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Metagenomic next-generation sequencing in the diagnose of pulmonary infection with airway complications in a lung transplant recipient

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ABSTRACT

We reported a case of a 60-year-old male with fever, cough, expectoration, and chest distress after right lung transplanted. Blood examination showed elevated C-reaction protein (CRP), white blood cell (WBC), and ammonia. Computed tomography (CT) revealed patchy high-density shadows and few pleural effusions in the transplanted lung. Bronchoscopy illustrated anastomotic fistula, and pseudomembrane and mucus plugs around the right main bronchial anastomosis. Carbapenem-resistant *Klebsiella pneumoniae*, *Ureaplasma urealyticum,* and *Aspergillus flavus* was successively detected by metagenomic next-generation sequencing (mNGS). Targeted antimicrobial agents were administered and patient was successfully discharged. Unfortunately, a year later, patient died of respiratory failure due to recurrent pulmonary infections.

1. Introduction

Lung transplant recipients was of a higher infection burden than other solid organ transplant recipients [1]. The rapidly and accurately detection of causative pathogens is vital to guide appropriate therapy and reduce mortality rate. mNGS represented as a promising method for diagnosis of infectious disease, for the sake of sequencing of almost all the nucleic acids in parallel [2,3]. In this study, a lung transplant recipient was reported to suffer from recurrent pulmonary mixed infections in the first-year post transplantation, with pathogens successfully identified by mNGS even when conventional diagnostics failed. This findings not only enabled us to understand the rare pathological changes caused by *U. urealyticum* such as formation of pseudomembrane and mucus plugs in airways, but also provided persuasive evidence for the final diagnosis.

2. Case presentation

On March 21, 2020, a 60-year-old male was admitted to the thoracic surgery department of Henan Provincial People's Hospital due

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to an acute exacerbation of wheezing, suffering from interstitial lung disease for 3 years and hyperglycemia for more than 4 years (Fig. 1). On March 23, Chest CT showed multiple diffuse reticular shadows, ground-glass opacities, and emphysematous bullae in both lungs (Fig. 2*a*). On March 26, the patient underwent single right lung transplantation. On March 27, the patient developed a fever of 38 °C. By bronchoscopy, a small amount of whitish-yellow mucus was observed. Carbapenem-resistant *K. pneumoniae* was found in BALF culture. Thus, tigecycline 100 mg (i.v. infusion, q12h) and ceftazidime/avibactam 2.5 g (i.v. infusion, q8h) were prescribed. Gradually, his body temperature returned to normal. On April 3, the patient started cough, expectoration, and chest distress, with whitish-yellow pseudomembrane at the bronchial anastomosis. On April 5, chest CT illustrated patchy high-density shadows and few pleural effusions in the lower lobe of the transplanted right lung (Fig. 2*b*). On April 7, the pseudomembrane around the bronchial anastomosis had already extended into the small airways where mucus plugs were formed (Fig. 3*a*). Serum 1,3-beta-D glucan assay (G test), serum galactomannan assay (GM test), and BALF smear were all negative. The patient was clinically diagnosed as airway-invasive aspergillosis for the typical mucosal lesions in the airways, and treated with voriconazole at a loading dose of 6 mg/Kg (400 mg, i.v. infusion, q12h). Considering the side effect of tigecycline, the antibiotic regimen was adjusted to ceftazidime/avibactam combined with polymyxin B. On April 11, linezolid (600 mg, iv, q12h) was added based on the results of blood tests (WBC 25 $\times$ 10^9/L, CRP 172 mg/L, PCT 1.6 ng/ml) and the presence of *Corynebacterium striatum* in sputum culture. On April 13, the patient had an increased chest tight, with further extension of the pseudomembrane in the airways and heavy build-up of mucus plugs (Fig. 3*b*). Meanwhile, blood tests: WBC 21 $\times$ 10^9/L, CRP 116 mg/L, PCT 1.5 ng/ml and tacrolimus 7 ng/ml (Fig. 4a). These findings still indicate Aspergillus infection, and thus, voriconazole and caspofungin was prescribed. On April 14, the blood trough concentration of voriconazole was 5.7 μg/ml, and then we changed maintenance dose into 4 mg/kg (200 mg, i.v. infusion, q12h) (Fig. 4b). On April 17, BALF mNGS resulted *U. urealyticum* (6416) and *C. striatum* (2625). And increased blood ammonia level (238 μmol/L) confirmed the *U. urealyticum* infection. Meanwhile, chest CT revealed decreased exudative lesions in both lungs, a small pleural effusion in right lung (Fig. 2*c*). Then, the anti-infective treatment was adjusted to meropenem, erythromycin (0.75g, iv, q8h) and caspofungin. Two days later, the blood ammonia level dropped to 125 μmol/L. On April 22, pseudomembrane and mucus plugs significantly decreased (Fig. 3*c*). On April 30, the patient developed shortness of breath again, accompanied by a cough with yellow sputum. On May 1, Chest CT revealed patchy high-density shadows in the transplanted right lung (Fig. 2*d*). On May 4, BALF mNGS implied mixed infections with *K. pneumoniae* (84387), *Acinetobacter baumannii* (21), *U. urealyticum* (14), and *C. striatum* (100010). Tigecycline, ceftazidime/avibactam, and polymyxin B were used. On May 7, Chest CT indicated signs of improvement (Fig. 2*e*), but the shortness of breath got worse, probably caused by build-up of pus-like secretions and formation of a bronchopleural fistula at the bronchial anastomosis (Fig. 3*d*). Yet BALF culture and smear all were negative, and there was one read for *A. flavus* in BALF mNGS. Considering *A. flavus* infection in the fistula of the bronchial anastomosis (Fig. 3*e and f*), the anti-infective treatment was adjusted to tigecycline, ceftazidime/avibactam, teicoplanin, azithromycin, voriconazole, and caspofungin. For the suspicion of acute rejection, panel-reactive

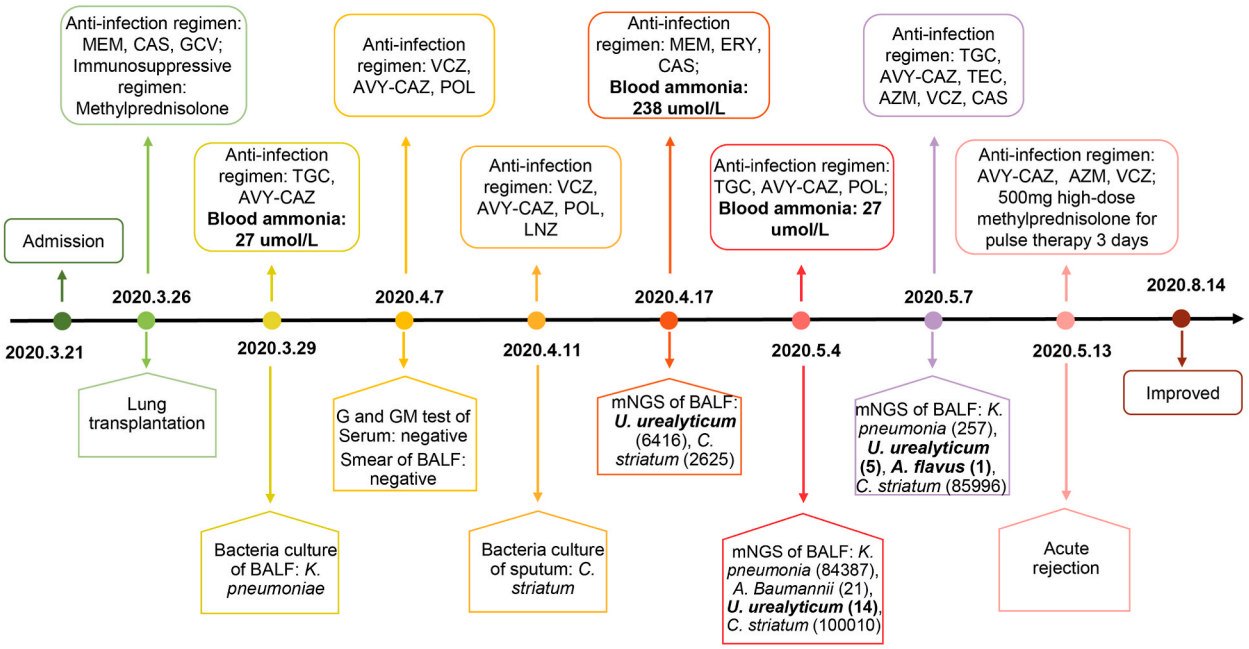

Fig. 1. Timeline of the clinical course of the patient and identification of causative pathogen. Abbreviations: MEM, meropenem; CAS, caspofungin; GCV, ganciclovir; TGC, tigecycline; AVY-CAZ, ceftazidime/avibactam; VCZ, voriconazole; POL, polymyxin B; LNZ, linezolid; ERY, erythromycin; TGC,tigecycline; TEC, teicoplanin; AZM, azithromycin; POS, posaconazole; AMB, amphotericin B; G test, (1, 3) -β-D-glucan, GM test, galactomannan; *K. pneumonia*, *Klebsiella pneumonia*; *C. striatum*, *Corynebacterium striatum*; *U. urealyticum*, *Ureaplasma urealyticum*; *A. Baumannii*, *Acinetobacter baumannii*; *A. flavus*, *Aspergillus flavus*; *P. jirovecii*, *Pneumocystis jirovecii*; *R. microspores*, *Rhizopus microspores*; HSV1, human herpes simplex virus type 1; CMV, human herpes simplex virus type 5.

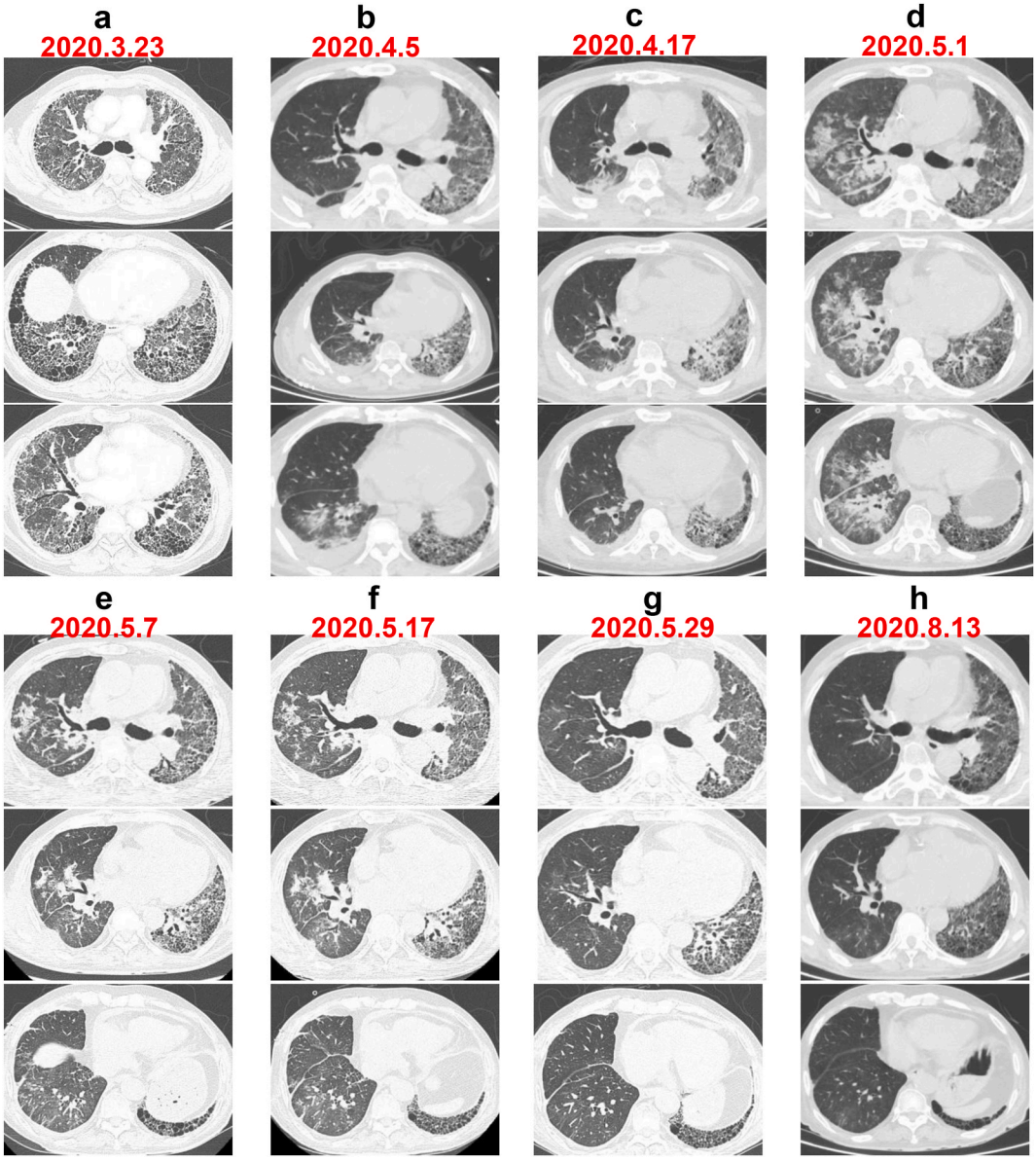

Fig. 2. Serial chest computed tomography (CT) scans of the patients. (a) March 23, 2020, before lung transplantation, multiple diffuse reticular shadows, ground-glass opacities, and emphysematous bullae in both lungs; **(b)** April 5, 2020, 10 days after lung transplantation, exudative lesions in the lower lobe of the right lung with a small pleural effusion, multiple diffuse reticular shadows in the left lung; **(c)** April 12, 2020, a small exudative lesions in the lower lobe of the right lung and decreased pleural effusion in the right lung, increased exudative lesion in the left lung; **(d)** May 1, 2020, multiple patchy shadows along bronchial tree in right lung; **(e)** May 7, 2020, after anti-infection treatment, multiple patchy shadows decreased in the right lung; **(f)** May 12, 2020, the exudation of the right lung slightly improved; **(g)** May 29, 2020, the exudation of right lung markedly improved; **(h)** August 13, 2020, no obvious lesions in the right lung.

antibody (PRA) testing was carried out. On May 12, dyspnea increased, PRA testing showed human leukocyte antigen-I (HLA-I) strongly positive with highest mean fluorescence intensity (MFI) value of 2025.75, and pulse therapy with methylprednisolone was started at an initial dose of 500 mg for 3 consecutive days, and then the dyspnea relieved. The oxygenation index climbed up to 400 mmHg, and chest CT also implied signs of improvement (Fig. 2*f*). Bronchoscopy revealed an increasing amount of pseudomembrane and pus-like secretions. It was suggested that infections of *A. flavus* and *U. urealyticum* were not well controlled, and the anti-infective treatment was then shifted to meropenem, doxycycline, voriconazole, and nebulized amphotericin B. On May 29, the exudation of right lung markedly improved (Fig. 2*g*). On June 22, there were still a lot of yellow remains of the abscess attached to the anastomosis of the right main bronchus (Fig. 3*g*). On August 13, the lesions in the right lung basically disappeared (Fig. 2*h*). On August 14, the fistula closed up at the anastomosis of the right main bronchus, where the lumen was narrowed by bronchial smooth muscle

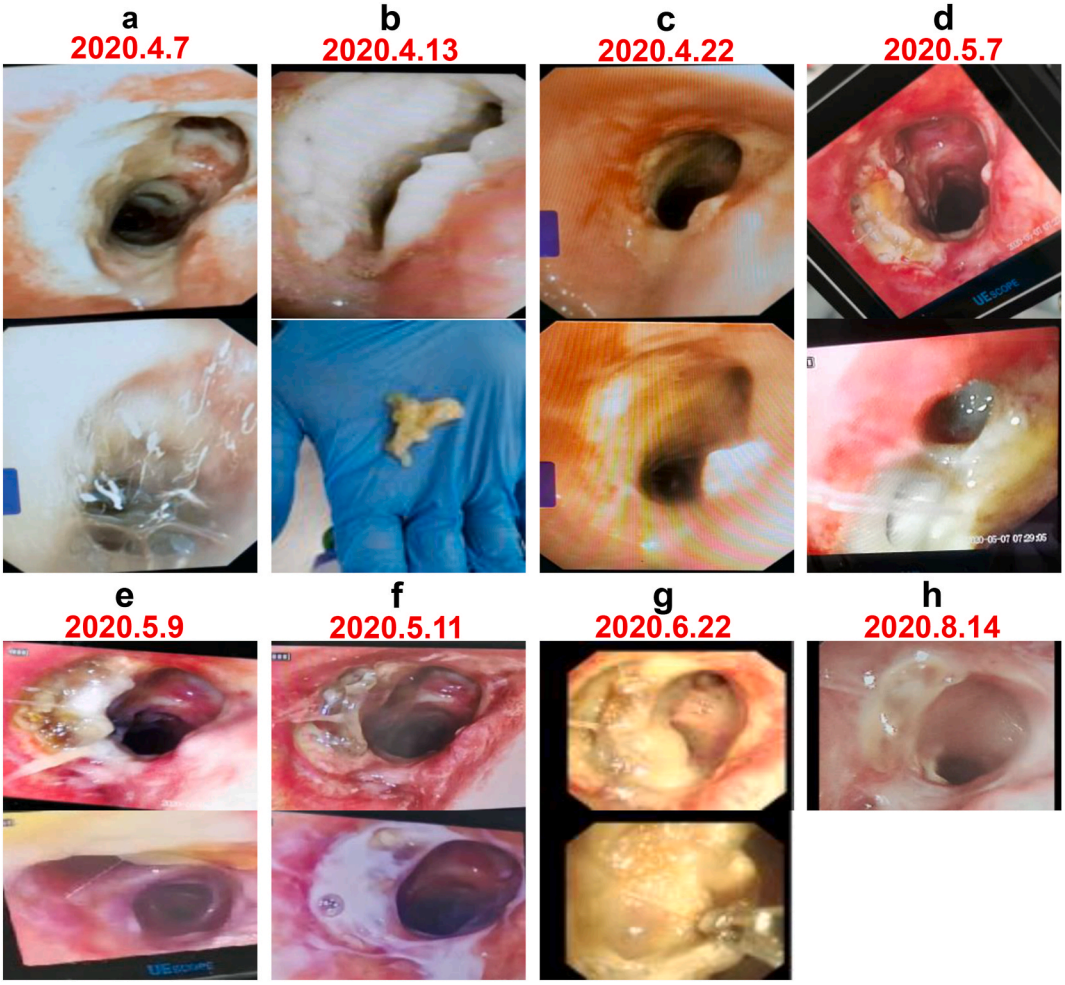

Fig. 3. Bronchoscope images of patient. **(a)** April 7, 2020, yellow-white pseudomembrane around the right main bronchial anastomosis extended into small airways; **(b)** April 13, 2020, yellow-white pseudomembrane around the right main bronchial anastomosis increased and mucus plugs formed in the small airways; **(c)** April 22, 2020, after erythromycin treatment, pseudomembrane around the right bronchial anastomosis and mucus plugs decreased; **(d)** May 7, 2020, a mass of yellow-white pseudomembrane formed in the right main bronchial anastomosis, and fistula formed in the internal side of anastomosis; **(e)** May 9, 2020, a mass of yellow-white pseudomembrane and pus blocked the right main bronchial anastomosis, and lung CT showed the defect in the right main bronchial airway wall; **(f)** May 11, 2020, a mass of yellow-white pseudomembrane and pus in the right main bronchial anastomosis was treated with amphotericin B lidocaine gel; **(g)** June 22, 2020, a mass of yellow pus and sputum was removed by the biopsy forceps; **(h)** August 12, 2020, mucosa was smooth and lumen contracture narrowed in the right main bronchial anastomosis.

contraction and covered by a small amount of pseudomembrane (Fig. 3*h)*. And then the patient was discharged. Unfortunately, on April 2021, we were informed that the patient died of respiratory failure due to recurrent pulmonary infections by the telephone follow-up.

3. Discussion

Airway complications mainly occur within 3 months after lung transplantation, which is one of the main causes affecting patients' prognosis [4]. Infection, surgical methods, and acute rejection are the most causative factors of airway complications post lung transplantation, which may lead to mucosa ischemia, mucosa necrosis, airway stenosis, and airway softening at the site of bronchial anastomotic [5–7]. The most serious complication is anastomotic rupture, causing patient death [5]. We reported this case of a male patient who developed twice airway complications after right lung transplantation with different infection etiology, different characteristics of airway mucosal injury, and different CT images.

In the first stage, pseudomembrane of the right main bronchial airway and mucus plug of the distal small airway were found by bronchoscopy on April 3 (9 days postoperatively), which occurred when anti-bacterial therapy was effective and inflammatory indexes were improved. It was considered to be caused by Aspergillus infection. Caspofungin was discontinued and changed to voriconazole on April 7. However, pseudomembrane and mucus plugs were increased and chest tightness was observed. Meanwhile, fungus was not

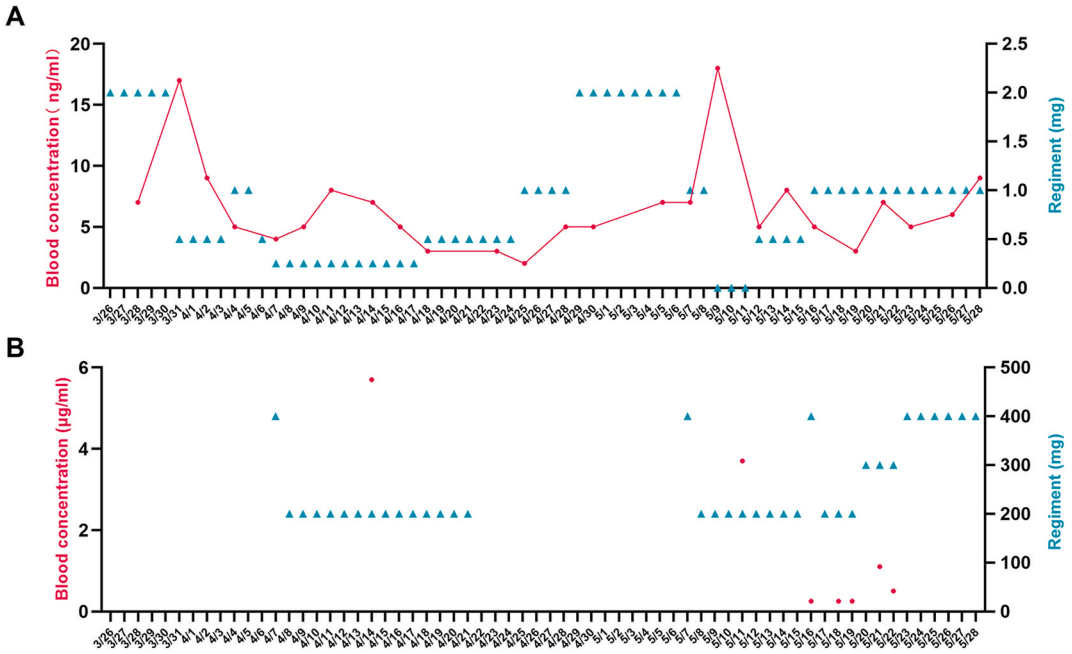

Fig. 4. The curve of blood concentrations and regiment of tacrolimus (A) and voriconazole (B).

detected by repeated conventional microbiology tests from April 13 to April 17. Voriconazole combined with caspofungin was used as an antifungal agent, but the airway lesions continued to worsen and the chest tightness worsened. On April 17, BALF mNGS detected *M. urealyticum*, and blood ammonia was increased to 238 μmol/l, which was the significant characteristics of *M. urealyticum* infection [8,9]. Erythromycin was given on April 17th, and blood ammonia was decreased to 125 μmol/l on April 20. On April 22, bronchoscopy found that pseudomembrane in the right main airway and mucus plugs in the distal small airways decreased significantly, meanwhile, chest tightness relieved significantly. According to the response to anti-infection treatment and bronchoscopy manifestations, *M. urealyticum* infection were considered. Thus voriconazole was discontinued and caspofungin was used to prevent fungal infection.

In the second stage, chest tightness appeared again on April 30, and chest CT suggested that the exudative lesions of transplanted lung increased. On the basis of continued treatment of *M. urealyticum*, bronchoscopy found that a large number of yellowish white pseudomembrane and pus moss covered at the right main bronchial airway anastomosis and distal segment. BALF mNGS results conveyed that the reads number of *M. urealyticum* significantly decreased. And the blood ammonia was significantly reduced, too. On May 7, bronchoscopy showed a large amount of yellowish white pseudomembrane and pus moss in the right main bronchial anastomosis, and a fistula was formed inside the anastomosis orifice. Chest CT illustrated a break at the right main bronchial anastomosis. BALF mNGS detected the sequence of *A. flavus*, and caspofungin plus voriconazole was given as antifungal therapy. Although there was an acute rejection, considering high-dose hormone shock treatment might lead to the aggravation of the anastomotic break lesion, hormone shock treatment was not carried out in the early stage. Nevertheless, the patient's dyspnea worsened. On May 13, 500 mg methylprednisolone shock was given for 3 days and then the dose was reduced, and the patient's chest tightness rapidly improved. However, the right main bronchial anastomotic fistula deepened, a large number of yellow pus moss attached to the surrounding mucosa. Intravenous antifungal therapy was continued, pus moss was cleaned under bronchoscopy, and amphotericin B was local applied at the anastomotic fistula. Anastomotic fistula healed, anastomotic lumen slightly contracted, and a small amount of white pseudomembrane covered mucosa after 3 months antifungal treatment.

The characteristics and symptoms of the airway lesions were significantly different in the two stages. In the first stage, airway mucosal lesions was mainly caused by *M. urealyticum*, which airway pseudomembrane formation and a larger number of mucus plugs blocking the distal small airway were the main characteristics. Meanwhile, chest tightness are obvious and blood ammonia increased. Symptoms can be improved in a short time after effective treatment. Airway mucosal lesions recovered quickly without mucosal ulcerative lesions. In the second stage, airway lesions was associated with acute rejection, bacterial infection, and especially Aspergillus infection. The lesions were characterized by airway anastomosis break, fistula formation, a large amount of pseudomembrane and pus moss adhering to the mucosa near the anastomosis. The distal airway mucus plugs were rare, and the treatment lasted for months. The patient's chest tightness was mainly associated with acute rejection.

Among infection-induced airway complications, Aspergillus infection is more common [10,11]. In contrast, airway mucosal lesions caused by *M. urealyticum* have rarely been reported. *M. urealyticum* is mostly associated with genitourinary tract infections and can cause bacteremia, central nervous system infections, and pneumonia in immunocompromised populations. Mycoplasma adhesive organelles and lipoproteins can assist in the formation of biofilms, adhering to the surface of respiratory columnar epithelial cells and protecting itself from removal by mucus cilia. However, the mechanism of *M. urealyticum* causing airway pseudomembrane lesions is

not clear. And *M. urealyticum* is usually sensitive to macrolides, fluoroquinolones and tetracycline antibiotics.

4. Conclusion

Patients after lung transplantation need to be alerted to airway complications with or without symptoms, and bronchoscopy can be used for early detection of airway mucosal lesions [12]. Infection is a common cause of airway complications, and it is of great important to apply mNGS to determine etiology, especially rare pathogens [13]. The characteristics of airway complications caused by different pathogens are different and need to be summarized by more clinical observations. In the course of treatment, the immunosuppressant tacrolimus and antifungal drug voriconazole drugs interact with each other. Thus, blood concentration testing is carried out in a timely manner to avoid drug toxicity or insufficient doses.

Ethics statement

This case report was approved by the Ethics Committee of Henan Provincial People's Hospital. And written informed consent for clinical details and images was obtained from the patient for publication of this case report.

Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article.

Data availability statement

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The study was planned and designed by XJZ, HML and HX. The original draft was prepared by HML and HX. LH and ZGZ collected and analyzed the data. The manuscript was revised by XJZ. All authors read and approved the final manuscript.

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