

PROF. OLIVIER BOYER (Orcid ID : 0000-0002-7591-307X)

DR. JÉRÉMIE MARTINET (Orcid ID : 0000-0002-3895-2731)

Corresponding author mail id:- jeremie.martinet@chu-rouen.fr

Medical algorithm: *Aspergillus fumigatus* components in the diagnosis of Allergic
Bronchopulmonary Aspergillosis

R. Lukaszewicz¹, G. Mahay¹, O. Boyer², J. Martinet²

¹ CHU Rouen, Department of Pulmonary, Thoracic Oncology and Respiratory Intensive Care, F-76000 Rouen, France

² Normandie Univ, UNIROUEN, Inserm U1234, CHU Rouen, Department of immunology and biotherapy, F-76000 Rouen, France

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Main text:

Allergic bronchopulmonary aspergillosis (ABPA) is a lung disease involving hypersensitivity to *Aspergillus fumigatus* (*Af*) antigens caused by repeated inhalation of spores in susceptible hosts. Its identification is sometimes difficult, and delayed diagnosis, or lack of treatment, leads to worsening lung function and major complications like pulmonary fibrosis or bronchiectasis.

ABPA was first described in 1952 by Hinson et al. in patients with asthma and frequent exacerbations (cough, dyspnoea) associated with recurrent infiltrates on chest radiography, blood hypereosinophilia and presence of *Af* in sputum. Since then, diagnostic criteria have been progressively updated (table 1), highlighting the involvement of type I (IgE-mediated) and type III (IgG-mediated) mechanisms, and specifying radiological aspect. Thus in 1977, Rosenberg and Patterson explained the importance of skin tests for *Af*, elevated total serum IgE level and precipitating antibodies (IgG) against *Aspergillus* antigens (1). The development of immunoassay technology led to use *Af*-specific IgE and IgG as an alternative to skin tests and precipitins (2). At the same time, many cases of ABPA were described in patients with cystic fibrosis, extending the diagnosis to this population (2). Currently, criteria commonly used are those proposed by ISHAM

(International Society of Human and Animal Mycology) in 2013, which remind us that allergic mechanisms are at the core of ABPA (3).

Evidence of *Af*-sensitisation is therefore necessary for the diagnosis of ABPA. It can be assessed either by skin tests for *Af*, or by *Af*-specific IgE immunoassay, with good sensitivity in both cases. In a study evaluating these diagnostic criteria in almost 400 asthmatics, sensitivity appeared better with *Af*-specific IgE at a threshold of 0.35 kU/L (100%) compared to skin tests (88-95%) (4), leading to update diagnostic criteria (5). ISHAM criteria were recently improved by modifying serum total IgE threshold (>500 UI/mL), excluding skin test, and using CT-scan instead of chest radiography (5).

The involvement of an associated IgG-mediated mechanism was included in Rosenberg and Patterson's criteria (1) with the presence of precipitating antigen for *Af*. *Af*-specific IgG immunoassay appears to be a good alternative, but its superiority compared to precipitins is still debated. A threshold at 27 mgA/L has been proposed on ImmunoCAP system (ThermoFisher®) (6), but there is no equivalence with EIA system (Bio-Rad®) for which *Af*-specific IgG threshold is usually defined at 10 UA/mL. Moreover, IgG levels may fluctuate and need to be adjusted according to age.

In addition to asthma and cystic fibrosis, some cases of ABPA have been described in chronic obstructive pulmonary disease (COPD), post-tuberculous fibrocavitary disease, and in more rare disorders such as MacLeod's syndrome or primary ciliary dyskinesia. All these diseases promote *Af* colonisation of the airways, which is the first step in the development of ABPA. Bearing in mind that other forms of aspergillosis (such as chronic pulmonary aspergillosis) could be accompanied by some features of ABPA, diagnostic criteria should probably be extended to all bronchopulmonary diseases leading to a structural alteration (figure 1).

Despite recent updates, criteria (3,5) have several limitations in clinical practice and ABPA remains difficult to assess. Abnormalities described on thoracic imaging (radiography or CT-scan) may fluctuate (opacities) or appear late (bronchiectasis, mucoid impaction, hyper-attenuated mucus), therefore normal thoracic imaging cannot rule out the diagnosis. Similarly, blood eosinophilia count is variable and is lowered by systemic corticosteroid therapy, which is the

main treatment during exacerbations. Finally, *Af*-specific IgE cannot distinguish sensitisation from allergy to *Af*. Molecular allergology, by evaluating IgE directed against *Af* components (rAsp), offers additional tools when ABPA is suspected. It enables the detection of sensitisation to some *Af* proteins which could be particularly involved in ABPA. In a meta-analysis evaluating the use of rAsp in 1700 patients with ABPA, positivity for either rAsp f1 or rAsp f3 showed a sensitivity of 96.7% in asthma and 93.3% in cystic fibrosis, while positivity for either rAsp f4 or rAsp f6 showed good specificity: 99.2% in asthma, 93.9% in cystic fibrosis (7). rAsp f2 showed good sensitivity for the diagnosis of ABPA in asthma (8). Thus, rAsp are particularly useful when there is a strong suspicion of ABPA while minor criteria are difficult to meet. Due to the possibility of cross-reaction with other fungal species, the interpretation of some rAsp should be done with caution. Indeed, rAsp f3 cross-react with *Penicillium*, *Candida*, *Malassezia*, and rAsp f6 with *Alternaria* and *Malassezia*. In these cases, especially if the other rAsp are negative, the diagnosis of allergic bronchopulmonary mycosis (ABPM) should be considered (9) (table 1).

Based on currently used criteria (3), and according to the latest data (5,7,9), we design an algorithm for ABPA highlighting the role of rAsp, and reminding the need to investigate ABPM (figure 1).

Allergic mechanisms play a predominant role in ABPA, and the negativity of the first-line biological assessment (total and *Af*-specific IgE) should allow to rule out this diagnosis, and to consider the possibility of ABPM if the radio-clinical presentation is typical. In case of sensitisation to *Af* and a high level of total IgE, several diagnoses must be considered: sensitisation to *Af* without allergy, bronchopulmonary disease (e.g. asthma or COPD) with allergy to *Af*, or true ABPA.

Then, secondary criteria must be investigated. This second-line assessment is more difficult to interpret because of the fluctuating nature of its parameters (radiological abnormalities, blood eosinophilia), sometimes leading to diagnostic and therapeutic delays.

Molecular allergology offers new tools to guide the clinician, and *Af* components play an important role in these situations. Thus, a third-line assessment using rAsp may be proposed when ABPA is strongly suspected and usual criteria cannot be met. Because of their good sensitivity, the negativity of rAsp f1, f2 and f3 make ABPA highly improbable and other diagnoses,

such as ABPM, should be considered. In case of positivity, rAsp f4 and f6 immunoassays should be performed to confirm the diagnosis of ABPA because of their good specificity (figure 1).

Allergenic component approach should be further investigated in order to confirm their utility in different geographic areas, ethnicities, and predisposing diseases. Their use can be a first step towards updating the diagnostic criteria.

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Table:

TABLE 1: Diagnostic criteria for ABPA from the first description to present day.

History of diagnostic criteria for allergic bronchopulmonary aspergillosis

Rosenberg and Patterson (1977) [†]	Cystic Fibrosis Foundation Consensus Conference (2003) [‡]	ISHAM group (2013) [§]	Saxena et al. (2021) [*]	Asano et al. (2021) [*]
- Asthma	- Asthma - Cystic fibrosis	- Asthma - Cystic fibrosis	- Asthma	- Asthma
Major criteria				ABPA/ABPM criteria
- Episodic bronchial obstruction. - Peripheral blood eosinophilia. - Immediate skin reactivity to <i>Aspergillus</i> antigen. - Precipitating antibodies against <i>Aspergillus</i> antigen. - Elevated serum total IgE level. - History of pulmonary infiltrates (transient or fixed). - Central bronchiectasis.	- (Sub)acute clinical respiratory degradation not attributable to another aetiology. - Total IgE level >500 IU/mL - Immediate skin reactivity to <i>Aspergillus</i> antigen or presence of serum IgE antibody to <i>Af</i> .	- Immediate skin reactivity to <i>Aspergillus</i> antigen or elevated <i>Af</i> -specific IgE level. - Total IgE level > 1000 IU/mL (a value of 500-1000 IU/mL may be acceptable if all other criteria are met).	- <i>Af</i> -specific IgE >0.35 kUA/L - Total IgE level > 500 IU/mL	- Current or previous history of asthma or asthmatic symptoms - Eosinophil count ≥500 cells/mm ³ - Total IgE level ≥417 IU/mL - Immediate cutaneous hypersensitivity or specific IgE for filamentous fungi ^{**} - Presence of precipitins or specific IgG for filamentous fungi ^{**} - Filamentous fungal growth in sputum cultures or bronchial lavage fluid ^{**} - Presence of fungal hyphae in bronchial mucus plugs - Central bronchiectasis on CT - Presence of mucus plugs in central bronchi, based on CT/bronchoscopy or mucus plug expectoration history - High attenuation mucus in the bronchi on CT
Minor criteria				
- <i>Af</i> in sputum (culture or microscopic examination). - History of expectoration of brown plugs or flecks. - Arthus reactivity (late skin reactivity) to <i>Aspergillus</i> antigen.	- precipitins to <i>Af</i> or presence of serum IgG antibody to <i>Af</i> . - new or recent abnormalities on chest radiography (infiltrates or mucus plugging) or CT chest (bronchiectasis) persisting after antibiotics and physiotherapy.	- Presence of precipitins or IgG antibodies against <i>Af</i> in serum. - Thoracic imaging findings consistent with ABPA. - Blood eosinophil count > 500 cells/μL in steroid naïve patients (may be historical).	- <i>Af</i> -specific IgG levels >27 mgA/L (ImmunoCAP [®]) - Bronchiectasis on CT chest - Eosinophil count >500 cells/μL	
Interpretation of criteria for the diagnosis of ABPA				
- Certain ABPA: if all of the major criteria are present. - Likely ABPA: if the first six of the major criteria are present. - Very unlikely ABPA: if serum IgE level is normal. Minor criteria may be an aid when the diagnosis is not certain.	All of the major criteria and at least one of the minor criteria must be met for the diagnosis of ABPA.	All of the major criteria and at least two of the minor criteria must be met for the diagnosis of ABPA.	All of the major criteria and at least two of the minor criteria must be met for the diagnosis of ABPA.	^{**} Filamentous fungi should be identical. Six criteria at least must be met for the diagnosis of ABPA (if filamentous fungous if <i>Af</i>) or ABPM (if filamentous fungous is not <i>Af</i>).

Af: *Aspergillus fumigatus*; ABPA: Allergic bronchopulmonary aspergillosis; ABPM: Allergic bronchopulmonary mycosis; ISHAM: International Society of Human and Animal Mycology; CT: computed tomography

[†]Based on Rosenberg, Patterson et al. « Clinical and Immunologic Criteria for the Diagnosis of Allergic Bronchopulmonary Aspergillosis. » *Ann Intern Med.* (1977).

[‡]Based on Stevens et al. « Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. » *Clin Infect Dis Off Publ Infect Dis Soc Am.* (2003).

[§]Based on Agarwal et al. « Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. » *Clin Exp Allergy J.* (2013).

^{*}Based on Saxena et al. « Which are the optimal criteria for the diagnosis of allergic bronchopulmonary aspergillosis? A latent class analysis. » *J Allergy Clin Immunol Pract* (2021).

^{*}Based on Asano et al. « New clinical diagnostic criteria for allergic bronchopulmonary aspergillosis/mycosis and its validation. » *J Allergy Clin Immunol Pract* (2021).

Figure legends:

Figure 1: Medical algorithm for allergic bronchopulmonary aspergillosis (ABPA)

Statement of Contribution:

RL collected and reviewed data, created medical algorithm and wrote the manuscript.

JM contributed to design the study, writing and critical review of the manuscript.

GM contributed to design the study and critical review of the manuscript.

OB critically reviewed the manuscript.

Medical algorithm for allergic bronchopulmonary aspergillosis

Are there predisposing conditions ?

- Asthma, Cystic fibrosis, COPD
- Damaged bronchopulmonary structure

Yes

Major criteria: are both present ?

- Serum Af-specific IgE level > 0.35 kUA/L
(or positive Af skin test if Af-specific IgE is not available)
- Serum total IgE > 500 IU/mL

Yes

Minor criteria: Are ≥2 present ?

- Presence of precipitins or IgG antibodies against Af in serum.
- Thoracic imaging findings on CT chest consistent with ABPA.
- Peripheral blood eosinophil count > 500 cells/μL (may be historical).

Yes

ABPA[†]

No

Consider using Af components[‡]

rAsp f1 or rAsp f2 or rAsp f3

+

rAsp f4 or rAsp f6

+

Repeat eosinophil count and thoracic imaging, and/or consider search for ABPM.

Probable ABPA

-

Improbable ABPA

Consider search for ABPM (≥6 criteria)*

- Current or previous history of asthma or asthmatic symptoms
- Eosinophil count ≥500 cells/mm³
- Total IgE level ≥417 IU/mL
- Immediate cutaneous hypersensitivity or specific IgE for filamentous fungi**
- Presence of precipitins or specific IgG for filamentous fungi**
- Filamentous fungal growth in sputum cultures or bronchial lavage fluid**
- Presence of fungal hyphae in bronchial mucus plugs
- Central bronchiectasis on CT
- Presence of mucus plugs in central bronchi (based on CT/bronchoscopy or mucus plug expectoration history)
- High attenuation mucus in the bronchi on CT

No

No ABPA

Af: *Aspergillus fumigatus*; ABPA : allergic bronchopulmonary aspergillosis; ABPM: allergic bronchopulmonary mycosis; COPD: chronic obstructive pulmonary disease; CT: computed tomography

+ : positivity for at least one rAsp; - : negativity for all/both rAsp; **Filamentous fungi should be identical.

[‡]Adapted from Agarwal et al. « Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. » *Clin Exp Allergy* (2013), revised by Saxena et al. « Which are the optimal criteria for the diagnosis of allergic bronchopulmonary aspergillosis? A latent class analysis. » *J Allergy Clin Immunol Pract* (2021).

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