Diagnostic yield of specific inhalation challenge in hypersensitivity pneumonitis

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ABSTRACT Reliable methods are needed to diagnose hypersensitivity pneumonitis. The aim of the study was to establish the diagnostic yield of specific inhalation challenge (SIC) in patients with hypersensitivity pneumonitis.

All patients with suspected hypersensitivity pneumonitis in whom SIC was performed (n=113) were included. SIC was considered positive when patients showed a decrease of >15% in forced vital capacity (FVC) or >20% in diffusing capacity of the lung for carbon dioxide, or a decrease of 10% to 15% in FVC accompanied by a temperature increase of 0.5°C within 24 h of inhalation of the antigen.

SIC was positive to the agents tested in 68 patients: 64 received a diagnosis of hypersensitivity pneumonitis and SIC results were considered false-positive in the remaining four patients. In the SIC-negative group (n=45), 24 patients received a diagnosis of hypersensitivity pneumonitis and SIC results were considered false-negative, and 21 patients were diagnosed with other respiratory diseases. The sensitivity and specificity of the test were 72.7% and 84%, respectively. Having hypersensitivity pneumonitis caused by an antigen other than birds or fungi predicted a false-negative result (p=0.001).

In hypersensitivity pneumonitis, positive SIC testing virtually confirms the diagnosis, whereas negative testing does not rule it out, especially when the antigenic sources are not birds or fungi.

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Introduction

Hypersensitivity pneumonitis is a disease with heterogeneous causes, resulting from an inflammatory pulmonary reaction of immunological origin in response to a wide variety of antigens that can provoke varying degrees of inflammation and destructuring of the lung parenchyma [1]. Currently, most authors agree that the diagnosis of hypersensitivity pneumonitis should be based on the criteria proposed by SCHUYLER and CORMIER [2]. The development and clinical presentation of hypersensitivity pneumonitis are influenced by several factors [3–6]. Genetic susceptibility may explain why one individual develops the disease, another individual with exactly the same exposure is only sensitised but remains healthy, and yet another will not even become sensitised [3, 4]. In this context, in which a process of sensitisation seems essential, the specific inhalation challenge (SIC) could be a tool for diagnosing this condition [5], especially bearing in mind the immunological and histopathological response elicited in affected patients [6].

Nevertheless, SIC currently lacks standardisation. Only four studies to date, all in the framework of bird fancier's lung, have attempted to establish the performance of the test [7–10]. The sensitivity and specificity values obtained in these studies were between 80% and 100%, but the small number of patients tested and, in particular, the fact that the manner of performing and interpreting the test substantially differed between studies may be the reasons why this diagnostic tool is not more widely used.

The aim of this study was to evaluate the performance of SIC for diagnosing hypersensitivity pneumonitis due to a variety of causal agents in a large number of patients with this condition.

Materials and methods

Study population

This was a retrospective, cross-sectional study including all patients older than 18 years with suspected hypersensitivity pneumonitis who underwent SIC in our centre (Hospital Universitari Vall d'Hebron, Barcelona, Spain) between June 1995 and December 2010. Some of these patients had been initially seen at other hospitals and were later referred to our centre to complete the diagnostic study.

Diagnostic protocol

In all patients, we analysed data from the clinical history and physical examination, as well as the results from the following additional tests: laboratory analyses with complete blood count, erythrocyte sedimentation rate, gammaglobulin, total G and E immunoglobulins, calcium level, 24-h urinary calcium, plasma angiotensin-converting enzyme and lactate dehydrogenase levels, as well as specific serum immunoglobulin G antibody determinations, chest radiograph, chest computed tomography, pulmonary function testing including spirometry, static lung volumes, and carbon monoxide diffusing capacity (*D*LCO), immediate and delayed hypersensitivity skin testing, bronchofibroscopy with bronchoalveolar lavage and/or transbronchial biopsy, and SIC. In the few cases in which a definite diagnosis could not be reached once these tests had been carried out, surgical lung biopsy was performed after individualised assessment of the indication [10].

Diagnostic confirmation

The definite diagnosis of hypersensitivity pneumonitis was established based on independent review of the patients' medical records by two pulmonologists with extensive experience in diffuse interstitial lung disease who were unaware of the SIC results. In addition to evaluation of the data at the time when SIC was performed, the two experts also assessed the patients' clinical follow-up data up to the time of writing. The diagnosis of hypersensitivity pneumonitis was established by the two specialists according to the same diagnostic protocol [10] based on the proposed criteria of SCHUYLER and CORMIER [2].

Antigen extract preparation for specific inhalation challenge

Commercialised extracts (Bial-Aristegui, Bilbao, Spain) from *Penicillium frequentans*, *Aspergillus fumigatus* and *Mucor mucedo* were used to study fungi [11]. The avian sera were prepared in our laboratory. Blood for avian serum extracts was collected from several birds and centrifuged; the serum protein concentration was measured by bicinchoninic acid assay (Pierce Chemicals, Rockford, IL, USA). The extracts for SIC to isocyanates and esparto were prepared as previously described [11, 12]. The SIC to metalworking fluid, dental prostheses and interferon were performed with the material provided by the patient to simulate the working conditions in a challenge room.

Specific inhalation challenge

SIC was performed in the hospital setting after obtaining the patient's written consent. In patients receiving anti-inflammatory medication (corticosteroids), the medication was discontinued at least 8 days before SIC was carried out. Patients were required to have a baseline forced vital capacity (FVC) >50% and

*D*LCO ⩾40% of the reference value [13] to be considered for SIC testing. The test was performed on an outpatient basis.

In 81 patients, SIC consisted of nebulisation of an extract of the suspected causal agent using a De Vilbiss 646 nebuliser (De Vilbiss CO, Somerset, PA, USA) from 1995 to 2001 or a Mefar MB3 dosimeter (Mefar, Ele H_2O; Medicali, Brescia, Italy) from 2001 to 2010, which release the solution during the first second of each inspiration. Patients were requested to inhale 2 mL of the suspected antigen at a dilution of 1:100 (0.01 mg·mL^{-1}) [10, 13]. FVC, forced expiratory volume in 1 s, *D*LCO, and the patient's temperature were recorded at baseline, 20 min after inhalation challenge, every hour thereafter for the next 8 h and at 24 h. Blood cell count, chest radiograph and O_2 saturation measurement were performed before and 8 h after inhalation. In all cases, SIC with a placebo solution (saline) was carried out 1 day before testing with the suspected antigen. None of the patients responded positively to the placebo solution.

The test was considered positive when any of the following responses was elicited [10, 13]. 1) FVC decrease of >15% or *D*LCO decrease of >20% as compared to baseline values. 2) 10% to 15% FVC decrease plus at least one of the following five criteria with respect to clinical status and baseline analytical values: white blood cell increase of 20%; O_2 saturation decrease of 3%; significant radiological changes; rise in body temperature of more than 0.5°C; or evident clinical symptoms (*e.g.* cough or dyspnoea). 3) FVC decrease <10% but with evidence of three or more of the previously mentioned clinical or analytical criteria. When the test proved negative, inhalation of a new antigen dilution of 1:10 (0.1 mg·mL^{-1}) was performed the next day following the same procedure.

In 32 patients, the antigens used were not soluble (isocyanates, wood dust, humidifiers, duvets, *etc.*), and the SIC was conducted by directly exposing the patient to the suspected causal agent in a challenge chamber [12, 14, 15]. When the previous result was negative, the exposure time was increased on successive days, up to a maximum exposure of 2 h per day. The clinical and functional response and the criteria to establish positivity were those mentioned above.

Pulmonary function testing

Pulmonary function testing was carried out on a MasterLab system (MasterLab, Jaeger, Germany) according to the guidelines of the European Respiratory Society [16, 17]. The forced spirometry reference values were those proposed for the Mediterranean population [18]. For the diffusion study, the single-breath diffusing capacity of the lung for carbon monoxide was used. The theoretical values were those proposed by the European Respiratory Society [17]. Starting in 2006, the European Respiratory Society/American Thoracic Society joint guidelines were used [19, 20].

Statistical analysis

The Mann–Whitney test and chi-squared test were applied to compare continuous and nominal variables, respectively, with a two-sided significance level of 0.05. The consistency of SIC was estimated by evaluating the sensitivity and specificity [21] of the method, the positive and negative predictive values [22], and the likelihood ratio of a positive and negative value [23] with 95% confidence intervals using the Wilson method [24]. Receiver operating characteristic (ROC) curves were constructed to determine the cut-off values that best differentiated between having the disease or not [25]. All analyses were done with SPSS, version 17 (Chicago, IL, USA) and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Of the original 136 patients with suspected hypersensitivity pneumonitis referred for SIC, 23 were excluded. The test was not performed in four patients due to a *D*LCO <40%, and in the other 19 no clinical data were available. Among the 113 subjects undergoing the test, SIC was positive to the agents tested in 68 patients, 64 of whom were diagnosed with hypersensitivity pneumonitis (group 1). The SIC was considered to yield false-positive results in the four remaining patients (group 2), who were diagnosed with smoking-related bronchiolitis, cryptogenic organising pneumonia due ortho-phenylenediamine exposure [26], sarcoidosis and idiopathic pulmonary haemosiderosis. In the SIC-negative group (n=45), 24 patients received a diagnosis of hypersensitivity pneumonitis and SIC results were considered false-negative (group 3), and 21 patients were diagnosed with other respiratory diseases: chronic obstructive pulmonary disease (n=5), idiopathic pulmonary fibrosis (n=4), bronchiectasis (n=4), bronchiolitis (n=3), silicosis (n=1), microscopic polyangiitis (n=1), sarcoidosis (n=1), nonspecific interstitial pneumonia (n=1), and asthma (n=1) (group 4) (fig. 1). None of the patients in group 4 presented symptoms or impaired lung function during SIC.

Demographic data and clinical characteristics of the total series and the group 1 and group 3 patients are summarised in table 1. Patient age and percentage of nonsmokers were significantly higher in group 3 than

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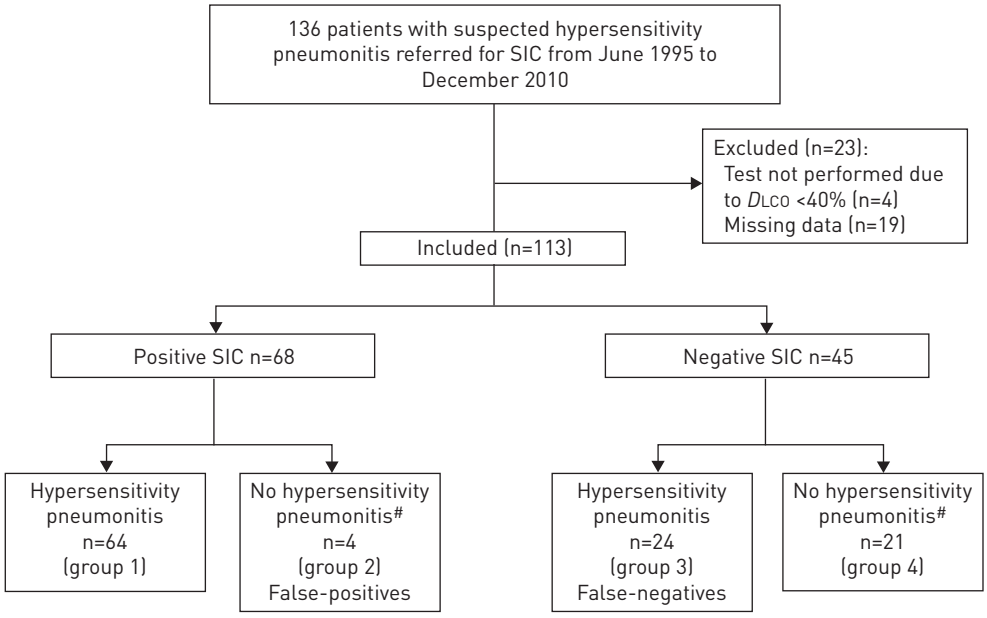

FIGURE 1 Flow chart of patient enrolment. SIC: specific inhalation challenge; *D*LCO: diffusing capacity of the lung for carbon monoxide. #: see diagnostics in text.

in group 1 (p=0.004 and p=0.003, respectively). In group 3, 58.3% of the hypersensitivity pneumonitis cases were not caused by avian or fungal proteins, whereas in group 1 only 11% of cases were not caused by these agents (p<0.001). The non-avian non-fungal agents causing these cases were isocyanates (n=3); esparto (n=2); wood (n=2) metalworking fluid (n=1), dental prosthesis (n=1) and interferon (n=1). In 11 patients, the causal antigen was not found. In these 11 patients, SIC was performed to several suspected antigens, but no positive results were recorded.

The sensitivity, specificity, and positive and negative predictive values of SIC according to the established criteria for positive status (FVC, *D*LCO and temperature) are shown in table 2, with 95% confidence intervals. The sensitivity and negative predictive value of the test were higher when only patients with hypersensitivity pneumonitis caused by avian or fungal proteins were assessed (table 2). The ROC curves in figure 2 show the most relevant differences in FVC, *D*LCO and temperature alone before and after SIC for predicting positive SIC status. Using the ROC curve, we found that a more than 10% FVC decrease achieved the most satisfactory sensitivity (45.5%, 95% CI 35.5–55.8) and specificity (88%, 95% CI 70–95.8) for the diagnosis of hypersensitivity pneumonitis. For *D*LCO, a post-SIC decrease greater than 15% resulted in a sensitivity of 45.5% (95% CI 35.5–55.8) and specificity of 76% (95% CI 56.6–88.5). Regarding temperature, an increase higher than 0.5°C after SIC achieved a sensitivity of 37.5% (95% CI 28.1–47.9) and specificity of 72% (95% CI 52.4–85.7).

Nine patients (8%) experienced severe reactions related to SIC testing. These included influenza-like symptoms in all cases, with fever up to 40°C in one patient, hypoxaemia with an arterial oxygen tension (P_{aO_2}) decrease of 10 mmHg in another patient, and the development of new radiological changes in another. All these severe reactions were transient, and only the three patients mentioned required administration of oral corticosteroids. None required hospital admission. As compared to patients who did not present severe reactions, these nine patients were younger (mean±SD age 39.56±13.07 *versus* 53.29±14.48 years; p=0.009) and pre-SIC *D*LCO was higher (94.28±17.44% *versus* 69.67±21.17% of theoretical value; p=0.005). There were no significant differences in the remaining variables analysed.

Discussion

The results of this study show that SIC could be useful as a tool for diagnosing hypersensitivity pneumonitis, with a positive predictive value of 94% and negative predictive value of 46.6% when considering the overall tests performed, and negative predictive value of 74.4% when including only tests performed with avian or fungal antigens. Of note, the likelihood ratios for positive and negative values, which are independent of the high prevalence observed in our sample, were >5 and <2, respectively. This is clearly an indication of the high utility of the combined diagnostic test to differentiate between patients testing positive and negative [23]. To our knowledge, this is the first study assessing the diagnostic yield of

TABLE 1 Demographic data and clinical characteristics of the study subjects

	Total	Group 1	Group 3
Subjects	113	64	24
Age years	52±14.8	50±14.6##	59.5±13.8##
Male	49 (43.4)	28 (43.8)	10 (41.7)
Exposure time (months) before SIC	170±168	171±167	159.6±179
Duration of symptoms (months) before SIC	37.2±45.5	42.5±52.5	22.82±19.2
Time (months) since last exposure	10.6±31.8	12.3±38.9¶¶	4.2±7.8¶¶
Smoking habit			
Smoker	19 (18.1)	13 (22)	2 (9.1)
Nonsmoker	56 (53.3)	24 (40.7)	18 (81.8)
Ex-smoker	30 (28.6)	22 (37.3)	2 (9.1)
Lymphocytes in BAL %	22.4±21.3	25±23.7	24±18.9
FVC before SIC %	79.3±16.6	78.75±18.2	75.7±13.8
FEV1 before SIC %	86.4±18.2	83.60±18.0	88±18.8
FEV1/FVC before SIC %	81.3±13.8	81.6±8.8	82.2±19.0
TLC before SIC %	86.9±16.6	86.4±19.1	85.7±12.0
***D*LCO before SIC %**	71.3±21.7	70±23.4	71.2±18.9
Chest CT			
Normal	10 (9.6)	6 (10)	2 (9.1)
Nodular	15 (14.4)	11 (18.3)	1 (4.5)
Reticular	5 (4.8)	3 (5)	2 (9.14.7)
UIP	19 (18.3)	9 (15)	7 (31.8)
Ground glass	39 (37.5)	24 (40)	10 (45.5)
Emphysema	6 (5.8)	2 (3.3)	0
Bronchiectasis	10 (9.6)	5 (8.3)	0
Antigen SIC			
Avian proteins	65 (57.5)	34 (53.1)	17 (70.8)
Fungi	30 (26.5)	23 (35.9)	4 (16.7)
Others	18 (15.9)	7 (10.9)	3 (12.5)
Final clinical diagnosis* **			
HP due to avian proteins	43 (38.1)	34 (53.1)	9 (37.5)
HP due to fungi	24 (21.2)	23 (35.9)	1 (4.2)
HP due to other antigens	21 (18.6)	7 (10.9)	14 (58.3)
No HP	25 (22.1)		

Data are presented as n, n (%), or mean±SD. SIC: specific inhalation challenge; BAL: bronchoalveolar lavage; FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; TLC: total lung capacity; *D*LCO: diffusing capacity of the lung for carbon monoxide; CT: computed tomography; UIP: usual interstitial pneumonia; HP: hypersensitivity pneumonitis. ##: p=0.004; ¶¶: p=0.003, group 1 *versus* group 3; ***: p<0.001.

SIC in a clinical practice setting in a large number of cases and with no restrictions on the agents causing hypersensitivity pneumonitis.

Although SIC has been used for the diagnosis of hypersensitivity pneumonitis since the 1960s, it was not until 1980 that HENDRICK *et al.* [7] attempted to determine the performance of this test in a study including 29 cases of suspected hypersensitivity pneumonitis due to avian antigens. The authors reported a sensitivity of 48% to 85% and a specificity of 95%. Also in the context of hypersensitivity pneumonitis due to avian antigens, RAMÍREZ-VENEGAS *et al.* [8] studied 17 patients with chronic hypersensitivity pneumonitis and 17 patients with other interstitial lung diseases and reported a sensitivity of 82% to 86% and specificity of 76% to 100%, whereas OHTANI *et al.* [9] found no false results in a study of 11 patients with bird fancier's lung and six control subjects. The largest study to date, also conducted in patients with bird fancier's lung, showed a sensitivity and specificity of 92% and 100%, respectively, in 59 patients with hypersensitivity pneumonitis, 30 healthy pigeon keepers, and 20 patients with other diffuse interstitial lung diseases [10].

One noteworthy finding in the present study was the elevated number of false-negative test results. In 24 patients with hypersensitivity pneumonitis, SIC results were negative. The first reason for this may be related to the fact that our study was based on regular clinical practice, whereas in the ones mentioned above [7–9], the study protocol may have had an effect on the results. In this sense, it may not have been completely appropriate to include as false-negative cases the 11 patients in whom the causal agent was not found. It may have been that the antigen used for SIC was simply not the one causing the disease. Second,

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TABLE 2 Diagnostic performance of specific inhalation challenge (SIC)

	All SIC	SIC to avian proteins and fungi
Subjects n	113	95
Prevalence %	78	73
Sensitivity %	72.7 (62.6–80.9)	85.1 (74.7–91.7)
Specificity %	84.0 (65.3–93.6)	86.2 (69.4–94.5)
PPV %	94.0 (85.8–97.7)	93.4 (84.3–97.4)
NPV %	46.6 (32.9–60.9)	71.4 (54.9–83.7)
LR+ %	4.6 (1.8–11.3)	5.32 (2.15–13.13)
LR- %	0.3 (0.23–0.5)	0.18 (0.10–0.32)

Data are presented with 95% confidence intervals. PPV: positive predictive value; NPV: negative predictive value. LR+: positive likelihood ratio; LR-: negative likelihood ratio.

and likely the determinant factor, is that all types of hypersensitivity pneumonitis were included, regardless of the causal agent. As would be expected, our group has considerable experience in performing SIC with avian proteins [10] and fungi [11, 27, 28], but much more limited practice with the less common causal agents of hypersensitivity pneumonitis. This was illustrated when only patients with hypersensitivity pneumonitis caused by exposure to birds or fungi were analysed: there were only 10 false-negative results and the sensitivity of the test rose to 85%. Finally, there are a high percentage of patients with usual interstitial pneumonia in the false-negative group (31.8%). These chronic fibrotic patients tend to have lower inflammatory cell infiltrates, indicating perhaps that the test may not be useful for this subgroup [29]. We do not know the effect that age and smoking habit can have on the positivity or negativity of the SIC.

One of the main problems for definite implementation of SIC in the diagnosis of hypersensitivity pneumonitis stems from the lack of a well-defined method for carrying out the test. This may also have contributed to the differences in false-negative rates. Three methods have been proposed for SIC. First, some authors hold that natural workplace or home exposure is a reasonable way to provoke symptoms or manifest deterioration of functional parameters in uncertain cases of hypersensitivity pneumonitis [4]. Secondly, it may be useful to try to reproduce the working or environmental conditions in a challenge chamber. This method has not been widely used [14, 30], and although it does not conclusively identify the causal agent, it may be easier to standardise and possibly safer. The third method consists of using an aerosolised extract material of the suspected causal agent. This may be the most commonly used method, with one of the first descriptions published by WILLIAMS [31] in 1963. It is the method we apply when the suspected agents are avian proteins or fungi [7–9]. It is important to mention that the SIC method used by our group is focussed on achieving the correct diagnosis while submitting the patient to the least possible

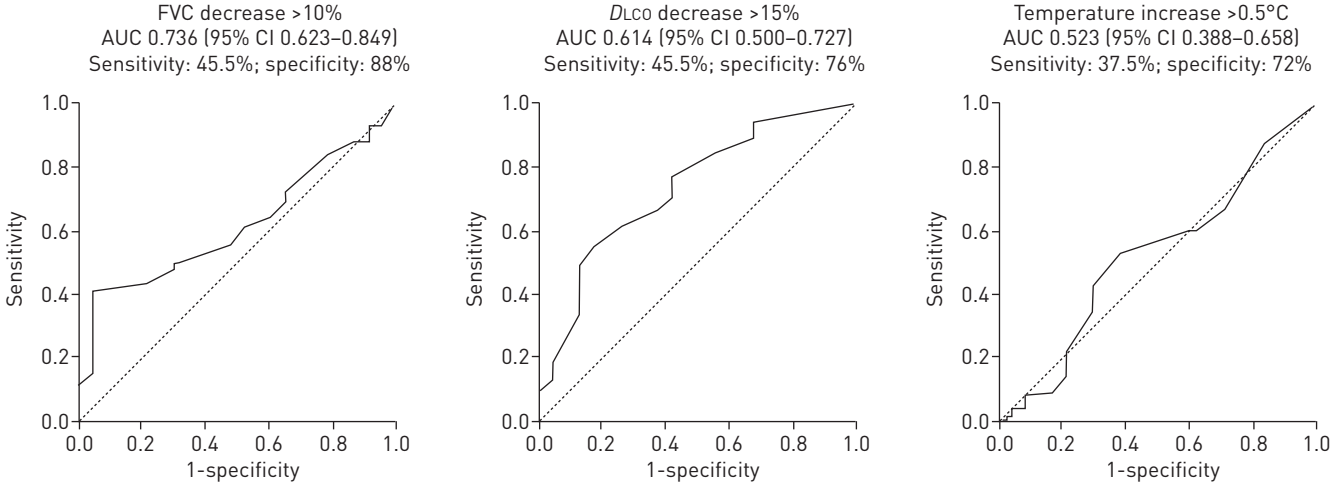

FIGURE 2 Receiver operating characteristic curves constructed to determine the most relevant differences in forced vital capacity (FVC), diffusing capacity of the lung for carbon monoxide (*D*LCO), and temperature before and after specific inhalation challenge for predicting hypersensitivity pneumonitis. AUC: area under the curve.

risk. Thus, the level of antigen exposure is probably lower than that used by other groups. For example, maximum exposure in the studies of OTANI *et al.* [9] was 680 μg of protein, whereas our group uses a maximum dose of 200 μg protein. The rationale is that fewer respiratory and systemic symptoms will be produced, and that possible pulmonary function abnormalities will be rapidly reversible.

Related with this last observation, another critical aspect is how to establish a positive outcome in SIC. Whereas HENDRICK *et al.* [7] and OTANI *et al.* [9] basically considered the test positive when symptoms and signs consistent with an influenza-like illness developed, other groups place more importance on objective measures than on clinical aspects. RAMÍREZ-VENEGAS *et al.* [8] considered the test positive when a decrease >16% in FVC, >3 mmHg in P_{aO_2}, >3% in arterial oxygen saturation, or a body temperature increase >0.5°C occurred. Our group, however, has assigned greater value to pulmonary function studies [10, 13]. When the criterion of >0.5°C body temperature increase is combined with an FVC decrease >15%, *D*LCO decrease >20%, or FVC between 10% and 15%, the test shows good sensitivity, particularly in the study of avian or fungal antigens. When these parameters were tested separately, specificities were good, but sensitivities were low. For this reason, we believe that combined assessment of these parameters provides the best diagnostic yield.

Another important aspect when recommending SIC in the diagnosis of hypersensitivity pneumonitis is the small number of associated adverse effects. Only nine patients experienced such effects, which were transient in all cases, and only three patients required oral corticosteroid administration. These secondary effects occurred in younger patients with higher baseline *D*LCO levels, an observation that should alert to the need for special precautions (*e.g.* decrease the dose of inhaled antigen and hospitalise the patient) in this patient group. The fact that complications presented in individuals with a nearly normal *D*LCO may be because these patients are more prone to note clinical changes than those in whom *D*LCO is already decreased and baseline symptoms may be present. It is important to mention that in our protocol, SIC is only contraindicated when the FVC and *D*LCO are less than 50% and 40%, respectively, of the theoretical value [13]. Some authors believe that SIC should not be performed under any circumstances because the test itself may sensitise the patient and cause illness [32]. This has not been observed in any patients undergoing SIC in whom a diagnosis other than hypersensitivity pneumonitis was ultimately established. Nor has it been observed in other studies in which healthy control subjects underwent SIC [7, 8, 10].

This study has the limitations of a retrospective design. Although all patients were studied according to the same protocol, SIC was performed for diagnostic purposes and not in the context of a study designed to determine its performance. This limitation may have been compensated by the fact that the definite diagnosis of the disease was established at the time of the study by two independent observers who took into consideration the patients' follow-up data in addition to the proposed diagnostic criteria for hypersensitivity pneumonitis [2, 33]. This fact practically assures the diagnosis and likely enables establishment of the usefulness of the test in actual clinical practice. Another possible limitation is the fact that the data for sensitivity and specificity were calculated in the context of diagnosis of hypersensitivity pneumonitis, and not in the context of the differential diagnosis of interstitial lung diseases. Future studies should be designed to determine the sensitivity and specificity of SIC for the differential diagnosis of interstitial lung diseases.

In conclusion, the results of the present study show that SIC could be useful in the diagnosis process of hypersensitivity pneumonitis. In fact, a positive result is practically diagnostic for this disease, with good sensitivity. It has also proven to be a safe test, with few adverse effects. Nonetheless, efforts should be made to standardise the way in which SIC is performed, particularly when the suspected causal agent is not avian or fungal proteins, and the interpretation of the results.

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