Heterogeneity of sensitization profiles and clinical phenotypes among patients with seasonal allergic rhinitis in Southern European countries-The @IT.2020 multicenter study

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ORIGINAL ARTICLE

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Heterogeneity of sensitization profiles and clinical phenotypes among patients with seasonal allergic rhinitis in Southern European countries—The @IT.2020 multicenter study

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Abstract

Background: Pollen allergy poses a significant health and economic burden in Europe. Disease patterns are relatively homogeneous within Central and Northern European countries. However, no study broadly assessed the features of seasonal allergic rhinitis (SAR) across different Southern European countries with a standardized approach.

Objective: To describe sensitization profiles and clinical phenotypes of pollen allergic patients in nine Southern European cities with a uniform methodological approach.

Methods: Within the @IT.2020 multicenter observational study, pediatric and adult patients suffering from SAR were recruited in nine urban study centers located in seven countries. Clinical questionnaires, skin prick tests (SPT) and specific IgE (sIgE) tests with a customized multiplex assay (Euroimmun Labordiagnostika, Lübeck, Germany) were performed.

Results: Three hundred forty-eight children (mean age 13.1 years, SD: 2.4 years) and 467 adults (mean age 35.7 years SD: 10.0 years) with a predominantly moderate to severe, persistent phenotype of SAR were recruited. Grass pollen major allergenic molecules (Phl p 1

Abbreviations: AIT, allergen immunotherapy; ARIA, allergic rhinitis and its impact on asthma; ATH, study center in Athens, Greece; CCD, cross-reactive carbohydrate determinants; CRD, component-resolved diagnosis; ED, etiological diagnosis; ESEP, Euroline Southern European Profile Test (Euroimmun Labordiagnostika, Lübeck, Germany); IQR, interquartile range; IST, study center in Istanbul, Turkey; IZM, study center in Izmir, Turkey; MAR, study center in Marseille, France; MES, study center in Messina, Italy; POR, study center in Porto, Portugal; ROM, study center in Rome, Italy; SAR, seasonal allergic rhinitis; SD, standard deviation; SPT, skin prick test; TIR, study center in Tirana, Albania; VAL, study center in Valencia, Spain.

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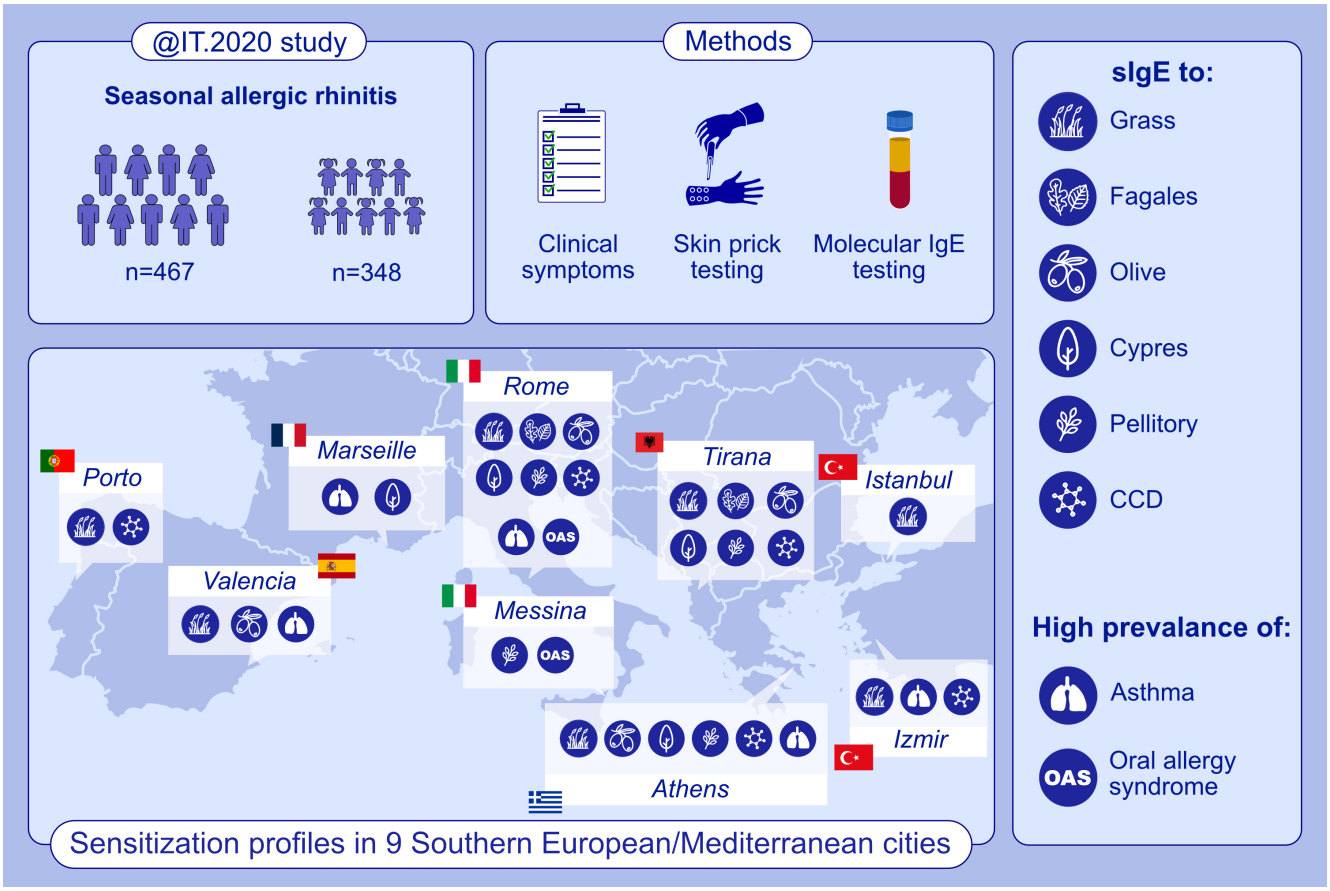
and/or Phl p 5) ranged among the top three sensitizers in all study centers. Sensitization profiles were very heterogeneous, considering that patients in Rome were highly poly-sensitized (sIgE to 3.8 major allergenic molecules per patient), while mono-sensitization was prominent and heterogeneous in other cities, such as Marseille (sIgE to Cup a 1: $n=55/80$, 68.8%) and Messina (sIgE to Par j 2: $n=47/82$, 57.3%). Co-sensitization to perennial allergens, as well as allergic comorbidities also broadly varied between study centers.

Conclusions: In Southern European countries, pollen allergy is heterogeneous in terms of sensitization profiles and clinical manifestations. Despite the complexity, a unique molecular, multiplex, and customized in-vitro IgE test detected relevant sensitization in all study centers. Nevertheless, this geographical diversity in pollen allergic patients imposes localized clinical guidelines and study protocols for clinical trials of SAR in this climatically complex region.

KEYWORDS

allergic rhinitis, component-resolved diagnostics, IgE, phenotypes, pollen allergy, sensitization



GRAPHICAL ABSTRACT

Within the @IT.2020 multicenter observational study, pediatric and adult patients suffering from seasonal allergic rhinitis were recruited in nine study centers from seven countries. Sensitization profiles were very heterogeneous but grass pollen major allergenic molecules were among the top three sensitizers in all study centers. Poly-sensitization was prevalent in Rome, Athens, and Tirana, while one single pollen was predominant in Valencia (olive), Marseille (cypress), Messina (pellitory), Porto, Istanbul, and Izmir (grass).

Abbreviations: CCD, Cross-reactive carbohydrate determinants; sIgE, specific immunoglobulin E; OAS, oral allergy syndrome.

1 | INTRODUCTION

Seasonal allergic rhinitis (SAR) is a widespread disease in Western industrialized countries.[1] Together with allergic asthma, respiratory allergic diseases affect an estimated 150 million citizens in the European Union.[2] Symptoms and effects of allergic rhinoconjunctivitis (runny/stuffy nose, itchy nose and eyes, sneezing, watery eyes, rhinitis-related disturbed sleep, etc.) do not only lower the individual quality of life but also affect patients' productivity.[3,4] Particularly patients with inadequate treatment, often based on self-medication with over-the-counter drugs, may unnecessarily suffer from symptoms and their loss in productivity has a significant economic impact.[5] Therefore, clinicians aim at finding the optimal disease management strategy for every patient, whether this implies avoidance, symptomatic treatment, or the only available causal treatment: allergen immunotherapy[6] (AIT).

Particularly for the prescription of AIT, it is essential to precisely identify the eliciting allergen to select the correct therapeutic agent(s).[7] This decision may be relatively easy in Central and Northern European countries, where pollination periods of most relevant pollen (grass and birch) are well temporally separated. In contrast, the same decision can be significantly more challenging in Southern European and Mediterranean countries where the spectrum of allergen exposure is much broader, as many plants pollinate simultaneously. In addition, the timing and quantity of pollen exposure clearly vary between different countries of the same Mediterranean,[8] climate zone.[9] As pollen exposure not only induces sensitization, but also triggers clinical symptoms, differences in both sensitization patterns and clinical phenotypes of SAR may be expected within this complex geographic area. To our knowledge, no study has yet focused on the description of sensitization profiles and clinical phenotypes of pollen allergic patients in several Southern European countries with a uniform methodological approach. However, a better understanding of potential similarities or differences among these distinct areas is essential to plan clinical and pharmacological studies as well as European prevention and treatment guidelines. Within the framework of the @IT.2020 multicenter observational study, patients suffering from SAR in nine Southern European cities were recruited to assess the value of component-resolved diagnosis (CRD) with a customized and validated multiplex molecular IgE test[10] in combination with mobile health technology. The aim of the present analysis is to investigate clinical and serological varieties and similarities of pollen allergic patients recruited in nine Southern European cities.

2 | MATERIALS AND METHODS

2.1 | Study population

The @IT.2020 Observational Longitudinal Multicentre Clinical Study was conducted in 2017 and 2018 to determine the impact of component-resolved diagnostics and mobile health on the diagnosis of SAR in Southern Europe. To this aim, pediatric and adult patients with a diagnosis of SAR were recruited in nine urban study centres located in seven Southern European countries (Porto, Portugal; Valencia, Spain; Marseille, France; Rome and Messina, Italy; Tirana, Albania; Athens, Greece; Istanbul and Izmir, Turkey). All study centres are specialized allergy clinics. Inclusion criteria were as follows: (i) age 10–18 years for children or 19–60 years for adults; (ii) a good understanding of the national language or one of the languages offered in the mobile study application (Allergymonitor®, TPS software production, Rome, Italy); (iii) availability of a smartphone; (iv) written informed consent. Exclusion criteria were as follows: (i) prior AIT for pollen allergies; (ii) any severe chronic disease; (iii) living further than 30 km away from the local aerobiological center's pollen trap.

2.2 | Study design

The prospective observational study consisted of two face-to-face study visits, a recruitment visit (T0) in winter 2017/spring 2018 including the collection of blood samples and questionnaires and a final visit (T1) after the pollen season 2018 including questionnaires. Between the study visits, participants were prescribed an individual monitoring period to record their allergy symptoms via the Allergymonitor® e-Diary. The timing of the prescribed period was selected according to the flowering period of the suspected eliciting allergen source. All participants or their guardians provided written informed consent and the study was approved by the local ethics committees. For more details on the methods, please see the *online repository*.

3 | RESULTS

3.1 | Study population

We examined 348 children (mean age 13.1 years, SD: 2.4 years) and 467 adults (mean age 35.7 years, SD: 10.0 years). All centers but Marseille included both pediatric and adult patients. Male gender was more frequent among pediatric patients (218/248, 62.6%) than among adults (223/467, 47.8%) and 457/815 (56.1%) participants reported a parental history of at least one allergic disease. Regarding disease severity, a persistent, moderate–severe phenotype of allergic rhinitis was the most frequent clinical phenotype of SAR (Table 1). Overall median age at disease onset was 11 years (IQR: 6–20) and median disease duration at inclusion was 9 years (IQR: 4–17). Patients in Rome reported the youngest age at onset (median 8 years, IQR: 5–14). Apart from Marseille (only adult patients), the longest disease duration was observed in Rome and Messina (median 10 years [both], IQR: 5–28 [Rome] vs. 5–17 [Messina]). The average number of allergic comorbidities ranged between a minimum of 0.5 (SD: 0.7) in Istanbul and a maximum of 1.7 (SD: 1.5) in Rome. The most frequently reported allergic comorbidities were urticaria, followed by atopic dermatitis and asthma. However, substantial prevalence variations could be observed among the different study

TABLE 1 Characteristics of the study population.

	Total ($n=815$)		POR ($n=102$)		VAL ($n=71$)		MAR ($n=80$)		ROM ($n=99$)		MES ($n=82$)		TIR ($n=93$)		ATH ($n=97$)		IST ($n=96$)		IZM ($n=95$)		Mean SMD
Male [*n* (%)]	441	54.1	56	54.9	36	50.7	40	50.0	54	54.5	45	54.9	52	55.9	64	66.0	49	51.0	45	47.4	0.12
Pediatric patients (age <19 years) [*n* (%)]	348	42.7	52	51.0	40	56.3	–		49	49.5	42	51.2	26	28.0	52	53.6	38	39.6	49	51.6	0.46
Age adult participants (years) [mean, SD]	35.3	10.2	34	10.0	34.6	8.7	39.2	10.0	39.3	10.3	34.0	10.0	30.3	7.7	34.1	10.1	32.7	10.5	39.0	10.4	0.38
Age pediatric participants (years) [mean, SD]	13	2.2	13.5	2.1	12.6	1.9	–		12.6	2.4	12.8	2.2	13.0	2.5	12.9	2.0	13.5	2.3	13.0	2.5	0.22
Family history																					
Parental history of allergy [*n* (%)]	457	56.1	56	54.9	43	60.6	42	52.5	53	53.5	57	69.5	47.0	50.5	59	60.8	52	54.2	48	50.5	0.14
Older siblings [*n* (%)]	415	50.9	45	44.1	39	54.9	45	56.3	48	48.5	46	56.1	52.0	55.9	46	47.4	46	47.9	48	50.5	0.11
Active smoking in the past 12 months [*n* (%)]	140	17.2	10	9.8	5	7.0	16	20.0	18	18.2	18	22.0	16.0	17.2	12	12.4	14	14.6	31	32.6	0.24
Passive smoking in the past 12 months [*n* (%)]	249	30.6	24	23.5	9	12.7	5	6.3	38	38.4	39	47.6	29.0	31.2	46	47.4	20	20.8	39	41.1	0.43
Allergic rhinitis																					
Age at onset (years) [median (IQR)]* (2 missings)	11	(6–20)	9	(5–18)	10	(8–16)	17	(10–30)	8	(5–14)	10	(5–15)	16	(9–23)	10	(6–15)	16	(9–23)	10	(6–22)	
Mean (SD)	14.0	10.6	12.6	10.4	13.2	9.5	20.5	14.0	10.5	8.0	11.3	8.4	16.4	9.6	11.7	7.7	17.0	11.9	13.9	11.4	0.36
Disease duration (years) [median (IQR)]* (2 missings)	9	(4–17)	8	(4–15)	6	(3–12)	15	(9–28)	10	(5–28)	10	(5–17)	8	(4–13)	8	(4–18)	7	(3–11)	8	(5–16)	
Mean (SD)	12.0	10.4	11.4	10.5	9.0	8.2	18.8	13.0	16.1	12.8	11.8	9.0	9.6	7.3	11.7	9.3	8.3	7.6	11.7	10.5	0.37

(Continues)

TABLE 1 (Continued)

	Total ($n=815$)		POR ($n=102$)		VAL ($n=71$)		MAR ($n=80$)		ROM ($n=99$)		MES ($n=82$)		TIR ($n=93$)		ATH ($n=97$)		IST ($n=96$)		IZM ($n=95$)		Mean SMD
Months/year with symptoms [mean, SD]	4.7	2.4	4.7	2.4	4.0	2.7	4.8	2.0	4.4	1.7	4.9	2.2	6.0	2.3	5.0	2.5	4.6	2.8	3.6	1.9	0.33
ARIA classification (severity and quality)																					
Mild intermittent [*n* (%)]	41	5.0	1	1.0	10	14.1	1	1.3	0	0.0	12	14.6	6	6.5	3	3.1	4	4.2	4	4.2	0.62
Mild persistent [*n* (%)]	60	7.4	2	2.0	16	22.5	2	2.5	7	7.1	9	11.0	2	2.2	2	2.1	15	15.6	5	5.3	
Mod./severe intermittent [*n* (%)]	152	18.7	31	30.4	15	21.1	15	18.8	3	3.0	27	32.9	10	10.8	14	14.4	14	14.6	23	24.2	
Mod./severe persistent [*n* (%)]	562	69.0	68	66.7	30	42.3	62	77.5	89	89.9	34	41.5	75	80.6	78	80.4	63	65.6	63	66.3	
Rhinitis sneezer/runner [*n* (%)]	540	66.3	74	72.5	42	59.2	63	78.8	89	89.9	55	67.1	60	64.5	56	57.7	53	55.2	48	50.5	0.45
Rhinitis blocker [*n* (%)]	148	18.2	16	15.7	21	29.6	9	11.3	10	10.1	14	17.1	8	8.6	28	28.9	20	20.8	22	23.2	
No information on type of rhinitis [*n* (%)]	127	15.6	12	11.8	8	11.3	8	10.0	–		13	15.9	25	26.9	13	13.4	23	24.0	25	26.3	
Other allergic comorbidities																					
Number of comorbidities [mean, SD]	1.0	1.2	0.9	1.2	1.2	1.3	1.2	1.3	1.7	1.5	1.2	1.3	1.1	1.1	1.0	1.2	0.5	0.7	0.8	0.9	0.31
Median (IQR)	1.0	(0,0-2,0)	0.5	(0,0-1,0)	1.0	(0,0-2,0)	1.0	(0,0-2,0)	2.0	(0,0-2,0)	1.0	(0,0-2,0)	1.0	(0,0-2,0)	1.0	(0,0-2,0)	0.0	(0,0-1,0)	0.0	(0,0-1,0)	
Asthma [*n* (%)]	302	37.1	20	19.6	22	31.0	36	45.0	44	44.4	34	41.5	15	16.1	50	51.5	29	30.2	52	54.7	0.36
Oral allergy syndrome (*n*[%|)	123	15.1	13	12.7	6	8.5	6	7.5	35	35.4	22	26.8	16	17.2	12	12.4	2	2.1	11	11.6	0.33
Anaphylaxis [*n* (%)]	49	6.0	8	7.8	6	8.5	3	3.8	7	7.1	5	6.1	10	10.8	4	4.1	–		6	6.3	0.05
Urticaria [*n* (%)]	194	23.8	12	11.8	14	19.7	26	32.5	39	39.4	25	30.5	37	39.8	21	21.6	4	4.2	16	16.8	0.38
Atopic dermatitis [*n* (%)]	179	22.0	28	27.5	20	28.2	25	31.3	31	31.3	19	23.2	22	23.7	14	14.4	10	10.4	10	10.5	0.25
Food allergy [*n* (%)]	107	13.1	12	11.8	15	21.1	11	13.8	19	19.2	7	8.5	14	15.1	13	13.4	11	11.5	5	5.3	0.05
Other [*n* (%)]	26	3.2	–		3	4.2	1	1.3	8	8.1	1	1.2	2	2.2	6	6.2	3	3.1	2	2.1	0.16

*According to Cohen values of 0.2, 0.5, and 0.8 represent small, medium, and large effect sizes.

TABLE 2 Skin prick test (SPT) results of the total study population and by center.

	Total ($n=815$)	POR ($n=102$)	VAL ($n=71$)	MAR ($n=80$)	ROM ($n=99$)	MES ($n=82$)	TIR ($n=93$)	ATH ($n=97$)	IST ($n=96$)	IZM ($n=95$)	Mean SMD*	
Number of positive SPT												
In total	2684	265	169	242	558	164	394	467.00	162	263	0.8	
Per patient, mean (SD)	3.3 (2.4)	2.6 (2.0)	2.4 (1.6)	3.0 (2.0)	5.6 (2.7)	2.0 (1.3)	4.2 (2.3)	4.8 (2.4)	1.7 (1.2)	2.8 (1.9)		
Per patient, median (IQR)	3 (1–5)	2 (1–3)	2 (1–3)	3 (1–4)	5 (4–8)	2 (1–2)	4 (2–6)	5 (3–7)	1 (1–2)	2 (1–3)		
Positive SPT per allergen; *n* (%)												
Grass	585 (71.8)	95 (93.1)	33 (46.5)	47 (58.8)	89 (89.9)	30 (24.6)	83 (89.2)	75 (77.3)	59 (61.5)	74 (74.7)	0.6	Highest
Olive	408 (50.1)	30 (29.4)	54 (76.1)	43 (53.8)	66 (66.7)	43 (35.3)	47 (50.5)	72 (74.2)	9 (9.4)	44 (46.3)	0.6	
Juniper ash (cypress)	306 (37.5)	7 (6.9)	20 (28.2)	65 (81.3)	73 (73.7)	2 (1.6)	34 (36.3)	51 (52.6)	24 (25.0)	30 (31.6)	0.8	
Pellitory	260 (31.9)	21 (20.6)	11 (15.5)	15 (18.8)	56 (56.6)	54 (44.3)	16 (17.2)	62 (63.9)	13 (13.5)	12 (12.6)	0.6	
Mugwort	209 (25.6)	21 (20.6)	7 (9.9)	12 (15.0)	50 (50.5)	7 (5.7)	40 (43.0)	41 (42.3)	13 (13.5)	18 (18.9)	0.4	
Plane tree	198 (24.3)	19 (18.6)	11 (15.5)	21 (26.3)	53 (53.5)	2 (1.6)	35 (37.6)	29 (29.9)	9 (9.4)	19 (20.0)	0.5	Lowest
Salsola kali	179 (22.0)	17 (16.7)	13 (18.3)	12 (15.0)	43 (43.4)	9 (7.4)	26 (28.0)	36 (37.1)	8 (8.3)	15 (15.8)	0.3	
Ragweed	177 (21.7)	20 (19.6)	5 (7.0)	8 (10.0)	36 (36.4)	6 (4.9)	39 (41.9)	31 (32.0)	17 (17.7)	15 (15.8)	0.4	
Fagales	208 (25.2)	23 (22.5)	3 (4.2)	13 (16.3)	51 (51.5)	4 (3.3)	58 (62.4)	33 (34.0)	7 (7.3)	16 (16.8)	0.6	
Alternaria	154 (18.9)	12 (11.8)	12 (16.9)	6 (7.5)	41 (41.4)	7 (5.7)	16 (17.2)	37 (38.1)	3 (3.1)	20 (21.1)	0.4	
Dog	250 (30.7)	32 (31.4)	21 (29.6)	26 (32.5)	62 (62.6)	7 (5.7)	20 (21.5)	37 (38.1)	13 (13.5)	22 (23.2)		
Cat	304 (37.3)	40 (39.2)	23 (32.4)	40 (50.0)	54 (54.5)	26 (21.3)	18 (19.4)	48 (49.5)	25 (26.0)	30 (31.6)		
House Dust Mite	392 (48.1)	65 (63.7)	42 (59.2)	41 (51.3)	60 (60.6)	55 (45.1)	52 (55.9)	30 (30.9)	32 (33.3)	15 (15.8)		

Note: Tests with a wheal size of 3 mm or greater were considered positive. Colors mark the level of prevalence for seasonal allergens (red = most prevalent, orange = second most prevalent, yellow = third most prevalent, green from dark to light mark ranks 4–10).

*Standardized mean difference.

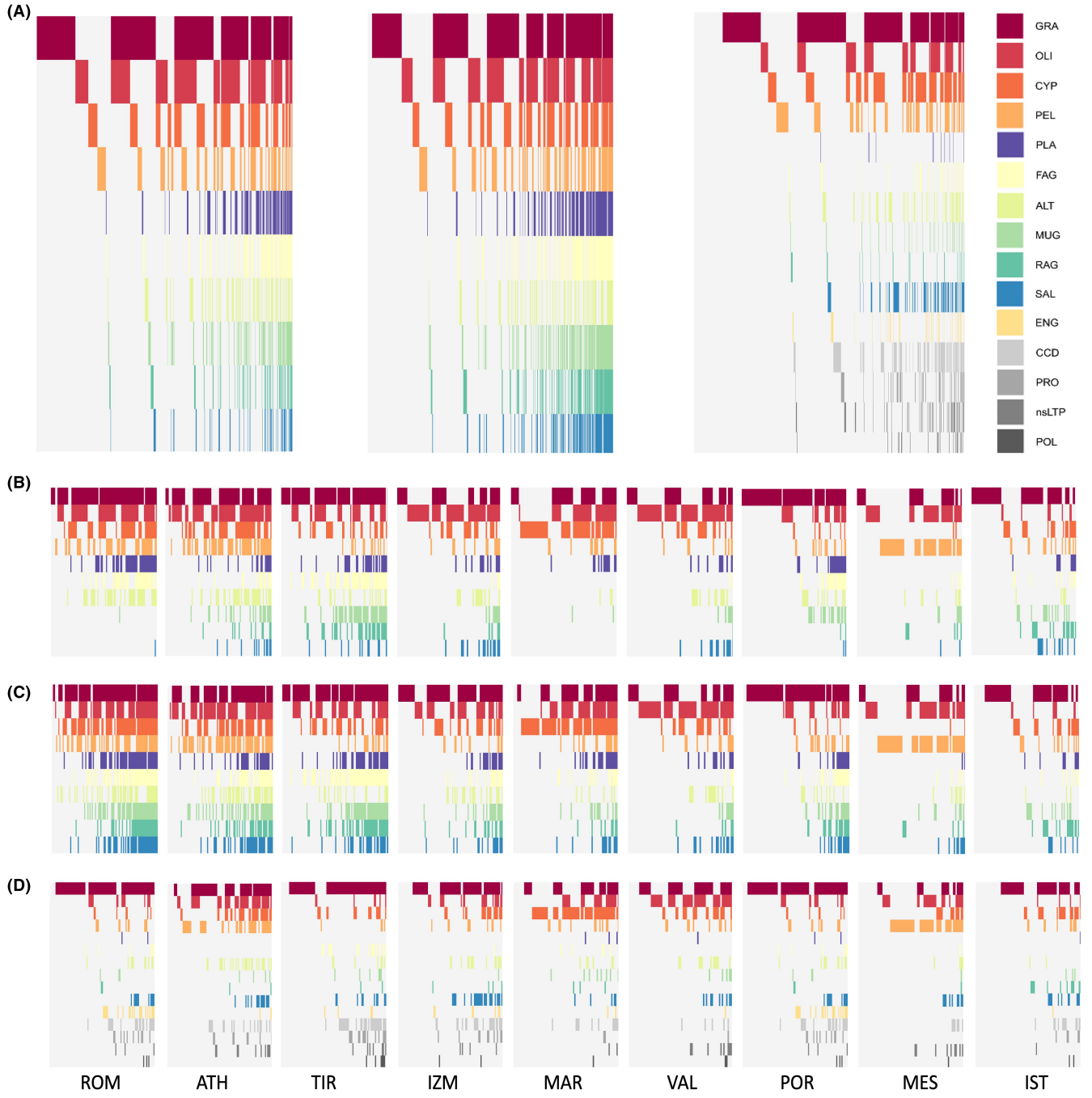


FIGURE 1 Heatmaps illustrate individual combinations of (A) etiological diagnoses based on clinical history, SPT and historical pollen Calendars (left) skin prick test (SPT) profiles (middle) and molecular IgE responses (right) of 815 patients with SAR; (B) etiological diagnoses of pollen allergy by study center; (C) SPT by study centre; (D) molecular IgE results by study centre. Molecular IgE results represent the presence of IgE antibodies toward the major allergenic molecule of each shown allergen, as well as common representatives for groups of panallergens (profilins, nsLTP, and polcalcins) and CCD. **GRA**: Phl p 1 and/or Phl p 5 and/or Cyn d 1), **CYP**: Cup a 1, **OLI**: Ole e 1, **PEL**: Par j 2, **SAL**: Sal k 1, **ALT**: Alt a 1, **FAG**: Bet v 1 and/or Que a 1 and/or Cor a 1), **ENG**: Pla l 1, **MUG**: Art v 1, **RAG**: Amb a 1, **PLA**: Pla a 1 and/or Pla a 2, **CCD**: marker for IgE against CCD, **PRO**: profilins Bet v 2 and/or Phl p 12, **nsLTP**: Ole e 7 and/or Art v 3, **POL**: polcalcins Bet v 4 and/or Phl p 7.

centers, with asthma being the most frequent comorbidity in Athens, Istanbul and Izmir, urticaria in Marseille, Rome, Messina, and Tirana and atopic dermatitis in Porto and Valencia (Table 1). Sensitization to perennial allergen sources (HDM, cat and dog) ranged between 8.5% (SPT to dog in Messina) and 67.1% (SPT to HDM in Messina) in individual centers. In the total population, the most frequent perennial sensitization was to HDM which was reflected in a positive SPT for 392/815 (48.1%) participants (Table 2).

3.2 | Skin prick test results of pollen-allergic patients in nine Southern European study centers

Overall, 2.716 positive SPT results were observed toward the 10 allergens tested, with an average of 3.3 (SD: 2.4) positive tests per participant. The frequency distribution of sensitization to individual allergens and the patients' sensitization profiles was highly heterogeneous among the 9 study centers (Figure 1C; Figure 2C). The strongest poly-sensitization was observed in Rome (total = 565, mean 5.7/patient, SD: 2.6), followed by Tirana (mean 4.2, SD: 2.3), Marseille (mean: 3.1, SD: 2.0), Izmir (mean: 2.8, SD: 1.9), Porto (mean: 2.6, SD: 2.0), Valencia (mean: 2.4, SD: 2.6), Messina (mean: 2.0, SD: 1.3), and finally Istanbul with the lowest frequency (mean: 1.8, SD: 1.2) (Figure 1C). Timothy and bermuda grass pollen was the most frequent sensitizer in all centers but Marseille, Valencia, and Messina (Figure 2C; Table 2). Beyond grasses, pellitory, olive,

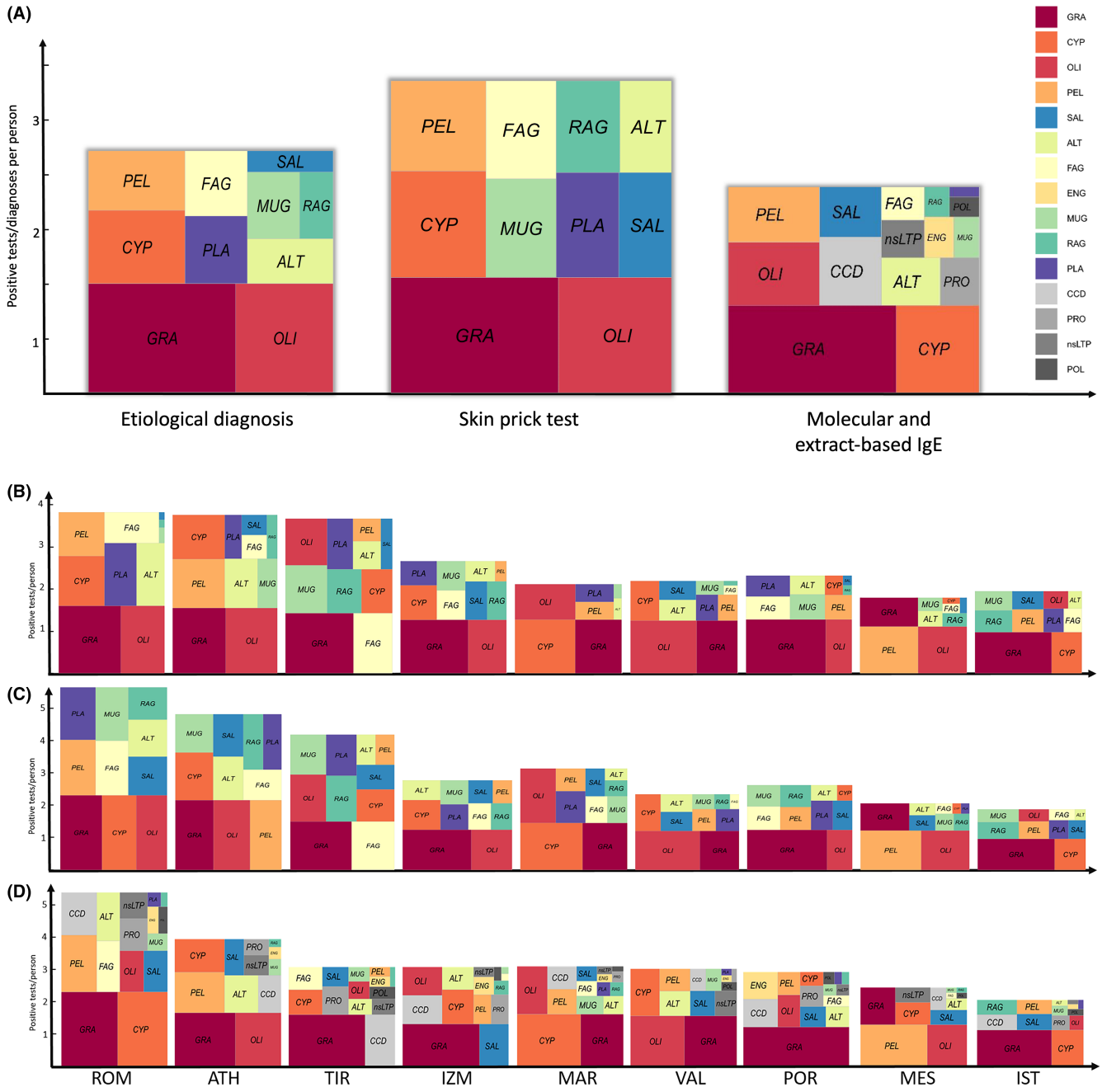


FIGURE 2 Tree maps indicating the prevalence of (A) etiological diagnoses (based on clinical history, SPT and historical pollen calendars), sensitization profiles (skin prickt test (SPT) and molecular IgE responses) of 815 patients with SAR; (B) etiological diagnoses; (C) SPT results; and; (D) IgE responses to allergenic molecules by centre. Test results are ordered by decreasing frequency of positive results from the left lower corner to the upper right corner; rectangle sizes are proportional to the number of positive results relative to the total of tested patients. Abbreviations in the legends refer to the allergen source and the following (major) allergenic molecules.

and cypress most frequently elicited an IgE response. Only 146 patients (17.9%) were mono-sensitized if evaluated by SPT only, while 171 (21.0%), 136 (16.7%), and 90 (11.0%) were sensitized to two, three, or four allergens, respectively. While timothy grass was the most important sensitizer in Porto, Rome, Tirana, Istanbul, and Izmir, olive pollen caused most positive SPT results in Valencia and Athens, cypress in Marseille and pellitory in Messina. Interestingly, pollen of *Fagales* plays a subordinate role in all study centers but Tirana. Overall, 267 (38.2%) patients exclusively reacted to seasonal airborne allergen sources, while the majority (537, 65.9%) also reacted to perennial allergen sources, such as house dust mites ($n=400$, 49.1%), cat ($n=309$, 37.9%), or dog ($n=254$, 31.2%) (Table 2). This combination of reactions to seasonal and perennial allergen sources, was most frequently observed in Rome, followed by Porto and Messina.

3.3 | IgE responses toward major and common cross-reactive allergenic molecules of seasonal airborne allergens

Overall, 1.883 positive IgE results were observed in the total study population, reflecting an average of 2.3 positive tests per participant (SD: 1.7) (Table 3). Molecular IgE profiles of the nine study centers exhibited basic agreement with the SPT results, combining a high prevalence of IgE toward the major allergenic molecules of grass pollen (Phl p 1, Phl p 5, and Cyn d 1), olive (Ole e 1) and cypress (Cup a 1) with a heterogeneous response toward other seasonal airborne allergens and common panallergens (Figure 1D). A majority ($n=418$, 51.3%) exhibited poly-sensitization with specific IgE toward ≥3 major allergenic molecules, while 135 (16.6%) patients showed a mono-molecular and 163 (20.0%) individuals an oligo-molecular (IgE toward two major molecules) response, respectively (Table 4). Regarding the presence of IgE toward the three tested groups of panallergens (profilins, nsLTP, and polcalcins), 90 patients (11.0%) were positive for molecules of one group, and 45 (5.5%) for allergens of two or all three groups. The highest prevalence of mono-molecular responses was observed in Messina, while oligo-sensitization was most frequent in Porto and patients from Rome were most frequently poly-sensitized (Table 4; Figure 2D). Interestingly, 62/815 (7.6%) participants exhibited no IgE in the ESEP test. These patients were observed mostly in Istanbul ($n=22$). And were characterized by low average number (2.0/patient, SD: 1.7) and diameter (4.2 mm, SD: 1.3) of positive SPT to seasonal allergens.

Patients with IgE to cross-reactive carbohydrate determinants (CCD) ($n=154$, 18.9%) had on average 6.8 (SD 3.7) positive SPT results, while those without IgE to CCD ($n=661$) had 4.8 (SD 3.1) ($p<.001$). Patients with a response to CCDs had also more positive IgE results toward allergen extracts (CCD+ 7.4 (SD 3.4)/CCD− 2.7 (SD 2.1), $p<.001$), panallergens (CCD+ 0.6 (SD 0.9)/CCD− 0.2 (SD 0.5) $p<.001$), and major allergenic molecules, (4.1 (SD 2.1/2.5) (SD 1.8) $p<.01$) than those with no IgE to CCDs.

For more information on the heterogeneity of individual IgE sensitization profiles as well as etiological diagnoses, and the numbers on positive test results related to a subsequent doctor's diagnosis please see the *online repository*.

3.4 | Severity, timing, and progression of SAR symptoms

While the majority (562/815, 69%) of patients in the total study population reported having moderate to severe symptoms of allergic rhinitis, this trend became particularly apparent in Rome (89.9%), Tirana (80.6%), and Athens (80.4%). Interestingly, these three study centers were those with the highest prevalence of positive SPT results (Figure 2C), positive IgE responses (Figure 2D) and doctor's diagnoses of pollen allergy to an individual source (Figure 2B).

The annual distribution of retrospectively assessed symptoms among all 815 patients reveals parallel trends for all centers (Figure 3A) with a peak in the months March–May and a second, lower increase in September. While the first peak matches the flowering periods of the most clinically relevant allergen sources (grass and olive), the second increase can be associated with late seasonal (e.g., pellitory, Alternaria) or even perennial allergen sources.[9] In centers with relevant sensitization to cypress (Marseille) an earlier increase of symptom prevalence was observed, matching again the respective flowering period. (Figure 3A).

When analyzing the duration of allergy symptoms in nine study centers, clear differences become apparent. While in some centers (e.g., Valencia, Marseille), most patients suffer from SAR symptoms for 2–3 months in the year, the majority of participants in other centers (e.g., Messina, Tirana) indicated being symptomatic during at least 4–5 months. Interestingly, 33/815 patients (4%) suffer from allergy symptoms throughout the entire year, most likely due to a sensitization toward perennial allergen sources (Figure 3B).

For an overview of results by study center, please see the *online repository*.

4 | DISCUSSION

In the @IT.2020 multicenter study on SAR, conducted in 815 patients (42.7% children) in study centers of nine Southern European/Mediterranean cities, we observed that: (i) SPT and IgE sensitization profiles vary greatly among clinical centers; (ii) clinical phenotypes, in terms of comorbidities and severity/frequency of allergy symptoms also vary between centers; (iii) despite the observed heterogeneity, a customized IgE test containing the most relevant seasonal airborne allergen extracts and molecules can detect sensitization in over 90% of the patients with SAR in the Mediterranean area. Altogether, our observations suggest that SAR may be a more heterogeneous, complex, and severe disease in Southern European/Mediterranean countries compared to Norther or Central Europe, thus requiring tailored diagnostics and clinical guidelines.

TABLE 3 Frequency of IgE antibodies toward allergen extracts and major allergenic molecules, panallergens and CCD.

		Total ($n=815$)		POR ($n=102$)		VAL ($n=71$)		MAR ($n=80$)		ROM ($n=99$)		MES ($n=82$)		TIR ($n=93$)		ATH ($n=97$)		IST ($n=96$)		IZM ($n=95$)		Mean SMD
IgE to extracts and molecules																						
	Total	5170		679		377		448		1068		349		613		683		363		590		
	Per patient mean (SD)	6.3	4.7	6.7	4.1	5.3	3.7	5.6	4.0	10.8	5.1	4.3	4.3	6.6	4.5	7.0	4.2	3.8	3.2	6.2	4.5	0.52
	Per patient median (IQR)	5	(3–9)	6	(4–8)	5	(2–7)	5	(3–8)	11	(6–15)	3	(2–5)	6	(4–8)	7	(4–10)	4	(1–6)	6	(3–9)	
Extracts	**Molecules**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**s**
Timothy grass		483	59.3	81	79.4	32	45.1	35	43.8	90	90.9	17	20.7	68	73.1	56	57.7	45	46.9	59	62.1	0.59
	Phl p1	462	56.7	85	83.3	25	35.2	27	33.8	82	82.8	18	22.0	68	73.1	48	49.5	49	51.0	60	63.2	0.61
	Phl p5	271	33.3	50	49.0	18	25.4	23	28.8	54	54.5	6	7.3	43	46.2	33	34.0	21	21.9	23	24.2	0.41
Bermuda grass		480	58.9	82	80.4	30	42.3	34	42.5	81	81.8	22	26.8	67	72.0	58	59.8	45	46.9	61	64.2	0.50
	Cyn d1	440	54.0	76	74.5	29	40.8	26	32.5	77	77.8	18	22.0	63	67.7	52	53.6	40	41.7	59	62.1	0.52
Olive		374	45.9	35	34.3	56	78.9	36	45.0	55	55.6	43	52.4	25	26.9	69	71.1	10	10.4	45	47.4	0.58
	Ole e1	209	25.6	15	14.7	40	56.3	24	30.0	20	20.2	28	34.1	7	7.5	49	50.5	4	4.2	22	23.2	0.53
Pellitory		216	26.5	17	16.7	13	18.3	14	17.5	47	47.5	50	61.0	6	6.5	43	44.3	10	10.4	16	16.8	0.52
	Par j2	185	22.7	11	10.8	10	14.1	12	15.0	41	41.4	47	57.3	4	4.3	40	41.2	10	10.4	10	10.5	0.52
Cypress		192	23.6	8	7.8	9	12.7	50	62.5	62	62.6	8	9.8	12	12.9	20	20.6	13	13.5	10	10.5	0.55
	Cup a1	264	32.4	7	6.9	20	28.2	55	68.8	76	76.8	13	15.9	16	17.2	33	34.0	23	24.0	21	22.1	0.65
Ragweed		191	23.4	24	23.5	7	9.9	15	18.8	41	41.4	13	15.9	24	25.8	19	19.6	24	25.0	24	25.3	0.24
	Amb a1	28	3.4	2	2.0	-	-	3	3.8	2	2.0	1	1.2	2	2.2	2	2.1	12	12.5	4	4.2	0.18
Salsola Kali		177	21.7	18	17.6	14	19.7	7	8.8	32	32.3	13	15.9	29	31.2	26	26.8	12	12.5	26	27.4	0.26
	Sal k1	114	14.0	11	10.8	9	12.7	5	6.3	20	20.2	9	11.0	10	10.8	14	14.4	11	11.5	25	26.3	0.19
Plane tree		167	20.5	20	19.6	12	16.9	12	15.0	43	43.4	6	7.3	33	35.5	15	15.5	8	8.3	18	18.9	0.33
	Pla a1	11	1.3	2	2.0	1	1.4	3	3.8	4	4.0	-	-	-	-	-	-	1	1.0	-	-	0.15
	Pla a2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00
English plantain		168	20.6	36	35.3	6	8.5	5	6.3	40	40.4	7	8.5	20	21.5	21	21.6	5	5.2	28	29.5	0.41
	Pla l1	44	5.4	20	19.6	1	1.4	2	2.5	6	6.1	-	-	4	4.3	3	3.1	1	1.0	7	7.4	0.26
Birch		131	16.1	17	16.7	7	9.9	10	12.5	39	39.4	8	9.8	24	25.8	9	9.3	4	4.2	13	13.7	0.31
	Bet v1	43	5.3	6	5.9	-	-	5	6.3	17	17.2	1	1.2	14	15.1	-	-	-	-	-	-	0.32
Oak		108	13.3	14	13.7	4	5.6	6	7.5	30	30.3	6	7.3	21	22.6	9	9.3	3	3.1	15	15.8	0.30

(Continues)

TABLE 3 (Continued)

Extracts	Molecules	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	s
	Que a1	28	3.4	3	2.9	-	-	4	5.0	9	9.1	1	1.2	10	10.8	-	-	-	-	1	1.1	0.25
Hazel	Cor a1	34	4.2	-	-	-	-	4	5.0	21	21.2	-	-	9	9.7	-	-	-	-	-	-	0.28
Alternaria		117	14.4	13	12.7	12	16.9	7	8.8	25	25.3	5	6.1	9	9.7	24	24.7	4	4.2	18	18.9	0.28
	Alt a1	102	12.5	10	9.8	11	15.5	6	7.5	23	23.2	4	4.9	7	7.5	25	25.8	2	2.1	14	14.7	0.30
Mugwort		93	11.4	13	12.7	6	8.5	10	12.5	24	24.2	4	4.9	12	12.9	11	11.3	3	3.1	10	10.5	0.21
	Art v1	38	4.7	3	2.9	5	7.0	8	10.0	7	7.1	1	1.2	6	6.5	4	4.1	3	3.1	1	1.1	0.17
Profilin	Bet v2	66	8.1	10	9.8	1	1.4	2	2.5	18	18.2	-	-	13	14.0	8	8.2	5	5.2	9	9.5	0.29
	Phl p12	22	2.7	3	2.9	-	-	-	-	13	13.1	-	-	3	3.2	1	1.0	-	-	2	2.1	0.22
Polcalcin	Bet v4	18	2.2	3	2.9	2	2.8	1	1.3	5	5.1	1	1.2	2	2.2	-	-	2	2.1	2	2.1	0.11
	Phl p7	20	2.5	3	2.9	2	2.8	1	1.3	5	5.1	1	1.2	5	5.4	-	-	1	1.0	2	2.1	0.14
nsLTP	Ole e7	17	2.1	-	-	2	2.8	-	-	4	4.0	2	2.4	1	1.1	6	6.2	-	-	2	2.1	0.17
	Art v3	48	5.9	3	2.9	6	8.5	2	2.5	14	14.1	7	8.5	7	7.5	6	6.2	1	1.0	2	2.1	0.21
CCD		154	18.9	20	19.6	5	7.0	11	13.8	31	31.3	6	7.3	30	32.3	17	17.5	12	12.5	22	23.2	0.30
		2618		343		187		224		549		164		324		341		198		288		
		3.2		3.4		2.6		2.8		5.5		2.0		3.5		3.5		2.1		3.0		

TABLE 4 Frequency of mono-, oligo-, and poly-sensitization, IgE to panallergens, and seasonal versus perennial allergens.

	Total ($n=815$)		POR ($n=102$)		VAL ($n=71$)		MAR ($n=80$)		ROM ($n=99$)		MES ($n=82$)		TIR ($n=93$)		ATH ($n=97$)		IST ($n=96$)		IZM ($n=95$)	
Mono-sensitized[a] [*n* (%)]	135	16.6	7	6.9	16	22.5	20	25.0	6	6.1	**28**	**34.1**	14	15.4	16	16.5	18	18.8	10	10.5
Oligo-sensitized[b] [*n* (%)]	163	20.0	28	27.5	18	25.4	15	18.8	10	10.1	19	23.2	19	20.9	18	18.6	18	18.8	18	18.9
Poly-sensitized[c] [*n* (%)]	**418**	**51.3**	**59**	**57.8**	**30**	**42.3**	**36**	**45.0**	**82**	**82.8**	18	22.0	**52**	**57.1**	**57**	**58.8**	**35**	**36.5**	**49**	**51.6**
Sensitized to one group of panallergens[d]	90	11.0	10	9.8	7	9.9	4	5.0	12	12.1	9	11.0	13	14.0	15	15.5	7	7.3	13	13.7
Sensitized to two or three groups of panallergens	45	5.5	6	5.9	3	4.2	1	1.3	20	20.2	1	1.2	7	7.5	3	3.1	1	1.0	3	3.2
IgE to seasonal aeroallergens exclusively [*n* (%)]	267	32.8	20	19.6	20	28.2	26	32.5	13	13.1	21	25.6	33	35.5	36	37.1	44	45.8	54	56.8
IgE to seasonal AND perennial allergens [*n* (%)]	537	65.9	80	78.4	51	71.8	52	65.0	86	86.9	60	73.2	60	64.5	61	62.9	46	47.9	41	43.2

Note: The most frequent results per center are marked in bold.

[a]IgE to one major molecule.

[b]IgE to two major molecules.

[c]IgE to three or more major molecules.

[d]Analyzed groups: nsLTP, profilins, polcacins.

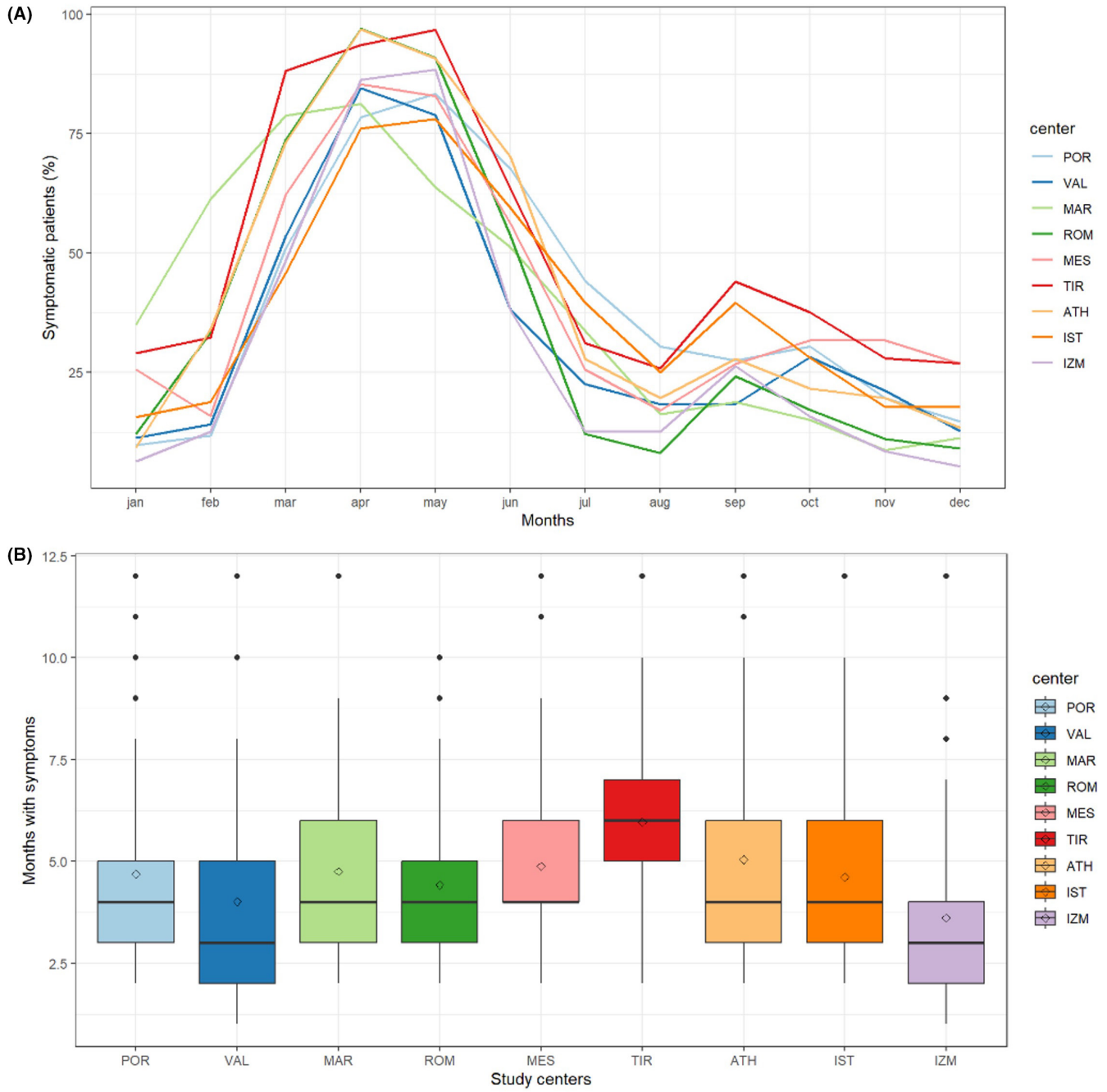


FIGURE 3 (A) Annual distribution and (B) length of allergic symptoms among 815 patients with SAR attending allergy centres of nine cities in seven Southern European and Mediterranean countries.

4.1 | Heterogeneity in pollen sensitization profiles

The spectrum of diversity in IgE profiles among patients of our study population ranged from a clear predominance of single allergen sources (e.g., cypress pollen in Marseille, grass pollen in Porto or pellitory in Messina) to a high degree of poly-sensitization in other study centers, like Rome, Athens or Tirana.

Overall, the most important seasonal airborne allergen source was **grass** pollen, an observation matching the results of previous studies in different regions of Southern Europe.[10–14]

Another frequent elicitor of SAR symptoms in the Mediterranean basin is **olive** pollen.[15] In our study, olive pollen ranged among the top three sensitizers being the second most important allergen source after grasses at population level and the main sensitizer in Valencia and Athens (Table 2). The high prevalence of olive pollen sensitization in Valencia matches a recent observation among children and adolescents from Murcia (distance to Valencia: approx. 200 km) reporting olive pollen to be the most frequent sensitizer in their cohort.[16] Interestingly, we observed a remarkably low frequency of IgE and/or positive SPT toward olive pollen in Istanbul. This reflects

recent findings of a study group analyzing relevant seasonal airborne allergen sources in the greater Istanbul area[17] and may be attributed to low exposure levels,[9] as olive trees are not frequently found in this urban region and surrounding.

Overall, our findings underline the increasing importance of **cypress** allergy in the Mediterranean region, where we observed three general patterns: (i) mono-sensitization to cypress (frequent in Marseille and, although less, in Istanbul); (ii) cypress as part of (broad) poly-sensitization (Rome, Tirana, Athens, Valencia, Ismir); and (iii) almost no relevant cypress pollen sensitization (Porto, Messina). In a study from 2012, including 6815 allergic patients being referred to an allergy clinic in Montpellier, the prevalence of cypress allergy was 20.7%, quite low compared to 68.8% observed in our study center in Marseille.[18] Besides differences in the study protocol (i.e., longer recruitment period in Montpellier than in the @IT.2020 study), possible explanations for the high prevalence of cypress pollen allergy have been sought in an increasing exposure, since cypress trees have been frequently planted as ornamental trees, but also in pollution levels as co-factors promoting allergenicity of cypress pollen.[19] Among 1278 patients with respiratory allergy in Barcelona, 15% had a positive SPT to cypress, 13% IgE to extracts, and 11% IgE to allergenic molecules of the participants.[20] This fits our observations in Valencia, although the study center is located more to the South along the Mediterranean coast. Similar prevalences for cypress pollen allergy were observed in Tirana, Istanbul, and Izmir. In Izmir, sensitization to cypress was higher than that observed in a local study completed in 2008, when mono-sensitization to cypress, confirmed by nasal allergen provocation test (NAPT), was rare.[21]

As previously described in regional studies, the allergenic relevance of ***Fagales*** allergens was also low in our study population where the highest prevalence of IgE to Bet v 1 was observed among poly-sensitized patients in Rome and no IgE to this major molecule could be detected in Valencia, Athens, Istanbul, and Izmir. In Italy a north–south gradient in the prevalence of specific IgE toward birch pollen allergens among adults was recently reported,[22] characterized by a decrease of sensitizations toward the major allergen Bet v 1 and a parallel increase of responses toward the panallergens Bet v 2 and Bet v 4. We also found a relative low prevalence of IgE to Bet v 1 in Rome and only 1 patient with IgE to Bet v 1 in Messina (Southern Italy) (Table 3). Moreover, birch pollen sensitized patients in Rome produced IgE to profilins and/or polcalcins in 18.2% of cases.

The prevalence of IgE and/or positive SPT results against **plane tree** allergens varied between centers as previously described in the literature.[23,24] Although contributing to the diversity of sensitization profiles, plane tree has no outstanding role regarding mono-sensitization and its clinical relevance is judged heterogeneously by study doctors in the different centers (Figure 2A–D).

Sensitization to **pellitory** was mostly observed in poly-sensitized patients in all study centers, with the outstanding exception of Messina, where pellitory stands out as the most frequently sensitizing pollen. Sensitization to other weeds (mugwort, *Salsola kali*, and ragweed) was highly **heterogeneous** among centers and mostly associated with poly-sensitization. Interestingly, the highest frequency of IgE toward the major ragweed allergen Amb a 1 was observed in Istanbul, where patients also recorded a distinct symptom peak during the ragweed pollen season (mid-August to end-September).

As expected, the differences in clinical phenotypes of pollen allergy among nine study centers reflected the heterogeneity of sensitization profiles. First, a higher degree of disease severity was generally related to higher frequencies of poly-sensitization with the largest proportion of patients suffering from moderate to severe, persistent allergic rhinitis in Rome, Tirana and Athens. An exception to this rule was observed in Marseille, where many patients also reported moderate to severe symptoms although mono-sensitized to cypress pollen. This may indicate a more severe clinical phenotype of cypress pollen allergy, also suggested by a younger age at disease onset (average 10 years) than observed in a previous local study.[25] Second, the frequency of comorbidities was also highly heterogeneous among study centers. The highest number of comorbidities per patient was observed in Rome, which was also the center with the highest degree of poly-sensitization. Surprisingly, centers with a high number of patients being parallelly sensitized to seasonal and perennial allergen sources (Rome, Porto), did not report a higher prevalence of allergic asthma. The clinical features of pollen-food allergy syndrome in the present study population have been reported elsewhere.[26] As expected, clinical heterogeneity was also observed in the timing and duration of symptoms reflecting the exposure times of the most relevant allergen sources for each center.

4.2 | Explanations for the heterogeneity of SAR among the study centers

Our study is, to our knowledge, the first comparing patients from different geographical regions of Southern European/Mediterranean countries with a standardized diagnostic approach, thus creating an overview of current sensitization profiles and their frequency in this area of the world. This heterogeneity should be explained and interpreted. First, an important role probably needs to be attributed to the strong differences in allergen exposure among our study centers.[9] In particular, cypress pollen is pre-dominant in Marseille and pellitory pollen reach high airborne concentrations over many months in Messina,[9,19] thus explaining the high prevalence, with a strong mono-sensitization fraction, of allergy to cypress and to pellitory in those two cities, respectively. Interestingly, 65.9% of the study population showed a co-sensitization to seasonal and perennial allergensources, most likely reflecting high levels of exposure to pets and HDM, which has been described previously in regions with subtropical climate.[27] However, differences in pollen exposure may not be the only explanation of differences in sensitization profiles among our study centers. A major role may be also played by the atopic propensity (poly-sensitization) of different populations, which seemed to be much higher in some centers (Rome, Tirana, Athens) than others (Istanbul, Marseille, Porto, Messina). Over 20 years ago,

the ISAAC study[1] had already shown major differences in the prevalence of allergic rhino-conjunctivitis in the Mediterranean countries, with the highest prevalences observed in France (12%–15%) and lowest in Albania (<5%). Our results partially reflect this hierarchy, suggesting that beyond allergen exposure, also different exposure to risk factors linked to westernization (hygiene, nutrition, pollution, and others) may determine different propensity of inhabitants to sensitization to multiple pollen, including those with a weaker allergenic power.[28] A third reason of heterogeneity can be linked to differences in the clinical settings of our study centers. Although all study centers were in hospitals, we cannot exclude that our results are influenced by a certain level of selection bias. The study population of each center is not representative of the general population of patients with SAR in the respective city, and this important limitation may have artificially generated part of the observed differences.

4.3 | Multiplex IgE testing for SAR in Southern Europe

A natural consequence of the above-described heterogeneity, in combination with previously observed trends toward polysensitization in Mediterranean countries, is the need for cost- and time-efficient multiplex IgE testing for pollen allergic patients. Although several commercially available test systems include a broad variety of relevant allergenic molecules of seasonal airborne sources, the interpretation of results and their clinical relevance is often difficult for clinicians. It is therefore essential to support the selection and interpretation of adequate IgE tests. When compared with micro- or macroarrays or, a customized test panel like the previously validated[29] ESEP test may provide an effective alternative, as it includes only the reagents needed to explain SAR and related pollen food allergy syndrome and excludes those relevant for other diseases. For more information on its performance in the present study, please see the *online repository*.

4.4 | Strengths and limitations

An important strength of our study is the standardized methodological approach in geographically and culturally diverse regions of the Mediterranean area. However, some limitations need to be considered. Firstly, as already discussed, the study population is not representative for the general populations in the respective countries. However, they depict a cross-section of patients suffering from SAR who attend specialized allergy centers. Second, the panel of examined allergens contained not only recombinant but also six native molecules which may lead to test positivity caused by IgE to CCD. Further, the test was broad but did not cover all potentially relevant seasonal airborne allergens. Therefore, sensitizations to less frequent allergens may be underestimated. On the other hand, the selection of allergens enabled the evaluation of a feasible diagnostic approach for different geographic regions without excessive effort for the participating clinicians and patients. Third, the presented results only cover a cross-sectional picture of pollen allergy and conclusions on a potential evolution over time can only be drawn with reference to previous studies.

5 | CONCLUSIONS

In conclusion, our multicenter study shows that pollen allergy is heterogeneous in terms of sensitization profiles but also clinical manifestations in different geographic regions of the same climatic zone. While a customized in-vitro test was able to detect the relevant sensitization for most of the included patients, it is important to acknowledge the high degree of diversity, particularly when developing guidelines or study protocols for pollen allergy in the Southern European/Mediterranean region.

AUTHOR CONTRIBUTIONS

Matricardi PM and Dramburg S wrote the study protocol, Dramburg S wrote the first manuscript draft, Uguz U performed the statistical analysis and reviewed the manuscript, all other co-authors contributed to data collection, data management and carefully reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

Salvatore Tripodi and Simone Pelosi are co-founder of TPS Software Production. Salvatore Tripodi reports funds from TPS Production.Simone Pelosi reports personal fees from TPS Software Production. Stefania Arasi reports honoraria from Ulrich, Abbott, DBV, funds from Stallergenes Greer, participation on boards of Novartis, Aimmune and WAO. Paraskevi Xepapadaki reports consulting fees from Novartis and honoraria from Galenica, Glaxo Smith Kline, Menarini, Novartis, Uriach, Nestle, Nutricia. Lucia Caminiti reports participation on board of Aimmune. Cansin Sackesen reports honoraria from UCB. Ozlem Goksel reports honoraria from UCB. Psarros Fotios reports honoraria from Takeda, Abbvie, CSL Behring, GSK, Astra Zeneca, participation board of Takeda. Ulas Uguz reports honoraria from UCB. Ana Margarida Pereira reports funds from Roxall group. João Fonseca reports funding and participation on board of Alerimune, Lda Porto. Laurie Pahus reports honoraria from Glaxo Smith Kline, Astra Zeneca, funds and participation on boards of from Glaxo Smith Kline, Astra Zeneca, Chiesi. Luis Delgado reports funds and honoraria from Thermo Fisher Diagnostics, Leti Pharma, Alerimune, Lda Porto, participation on boards of Laboratorios Vitoria SA, Alerimune, Lda Porto. Mariana Couto reports grants from Roche, funds from EAACI.

All other authors report no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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

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