Laryngeal Aspergillosis-Clinical, Diagnose and Treatment

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Introduction

It was long after scientist identified and named the fungus, Aspergillus, in 1729. Generally, the Aspergillus is widespread in natural and indoor environment. By far, several hundred species of the Aspergillus genus have been identified by mycologist, most are harmless to human. But we have realized some species, for instance, Aspergillus fumigatus, A. flavus and A. niger, can cause the infection of different parts of human body and develop corresponding symptoms.

Until 1969, Rao PB [1] reported the first case of laryngeal aspergillosis in English literature. Unfortunately, up to now, the certain etiological factors are still unconfirmed, many possible factors are taken into consideration. Any reason that causes immunocompromise, as advanced age, diabetes, leukemia, chronic obstructive pulmonary disease, decline of CD4 lymphocyte cells, HIV infection and so on, can be underlying aetiological factor. But besides that, laryngeal aspergillosis has also been reported in immunocompetent patients in several cases, the pathogenesis still remains obscure. It is thought that the damage of local mucosal membrane barrier or local dysbacteriosis, which contributes to the colonization of Aspergillus spores, plays a very important role. Potential etiological factors include long-term use of steroid (inhaler) or antibiotics, vocal abuse, oral sex (fellatio), radiotherapy and so on.

Up to now, no epidemiological data is available. There are less than one hundred cases have been reported, and all these have used “very rare”, or even “extremely rare” to describe the very low incidence. Therefore this disease is often ignored or mis-diagnosed at the beginning. In general, more and more cases have been reported in recent years, indicates the increase in incidence. Moreover, females between the ages of 20-40 years have a much higher incidence than males in the past decade [2].

Clinical Manifestation

Generally, the symptoms onset acutely or in a relatively short period of time. In most cases, progressive hoarseness is the most common and prominent persistent symptom, sometimes even the only one. A few patients accompany by fever, coughing or tachypnea at the early stage. The hoarseness can last for several weeks, even months, if the patient doesn’t receive proper treatments. The appearances of dysphonia, dysphagia and rare acute airway obstruction are usually presented in severe cases which should be highly concerned.

Because lack of specific symptoms, laryngeal aspergillosis is often misdiagnosed with other diseases which can also cause hoarseness, such as acute/chronic laryngitis, vocal nodule/polyp and laryngeal tuberculosis/tumor. The following improper treatments, especially the use of steroid or antibiotics, can aggravate the symptoms and lead longer disease course. Therefore, early diagnosis is very important.

Once laryngeal aspergillosis is suspected, clinic history should be taken very carefully. Any possible etiological factors mentioned above should be considered, including any underlying diseases which cause immunocompromise, history of drug use, profession and lifestyle of patient with vocal abuse. Some
privacy issues, for example, the history of oral sex, can’t be avoided. But more often, these only suggest the possible of laryngeal aspergillosis, and can’t directly diagnose the disease without further examination.

**Auxiliary Examination**

But even careful history-taking and body examination are performed, differential diagnosis is still difficult. Appropriate auxiliary examinations are very important for diagnosis and therapy. We recommend following examinations:

1. First of all, indirect laryngoscopy should be performed once the disease is suspected. Exploration of the larynx allows physicians to detect any neoplasm on epiglottis, vocal cord, ventricle or other parts. Whitish plaque on vocal cord and adjacent sites of larynx is the commonest finding in all cases (figure 1 a, b, c) [2-4]. Direct laryngoscopy then should be carried out to obtain biopsy specimen.

2. The specimen should be tested by a series of examinations, including microscopy of KOH preparation (quickest and easiest) (figure 2) [2-4], pathology (figure 3) [2-4] and scanning electron microscopy (if possible) (figure 4) [2-4]. Detections of characteristic hyphae branching at 45° angles, distinguished from other fungi (especially candida), highly support the diagnosis. It should be noted that negative findings due to improper sampling can’t thoroughly exclude the disease and positive findings may mask the underlying malignant tumor in larynx [5]. In routine hematoxylin and eosin stain (HE) could find the pathogenic hyphae in tissue. Periodic Acid-Schiff stain (PAS) and Grocott Methenamine Silver stain (GMS) which help to revealed hyphae and improve positive rate are highly recommended in pathology. Acanthosis and pseudoepitheliomatous hyperplasia often lead to a mistaken diagnosis of malignancy or premalignant condition [6].

3. Culture and identification of the fungus are also necessary, regardless of positive or negative results of the microscopy and pathology. It’s very important to differentiate with other laryngeal mycosis. Be careful to avoid pollution of the specimen. Both traditional methods such as slide culture, biochemical test and non-culture identification by molecular biological technique are recommended. Drug sensitivity tests will be a plus if possible. *A. fumigatus* has been isolated in most cases as the main pathogen in the disease. Velvety and powdery colonies (figure 5) develop on Sabouraud Dextrose Agar (SDA), at 28 °C. Microscopic examination of a slide culture (figure 6) and scanning electron microscopy (SEM) observation (figure 7) show subdavate vesicles, like a handstand flask, bearing phialides in one series, and closely packed with the axis roughly parallel to the axis of the stalk; conidiogenous cells are uniseriate and only on the upper two-thirds of the vesicle. DNA sequencing is used to confirm the identification as described in literatures [2-4]. Extract genomic DNA and amplify the intergenic transcribed spacer (ITS) regions flanking the 5.8S region of the rDNA by PCR. The ITS sequence is used for a BLAST search in GenBank, with high homology to *A. fumigatus*. Exocrine enzymatic activity tests of the isolates have been carried out by the semi-quantitative Api-Zym system [3-4]. They all show very strong activity of Alkaline phosphatase, Acid phosphatase, Naphtol-AS-BI-phosphohydrolase, β-glucosidase and N-acetyl-β-glucosaminidase, which might be related to their pathogenicity.

4. G test and GM test (for the *Aspergillus*) can be performed, positive results highly suggest the infections of invasive fungus. So far, little data has been reported in laryngeal aspergillosis. Lesions are very focal in most cases, G test and GM test may be negative. Positive results should be concerned with a possibility of other invasive aspergillosis, especially pulmonary aspergillosis.
Figure 3. Histopathological examination showed many hyphae deep within the vocal cord tissue (marked by arrows) and aligned along the vessel (marked by *). The hyphae had septate branches at 45° angles, which suggested Aspergillus infection. The tissue was destructuralized with neutrophils infiltration (marked by triangle symbol) (H&E, a,b X100, c,X400).

Figure 4. Scanning electron microscopy (SEM) of the true vocal cord before treatment, with crowded hyphae covering and passing throughout the damaged tissue. The characteristic hyphae branching at 45° angles and disorganized tissue with scattered red blood cells are impressive.

Figure 5. The culture of the isolate *Aspergillus fumigatus* on Sabouraud Dextrose Agar (SDA, at 28 °C) showed that the velvety and powdery colonies with a white border.
There are two main treatments for laryngeal aspergillosis in the reported cases: antifungal therapy and/or local resection. Amphotericin B and itraconazole, both of which have high antifungal activities of the *Aspergillus*, are recommended as the first-line antifungal drugs. Amphotericin B, usually associated with significant adverse effects or expensive of its lipidosome, is often used in severe hospitalized patients. In our experience, oral itraconazole is more suitable for outpatient treatment [2-4]. The recommended dose is 200mg twice a day in adults and 5mg/kg/d in children. It's very important to notice that itraconazole should be taken with whole milk or oily soup to improve its absorption. We experienced one severe case with itraconazole oral solution at 200 mg (20 ml) twice a day (keep the solution in mouth for a moment, then swallow slowly) for 5 days, followed by itraconazole capsules at 200 mg twice a day for another 25 days. Moreover, other antifungal drugs like voriconazole can also achieve a good therapeutic effect, but fluconazole should not be used because of intrinsic drug resistance of the *Aspergillus*. Before antifungal therapy, make sure there is no contraindication, such as hepatic or renal dysfunction.

The course of antifungal therapy usually lasts for weeks, depending on the remission of signs and symptoms. Therefore regular follow-up, including examinations of hepatic/kidney function and laryngoscopy, is quite necessary. The disappearances of neoplasm, local mucosa swelling and hyperemia (figure 1 d, e, f), accompanied by remission of hoarseness and other symptoms, indicate the treatment is effective. Generally, adequate course of treatment should be more than four weeks. Local resection is another approach, especially for those with...
Indirect laryngoscopy

- 

other diseases

+ 

direct laryngoscopy

specimen taken from neoplasm

Microscopy (KOH), SEM, pathology

- 

culture and identification (avoid pollution)

- 

other diseases

+ 

+ 

diagnose laryngeal aspergillosis

antifungal therapy

local resection

other auxiliary examinations to evaluate the patient

adjuvant therapy and treatment for underlying diseases

follow up

Figure 8. The diagnostic pathway of laryngeal aspergillosis.
contraindications of antifungal drugs or small focal lesions. Successful treatments have been reported in a few cases. It can be carried out by fibrolaryngoscope or direct laryngoscopy. Local resection combined with antifungal therapy is thought more effective than antifungal drugs alone.

Besides, vocal rest, which helps to alleviate the mucosal edema, should be emphasized to patients. Avoid any potential risk factors such as use of steroid or antibiotics. Any underlying diseases, especially those cause immunocompromise, should be treated at the same time. Immunological therapy enhancing the immune function can be an adjunctive treatment.

Generally, the prognosis of laryngeal aspergillosis is very good in most patients after proper and adequate-duration treatments. So far, there are no recurrent cases. Two death cases have been reported. One patient with subglottal aspergillosis and severe aplastic anemia ultimately died of pulmonary aspergillosis [7], another with laryngeal aspergillosis and precursor B-cell acute lymphoblastic leukemia died of severe respiratory distress [8], although both of them had received regular and adequate antifungal therapy. It suggests the laryngeal aspergillosis can be refractory and fatal in severe case.

We recommend a helpful algorithm for the diagnosis and treatment of laryngeal aspergillosis (figure 8).

Prospect

Although lack of accurate data, the number of reported cases has increased rapidly in the past twenty years, indicates an increased incidence, and even higher in the future can be predicted [9]. It may attribute to potential risk factors become more common in the population, for example, aging, increased use of steroid or antibiotics and increased practice of oral sex. Thus more attention should be paid on this rare but rapidly increased disease. Physicians should be alert to any suspected case with hoarseness, particularly in immunocompetent patients who are easily overlooked.

References


