LABORATORY SUPPORT IN CHRONIC FUNGAL RHINOSINUSITIS NON-IGE-MEDIATED

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[Il supporto del laboratorio nella rinosinusite cronica fungina non IgE mediata]

ABSTRACT

Chronic rhinosinusitis (CRS) is a pathology first described many years ago and is now divided into invasive and non-invasive, which are then subdivided into the typical picture of the "fungus ball" and Allergic fungal rhinosinusitis (AFRS). There are many fungal species implicated in CRS even if the most frequently isolated mycetes belong to the Aspergillus *family and to some demaziacei fungi. Considering the role that the regulation of the Th response has in the pathogenesis of chronic rhinosinusitis with nasal poliposi, the aim of this study was to show the role of some laboratory examinations for aspergillosis at the rhinosinusal level in 12 patients affected by poliposi who underwent surgery.*

*For each patient the following were dosed: IgG, IgM and IgA anti-*Aspergillus *in indirect immunofluorescence with and without serum absorption, the serum dosages of the precipitin in agarose gel, mycological examination of the mucin and of the bioptic fragments and finally, the microscopic examination of the mucin for the presence of granulocytes, neutrophils and eosinophils. From the comparison of the antibody titers of IgG and IgM anti-*Aspergillus *in IFA with and without serum absorption with spores of* Penicillium *sp. and A.* flavus*, it was seen that, even if not in all patients, a decrease of the antibody titers on the absorbed serum. Only in one patient, clinically diagnosed as having chronic fungal rhinosinusitis non-IgE-mediata, was an IgG titer equal to 640 found, notwithstanding surum absorption, the positivity to IgM and IgA and the isolation in culture of* Aspergillus flavus *mucin. In conclusion, considering the low number of patients examined, further studies are needed to evaluate the role and usefulness that these serum tests could have for patients affected by chronic fungal rhinosinusitis.*

Key words: *Chronic rhinosinusitis, Fungi,* Aspergillus*, Antibodies.*

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Introduction

Rhinosinusitis is commonly defined as a clinical manifestation characterized by inflammation of the mucosa of the nose and of the paranasal sinuses. There are numerous causes such as viral, bacterial and fungal infections, allergic reactions, but also, in many patients, an apparently idiopathic manifestation[1].

The incidence and the prevalence of rhinosinusitis are increasing and it has been estimated that each year, in the USA, about 31 million patients are affected by this pathology[1]. The two principal typologies of rhinosinusitis are the acute form and the chronic form, even if others have been described such as sub-acute, the recurrent acute form, acute exacerbations of the chronic and the nosocomial forms.

The problems relative to the classification of rhinosinusitis have been discussed for some time now and a consensus on definitions of the various clinical forms has been reached, as well as on the criteria that underlie them. In particular, various clinical pictures have been proposed such as acute rhinosinusitis from presumed bacterial etiology, chronic rhinosinusitis without polyps, chronic rhinosinusitis with polyps and allergic rhinosinusitis of fungal origin.

A useful classification for differential diagnosis of all forms of rhinosinusitis was also proposed, even if many doubts have remained regarding the role that fungi can have in the development of chronic rhinosinusitis (CRS).

CRS was first described many years ago, but since 1970 it has been accurately studied from the

mycological point of view[2]. There are many fungal species involved in CRS even if the most frequently found mycetes belong to the genus Aspergillus and to some demaziacei fungi[2,3,4,5]. The genus *Aspergillus* is classified in 6 subgenera that are in turn subdivided into various sections that include the relative species. These are ubiquitous mycetes that normally live in the soil, on decomposing vegetation and on organic debris. Their spores are easily dispersed in the air and, due to their dimensions, can be easily inhaled. Of the approximately 250 known species, more or less 20 have been indicated as pathogens for humans[2].

In a study relative to mycotic rhinosinusitis the authors reported a positivity for *Aspergillus* at the mycological test of 42% of the patients in which mycotic rhinosinusitis was diagnosed, with a prevalence of *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*, normally isolated from paranasal sinuses[6].

Chronic mycotic rhinosinusitis has been divided into invasive and non-invasive. The non-invasive form has been subdivided in the typical picture of the "fungus ball" and allergic fungal rhinosinusitis (AFRS)[1]. In fungal rhinosinusitis the species of *Aspergillus* can colonize the cavity, such as the paranasal sinuses, with formation of hyphae. The patients, in this stage, are often asymptomatic but over time the fungal mass increases and most people develop clinical manifestations.

AFRS would be considered a subclass of CRS where the patient, in most cases, presents an evident allergy to fungal colonization of their mucin. Physiopathological studies regarding AFRS are based on the premise that IgE mediated allergy to one or more fungi leads to a larger number of eosinophils in tissues and thus to the allergic phase of the inflammation[1,7]. The patient with AFRS normally shows some characteristics such as an increased production of eosinophilic mucin containing "colonizing" fungal hyphae, nasal polyps, and specific characteristics on radiological examination, immunocompetence and allergy to fungi that colonize the nasal cavity.

In a study carried out on patients with allergic fungal rhinosinusitis, the role that the fungal colonization of the mucus of the sinuses can play in the pathogenesis of CRS was identified. Some patients, in fact, showed positive fungal culture of nasal wash, presence of eosinophils in the mucus of the sinuses but absence of allergy to IgE-mediated fungi. Therefore, for these cases it was suggested that the term allergic fungal rhinosinusitis should be substituted with eosinophilic fungal rhinosinusitis[1]. These patients showed sensitivity and immunitary activation in response to fungal colonization in the mucus of the nasal sinuses. This process could be responsible for the production of cytokines that activate eosinophils leading to CRS.

It is clear that induction of the adaptive response of the Th cells is controlled by a group of receptors (Toll-like receptors, TLRs) present on the cells of the innate immune system. All these are able to activate stereotypic responses such as inflammation, but each TLR can also induce specific programs in the cells of innate immunity tailored to particular pathogens[8].

Also the humeral response is controlled by the Th response, in fact, the production of antibodies against specific antigens requires the interaction of B lymphocytes with helper T lymphocytes other than the presence of lymphokines. Also the class of immunoglobulins produced is induced by the combination of lymphokines secreted by helper T cells. Considering the role that the regulation of the Th response has in the pathogenesis of the chronic rhinosinusitis with nasal polyposis, the aim of this study was to show the role of some laboratory test in aspergillosis at the rhinosinusal level in subjects affected by polyposis who had undergone surgery.

Materials and methods

Patients

Twelve patients affected by nasal polyposis and due to undergo surgery at the ENT Clinic at the A.O.U. Policlinico-Vittorio Emanuele of Catania, were included in the study.

Laboratory tests

For each patient, other than the routine tests before surgery, the following were carried out: serum dosage of IgG, IgM and IgA anti-*Aspergillus* in indirect immunofluorescence with and without absorbtion with spores of *Penicillium lanosum* Westling and *Aspergillus flavus* 136/M; the serum dosage of precipitin in agarose gel against metabolic antigens of *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger* and *A. nidulans*; microscopic and culture examinations to identify and isolate mycetes of the mucin and bioptic fragment and finally, microscopic examination of the mucin to identify granulocytes, neutrophils and eosinophils. Also total IgE was measured as well as the histopathological spec-

imen of the bioptic fragment.

Serum dosage of IgG, IgM and IgA anti-*Aspergillus *in IFA

Setting up sensitive slides with spores and germinative tubules of *Aspergillus flavus* 136/M, species used following a notable diffusion in our region, required various experiments to limit the formation of mycitic masses that could interfere with the reading of the results, and obtain a suitable germination of the spores and a appropriate quantity of fungal cells in each well of the slide. To limit cross-over reactions with *Penicillium* and the absorption of antibodies against epitopes of the spores of *A. flavus* 136/M, so as to differentiate better the reaction against common or exclusive epitopes the hyphae, all the serums were absorbed with spores of *Penicillium lanosum* Westling and with spores of *Aspergillus flavu*s.

Serum dosage of the anti-*Aspergillus *precipitin in agarose gel (DD)

For the investigation of precipitin in agarose gel, antigenes of *A. fumigatus metabolico*, *A. fumigatus somatico*, *A. flavus*, *A. nidulans*, *A. niger* and *A. terreus* were used.

Mycological examination of mucin and biopsy

The mucin pellet obtained after centrifugation at 3000 rpm for 10 min, was fluidified by means of N-acetil-L-cistein (NAC) in the ratio 1:1, observed at the microscope and seeded onto Agar Sabouraud Dextrose Agar (SC) dishes. The bioptic material was cut up with a sterile scalpel and, in part, clarified with KOH for the microscopic observation and the other part was seeded onto SC. The dishes were incubated at 30 °C for at least 10 days and, for the positive samples, standard mycete identification techniques were used[9].

Microscopic examination of the mucin to detect granulocytes, neutrophils and eosinophils

With an aliquot of the mucin sediment slides were prepared that were then coloured by Gram staining and Giemsa May-Grünwald, following manufactures' instructions.

Results

From the comparison of the antibody titers of IgG and IgM anti-Aspergillus in IFA with and without absorption of the serum with spores of *Penicillium* sp. and A. flavus, it was seen that, even if not in all the patients, there was a decrease of the antibody titers of the absorbed sera (Table 1). In particular, a notable decrease of the antibody titers was seen for IgG and for the sera adsorbed with spores of *Aspergillus*. More precisely, for IgG an average decrease of the antibody titer on sera absorbed with spores of *Penicillium* and *Aspergillus* of 0.9 and 1.6 respectively. For IgM the average value was 0.8 on sera absorbed with spores of Penicillium and 1.1 on those absorbed with spores of *Aspergillus*. Bearing in mind the results obtained from a previous study in which the anti-*Aspergillus* antibody response was evaluated on a healthy population[10], we believe that our results were within an interval of normality (20-160) as regards the titers of IgG obtained after absorption with spores of *Penicillium*. Only one patient had a titer of 640 notwithstanding the absorbtion of the serum. For IgM, not having any reference values for the healthy population, it was difficult to interpret the significance of the positivity seen in 9/12 patients, it was also difficult to establish the values of the antibody titers to use in our population under study. Regarding the results of IgA, all results were negative except for the patients with an elevated IgG anti-*Aspergillus* titer.

Also the detection of precipitin by means of double diffusion was negative for all the antigens tested.

Finally, as regards the results of the mycological test on mucin, only in the patients with an elevated IgG anti-*Aspergillus* antibody titer, was Aspergillus group flavus isolated. All the microscope and culture tests of the biopsies were negative. This result was confirmed by the histopathological examination of all the samples were there was the absence of fungal elements as well as the presence of an eosinophilic tissue infiltrate. The investigation of granulocytes and eosinophils in the mucin was positive always for the patients with isolation of A.flavus of the mucin and with an elevated IgG and positive IgA. The study of total IgE was within limits for all patients, that is < 240 ng/ml.

Conclusions

Even if the population size was small, from our results, we believe that chronic rhinosinusal pathologies with colonization by Aspergillus are relatively low (8.3 %) and less than those reported by other authors[2, 11].

Patient	IgG			IgM		
	Absorption of Penicillium	Absorption of A.flavus	Without Absorption	Absorption of Penicillium	Absorption of A.flavus	Without Absorption
1	1/160	1/20	1/320	1/20	Negative	1/80
2	1/160	1/80	1/160	1/40	1/20	1/40
3	1/20	1/40	1/80	Negative	Negative	Negative
4	1/20	Negative	1/40	Negative	Negative	Negative
5	1/80	1/80	1/160	1/20	1/20	1/40
6	1/160	1/160	1/160	1/40	1/40	1/40
7	1/80	1/40	1/160	1/80	1/40	1/80
8	1/40	1/40	1/80	1/40	1/80	1/160
9	1/640	1/640	1/1280	1/20	1/20	1/40
10	1/20	Negative	1/80	1/40	1/20	1/40
11	1/160	1/40	1/320	Negative	Negative	1/20
12	1/160	1/160	1/160	Negative	Negative	Negative

Table 1: Antibody titers of IgG and IgM with and without serum absorption.

The sierological and culture data obtained were in agreement with the histological data. The only patient, with a positive cultural examination of the mucin for Aspergillus, IgA positive and IgG with a titer of 640 after absorption of the serum, could be clinically explained as being affected by chronic fungal rhinosinusitis non-IgE-mediated[12]. Probably the low frequency of fungal isolation from mucin that characterized our study is due to the method of collection. Contrary to what Ponikao suggested that there si an elevated probability of contamination, this took place in an operating room, before surgery, with a procedure of washing and aspiration to guarantee a minimum risk of environmental contamination.

Considering the low number of patients examined, further studies are needed to evaluate the role and usefulness that these serum tests could have for patients affected by chronic fungal rhinosinusitis.

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