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 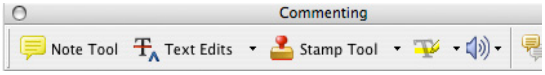

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 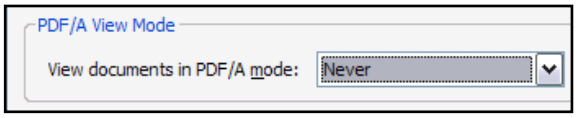

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 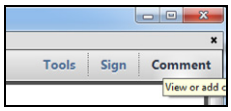

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 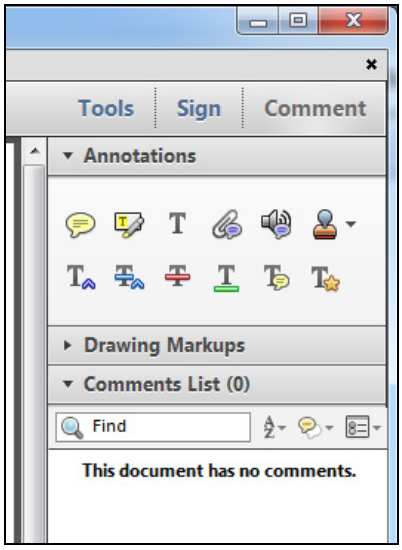
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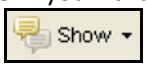
Review	To review your changes, click on the 'Show' button  on the Commenting tool bar. Choose 'Show Comments List'. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.	Your changes will appear automatically in a list below the Comment tool bar. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.
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Letter to the Editor

Allergic fungal rhinosinusitis—more than a fungal disease?

To the Editor:

Allergic fungal rhinosinusitis (AFRS) is a noninvasive form of fungal disease mostly caused by *Aspergillus* species.[1] Patients with AFRS commonly present with chronic rhinosinusitis with nasal polyps (CRSwNP), inhalant atopy with specific IgE antibodies present in serum against *Aspergillus* species, elevated total serum IgE, and sinus-obstructing viscoelastic eosinophil-rich material called "allergic mucin," containing sparse numbers of fungal hyphae.[1] This constellation of findings is shared with allergic bronchopulmonary aspergillosis. Characteristic computed tomography findings including complete opacification of at least 1 paranasal sinus, a typical heterogeneity of the signal within involved sinuses, and the expansion and attenuation/erosion of the bone with displacement of adjacent anatomic compartments can be seen in AFRS (see Fig E1 in this article's Online Repository at www.jacionline.org).[1]

There is no doubt that fungi can function as allergens and induce an allergic IgE-mediated reaction; allergic disease should generally be considered a superimposed problem that contributes with a variable but relatively mild impact on the inflammation seen in most patients with CRS.[2] In that line of thinking, *Aspergillus* species may induce the formation of specific IgE antibodies to fungal allergens; however, the exorbitant elevations in serum total IgE level that is typical for AFRS cannot be explained. We therefore hypothesized that another stimulus with superantigenic properties is involved in aspergillus-related airway disease with high IgE production.[3]

Superantigens from *Staphylococcus aureus* are possible candidates for such an effect and have been demonstrated to be involved in upper and lower airway disease, preferentially amplifying T_H2-biased inflammatory reactions in CRS with nasal polyps and asthma, and inducing a local mucosal production of high concentrations of polyclonal IgE and IgG antibodies.[4] The mechanisms in AFRS could be similar, with *Aspergillus* preparing the ground for the impact of superantigens by breaking the epithelial barrier and initiating a T_H2 bias. *S aureus* could profit from the disturbed barrier and the weakened innate response of the sinus mucosa, partially created by this T_H2-bias, leading to the alternative activation of macrophages with a decrease in phagocytotic and intracellular killing properties for the germs, and symbioses with *Aspergillus* species to elicit a polyclonal B- and T-cell activation.[5] A recent retrospective review showed that *S aureus* was significantly more prevalent in the AFRS group compared with other subsets of CRSwNP.[6]

Here we investigated serum samples of AFRS (n = 17), CRSwNP (n = 13), and healthy control (n = 12) patients, sampled in Riyadh, Saudi Arabia, with AFRS being abundant in this region. AFRS was diagnosed according to the clinical criteria of Schubert.[7] *Aspergillus fumigatus* was found in the nasal samples of patients with AFRS, but not in CRSwNP subjects or controls; concentrations of serum total IgE, specific IgE antibodies to the classical *S aureus* superantigens (SE-IgE), specific IgE antibodies to *Aspergillus* species (m3-IgE), and eosinophil cationic protein were measured by using the CAP system (Phadia) Q1 as detailed earlier.[6] We furthermore studied formalin-fixed tissue removed from the sinuses in 3 subjects with AFRS by in situ hybridization (PNA-FISH) by using panfungal and *S aureus*–specific probes as detailed before.[8] Q2

In 13 of 17 sera from subjects with AFRS, but not in control or CRSwNP subjects, specific IgE to *A fumigatus* (m3-IgE) could be detected (median and interquartile range, 8.7 kU/L [2.3-12.9 kU/L]); total IgE level was strongly elevated above normal values in patients with AFRS (median [interquartile range], 1220 [226-3287] kU/L). Sixteen of 17 subjects with AFRS also expressed SE-IgE in serum (3.5 [0.6-7.2] kU/L). There was a

TABLE I. Serum total and specific IgE concentrations (median = IQR) of patients with AFRS versus non-AFRS CRS and control subjects

	CRSwNP	AFRS	Control	*P* value	
				AFRS vs CRSwNP	AFRS vs control
Samples, n	13	17	12		
IgE (kU/L)				<.005	<.0001
Median	220.0	1220.0	36.7		
IQR	96.6-448.0	226.8-3287.5	17.7-63.0		
ECP (μg/L)				NS	NS
Median	10.2	21.7	9.3		
IQR	6.1-29.4	9.9-49.0	4.9-31.8		
SE-IgE (kUA/L)				NS	<.004
n	10/13	16/17	3/12		
Median	0.4	3.5	0.5		
IQR	0.3-0.72	0.64-7.2	0.24-0.93		
m3-IgE (kUA/L)				<.0001	<.0001
n	0/13	13/17	0/12		
Median		8.7			
IQR		2.3-12.9			

ECP, Eosinophil cationic protein; *IQR*, interquartile range; *NS*, not significant.

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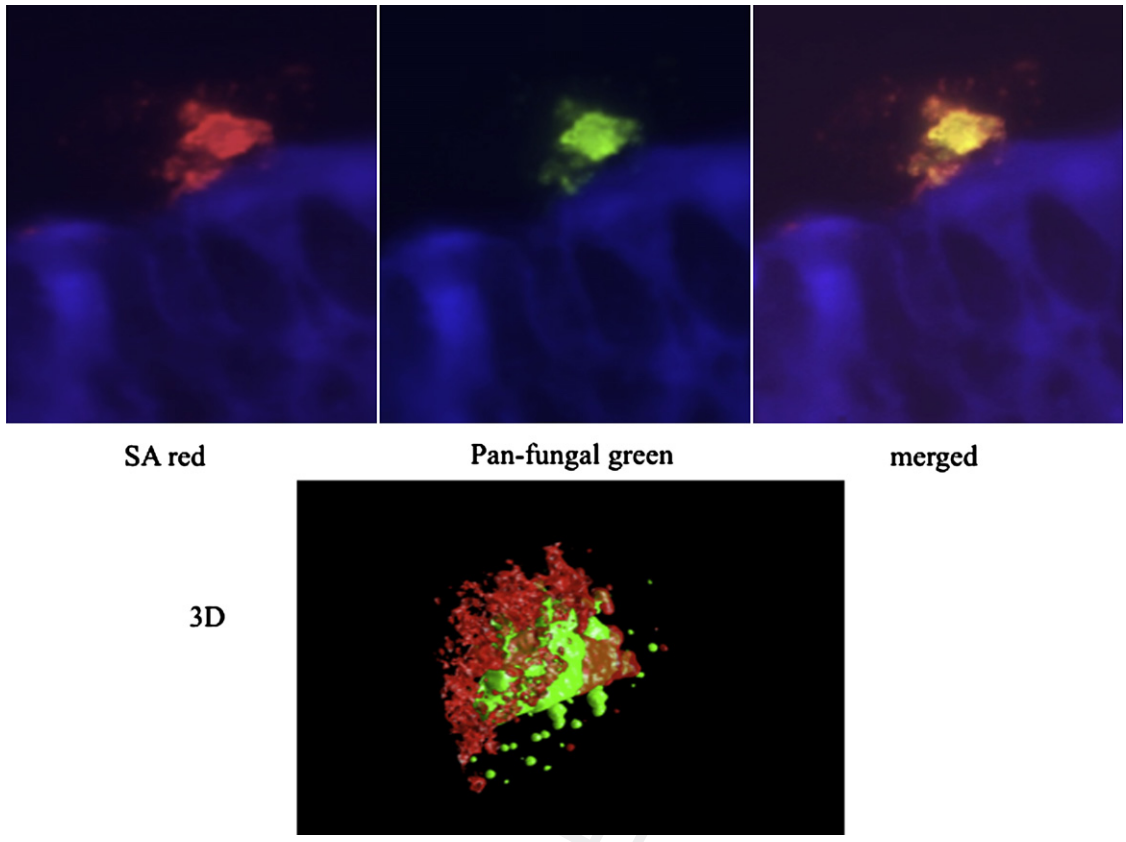

FIG 1. In situ hybridization (PNA-FISH) of a biofilm-like structure on the epithelium of paranasal sinus mucosa in a patient with AFRS. *Red*: *Staphylococcus aureus*; *green*: fungus (*Aspergillus fumigatus* according to culture). Confocal microscopy, magnification ×63. *PNA-FISH, XXX-XXXX.*

significant and strong correlation of SE-IgE with total IgE ($r = 0.78$; $P < .002$), whereas m3-IgE did not correlate with total IgE ($r = 0.31$; $P = .37$). However, the co-expression of *A fumigatus*–specific IgE antibodies in AFRS did amplify the total IgE concentrations significantly versus CRSwNP. Serum eosinophil cationic protein tended to be higher in patients with AFRS than in control patients (21.7 vs 9.3 μg/L), but differences did not reach statistical significance. For all results, please see Table I. [T1]

In all 3 tissue samples, we could discover biofilms adherent to the epithelium, carrying fungus and *S aureus* together. A representative confocal microscopy, showing the staining with the fungal probe (*Aspergillus* species has been demonstrated in the same patients by culture), the probe for *S aureus*, and the three-dimensional reconstruction of the combination of both is depicted in Fig 1. [F1-4/C]

On the basis of our findings of *S aureus* coexisting with *Aspergillus* species within the sinuses, and the presence of SE-IgE in the sera of nearly all subjects with AFRS, significantly correlating with the total IgE (in contrast to m3-IgE, which does not correlate with total IgE), we hypothesize that *S aureus* may play a crucial role in AFRS. We estimate that *S aureus* synergizes with or makes use of *Aspergillus* species in creating a T_H2 tissue signature, and adds its superantigenic activities to the disease, resulting in the high total IgE concentrations typically found in AFRS.[9] In this way, *Aspergillus* species and *S aureus* benefit from each other's potential to overcome the mucosal barrier, bias the immune system, and cause the fulminant characteristics of AFRS.

This hypothesis is supported by the published literature demonstrating a role for *S aureus* superantigens in upper airway disease, specifically in adult nasal polyp disease unrelated to AFRS.[4] The presence of SE-IgE in 10 of the 13 patients with CRSwNP and a total IgE concentration of 220 kU/L in this investigation is in line with former findings.[3,10] Also, in CRSwNP, we reported a T_H2 signature of the mucosal inflammation, which is amplified by *S aureus* enterotoxins resulting in high total IgE tissue and serum concentrations and an increase in the degree of eosinophilic inflammation. The involvement of *S aureus* and its superantigenic activities in AFRS and possibly allergic bronchopulmonary aspergillosis does open a new insight into the pathophysiology of this disease, may have an impact on our diagnostic tools in aspergillus-related airway disease, and finally may result in new therapeutic options including anti-IgE strategies.[10]

The role of *S aureus* in the disease needs to be demonstrated by the local presence of *S aureus*–derived enterotoxins and *S aureus*–specific interventions in comparison to antifungal therapeutic approaches. The contribution of the polyclonal IgE antibodies in patients with AFRS needs to be studied by making use of anti-IgE strategies.

Q8 Q3 Q4 Ghent, Belgium
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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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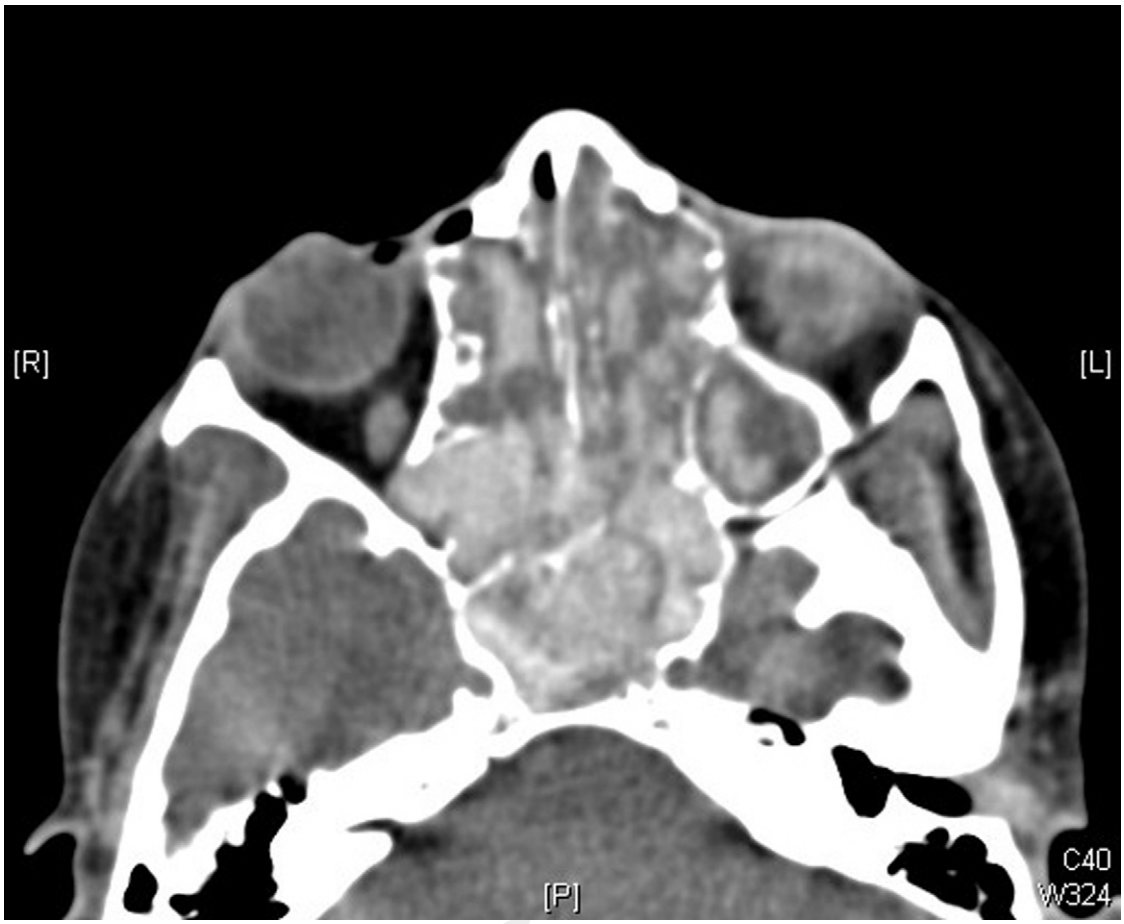

FIG E1. Typical computed tomography scan of AFRS with complete opacification of the paranasal sinuses, typical heterogeneity of the mucosal signal, and displacement of adjacent anatomic structures.

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