Priftanji, A., Psarros, F., Sackesen, C., Sfika, I., Suarez, J., Thibaudon, M., Travaglini, A., Tripodi, S., Verdier, V., Villella, V., Xepapadaki, P., Yazici, D., Matricardi, P.M., Dramburg, S.. “Prevalence and Clinical Characteristics of Pollen Food Syndrome in Children from 7 Southern European Countries: Results from the @IT2020 Multicenter Study”. Pediatric Asthma and Allergy Meeting 2019. Poster. Abstract D53. Florenz, IT, 17.-19.10.2019.

Lipp T, Kopf A. “Benchmarking der schmerzmedizinischen Kompetenzen von Ärzten in Weiterbildung an der Charité – Universitätsmedizin Berlin – Campus Benjamin Franklin“. Deutscher Schmerzkongress 2015. Poster. Abstract 298. Mannheim, DE, 14.-17.10.2015.

Note of Thanks

My heartfelt thanks go to my mentors, PD Dr. med. Paolo Matricardi and Dr. med. Stephanie Dramburg, for sharing their experience and wisdom and giving me their support. Furthermore, I would like to thank all members of the @IT.2020 Multicenter Study Team for the great collaboration and insights.

Many thanks to my family and friends for supporting me during this important and inspiring phase of my life.