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INVASIVE PULMONARY ASPERGILLOSIS AFTER HEART TRANSPLANTATION

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Objective: to assess the incidence, determine the peculiarities of the course of invasive pulmonary aspergillosis (IPA) and identify risk factors for IPA in heart transplant recipients. **Materials and methods.** From January 2010 to December 2019, 137 heart transplantations (HT) were performed: mean age 46 ± 14 years; male 102 (74%) and female 35 (26%). All patients received a three-component immunosuppressive therapy: calcineurin inhibitors, mycophenolate mofetil (MMF) and Glucocorticoid (GCs). Induction therapy consisted of Basiliximab (81%, n = 111) and antithymocyte immunoglobulin (15%, n = 20). A retrospective analysis of patients with identified post-HT invasive IPA was performed; risk factors for IPA were assessed. In patients with early IPA, the length of stay in the intensive care unit (ICU), the duration of mechanical ventilation, and the initial severity of the condition were studied. All patients with suspected pneumonia underwent bronchoscopy with examination of bronchoalveolar lavage (BAL) and chest computed tomography (chest CT scan). **Results.** During the follow-up, there were 58 episodes of pneumonia, of which 16 (28%) were IPA (age 33 to 64 years). All patients had a target level of immunosuppressive drugs concentration in blood; basiliximab was used as induction therapy in 15 of 16 patients. Half of the recipients developed IPA in the early post-HT period (less than 3 months after HT), in the rest (n = 8) – at a later date (3 months to 1 year after HT). The diagnosis was verified: 14 out of 16 patients showed an increase in the Aspergillus antigen positivity in the BAL to 7.2 (2.8 ± 1.6); chest CT scan revealed specific changes. In two patients, there were no diagnostic criteria for IPA, but the diagnosis was made based on the results of histological examination after resection of the left lower lobe of the lung. All patients received voriconazole therapy for 2 to 6 months, their immunosuppressive therapy was adjusted (tacrolimus and MMF dose adjustment) and their white blood cell count was monitored. Complete cure of the disease was achieved in 13 (81%) patients. Two patients died within 30 days after HT in the intensive care unit, one died from urogenital diseases caused by bacterial flora and leading to urosepsis, 4 months after IPA treatment was initiated. All patients had risk factors for IPA: taking immunosuppression, including GCs (n = 16), prolonged ICU stay (n = 14), inotropic support exceeding 2 days in the early post-transplant period (n = 10), cachexia during HT (n = 6), leukopenia (n = 9) and neutropenia (n = 14). **Conclusion.** In heart transplantat recipients, the incidence of IPA among respiratory tract infections is 28%. The risk of developing IPA was highest during the first year following HT. In the majority of recipients, the disease was detected at the early stages; diagnosis required surgical intervention in 12% of cases. A decrease in the risk of developing IPA was associated with correction of the following risk factors for this disease in all patients: volume of immunosuppressive therapy during the first year after transplantation and prevention of the development of neutropenia as a marker of infectious complications or immunosuppression overdose. Early diagnosis of IPA allowed for initiation of timely specific therapy in most recipients and achievement of a positive effect in 80% of them.

Keywords: heart transplantation, infectious complications, invasive pulmonary aspergillosis.

INTRODUCTION

The number of heart transplant (HT) recipients is increasing every year. Infectious diseases are the most frequent complications and the leading cause of mortality in the post-transplant period. The number of heart recipients is increasing every year. Infectious diseases are the most frequent complications and the leading cause of post-transplant mortality [1, 2].

Invasive pulmonary aspergillosis (IPA) is an opportunistic fungal infection that can develop within the first year after HT (most commonly within <1 to 3 months after surgery) and can be fatal [3, 4]. When pneumonia develops in patients regardless of the timing after heart transplantation, it is shown to exclude its fungal genesis. Currently, there are no data on the incidence of IPA in the post-transplant population [4, 5]. Some studies

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have reported that this complication predominates in men (84%), whose average age is 43 years, one third of which has ischemic congestive heart failure (CHF) [4, 6]. Patients usually complain of fever and cough, but clinical manifestations may be absent in the initial stages of the disease [4, 6]. Laboratory findings may include leukopenia and neutropenia up to agranulocytosis [4, 6, 7]. Radiological findings include infiltrative changes, and chest CT scan may show "frosted-glass" changes, which is typical for various respiratory diseases of viral or fungal origin [4, 6, 7]. Absence of clinical symptoms at the initial stages of the disease and the risk of developing infectious complications caused by mixed flora (bacterial + fungal, viral + fungal) often lead to untimely diagnosis and increases the mortality rate.

Objective: to assess the incidence, features of the course and identify risk factors for the development of pulmonary aspergillosis in HT recipients.

MATERIALS AND METHODS

From January 2010 to December 2019, 137 orthotopic HTs were performed using the bicaval technique at Almazov National Medical Research Centre, St. Petersburg, Russia. This included 102 men (74%) and 6 children (5 of them were female) 10–16 years old (median 15 years). The mean age of the recipients was 46 ± 14 years.

During the first year after HT, all patients received three-component immunosuppressive therapy: calcineurin inhibitors (FK-506), mycophenolate mofetil (MMF)/everolimus, and glucocorticoids (GCS). During the first year after HT, the volume of glucocorticoids was gradually reduced to complete cancellation. In the case of development of leukopenia and/or neutropenia against the background of GCS reduction, the volume of MMF therapy also decreased. Induction therapy was represented by Basiliximab (81%, n = 111) and antithymocyte immunoglobulin (15%, n = 20). Along with immunosuppressive therapy, pneumocystis pneumonia prophylaxis was carried out for 6 months (sulfamethoxazole + trimethoprim together with folic acid), antifungal (nystatin for 1 month) antiviral (valganciclovir for 1 year) therapy was used. Prophylactic therapy in all patients included statins (atorvastatin at a dose of up to 20 mg/day), calcium preparations together with cholecalciferol, proton pump inhibitors, and antihypertensive, antiarrhythmic, etc. therapy was prescribed as indicated.

This study was carried out in accordance with the principles of the Declaration of Helsinki. For the period from January 2010 to December 2019, a retrospective analysis of clinical and laboratory characteristics in patients with post-HT IPA was carried out, its risk factors were evaluated. In patients with early IPA, the initial severity of the condition, length of stay in the intensive care unit (ICU), and duration of mechanical ventilation were studied. All patients with suspected pneumonia underwent chest CT scan and bronchoscopy, followed by bronchoalveolar lavage (BAL): culture, cytology, Aspergillus antigen, cytomegalovirus infection, and pneumocystis. Laboratory diagnostics included evaluation of inflammation markers (clinical blood count with WBC count and C-reactive protein (quantitative) over time, polymerase chain reaction to cytomegalovirus and Epstein–Barr virus, blood culture, general analysis and sputum culture, general analysis, and urine culture, and if fungal infection was suspected (development of leukopenia and/or neutropenia), a blood test for galactomannan was performed. Immunosuppressive therapy was adjusted: in patients with confirmed IPA, mycophenolate mofetil therapy was reduced or, in the case of bacterial infection, it was temporarily canceled, and serum tacrolimus levels corresponded to the target values, according to the timing after HT (8–12 ng/mL).

The data were statistically processed using SPSS 21.ORU. Mean values and standard deviation (M ± SD) were calculated. In the case of a small sample ($n < 20$), the data were presented as medians (Me), [minimum and maximum values]. With a distribution other than normal, the nonparametric Mann–Whitney method was used to assess the differences; the data are presented as medians (Me), [25th and 75th percentiles]. Fisher's exact test was used to assess differences in qualitative parameters. The criterion for statistical significance of the obtained differences was $p < 0.05$.

RESULTS

During the follow-up, 58 episodes of pneumonia were recorded among all patients who underwent HT, of which 16 (28%) people were diagnosed with IPA.

General characteristics of patients. The age of these patients ranged from 33 to 64 years (Me 55 years). The patients who underwent IPA had characteristic risk factors for fungal infections: 6 had cardiac cachexia at the time of HT, all had ongoing immunosuppressive therapy, including GCS (n = 16), leukopenia (n = 9), and neutropenia (n = 14), inotropic support lasting more than 2 days in the early postoperative period (n = 10) and long stay in the ICU (n = 14).

Basiliximab was used as induction therapy in 15 out of 16 patients, and antithymocyte globulin was used in one patient. Half of the recipients developed IPA early after HT (less than 3 months after HT), while the rest (n = 8) developed IPA between 3 months and 1 year after HT. None of the patients developed IPA for more than 1 year after HT. In the early postoperative period, an extracorporeal membrane oxygenation (ECMO) system was installed in one patient due to right ventricular heart failure [8], against the background of which pneumonia of mixed (bacterial + fungal) origin was detected. In 14 out of 16 patients, there was increased Aspergillus antigen positivity in BAL (from 1.45 to 10.2) and chest CT scan showed "frosted-glass" changes. In two patients no

effect of antibiotic therapy, laboratory and instrumental criteria for IPA were absent, and transthoracic resection of the lung lobe was performed for differential diagnosis with oncopathology due to anatomical inaccessibility for transbronchial biopsy. The IPA diagnosis was verified by histological examination. Characteristic histological changes are shown in Fig.

After detection of IPA, all patients were treated with voriconazole for 2 to 6 months (initially by intravenous administration of 400 mg × 2 times a day, and then orally after normalization of the CRP level).

Features of immunosuppressive therapy. At the time of IPA development, all patients were receiving tacrolimus, MMF, and glucocorticoid therapy; immunosuppressive drugs concentrations in blood were targeted. When pneumonia was detected, MMF was temporarily canceled, then the timing of the resumption of such therapy was determined individually, taking into account the clinical symptoms (absence of fever), level of inflammation markers and under control of heart graft function. Taking into account drug interactions after antimycotic therapy, all patients required a reduction of the tacrolimus dose by at least 2–3 times, and after the end of voriconazole, the tacrolimus dose was increased again to achieve the target blood concentration values, followed by regular laboratory monitoring. In three patients, due to the development of agranulocytosis, the mycophenolate mofetil dose was temporarily canceled or reduced, and colony-stimulating factors were used until the transition through nadir.

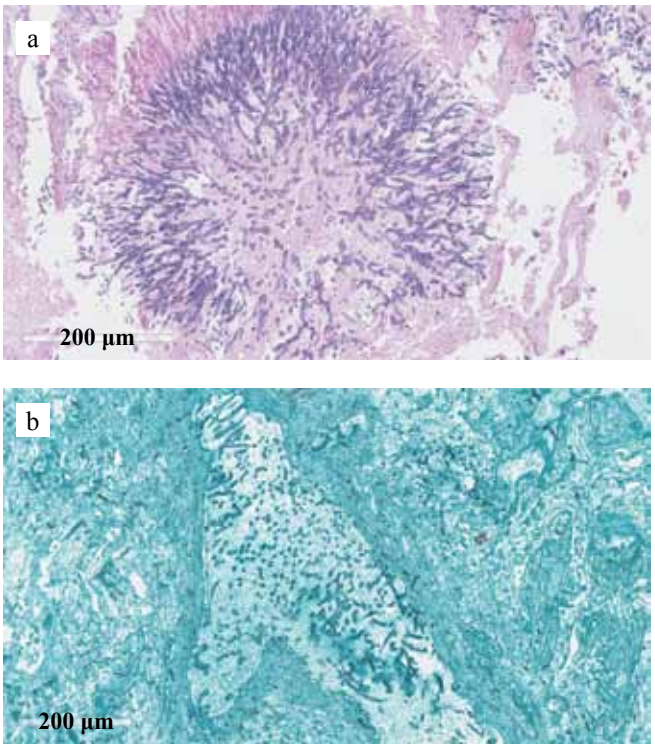

Fig. Aspergillosis with dichotomous branching of septate hyphae: A. H&E Stain, 200×. B. Grocott's stain, 200×

Every 1–2 weeks from the beginning of the infectious process, the heart graft function was assessed – ECG (every week), ECHO (at least once in 2 weeks). None of the patients had any cardiac graft function damage during IPA; therefore, the endomyocardial biopsy (EMB) was postponed until the acute infectious process was relieved and was performed after normalization of inflammatory markers in the absence of fever, no earlier than 2 months after IPA development.

The disease was completely cured in 13 (81%) patients within six months. Two patients died within 30 days after HT from infectious complications of bacterial genesis followed by development of sepsis. Another patient died 4 months after the start of IPA treatment from urosepsis. None of the convalescents had recurrent IPA in the long-term after heart transplantation.

DISCUSSION

When infectious complications develop in patients after heart transplantation, an examination should always be conducted to find the etiology, with mandatory exclusion of the presence of mixed flora. With persisting radiological changes, even if there is a positive effect (reduction or normalization of C-reactive protein (CRP) levels, absence of fever) against the background of standard antibiotic therapy for the treatment of pneumonia, continuation of the examination is always indicated in order to exclude non-bacterial genesis of the process, for example, cytomegalovirus or coronavirus infection, aspergillosis, pulmonary mycosis or toxic everolimus-associated alveolitis. Aspergillus is found in cultures of sputum (8–34%) and BAL (45–62%) [11], and its detection in the early stages of the lesion allows arresting the process without reducing lung function and increases patient post-HT survival.

Common risk factors for IPA are steroid-dependent asthma or chronic obstructive pulmonary disease [14], but none of the described cohort of patients had such comorbidities. P. Munoz et al. and T. Pelaez et al. distinguish another risk factor – diabetes mellitus [5, 14]. According to our results, 63% (n = 10 of 16) of patients diagnosed with IPA had type 2 diabetes mellitus or post-transplant diabetes. Other risk factors for IPA in solid organ recipients are cytomegalovirus infection, use of mechanical circulatory support and/or renal replacement therapy in the early postoperative period [5, 11].

Reducing the risk of IPA may be associated with adjusting the volume of immunosuppressive therapy during the first year after transplantation, preventing neutropenia and normalizing nutritional status, which are reversible risk factors for this disease. An overdose of immunosuppressive therapy increases the risk of post-transplant complications, especially infectious ones. None of the patients in the described group were at risk of developing IPA in the long term after surgery (more than 1 year), which can be associated with reduced

volume of immunosuppressive therapy over time: decrease in GCS up to complete cancellation, timing after introduced induction therapy and a decrease in calcineurin inhibitor concentrations. Given the drug interactions of antimycotic drugs, regular laboratory monitoring of the concentrations of immunosuppressive drugs (to maintain target values), clinical blood count with WBC count (as a marker of inflammation and to assess tolerance of optimal doses of antiproliferants) and CRP, as well as radiological monitoring of chest organs (X-rays, CT) help to reduce the risk of infectious complications and ensure detection of post-transplant diseases [4, 9, 10].

Given the immunocompromised status of patients, characterized by atypical symptoms of infectious processes (including absence of fever), laboratory and imaging examination results should be evaluated taking into account the anamnesis. For example, "normalization" of the WBC count at initial chronic leukopenia may reflect the accession of bacterial flora, and changes in WBC counts combined with increased CRP in the long-term after HT period may require the exclusion of oncopathology, etc. [9, 11–12]. Meanwhile, "frosted-glass" changes in the lung tissue, characteristic of fungal and viral pneumonia, can persist in the long-term period after the relief of a previous IPA or pneumonia caused by SARS-CoV-2, etc. Another peculiarity of radiological dynamics of the process is that the volume of pulmonary infiltrates may even increase during the first 7–10 days of antifungal therapy, but such dynamics are a predictor of granulocyte recovery [13].

The drugs of choice for the treatment of IPA are amphotericin B, caspofungin, and voriconazole [4, 11, 13]. Survival rates were higher in patients treated with voriconazole compared to those treated with amphotericin B (70.8% and 57.9%, respectively) [4, 11]. The standard regimen for prescribing voriconazole in patients after solid organ transplantation is 6 mg/kg, 2 doses intravenously, then 4 mg/kg intravenously every 12 hours, with the dynamic transition to oral administration of the drug, 200 mg/day every 12 hours. In patients taking calcineurin inhibitors (cyclosporin, tacrolimus), it is necessary to consider drug interactions when adding antimycotic agents to therapy, which lead to increased concentrations of immunosuppressive drugs in a short time. This requires a reduction in cyclosporine and tacrolimus doses from the very first day of administration of antimycotic drugs [11, 13]. The minimum duration of antimycotic therapy in immunocompromised patients is 6–12 weeks, but the duration of conservative therapy in each case is determined individually and depends on the time of resolution of the pulmonary lesion [10, 13, 15]. Surgical resection of tissues infected by Aspergillus can be considered as an adjunct to conservative treatment in patients with involvement of large vessel lesion, development of pericarditis and/or pleurisy [13, 15].

According to reports, mortality from IPA is 30–40% [4, 13]. In our present study, out of 16 recipients diagnosed with IPA, 3 died (19%), and IPA had not been cured at the time of death. However, those who died had other infectious complications in addition to IPA (mixed infection: bacterial and fungal.

CONCLUSION

In post-HT patients, the incidence of IPA among respiratory tract infections was 28%. The highest risk of IPA was in the first year after HT. In the majority of recipients, the disease was detected at the initial stages; in 12% of cases, surgical intervention was required for diagnosis. A decrease in IPA risk was associated with correction of the following risk factors for this disease in all patients: volume of immunosuppressive therapy during the first year after transplantation and prevention of the development of neutropenia as a marker of infectious complications or an immunosuppression overdose. Early diagnosis of IPA made it possible to initiate timely specific therapy in the majority of recipients and achieve a positive effect in 80% of them.

The authors declare no conflict of interest.

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