Clinical Features of Microbial Reactivation in Early Onset of Severe Pneumonia by Metagenomic Next-Generation Sequencing in Intensive Care Units, a Multicenter, Retrospective Study

Lingtong Huang
Zhejiang University School of Medicine
Lisha Pang
Tongde Hospital of Zhejiang Province
Peng Sheng
the First Hospital of Jiaxing
Yanqiu Wang
Hangzhou Hospital of Traditional Chinese Medicine
Fan Yang
Zhejiang University School of Medicine
Huili Yu
Zhejiang University School of Medicine
Ning Zhang
Lishui City People's Hospital
Hongliu cai
Zhejiang University School of Medicine
Lingling Tang
Shulan (Hangzhou) Hospital, Zhejiang Shuren University of Shulan International Medical College
Xueling Fang (xuelingfang@zju.edu.cn)
Zhejiang University School of Medicine

Research Article

Keywords:

Posted Date: April 4th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1506074/v1

Abstract

Purpose: This study aims to assess the clinical features of microorganism reactivation in the early onset of severe pneumonia in ICU patients.

Methods: A total of 97 patients' clinical data was collected retrospectively from intensive care units of five teaching hospitals in China from June 2018 to July 2021 followed by metagenomic next-generation sequencing (mNGS) of the bronchoalveolar lavage fluid (BALF) at the onset of severe pneumonia.

Results: A total of 43 pathogenic microorganisms were detected in 97 patients with severe pneumonia by mNGS, where CMV (21.6%), HSV-1 (18.6%), and Pneumocystis (14.4%) were the most common reactivated microorganisms in the lungs of patients with severe pneumonia. In the present study, reactivation was considered when mNGS detected CMV, HSV-1, or Pneumocystisc DNA. A total of 11 patients (11.3%) had two or more reactivated microorganisms, and the overall rate of microbial reactivation was 40.2%. After adjusting for the risk of confounding and competition, one or more reactivations of CMV, HSV-1, and Pneumocystis resulted in an overall increase of 2.381 folds of mortality (95% CI: 1.198-4.733).

Conclusion: This study showed that CMV, HSV-1, and Pneumocystis were the most common reactivated microorganisms in the lungs of patients in the ICU at the onset of severe pneumonia. Reactivation of two or three microorganisms is common and is associated with an increased risk of mortality.

Introduction

Severe pneumonia is one of the leading causes of death in ICU and is often accompanied by the reactivation of cytomegalovirus (CMV) [1], herpes simplex virus (HSV) [2], and Pneumocystis (PC) [3] during the course of the disease treatment. Evidence suggests that CMV reactivation was always assessed using serum samples [4, 5]. On the other hand, the reactivation of HSV-1 and PC is often identified by RT-PCR in bronchoalveolar lavage fluid (BALF) [6, 7]. A poor prognosis is often associated with the reactivation of CMV, HSV-1 or PC [8–10]. Currently, few studies have evaluated the reactivation of these three microorganisms in the lungs simultaneously and their contribution to the mortality of patients with severe pneumonia in the ICU.

Metagenomic next-generation sequencing (mNGS) is a commonly used technique for the unbiased detection of pathogenic microorganisms in the ICU [11, 12]. Compared to RT-PCR and other techniques, mNGS can comprehensively detect the reactivation of CMV, HSV-1, and PC in the lungs. In the present study, the BALF of ICU patients with severe pneumonia was analyzed by mNGS. The results indicated that CMV, HSV-1, and PC were the common reactivated microorganisms in the lungs of these patients. Therefore, this study aimed to assess the clinical characteristics of reactivation of these three microbes and their association with mortality in patients with severe pneumonia.

Methods

Study design and patient population

In this multicenter retrospective study, mNGS detection was performed on the bronchoalveolar lavage fluid of 97 patients with severe pneumonia admitted to the ICU in five teaching hospitals including the First Affiliated Hospital of Zhejiang University School of Medicine, Tongde Hospital of Zhejiang Province, the First Hospital of Jiaxing, Hangzhou Hospital of Traditional Chinese Medicine and Lishui City People's Hospital. The data were collected from June 2018 to July 2021. The ethics committees of the five participating institutions approved the study protocol. Since the research involved retrospective data, written informed consent was waived off. The data of only those patients were retrieved who were of age over 18 years, were transferred to ICU, and diagnosed with severe pneumonia. Reactivation was defined as the presence of CMV, HSV-1, or PC in the lung as detected by mNGS. Patients were considered immunocompromised if they had peripheral blood neutropenia < 0.5×10^9/L for 10 days after admission, were taking immunosuppressive drugs within 30 days before mNGS test, such as tacrolimus, cyclosporine, mycophenolate mofetil, or monoclonal antibodies such as rituximab, and with a history of AIDS, hematological tumors, or transplant. Due to the subjectivity of infection diagnosis, we only looked for objective evidence of CMV, HSV-1, and PC reactivation in this study without evaluating whether it was an infection.

Data collection

For all included patients, the demographic data like gender, age, days of ICU stay, days of mechanical ventilation, use of ECMO, days from admission to mNGS testing, PaO_2/FiO_2 at mNGS testing, procalcitonin, surgery before admission history, community-acquired pneumonia, immunosuppressive status, and patient outcomes were recorded. To assess disease severity, the APACHE II and SOFA scores were also calculated when the patients were admitted to ICU and on the day of mNGS testing.

mNGS assay of BALF

Low-speed centrifugation (1500×g for 20 min) was used to remove human cells in BAL. The samples were then homogenized using bead-beating followed by DNA extraction using IngeniGen DNA Extraction Kit (IngeniGen XMK Biotechnologies, Inc., Zhejiang, China). IngeniGen DNA Library Prep Kit was used to prepare the DNA libraries according to the manufacturer's instructions. Briefly, the DNA was fragmented, and Illumina-compatible adaptors were added to the fragmented DNA simultaneously by a tagment enzyme. The library was purified by magnetic beads and then amplified by 15 PCR cycles. Samples were then homogenized using bead-beating followed by RNA extraction using IngeniGen RNA Extraction Kit (IngeniGen XMK Biotechnologies, Inc., Zhejiang, China). RNA libraries were constructed using the Ingenigen XMKbio RNA-seq Library Prep Kit (IngeniGen XMK Biotechnologies, Inc., Zhejiang, China). Briefly, DNase was used to remove residual human DNA, and the RNA was fragmented, followed by double-strand cDNA synthesis, end-repair, dA-tailing, and adapter ligation.

Sequencing was performed on the Illumina MiniSeq (Illumina, San Diego, CA) using 2X75bp chemistry. Negative control was added to each run to detect background contaminants, and internal control was

added to each sample to monitor the entire procedure. Data analysis was performed using IngeniSeq MG (IngeniGen XMK Biotechnologies, Inc., Zhejiang, China), a proprietary automated shotgun metagenomics analysis platform for pathogen detection. Briefly, human and other contaminant sequences were extracted from the raw data. Then, the sequences were duplicated and matched against a curated database consisting of more than 20,000 microbial reference genomes. The resulting hits were again filtered by a proprietary algorithm to remove background contaminants that may appear during sample processing and library preparation. This resulted in a final report of detected pathogens.

Statistical analysis

Statistical analysis was performed using SPSS statistical package 26. Data were first summarized using standard descriptive statistics. Mann–Whitney U and Chi-square tests were used to analyze the clinical characteristics. Cox proportional hazards models, hazard ratios (HR), and confidence intervals (CI) were estimated to identify predictors of mortality or survival in-hospital. Kaplan–Meier analysis was performed to determine patient survival in the non-reactivation and reactivation groups. Unadjusted HR was reported, $p < 0.05$ were considered statistically significant. Figures were prepared using GraphPad Prism 8 or R 4.1.1.

Results

mNGS results

A total of 43 reported pathogenic microorganisms were detected in all 97 patients with severe pneumonia. The top five microorganisms were found to be CMV, *Klebsi Pneumoniae*, HSV-1, PC, and *Acinetobacter Bowman* (Fig. 1A). The frequencies of CMV, HSV1 and PC were found to be 21(21.6%), 18 (18.6%), and 14 (14.4%), respectively. In addition, 2 or 3 microbial reactivations occurred in 11 of 39 (28.2%) patients with reactivation (Fig. 1B). In the present study, only objective evidence of CMV, HSV, and PC reactivation was observed without examining whether the infection had occurred or not. Therefore, patients were classified into two groups i.e., patients with reactivation of CMV, HSV, or PC and the non-reactivation group.

Patient characteristics

In this study, the data of 97 patients with severe pneumonia admitted to the ICU of the five teaching hospitals in China were included. The median age of participants was found to be 65 years with an IQR value of 53 to 73 years, where a total of 57 (58.8%) were male patients. Participants were classified based on either presence or absence of reactivation of CMV, HSV-1, PC, where the non-reactivation group had a total of 58 patients, and the reactivation group had 39 patients (Table 1).

Table 1
Clinical characteristics.

	Total (n = 97)	Non-reactivation (n = 58)	Reactivation (n = 39)	P-value
Age, years	65 (53–73)	63 (52.75-74)	66 (53–71)	0.99
Male gender (%)	57 (58.8)	34 (58.6)	23 (59)	1
Hospital stay, day	26 (14.5–47)	26.5 (16–53)	24 (13–45)	0.40
ICU stay, day	16 (10-32.5)	17 (10–30)	13 (9–39)	0.67
Surgical before transfer to ICU (%)	13 (13.4)	9 (15.5)	4 (10.3)	0.55
CAP (%)	60 (61.9)	38 (65.5)	22 (56.4)	0.40
Immunosuppression (%)	36 (37.1)	12 (20.7)	24 (61.5)	< 0.001
Mechanical Ventilation, day	12 (6–26)	11.5 (6-26.25)	12 (5–24)	0.74
ECMO (%)	16 (16.5)	6 (10.3)	10 (25.6)	0.06
PCT, ng/mL	0.7 (0.24–5.485)	0.79 (0.285-9.1)	0.5 (0.15–1.9)	0.12
SOFA score at transfer to ICU	8 (4.5–12)	8 (4-12.25)	7 (5–12)	0.89
APACHE II score at transfer to ICU	18 (13-25.5)	19 (13–26)	17 (13–25)	0.52
NGS testing of Balf				
Days after admission to hospital, day	3 (2–11)	3 (2-9.5)	4 (1–14)	0.45
PaO_2/FiO_2	180 (126-261.5)	205.5 (133.3–288)	160 (105–215)	< 0.05
SOFA score at testing	8 (5–12)	8 (5–12)	8 (6–12)	0.89
APACHE II score at testing	19 (15-25.5)	20 (15–26)	18 (15–25)	0.93
Hospital mortality (%)	51 (52.3)	25 (43.1)	26 (66.7)	< 0.05

Mann–Whitney U test and the Chi-square test were used to analyze the clinical characteristics, *p*-values < 0.05 were considered statistically significant. Abbreviation: CAP, community-acquired pneumonia; ECMO, extracorporeal membrane oxygenation, PCT, procalcitonin; SOFA, sequential organ failure; APACHE II, acute physiology and chronic health evaluation II.

The two groups of patients were not significantly different in terms of age (63 vs. 66, $p = 0.99$), gender (58.5% vs. 59%, $p = 1$), length of hospital stay (26.5 vs. 24, $p = 0.4$), and length of stay in ICU (17 vs. 13, *p*

= 0.4), days of ventilator use (11.5 vs. 12, $p = 0.74$), time from admission to mNGS test (3 vs. 4, $p = 0.45$), and surgery before admission to the ICU (15.5% vs. 10.3%, $p = 0.55$. In non-reactivation group, 38 (65.5%) patients had severe community acquired pneumonia whereas 22 (56.4%) patients in the reactivation group had severe pneumonia. However, when both groups were compared, no significant differences were found ($p = 0.4$).

ECMO was administered to ten patients in the reactivation group (25.6%) compared to only six patients (10.3%) in the non-reactivation group. However, the difference was not statistically significant ($p = 0.06$). Procalcitonin is a biomarker of bacterial infection, and procalcitonin levels between both groups were compared, which were found to differ insignificantly (0.79 ng/mL vs. 0.5 ng/mL, $p = 0.12$). To assess the extent of severity of these patients, SOFA and APACHE II scores were estimated at the time of admission and on mNGS testing. There was no statistically significant difference between the SOFA (8 vs. 7, $p = 0.89$), APACHE II (19 vs. 17, $p = 0.52$) at admission or SOFA (8 vs. 8, $p = 0.89$), APACHE II (20 vs. 18, $p = 0.93$) at mNGS testing. However, 24 of the patients (61.5%) in the reactivation group had an immunosuppressed state, which is significantly higher than 12 of the patients (20.7%) in the non-reactivation group ($p < 0.001$). In the reactivation group, PaO_2/FiO_2 was detected by mNGS. When the levels of PaO_2/FiO_2 in both groups were compared, significantly lower levels were detected in the reactivation group (160 vs. 205.5, $p < 0.05$). Consequently, mortality in the reactivation group was significantly higher than in the non-reactivation group (66.7% vs. 43.1%, $p < 0.05$).

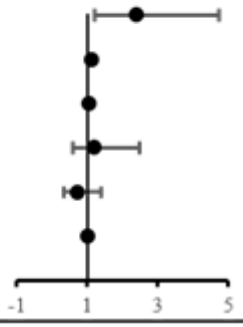
The subgroup analysis was also performed on the reactivation of CMV, HSV-1, and PC. For 2 or 3 microbial reactivations, they were summarized into the corresponding subgroups and the calculations were repeated (Supplement). Almost all patients (92.9%) in PC group were immunosuppressed before mNGS testing. Patients in all three groups had similar clinical characteristics. PC group had longer ICU stay days (17.5, IQR 10.75–46.25) and had the highest mortality rate (71.4%), while HSV-1 group had the lowest mortality rate (50%). The clinical characteristics in the subgroup analysis were not statistically different because of the small sample size and overlapping patients.

Associations with Clinical Outcome

Survival curves were plotted to observe the survival status of the patients in both groups (Fig. 2). Kaplan-Meier curves indicate significantly higher mortality rates in the reactivation group with unadjusted influence versus the non-activation group (unadjusted HR 1.851 [95% Confidence Interval 1.036–3.309], $p = 0.026$). The results of multivariate analysis using the Cox regression model are presented in Table 2. After a prior adjustment for age, sex, immunosuppressive status, APACHE II score at the time of the mNGS test, SOFA score, community-acquired pneumonia, PaO2/FiO2, the association remained significant. Reactivation status was identified as an independent risk factor for death in patients with severe pneumonia (adjusted HR, 2.381; 95% CI: 1.198–4.733; $p = 0.013$) (Table 2). It is critical to observe that a larger sample was needed to analyze the clinical prognosis of different subgroups.

Table 2. The results of multivariable analyses with the Cox regression model

	HR (95% CI)	P
Reactivation	2.381 (1.198-4.733)	0.013
SOFA	1.127 (1.039-1.223)	0.004
APACHE II	1.042 (1.001-1.084)	0.045
Immunosuppression	1.200 (0.581-2.475)	0.622
CAP	0.712 (0.363-1.396)	0.322
PaO_2/FiO_2	0.998 (0.995-1.002)	0.349



The results of multivariable analyses with the Cox regression model, P-values < 0.05 were considered statistically significant.

Abbreviations SOFA, sequential organ failure; APACHE II, acute physiology and chronic health evaluation II; CAP, community-acquired pneumonia.

Discussion

In the present multicenter study, mNGS was used to examine BALF of patients with severe pneumonia in the early stage of infection. The results of our study indicate that the incidence of HSV-1, CMV, and PC reactivation was higher in the lungs (Fig. 1A).

There are few reported studies on the reactivation of lung microbes in critically ill pneumonia patients in the ICU. Recent studies on HSV-1 and CMV reactivation used blood samples to detect viral reactivation. However, blood samples are not a reliable tool for assessing viral reactivation in the lungs. In addition, most studies failed to simultaneously assess HSV-1, CMV, PC reactivation, and clinical characteristics of patients, like most previously conducted studies utilized only a single RT-PCR for detection [13, 14].

In the present study, the mNGS analysis indicated the highest incidence of HSV-1, CMV, or PC reactivation in the lungs. However, studies that evaluate HSV-1, CMV, or PC reactivation alone may have certain limitations due to the absence of adjustments for confounding factors such as simultaneous reactivation. In the present study, due to the small sample size, the effect of simultaneous reactivation of CMV, HSV-1, and PC on the clinical prognosis of patients was determined, and no statistical analysis was performed on subgroups. Approximately 400 samples are needed for a study to assess how these three microbial reactivations affect patient mortality accurately.

Approximately 40.2% of patients had a reactivation of CMV, HSV, or PC on the third day (IQR, 2–11 days) after admission. According to Ong et al., the incidence of herpes viremia increased proportionally with the length of hospital stay, and on day 28, almost all patients had herpes viremia [14]. As part of this study, mNGS was performed on the BALF at the beginning of the patient's illness. However, no continuous monitoring of the reactivation of these three microorganisms in the lungs was carried out, so there is a chance that the incidence of reactivation was underestimated. However, previous evidence reports that

multiple herpesvirus reactivation is common in critically ill patients in ICU [15]. Among our study participants, 11.3% had two or more reactivations of microbial infections in the lungs. However, in the present study, a single mNGS assay result was presented. May be due to the high frequency of simultaneous reactivation of CMV, HSV-1 and PC, few studies of the effect of ganciclovir alone to prevent CMV reactivation on patient outcomes have achieved statistical significance [13]. A combination of CMV, PC, and HSV-1 reactivation prevention may reduce mortality in patients with severe pneumonia in the ICU, but randomized controlled trials are needed for confirmation. Our study showed evidence that the simultaneous reactivation of multiple microorganisms needs to be considered in the design of clinical trials.

Conclusion

This study showed that CMV, HSV-1, and PC were the most common reactivated microorganisms in the lungs of patients with the onset of severe pneumonia admitted to ICU. Reactivation of two or three microorganisms is common, and the existence of these microbial reactivations was associated with an increased risk of mortality.

Declarations

Ethical Approval

The ethics committees of the five participating institutions including the First Affiliated Hospital of Zhejiang University School of Medicine, Tongde Hospital of Zhejiang Province, the First Hospital of Jiaxing, Hangzhou Hospital of Traditional Chinese Medicine and Lishui City People's Hospital approved the study protocol. Since the research involved retrospective data, written informed consent was waived off.

Consent for publication

All authors agreed to publish this manuscript.

Data Availability Statement

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

Competing interests

We declare no competing interests.

Fundings

The work was supported by the Medical and Health Science and Technology Plan Project of Zhejiang Province (grant # WKJ-ZJ-2110 to HLC) and the National Science & Technology Major Project of China (grant #2017ZX10204401 to LLT).

Author Contributions

XLF, LTH, LLT designed the clinical trial. LTH, LSP, HLC, PS, YQW, NZ collected clinical data from each center. LTH, FY, HLY perform statistical analysis of the data. XLF and LTH drafted the manuscript, prepared the figures and critically reviewed the final manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgements

Not applicable.

References

1. Imlay H, Dasgupta S, Boeckh M, Stapleton RD, Rubenfeld GD, Chen Y, Limaye AP: Risk Factors for Cytomegalovirus Reactivation and Association With Outcomes in Critically Ill Adults With Sepsis: A Pooled Analysis of Prospective Studies. The Journal of infectious diseases 2021, 223(12):2108–2112.
2. Hagel S, Scherag A, Schuierer L, Hoffmann R, Luyt CE, Pletz MW, Kesselmeier M, Weis S: Effect of antiviral therapy on the outcomes of mechanically ventilated patients with herpes simplex virus detected in the respiratory tract: a systematic review and meta-analysis. Critical care 2020, 24(1):584.
3. Azoulay E, Roux A, Vincent F, Kouatchet A, Argaud L, Rabbat A, Mayaux J, Perez P, Pene F, Nyunga M *et al*: A Multivariable Prediction Model for Pneumocystis jirovecii Pneumonia in Hematology Patients with Acute Respiratory Failure. American journal of respiratory and critical care medicine 2018, 198(12):1519–1526.
4. Limaye AP, Kirby KA, Rubenfeld GD, Leisenring WM, Bulger EM, Neff MJ, Gibran NS, Huang ML, Santo Hayes TK, Corey L *et al*: Cytomegalovirus reactivation in critically ill immunocompetent patients. Jama 2008, 300(4):413–422.
5. Limaye AP, Stapleton RD, Peng L, Gunn SR, Kimball LE, Hyzy R, Exline MC, Files DC, Morris PE, Frankel SK *et al*: Effect of Ganciclovir on IL-6 Levels Among Cytomegalovirus-Seropositive Adults With Critical Illness: A Randomized Clinical Trial. Jama 2017, 318(8):731–740.
6. Heimes E, Baier M, Forstner C, Weis S, Bauer M, Fritzenwanger M, Scherag A, Pletz MW, Kesselmeier M, Hagel S: Effect of Antiviral Therapy on the Outcome of Mechanically Ventilated Patients With Herpes Simplex Virus Type 1 in BAL Fluid: A Retrospective Cohort Study. Chest 2020, 158(5):1867–1875.
7. Azoulay E, Russell L, Van de Louw A, Metaxa V, Bauer P, Povoa P, Montero JG, Loeches IM, Mehta S, Puxty K *et al*: Diagnosis of severe respiratory infections in immunocompromised patients. Intensive

care medicine 2020, 46(2):298–314.

8. Papazian L, Hraiech S, Lehingue S, Roch A, Chiche L, Wiramus S, Forel JM: Cytomegalovirus reactivation in ICU patients. Intensive care medicine 2016, 42(1):28–37.
9. Linssen CF, Jacobs JA, Stelma FF, van Mook WN, Terporten P, Vink C, Drent M, Bruggeman CA, Smismans A: Herpes simplex virus load in bronchoalveolar lavage fluid is related to poor outcome in critically ill patients. Intensive care medicine 2008, 34(12):2202–2209.
10. Schmidt JJ, Lueck C, Ziesing S, Stoll M, Haller H, Gottlieb J, Eder M, Welte T, Hoeper MM, Scherag A *et al*: Clinical course, treatment and outcome of Pneumocystis pneumonia in immunocompromised adults: a retrospective analysis over 17 years. Critical care 2018, 22(1):307.
11. Huang L, Zhang X, Fang X: Case Report: Epstein-Barr Virus Encephalitis Complicated With Brain Stem Hemorrhage in an Immune-Competent Adult. Frontiers in immunology 2021, 12:618830.
12. Peng JM, Du B, Qin HY, Wang Q, Shi Y: Metagenomic next-generation sequencing for the diagnosis of suspected pneumonia in immunocompromised patients. The Journal of infection 2021, 82(4):22–27.
13. Papazian L, Jaber S, Hraiech S, Baumstarck K, Cayot-Constantin S, Aissaoui N, Jung B, Leone M, Souweine B, Schwebel C *et al*: Preemptive ganciclovir for mechanically ventilated patients with cytomegalovirus reactivation. Annals of intensive care 2021, 11(1):33.
14. Ong DSY, Bonten MJM, Spitoni C, Verduyn Lunel FM, Frencken JF, Horn J, Schultz MJ, van der Poll T, Klein Klouwenberg PMC, Cremer OL *et al*: Epidemiology of Multiple Herpes Viremia in Previously Immunocompetent Patients With Septic Shock. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 2017, 64(9):1204–1210.
15. Hraiech S, Bonnardel E, Guervilly C, Fabre C, Loundou A, Forel JM, Adda M, Parzy G, Cavaille G, Coiffard B *et al*: Herpes simplex virus and Cytomegalovirus reactivation among severe ARDS patients under veno-venous ECMO. Annals of intensive care 2019, 9(1):142.

Figures

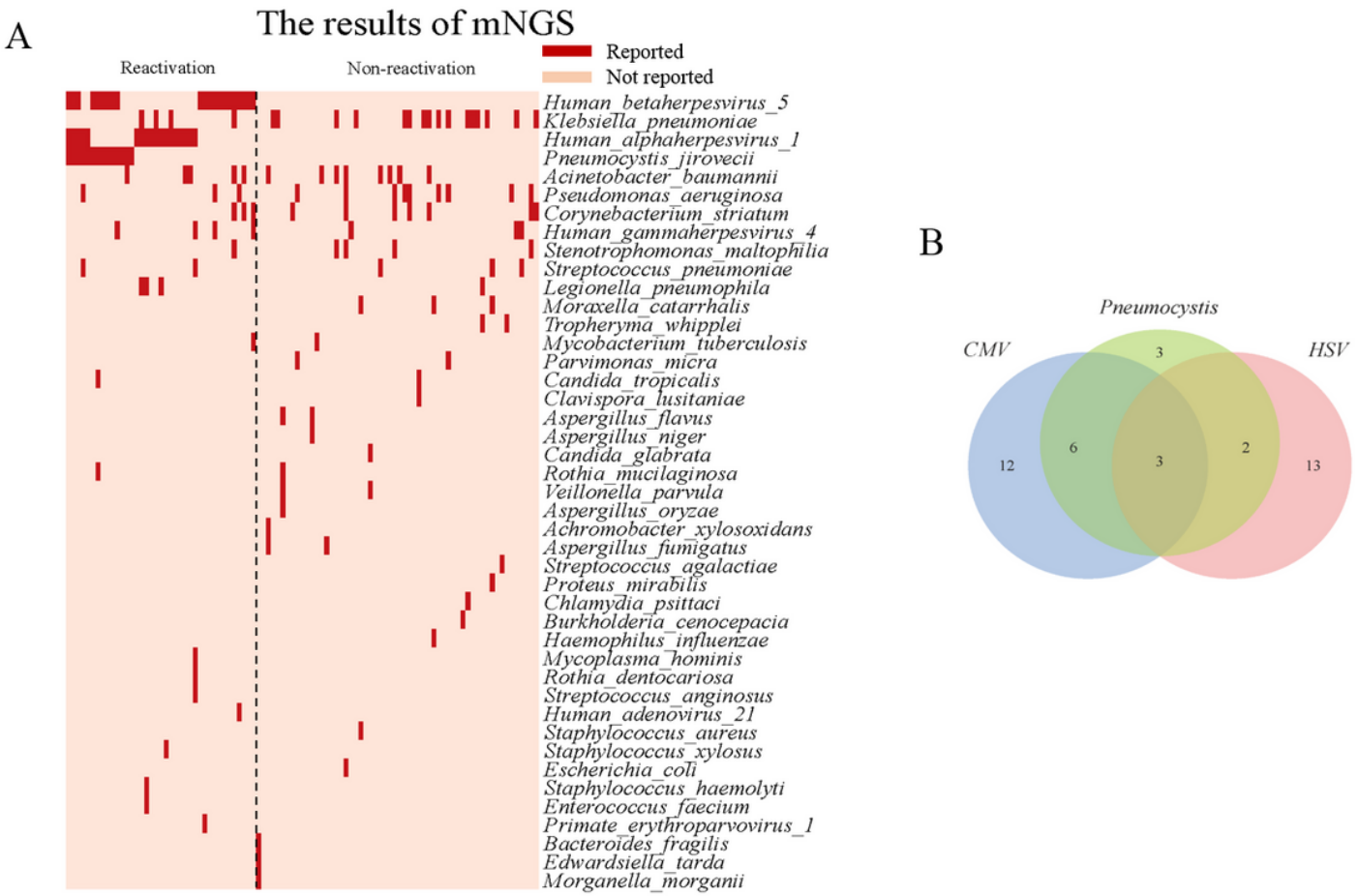


Figure 1

mNGS results of the BALF. (A) Results of mNGS. The red marker in the figure represents microorganisms that were detected and defined as pathogenic by a proprietary algorithm. (B) Venn diagram showing the overlap of reactivation of CMV, HSV-1, and Pneumocystis in a patient's lungs.

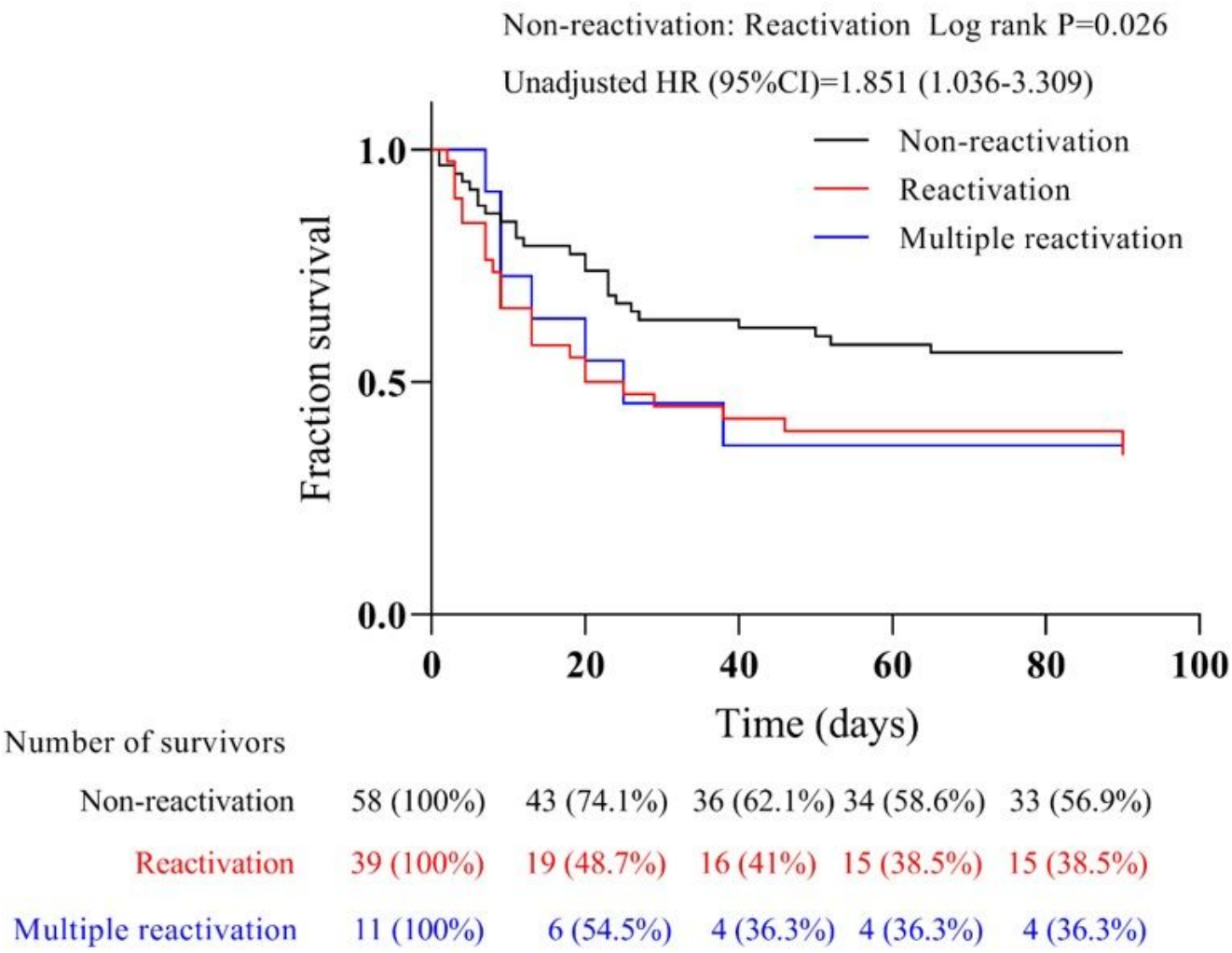


Figure 2

Kaplan–Meier curve of Reactivation, Non-reactivation, muti-reactivation group. The number of surviving patients at different time points is provided in the figure.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary.png