

Comparison of skin prick tests with specific serum immunoglobulin E in the diagnosis of fungal sensitization in patients with severe asthma

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Summary

Background It has been shown that patients with allergic bronchopulmonary aspergillosis (ABPA) and patients with severe asthma with fungal sensitization (SAFS) can benefit from antifungal therapy. It is not known whether allergy skin prick tests (SPT) or specific IgE tests are more sensitive in the identification of patients who are sensitized to fungi and who are therefore candidates for antifungal therapy.

Objectives To compare SPT and specific serum IgE tests for fungal sensitization in patients with severe asthma.

Methods We have undertaken SPT and specific serum IgE tests to six fungi (*Aspergillus fumigatus*, *Candida albicans*, *Penicillium notatum*, *Cladosporium herbarum*, *Alternaria alternata* and *Botrytis cineria*) and specific serum IgE test for *Trichophyton* in 121 patients with severe asthma (British Thoracic Society/SIGN steps 4 and 5).

Results Sixty-six percent of patients were sensitized to one or more fungi based on SPT and/or specific serum IgE results. Positivity to SPT and/or specific serum IgE was as follows: *A. fumigatus* 45%, *C. albicans* 36%, *P. notatum* 29%, *C. herbarum* 24%, *A. alternata* 22%, *B. cineria* 18%, *Trichophyton* 17% (specific serum IgE only). Concordance between the tests was 77% overall but only 14–56% for individual fungi. Twenty-nine (24%) patients were sensitized to a single fungus and seven (6%) were sensitized to all seven fungal species. Fifty percent of patients were sensitized to fungal and non-fungal extracts, 21% were sensitized only to non-fungal extracts, 16% were sensitized only to fungal extracts and 13% had no positive tests.

Conclusion This study is consistent with previous reports that fungal sensitization is common in patients with severe asthma. At present, it remains necessary to undertake both SPT and specific serum IgE testing to identify all cases of fungal sensitization. This may be important in the identification of patients with ABPA and SAFS who may benefit from antifungal therapy.

Keywords allergy, asthma, fungal sensitization, skin prick test, specific serum IgE test
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Introduction

It is estimated that over 300 million people world-wide have asthma placing a large burden on the patients, their families and healthcare systems [1]. In 2004 Asthma UK [2] estimated that 8 million people in the United Kingdom will have a diagnosis of asthma at some point in their life and that out of the 5.1 million people being treated, 2.6 million have regular serious debilitating symptoms. For approximately 250 000 UK asthma patients, existing asthma medicines are not sufficient to adequately control their symptoms. These patients are those who need frequent hospital admissions

and who rely on high-dose inhaled corticosteroids and frequent or continuous oral steroids (steps 4 and 5 of the BTS-SIGN Asthma Guidelines) [3].

The European Community Respiratory Health Survey (ECRHS) has shown that 5% of 11 355 European adults aged 20–44 had current asthma and 36% of this random population sample were atopic as defined by having one or more positive skin prick tests (SPT) [4]. Of these European adults, 4.4% were sensitized to *Alternaria* and 2.3% were sensitized to *Cladosporium* compared with 21% sensitized to house dust mite (HDM), 17% sensitized to grass pollen and 10% sensitized to cat. This suggests that

fungal sensitization is much less common than other sensitizations in the general population in Europe.

However, fungal sensitization seems to be much more prevalent in populations of patients with severe asthma. Approximately 25% of adult asthma patients referred to specialists and up to 75% of patients with severe asthma requiring multiple hospital admissions are sensitized to one or more fungi [5, 6]. It is known that patients with one specific type of fungal sensitization [allergic bronchopulmonary aspergillosis (ABPA)] can benefit from treatment with antifungal agents [7–9] and it has recently been demonstrated by our group that patients with severe asthma with fungal sensitization (SAFS) may also benefit from antifungal therapy [10]. Accurate diagnosis of fungal sensitization in patients with severe asthma has become increasingly important because of the therapeutic implications for patients with ABPA or SAFS.

SPT and specific serum IgE tests are the most common methods used for diagnosis of sensitization to common inhaled allergens [11]. SPT involves the direct application into the skin of commercially prepared solutions to detect the presence of an IgE antibody response with a 95% accuracy in predicting negative results [12]. Accuracy for positive results is less at 50–60%, although these variations depend on the reagent and manufacturer used, as well as the skill of the tester, potency of solutions, interpretation of results and patient use of antihistamines or steroids [12–15]. There is also substantial geographic variation in the prevalence of sensitization to aeroallergens, including fungi [4, 15].

In vitro measurement of specific IgE antibodies can be useful in patients who cannot undergo SPT (those with eczema or taking antihistamine therapy). It is more costly than SPT and its use should be justified [16]. Smits *et al.* [14] found that only 43% of patients reacted to both SPT and specific serum IgE tests when tested for common aeroallergens and foods. They recommended the use of both tests to gain a definitive diagnosis, as not all sensitivities will be identified with the use of one alone. It has been reported that SPT are more sensitive but less specific than serum IgE tests to diagnose allergy in subjects with asthma or rhinitis [15]. It has also been reported that the CAP system for specific serum IgE testing has higher sensitivity than RAST tests with comparable specificity [17]. Because of these discrepancies, the use of specific serum IgE tests to support findings of SPT and clinical history is widely advocated [12, 15, 18].

As neither SPT nor specific serum IgE testing to fungi has been prospectively evaluated in severe asthma or SAFS, we undertook the present study as a component of the screening for inclusion in a randomized, placebo-controlled study of antifungal therapy for SAFS [10]. The present report describes in detail the methodology that was used in the above study to confirm fungal sensitiza-

tion. We prospectively compared the SPT and specific serum IgE results of adults with severe asthma.

Methods

Patients with asthma classified as BTS-SIGN category 4 (poor asthma control on moderate dose inhaled steroid therapy and add-on therapy) or step 5 (continuous or frequent use of oral steroids) were assessed at four university hospital sites in Manchester and Lancashire [3]. Patients with known ABPA were excluded from the study as they were not eligible for inclusion in the FAST trial [10]. Referrals were made by general practices, tertiary referrals and self-referral from press coverage. All patients gave informed consent for testing. Ethical approval was obtained from the UK ethics service (Central Office of Research Ethics Committees) and the Multi-centre Research Ethics Committee).

Screening for fungal sensitization was undertaken as the first step in enrolment in a placebo-controlled study of itraconazole therapy for patients with SAFS [10]. SPT and specific IgE was tested for *Aspergillus fumigatus*, *Alternaria alternata* (*tenius*), *Candida albicans*, *Cladosporium herbarum*, *Penicillium chrysogenum* (*notatum*), *Botrytis cinerea* and *Trichophyton species* (specific serum IgE only).

The Phadia (previously Pharmacia) CAP system was used to quantify total and specific IgE levels (Phadia Ltd, Uppsala, Sweden). A positive test was taken as a measurement >0.4 kIU/L and total IgE was considered to be elevated if the value was >100 kIU/L.

Percutaneous SPT with reagents and lancets from Allergopharma (Reinbek, Germany) was carried out by two research nurses using a standardized protocol. SPT and specific serum IgE testing were evaluated for the same range of fungi with the exception of *Trichophyton* which was not included in the SPT. SPT was also undertaken with timothy grass, HDM, cat and dog to determine the overall level of atopy in the studied population. A positive control solution of 0.1% histamine and negative solution of 0.9% saline were used. Weal size was measured at 15 min, weals with diameter at least 3 mm greater than the negative control were taken as 'positive' [19]. Any patient on antihistamine therapy was asked to omit it for at least 48 h before testing.

Results were entered into an Excel database and analysed using Excel and SPSS

Results

One hundred and twenty-one patients with severe asthma (48 male, 73 female, mean age 49.4 years and range 18–79) met clinical criteria for screening for inclusion in the FAST study of itraconazole treatment for SAFS [10]. All of these patients had SPT and specific serum IgE tests as part of the screening and recruitment phase of this study.

Table 1. Overall skin prick test (SPT) and specific serum IgE results for fungal reagents

	Number of patients screened	SPT and specific serum IgE both positive number (%)	SPT and specific serum IgE both negative number (%)	≥ 1 SPT positive but specific serum IgE all negative number (%)	≥ 1 specific serum IgE positive but SPT all negative number (%)
BTS level 4	108	48 (44.4%)	35 (32.4%)	11 (10%)	14 (13%)
BTS level 5	13	4 (31%)	6 (46%)	1 (8%)	2 (15%)
Total	121	52 (43%)	41 (34%)	12 (10%)	16 (13%)

$P = 0.74$ by Fisher's exact test.

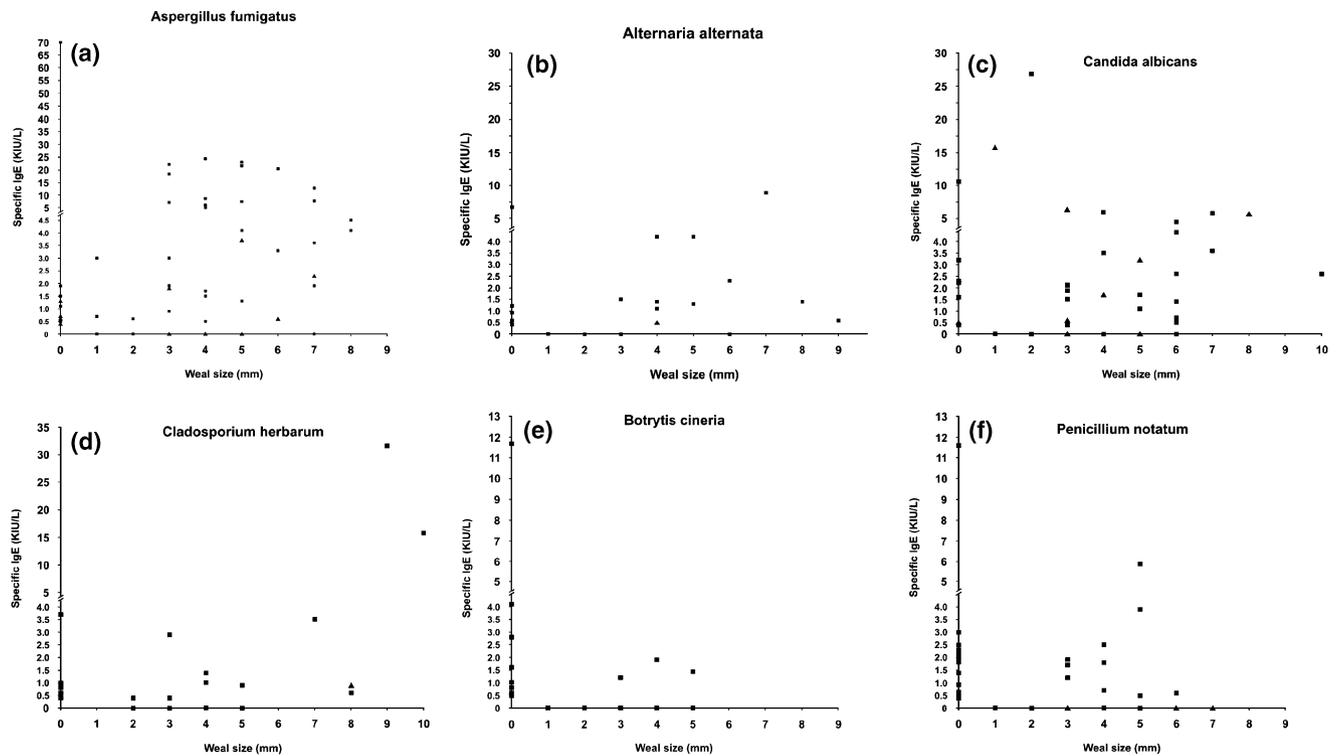


Fig. 1. Specific IgE levels (vertical axis) compared with skin prick test (SPT) weal size (horizontal axis). (a) *Aspergillus fumigatus* Spearman correlation -0.585 ($\rho < 0.001$). (b) *Alternaria alternata* (*tenius*) Spearman correlation -0.519 ($\rho < 0.001$). (c) *Candida albicans* Spearman correlation -0.531 ($\rho < 0.001$). (d) *Cladosporium herbarum* Spearman correlation -0.512 ($\rho < 0.001$). (e) *Botrytis cineria* Spearman correlation -0.189 ($\rho = 0.038$). (f) *Penicillium chrysogenum* (*notatum*) Spearman correlation -0.257 ($\rho = 0.004$). \blacktriangle depicts patients who were sensitized to only one fungus. Positive IgE > 0.4 kIU/L. Positive SPT – weal size 3 mm or more.

The total IgE levels ranged from 8 to 3189 kIU/L, with a median of 200 kIU/L. Forty-three patients had normal total IgE levels (< 100 kIU/L), 51 had levels 100–500 kIU/L, 18 had levels > 500 kIU/L and nine had levels > 1000 kIU/L.

Sixty-six percent (80/121) of subjects were sensitized to one or more fungi by SPT or specific serum IgE tests or both. Table 1 and Fig. 1 summarize the overall results of allergy SPT and specific serum IgE results. Weal size varied from 3 to 10 mm. The total IgE level of these fungal-sensitized patients varied from 24 to 3189 kIU/L. SPT and specific serum IgE tests agreed, positively or negatively in 93 (77%) of patients but 28 patients (23%) had discordant SPT and specific serum IgE results. Skin prick weal size increased proportional to the level of specific IgE for all fungi except *Penicillium* and *Botrytis* (Fig. 1).

Of the patients with at least one positive SPT or specific serum IgE result, the test results for the individual reagents are shown in Table 2. On average, 30% of patients were sensitized to each individual fungal extract (positive SPT or specific IgE) with 12% having positive responses to both tests, 7% had positive SPT only and 11.5% had specific IgE only. The number of positive specific IgE tests to individual fungi was greater than the number of positive SPT results for five of the six fungal species for which both tests were conducted. The concordance rates for individual fungi ranged from 14% for *Botrytis* to 56% for *Alternaria* and the average was only 40%. In addition 21 patients (17.3%) had specific serum IgE antibodies to *Trichophyton* (no SPT done). Seven patients were sensitized to all seven fungi tested and 29/121 (24%) patients were sensitized to only

Table 2. Skin prick test (SPT) and specific serum IgE results for individual fungi

	SPT or specific IgE test positive	SPT and specific IgE both positive	SPT positive but specific IgE negative	Specific serum IgE positive but SPT negative	Concordance per species among positive results
<i>Aspergillus</i>	54 (45%)	29 (24%)	10 (8%)	15 (12%)	54% (29/54)
<i>Candida</i>	43 (36%)	22 (18%)	9 (7%)	12 (10%)	51% (22/43)
<i>Penicillium</i>	35 (29%)	10 (8%)	10 (8%)	15 (12%)	29% (10/35)
<i>Cladosporium</i>	29 (24%)	10 (8%)	11 (9%)	8 (7%)	35% (10/29)
<i>Alternaria</i>	27 (22%)	15 (12%)	4 (3%)	8 (7%)	56% (15/27)
<i>Botrytis</i>	22 (18%)	3 (2%)	8 (7%)	11 (9%)	14% (3/22)
Mean	35 (30%)	15 (12%)	9 (7%)	12 (10%)	40%

Table 3. Number of subjects sensitized to one or more fungi

Number of fungi to which each patient reacted	0	1	2	3	4	5	6	7
Number of patients	41	29	11	11	12	7	3	7
Percent of patients	34	24	9	9	10	6	2	6

one fungus (Table 3). Of the 29 patients who were sensitized to a single fungal species, 13 subjects (45%) were sensitized to *Aspergillus*, eight (28%) were sensitized to *Candida*, three were sensitized to *Trichophyton* (10%) and five subjects had an isolated response to *Alternaria* (one), *Cladosporium* (one) or *Penicillium* (three). No patient was sensitized only to *Botrytis*.

Overall 19 patients (16%) were solely allergic to fungi with no response to other aeroallergens such as grass or HDM. Twelve of these 19 patients with isolated fungal sensitization were sensitized to a single fungus (eight *Aspergillus*, two *Candida*, one *Trichophyton* and one *Penicillium*).

One patient was found to have definite ABPA with positive *Aspergillus* precipitins in addition to positive SPT and specific IgE. All other patients had negative *Aspergillus* precipitins. Of the nine patients with serum IgE exceeding 1000 kIU/L, values varied from 1000 to 3189 kIU/L. Of these patients, one had negative SPT and specific serum IgE to all fungi tested, one negative specific IgE tests, but a positive SPT to *Candida* and *Botrytis*, and the remainder positive specific serum IgE tests to one or more fungi including six to *Aspergillus* and one to *Candida* only. However, the *Aspergillus* IgE test values varied substantially, from 0.6 to 70 kIU/L, with other fungal IgE tests being very high in individual patients. Examples include a total IgE of 1200 with a *Trichophyton* IgE of 20.1 kIU/L (*Aspergillus* 1.9 and a positive *Aspergillus* SPT); another a total IgE of 1800, a *Trichophyton* IgE of 42.9 kIU/L (*Aspergillus* 1.5) and two patients with only marginally elevated IgE to all fungi (up to 2.3 kIU/L), with total IgEs of 1600 and 2700 kIU/L. Thus one patient almost certainly had ABPA and five had serology consistent with it, but three would probably be better defined as

Table 4. Sensitization to fungal and non-fungal reagents

Sensitization	Number	%
Fungi only	19	16
Non-fungal reagents only	25	21
Both	61	50
Neither	16	13

having allergic bronchopulmonary mycosis, one probably to *Candida* and two to *Trichophyton*.

Overall 71% (86/121) of subjects were sensitized to non-fungal aero-allergens such as HDM (63), cat (50), dog (29) and timothy grass (56). Of these 86 subjects, 61 were also sensitized to fungi and 25 patients were atopic but not sensitized to fungi (Table 4).

Discussion

This study on a selected population of UK patients found that atopic sensitization (both fungal and non-fungal) was common among patients with severe asthma who were screened for possible recruitment to a trial of antifungal therapy. Although patients with known ABPA were excluded from this study, *A. fumigatus* was still the most frequent fungus to which sensitization was detected with *C. albicans* the second commonest. The number of cases of confirmed ABPA at each centre is not documented but the number of cases of ABPA known to the investigators is substantially lower than the number of cases of SAFS identified at each centre.

Overall, 66% of our patients with severe asthma were sensitized to one or more fungi based on SPT or specific serum IgE testing or both. Most of these patients (64%) were sensitized to (or cross-reacted to) more than one fungal species and the majority (76%) of those with fungal sensitization were also sensitized to non-fungal reagents such as HDM. This is the biggest study of fungal sensitization in patients with severe asthma (BTS-SIGN steps 4 and 5) and the first study to show that fungal sensitization was similar at both levels of asthma severity. The proportion of

patients with severe asthma who were sensitized to fungi in the present study (66%) was similar to that reported in a smaller study from New Zealand (54%), and the number with *Aspergillus* sensitivity in the present study (45%) was similar to that reported recently in patients with severe asthma admitted to an intensive care unit in India (51%) [20, 21]. Importantly, 16% of our patients were solely allergic to fungi and not sensitized to other common aeroallergens. These patients would have been described as 'non-atopic' if a standard set of allergy tests did not include fungal reagents.

Most of the patients in this study were already attending the severe asthma clinics at four University hospitals and others were recruited from a primary care setting. It is likely that these patients were representative of patients with severe asthma in North West England but it is probable that sensitization to other fungi might be found at other geographical locations, depending on the prevailing fungal aerobiology in each area. For example, the ECRHS showed varying levels of sensitization to *Alternaria* and *Cladosporium* in the general population in different parts of Europe [4].

Documentation of fungal sensitization is important because fungal spores are present in external and internal environments and are smaller than pollen grains making them easy to inhale. Allergy to fungi is associated with increased asthma symptoms and severity, increased asthma admissions (including intensive care admissions) and even death [5]. A previous Manchester study showed that 75% of patients with multiple hospital admissions for asthma were sensitized to one or more fungi and the excess admissions tended to occur during the late summer-autumn season when mould spore counts in the United Kingdom are at their highest [6].

Although 77% of patients in the present study had concordant SPT and specific serum IgE tests for fungal extracts, 23% had discordant results. This was an expected finding as the reagents were from different manufacturers who used different processing methods and the biochemistry of fungal allergens is poorly understood at present with the result that there is a lot of batch-to-batch variation in fungal extracts. It is disappointing that the standardization of reagents for testing for IgE-mediated fungal sensitization has lagged behind other areas of IgE testing in the past three decades. For example, a comparison of SPT and RAST tests for non-fungal sensitization in 1976 reported 83% agreement between SPT and specific IgE testing while the level of agreement for fungal sensitization in a 1985 report was only 62% and we found 77% concordance for fungal sensitization in 2007 [22, 23]. Although the CAP system represents progression from the previous RAST systems, the extracts that are used to produce the CAP reagents are not available for the production of SPT reagents.

The level of agreement between SPT and specific IgE tests for individual fungi has improved even less over recent decades. We found only 54% concordance between

SPT and specific IgE testing for *Aspergillus* sensitization despite the long-established importance of making an accurate diagnosis of ABPA and we found concordance levels of only 29% for *Penicillium* and 14% for *Botrytis*. Angrisano and colleagues reported 66% correlation between SPT and RAST tests for *Alternaria* in a group of 253 children in 1987 but Negrini's study published in 2000 reported that only 33% of patients with positive *Alternaria* SPT had a positive RAST test and we found only 56% concordance between SPT and specific IgE for *Alternaria* in 2007 [24, 25]. Most previous studies have found a greater number of positive SPT than specific IgE tests but our patients had slightly more positive specific IgE tests, possibly reflecting an improvement in the sensitivity of this technique over the past few decades.

It has recently been reported that the relationship between specific IgE level and SPT for *Cladosporium* was weak despite using reagents from the same manufacturer for both tests and we found only 35% concordance for this reagent in our study [15]. Furthermore mould allergens are poorly characterized at present, for example, >60 IgE binding proteins have been identified in *A. fumigatus* [26]. Each fungal species produces many different proteins which are capable of stimulating an IgE response [27]. The discordance rates for individual fungal reagents ranged from 46% to 86%. We would have missed 19% of cases of *Aspergillus* sensitization if we had used specific serum IgE tests alone and we would have missed 28% if we had used SPT alone, thus potentially missing the diagnosis of ABPA and SAFS in many cases if SPT alone or specific serum IgE alone were used to screen patients for these conditions. It is likely that slightly different results would have been obtained if we had used SPT reagents or specific serum IgE reagents from a different manufacturer.

Part of the problem is that some allergies are caused by sensitization to a few proteins or even a single molecule but others are caused by diverse and complex allergen expression [28]. Furthermore, it has been reported in the case of HDM allergy confirmed by bronchial provocation tests that SPT were more sensitive than specific IgE tests but the latter was more specific [29]. The discrepancies between skin tests and specific IgE tests are even greater if intradermal tests are used [30]. Despite this lack of progress, in recent decades, it is hoped that the new techniques based on cloning of fungal allergens and possibly the use of tandem mass spectrometry will allow improvement in this important field in the near future [28, 31].

Although some of the discrepancy between skin tests and specific IgE tests is likely to be technical in nature, it is also likely that some of the differences are due to the diversity in the physical expression of atopy in different individuals and populations. Studies in Kenya have shown that rural African children have fewer skin test responses than urban African children and bronchial hyper-reactivity (exercise-induced bronchospasm) was correlated to

skin test atopy in the urban Kenyan children but not in the rural children [32].

It is important to diagnose ABPA because of the distinctive clinical features and because of the established response to antifungal treatment [7–9]. However, some patients with ABPA do not have severe asthma and it has recently been shown that patients with SAFS may also benefit from antifungal treatment [10]. By definition, all patients with SAFS have severe asthma and our results suggest that the results of fungal sensitization tests may have therapeutic consequences for about 25% of patients with severe asthma and 36% of those with positive results [10]. This increases greatly the importance of not missing fungal sensitization. It is likely that fungal allergens will be better characterized and better standardized in the future and this may allow testing with a single modality. However, because of the error rate of both modalities (SPT and specific serum IgE) when used in isolation at the present time, we recommend the use of both tests for patients with severe asthma that does not respond to treatment at steps 1–3 of the BTS-SIGN Asthma Guidelines. The use of both test modalities should identify the small proportion of asthma patients with ABPA and the higher proportion of asthma patients who have SAFS, which we have found to be a common, important and largely neglected clinical problem for which therapeutic options are now available. Future progress in the diagnosis and management of fungal asthma will depend on more accurate characterization of fungal allergens and the preparation of better test reagents using new technologies. The development of recombinant allergens for the diagnosis of ABPA represents an exciting area of research in this field [33].

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Conflicts of interests: The authors have no conflicts of interests.

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