Archivos de Bronconeumología 58 (2022) 529–530

Sociedad Española de Neumología y Cirugía Torácica SEPAR

ARCHIVOS DE Bronconeumología

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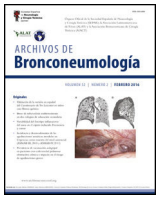

Editorial

Birds of a Feather Precipitate Together

Establishing a diagnosis of HP can be challenging, particularly when an inciting antigen goes unidentified, is not identified with confidence, or when there are multiple potential exposures and it is unclear which if any, is causing disease. Recent societal guidelines provide evidence-based recommendations for diagnosing HP, emphasizing the importance of identifying an inciting antigen.[1,2] Interestingly, the two guidelines provide differing recommendations regarding the use of specific tools for antigen identification, namely serum specific immunoglobulins (SSIgGs). A recent workshop report also provides an evidence review and semi-quantitative evaluation of SSIgG performance to support antigen identification in patients with suspected HP.[3] SSIgGs are not available at all centers, are largely non-standardized, and demonstrate mediocre performance characteristics. Both HP guideline committees identified a lack of robust data informing the SSIgG recommendations, which were based on few studies and low-quality evidence.[4] Yet, in the evaluation of patients with suspected HP, they remain a frequently used tool providing serological information on exposure and immunological reactivity to specific antigens associated with HP.

Birds and feathers are nearly ubiquitous environmental exposures, and Bird-Fanciers Lung (BFL) remains the most prevalent reported form of disease.[5] Feather-associated HP has been increasingly reported due to feather exposure in bedding and furniture. Given their often-indolent nature and less overt status as a cause of HP, feathers can be difficult to identify as an etiology. In such scenarios, a serological test could prove informative to support a diagnosis of HP and/or identify the culprit antigen.

In this pilot study by Rouzet and colleagues,[6] the authors sought to assess the role of the recombinant antigens r-PROE and r-IGLL1 in the diagnosis of Feather Duvet Lung (FDL). These proteins are involved in either the digestive or immune systems of birds and have been found in large quantity in droppings and blooms,[7] with both having been used as serological tests supporting BFL diagnosis. The study population included patients with BFL ($n = 15$), FDL ($n = 31$), exposed controls ($n = 15$) and unexposed controls ($n = 15$). Specific IgG antibodies against r-PROE and r-IGLL1 were analyzed using ELISA, with interpretations blinded to the clinical status of the patients. Each ELISA was performed twice with an index calculated, and results compared with clinical and demographic data. FDL patients were more likely ever smokers compared to the other groups, and had more chronic/fibrotic HP compared to those with BFL. Patients with BFL had higher antibodies to r-PROE and r-IGLL1 compared to FDL patients or controls. FDL patients did not show higher r-IGLL1 antibody levels compared to controls, but did show significantly higher r-PROE antibody levels compared to both control groups. In receiver operating curves, the optimal index cutoff of r-PROE provided 74% sensitivity and 86.7% specificity to differentiate FDL patients from controls. To differentiate BFL from FDL, index cut-offs were proposed that would favor birds or feathers as the etiology of the patient's HP. The authors present an algorithm to guide clinical use of these tests, proposing an index value of positivity that may be useful to interpret r-PROE and r-IGLL1 ELISA results in case of exposure to both feathers and birds.

The originality of the technique presented in this paper is standardized ELISA using recombinant antigens instead of purified mixtures of feather or droppings, whose performance may vary from one batch to another. Immuno-proteomic approaches, as used here, allow the selection of proteins of interest, expressed only in patients and not in exposed controls. The DNA sequence coding for the selected protein is inserted into a plasmid; after transforming bacteria (usually *E. coli*) with this plasmid, a large quantity of the specific protein is produced. This innovative way to produce antigens assures high standardization and optimal inter-batch reproducibility. Recombinant antigens differ from purified antigens by their nature and mode of production, showing different performance characteristics. Purified antigen contains many proteins from bird material, and should be more sensitive to detect patients exposed to many type of birds including duck, poultry or parrots. The recombinant antigens r-PROE and r-IGLL1 each contain a single protein identified in pigeon only, which is more specific and thus more restrictive to pigeon exposure. The production of recombinant antigens requires upfront costs, specific proteomic approaches available only at specialized centers, and are not yet commercialized for BFL diagnosis. They are an attractive potential tool however, given their high specificity and standardization.

This paper has several strengths. The study population is well characterized, with known exposures. The study team is highly experienced using these tests and in studying HP, and the test is one of clinical importance to the field. Yet some issues remain that may attenuate broad uptake of such a test. All studies of serological tests have yet to demonstrate their additive discriminative value beyond a positive exposure history, and it is unclear how this fits into the diagnostic approach from a Bayesian perspective that incorporates pre-test disease probability. The clinical need for these

DOI of original article: https://doi.org/10.1016/j.arbres.2021.02.011

https://doi.org/10.1016/j.arbres.2021.05.012

tests is to differentiate patients with HP from patients with other lung diseases, to identify a potential culprit antigen when one was not clinically identified, or to identify which of birds or feathers are causing HP in patients exposed to both. In these settings, test results may lead to change in clinical management, treatment and prognosis. The performance characteristics reported here are moderate, suggesting that the test should not necessarily be used in isolation, but rather incorporated into a multi-dimensional approach. The authors propose an approach in their figure, which serves as a starting point for such clinical integration. In spite of limitations, the technique used here should motivate the widespread use of ELISA using recombinant antigens over traditional tests. Generalization of standardized ELISA using recombinant antigens for all HP forms in the future could avoid variability across batches and laboratories.

Future work should incorporate this and similar tools to determine their additive discriminative value in identifying feathers and birds as the cause of HP, and/or to diagnose HP in real world contexts (i.e. differentiating from other mimics of HP). Standardizing the approach to serological tests and increasing their accessibility will help define their role. Access to commercialized recombinant antigens or ELISA plates with a panel of recombinant antigens pre-coated for immediate use could help transform exposure assessment in the clinic, improving diagnostic accuracy and hopefully, patient outcomes.

Funding

No funders pertaining to this article.

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