

New tools for preoperative diagnosis of allergic fungal sinusitis? A prospective study about 71 patients

1 | INTRODUCTION

Allergic fungal sinusitis (AFS) is a subgroup within noninvasive fungal chronic rhinosinusitis. Its paraclinical diagnosis is based on a « gold standard »: rhinosinusitis evolving for at least 12 weeks or persisting on CT scan, allergic mucin seen during surgery, identification of Charcot-Leyden crystals or eosinophils aggregates in pathology, fungal hyphae on direct examination or culture.¹

Our objective was to search for clinical, radiological, biological, immunological and mycological criteria permitting AFS preoperative diagnosis, within a population operated on for CRS.

2 | MATERIALS AND METHODS

We conducted a prospective, open, monocentric study on the clinical, radiological, immunological, mycological and pathological aspects of CRS.

The study was approved by the institutional review board proposed to all patients receiving surgical indication for CRS in our university hospital. Briefly, inclusion criteria were age ≥ 18 years, planned surgery for CRS, having given their written consent to participate in the study. Exclusion criteria were proven cystic fibrosis, mental or motor impairment preventing understanding or cooperation.

Anonymised data were collected. Epidemiological data were age, sex, proven allergy, asthma, aspirin intolerance, local and systemic corticosteroid intake, immunocompromised status. A 60-point symptom score was used containing the 10 following functional signs: anosmia, cacosmia, maxillary or frontal sinus pain, nasal obstruction, anterior clear or purulent rhinorrhea, epistaxis and sneezing. Lund and Mackay endoscopic score was recorded.

Blood tests included: blood count with neutrophil (NPN) and eosinophil (EoPN) granulocytes, C-reactive protein (CRP), determination of total immunoglobulin E (IgE) (standard <100 kIU/L),¹ of specific IgE (sIgE) for *Aspergillus fumigatus* and *Alternaria alternata* (<0.10 kUA/L)¹ and of total tryptasemia (<11.5 μ g/L).² In the event of positive sIgE for *Aspergillus* or *Alternaria*, the molecular profile (IgE response to individual allergens) was performed. *Aspergillus* serology was also analysed using ELISA (Sérion kit, Orgentec, Trappes, France) and immunoelectrophoresis (IEP) (Sebia, Evry, France). The positivity threshold using ELISA was 70 IU/mL. IEP was considered positive if one or more precipitation arcs formed.

CT scan (unenhanced unless suspected cerebro-orbital complication or differential diagnosis with a tumour) and MRI (T1, T2, T2 with fat saturation, T1 with gadolinium injection, diffusion and perfusion) were performed. A senior radiologist, expert in ENT pathology, interpreted both CT and MRI to establish the probability of AFS or fungus ball (FB), without any knowledge of the patient diagnosis. A Likert scale rated from 1 (unlikely diagnosis) to 5 (highly likely diagnosis) was used for the CT scan, and then for MRI. For CT, criteria for this AFS probability score were ³ bilateral, asymmetric and diffuse paranasal involvement, total and heterogeneous sinus opacification, suspicion of bony erosion or osteolysis of the sinus walls and for MRI, multiple paranasal sinus signal void on T2W images.

In the pathology laboratory, after formalin fixation, morphological investigation was performed with the Gomori-Grocott stain, searching for fungal hyphae in the removed sinus content and in the mucosa (search for invasivity). The presence of EoPN aggregates and of Charcot-Leyden crystals was noted.

Mycological examination was performed by direct microscopic examination after staining with lactophenol blue. Another biopsy fragment was cultured on Sabouraud gentamicin-chloramphenicol agar (Oxoid, Dardilly, France) and incubated for up to 10 days at 27°C in a normal atmosphere. As soon as a fungal colony developed, it was identified by MALDI-TOF mass spectrometry after pre-processing with formic acid and acetonitrile. A real-time polymerase chain reaction (PCR) specific to *Aspergilli* was carried out on another fragment.

Finally, patients were classified into three diagnostic groups: fungal chronic rhinosinusitis -FCRS (combining FB, AFS and indolent invasive fungal sinusitis (IIFS)) or non-FCRS with polyps (CRSwNP) or without polyps (CRSsNP). FCRS diagnoses were assessed in the event of fungal hyphae by direct examination or by positive culture.⁴ Within this group, AFS was assessed by the presence of allergic mucin, Charcot-Leyden crystals or EoPN aggregates, and the absence of mucosal or bone invasiveness by fungal hyphae. EoPN aggregates, as well as by the absence of mucosal invasiveness. IIFS was assessed by mucosal or bone invasiveness by fungal hyphae.⁴ Non-FCRS was assessed by the absence of direct fungal hyphae or a negative culture. In this group, CRSwNP was assessed by known history of sino-nasal polyposis or bilateral polyps observed in the middle meatus by endoscopy.⁵ Otherwise, they were classified as CRSsNP.⁵

Statistical analysis was carried out using Excel and R software. Clusters were obtained by multivariate unsupervised analysis and compared with variables of interest to understand the significance of their diagnosis. Logistic regression was used in order to build diagnostic trees by CART method.

3 | RESULTS

Seventy-five patients were included between February 2016 and February 2017, of whom four cystic fibrosis patients were excluded from the analysis. Seventy-one patients (mean age 53 years [18-87], 39 females) undergoing surgery were analysed. The fungal sinusitis group comprised 31 patients (7 AFS, 24 FB, 0 IIFS). Relevant descriptive results are presented in Table 1 (qualitative variables) and Table 2 (quantitative variables).

Unsupervised multivariate analysis of qualitative variables clearly distinguished four clusters. Each cluster corresponded to a precise diagnosis: cluster 1 to FB, cluster 2 to AFS, cluster 3 to CRSsNP and cluster 4 to CRSwNP, thus constituting endotypes (Figure 1A). The AFS endotype was defined by allergy ($P = 3.10^{-5}$), unilateral or bilateral pansinusitis ($P = 0.009$), headache ($P = 0.04$), fungal material and allergic mucin observed during surgery ($P = 0.006$).

Three clusters were obtained by multivariate analysis of quantitative variables (Figure 1B). Cluster 3 corresponded to CRSwNP with 5/7 AFS patients and was characterised by a mean symptom score of 2/60 ($P = 1.10 \cdot 10^{-10}$), a mean endoscopic score of 3.7/10 ($P = 3.10 \cdot 10^{-6}$), 2.6 affected sinuses ($P = 1.10 \cdot 10^{-5}$), a mean diagnostic probability score of 2.4/5 on CT scan ($P = 2.10 \cdot 10^{-4}$) and of 3/5 on MRI ($P = 9.10 \cdot 10^{-7}$), mean total IgE of 322 kIU/L ($P = 1.10 \cdot 10^{-4}$), and *Aspergillus*- or *Alternaria*-specific IgE of, respectively, 1.8 and 2.2 kUA/L ($P = 5.10 \cdot 10^{-3}$ and $P = 2.10 \cdot 10^{-2}$).

A diagnostic tree associating radiologic and immunologic criteria showed that total IgE greater than 517 kIU/L confirmed AFS diagnosis (Figure 2A). Below this threshold, if specific IgE was greater than 0.10 kUA/L, AFS was diagnosed in 66% of cases. If the diagnostic threshold of total and specific IgE was not reached and the probability score was less than 3/5 on CT scan, AFS was ruled out.

On another diagnostic tree (Figure 2B), a MRI diagnostic probability score for AFS lower than 3/5 excluded 86% of AFS patients (1 false-negative among 7 AFS). Associating diagnostic probability scores of CT scan and MRI superior or equal to 3/5 identified 71% of AFS patients (5/7) with 3 false positives (0.05% of non-AFS patients).

4 | DISCUSSION

4.1 | Synopsis of key

Our study suggests that:

- A total IgE value >517 kIU/L confirms AFS diagnosis.
- A total IgE value <517 kIU/L, a specific IgE value <0.10 kUA/L or a CT scan probability score <3/5 rule out AFS diagnosis.

Keypoints

- Definitive diagnosis of allergic fungal sinusitis is confirmed by pathologists postoperatively. We aimed at identifying preoperative predictive factors for allergic fungal sinusitis (AFS) diagnosis in order to support the surgical strategy that requires a complete surgical debridement of the fungal material, thus differing from classical endoscopic surgery for chronic rhinosinusitis (CRS). The confirmation of systemic corticotherapy indication and its rapid postoperative introduction reduces the risk of relapse.
- Our study was open, monocentric and prospective. We included consecutively 75 patients for CRS with surgical indication during one year, and four were excluded due to cystic fibrosis.
- We demonstrated the efficiency of CT scan, MRI and laboratory examinations for the diagnosis of AFS.
- We found that allergic status, unilateral or bilateral pansinusitis involvement, headache, intraoperative observation of mucin and fungal material, total IgE ≥ 517 kIU/L and positive-specific anti-*Aspergillus* IgE, the uncertain to high likelihood for AFS diagnosis at CT and MRI were predictive factors.
- In conclusion, preoperative diagnosis of allergic fungal sinusitis requires sinus MRI and CT, total and anti-*Aspergillus* IgE to be included in the initial work-up prescribed by ENT specialists for atypical or refractory CRS with or without polyps.

4.2 | Strengths of the study

This is the first prospective study highlighting all preoperative diagnostic criteria for the diagnosis of AFS. The study lasted 12 months. We obtained a statistically robust endotype for AFS with the clusters method.

4.3 | Comparison with other studies

Among included 71 patients, 9.8% had AFS which is comparable to data in other studies.^{6,7} Clusters method indicates that an AFS endotype can be defined by: allergic background, unilateral or bilateral pansinusitis, headache, allergic mucin and fungal material observed during surgery. Similarly, Serrano et al,⁷ in multivariate analysis, found perioperative allergic mucin, maxillary sinus localisation and the presence of *Aspergillus*-specific IgE to be significant indicators.

Analysis of the quantitative variables associated the majority of AFS (5/7, 71%) with CRSwNPs. However, none of these five AFS had polyps on endoscopy. Therefore, we speculate on the existence of a relationship between CRSwNP and AFS. A patient with all the

TABLE 1 Descriptive and univariate analyses of qualitative variables

	Total population		AFS group		Non-AFS group		P value
Qualitative variables	Effective	%	Effective	%	Effective	%	
Sex							
Females	39	54.9	6	86	33	52	0.12
Males	32	45.1	1	14	31	48	
Surgical history							
ENT	13	18.3	2	29	11	17	0.68
Sinus in particular	13	18.3	1	13	12	19	
Other	31	43.7	2	29	29	45	
None	14	19.7	2	29	12	19	
Medical history							
Immunodepression	15	21.1	2	29	13	30	0.68
ENT	3	4.2	0	0	3	5	
Samter's triad	7	9.9	1	14	6	9	
None	46	64.8	4	57	42	66	
Nasal polyposis	15	21.1	1	14	14	22	1.00
Allergies	23	32.4	4	57	19	30	0.20
Asthma	9	12.7	1	14	8	13	1.00
Headaches	11	15.5	4	57	56	88	0.07
Diplopia	11	15.5	2	29	9	14	0.30
Positive <i>Aspergillus</i> serology	7	9.9	2	40			
Unilateral sinusitis	58	81.7	7	100	51	80	0.33
Sinus involved							
Maxillary alone	14	19.7	0	0	28	44	0.01
Anterior ^a	28	39.4	1	14	13	20	
Posterior ^b	16	22.5	2	29	14	22	
Total	13	18.3	4	57	9	14	
Mucin and fungal material observed during surgery	8	11.3	3	43	5	8	0.03
Pus observed during surgery	45	63.4	4	57	41	64	0.70
Polyps observed during surgery	18	25.4	2	29	16	25	1.00
Fungal hyphae found on direct pathological examination	26	36.6	5	71	21	33	0.09
Charcot-Leyden crystals observed	5	7.0	4	57	1	2	
Fungal hyphae found on direct mycological examination	13	18.3	2	29	11	17	0.60
Positive mycological culture	12	16.9	5	71	7	11	0.00
PCR	20	28.2	3	43	17	27	0.39
Positive sequencing (/14)	7	50.0	0	0	7	50	
Fungal species identified							

(Continues)

symptoms of a CRSwNP but without polyps could therefore be suspected of AFS. These data should be compared with published data classifying AFS among diseases with polyps.⁶

The diagnostic tree of radiology alone showed that the combination of an MRI with CT would increase sensitivity and diagnostic specificity. AFS is multisinusual and evolves in a highly inflammatory context where the mucin is difficult to diagnose on CT scan as it occupies the entire sinusual space and conceals fungal content. MRI

provides a clearer distinction between the fungal content and the mucin, resulting in a T2 signal void.⁸ Eggesbo et al⁹ reported the benefits of MRI in soft tissue characterisation differentiating pyocoele, FCRS, neoplastic process, or an orbital and cerebral complication.

The importance of immunology in the diagnosis of AFS is established on a diagnosis tree capable of providing certain confirmation or invalidation of AFS diagnosis. Total and specific IgE and CT scan are complementary. Agarwal et al¹⁰ confirmed the importance of specific

TABLE 1 (Continued)

Qualitative variables	Total population		AFS group		Non-AFS group		P value
	Effective	%	Effective	%	Effective	%	
<i>Aspergillus</i>	21	29.6	3		18		>0.05
<i>Aspergillus flavus</i>	7	9.9	1		6		
<i>Aspergillus fumigatus</i>	6	8.5	1		5		
<i>Aspergillus niger</i>	7	9.9	0		7		
<i>Aspergillus terreus</i>	1	1.4	1		0		
<i>Aspergillus tubinensis</i>	1	1.4	1		0		
<i>Fusarium proliferatum</i>	1	1.4	0		1		
<i>Rhizopus oryzae</i>	1	1.4	1		0		
<i>Scedosporium apiospermum</i>	1	1.4	0		1		
<i>Schizophyllum commune</i>	1	1.4	1		0		
Unidentified	7	9.9	2		4		

The pus was analysed for each operated patient finding one to three different bacteria each time. We did not obtain any significant results with respect to the diagnostic groups.

P values <0.05 were considered significant.

^aMaxillary, anterior ethmoid and frontal sinuses.

^bPosterior ethmoid and sphenoid sinuses.

TABLE 2 Descriptive and univariate analyses of quantitative variables

Quantitative variables	Total population		AFS Group		Non-AFS group		P value
	Median	Min;Max	Median	Min;Max	Median	Min;Max	
Age	55	(18, 87)	48	(44, 65)	56	(18, 87)	0.57
Symptom score /60	8	(0, 38)	12	(6, 38)	8	(0, 34)	0.10
Anosmia	0	(0, 6)	0	(0, 6)	0	(0, 6)	
Cacosmia	0	(0, 6)	0	(0, 6)	0	(0, 4)	
Maxillary pain	0	(0, 6)	0	(0, 6)	0	(0, 6)	
Frontal pain	0	(0, 6)	1	(0, 6)	1	(0, 6)	0.07
Other pain	0	(0, 6)	0	(0, 6)	0	(0, 6)	
Nasal obstruction	2	(0, 6)	2	(0, 6)	1	(0, 6)	
Watery rhinorrhea	0	(0, 6)	0	(0, 6)	0	(0, 6)	
Purulent rhinorrhea	0	(0, 6)	0	(0, 4)	0	(0, 6)	
Posterior rhinorrhea	0	(0, 6)	0	(0, 6)	0	(0, 6)	
Nose bleeding	0	(0, 6)	0	(0, 2)	0	(0, 6)	
Endoscopic score /10	2	(0, 7)	3	(0, 5)	1	(0, 7)	0.10
CT diag. AFS	2	(1, 5)	3	(2, 4)	2	(1, 3)	0.00
IRM diag. AFS	2	(1, 4)	4	(4, 5)	1	(1, 5)	0.00
Number of involved sinuses	1	(1, 8)	4	(1, 4)	1.0	(1, 8)	0.00
EoPN (G/L)	0.2	(0.0, 3.6)	0.5	(0.1, 3.6)	0.2	(0.0, 2.1)	0.04
NPN (G/L)	4.4	(0.0, 16.6)	4.5	(1.0, 6.6)	4.3	(3.3, 16.6)	0.92
CRP (mg/L)	3.1	(0.1, 77.0)	3.3	(0.0, 7.7)	3.1	(0.0, 77.0)	0.85
Total IgE (kIU/L)	66.2	(3.7, 1259)	689.0	(119, 1259)	53.8	(3.7, 517)	0.00
slgE anti-Af (kUA/L)	0.0	(0, 25.0)	1.0	(0.0, 25.1)	0.0	(0.0, 0.37)	0.00
slgE anti-Aa (kUA/L)	0.0	(0, 14.0)	3.2	(0.0, 14.0)	0.0	(0.0, 0.0)	0.00
Tryptasemia (µg/L)	4.3	(1.1; 21.3)	4.3	(1.7; 5.4)	4.3	(1.1; 6.4)	0.47

P values <0.05 were considered significant

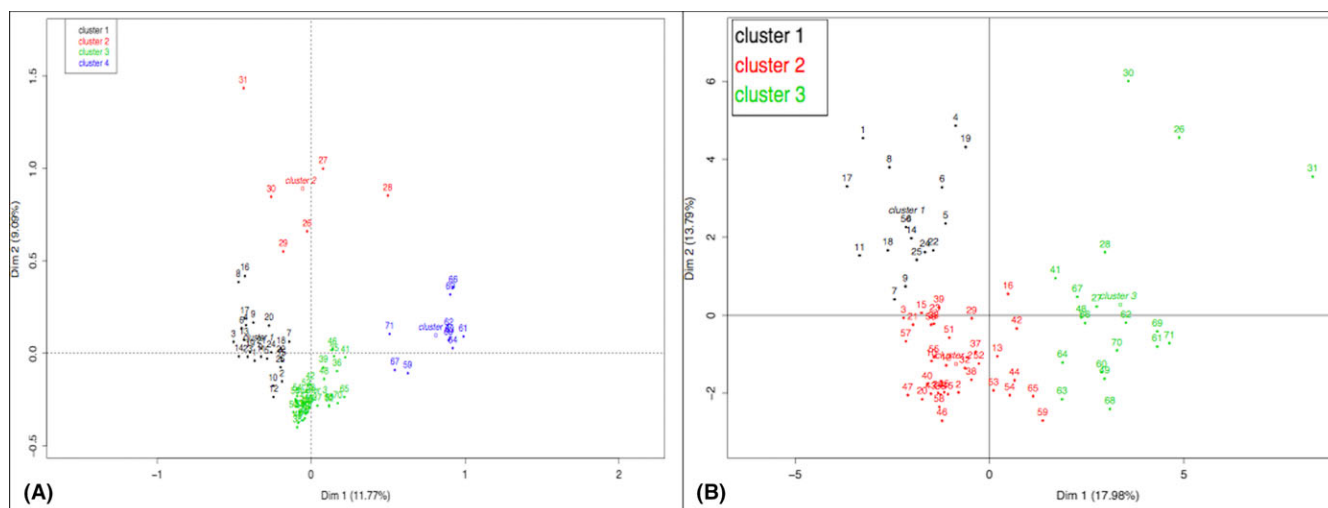


FIGURE 1 Clusters. A, Clusters of qualitative variables constituting endotypes. Cluster 1: contains all and only fungal balls; Cluster 2: contains all and only allergic fungal sinusitis; Cluster 3: contains all and only chronic rhinosinusitis without nasal polyps; Cluster 4: contains all and only chronic rhinosinusitis with nasal polyps. B, Clusters of quantitative variables. Cluster 1: contains all and only fungal balls; Cluster 2: contains all chronic rhinosinusitis without nasal polyps and two allergic fungal sinusitis; Cluster 3: contains all chronic rhinosinusitis with nasal polyps and five allergic fungal sinusitis. CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; FCRS, fungal chronic rhinosinusitis; Non-FCRS, non-fungal chronic rhinosinusitis

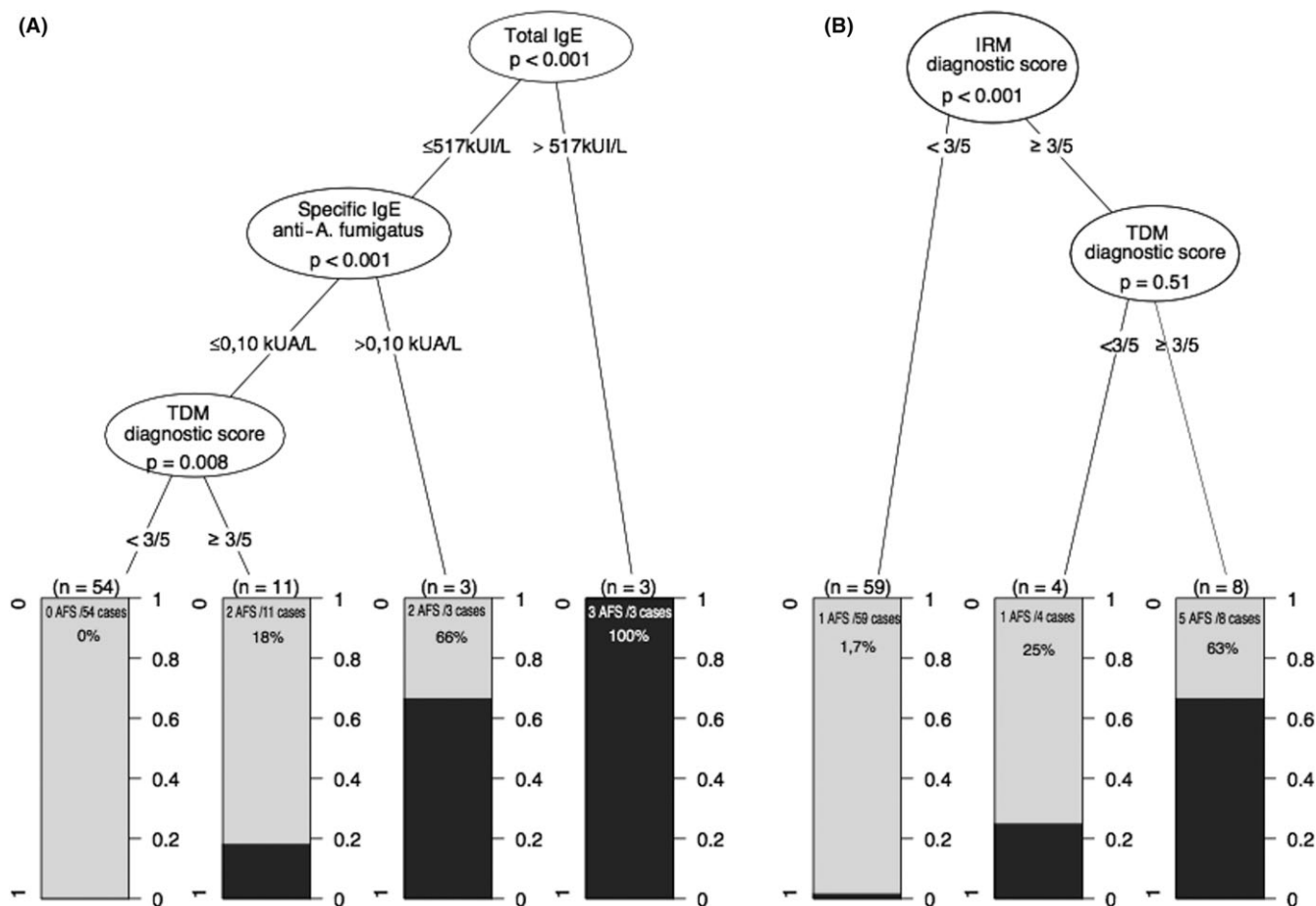


FIGURE 2 Diagnostic tree with radiological and immunological examinations for FCRS preoperative diagnosis. A, Diagnostic tree with immunological and radiological examinations. B, Diagnostic tree with radiological examinations alone. *A. fumigatus*, *Aspergillus fumigatus*; CT, computerised tomography; IgE, Immunoglobulin E; MRI, magnetic resonance imaging

IgE for the diagnosis of AFS. It is difficult to diagnose all AFS by the specific IgE anti-*Aspergillus* and *Alternaria*. However, *Aspergillus* was found to be the most common fungus in our series (65%). At low IgE or positive IgE rates for the two germs, the search for molecular IgE profiles (Asp f 1, 2, 3, 4, 6 or Alt a 1) confirmed true sensitisation. The study by Collins et al⁴ on specific IgE assays in intraoperative allergic mucin demonstrated the presence of specific IgE in mucin in all patients with specific IgE serology. The present study deals only with patients that have been operated for CRS. To strengthen these results, prospective studies need to be conducted on all CRS patients.

CONFLICT OF INTERESTS

None to declare.

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REFERENCES

- Bent JP, Kuhn FA. Diagnosis of allergic fungal sinusitis. *Otolaryngol Head Neck Surg.* 1994;111(5):580-588.
- Klossek J, Serrano E. *Les Mycoses en ORL*. Paris: France: Société française d'oto-rhino-laryngologie et de chirurgie de la face et du cou; 2003.
- Mossa-Basha M, Ilica AT, Maluf F, Karakoc O, Izbudak I, Aygun N. The many faces of fungal disease of the paranasal sinuses: CT and MRI findings. *Diagn Interv Radiol.* 2013;19(3):195-200.
- Collins M, Nair S, Smith W, Kette F, Gillis D, Wormald PJ. Role of local immunoglobulin E production in the pathophysiology of noninvasive fungal sinusitis. *Laryngoscope.* 2004;114(7):1242-1246.
- Safirstein BH. Allergic bronchopulmonary aspergillosis with obstruction of upper respiratory-tract. *Chest.* 1976;70(6):788-790.
- Fokkens WJ, Lund VJ, Mullol J, et al. European position paper on rhinosinusitis and nasal polyps 2012. *Rhinology.* 2012;50:1-298.
- Serrano E, Percodani J, Uro-Coste E, et al. Value of investigation in the diagnosis of allergic fungal rhinosinusitis: results of a prospective study. *J Laryngol Otol.* 2001;115(3):184-189.
- Aribandi M, McCoy VA, Bazan C. Imaging features of invasive and noninvasive fungal sinusitis: A review. *Radiographics.* 2007;27(5):14.
- Eggesbo HB. Radiological imaging of inflammatory lesions in the nasal cavity and paranasal sinuses. *Eur Radiol.* 2006;16(4):872-888.
- Agarwal R, Bansal S, Chakrabarti A. Are allergic fungal rhinosinusitis and allergic bronchopulmonary aspergillosis lifelong conditions? *Med Mycol.* 2017;55(1):87-95.

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Analysis of inner ear malformations associated with a facial nerve anomaly in 653 children fitted with a cochlear implant

1 | INTRODUCTION

Cochlear implantation (CI) can be a real challenge in case of inner ear anomalies, which occur in 20% of congenital hearing loss.¹

Hence, several surgical risks are increased as follows: perilymph or cerebrospinal fluid leak, electrode array misplacement and facial nerve injury. Pre-operative radiological evaluations with high-resolu-