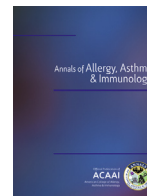




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Component-resolved diagnostics in vernal conjunctivitis

Alicia Armentia, MD, PhD^{*}; Beatriz Iglesias, Opt, PhD[†]; Darío Iglesias, MD, PhD[‡];
Javier A. Montero, MD, PhD[‡]; Eugenia Sanchís, MD, PhD^{*}; Blanca Martín, MD[§];
Delia Fernández, BD, PhD^{||}; Fé Munoz, MD, PhD[¶]; and Jesus Bermejo, MD, PhD[¶]

^{*} Allergy Unit, Rio Hortega University Hospital, Valladolid, Spain

[†] Ophthalmologist, Madrid, Spain

[‡] Ophthalmology Unit, Rio Hortega University Hospital, Valladolid, Spain

[§] Research Unit, Rio Hortega University Hospital, Valladolid, Spain

^{||} Department of Biodiversity and Environmental Management, University of Leon, Spain and Institute of Atmospheric Sciences and Climate, Bologna, Italy

[¶] Research Unit, Clinic University Hospital, Valladolid, Spain

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ABSTRACT

Background: Conventional diagnostic tests in allergy are insufficient to clarify the origin of vernal conjunctivitis (VC).

Objectives: To evaluate IgE-mediated hypersensitivity by component-resolved diagnosis (CRD) in tears and serum from patients with VC and to evaluate how to treat patients with identified triggering allergens by specific immunotherapy.

Methods: Patients were divided into 3 groups: (1) patients with VC (25 patients), (2) patients allergic to grass pollen with seasonal allergic conjunctivitis (AC) (50 patients), and (3) healthy blood donors (50 patients). If triggering allergens were detected, specific conventional immunotherapy was administered for 1 year.

Results: Twenty-five patients with VC were evaluated. The identified triggering allergens were n Lol p 1 (11 patients), n Cyn d 1 (8 patients), group 4 and 6 grasses (6 patients), and group 5 grasses (5 patients). Prick test and pollen IgE test results were positive in one patient. Clinical improvement was observed in 13 of the 25 patients with VC after 1 year of specific immunotherapy.

Conclusion: CRD seems to be a more sensitive diagnostic tool compared with prick test and IgE detection. Specific CRD-led immunotherapy may achieve clinical improvements in patients with VC.

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Introduction

The terms *ocular allergy* and *allergic conjunctivitis* (AC) include a broad group of diseases caused by specific and nonspecific immunologic mechanisms. These terms are frequently used to describe any immunologic process that affects the ocular surface, regardless of the mechanism involved and the mediation of IgE.^{1–4} AC may affect more than 40% of the general population.^{5–16} Ocular symptoms may be the most frequent allergic manifestation in children.¹ The 2004 World Allergy Organization Nomenclature Committee Report¹⁰ specified that IgE-mediated AC is usually accompanied by allergic rhinitis and might therefore be called *allergic rhinoconjunctivitis*. In 2007, Bielory et al¹⁷ proposed a new classification of the spectrum of AC considering different immunopathogenic mechanisms that might explain the overlapping of different

entities: IgE-mediated allergic processes (seasonal and perennial AC); conditions with mixed pathogenic mechanisms that involve lymphocytes, mast cells, and eosinophils, such as vernal conjunctivitis (VC) and atopic keratoconjunctivitis; and conditions with a predominantly lymphocytic hyperactivity (contact dermatitis-conjunctivitis, giant papillary conjunctivitis).

AC is often the first clinical expression of allergic disease that might be susceptible of early specific management if the cause were known. Conjunctivitis is the first symptom of allergic disease in up to 32% of children, mostly associated with rhinitis,¹ and this provides a window in which specific treatment may be useful. VC is a form of chronic conjunctivitis that mainly affects children living in temperate areas with a strong family history of allergies. It is most common in young males and usually occurs during spring and summer.¹⁷ The etiologic diagnosis is difficult to determine.^{13,15} Routine diagnostic tests (prick test, specific IgE determination) do not resolve the etiologic diagnosis of VC, and therefore there is no truly specific treatment.⁶ Component-resolved diagnosis (CRD) uses multiple molecular microarray techniques to improve the

Reprints: Alicia Armentia, MD, PhD, Allergy Unit, Rio Hortega University Hospital, C/ Dulzaina 2, 47012 Valladolid, Spain; E-mail: aliciaarmentia@gmail.com.

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diagnosis of allergy-related diseases for which conventional techniques are not efficient.^{9,18–22} Recent immunohistochemical and clinical studies suggest that IgE-dependent and independent IgE mechanisms are involved in the immunopathogenesis of VC, in which various inflammatory cells, including a different subpopulation of T cells, play an active role.⁶ Although endocrine, genetic, neurogenic, environmental, and social factors have been identified, the cause remains unknown, and in many cases the prognosis is poor.

We hypothesized that the localization of the hypersensitive response in VC is responsible for the low efficacy of routine diagnostic tests. The aim of this study is to evaluate the IgE-mediated allergic hypersensitivity to aeroallergens by CRD in patients with VC, AC, and healthy controls.

Methods

We made a clinical diagnosis of VC based on symptoms and eye exploration, including irritated, red, and itchy eyes, with burning sensation, excessive tearing, swollen eyes (especially the area around the edge of the cornea), cobblestoning of the eyelids, and presence of Hanta dots. These symptoms were present in all patients, most often in the spring and summer. Clinically, cobblestoning of the eyelids in our patients was more prevalent than Hanta dots. All participants gave their written informed consent to participate in the study. The study was evaluated by the Clinical Research Ethics Committee of the Rio Hortega University Hospital. The study adhered to the CONSORT guidelines and the Declaration of Helsinki principles.

We have designed a comparative longitudinal study after performing a careful literature search using methodologic filters (Clinical Queries, SUM Search, Grade, Cochrane Library). Patients diagnosed as having VC were selected from the database of the Department of Ophthalmology at the Rio Hortega University Hospital. For each patient with VC, 2 control patients with grass pollen AC of comparable severity were selected from the database of the Allergy Clinic, Rio Hortega University Hospital. Grass pollen conjunctivitis was defined by positive prick test result, specific IgE, and allergologic and ophthalmologic clinical criteria. Pollen sensitization was defined by the presence of the following 3 diagnostic criteria: one or more positive skin test results to pollen, CAP (IgE) positive titers greater than 0.35 IU/mL to these allergens, and positive conjunctival specific provocation result.²¹

All patients were studied simultaneously using the same diagnostic tests during the same period. A control group of healthy, nonsmoking controls were randomly selected by the Blood Donation Unit, Sanidad Castilla y León. Two healthy controls were selected for each patient with VC.

Conventional prick tests were performed with commercial allergens and with purified proteins of native (n) and recombinant (r) allergens. Prick tests were performed according to the standards of the European Academy of Allergy and Clinical Immunology. After placing a drop of each allergen in the anterior forearm, a small puncture that did not reach the dermis was made through the drop with a lancet. The result was read after 15 minutes. A wheal with a diameter of 3 mm or larger was considered a positive result. Each allergen was tested twice. A standard battery of aeroallergens and foods, including pollen (trees, grasses, weeds, and flowers), mites (*Dermatophagoides* and storage mites), fungi, antigens to animals, and common foods (ALK-Abelló, Madrid, Spain) was tested.

CRD was performed on tears and serum using the ISAC 112 panel (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. CRD (IgE) titers greater than 0.3 ISU were considered positive results.

A total of 100 μ L of tears were collected from the right eye of each patient using a capillary tube and were immediately frozen

at -70°C until the test was performed. The response to 112 proteins of different origins was measured, and the response due to cross-reactivity with other allergens (profilin, cross-reactive carbohydrate determinants, polcalcins) was assessed.

Patients were advised to avoid the identified causative allergen. Usual symptomatic treatment, such as tear substitutes, antihistamine (azelastine drops) or anti-inflammatory drops, and mild steroid drops, was allowed.

Conventional depot immunotherapy (ALK-Abelló, Hørsholm, Denmark) was initiated in patients in whom CRD revealed hypersensitivity to allergens for which there is effective immunotherapy. The treatment was maintained during 1 year, and its effectiveness was evaluated in spring and summer 2014. Clinical eye examinations were performed on a 6-month basis. Patients were considered to have a favorable evolution if clinical signs and symptoms improved or disappeared and the conjunctival examination findings were normal 1 year after the initiation of immunotherapy. A diary record was used to assess quality of life, the number and severity of symptoms, and the dosage of medication required at baseline and after 6 and 12 months. Efficacy was evaluated using the Average Rhinoconjunctivitis Total Symptom Score.⁸ Patients were considered to have a favorable evolution if, 11 year after the initiation of immunotherapy, clinical signs and symptoms improved or disappeared and the slitlamp examination revealed no signs of active inflammation.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software, version 15.0 (SPSS Inc, Chicago, Illinois). The χ^2 test was used to analyze qualitative data. The *t* test for nonpaired data and analysis of variance were used to compare mean values of parametric data. The Fisher exact test was used when the number of cells with expected values of 5 or more was greater than 20%. Nonparametric data were analyzed using the Mann-Whitney test (for 2 groups) or the Kruskal Wallis H test (for >2 groups). A multivariate analysis was performed using an adjusted multinomial logistic regression model.

Results

Twenty-five patients with VC (14 girls and 11 boys), 50 patients with AC (23 girls and 27 boys), and 50 healthy controls (15 girls and 35 boys) were studied. The global mean (SD) age was 28 (13) years (31.6 [11.5] years for healthy controls, 25.8 [10.3] years for AC patients, and 27.6 [19.5] for VC patients). Mean age was not significantly different among the three groups ($P = .08$, *t* test for unpaired data). Although VC affects younger people, the mean (SD) age in this group was 27.6 (19.5) years, which is explained by the fact that due to the infrequency of these patients in our surgery, we selected all patients who sought treatment at our allergy unit in the last 23 years. Some patient had VC since they were children but were adults in the moment of study.

AC occurred more frequently in males, and VC was more frequent in females, even though the differences were not statistically significant ($P = .07$, χ^2 test).

Table 1, 2, and 3 give the results of serum CRD and conventional IgE and prick techniques. Tear CRD test results were negative. Group 1 allergens of grasses (pol 1), which include the β -expansins nLol p 1 from *Lolium perenne* (the predominant grass pollen allergen in our setting²³), and rPhl p 1 from *Phleum pratense* were the most frequent among patients with VC and AC (11 of 25 and 36 of 50, respectively). Nine patients with VC responded to nCyn d 1 (5 to group 5 [pol 5] and 3 to group 4 [pol 4]), 2 patients responded to nDer p 1 and nDer p 2 (major mite allergens), and 2 patients responded to lipidic transport proteins of hazelnut (rCor a 8) and peach (nPru p 3).

Table 1
Results of component-resolved diagnosis

Array ^a	No. of positive arrays			P value (χ^2 test)
	Healthy controls (n = 50)	AC patients (n = 50)	VC patients (n = 25)	
pol 1	1	36	11	<.001
pol 2	0	34	2	<.001
pol 4	0	21	3	<.001
pol 5	0	6	5	.009
pol 6	0	14	3	<.001
nCyn d 1	0	17	9	<.001
nDer p 1	0	1	2	.10
nDer p 2	1	1	2	.31
rAlt a 1	0	2	1	.36
rAni s 1	2	3	0	.46
nPru p 3	0	1	2	>.99
rCor a 8	0	2	2	.16
nArt v 3	0	2	2	.16

Abbreviations: AC, allergic conjunctivitis; n, native allergen; r, recombinant allergen; VC, vernal conjunctivitis.

^apol 1 indicates group 1 of pollen (include the β -expansins nLol p 1 from *Lolium perenne* and rPhl p 1 from *Phleum pratense*); pol 4, 5, 6, rPhl p 5 ribonuclease and rPhl p 6 from *Phleum pratense*; nCyn d 1, group 1 of *Cynodon dactylon* (Bermuda grass pollen); nDer p 1 and nDer p 2, cysteine proteases of *Dermatophagoides pteronyssinus* and *farinae*, respectively; rAlt a 1, acidic glycoprotein of *Alternaria alternata*; rAni s 1, serin-protease inhibitor of *Anisakis simplex*; nPru p 3, peach lipid transfer protein; rCor a 8, hazelnut lipid transfer protein; and nArt v 3, mugwort lipid transfer protein.

Pollen prick test results were positive in only 2 patients with VC (positivity to *Lolium*, *Cynodon*, and *Olea*). *Lolium* and *Cynodon* IgE were detected in 4 and 3 patients with VC, respectively. Prick results are given in Table 2.

The relative sensitivity and specificity of the 3 diagnostic tests area given in Table 4. CRD had a better sensitivity (48%) than the prick test and IgE determination (8% and 16%, respectively) and a similar specificity (98%, 96%, and 100%, respectively).

Table 2
Prick test results

Prick test	No. of positive prick test results			P value (χ^2 test)
	Healthy controls (n = 50)	AC patients (n = 50)	VC patients (n = 25)	
<i>Lolium pratense</i>	2	44	2	<.001
<i>Cynodon dactylon</i>	1	20	2	<.001
<i>Olea europaea</i>	0	14	2	<.001
<i>Platanus species</i>	0	12	1	<.001
<i>Cupressus arizonica</i>	0	4	0	.045
<i>Phleum pratense</i>	0	16	0	<.001
<i>Artemisia vulgaris</i>	0	7	0	.004
<i>Chenopodium</i>	0	9	1	.003
Cat	0	2	0	.22
<i>Dermatophagoides pteronyssinus</i>	0	1	1	.41
<i>Dermatophagoides farinae</i>	1	1	0	.78
<i>Alternaria alternata</i>	0	2	0	.22
Wheat	0	2	0	.22
Barley	0	2	0	.22
Rye	0	2	0	.22
Peanut	0	2	0	.22
Hazelnut	0	3	0	.10
Pine nut	0	2	0	.22
Legume	0	2	0	.22
Peach	0	3	0	.10
<i>Anisakis simplex</i>	3	2	0	.46
Tomato	0	2	0	.22
Almond	0	2	0	.22
Apple	0	2	0	.22

Abbreviations: AC, allergic conjunctivitis; VC, vernal conjunctivitis.

Table 3
Positive results of specific IgE tests

IgE	No. of positive specific IgE test results			P value (χ^2 test)
	Healthy controls (n = 50)	AC patients (n = 50)	VC patients (n = 25)	
<i>Lolium perenne</i>	0	25	4	<.001
<i>Cynodon dactylon</i>	0	12	3	.001
<i>Olea europaea</i>	0	6	1	.03
<i>Platanus species</i>	0	6	1	.03
<i>Phleum pratense</i>	0	9	0	.001
<i>Artemisia vulgaris</i>	0	4	0	.045
<i>Chenopodium</i>	0	4	1	.12
<i>Alternaria alternata</i>	0	2	0	.22
Egg white	0	0	2	.02
Hazelnut	0	4	0	.045
Pine nut	0	2	0	.22
Peach	0	7	0	.004
<i>Anisakis simplex</i>	2	2	0	NS
Almond	0	2	0	.22

Abbreviations: AC, allergic conjunctivitis; NS, no significance; VC, vernal conjunctivitis.

Allergen specific immunotherapy was indicated in 37 patients with AC (all with grass pollen) and in 13 with VC (11 with grass pollen and 2 with *Dermatophagoides*). All these patients completed 1 year of treatment. All 13 patients with VC and 36 patients with AC improved ($P < .001$). Avoidance of the allergen alone produced a significant improvement only in patients with VC ($P < .05$; Table 5).

All the patients with AC or VC improved after immunotherapy based on a validated score, including clinical, therapeutic, and quality of life data. Inflammation signs, cobblestoning of the eyelids, and Hanta dots improved or disappeared, as well as symptoms and needed medication. The quality-of-life score was better after 2 years of treatment in patients with AC compared with VC, but the difference did not reach statistical significance.

Usual symptomatic treatments, such as tear substitutes, antihistamine (azelastine) or anti-inflammatory drops, and mild steroidal drops, were allowed, but after 1 year of immunotherapy only 1 VC patients needed tear substitutes.

Discussion

VC is a specific form of AC. This disease is important because this condition can be irritating and treatment is difficult with medications alone. Many patients have severe symptoms, including burning, dry eyes, and scarring. It is a condition difficult to control with conventional medications, and the understanding of the pathogenesis is poor. In our study, the condition had not changed in patients who had VC since childhood, and many of these patients had conjunctivitis but not rhinitis.

The role of IgE in the pathogenesis of this condition is important but represents only one facet of VC. Various forms of lymphocyte subtypes and eosinophils are predominant in the conjunctiva. Recent immunohistochemical and clinical studies suggest that IgE-dependent and independent IgE mechanisms are involved in the immunopathogenesis of VC, in which various inflammatory

Table 4
Sensitivity and specificity to pollen for each diagnostic method

Diagnostic method	No. of positive results		Sensitivity, % (95% CI)	Specificity, % (95% CI)
	VC patients (n = 25)	Controls (n = 50)		
Skin prick test	2	2	8 (0–20.6)	96 (59.6–100)
IgE test	4	0	16 (0–30.4)	100 (100–100)
CRD	12	1	48 (26.4–69.6)	98 (93.1–100)

Abbreviations: CI, confidence interval; CRD, component-resolved diagnosis; VC, vernal conjunctivitis.

Table 5

Clinical course of patients after 1 year of treatment with immunotherapy and/or avoidance of allergens detected in the arrays

Group	No. of patients by outcome		Total No. of patients	P value
	Unfavorable	Favorable		
Control group (no treatment) (n = 50)	0	50	50	
AC patients (n = 50)				<.001
No treatment	8	0	8	
Avoidance	5	0	5	
Immunotherapy	0	30	30	
Both	1	6	7	
VC patients (n = 25)				<.001
No treatment	11	0	11	
Avoidance	0	1	1	
Immunotherapy	0	9	9	
Both	0	4	4	
Total				<.001
No treatment	19	50	69	
Avoidance	5	1	6	
Immunotherapy	0	39	39	
Both	1	10	11	

Abbreviations: AC, allergic conjunctivitis; VC, vernal conjunctivitis.

cells, including a different subpopulation of T cells, play an active role.¹⁷

Patients who had been diagnosed as having VC were compared with patients who had grass pollen AC and with healthy controls. CRD were performed in all these patients and controls.

Up-to-date molecular analysis or CRD with recombinant and native allergens has not been performed in VC patients. We have found that this technique is more efficient in these conditions than skin prick tests and specific IgE determination.

The results of this study indicate that CRD detects more patients compared with the prick test and specific IgE determination. Eleven of 25 patients with VC responded to nLol p1, the predominant allergen in our environment. Levels of specific IgE to *Lolium* were only positive in 4 patients.

Specific immunotherapy achieved clinical improvement in patients with VC.

We were unable to perform tear CRD. Even though VC is considered a local reaction and the concentration of allergen specific IgE may be higher in tear than in serum, the results of the first arrays were negative, and this technique was abandoned. As far as we know, no tears testing for CRD allergens have been performed, although in previous studies, stationary-phase antibody arrays using tears were useful for the screening of various cytokines, growth factors, and matrix metalloproteinase (MMP). These tests have previously identified unreported factors in the tears of VC patients, including MMP-3 and MMP-10, and multiple proteases, growth factors, and cytokines, which may all play an important role in the pathogenesis of conjunctival inflammation.¹³ However, the concentration of inflammatory mediators may be decreased by the tear dilution caused by lacrimation reflex induced by tear collection.²⁴ The literature recommends obtaining basal tears under topical anesthesia for determination of components^{24,25}; however, this technique becomes even more difficult in eyes with VC and associated dry eye syndrome.²⁶

Serum CRD revealed sensitization to native and recombinant allergens. Eleven patients with VC and 36 patients with AC responded to nLol p 1, the predominant allergen in our local environment.²³ These results may be particularly relevant because in our environment grass pollen represents only 10% to 18% of total annual pollen, even though Lol p 1 appears in the atmosphere for a sustained period (April to July) and in significant amounts (allergen/grass ratio = 2.8), almost twice as much as the group 5 grass allergens (Lol p 5, 1.7) and far greater than Pla a 1 (0.2) from

the plane tree, the major pollen allergen in the city. Therefore, aeroallergen Lol p 1 is, a priori, a major risk factor for patients with AC and other respiratory problems, as indicated in environmental studies from other areas.^{23,27}

The molecular microarray analysis was useful in the diagnosis and treatment of our patients and allowed us to perform a more restricted allergen elimination and specific immunotherapy. Directed avoidance therapy (in the case of a food or avoidable allergen) or conventional specific immunotherapy (in the case of mites and pollens) was administered. After 1 year of treatment, avoidance therapy produced no significant results. Avoidance therapy does not seem to be useful in patients with AC, suggesting that avoiding outdoor exposure conjunctivitis does not improve the conjunctivitis in this group of patients.²⁸ However, in our series, combined avoidance and specific immunotherapy resulted in a significant improvement in both AC and VC patients. Specific immunotherapy resulted in improvement in terms of quality of life and VC symptoms. Thirteen patients with VC who received specific immunotherapy guided by the results of the CRD had definite improvement.

Studies have found that immunotherapy directed against recombinant allergens is both effective and safe^{9,18–22} and permits the use of hypoallergenic forms with great immunogenic potential. It is necessary to demonstrate that these formulations have the same power as the complete natural allergens and that the panel of recombinant allergens includes all the epitopes recognized by B and T lymphocytes. In the case of grasses, a panel of 5 recombinant allergens (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b)²¹ and the panel plus Phl p 6¹⁸ significantly reduce symptoms and induce an intense specific IgE-mediated response against these allergens. CRD may help provide a more accurate immunotherapy for complex pollination.²⁹

Among the limitations of this study is that the validity and feasibility may be compromised by the fact that the study only detected possible causal allergens in patients with conjunctivitis and an atopic background. We were unable to determine whether the response to a food epitope would have clinical value or not because subclinical sensitization (ie, without a clear correlation of the disease) has been described.

In conclusion, CRD techniques were highly specific and allowed the diagnosis of more patients than the prick test plus IgE determination. Serum testing with CRD can be a useful tool to guide specific immunotherapy if patients cannot avoid the antigen. The study indicates that VC can be treated with specific allergen immunotherapy, which is an important step in the treatment of this difficult condition. CRD-led immunotherapy in patients with VC and AC resulted in objective and subjective improvements without the need for symptomatic treatment.

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