Methodologic issues concerning Stachyhemolysin and Stachyrase-A as clinical biomarkers

Dear Editor,

We are writing about the recently published article "Antibodies against *Stachybotrys chartarum* extract and its antigenic components, Stachyhemolysin and Stachyrase-A: a new clinical biomarker" [1]. The author states that his hypothesis is "that environmental exposure to Stachyhemolysin and Stachyrase-A may result in antibody production against these mold antigens." There are numerous methodologic deficiencies in this paper which preclude drawing any conclusions regarding the utility of anti-stachyhemolsyin and anti-stachyrase-A antibodies as biomarkers.

First, and most basically, it is unknown if the sera that were tested for these antibodies came from individuals with documented exposure to *Stachybotrys chartarum*, i.e. persons who had spent considerable time in an environment with significant Stachybotrys chartarum contamination. Because there are no acceptable biomarkers of exposure to *Stachybotrys chartarum* in humans [2], it is impossible to know if the patients had any *Stachybotrys chartarum* exposure. The sera used are all from persons with very high IgE levels and positive for IgE to different mold extracts, although which mold extracts are not reported. These individuals would not be representative of the general population who would be exposed to *Stachybotrys chartarum.* The author suggests that the observed antibody reactivity to their antigen preparations make them possible clinical markers without showing the reactivity of other mold-sensitive but *Stachybotrys*-negative sera or showing any data on the species-specificity of the antibodies they detect. There is enormous cross-reactivity among IgG and IgM from related and even unrelated fungal species [3], making it impossible to determine whether their primary sensitization was to *Stachybotrys chartarum.*

Second, there are no controls, or unexposed sera tested as comparison. It appears that the author is using the presence of IgG to *Stachybotrys chartarum* as his "gold standard" for exposure; however, this has been shown not to be a useful marker of exposure [2]. Indeed, the author states that IgG and IgE to *Stachybotrys chartarum* may be the result of cross-reactivity between other fungi. In fact, four specimens with IgG to *Stachybotrys chartarum* extract were negative for IgG to Stachyhemolysin and Stachyrase-A, and vice versa. Similar results were found for IgE. While the author rightly attributes this to cross-reactivity from other fungi there is no way to determine whether any of the reactivity he reports is *Stachybotrys* specific or cross-reactivity from other fungi. We refer the author to a monograph reviewing methods for the design of epidemiologic studies with immunotoxic endpoints [4].

Third, there are several issues with the test methods and their interpretations. For example, the author states that optical density greater than 4 times the background was considered positive, while in the Figure 2 legend he says 4 times the standard deviation is positive. Figure 2 also suggests that both the IgG and IgE ELISAs have the same levels of variance and mean optical density values for anti-HSA although the sera were diluted 1:2 for IgE and 1:100 for IgG, a finding we feel is statistically improbable. Further, in reviewing the reference cited for the isolation of Stachyrase-A [5], the authors of that paper state that only a short (ATQTGA) N-terminal sequence could be identified; there was difficulty in obtaining longer sequences. Did the Stachyrase-A N-terminal sequence used by Vojdani [1], contain only 6 amino acids? A simple BLAST search reveals at least 20 proteins that contain an identical sequence, none of which are Stachyrase-A.

Based on the findings from the 50 subjects (patients in the Houston and Los Angeles areas) in this article and 139 blood donors (presumably from the Kansas City, MO area) [6], the authors state "It appears that about 10% of the general population and 27.4% to 42% of symptomatic mold exposed individuals have IgE antibodies to *S. chartarum* antigens." We don't believe this limited (both geographically and by size) meta-analysis can be representative of the general US population or warrant such a conclusion.

In closing, we feel there are significant problems with this paper which preclude the author's conclusions that antibodies to Stachyhemolysin and Stachyrase-A are new clinical biomarkers of exposure to *Stachybotrys chartarum.*

The findings and conclusions in this letter to the editor are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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Sincerely,

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