Impact of donor lung pathogenic bacteria detected by next-generation sequencing on early post-transplant outcomes in lung transplant recipients

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Abstract

Background: The effect of donor lung pathogenic bacteria on the prognosis of lung transplantation is not clear. We used the technique of next-generation sequencing (NGS) to detect the pathogenic bacteria from the lower respiratory tract and analyzed whether the colonized bacteria of donor lung affect the outcomes of lung transplantation.

Methods: All patients who underwent lung transplantation from March 2018 to June 2018 at the Wuxi People's Hospital affiliated to Nanjing Medical University were included in this study. Twelve cases of donor lung were obtained, and 17 lung transplants were performed, including 12 single lung transplantation and 5 bilateral lung transplantation. The colonized bacteria in the lower lobe tissue of donor lung were detected by NGS, and the bacteria culture method was used to detect the bacteria in the airway secretion before and after the operation. The information of extracorporeal membrane oxygenation (ECMO) support time, mechanical ventilation time, intensive care unit (ICU) stay time,duration of fever and hospital length of staywere collected for prognostic analysis.

Results: Compared with bacterial culture methods, the positive rate of bacteriaby using NGS in the lungs were higher (52.9% vs 41.2%). Among the patients who had detected bacteria by NGS in donor lungs before surgery, only one patient (1/9) developed the same bacteria after lung transplantation.Either NGS or bacterial culture methods, there is no association between the colonized colonization bacteria in donor lungs and the patient outcomes of immediate posttransplant period.

Conclusion: The detection of bacteria by using NGS is more sensitive than normal bacterial culture. The colonized bacteria in different parts of the lung are inconsistent. There is no association between the colonized bacteria in donor lungs and short-term outcome of lung transplantation patients.

Background:

In China, because of the insufficient understanding of organ donation and donors with endotracheal intubation or tracheostomy and mechanical ventilation in the intensive care unit for a long time, the donor lungs have been often infected when donated. Therefore, the selection of donor lungs is very important. Even though we had use the lung selection criteria of the Chinese Lung Transplantation Data Center to exclude infection of donor lungs, but the colonized bacteria in donor lungs are still existent.

Some studies have showed that traditional bacterial culture methods are not sensitive enough. Using routine bacterial culture methods in patients with community-acquired pneumonia, approximately 20% of children and 60% of adults are unable to detect pathogens[1, 2]. Therefore, a new detection method is needed to clarify the colonized bacteria in lungs.

High-throughput pathogenic microbial gene detection technology (also named as Next Generation Sequencing, NGS) is a new technology developed in recent years, which can improve the detection rate of pathogens[3–7]. A randomized, double-blind, prospective study suggests that the NGS is highly

consistent with traditional methods and is more reliable in terms of negative diagnosis. In 101 patients, NGS and traditional bacterial culture methods were used to detect colonized bacteria in lungs. 72 patients (63 negative and 9 positive) had the same results, but the positive rate of NGS in the general population is higher than the traditional methods[8].

At present, there is no conclusion of whether colonized bacteria in donor have an effect on the prognosis of lung transplant patients. Therefore, we used NGS to detect the colonized bacteria in donor lungs, for understanding the current status of colonized bacteria in donor lungs by NGS, and clarify whether the early prognosis of lung transplant recipients is associated with the colonization of donor lungs.

Material And Methods:

Donor characteristics.

Donor lungs inclusion criteria: a. Age <60 years old, smoking history <20 packs/year. b. No chest injury. c. Continuous mechanical ventilation < 1 week. d. PaO2≥300 mmHg (FiO2 = 100%, PEEP = 5 cmH_2O). e. X-ray or CT shows that the lung field is relatively clear. f. There is no abscess secretion in the lung bronchus at all levels though bronchoscopy.

Donor lungs exclusion criteria: a. Age >60 years old, smoking history > 20 packs/year. b. Chest trauma and lung contusion. c. Continuous mechanical ventilation > 1 week. d. PaO_2<300 mmHg (FiO_2 = 100%, PEEP = 5 cmH_2O). e. X-ray or CT shows that the lung field is infected. f. There are purulent secretions at bronchoscopy in the the donor lower airways.g. The percentage of white blood cells, neutrophils, C-reactive protein, and procalcitonin increased gradually compared with the onset of disease. h. The donor's body temperature is higher than normal. i. Blood culture is positive.

Sample collection and preservation.

According to the National Lung Transplantation Data Center for donor lung selection criteria, this study obtained 12 donor lungs. 17 lung transplantations were performed, including 12 single lung transplantation and 5 lung transplantation from March 2018 to June 2018 at the Wuxi People's Hospital affiliated to Nanjing Medical University (Wuxi, China). This study included 12 donor lung tissue samples which were biopsy with a cutting suturing device and the basal segment of the lower lobe, the size of tissues was about 0.5 cm × 0.5 cm. The lung tissue samples were frozen in solid carbon dioxide immediately. NGS is used for bacterial detection of the donor lungs (Huada Gene Company). At the same time, we also took bronchial secretions under aseptic conditions for aerobic and anaerobic conditions bacteria culture. This study was authorized and monitored by the Wuxi People's Hospital affiliated to Nanjing Medical University Ethics Committee, and each patient signed an informed consent form.

Sample detection

Based on High-throughput pathogenic microbial gene detection technology (Next Generation Sequencing, NGS), the nucleic acid sequence of the pathogenic microorganism in the sample was analyzed, and the microorganism was identified by comparing with the nucleic acid sequence of the existing microorganism in the database. The detection process includes: nucleic acid extraction, library construction, sequencing, information analysis, report interpretation and etc.

Tissue sample: Sample Processing and DNA Extraction

Tissue sample from patient was collected and cut into small pieces according to standard procedures. 1.5mL microcentrifuge tube with 0.7mL lysis buffer and pieces of tissue sample and 1g 0.5mm glass bead were attached to a horizontal platform on a vortex mixer and agitated vigorously at 2800-3200RPM for 30 min. 0.3mL sample was separated into a new 1.5mL microcentrifuge tube and DNA was extracted using the TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH) according to the manufacturer's recommendation.

Construction of DNA libraries

Then, DNA libraries were constructed through DNA-fragmentation□end-repair, adapter-ligation and PCR amplification. Agilent 2100 was used for quality control of the DNA libraries. Quality qualified libraries were sequenced by BGISEQ-50 platform[9].

Sequencing and bioinformatic analysis

High-quality sequencing data were generated by removing low-quality, and short (length < 35bp) reads, followed by computational substraction of human host sequences mapped to the human reference genome (hg19) using Burrows-Wheeler Alignment[10].The remaining data by removal of low-complexity reads were classified by simultaneously aligning to four Microbial Genome Databases, consisting of viruses, bacteria, fungi, and parasites.

The classification reference databases were downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/). RefSeq contains 4,061 whole genome sequence of viral taxa, 2,473 bacteral genomes or scaffolds, 199 fungi related to human infection, and 135 parasites associated with human diseases.

Statistical analysis

Descriptive statistics were computed for the overall sample and stratified by presence of positive bacteria detected by NGS or bacterial culture positive on donor lung samples. Mean ± standard deviation (SD) or median (interquartile range (IQR) was used for describe the continuous variables. We used *t* test/ANOVA or non-parametric Wilcoxon-Mann-Whitney (for continuous variables) and chi-squared or Fisher's Exact test for categorical variables to compare the difference between two groups. The significance level was set at 0.05. SPSS 19.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for statistical analysis.

Results:

1. Clinical and microbiological characteristics.

Table 1 details the basic characteristics of the study patients. Of the 17 patients in the overall cohort, there were 10 males and 7 females who underwent lung transplantation. Among them, 6 males and 3 females had detected colonized bacteria in donor lung by using NGS. The average age of the overall patients was 52.18 ± 9.5 years, the youngest recipients and the oldest recipients were 31 years old and 66 years old. The average age of patients who received donor lungs with NGS detected colonized bacteria was 51.33 ± 10.84 years old, with an age range of 31-65 years. On the contrary, the average age of patients who received donor lungs without NGS detected colonized bacteria was 55.25 ± 7.92 years old, with an age range of 47-66 years. Of the 17 lung transplantations, 12 were single lung transplantation and 5 were bilateral lung transplantation. 10 of the 12 donor lungs of the single lung transplantation patients were derived from five donors. In the donor lungs of lung transplant recipients, 7 of the single lung transplantation patients had detected colonized bacteria by using NGS (7/12), and 2 of the donor lungs of the bilateral lung transplantation were found have colonized bacteria (2/5). In this study, the primary disease of lung transplantation patients were interstitial lung disease (76.5%) and chronic obstructive pulmonary disease (COPD) (23.5%) (Table 1).

2. Classification of lung colonized bacteria detected by different methods.

We also analyzed the types of lung colonized bacteria which detected by NGS, or the types of bacteria cultured from lung airway secretions from donor lungs and post-transplantation lungs. We found that the proportion of bacteria detected by NGS in donor lungs is 52.9%, the proportion of bacteria detected by bacterial culture in donor lungs is 35.3%. Only 5 (29.4%) cases of all samples, the bacteria detected by NGS in donor lungs with the tissues from the lower part of the lungs were identical to the bacteria cultured in the bronchial secretions of the lungs. This shows that in most cases, the colonized bacteria of different parts of the lungs is not consistent, and it is more sensitive to use NGS to detect bacteria than Classical bacterial culture method (Table 2).

Of the 17 patients, 9 (52.9%) patients detected bacteria by sputum culture after operation. In 5 (29.4%) cases, the bacterial detected by NGS were compared with the sputum culture results of post-operation, and only 1 (11.1%) of the patients who detected bacteria by using NGS before operation had the same postoperative bacteria after lung transplantation. This indicates that the bacteria in lung after lung transplantation are not mainly derived from colonized bacteria in the donor lung before operation, and may be more closely related to secondary infection (Table 2).

3. The transform of colonized bacteria in lungs before and after operation.

For the classification and analysis of the bacterial species which detected under different conditions, we found that the NGS could detected more bacteria in the donor lungs, and those bacteria are not specific. The most common type of bacteria found in donor lung bacterial cultures is *Acinetobacter baumannii*.

But the types of cultured bacteria after surgery were significantly different than the donor lungs before operation. Among them, *A. baumannii* is the most important infectious bacteria (50%), followed by *Klebsiella pneumoniae* (25%) and *Candida albicans* (16.7%). This result is consistent with other reports. This further demonstrated that the bacteria in the lungs after lung transplantation derived from secondary infections, there is no correlated with colonization of bacteria in the donor lungs (Table 3).

4. Patient outcomes.

We also collected the clinical information and analyzed whether the colonized bacteria in donor lungs affect the outcome of patients. Divided patients into colonized bacteria group (the colonized bacteria in donor lung was positive detected by NGS) and without colonized bacteria group (the colonized bacteria in donor lung was negative detected by NGS). There was no difference in extracorporeal membrane oxygenation (ECMO) support time, mechanical ventilation time, intensive care unit (ICU) stay time, duration of fever and the number of days in hospital between those two groups (Table 4). While divided patients into patients with culture bacteria group (the colonized bacteria in donor lung was positive detected by culture bacteria) and patients without culture bacteria group (the colonized bacteria in donor lung was negative detected by culture bacteria). There was also no correlation between the colonized bacteria in the upper donor lungs and the prognosis of the patients (Table 5). This indicates that there is no significant correlation between the presence of colonized bacteria in donor lungs and the short-term prognosis of the patients.

Discussion:

A plenty of patients in the Intensive Care Unit (ICU) have a multi-drug resistant bacteria infection risk due to the abuse of antibiotics and the increase of invasive procedures, especially the carbapenem-resistant K. pneumoniae and other carbapenem-resistant Enterobacteriaceae[11, 12]. It was reported that the donors could be infected by multi-drug resistant nosocomial bacteria in two days, and spread those bacteria to recipients[13]. Most of donor organs are donated by brain-dead patients from the ICU, it is common that the donor lungs had been infected and colonized by bacteria, leading to an increase in donor-derived infections. In the United States, the total number of donor-derived disease transmission events on the Organs Access and Transplant Network is also increasing year by year, with donor-derived infections accounting for 71%. And the probability of different diseases is transmitted from the donor to the recipient is different. The probability of malignant tumors, viruses, bacteria, fungi, and parasites caused by donor organs is 67%, 46%, 34%, 29%, and 17%. Compared with other organ transplantation, there are 80% of lung transplant recipients were infected when they received an organ from a same donor who had communicable disease[14].One research had reported that 18 (10.5%) donors were fixed or infected by carbapenem resistant Gram-negative bacteria in 170 donors who met the transplant criteria in 10 hospitals in Italy during January 1, 2012 to December 31, 2013, but those bacteria were not found during donation of organs and transplantation[15]. Therefore, the screening for colonized bacteria or infected pathogens in donor lungs is particularly important.

In this study, we currently receive donor lungs in accordance with the donor lung selection criteria of National Lung Transplantation Data Center, based on medical history, chest X-ray or chest CT, fiberoptic bronchoscopy, and blood cell counter, C-reactive protein, procalcitonin and other tests to comprehensively assess the infection of the lungs, and exclude the lungs when it was infected. However, we can still detect pathogenic microorganisms from the lungs that meet the standards for lung utilization. We define them as colonized bacteria. Traditional methods for detecting colonized bacteria include bronchial secretions or alveolar lavage fluid. In this study, we not only used bronchial secretions but also lung tissue pathogenic microbial gene detection technology to detect lung-fixing bacteria, we hope to comprehensively evaluate the different distribution of colonized bacteria of the donor lungs and the prognosis of recipient.

It is still unclear whether the pathogenic microorganisms in the donor lungs affect the prognosis of lung transplant recipients. Bonde had reported that 57 of the 64 donors (89%) had positive bacteria for bronchial secretion culture, the study had shown that the colonization of pathogenic microorganisms in donor lungs is common. And multi-factor analysis of pneumonia after lung transplantation found that there is no significant correlation between the culture results of bronchial secretions and pneumonia after lung transplantation. Even more most clinical infections of the recipients were not directly related to the presence of donor organisms. It appears to be no correlation between organisms identified on donor cultures and pathogenic organisms infected with receptors[16]. Ahmad's study showed that 32 lung transplant recipients, of which 20 (63%) were positive for bronchial secretion culture, 12 (37%) were culture-negative, and the bronchial secretion positive group had a longer mechanical ventilation time than the negative group. But there was no significant difference of the 30-day survival rate and the incidence of grade 3 preimplantation genetic diagnosis (PGD) between the two groups[17]. A study by Avlonitis retrospectively analyzed the culture of lung alveolar lavage fluid in 115 lung transplant recipients, including 53 positive (46%) and negative 62 (54%) for pulmonary alveolar lavage fluid. In the culture-positive recipients, the average tracheal intubation time and ICU intensive time were higher than the negative group, and the 6-month, 1-year, and 2-year survival rates were lower than the negative group[18].

In this study, the lung colonized bacteria were detected by NGS of lung tissue and bronchial secretion culture. The results of the two methods were not identical may due to different sample. More pathogenic microorganisms could be detected in the NGS group than traditional culture group, and the precise method of gene detection could reflect the distribution of bacteria for donor lung more comprehensive. Although the patients were grouped according to NGS test results or bronchial secretion culture results, the results showed that there was no difference in extracorporeal membrane oxygenation (ECMO) support time, mechanical ventilation time, intensive care unit (ICU) stay time, duration of fever and the number of days in hospital between positive group and negative group. This indicate that neither the bronchial colonized bacteria nor the lung tissue colonized bacteria in donor lungs has affected the early prognosis of lung transplant recipients.

Due to the shortage of standard donor lungs, the use of expanded standard donor lungs was increased in recent years, it was defined as expanded standard donor lungs when bronchoscopy revealed purulent

secretion or sputum culture found bacteria[19, 20]. However, whether the expansion of the standard for donor lungs has an impact on the prognosis of recipients is still inconclusive[20, 21]. In fact, positive sputum bacteria culture results could be caused by lung infection or colonized bacteria. This study shows that the donor lungs which had colonized bacteria can also be used for lung transplantation. If there is no clear evidence for lung infection, pathogenic microorganisms in sputum or bronchial secretions should be defined as colonized bacteria. There is no significant correlation between the presence of colonized bacteria in donor lungs and the short-term prognosis of the patients. It is significant for further research on the stratification and definition of donor lung expansion criteria.

In our research, the colonized bacteria in donor lungs did not cause postoperative pulmonary infection in lung transplant recipients, it may due to the prophylactic application of antibiotics. Researches have shown that postoperative targeted antibacterial agents could prevent recipient infections which caused by multidrug-resistant Gram-negative bacilli in lungs[22, 23]. In this study, the pathogenic microorganisms of the recipients were mainly common with the bacteria in the ICU of our center, and there was no correlation with the colonized bacteria of the donor lungs. This may be due to colonized bacteria of the donor lungs causing subclinical infection and lung injury, making the lung susceptible to infection by different pathogenic microorganisms of the recipient[24, 25].

There are also some shortcomings in this research. Such as the number of study cases is not enough, the follow-up time is relatively short, the effect of lung colonization on long-term prognosis of lung transplant recipients has not been observed. Further confirmation is needed for large sample sizes and long-term follow-up studies.

Conclusion:

The incidence of donor-derived infections is very high. Accurate screening of donor-derived pathogen or colonized bacteria is very important to reduce the risk of infection of the recipient. NGS is a new technique for detecting pathogens. We found that it is more sensitive to pathogens than traditional bacterial cultures. In our study, we do not found that the presence of donor colonized bacteria would affect the early prognosis of recipients. This finding provides the possibility to expand the criteria of donor lung and use partially infected donors. In the future, large-scale clinical studies are needed to further confirm this conclusion and its impact on the long-term prognosis of recipients.

Abbreviations:

NGS: Next Generation Sequencing; ICU: Intensive Care Unit; PGD: preimplantation genetic diagnosis; ECMO: extracorporeal membrane oxygenation;

Declarations:

Ethics approval and consent to participate

Ethics approval of this study was obtained from the Ethical Committee of the Wuxi People's Hospital affiliated to Nanjing Medical University.

Conflicts of interest

The authors have declared that no conflicts of interest exist.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Consent for publication

Written informed consent was obtained from the patients for publication of this article.

Authors' contributions

L.D and Z.J designed and performed the study, W.B, L.F, Y.S.G and W.H.M collected and analyzed data. L.D, Z.J, L.J and W.X wrote the paper. C.J.Y, H.W.L and C.Y supervised the clinical research and revised the manuscript. All authors approved the final manuscript.

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Tables

Table 1. Patient demographics.

ent characteristics	Overall cohort	Patients with colonized bacteria*	Patients without colonized bacteria*
	n = 17	n = 9 (52.9%)	n = 8 (47.1%)
der, n (%)			
le	10 (58.8)	6 (66.7)	4 (50)
male	7 (41.2)	3 (33.3)	4 (50)
an (SD)	53.18 9.5	51.33 10.8	55.25 7.9
dian (min, max)	53 31,66	53 31,65	55 47 66
e of transplant, n (%)			
ngle lung	12 (70.6)	7 (77.8)	5 (62.5)
ateral lung	5 (29.4)	2 (22.2)	3 (37.5)
erlying condition, n (%)			
)	13 (76.5)	6 (66.7)	7 (87.5)
PD	4 (23.5)	3 (33.3)	1 (12.5)

* The colonized bacteria was detected by NGS.

Table 2. Bacteria detected by different methods for each lung transplant patient.

Case number	Bacteria detected by using NGS from donor lungs	Bacteria cultured from donor lungs	Bacteria cultured after lung transplantation
1	Non	Non	*Acinetobacter baumannii*
2	*Acinetobacter baumannii*, *Gordon*	Non	Non
3	*Campylobacter*, *Haemophilus parainfluenzae*	Non	*Klebsiella pneumoniae*
4	*Campylobacter*, *Haemophilus parainfluenzae*	Non	*Klebsiella pneumoniae*
5	*Rothia*	Non	*Acinetobacter baumannii*
6	Non	Non	Non
7	Non	Non	Non
8	*Streptococcus pyogenes*,	*Serratia*, *Klebsiella oxytosus*	Non
9	*Corynebacterium ammoniagenes*, *Pseudomonas sphaeroides*, *Stenotrophomonas maltophilia*	*Serratia*, *Klebsiella oxytosus*	*Acinetobacter baumannii*, *Klebsiella pneumoniae*
10	Non	*Acinetobacter baumannii*	Non
11	Non	*Acinetobacter baumannii*	*Acinetobacter baumannii*, *Klebsiella pneumoniae*
12	*Candida albicans*	*Candida albicans*	Non
13	*Candida albicans*	*Candida albicans*	*Acinetobacter baumannii*
14	Non	Non	Non
15	Non	Non	*Candida albicans*, *Pseudomonas aeruginosa*
16	*Acinetobacter baumannii*, *Acinetobacter junii*, *Staphylococcus aureus*, *Corynebacterium*	*Acinetobacter baumannii*	*Acinetobacter baumannii*
17	Non	Non	Non

Table 3. Bacterial species detected by different methods.

:	Number of bacteria detected by using NGS from donor lungs, n (%)*, Totle n = 14	Number of bacteria cultured from donor lungs, n (%)*, Totle n = 6	Number of bacteria cultured after lung transplantation, n (%)*, Totle n = 12
:ter	2 (14.3)	3 (50)	6 (50)
bicans	1 (7.1)	1 (16.7)	2 (16.7)
	0 (0)	0 (0)	3 (25)
e			
nas	0 (0)	0 (0)	1 (8.3)
a			
	1 (7.1)	0 (0)	0 (0)
cter	1 (7.1)	0 (0)	0 (0)
lus	1 (7.1)	0 (0)	0 (0)
nzae			
	1 (7.1)	0 (0)	0 (0)
cus	1 (7.1)	0 (0)	0 (0)
terium	1 (7.1)	0 (0)	0 (0)
enes			
nas	1 (7.1)	0 (0)	0 (0)
es			
omonas	1 (7.1)	0 (0)	0 (0)
a			
:ter junii	1 (7.1)	0 (0)	0 (0)
ccus	1 (7.1)	0 (0)	0 (0)
terium	1 (7.1)	0 (0)	0 (0)
	0 (0)	1 (16.7)	0 (0)
	0 (0)	1 (16.7)	0 (0)

*Percentages may not add up to 100% due to rounding.

Table 4. Patient outcomes. (Patients with/without colonized bacteria).

outcomes	Overall cohort n = 17	Patients with colonized bacteria*, n = 9 (52.9%)	Patients without colonized bacteria*, n = 8 (47.1%)	*P*-value
support time (hours)				
SD)	23.8 (50.9)	10.8 (13.0)	38.4 (72.6)	0.322
on mechanical ventilation				
SD)	73.3 (67.6)	67.1 (72.8)	80.3 (65.4)	0.701
y time				
SD)	112.6 (59.8)	98.4 (64.2)	128.5 (53.8)	0.311
ime in one month after surgery				
SD)	4.94 (3.9)	4.22 (3.9)	5.75 (4.1)	0.444
hospital (days)				
SD)	41.2 (46.9)	47.6 (25.1)	43.4 (68.4)	0.882

Table 5. Patient outcomes (Patients with/without culture bacteria).

outcomes	Overall cohort n = 17	Patients with culture bacteria, n = 7 (41.2%)	Patients without culture bacteria, n = 10 (58.8%)	*P*-value
support time (hours)				
SD)	23.8 (50.9)	12.1 (13.7)	31.9 (65.6)	0.375
on mechanical ventilation				
SD)	73.3 (67.6)	81.3 (77.9)	67.7 (63.1)	0.710
y time				
SD)	112.6 (59.8)	119.1 (67.5)	108.0 (57.0)	0.728
ime in one month after surgery				
SD)	4.94 (3.9)	3.85 (2.8)	5.70 (4.5)	0.322
hospital (days)				
SD)	41.2 (46.9)	28.8 (25.2)	56.9 (57.6)	0.997