

FUNDING INFORMATION

Center for Data Analysis, Visualization and Stimulation (DAVIS);
Vontobel-Stiftung

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

Debbie J. Maurer^{1,2} 

Elena Barletta^{3,4}

Anja Heider³

Nino Stocker³

Alexandra Wallimann¹

Michael Villiger^{1,5}

Beat Villiger^{1,6}

Katja Bärenfaller^{3,4} 

Cezmi A. Akdis^{1,3} 

Walter Kistler^{1,3} 

¹Swiss Research Institute for Sports Medicine (SRISM), Davos,
Switzerland

²Division of Sports and Exercise Medicine, Department of Sport,
Exercise and Health, University of Basel, Basel, Switzerland

³Swiss Institute of Allergy and Asthma Research (SIAF),
University of Zurich, Davos, Switzerland

⁴Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland

⁵Department of Sports Medicine, Hospital Davos, Davos,
Switzerland

⁶Medical Center Bad Ragaz, Bad Ragaz, Switzerland

Correspondence

Walter Kistler, Davos Hospital, Department of Sports
Medicine, Promenade 4, 7270 Davos Platz, Switzerland.

DOI: 10.1111/all.15413

Local production of broadly cross-reactive IgE against multiple fungal cell wall polysaccharides in patients with allergic fungal rhinosinusitis

To the Editor,

Allergic fungal rhinosinusitis (AFRS) is a subset of chronic rhinosinusitis characterized by fungal allergy.¹ Serum IgE of AFRS patients often exhibits broad reactivity to multiple fungi, including *Alternaria alternata* and *Aspergillus fumigatus*.² However, the nature of such

ORCID

Debbie J. Maurer  <https://orcid.org/0000-0002-8543-1998>

Katja Bärenfaller  <https://orcid.org/0000-0002-1904-9440>

Cezmi A. Akdis  <https://orcid.org/0000-0001-8020-019X>

Walter Kistler  <https://orcid.org/0000-0002-9289-6953>

REFERENCES

1. Hull JH, Loosemore M, Schwellnus M. Respiratory health in athletes: facing the COVID-19 challenge. *Lancet Respir Med*. 2020;8(6):557-558.
2. Nieman DC. Exercise is medicine for immune function: implication for COVID-19. *Curr Sports Med Rep*. 2021;20(8):395-401.
3. Maurer DJ, Liu C, Xepapadaki P, et al. Physical activity in asthma control and its immune modulatory effect in asthmatic preschoolers. *Allergy*. 2022;77(4):1216-1230.
4. Orysiak J, Witek K, Zembron-Lacny A, Morawin B, Malczewska-Lenczowska J, Sitkowski D. Mucosal immunity and upper respiratory tract infections during a 24-week competitive season in young ice hockey players. *J Sports Sci*. 2017;35(13):1255-1263.
5. Suhre K, Sarwath H, Engelke R, et al. Identification of robust protein associations with COVID-19 disease based on five clinical studies. *Front Immunol*. 2021;12:781100.
6. Fernández-Castañeda A, Lu P, Geraghty AC, et al. Mild respiratory SARS-CoV-2 infection can cause multi-lineage cellular dysregulation and myelin loss in the brain. *bioRxiv*. 2022.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Soichiro Haruna and Kazuya Takeda contributed equally to this work.

© 2022 European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd.

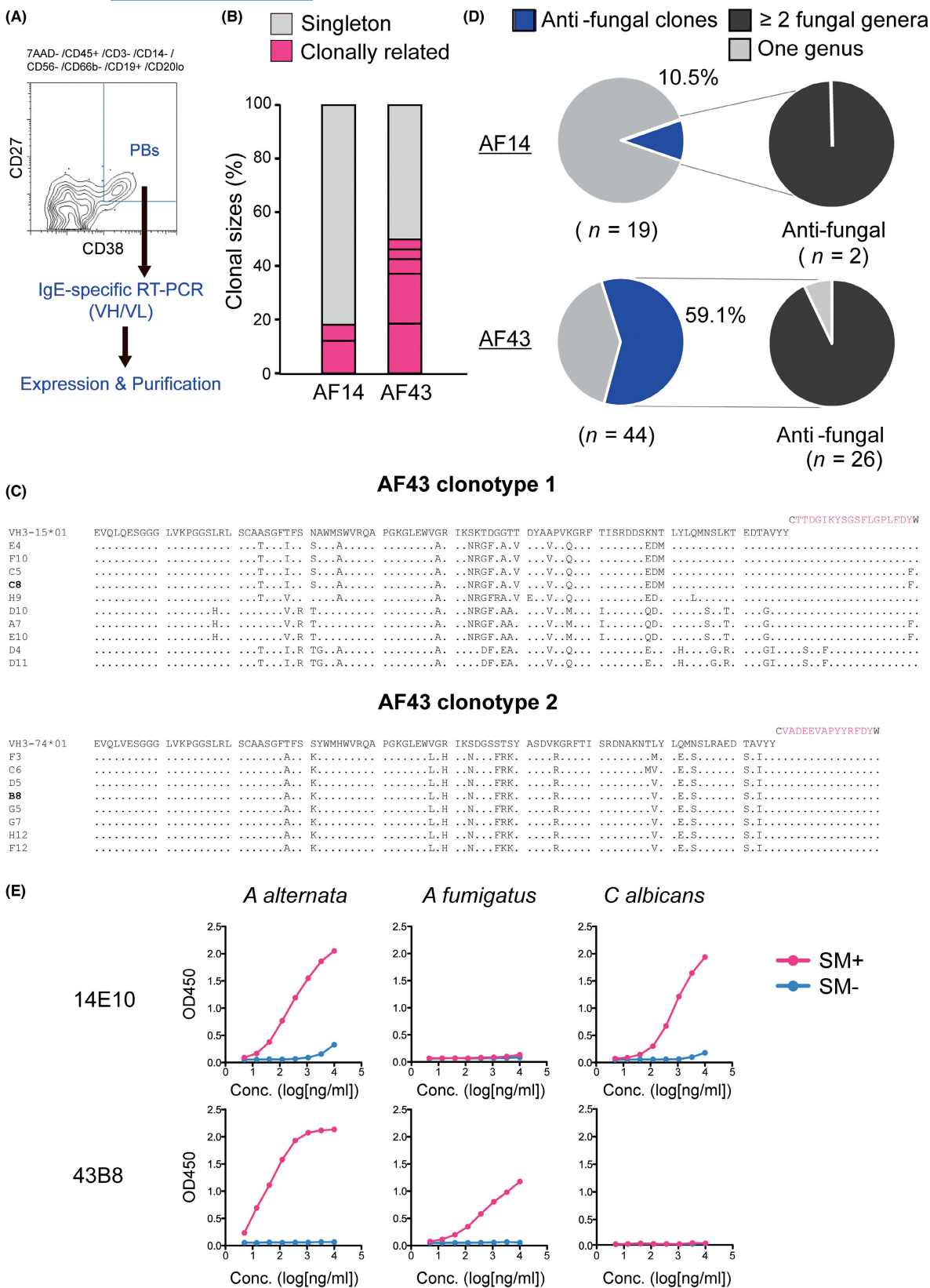


FIGURE 1 Anti-fungal IgE monoclonal antibodies (mAbs) from nasal polyps of AFRS patients are cross-reactive to different fungal genera. (A) Gating strategy for mAb cloning of IgE expressed in nasal polyp-residing plasmablasts (PBs). (B) Clonotype size of IgE. $N = 2$; AF14, $n = 33$; AF43, $n = 44$. (C) VH amino acid sequences of the representative IgE clonotypes isolated from a donor, AF43. (D) Proportion of anti-fungal clones in mAbs isolated from nasal polyps-residing PBs of the indicated donors. $N = 2$; AF13, $n = 19$; AF43, $n = 44$. (E) The anti-fungal reactivity is SHM-dependent. Binding activities of mAbs 14E10 and 43B8 (SM+) and their germline revertants (SM-) to whole fungal lysates from *Alternaria alternata*, *Aspergillus fumigatus*, and *Candida albicans* in ELISA are shown. Representative results from more than two independent experiments are shown

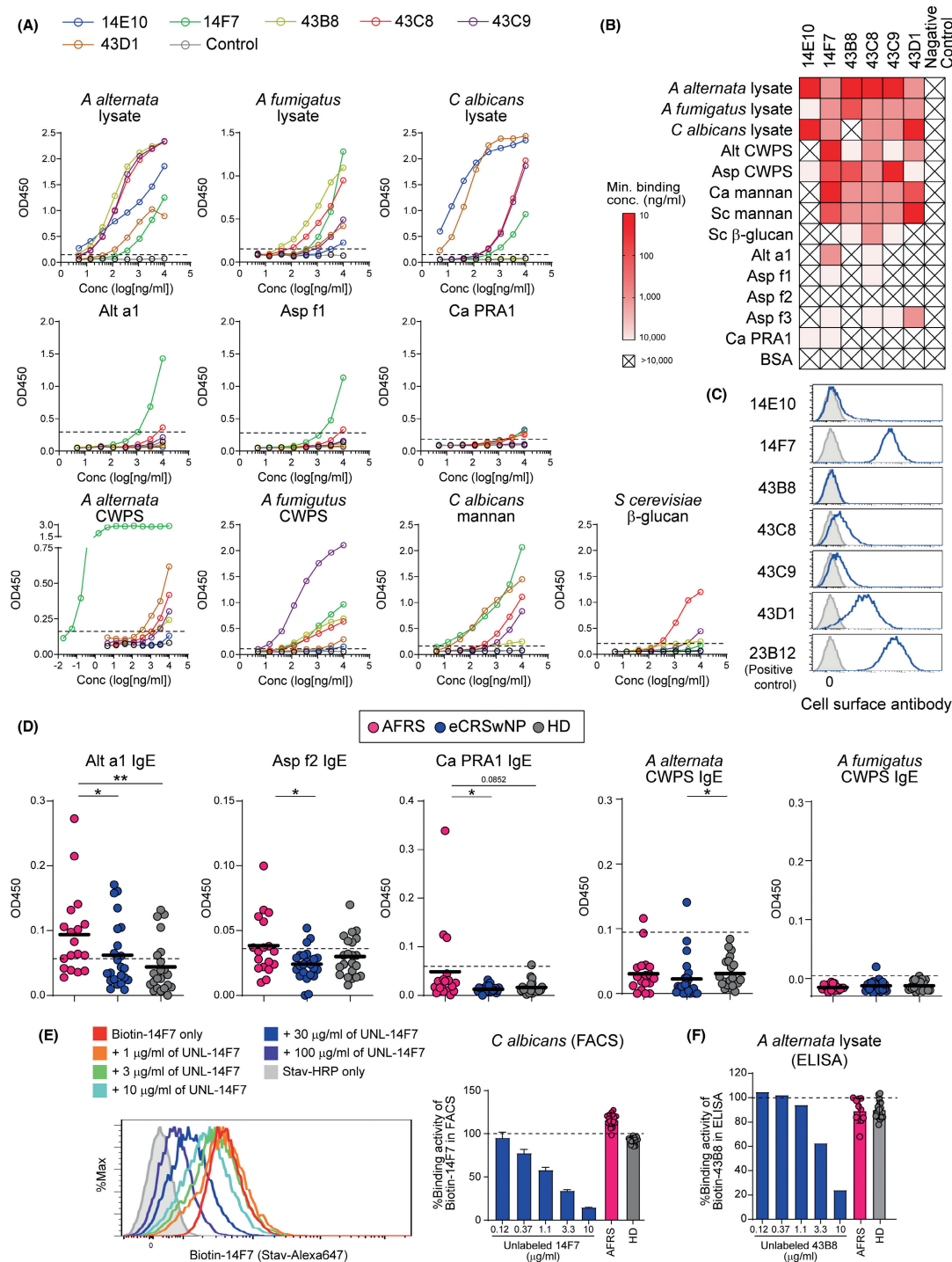


FIGURE 2 Anti-fungal cross-reactive IgE targets cell wall polysaccharides. (A and B) Reactivities of anti-fungal mAbs derived from nasal tissue-residing IgE⁺ plasmablasts with ELISA. Ca PRA1, *C. albicans*-derived PRA1; CWPS, cell wall polysaccharide; Ca mannan, *C. albicans*-derived mannan; Sc mannan and β -glucan, *Saccharomyces cerevisiae*-derived mannan and β -glucan. (C) Histogram of *C. albicans* stained with anti-fungal mAbs (10 μ g/mL) detected by flow cytometry. Grey histogram indicates cells stained with a negative control mAb. 23B12 was an anti-*Candida* mAb and used for positive control of the assay. (D) Titers of serum IgE Abs against major fungal allergens and CWPS. AFRS, $N = 18$; eCRSwNP, $N = 20$; HD (healthy donors), $N = 22$. (E) Competitive flow cytometry assay. *C. albicans* was pre-incubated with 1:30-diluted sera from AFRS ($N = 11$) and healthy donors (HD, $N = 17$) and then incubated with 3 μ g/mL of biotinylated mAb 14F7. Bound biotinylated 14F7 was detected by Alexa Fluor 647-conjugated streptavidin. Unlabeled 14F7 at indicated concentrations was used for positive control. The Δ MFI of biotinylated 14F7 without competitor serum was set to 100%. (F) Competitive ELISA using *A. alternata* lysate. Diluted sera (1:30) from AFRS ($N = 11$) and healthy donors (HD, $N = 17$) were pre-incubated in ELISA plates coated with *A. alternata* lysate. After 30-min-incubation, biotinylated mAb 43B8 was added. Bound biotinylated 43B8 was detected by HRP-conjugated streptavidin. Unlabeled 43B8 at indicated concentrations was used for positive control. The background subtracted OD₄₅₀ value of biotinylated 43B8 without competitor serum was set to 100%. (A–F) Representative results from three independent experiments are shown

nasal polyps of patients with AFRS and characterized their antigen reactivity.

In our cohort, AFRS patients had higher serum antifungal IgE and IgG titers than patients with eosinophilic chronic rhinosinusitis with nasal polyps (eCRSwNP) and healthy individuals (Figure S1A,B and Table S1). More than 55% of AFRS patients had serum IgE reactivity to at least two different fungal genera (Figure S1C), indicating that these patients were sensitized to multiple fungal genera.

As previously reported for eCRSwNP,³ IgE-expressing cells were observed in germinal center (GC) B-like cells and PBs of the mucosal tissues from patients with AFRS (Figure S2A–D and Table S4). We cloned IgE and Igκ or Igλ variable regions from mRNA of single nasal polyp-residing PBs isolated from two AFRS patients, AF14 and AF43 (Figure 1A and Table S5). Among the IgE sequences obtained, 20% and 50% were clonally related in AF14 and AF43, respectively (Figure 1B and Table S6). Clonally related anti-fungal immunoglobulins had shared somatic mutations as exemplified by sequences shown in Figure 1C. After subcloning of these variable region sequences into a human IgG1 expression vector, we obtained 19 recombinant mAbs derived from AF14, and 44 mAbs derived from AF43. About 11% of clones in AF14 and 59% of clones in AF43 reacted to at least one fungus (Figure 1D). More importantly, the vast majority of these anti-fungal clones were reactive to multiple genera of fungi (Figure 1D and Table S6). Such multi-fungal reactivities were highly dependent on somatic mutations (Figure 1E).

Then, representative mAbs selected from distinct clonal lineages were tested for their reactivity to fungal components. Most of the tested mAbs (5 of 6) turned out to be reactive to cell wall polysaccharides (CWPS) derived from different fungal genera including *Candida albicans*-derived mannan and *Saccharomyces cerevisiae*-derived β-glucan (Figure 2A,B). Indeed, they could bind to the surface of *C. albicans* as well (Figure 2C). On the other hand, these clones did not show significant binding to major fungal allergens such as Alt a1, Asp f1 and Ca PRA1. 14F7, a strong CWPS binder, also reacted weakly to Alt a1 and Asp f1 (Figure 2A). Although the exact mechanism by which this clone binds to protein components remains unclear, such weak binding may be due to polyreactivity that has been observed in a subset of mAbs against certain viruses.⁴ Interestingly, AFRS patients did not show higher serum IgE titers against fungal CWPS compared to eCRSwNP patients and healthy individuals, although their serum IgE displayed significantly higher reactivity to major fungal allergens, Alt a1, Asp f2 and Ca PRA1 (Figure 2D). We performed competitive binding assay using patients' sera as competitors for binding of mAbs to fungi. Sera from AFRS patients did not show any inhibitory effects on binding of biotinylated 14F7 to *C. albicans* in flow cytometry (Figure 2E). Furthermore, the binding activity of biotinylated 43B8 to *A. alternata* lysate was not substantially inhibited by sera from AFRS patients in ELISA (Figure 2F). These results indicate that serum anti-fungal Abs target different antigenic determinants from nasal polyp-derived IgE Abs in AFRS patients.

Fungal antigens targeted by cross-reactive IgE Abs are often intracellular housekeeping proteins presumably derived from dead

fungal cells and are not harmful to hosts.⁵ In this study, we characterized AFRS-associated IgE Abs at the clonal level and revealed that nasal mucosa-derived anti-fungal IgE mAbs target surface CWPS in AFRS patients like protective IgG Abs induced during fungal infection.^{6,7} Unexpectedly, the IgE Abs generated in the nasal mucosa do not appear to contribute to the reactivity of serum IgE in AFRS patients. Thus, anti-fungal IgE Abs in the nasal mucosa and sera appear to be generated from distinct responses in AFRS.

Previously, we have reported that local IgE in the nasal polyps of patients with eCRSwNP targets surface determinants of nasal resident bacteria.³ In addition, extensive class switching from IgG and IgA₁ to IgE has been observed in the nasal mucosa, suggesting that protective Ab responses to bacterial infection might be converted into allergic reactions in eCRSwNP.³ Together with the current study, the local IgE Ab response in allergic nasal mucosa might be initiated by a protective Ab response against local bacterial and fungal infections unlike other allergic diseases where IgE Abs are generated against non-harmful environmental substances.

ACKNOWLEDGEMENTS

We would like to thank all members of Laboratory of Immune Regulation, Immunology Frontier Research Center, Osaka University for their help on various aspects in this research.

FUNDING INFORMATION

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (grant no. 19K18753 to K.T.), and grants from the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to S.S.) and Kishimoto Foundation (to H.K.).

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

Soichiro Haruna¹

Kazuya Takeda^{1,2}

Marwa Ali El-Hussien¹

Yohei Maeda³ 

Masaki Hayama³

Takashi Shikina⁴

Katsumi Doi²

Hidenori Inohara³

Hitoshi Kikutani¹

Shuhei Sakakibara¹ 

¹Laboratory of Immune Regulation, Immunology Frontier Research Center, Osaka University, Osaka, Japan

²Department of Otolaryngology, Faculty of Medicine, Kindai University, Osaka, Japan

³Department of Otorhinolaryngology-Head and Neck Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

⁴Senri Nose Clinic, Osaka, Japan

Correspondence

Hitoshi Kikutani and Shuhei Sakakibara, Laboratory of Immune Regulation, Immunology Frontier Research Center, Osaka University, Osaka, Japan.

Emails: kikutani@biken.osaka-u.ac.jp (H.K.); sakakibara@ifrec.osaka-u.ac.jp (S.S.)

ORCID

Yohei Maeda  <https://orcid.org/0000-0003-1510-9191>

Shuhei Sakakibara  <https://orcid.org/0000-0003-3157-5870>

REFERENCES

1. Fokkens WJ, Lund VJ, Hopkins C, et al. European position paper on rhinosinusitis and nasal polyps 2020. *Rhinology*. 2020;58(Suppl S29):1-464.
2. Dykewicz MS, Rodrigues JM, Slavin RG. Allergic fungal rhinosinusitis. *J Allergy Clin Immunol*. 2018;142(2):341-351.

3. Takeda K, Sakakibara S, Yamashita K, et al. Allergic conversion of protective mucosal immunity against nasal bacteria in patients with chronic rhinosinusitis with nasal polyposis. *J Allergy Clin Immunol*. 2019;143(3):1163-1175 e1115.
4. Mouquet H, Nussenzweig MC. Polyreactive antibodies in adaptive immune responses to viruses. *Cell Mol Life Sci*. 2012;69(9):1435-1445.
5. Cramer R, Garbani M, Rhyner C, Huitema C. Fungi: the neglected allergenic sources. *Allergy*. 2014;69(2):176-185.
6. Casadevall A, Feldmesser M, Pirofski LA. Induced humoral immunity and vaccination against major human fungal pathogens. *Curr Opin Microbiol*. 2002;5(4):386-391.
7. Rudkin FM, Raziunaite I, Workman H, et al. Single human B cell-derived monoclonal anti-Candida antibodies enhance phagocytosis and protect against disseminated candidiasis. *Nat Commun*. 2018;9(1):5288.

SUPPORTING INFORMATION

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

DOI: 10.1111/all.15420

Fast challenge tests with gadolinium-based contrast agents to search for an alternative contrast media in allergic patients

To the Editor,

Gadolinium-based contrast agents (GBCA) are used in contrast-enhanced magnetic resonance imaging. Hypersensitivity reactions (HSR) to GBCA are scarce, with an incidence of 0.07% and a recurrence rate of 30%, being urticaria the most common presentation (91%), with a 0.52/10000 rate of severe reactions reported.¹

Recommendation of an alternative GBCA without a confirmed tolerance is hazardous, due to a high cross-reactivity between them.² Moreover, premedication is not enough,¹ showing a rate of breakthrough reactions overall of 39%.³

There is a growing tendency to perform allergy studies to achieve a safe recommendation in case of a HSR to GBCA. Negative predictive value of skin tests to GBCA has been estimated in 84%.¹ Thus, more than 10% of patients could react using an alternative negative skin-tested GBCA, and good tolerance to GBCA should be confirmed through a single-blind placebo-controlled drug challenge test (DCT).⁴ Furthermore, given that nephrogenic systemic fibrosis is more frequent in case of end stage renal failure, kidney function should be checked before performing a challenge test with GBCA. They are usually performed at graded administrations, and with observation periods between doses.^{1,5} But since GBCA is usually given in bolus, results at slow rates cannot be extrapolated to further administrations. Avoiding this limitation, we have developed a protocol for a prospective study searching the tolerance of an alternative GBCA, with a fast DCT approaching the rates used in clinical

practice. The same protocol was applied for both immediate and delayed HSR.

After obtaining signed informed consent from the patients, skin prick tests (SPT) with undiluted macrocyclic GBCA commercial solutions were done. When SPT results at 20 min were negative, intradermal tests (IDT) with 1:10 dilutions were performed (with readings at 20 min, and additionally at 24 h in case of a delayed reaction).

A fast DCT with negative skin-tested GBCA was performed later, according to our methodology to study HSR to iodinated contrast media,⁶ and using a volumetric pump Alaris® GW (BD®). Doses were 0.1 mg/kg for Gb and 0.2 mg/kg for Ga. One-third of total dose of Ga was administered at a rate of 120 cc/h and, immediately after, the remaining 2/3 at 80 cc/h. In case of Gb, infusion rates were half those of Ga, i.e., 1/3 at 60 cc/h and 2/3 at 40 cc/h. Total infusional time was 8 min. All patients had a normal renal function before the challenge test. Well-tolerated GBCA was recommended for subsequent examinations, and its tolerance was recorded if it was used later.

Recruitment period was between May 2018 and December 2021. Characteristics and study results of enrolled sixteen patients are summarized in Table 1. They were 12 women and 4 men, median age of 45.5 years (range 28–73). Adverse reactions to GBCA were immediate in 13 patients (12 urticaria and 1 anaphylaxis), and delayed exanthema in the remaining 3. Gb was involved in 11 reactions, and unknown GBCA in the other 5. Most of the patients (14/16) had been previously exposed to GBCA.