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ORIGINAL PAPER

nCup a 1 as a marker of allergy to cypress pollen

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Abstract The increase in polysensitisations among allergic patients has led us to search for suitable means of diagnosis for identifying true sensitisation, and distinguishing true sensitisation from cross-reactivity. Cross-reactive carbohydrate determinants (CCDs) present in glycoproteins from cypress pollen extracts have been linked with such cross-reactivity, particularly in in vitro assays. The application of component-resolved diagnosis using recombinant allergens makes it possible to identify true allergens. The problem arises when the allergen available for the usual diagnostic methods, which are used as a reference for the diagnosis of allergy to cypress pollen nCup a 1, is a native allergen. The aim of the study was to validate the native allergen nCup a as a marker of true sensitisation to cypress pollen. The sera of 96 subjects with a proven allergy to cypress pollen were analysed. We then quantified IgE specific to *Cupressus arizonica* and to nCup a 1 and also analysed the CCDs in subjects sensitised to several tree pollen allergens, presenting with MUXF3-specific IgE. Results revealed that there is a statistically significant correlation between conventional diagnostic techniques used to determine allergy to cypress pollen (SPT and IgE *Cupressus arizonica*) and sensitisation to nCup a 1. CCD quantification in subjects sensitised to several tree pollen antigens showed that these did not interfere with our results. We validated the native *Cupressus arizonica* allergen, nCup a 1, as a marker of allergy to cypress pollen in our population.

Keywords Cypress pollen allergy · *Cupressus arizonica* · Pectate lyase · Component-resolved diagnosis · Cup a 1

Abbreviations

CCDs	Cross-reactive carbohydrate determinants
CRD	Component-resolved diagnosis
SPT	Skin prick test

1 Background

Although allergy to grass pollen is the most common seasonal respiratory allergy, sensitisation to cypress pollen has dramatically increased in recent years and is currently the primary cause of winter pollinosis, particularly in North America, Mexico, Japan and Middle-Eastern and Mediterranean countries. The

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prevalence of allergy to cypress pollen in a population will vary according to the area. In a recent study, Sposato et al. (2014) found that 62.9, 16.1 and 32.7% of patients living in central, northern and southern Italy, respectively, were sensitised to cypress. Repeated cross-sectional studies conducted over different time intervals have shown an increase in the percentage of allergies to cypress pollen in the Mediterranean area. This increase has been associated with genetic factors, and greater exposure to cypress pollen due to the use of cypress plants for ornamental purposes or as a windbreak (Charpin et al. 2017). There are consistent correlations between exposure to Cupressaceae pollen and the presence of sensitisation and allergy (Yoshida et al. 2013). In Japan, the prevalence of allergy to Japanese cedar (*Cryptomeria japonica*) is 13.1% (Okuda 2003).

Cupressaceae are gymnosperms that are widespread around the world, both in the northern and southern hemispheres and on all the continents except Antarctica, and are the most important family of gymnosperms in terms of allergology. The genera that primarily contribute to pollinosis are those from the Cupressoideae (*Cupressus, Juniperus* and *Thuja*) and Taxodioideae (*Cryptomeria* and *Taxodium*) sub-families (Asam et al. 2015).

Cupressaceae are pollinated via the wind, whereby they release large amounts of pollen, though the success of pollination can vary greatly depending on the meteorological conditions. Pollination can begin in October and stretch until as long as May, according to the species and region (Perez-Badia et al. 2011; Boi and Llorens 2013; Docampo et al. 2007; Hidalgo et al. 2003). Furthermore, being fairly small in size, Cupressaceae pollen can be transported long distances by the wind (Mohanty et al. 2017).

The predominant clinical manifestation of cypress pollinosis is rhinitis, commonly combined with conjunctivitis described as very intense. The prevalence of asthma is lower but varies according to the series (Sposato et al. 2014; Charpin et al. 2017).

To date, four groups of Cupressaceae allergens have been described, with groups 1 (pectate lyase) and 2 (polygalacturonase) being indexed as major allergens, and groups 3 (thaumatin-like proteins) and 4 (calcium-binding proteins) as minor allergens. There are descriptions of other allergens, though nowadays these are not as relevant. These four groups of Cupressaceae allergens are largely homologous, with descriptions of a large degree of cross-reactivity between them (Matricardi 2016; Charpin 2017).

The different types of Cupressaceae pectate lyase contain the number 1 as part of their WHO/IUIS nomenclature (Cry j 1, Cup s 1, Cup a 1, Jun a 1, etc.) and are thought to be the most predominant allergens, causing sensitisation in almost 100% of patients who are allergic to cypress pollen (Alisi et al. 2001; Aceituno et al. 2000; Arilla et al. 2004). They have molecular masses of around 43 kDa, with various neutral isoforms glycated to acids (Aceituno et al. 2000). Pectate lyase is an enzyme that breaks the polysaccharide chains of D-galacturonic acid. Within a grain of pollen, it is involved in tissue modelling and the growth of pollen tubes. Group-1 allergens have a large degree of cross-reactivity among Cupressaceae species, given that they share 75–97% of their sequence identity. Cup s 1, Cup a 1 and Jun a 1 are the most similar, while Cry j 1 is the most dissimilar (Charpin et al. 2017).

Pectate lyase has also been found in other pollens, such as those of *Ambrosia* (Amb a 1) and *Artemisia* (Art v 6), though there is no cross-reactivity between these pollens (Pichler et al. 2015).

Allergy diagnosis via traditional methods, such as a skin prick test (SPT) and/or the quantification of IgE specific to the whole extract, provides very limited information regarding the true nature of allergic problems and their clinical, therapeutic and prognostic implications. Crude biological extracts contain a varied, very heterogeneous mixture of proteins, glycoproteins and polysaccharides, obtained from an allergenic source. Some of these have allergenic potential, while others do not, making standardisation difficult. Component-resolved diagnosis (CRD) with natural or recombinant allergens represents a huge leap forward in terms of qualitative data collection, and diagnostic precision is significantly improved by the use of CRD in conjunction with a review of patients' clinical histories and other in vivo and in vitro diagnostic methods (Valenta et al. 2007; Canonica et al. 2013), thus allowing us to make considerable advances in the diagnosis and treatment of allergic patients. CRD can also improve the indication and selection of allergens suitable for a specific immunotherapy, since identifying the allergen causing the illness is necessary before a particular immunotherapy may be prescribed (Tripodi et al. 2012).

At the close of the twentieth century, some authors began to report that cross-reactive carbohydrate determinants (CCDs)-specific IgE interferes with reactivity to *C. arizonica* pollen components. They also supported the idea that the epitopes of CCDs, as well as panallergens such as profilin and calcium-binding proteins, could influence in vitro and in vivo diagnosis and therefore, therapeutic strategies (Afferni et al. 1999). The same authors report that these CCDs can cause false positive results for nCup a 1 (being a purified or native natural allergen), given that they have the capacity to bind, in vitro, to IgE in the *C. arizonica* pollen extract (Iacovacci et al. 2002). *Cupressus sempervirens* polygalacturonase (Cup s 2) has recently been identified as the major CCD-carrying allergen in this pollen, to the extent that Cup s 2 might be associated with the highest prevalence of reactivity to cypress pollen extract-specific IgE due to the interference of CCDs (Shahali et al. 2017).

In Europe, Cup a 1 is in fact the reference allergen for diagnosis of allergy to cypress pollen (Scala et al. 2010; Matricardi 2016). To date, there is still no efficient procedure for cloning and expressing recombinant Cupressaceae allergens on diagnostic platforms using current components. As a result, the purified or native natural allergen, nCup a 1, is used, though this gives rise to a potential lack in specificity because of CCDs. When interpreting these results, other glycated molecules must be taken into account, such as nJug r 2, nPhl p 4 or the CCD MUXF3 (Barber et al. 2015). In light of this, the EAACI Molecular Allergology User's Guide (Matricardi 2016) proposes the quantification of bromelain (Ana c 2) or the purified N-glycan from bromelain, MUXF3, in the event of reactivity to IgE specific to multiple tree pollens, in order to rule out the possibility of reactivity to CCD.

The objective of this study was to validate the native allergen nCup a 1—the allergen available to us for CRD—as a marker of true sensitisation to cypress pollen.

2 Materials and methods

This is an analytical, observational and a cross-sectional study involving the prospective collection of primary variables.

2.1 Participant selection and SPTs

Ninety-six subjects of both sexes who had attended an allergology consultation at Burgos University Hospital, Spain, between January 2015 and March 2016, with a diagnosis of allergy to cypress pollen, were consecutively included in the study. Informed consent was obtained from all individual participants included in the study. The inclusion criteria were subjects presenting with symptoms of pollinosis (conjunctivitis and/or rhinitis and/or asthma) during Cupressaceae pollination periods, determined by pollen counts and a positive SPT for *Cupressus arizonica* and/or *Cupressus sempervirens* extract. We also incorporated in the study SPTs with *Juniperus oxycedrus* extract due to the abundance of trees from the *Juniperus* genus in our area. In addition, we administered SPTs with other pollens (from grass, trees and weeds) and other allergens like mite, molds and animal dander.

2.2 Pollen counts

The pollen counts in the atmosphere from 2001 to 2016 inclusive were analysed for Cupressaceae taxa, as well as for the pollens to which subjects were sensitised during SPTs. According to SPT with others pollens results (Table 2), these pollens were from the following families: Poaceae (grass pollen), *Plantago, Platanus* and Oleaceae (*Fraxinus* and *Olea*). We did this to ensure that the symptoms of pollinosis exhibited by subjects during the Cupressaceae pollination period were not triggered by another pollen to which they could be sensitised.

A Hirst volumetric trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England) was used. The sampling methodology was carried out in accordance with the recommendations of the Spanish Society of Allergology and Clinical Immunology (Subiza et al. 1995; Subiza and Jerez 1995), whose website provides publically published information in this regard.

2.3 Laboratory test

Titres of IgE specific to the whole extract of *Cupressus arizonica* (IgE *C. arizonica*) and IgE specific to nCup a 1 (IgE nCup a 1) were quantified using the ImmunoCAP Phadia 250 (Thermo Fisher Scientific, Uppsala, Sweden). IgE titres were considered to be positive if they measured $\geq$ 0.35 kU/l, and negative if they measured less.

We followed the recommendations of the EAACI Molecular Allergology User's Guide (Matricardi 2016) in the event of reactivity to IgE specific to multiple tree pollens, in order to rule out the possibility of reactivity to CCDs. MUXF3 was detected in 17 subjects in this position, namely those with positive SPTs for *Cupressus arizonica* and other pollens from Oleaceae and/or *Platanus* trees.

2.4 Statistical analysis

For the statistical analysis, data were processed using the statistics software IBM SPSS Statistics 19.0.

A descriptive analysis of the sample was carried out, providing means (standard deviation), medians (interquartile range) and frequency (percentage) according to the characteristics and distributions of the variables. We analyse SPT, IgE *C. arizonica* and IgE Cup a 1.

To determine the relationship between the three tests that we allow to decide if a patient is allergic to cypress pollen or not, the correlation between them was calculated. The concordance between them was also evaluated using the Cohen´s kappa coefficient, when is possible.

The following step involved using ROC (receiver operating characteristic) curves to determine the predictive power of the conventional diagnostic tests (SPTs and IgE to *C. arizonica*), taking molecular diagnosis (nCup a 1) as the gold standard. ROC curves allow us to determine the optimum cut-off point for the sensitivity/specificity binomial. The area under the curve (AUC) calculation allows us to determine the predictive power of each test to correctly diagnose allergic subjects, in this case to cypress pollen. The gold standard used to define true positive and negative subjects allergic to cypress pollen is the nCup a 1 test, where subjects with test titres of ≥ 0.35 kU/l were considered to be positive and those with test titres of < 0.35 kU/l were considered to be negative.

3 Results

3.1 Subject sample: baseline participant characteristics

Ninety-six predominant female (60%) subjects were included, aged between 6 and 74 years. The mean and standard deviation of the age of subjects was 32.6 ± 15.6 years. All subjects were suffering from rhinitis, while all except five were also suffering from conjunctivitis. Thirty-five subjects (36.5%) had symptoms of asthma.

3.2 Pollen counts

Figure 1 shows the major distribution of Cupressaceae pollen and the others pollens during its pollination between 2001 and 2016. The graph shows that the majority of Cupressaceae pollen is produced between January and mid-April. The major distribution of Poaceae (Gramineae) pollen during its pollination appears in our atmosphere primarily between in months of May and June, in some years extending into the first fortnight of July.

When we compare the pollination graphs for the Cupressaceae and Poaceae taxa, we see that their timings are different; Cupressaceae pollination is between January and April, whereas Poaceae pollination is between May and July.

Pollination of the *Plantago* and *Olea* taxa begins simultaneously with that of Poaceae, in May, which also coincides with the main period in May and June.

Pollination of the *Platanus* and *Fraxinus* taxa occurs at the start of spring, but with very low levels in the atmosphere in our area.

3.3 Skin prick tests

SPTs were carried out in 96 subjects with extracts of *Cupressus arizonica*, *Cupressus sempervirens* and *Juniperus oxycedrus*. The mean papule size was similar for all three (Table 1). According to SPT to others pollens, patients were sensitised to Poaceae (grass pollen), *Plantago, Platanus* and *Olea* (Table 2). We found 34 patients monosensitised to cypress pollen. There are two large groups related to the SPT results added to their sensitisation to cypress pollen: those that are sensitised to grass pollen ($n = 59$, 61.5%) and those that do not. A number of 23 patients (23.95%) are sensitised to three or more pollens (Table 3).

3.4 Quantification of IgE to *C. arizonica* and nCup a 1

Only 95 subjects were diagnosed via conventional methods due to one death. IgE to *C. arizonica* and

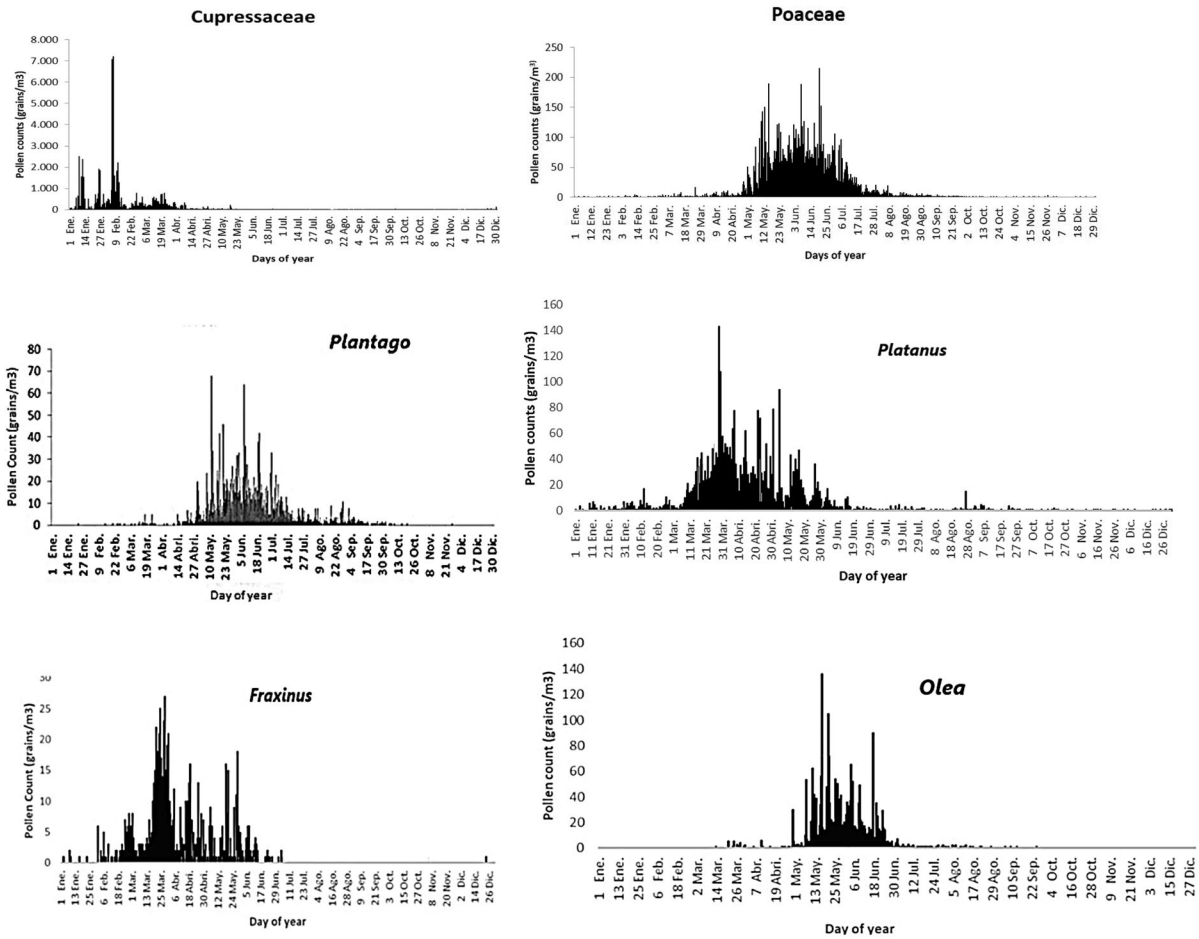

Fig. 1 Pollen counts. Major distribution of Cupressaceae, Poaceae, *Plantago*, *Platanus* and Oleaceae (*Fraxinus* and *Olea*) pollen during its pollination period. Daily airborne pollen concentration data cover a 16-year period of 2001–2016

Table 1 Description of SPTs for *Cupressus arizonica*, *Cupressus sempervirens* and *Juniperus oxycedrus* extract

	n	Mean (SD)	Median (Q1, Q3)	Min, max	Missing	Positive *n* (%)
SPT *C. arizonica*	96	6.8 (1.9)	7.0 (5.0, 8.0)	3, 12	0	96 (100.0%)
SPT *C. sempervirens*	96	7.7 (2.8)	7.0 (6.0, 9.0)	4, 20	0	96 (100.0%)
SPT *J. oxycedrus*	96	6.1 (1.8)	6.0 (5.0, 7.0)	4, 10	0	96 (100.0%)

Table 2 Description of SPTs for other pollens: Poaceae, *Plantago, Platanus* and *Olea*

	Total (n = 96)	
Poaceae	*n* (%)	59 (61.5%)
Olea	*n* (%)	15 (15.6%)
Plantago	*n* (%)	12 (13.1%)
Platanus	*n* (%)	6 (3.1%)

nCup a 1 was quantified. The results showed that 92 subjects (96.8%) had positive levels of IgE *C. arizonica* and three had negative levels. Only one subject tested negative for Cup a 1, the same subject who tested negative for IgE *C. arizonica* (Table 4).

Table 3 Description of SPTs (Total $n = 96$). Thirty-four patients were monosensibilised to cypress pollen. More than half of the patients ($n = 59$, 61.5%) were sensitised to grass pollen (Poaceae). Twenty-three patients (23.95%) were sensitised to three or more pollens

	n (%)
C Arizonica/Sempervirens	34 (35.41%)
C Arizonica/Sempervirens and *Olea*	1 (1.04%)
C Arizonica/Sempervirens and *Plantago*	1 (1.04%)
C Arizonica/Sempervirens, Plantago and Platanus	1 (1.04%)
C Arizonica/Sempervirens and Poaceae	39 (40.62%)
C Arizonica/Sempervirens, Poaceae and *Plantago*	6 (6.25%)
C Arizonica/Sempervirens, Poaceae and *Olea*	5 (5.20%)
C Arizonica/Sempervirens, Poaceae, *Olea* and *Plantago*	5 (5.20%)
C Arizonica/Sempervirens, Poaceae, *Olea* and *Platanus*	4 (4.16%)

Table 4 Description of IgE: IgE to *C. Arizonica* whole extract and IgE to nCup a 1

	n	Mean (SD)	Median (Q1, Q3)	Min, Max	Missing	Positive *n* (%)
IgE *C. Arizonica*	95	15,732 (22,421)	6860 (2700, 16,400)	0.07, 100.00	1	92 (96.8%)
IgE nCup a 1	95	24,536 (29,968)	12,300 (5100, 28,100)	0.01, 100.00	1	94 (98.9%)

3.5 Quantification of MUXF3-specific IgE

Among the 17 subjects in whom MUXF3 was detected, highly positive levels (17.20 kU/l) were detected in only one subject. However, this subject also had high levels of nCup a 1, measuring 45.2 kU/l, showing that this subject was clearly sensitised to cypress pollen.

3.6 Comparison of IgE quantification and SPTs

We compared IgE *C. arizonica* with nCup a 1 and SPTs for *C. arizonica* and *C. sempervirens* (Table 5).

In order to establish the relationship that exists between the three tests that would allow us to decide whether or not a subject is allergic to pollen, the correlation between them was calculated in the first instance (Table 6).

The correlation between IgE to *C. arizonica* and the SPT was statistically significant for both *C. arizonica* and *C. sempervirens*, although the correlation coefficients were relatively low, as shown in Table 6. The relationship between IgE to *C. arizonica* and nCup a 1 was not only statistically significant, but also correlated to a very large degree. Due to the limited number of subjects testing negative in both tests, the degree of agreement between both diagnoses, measured via the Cohen's kappa coefficient, amounted to a value of 0.492.

Dispersion diagrams of the various variable pairs (IgE *C. arizonica,* SPT to *C. arizonica* and *C. sempervirens* and nCup a 1), with logarithm transformation of the variables visually, show the degree of correlation of them (Fig. 2).

3.7 Results of ROC curves and AUC to SPTs and IgE to *C. arizonica* (Fig. 3, Table 7)

The estimated ROC curve for IgE *C. arizonica* gives an AUC of 1; in other words, its predictive power is perfect. The optimum cut-off point for 100% sensitivity and specificity would consider subjects with titres of ≥ 0.23 kU/l to be positive, since this perfectly differentiates nCup a 1-positive and nCup a 1-negative patients.

The estimated ROC curve for SPTs for *C. arizonica* gives an AUC of 0.9415 (CI 95%; 0.9056, 0.9774) and therefore has very good predictive power. The estimated optimum cut-off point is 4 mm; subjects with a SPT for *C. arizonica* giving rise to a papule

Table 5 Comparison of IgE quantification (*C. arizonica* with nCup a 1) and SPT for *C. arizonica* and *C. sempervirens*

		Positive IgE *C. arizonica*	Negative IgE *C. arizonica*
SPT *C. arizonica*			
Positive	*n* (%)	92 (100.0%)	3 (100.0%)
SPT *C. sempervirens*			
Positive	*n* (%)	92 (100.0%)	3 (100.0%)
nCup a 1			
Positive	*n* (%)	92 (100.0%)	2 (66.7%)
Negative	*n* (%)		1 (33.3%)
		Positive nCup a 1	Negative nCup a 1
SPT *C. arizonica*			
Positive	*n* (%)	94 (100.0%)	1 (100.0%)
SPT *C. sempervirens*			
Positive	*n* (%)	94 (100.0%)	1 (100.0%)
		Positive SPT *C. arizonica*	Negative SPT *C. arizonica*
SPT *C. sempervirens*			
Positive	*n* (%)	96 (100.0%)	0

more than 4 mm in diameter were considered positive. At the optimum cut-off point, sensitivity would be 89.4% and specificity perfect.

The estimated ROC curve for the SPT for *C. sempervirens* gives an AUC of 0.8457 (CI 95%; 0.7828, 0.9087) and therefore has a good predictive power. The estimated optimum cut-off point is 5 mm; subjects with a SPT for *C. sempervirens* giving rise to a papule more than 5 mm in diameter were considered to be positive. At the optimum cut-off point, sensitivity would be 77.7% and specificity perfect.

The three AUCs defined by the ROC curves are significantly different from a test with no predictive power (in case of AUC = 0.5). A comparison was also carried out of the AUCs of the three tests, two by two (Table 7). We found significant differences between the AUCs of the three tests, as follows:

The AUC for IgE *C. arizonica* is significantly higher than that of the SPT for *C. arizonica* ($p < 0.0001$).

The AUC for IgE *C. arizonica* is significantly higher than that of the SPT for *C. sempervirens* ($p = 0.0001$).

The AUC for the SPT for *C. arizonica* is significantly higher than that of the SPT for *C. sempervirens* ($p = 0.0017$).

4 Discussion

Improving diagnostic precision in patients allergic to pollen is a clinical challenge facing specialists, particularly in areas with several co-existing allergenic pollens and a large number of polysensitised patients, as is the case in Mediterranean countries. In such cases, it is important for the clinician to know whether a patient is co-sensitised to various allergen sources or whether sensitisation is caused by cross-reactive components.

Allergen extract-based diagnostic tests can reveal sensitisation to various allergens present in a pollen. In some cases, sensitisation is specific to the allergen source, whereas in others, it occurs due to cross-reactivity to other unrelated allergen sources. As a result, it can be difficult to identify the allergen responsible for the illness using this type of technique, particularly when patients are sensitised to more than one allergen, as is becoming increasingly common. CRD can improve diagnostic accuracy (specificity) and differentiate cross-reactivity from true co-sensitisation (Valenta et al. 2007).

In our study, we found that the native *C. arizonica* allergen, nCup a 1, is sufficient to diagnose allergy to

Table 6 Relationship between IgE *C. arizonica* with SPT (*C. arizonica* and *C. sempervirens*) and nCup a 1. There is a statistically significant correlation between IgE to *C. arizonica* and the SPT for both *C. arizonica* and *C. sempervirens*, and higher between IgE to *C. arizonica* and nCup a 1

	n	Correlation (CI 95%)	*p* value (agreement)	Kappa coefficient (CI 95%)
SPT *C. arizonica*** and IgE *C. arizonica* *	95	0.267 (0.069, 0.444)	0.0088	–
SPT *C. sempervirens** ** and IgE *C. arizonica* *	95	0.299 (0.104, 0.472)	0.0031	–
nCup a 1* and IgE *C. arizonica* *	95	0.977 (0.966, 0.985)	< 0.0001	0.492 (− 0.108, 1.000)
SPT *C. arizonica*** and nCup a 1*	95	0.300 (0.105, 0.473)	0.0029	–
SPT *C. sempervirens** ** and nCup a 1*	95	0.310 (0.115, 0.481)	0.0021	–
SPT *C. sempervirens** and SPT *C. arizonica***	96	0.650 (0.517, 0.753)	< 0.0001	–

*A log-transformed variable was used for standardisation purposes

**An estimation of the Cohen's kappa coefficient is not possible due to the absence of negative values in this variable

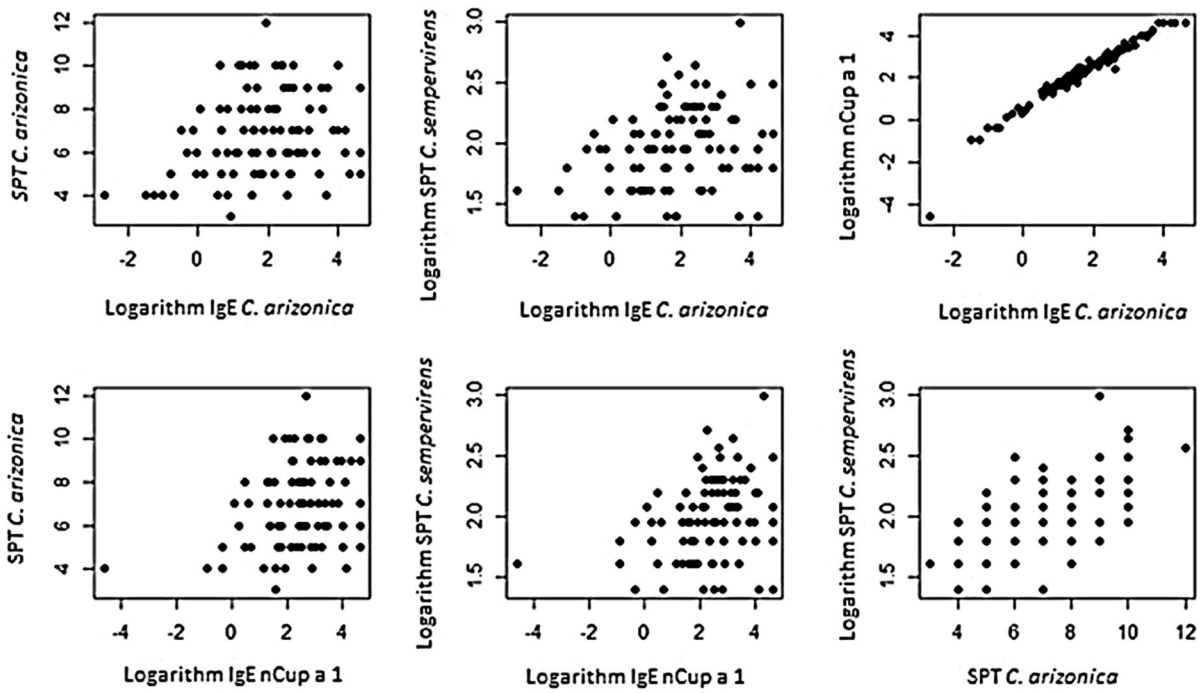

Fig. 2 Dispersion diagram of the various variable pairs (IgE *C. arizonica,* SPT to *C. arizonica* and *C. sempervirens* and nCup a 1) from Table 5, with logarithm transformation of the variables used for the test

cypress pollen, without any interference from other types of sensitisation.

4.1 Validation of the subject group

There is no doubt that the most important tool for evaluating immediate IgE-mediated hypersensitivity reactions is the patients' clinical history. Symptoms compatible with an allergic reaction, considered together with other factors, such as in cases of pollinosis in the environment in which the patient lives, guide us towards a correct diagnosis. SPTs can provide immediate information on sensitisation due to allergen-specific IgE (Bousquet et al. 2012). Specific IgE tests of the serum can test sensitisation to whole allergen extracts or to individual allergen components

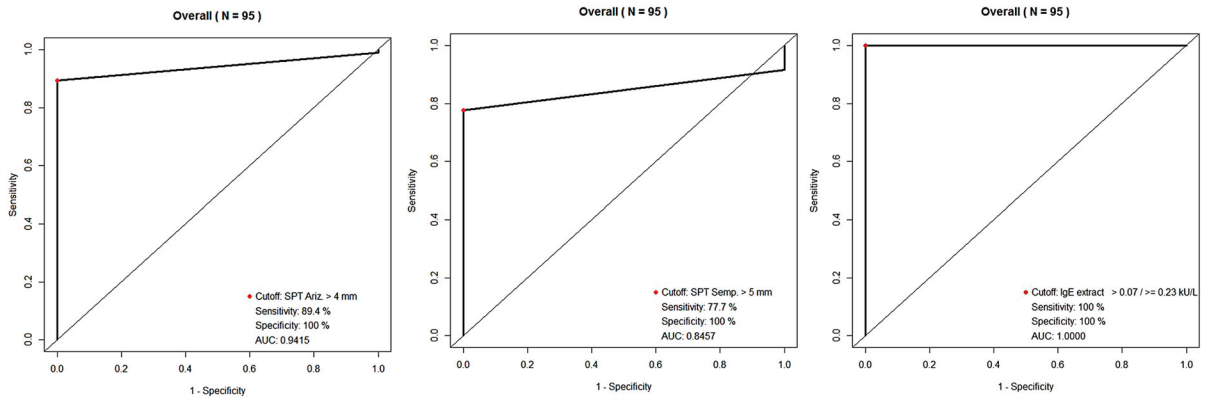

Fig. 3 ROC curve for SPT to *C. arizonica*, SPT to *C. sempervirens* and IgE to *C. arizonica*

Table 7 Comparison of the different AUCs, two by two

		Total with nCup a 1 value (n = 95)
Estimation of AUCs. Comparisons of AUCs, two by two		
IgE *C. arizonica*—test has no predictive power	Dif. AUC (CI 95%)	0.5000 (–, –)
	p value	–
SPT *C. arizonica*—test has no predictive power	Dif. AUC (CI 95%)	0.4415 (0.4056, 0.4774)
	p value	< 0.0001
SPT *C. sempervirens*—test has no predictive power	Dif. AUC (CI 95%)	0.3457 (0.2828, 0.4087)
	p value	< 0.0001
IgE *C. arizonica*—SPT *C. arizonica*	Dif. AUC (CI 95%)	0.0585 (0.0226, 0.0944)
	p value	0.0014
IgE *C. arizonica*—SPT *C. sempervirens*	Dif. AUC (CI 95%)	0.1543 (0.0913, 0.2172)
	p value	< 0.0001
SPT *C. arizonica*—SPC *C. sempervirens*	Dif. AUC (CI 95%)	0.0957 (0.0359, 0.1555)
	p value	0.0017

(Stiefel and Roberts 2012). The disadvantage of IgE sensitisation tests is that they determine the presence of specific IgE (sensitisation), but not the clinical presence of allergy. Nevertheless, when used by doctors trained to interpret them in the light of a complete clinical history, they are useful for supporting the clinical diagnosis of allergy and identifying the allergens responsible for the reaction (Roberts et al. 2016).

Our inclusion criteria for the study, in order to obtain a group of subjects allergic to cypress pollen, were subjects presenting with symptoms of pollinosis during the Cupressaceae pollination period, and a positive SPT for *C. arizonica* and/or *C. sempervirens*. As in other regions, the risk of suffering from polysensitisation could interfere with the group of subjects. As a result, we analysed the counts of pollens to which subjects were sensitised (determined via positive SPTs) from 2001 to 2016, inclusive. We concluded that sensitisation to other pollens did not prevent a suitable selection being made of our subjects.

4.2 Relationship between SPT for *C. arizonica,* IgE *C. arizonica* and nCup a 1

We evaluated the correlation between conventional techniques for diagnosing allergy to cypress pollen (SPTs and IgE *C. arizonica* whole extracts) and sensitisation to nCup a 1 (molecular component).

As well as being statistically significant, the relationship between IgE *C. arizonica* and nCup a 1 was found to be very strong (0.977). Due to the limited number of subjects with a negative result in both tests, the degree of agreement between both diagnoses, measured via the Cohen's kappa coefficient, amounted to 0.492. The correlation was also good between nCup a 1 and SPTs for both *C. arizonica* and *C. sempervirens*.

The correlation between IgE *C. arizonica* and the SPT for both *C. arizonica* and *C. sempervirens* is statistically significant, although the correlation coefficients are relatively low, as shown in Table 6. Nevertheless, the correlation between both SPTs is high. However, due to the absence of any negative values in the variable, it was not possible to estimate the Cohen's kappa coefficient.

According to our inclusion criteria, we began by including patients with a clinical history of pollinosis during the Cupressaceae pollination period and positive SPTs for *C. arizonica* and *C. sempervirens*, diagnosed with allergy to cypress pollen, by us, in our department. An analysis of SPTs, to both *C. arizonica* and *C. sempervirens*, and nCup a 1 found the correlation between them to be statistically significant. We can therefore confirm that nCup a 1 is a marker of sensitisation to cypress pollen in our population.

Two subjects with positive nCup 1 titres had negative titres of IgE C. arizonica. ImmunoCAP testing of the whole extract was probably not enough to generate a positive signal in these subjects, compared to the purified nCup a1.

We have come to the conclusion that, in our population, the selected subjects appear to be sensitised predominantly to Cup a 1 and not to other *C. arizonica* allergens, and a positive result for nCup a 1 is enough to establish a diagnosis of allergy to cypress pollen, without any current need to screen for other cypress pollen allergens. As Domínguez-Ortega et al. (2016) believe, according to our data, immunotherapy treatment with Cup a 1 alone could be effective to treat subjects in our population.

4.3 Interpretation of MUXF3 CCD titres

Among the subjects sensitised to other tree pollens, we found only one with positive MUXF3-specific IgE levels. This subject also had high levels of Cup a 1 (45.2 kU/l), which suggests that this subject was sensitised to cypress pollen. These data allow us to conclude that CCDs did not interfere with our results.

4.4 Interpretation of cases of polysensitisation

When comparing the results of SPTs with the atmospheric pollen counts, we found that a high percentage of our subjects are sensitised to grass pollen. However, grass pollination does not coincide with Cupressaceae pollination, and the SPT results were therefore attributed to co-sensitisation. The same situation applies to *Plantago*, although the number of sensitised patients was much lower; its pollination period overlaps with that of grass and therefore does not coincide with that of Cupressaceae. Cross-reactivity has been described between *Plantago*, *Olea* and grass pollens (Sousa et al. 2014) and the presence of Pla a 1 in the atmosphere with Oleacea (González Parrado et al. 2014).

Pollen counts of *Olea* showed us that its pollination period does not coincide with that of Cupressaceae and that it is present in the atmosphere in very small quantities. The pollen counts of other Oleaceae gena such as *Fraxinus,* which pollinates between winter and spring, are also low.

Very few subjects (a total of six) were sensitised to *Platanus*. Although there have been descriptions of the presence of Pla a 1 in the atmosphere, this appears to be independent of *Platanus* pollen counts during the same period. Furthermore, this aeroallergen does not coincide with the pollination of Cupressaceae but instead appears to coincide with the pollination of *Quercus*, *Betula* or the Salicaceae family (Fernández-González et al. 2010). Its low atmospheric levels, its pollination period in Burgos (short and separate from that of Cupressaceae) and the small number of subjects lead us to believe that cases of sensitisation to *Platanus* are not relevant to our study.

As a result, we believe that, due to the aerobiological characteristics of our area (i.e. the pollination periods and atmospheric pollen counts here), sensitisation to grass, Oleaceae, *Plantago* and *Platanus* pollens were caused by co-sensitisation or cross-reactivity and were therefore unconnected with allergy to cypress pollen.

5 Conclusion

We validated the native *C. arizonica* allergen, nCup a 1, as a marker of allergy to cypress pollen in our population; as a tool to be included in future diagnostic algorithms aimed at selecting suitable treatments, primarily immunotherapies (immunotherapy with Cup a 1 for allergies to cypress pollen), thus applying the principles of precision or personalised medicine to our allergic patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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