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Original article

Utility of immunological tests for bird-related hypersensitivity pneumonitis

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ABSTRACT

Background: The reaction of specific antibodies and sensitized lymphocytes to antigens is important in hypersensitivity pneumonitis (HP). However, there are no known studies evaluating the utility of the lymphocyte proliferation test (LPT) or specific antibodies to avian antigens in diagnosing bird-related HP. In this study, we examined the sensitivity and specificity of these two tests.

Methods: Patients with acute bird-related HP ($n=10$), chronic bird-related HP ($n=35$), acute summer-type HP ($n=14$), and other interstitial pneumonia (IP) ($n=76$) were evaluated. The optimal cutoff values were determined by receiver operating curve (ROC) analyses of specific antibodies in serum and bronchoalveolar lavage fluid (BALF), and by conducting the LPT on mononuclear cells in peripheral blood and BALF.

Results: The sensitivity and specificity of the antibodies were 80–100% and 92–100% in acute bird-related HP, and 26–79% and 73–93% in chronic bird-related HP, respectively. The sensitivity and specificity of the LPT were 50–100% and 100% in acute bird-related HP, and 46% and 91% in chronic bird-related HP, respectively.

Conclusions: Specific antibodies and the LPT are quite useful for diagnosing acute bird-related HP. The presence of specific antibodies in BALF and the results of LPT with peripheral blood mononuclear cells are particularly useful for diagnosing chronic bird-related HP.

1. Introduction

Hypersensitivity pneumonitis (HP) is an immune-mediated lung disease triggered by the inhalation of a wide variety of antigens [1], and its clinical presentation is classified into acute, subacute, and chronic [2]. However, it can be difficult to distinguish subacute HP from acute HP based on symptoms and imaging alone; therefore, the acute and chronic classifications of HP are more appropriate [3]. Various agents, including fungi, bacteria, animal proteins, and chemicals, are known to incite HP. An epidemiologic study of acute HP in Japan demonstrated that summer-type HP accounted for

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74% of all HP cases (621 cases), whereas bird-related HP accounted for only 4% [4]. By contrast, an epidemiologic survey of chronic HP in Japan demonstrated that summer-type HP and bird-related HP were similarly prevalent, accounting for 28% and 20% of all cases, respectively [5]. Avian antigens and *Mycobacterium avium* complex in hot tub water were the first and second most common causes of HP (both acute and chronic) in 64 (75%) of 85 patients at rates of 34% and 21% according to Hanak et al. respectively. Farmer's lung disease accounted for 11% of cases, and 9% of cases were related to household mold exposure [6].

Immune-complex-mediated lung injury (type III allergy) is thought to be involved in the acute phase of HP based on the following observations: (1) provocation symptoms emerge 4–6 h after patients leave the hospital or following inhalation challenge with culture filtrate [7]; (2) the skin test reaction peaks at 6 h, showing an Arthus-like reaction; and (3) the presence of immune complexes, activated complement fragments, and increased neutrophils in bronchoalveolar lavage fluid (BALF) 2 days after the onset of acute episodes [8,9]. The chronic phase of HP follows the acute phase within a short time through cell-mediated immunity (type IV allergy). The BALF of HP patients contains elevated numbers of CD4+ and CD8+ T cells, especially the latter, and stimulation of these cells with anti-CD3 enhances IFN-γ production [10]. Recent studies have demonstrated that CD8+ T cells do not always predominate over CD4+ T cells in the BALF of HP patients [11,12]. Several studies report that CD8+ T cells are associated with the acute phase, while CD4+ T cells predominate during chronic HP [13–18].

Many reports have challenged the value of serum precipitins for diagnosing HP [19–22]. Lacasse et al. identified serum precipitins as a significant predictor of HP, regardless of antigen exposure (odds ratio 5.3 [95% confidence interval 2.7–10.4]) [21]. A recent study by Fenoglio et al. found that serum precipitins were useful for diagnosing acute HP (predictive negative value, 81–88%; predictive positive value, 71–75%) [22]. Antibodies against avian antigens are reportedly positive in bird-related HP [23–26], but the cutoff values, sensitivity, and specificity, have not been well studied. Although the lymphocyte proliferation test (LPT) is thought to reflect the reactivity of lymphocytes to antigens *in vivo*, its utility remains unknown. In this study, we measured the antibodies against avian antigens and determined the lymphocyte proliferation response to these antigens in bird-related HP to study their diagnostic utility.

2. Materials and methods

2.1. Patients

This protocol was approved by the institutional review board (Approval date: April 25, 2013; Approved #: 1475). The requirement for informed consent was waived. The medical records of patients with bird-related HP, acute summer-type HP, and other interstitial pneumonia (IP) admitted between March 1996 and May 2008 were reviewed. A total of 45 patients with bird-related HP, comprising 10 with the acute type and 35 with the chronic type, were evaluated. Fourteen patients with acute summer-type HP and 76 patients with other IP were evaluated as controls for acute and chronic bird-related HP, respectively.

The diagnosis of acute and chronic bird-related HP was based on clinical, radiological, and histological criteria, as previously described [4,5]. All chronic cases underwent a surgical lung biopsy and tested positive on the inhalation challenge test for diagnostic confirmation.

Clinical data, smoking history, serum C-reactive protein (CRP) concentration, and data related to LDH, Krebs von den Lungen 6 (KL-6), surfactant protein D (SP-D), and pulmonary function, including vital capacity (VC) and diffusing capacity for carbon monoxide (DL_{co}), were collected.

2.2. Antibody measurement

IgG and IgA antibodies against avian antigens were detected using enzyme-linked immunosorbent assay (ELISA) as previously described [27]. Each polystyrene plate well (Immulon 2; Dynatech Laboratories Inc., Alexandria, VA, USA) was coated with 100 μL of 1 μg/mL pigeon dropping extract (PDE) in a carbonate buffer (pH 9.6) overnight at 4 °C. The wells were washed three times with phosphate-buffered saline containing 0.05% Tween-20 (PBST) and treated with 0.5% bovine serum albumin–PBST to block the free binding surfaces of the wells. The serum samples were diluted 1:400 and the BALF 1:8; then, 100 μL aliquots of the samples were added to each well and incubated at 37 °C for 1 h. After washing the wells with PBST, 100 μl of a 1:1000 dilution of goat anti-human IgG or anti-human IgA coupled to horseradish peroxidase (Cappel, Malvern, PA, USA) was added, and the mixture was incubated at room temperature for 1 h. The wells were washed again, the substrate (o-phenylenediamine and hydrogen peroxide [0.15% vol/vol]) added, and the color change was measured in an ELISA reader fitted with 490 and 655 nm filters.

2.3. LPT measurement

Venous blood (20 mL) was collected into sterile tubes containing heparin. Peripheral blood mononuclear cells (PBMC) were separated by standard density gradient centrifugation using Separate-L (Muto; Tokyo, Japan). The total mononuclear cell fraction was washed twice with RPMI 1640 medium (Gibco; Grand Island, NY, USA) and re-suspended to 2×10^6 cells/mL in RPMI 1640 supplemented with 10% fetal calf serum, penicillin, and streptomycin. PBMC or BALF cells (2×10^5 cells) were cultured in triplicate with a 1:100 dilution of pigeon sera in 96-well flat-bottom plates. Preliminary experiments showed these quantities of antigen to be optimal. The plates were incubated for 5 days at 37 °C in a 5% CO_2 incubator and pulsed with ^{3}H-thymidine for the final 16 h of culture. The cells were harvested, and the incorporation of ^{3}H-thymidine was estimated by counting the radioactivity with a β-counter. The results were expressed as the stimulation index (SI), i.e., the geometric mean counts per minute of cultures stimulated with pigeon sera divided by the geometric mean counts per minute of un-stimulated cultures as a control (medium only) [27].

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