

tinued even after 6 weeks of omeprazole therapy. Subsequently, the patient underwent esophagogastroduodenoscopy, which revealed mid esophagitis with narrowing compatible with EE, confirmed by 5 biopsies (23 eosinophils per high-power field, on average), and an allergy evaluation was performed in our allergy unit, confirming HE allergy (skin prick test positive for HE, 6×2 mm; atopy patch test, negative; Gal d 1 with ImmunoCap, 1.92 kUA/L; Gal d 2, 0.41 kUA/L).

After adhering to an egg-free diet and 3 months of oral steroid therapy (methylprednisolone, 1.5 mg/kg daily) (Figure 1), a clear clinical improvement was observed; therefore, egg avoidance was continued for 1 year during which no EE symptoms occurred.

Follow-up esophagogastroduodenoscopy was performed 1 year later, which revealed complete resolution of endoscopic features and histologic improvement (3 eosinophils per high-power field at the biopsy specimen).

Our clinic case findings are as follows: (1) more complex or mixed allergic mechanisms characterize EE; therefore, non-IgE-mediated allergies also need to be assessed; (2) given the correlation between food allergy and EE in pediatric patients, an elimination diet is confirmed to be a valuable therapeutic tool for reducing symptoms; in adults, however, this approach is mostly ineffective; (3) SOTI seems to alter the natural food allergy history by increasing the threshold dose, eliciting anaphylaxis and inducing, as a result of continuous exposure to the causative food, the development of EE; (4) when starting SOTI in patients with a history of food allergy, which requires, if tolerated, regular assumption of the specific food, monitoring not only the adverse effects but also the possible development of other allergic disorders, such as EE, is recommended.

ACKNOWLEDGMENTS

We acknowledge M. T. Valenti, S. Peveri, I. Morelli, and E. Olivieri.

ERMINIA RIDOLO*

GIAN LUIGI DE ANGELIS†

PIERPAOLO DALL'AGLIO‡

*Department of Clinical Sciences

†Department of Paediatrics

‡Department of Clinical Medicine, Nephrology, and Health Sciences

University of Parma

Parma, Italy

1. Straumann A, Simon HU. Eosinophilic esophagitis: escalating epidemiology? *J Allergy Clin Immunol*. 2005;115:418–419.
2. DeBrosse CW, Collins MH, Buckmeier Butz BK. Identification, epidemiology, and chronicity of pediatric esophageal eosinophilia, 1982–1999. *J Allergy Clin Immunol*. 2010;126:112–119.
3. Sugnam KKN, Collins JT, Smith PK, et al. Dichotomy of food and inhalant allergen sensitization in eosinophilic esophagitis. *Allergy*. 2007;62:1257–1260.
4. Simon D, Straumann A, Wenk A, et al. Eosinophilic esophagitis in adults: no clinical relevance of wheat and rye sensitizations. *Allergy*. 2006;61:1480–1483.
5. Furuta GT, Liacouras CA, Collins MH, et al; First International Gastrointestinal Eosinophil Research Symposium (FIGERS) Subcommittees. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology*. 2007;133:1342–1363.
6. Hofmann AM, Scurlock AM, Jones SM, et al. Safety of a peanut oral immunotherapy protocol in children with peanut allergy. *J Allergy Clin Immunol*. 2009;124:286–291.
7. Narisety SD, Skripak JM, Steele P, et al. Open-label maintenance after milk oral immunotherapy for IgE-mediated cow's milk allergy. *J Allergy Clin Immunol*. 2009;124:610–612.

COMPARISON OF DOUBLING AND QUADRUPLING METHACHOLINE CONCENTRATION REGIMENS USING THE TIDAL VOLUME METHOD

The methacholine challenge is commonly used to measure airway responsiveness. Two doubling concentration methods have been outlined by the American Thoracic Society: the tidal breathing method and the dosimeter method.¹ Quadrupling concentrations are an option with the dosimeter method as a time-saving measure.¹ We reasoned that the tidal volume method could also be performed with quadrupling concentrations and performed a study to compare doubling and quadrupling concentrations.

Fifteen nonpregnant, nonlactating adults with asthma but no allergic or infectious exacerbations within more than 4 weeks were recruited to perform 2 methacholine challenges in random order at the same time of day within 1 week, one with doubling and one with quadrupling concentrations of methacholine. The study was approved by the University of Saskatchewan Ethics Committee, and signed informed consent was obtained. Repeatability (2–8 weeks) data from our laboratory in 95 patients, similar to published data,² reveal that 7 study patients gives a more than 90% power to detect a half-concentration difference in provocation concentration that caused a decrease in forced expiratory volume in 1 second (FEV₁) of 20% (PC₂₀); this is the minimum clinically significant difference. Long-acting (n = 2) and short-acting (n = 5) β -agonists were withheld for 48 and 8 hours, respectively; no patients were using inhaled corticosteroids or other controllers. The FEV₁ was more than 70% predicted. Caffeine was avoided the day of the study.

The doubling concentration methacholine challenge was performed as outlined.¹ Methacholine was delivered with a Bennett Twin jet nebulizer (Puritan Bennett Corporation, Carlsbad, California) calibrated to deliver 0.13 mL/min. Study participants wore a nose clip and inhaled the aerosol via a face mask. The FEV₁ was measured in triplicate initially and was repeated 30 and 90 seconds after inhalation. Doubling concentrations of methacholine were then inhaled for 2 minutes at 5-minute intervals (between commencement of inhalations) and the FEV₁ measured at the same times. Available methacholine concentrations varied from 0.06 to 64 mg/mL. The percentage decrease in FEV₁ was calculated from the lowest post-saline FEV₁ to the lowest postmethacholine FEV₁.³ The test was stopped when FEV₁ had decreased by 17% or more.⁴ The PC₂₀ was interpolated⁵ or extrapolated⁶ from the log concentration vs response curve using algebraic formulas.^{5,6} Postmethacholine breathlessness was assessed using the Modified Borg Dyspnea Scale.⁷ The quadrupling concentration challenge was performed using the following concentrations: 0.06, 0.25, 1, 4, 16, and 64 mg/mL. The starting concentration, which depended on the patient's known responsiveness, was identical for the doubling method. The quadrupling challenge was stopped at an FEV₁ decrease of 15% or more to reduce the risk of severe bronchoconstriction with a 4-fold dose increase. The PC₂₀ values were log transformed, and data were analyzed with the paired *t* test with a computerized program (STATISTIX for Windows; Analytical Software, Tallahassee, Florida).

The 15 patients were a mean of 33.9 years old and 68.9 in tall, and their mean FEV₁ was 91.9% predicted. Five patients were female.

Disclosures: Authors have nothing to disclose.

© 2011 Published by Elsevier Inc. on behalf of American College of Allergy, Asthma & Immunology.
doi:10.1016/j.anai.2010.10.016

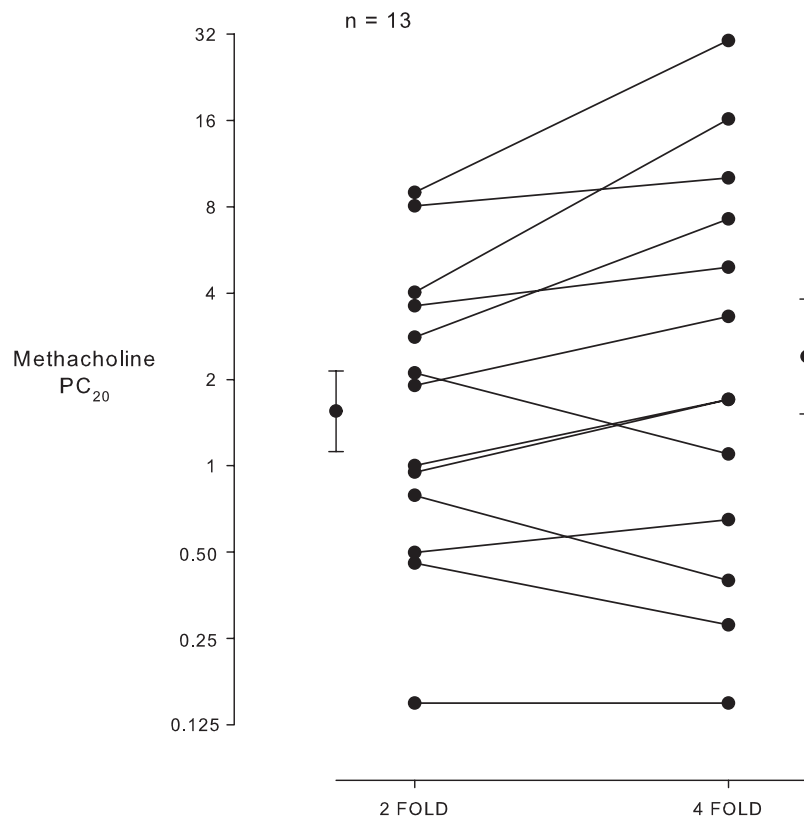


Figure 1. Concentration of methacholine causing a 20% decrease in forced expiratory volume in 1 second for 13 patients using both doubling and quadrupling methacholine protocols.

Two patients who were tested outside their pollen season had a dose-response plateau and were not analyzed, whereas the remaining 13 were included.

The PC₂₀ data are shown in Figure 1. There was a nonsignificant ($P = .10$) trend for the PC₂₀ to be higher with quadrupling step-ups. Two patients had a PC₂₀ with the quadrupling regimen that was twice that of the doubling regimen. One of these 2 patients had a negative test result only with the quadrupling method. There was a statistically significant larger reduction of FEV₁ (30% vs 22%, $P = .02$) and significantly more dyspnea (Borg score of 3.7 vs 2.6, $P = .04$) with the quadrupling regimen compared with the doubling regimen. Three patients in the quadrupling regimen and no patients in the doubling regimen had severe dyspnea (Borg score of ≥ 5). By omitting concentrations as in the quadrupling regimen, we saved on average less than 1 methacholine concentration and less than 2 minutes per methacholine challenge.

These data indicate that the tidal breathing method can be used safely (with the caveat to stop at a $>15\%$ FEV₁ decrease) with quadrupling concentration step-ups, producing clinically equivalent results. The quadrupling method resulted in small time savings, modestly greater bronchoconstriction, and slightly greater discomfort.

The trend to higher quadrupling PC₂₀ values was expected because of less cumulative effect than that seen with the doubling concentrations.⁷ Possible changes in the methacholine distribution within the airways may also contribute to differences. We

have validated that stopping methacholine inhalations at a 17% or greater decrease produces the same result for extrapolated PC₂₀ and avoids a 35% to 40% FEV₁ decrease.^{4,6} We extended this to 15% for the quadrupling concentrations because for safety and comfort we did not wish to quadruple (or more) the FEV₁ decrease to the range of 60% to 80%. Nevertheless, we saw lower FEV₁ values resulting in increased dyspnea because of overshooting the 20% FEV₁ decrease. These results lead us to conclude that, although the tidal breathing method can be used with quadrupling concentration step-ups, the small time saving does not favor the use of quadrupling methacholine concentrations. Other measures that can be used to shorten the duration of the methacholine challenge include the appropriate choice of a starting concentration and the omission of occasional concentrations when there has been a less than 5% decrease in FEV₁ from the previous concentration.⁸

GREGORY E. PETERS, MD, FRCP(C)
BETH E. DAVIS, PhD
DONALD W. COCKCROFT, MD, FRPC(C), FACCPC

Division of Respiriology Critical Care and Sleep Medicine
Department of Internal Medicine
University of Saskatchewan
Saskatoon, Saskatchewan, Canada
don.cockcroft@usask.ca

1. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing 1999. *Am J Respir Crit Care Med*. 2000;161:309–329.
2. Inman MD, Hamilton AL, Kerstjens AM, Watson RM, O'Byrne PM. The utility of methacholine airway responsiveness measurements in evaluating anti-asthma drugs. *J Allergy Clin Immunol*. 1998;101:342–348.
3. Davis BE, Cockcroft DW. Calculation of provocative concentration causing a 20% fall in FEV₁: comparison of lowest vs. highest post-challenge FEV₁. *Chest*. 2000;117:881–883.
4. Jokic R, Davis EE, Cockcroft DW. Methacholine PC₂₀ extrapolation. *Chest*. 1998;114:1796–1797.
5. Cockcroft DW, Murdock KY, Mink JT. Determination of histamine PC₂₀: comparison of linear and logarithmic interpolation. *Chest*. 1983;84:505–506.
6. Davis BE, Cockcroft DW. Extrapolation of methacholine PC₂₀. *Chest*. 2002;122:1499–1500.
7. Burdon JGW, Juniper EF, Killian KJ, Hargrave FE, Campbell EJM. The perception of breathlessness in asthma. *Am Rev Respir Dis*. 1982;126:825–828.
8. Cockcroft DW, Marciniuk DD, Hurst TS, et al. Methacholine challenge: test-shortening procedures. *Chest*. 2001;120:1857–1860.

BACTERIOSTATIC AGENTS AND STERILITY REQUIREMENTS FOR ALLERGEN IMMUNOTHERAPY

Phenol and glycerin are common bacteriostatic preservatives added to allergen extracts. United States Pharmacopeia guidelines, Immunotherapy 2007 Practice Parameters, and the American Academy of Allergy Asthma & Immunology/American College of Allergy,

Asthma and Immunology/Joint Council of Allergy, Asthma and Immunology (JCAAI) guidelines do not specify recommended concentrations of phenol or glycerin.^{1–3} The Joint Council of Allergy, Asthma and Immunology and the American Academy of Otolaryngic Allergy (AAOA) have recommended that “allergen extract dilutions must be bacteriostatic, meaning they must contain phenol concentrations of at least 0.25%, or if phenol concentration is less than 0.25%, the extract must have a glycerin concentration of at least 20%.”⁴ To our knowledge, no published studies support the use of phenol concentrations of at least 0.25%, or if less than that, glycerin concentrations of at least 20%. Limited studies have been published on the sterility of immunotherapy.⁵ Immunotherapy vials are multidose vials kept for up to 1 year, with the potential for multiple manipulations over that time. To our knowledge, no reports have been made of patients developing infections from immunotherapy injections. We investigated the potential for microbial growth in expired allergenic extracts previously used in patient care and studied the effects of using recommended and lower than recommended concentrations of phenol and glycerin on microbial growth in allergenic extracts.

Fifty expired immunotherapy vials from one institution were collected from patients undergoing immunotherapy. The phenol and glycerin concentrations in each expired vial were calculated. Albumin saline with 0.4% phenol (Allergy Laboratories, Oklahoma City, OK) was used for diluent. Phenol concentrations ranged from 0.12% to 0.4%, and glycerin concentrations ranged from 0% to 35% (Fig. 1). Forty-four vials contained greater than 0.25% phenol. Six vials contained less than 0.25% phenol but at least 20% glycerin. An additional 50 expired immunotherapy vials, from a different institution, were collected for culture purposes only. All 100 expired vials were sent for bacterial culture in thioglycolate broth, as well as in blood agar and anaerobic plates.

Disclosures: Authors have nothing to disclose.

Funding Sources: Financial support was provided by the Mayo Clinic Allergy Division discretionary funds.

© 2011 American College of Allergy, Asthma & Immunology.

Published by Elsevier Inc. All rights reserved.

doi:10.1016/j.anai.2010.10.024

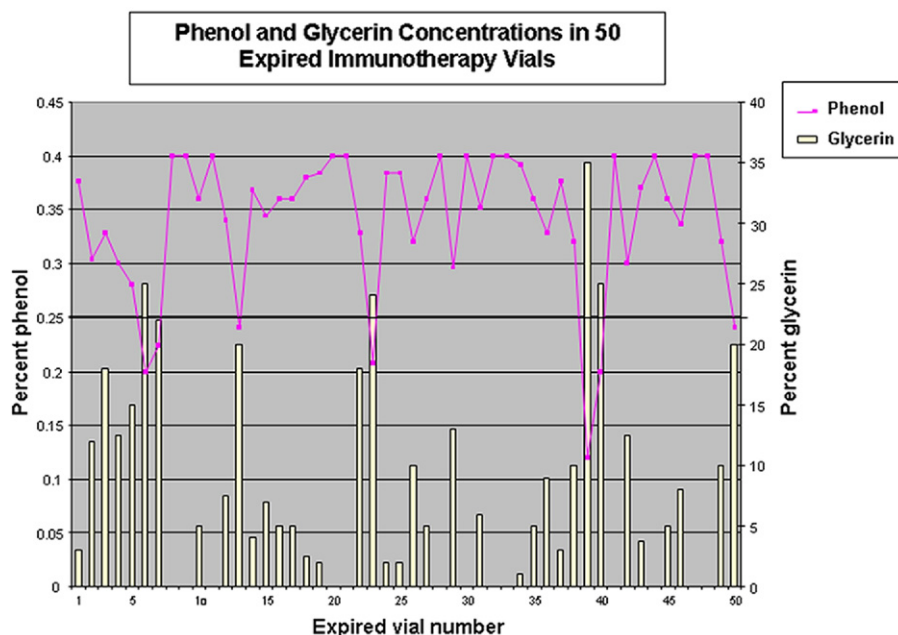


Figure 1. Phenol and glycerin concentrations in 50 of the expired patient immunotherapy vials. All expired immunotherapy vials met the JCAAI and AAOA recommendations of the use of at least 0.25% phenol, or if the phenol concentration was less than 0.25%, the extracts contained glycerin concentrations of at least 20%.