

COMMENTARY

Fungus and chronic rhinosinusitis: Weighing the evidence

Richard R. Orlandi, MD, and Bradley F. Marple, MD, Salt Lake City, UT; and Dallas, TX

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ABSTRACT

The hypothesis that fungus causes most, if not all, cases of chronic rhinosinusitis (CRS) has been debated for over a decade. Many opinions and interpretations have been rendered, but it is the objective data that speaks the loudest. The debate simply boils down to a core tenet of the scientific method: Can the data be independently replicated? If so, our patients benefit as new treatments are developed. If not, then the hypothesis must be discarded and new lines of research pursued.

Initial clinical trials supporting the fungal hypothesis have not been replicated in recent years by independent investigators. An attempt to independently replicate the basic science foundation of this hypothesis has also failed in a more heterogeneous group of CRS patients. The data can be dissected, reanalyzed, and reinterpreted and myriad arguments can be put forth. But an unbiased review of the data demonstrates that nearly every researcher outside of the original proponents of the fungal hypothesis has failed to replicate their work. The weight of the evidence is increasingly tipping the scales away from this theory.

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Is Fungus the Cause of Chronic Rhinosinusitis?

The debate regarding fungus as the unifying etiologic agent in chronic rhinosinusitis (CRS) has raged for over a decade. The commentary by Ponikau et al¹ in this issue of *Otolaryngology–Head and Neck Surgery* is the latest installment in this long-running controversy. Data produced almost exclusively by this group have led them to propose that “most, if not all, chronic rhinosinusitis conditions have a fungal etiology.”² Starting with an initial article by Ponikau et al in 1999,³ these researchers have put forth data and others have dissected that data and, in some cases, put forth additional refuting data.^{4,5} Efforts have been made to translate this “fungal hypothesis” to the bedside, with

initial open-label antifungal treatments showing promise in treating CRS. Unfortunately, these same results could not be replicated by other researchers in subsequent randomized placebo-controlled trials.^{6,7}

Failure of antifungal medications may not necessarily disprove fungus as the major etiologic factor but instead could be explained by ineffective dosage or delivery.⁸ However, the failure of these trials suggests the need for more study to better answer the myriad questions that they raise. It is natural, therefore, to reexamine the basic science underpinnings of the fungal hypothesis. Specifically, the finding of Shin et al⁹ that nearly 90 percent of CRS patients had non-IgE-mediated elevated interleukin-5 (IL-5) responses to *Alternaria alternata* extract has remained arguably the most compelling evidence that fungus may indeed be a major etiologic factor in CRS. By leveraging the experience of some of the authors of the original work, Orlandi et al¹⁰ recently attempted to duplicate the methods and results of Shin et al as far as we were able, with one exception. In order to test the universality of fungus as a possible etiologic agent, a more heterogeneous group of CRS patients was purposely chosen. Interestingly, not only did we produce results that differed from the Shin et al report in these more heterogeneous patients, but some of these results were directly opposite of the original findings.

Heterogeneous Sample

It is the use of this heterogeneous sample that is a major criticism of the Orlandi et al results. As pointed out in our article, we chose a sample size based on the assumption that all CRS patients would respond similarly based on the Shin et al data, yet our results demonstrated quite the opposite. In their commentary, Ponikau et al state that the presence of patients with classically defined allergic fungal rhinosinusitis (AFRS) “stacks the deck” in favor of disproving the fungal hypothesis, because “most [CRS] patients do not fulfill the criteria for AFRS.” We found this statement confusing inasmuch as the same group’s 1999 article states, “the diagnostic criteria for AFS are present in the majority of patients with CRS.”³ We purposely chose a diverse group of patients with CRS, includ-

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ing AFRS, specifically to test the universality of the fungal hypothesis. If a fungus is the cause of “most, if not all” cases of CRS, and especially if the “majority of patients with CRS” fulfill AFRS diagnostic criteria, how can including AFRS patients “stack the deck?” Inclusion of patients with classically defined AFRS simply accentuates the heterogeneity expressed within CRS. Further, if the findings of Shin et al had held true in this intentionally diverse population of patients, then our study would have served as a piece of outside corroborating information supportive of the universal fungal hypothesis. Unfortunately, it did not.

Let us nevertheless assume that the AFRS patients in some way skewed the results of our study, and, therefore, let us exclude these patients from the analysis. This exclusion then leaves the five Utah CRS patients, only one of whom had a positive IL-5 response. Comparing the 20 percent IL-5-positive Utah CRS patients to the 89 percent IL-5-positive patients in Shin et al, there is a 0.8 percent chance they represent the same populations (Fisher exact test). That fact bears restating. Statistically, there is a 99.2 percent chance that a true difference between the Shin et al results and the Orlandi et al results exists. But it will be claimed that such a comparison is underpowered. Power analysis actually shows a standard 80 percent chance of detecting a difference smaller than the 69 percent difference that was actually found.

As is pointed out in the Orlandi et al article, one should be very cautious about drawing definitive conclusions from small sample sizes. Throwing out an entire theory based solely on a study of 10 patients may be unwise. Nevertheless, the data have sufficient validity to cast a shadow of doubt upon the universality of the fungus hypothesis. Inasmuch as CRS affects millions of patients, calling for entirely new treatment paradigms based on a study of 18 patients with no further substantiation appears equally unwise. Clearly, another attempt to replicate these data by additional independent researchers is needed.

Heterogeneous Fungal Extracts

Such replication is complicated by the unavailability of one of the reagents. After embarking upon our attempt to exactly replicate the Shin et al work, it became clear that the lot of *Alternaria alternata* extract used by Shin et al was no longer available. For this reason, we utilized two additional *Alternaria alternata* extracts. We chose to use two because we recognized that the proteins contained within an extract may differ from one lot to another. In their commentary, Ponikau et al raise this extract issue as a major concern. It is indeed possible that the two extract lots used in the Orlandi et al study did not contain some as-yet-unknown unique immunologic trigger that is commonly produced by *Alternaria*. Conversely, the lot used by Shin et al may have been unique in containing this unidentified substance. We tested three vials of *Alternaria alternata* extract before settling on the two we used in the study. It is not clear from the Shin et al article how many were tested before settling on the one used in their experiments. Additional work in analyzing larger numbers of *Alternaria* extracts will need to be performed in

order to resolve this discrepancy. Nevertheless, a universal response to fungal antigens that plagues millions of CRS patients worldwide would logically be expected to be provoked by more than just one unique vial of *Alternaria alternata* extract. To our knowledge, no confirmation of the Shin et al results using different extract lots has been published or presented.

Heterogeneous Interpretations

Ponikau et al point out that there are similarities between the two studies and that the Orlandi et al data somehow support the Shin et al findings. We don't necessarily agree with their interpretation of the data but will leave it to the reader to make his or her own assessment. It is true that there were reactions to the *Alternaria alternata* extract seen in our patients. It is not surprising that the immune system reacted to a foreign substance. As a positive control, we exposed the peripheral blood mononuclear cells (PBMCs) to phorbol myristate acetate (PMA), concanavalin A (ConA), and phytohemagglutinin (PHA). We found CRS patients' PBMCs to have an enhanced IL-5 response to PMA and PHA. Yet, we don't hypothesize that these substances are the universal cause of CRS.

Shin et al found their IL-5 production to be independent of IgE and instead to correlate with IgG. They postulated an as-yet-undiscovered dysregulated immune response that was IgE-independent. Conversely, Orlandi et al found a very good correlation between IL-5 and IgE, but found no correlation between IL-5 and IgG. In their commentary, Ponikau et al criticize the inclusion of controls in the correlation analysis of IgG and IL-5 as the reason no correlation was found. It is easy enough to remove the controls and reanalyze the data. The results again show no correlation for either *Alternaria alternata* 1 extract ($P = 0.92$, $r = -0.05$) or *Alternaria alternata* 2 extract ($P = 0.49$, $r = 0.27$).

Unfortunately, no matter how the Orlandi et al data are dissected, reanalyzed, or reinterpreted, fungus just doesn't appear to be the universal cause of CRS.

Conclusions

Scientific discoveries, especially revolutionary ones, require verification by replication. This is the essence of the scientific method. Research publications must be presented with sufficiently detailed methodology so that the data can be objectively replicated. We followed the published methods as best we could with the assistance of some of the original researchers and were unable to reproduce the results described in Shin et al.

A better understanding of the etiology of CRS is crucial in making improvements in our patients' well-being, and discovering a single etiologic agent would be a huge advance. In vitro data, largely from one research group, have implicated fungus as an etiologic factor in CRS. One clinical trial from the same research group showed small, if any, clinical improvement with antifungal therapy. Unfortunately, both the clinical trial and the in vitro results have

failed attempts at replication. The antifungal therapy results could not be replicated in multiple randomized clinical studies performed by others. The first published attempt to independently replicate a significant *in vitro* underpinning of the fungal hypothesis, with assistance from some of the original authors, has also failed.

At the end of the day, we are each left to interpret the data for ourselves. Finding a treatable universal single etiology of CRS would be a life-altering advance for scores of millions of patients worldwide. All of us who take care of these patients know all too well the burden this disease places on them and their families. With our patients' welfare as our primary interest, all otolaryngologists would celebrate the discovery of such a universal etiology.

To be clear, if fungus were ever proven to be the major etiology of CRS and this knowledge resulted in improved treatments for our patients, we would welcome such an advance. But as physicians, we must also caution our patients from chasing down rabbit holes for treatments that won't work, especially those that are costly and have significant side effects. More than 10 years have come and gone while we have debated fungus as an etiology—or *the etiology*—of CRS. An objective weighing of the evidence, both clinical and basic science, must be done for the benefit of our patients. Unfortunately, the balance of available evidence appears to be tipped significantly against fungus as a major etiology of CRS.

Author Information

From the Division of Otolaryngology–Head and Neck Surgery, University of Utah (Dr. Orlandi), Salt Lake City, UT; and the Department of Otolaryngology–Head and Neck Surgery, University of Texas Southwestern at Dallas (Dr. Marple), Dallas, TX.

Corresponding author: Richard R. Orlandi, MD, Division of Otolaryngology–Head and Neck Surgery, University of Utah, 50 North Medical Drive, 3C120, Salt Lake City, UT 84132.

E-mail address: richard.orlandi@hsc.utah.edu.

Author Contributions

Richard R. Orlandi, analysis of data, drafting, final approval; **Bradley F. Marple**, analysis of data, revising, final approval.

Disclosures

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