AMERICAN ACADEMY OF OTOLARYNGOLOGY–HEAD AND NECK SURGERY FOUNDATION

OTO Open
2021, Vol. 5(1) 1–5

DOI: 10.1177/2473974X20986569
http://oto-open.org
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Cross-reactivity in Skin Prick Test Results of Members Within Pooideae Subfamily

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Abstract

Objective. Molecular similarities of grass pollen antigens have led to the view that cross-reactivity exists within members of the Pooideae subfamily of grasses. This has resulted in testing for only the most antigenically representative member of Pooideae, Timothy grass (*Phleum pratense*), despite little literature to support the claim that *Phleum* is the most representative member or that in vitro cross-reactivity correlates with in vivo cross-reactivity. The aim of the study was to determine if patients with allergic rhinitis symptoms and positive skin prick test results to meadow fescue (*Festuca pratensis*) also have positive results to Timothy grass.

Study Design. Retrospective cross-sectional study.

Setting. Tertiary care center in middle Missouri.

Methods. A retrospective chart review identified patients $\geq$12 years old with a diagnosis of allergic rhinitis who underwent skin prick testing between March 2016 and July 2018, by using a search with *CPT* code 95004 (*Current Procedural Terminology*). Positive skin prick test results were based on wheal produced $\geq$3 mm than the negative control.

Results. After review of 2182 charts, 1587 patients met criteria to test for *Phleum* and *Festuca*. In total, 1239 patients had a positive result for *Phleum* or *Festuca*. Of these, 479 (38.6%) tested positive for *Festuca* alone, while 342 (27.6%) and 418 (33.7%) tested positive for *Phleum* alone and *Phleum* + *Festuca*, respectively.

Conclusion. Clinical cross-reactivity among Pooideae members may not be as complete as traditionally thought. *P pratense* may not be the most antigenically representative subfamily member, and other grasses may need to be included in skin prick testing.

Keywords

allergies, allergic rhinitis, skin prick test, immunotherapy, Pooideae, *Phleum*, *Festuca*, grass pollen

Received October 26, 2020; accepted December 2, 2020.

Allergy, presenting clinically as allergic rhinitis (AR), affects up to 14.2% of adults in developed countries and an estimated 500 million people worldwide.[1,2] AR typically presents with sneezing, rhinorrhea, and nasal congestion.[3] Symptoms result from immediate responses to antigen, mediated by IgE, and chronic inflammation via allergen-specific T cells. Skin prick tests (SPTs) and serum IgE levels, correlated with clinical signs and symptoms, are used for additional diagnostic workup for AR. Symptoms caused by AR can cause a considerable decrease in work productivity and presenteeism, which has been identified as the largest contributor to the economic burden of AR.[4] The standard medical treatment for AR includes intranasal steroids, intranasal antihistamines, oral antihistamines, and nasal saline rinses. However, with allergen avoidance, allergen-specific immunotherapy is the only treatment modality shown to alter the natural course of allergic disease.[5]

Because of this, immunotherapy based on patients' specific allergens has become a common treatment for AR.[6] When patients experience allergy symptoms to only a few allergens, the choice of what antigens to include in their treatment is straightforward. However, when symptoms are caused due to numerous allergens, as is the case with 60% to 80% of patients with AR,[7] choosing the combination of antigens to include in their treatment can become complicated.[6,8,9] To simplify this, antigens have been extensively studied to determine the best way to maximize therapy

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This article was presented at the American Rhinological Society 65th Annual Meeting; September 13-14, 2019; New Orleans, Louisiana, USA.

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while minimizing the number of antigens required to adequately treat patients.

Grass pollens, from allergen groups 1 and 5 in particular, account for an extensive burden of disease, as an estimated 30% to 70% of patients with AR are sensitized to grass pollen.[10-12] Molecular similarities of grass pollen have led to suspicions that cross-reactivity exists within members of similar species.[8,13] Of the Pooideae subfamily of grasses, *Phleum pratense* has been identified in previous studies as the most antigenically representative member; thus, its extract alone has been proposed as being sufficient for predicting immunotherapy success (marked by an increase in allergen-specific IgG) from a diagnostic and therapeutic standpoint.[13]

A correlation between immunotherapy-induced IgG4 levels and levels of *P pratense* extract has been shown, in addition to evidence of T-cell cross-reactivity toward group 1 allergens.[2] Other studies have demonstrated cross-reactivity among Pooideae species by using 3-dimensional modeling of grass group 1 allergens, amino acid sequence, and IgE binding inhibition assays.[3,14] These results indicate that patient IgE may be directed primarily toward common epitopes, implying that treatment with simply 1 Pooideae species extract may affect clinical allergic responses from other members in the subfamily. However, in vitro cross-reactivity may reflect in vivo allergy testing results to a lesser degree than previously thought. Indeed, a correlation between allergy test results and in vitro assays has not been demonstrated consistently.[15] Differences in allergen-specific T- and B-cell responses have been found, in addition to the absence of an association between serum IgE responses and B- and T-cell proliferation.[16,17] Thus, despite molecular similarities supporting cross-reactivity among members of the Pooideae subfamily, which has led to the common practice of testing only for *P pratense*, data are conflicting or lacking to support the assumption of cross-reactivity in vivo. Similarly, there is little evidence to support *P pratense* being the most representative member of Pooideae.

If *P pratense* is the most representative of the Pooideae grass family and cross-reactivity is as extensive as currently thought, then skin testing with *P pratense* and a close member of that subfamily should demonstrate strong concordance between them. With this study, the aim was to determine if patients with AR symptoms during grass season with positive SPT result to meadow fescue (*Festuca pratensis*) also have a positive SPT result to the previously identified representative member of the Pooideae subfamily, Timothy grass (*P pratense*).

Methods

This study was approved by the University of Missouri Institutional Review Board (2007840). A retrospective review of the electronic health record at a single tertiary care center from March 1, 2016, to July 31, 2018, was performed to identify patients aged ≥12 years with a diagnosis of AR with symptoms during grass season who underwent skin prick testing, by using a search of *CPT* code 95004 (*Current Procedural Terminology*). All patients were tested with Multi-Test II devices (Lincoln Diagnostics) with panels including allergenic extracts (from Stallergenes-Greer and ALK) specific for the central Missouri area, including *Phleum* as the representative of the Pooideae subfamily; however, *Festuca* grasses have become more prominent in the surrounding areas. *Festuca* was added to testing panels on a trial basis. Positive results on skin prick testing to *Phleum, Festuca*, or both were based on recorded wheal size ≥3 mm when compared with the negative control prick test (50% glycerin), given that it was a satisfactory test with the positive histamine control prick test ≥7 mm when compared with the negative glycerin control.

Table 1. Skin Prick Test Results (N = 1587).[a]

	Positive result
Tested for *Phleum* and *Festuca*	1239 (78.1)[b]
Positive for	
Phleum only	342 (27.6)
Festuca only	479 (38.6)
Phleum+*Festuca*	418 (33.7)

[a]Results are presented as No. (%).
[b]Positive result for at least 1 antigen. Negative to both antigens: n = 348 (21.9%).

Results

Overall, 2182 patient charts were identified and reviewed. It was determined that 1587 records met criteria and included testing for *Phleum* and *Festuca*. Of these patients, 1239 had at least 1 positive test result to *Phleum* or *Festuca. Phleum*-only positive cases included 342 (27.6%) patients, while a surprising 479 (38.6%) were positive to *Festuca* only. It was noted that 418 (33.7%) patients were positive to both *Phleum* and *Festuca* on skin prick testing (**Table 1**).

Among the patients who had a positive SPT result to *Phleum*, 45% had a positive result only to *Phleum*, while 55% had positive results to *Phleum* and *Festuca*. For patients with a positive test result to *Festuca*, interestingly 53.4% had a positive result to *Festuca* only, as compared with 46.6% who had a positive result to *Festuca* and *Phleum*.

To determine if these numbers of *Festuca*-only results were statistically significant, the expected *Festuca* count was calculated (**Figure 1**). If a *Phleum*-positive test is completely predictive of a *Festuca*-positive test as previous literature reports, then a *Festuca*-only test result happens under 1 of 2 scenarios: (1) the patient is truly sensitized, with false-negative *Phleum* and true-positive *Festuca* test results, or (2) the patient is not sensitized, with true-negative *Phleum* and false-positive *Festuca* test results. **Table 2** shows the expected number and 95% CIs of *Fescue*-only test results that should occur if the *Phleum*-positive test is completely predictive of a *Festuca*-positive result.

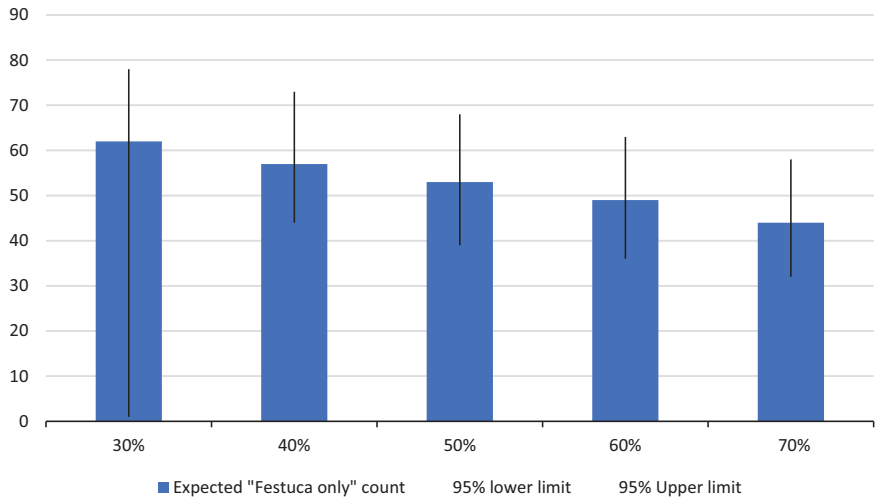

Figure 1. Expected number of *Festuca*-only test results based on the prevalence of grass allergy in the tested population, according to the sum of the false positives for *Festuca* and false negatives for *Phleum*.

Table 2. Expected *Festuca*-Only Test Results Based on the Prevalence of Grass Allergy.[a]

Prevalence, %	Expected count[b]	95% CI
30	62	47-78
40	57	44-73
50	53	39-68
60	49	36-63
70	44	32-58

[a]Test results depend on the prevalence of an allergy in the test population and the reliability of the tests.

[b]Expected count if the *Phleum* test is completely predictive of a *Festuca*-positive result. This is derived from the sum of the estimation for the false negatives of *Phleum* and the false positives of *Festuca*, according to these formulas: false negatives = population × prevalence of allergy × false-negative test result × true-positive test result; false positives = population × (1 − prevalence of allergy) × true-negative test result × false-positive test result.

Statistical Analysis

The expected count and 95% CI of *Festuca*-only test results were estimated empirically in a simulation study performed with R version 3.6.0 (R Foundation for Statistical Computing). In this study, the expected number of *Festuca*-only test results was assumed to depend on the underlying prevalence of fescue allergy in the test population and on the reliability of the allergy tests. In the simulations, we assumed a 30%-70% prevalence of *Festuca* allergy in the test population, based on the literature, in accordance with estimates for the proportion of individuals with an allergy who present with symptoms.[10,11] In addition, we conservatively assumed the tests to have a 2% false-negative rate and 5% false-positive rate according to materials from the test company (Lincoln Diagnostics, Inc). Using these assumptions, we simulated 10,000 studies of 1587 individuals similar to the original study. The mean and 95% quantiles of the simulations were compared with the actual number of *Festuca*-only cases observed in the original study. The maximum number of expected *Festuca*-only results was 62 (95% CI, 47-78). According to this analysis, it is statistically impossible to obtain the results of 479 patients with *Festuca*-only SPT results based solely on chance.

Discussion

Unexpectedly, a higher percentage of patients showed a positive test result to *Festuca* only when compared with *Phleum* only. Historically, *Phleum* has been identified as the representative member of the Pooideae subfamily.[13] Recommendations have been made to test for only this member of Pooideae to identify all sensitizations to antigens within this subfamily. However, these results suggest that *Phleum* may not be the most antigenically representative within Pooideae, as there was such a high percentage of patients who were sensitized to *Festuca* only.

A much higher-than-expected 53.4% of patients with *Festuca* positivity were not sensitized to *Phleum*. Based on previous in vitro data, this result should not have been seen. If a *Phleum*-positive test were completely predictive of a *Festuca*-positive test, as historically thought, the percentage of *Festuca*-only cases should have been considerably less. In vivo reactivity of members of the Pooideae subfamily has the potential to be very different when compared with in vitro reactivity.

If *Festuca* is not tested, it is possible that almost 40% of patients with grass pollen allergy symptoms will not have their antigen identified. Missing an antigen that causes significant symptoms can significantly affect the treatment of allergy patients, including frustration, continued AR symptoms, and noncompliance. With a goal of maximizing therapy while minimizing antigens in immunotherapy vials, educated choices must be made about which antigens to test

to ultimately include in the vial. However, 40% of patients is a substantial amount that cannot be ignored.

These results join a growing body of literature that suggests that cross-reactivity may not be as straightforward as previously thought, including a combination of specific and cross-reactive T-cell and antibody responses.[18] Additionally, this study adds to the literature suggesting that the most effective method to induce tolerance with immunotherapy is to include the specific allergens causing clinical symptoms rather than the antigen of a member of the same subfamily.[18,19] Indeed, testing for *Festuca* is not unnecessary and can have significant clinical implications.

It is important to note that this study has some limitations. It is based on the population of patients in mid-Missouri. *Festuca* is actually an invasive species in this region and quite prevalent.[20] It has the potential to overgrow, causing increased exposure in patients in this region when compared with other areas of the United States and the world. Additionally, this study is a retrospective review and does not allow for follow-up of symptoms based on immunotherapy treatment developed from positive SPT results. Prospective studies are needed in multiple patient populations to determine the in vivo results of skin prick testing, as well as the clinical symptoms and treatment, of patients who are sensitized to members of the Pooideae subfamily.

Conclusions

The cross-reactivity among Pooideae members may not be as complete as traditionally thought. *P pratense* may not be the most antigenically representative Pooideae subfamily member. Additional grass pollens, other than *Phleum* alone, may need to be included in skin prick testing to avoid missing clinically symptomatic sensitivities in patients with AR due to grass pollen. Additional prospective studies are required to accurately determine in vivo data about potential cross-reactivity among members of the Pooideae subfamily.

Acknowledgments

We acknowledge Steve Mortimer, MBA, for assistance with statistical analysis.

Author Contributions

Brette C. Harding, data collection, data analysis, manuscript drafting and editing, final manuscript approval; **Brian P. Kinealy**, data collection, data analysis, manuscript drafting and editing, final manuscript approval; **Christine B. Franzese**, study concept design, data analysis, manuscript editing, final manuscript approval.

Disclosures

Competing interests: Christine B. Franzese is a member of the advisory board of ALK. She also is a member of the speakers bureau of ALK, AstraZeneca, GSK, Optinose, Regeneron, and Sanofi. She has research funding from ALK, Novartis, Optinose, Merck, Shionogi, and Regeneron.

Sponsorships: None.

Funding source: None.

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