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LETTER TO THE EDITOR

Infection complications in febrile chimeric antigen receptor (CAR)-T recipients during the peri-CAR-T cell treatment period examined using metagenomic next-generation sequencing (mNGS)

Dear Editor,

Chimeric antigen receptor-engineered (CAR)-T cell therapy has achieved unprecedented efficacy on refractory/relapsed B-cell malignancies [1]. Yet, CAR-T recipients are highly susceptible to infection due to the immunodeficiency caused by B-cell aplasia and the pretreatment with chemotherapy. However, due to the systematic use of empirical broad-spectrum antibiotics and immunosuppressors to control cytokine release syndrome (CRS) reaction, microbiological diagnosis of infection has remained challenging in CAR-T recipients.

Currently, metagenomic next-generation sequencing (mNGS) is commonly used in clinical microbiological diagnosis and is partially suitable for the rare, novel, and atypical etiologies of complicated infectious diseases [2]. However, the use of mNGS in CAR-T recipients has been rarely reported. Most bacterial, fungal, and viral infections are complicated with fever. In this study, we combined mNGS with conventional methods to investigate the infection complications in febrile CAR-T recipients during the peri-CAR-T cell treatment period (Supplementary Materials and Methods).

The diagram of patient enrollment is shown in Supplementary Figure S1. We enrolled 102 febrile patients who received CAR-T cell therapy in different trials at Tongji Hospital of Huazhong University of Science and Technology (Wuhan, China) between March 2019 and May 2021. The patients were classified into two groups according to the Penn Grading System of CRS: 76 patients in the mild CRS group (CRS 1-2) and 26 patients in the severe CRS group (CRS 3-5).

The baseline clinical characteristics, including previous infection and prophylaxis, were similar in the mild CRS and severe CRS groups (Supplementary Tables S1-S2). However, after lymphodepletion and CAR-T cell infusion, patients with severe CRS had earlier fever onset, were more likely to suffer from CAR-T cell-related encephalopathy syndrome (CRES), and received more immunosuppressive treatment.

The criteria for infection events are defined in the Supplementary Materials and Methods. Infection events emerged after chemotherapy pretreatment, and continued to grow with time after CAR-T cell infusion (Figure 1A). The first infection event was detected at a median of 5 days after CAR-T cell infusion (range, -11 to 30 days). Eighty percent of first infection events were detected within 15 days after CAR-T cell infusion. Bacterial infections were more common than fungal infections (Figure 1B-C), while viral infections were the most common infections (Figure 1D).

Of the 102 patients, 77.45% showed evidence of infection (Figure 1E). For infection density, febrile CAR-T cell recipients were on average infected with 1.72 types of microbe (Figure 1F). Combining the findings of mNGS and conventional tests, 175 infection events were detected, including 41 bacterial infections, 10 fungal infections, and 124 viral infections (Figure 1G).

Since different CRS stages often represent different immunological states, we compared the microbe distribution pattern between the mild and severe CRS groups. Invasive fungal infections were more commonly detected in patients with severe CRS (Figure 1H). The infection probability for fungi was 5.26% in the mild CRS group and 23.08%

Abbreviations: ASCT, autologous stem cell transplantation; BK virus, *Human polyoma virus 1*; CAR-T, Chimeric antigen receptor-engineered T cell therapy; cHL, classical Hodgkin lymphoma; CMV, Human beta herpes virus 5; CRES, CAR-T cell-related encephalopathy syndrome; CRS, cytokine release syndrome; EBV, Human gamma herpes virus 4; HBV, Hepatitis B virus; HCT, hematopoietic stem cell transplant; mNGS, metagenomic next-generation sequencing; r/r B-ALL, refractory/relapsed B-cell acute lymphocytic leukemia; *S. maltophilia*, *Stenotrophomonas maltophilia*; TB, *Mycobacterium tuberculosis*

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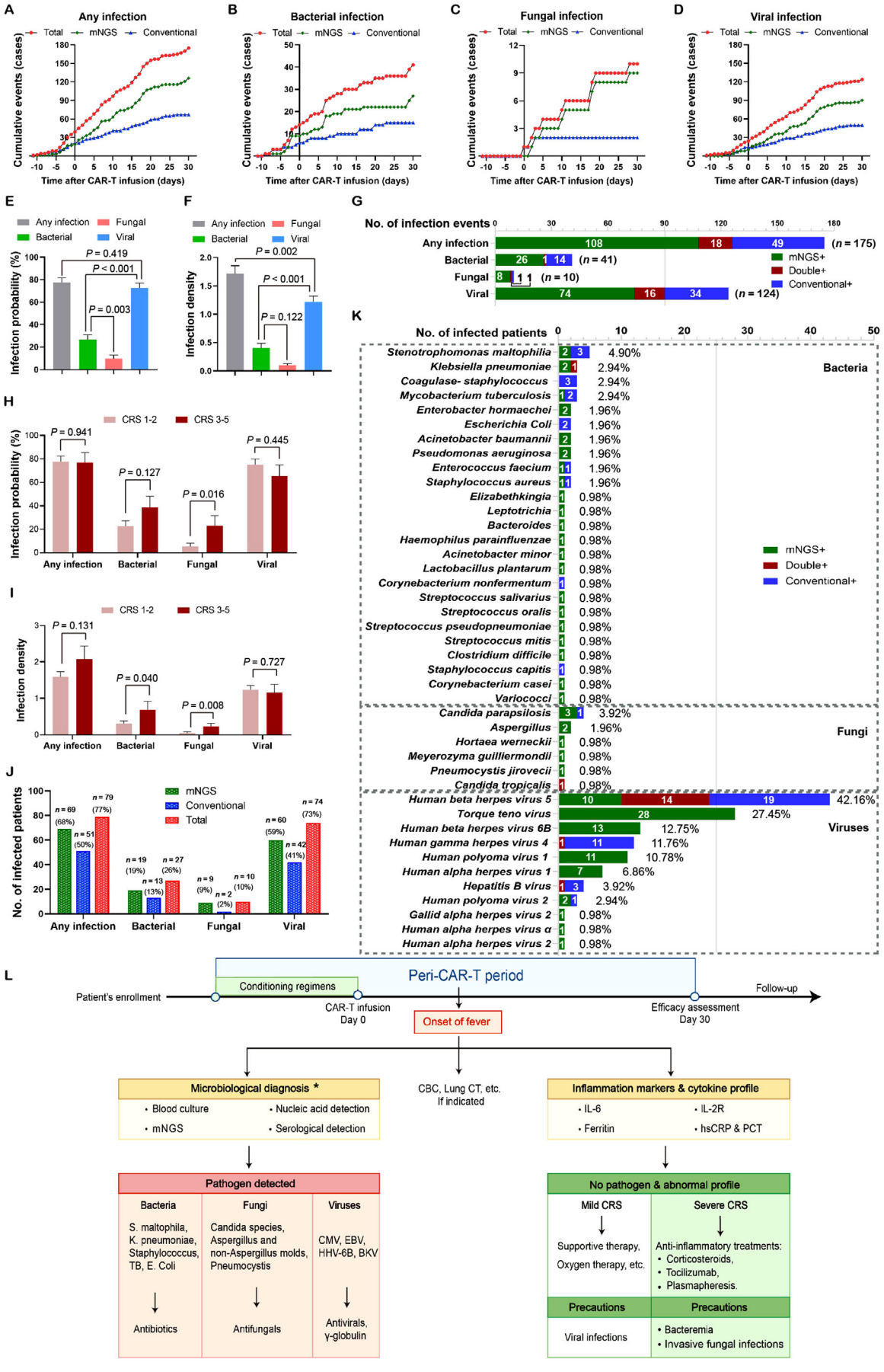
A
Any infection
B
Bacterial infection
C
Fungal infection
D
Viral infection
Total
mNGS
Conventional
Cumulative events (cases)
Time after CAR-T infusion (days)
E
F
Any infection
Fungal
Bacterial
Viral
Infection probability (%)
Infection density
P = 0.419
P < 0.001
P = 0.003
P = 0.002
P < 0.001
P = 0.122
G
No. of infection events
Any infection 108 18 49 (n = 175)
Bacterial 26 1 14 (n = 41)
Fungal 8 1 1 (n = 10)
Viral 74 16 34 (n = 124)
mNGS+
Double+
Conventional+
H
CRS 1-2
CRS 3-5
P = 0.941
P = 0.127
P = 0.016
P = 0.445
Any infection
Bacterial
Fungal
Viral
I
P = 0.131
P = 0.040
P = 0.008
P = 0.727
J
No. of infected patients
mNGS
Conventional
Total
n = 69 (68%)
n = 51 (50%)
n = 79 (77%)
n = 19 (19%)
n = 13 (13%)
n = 27 (26%)
n = 9 (9%)
n = 2 (2%)
n = 10 (10%)
n = 60 (59%)
n = 42 (41%)
n = 74 (73%)
K
No. of infected patients
Stenotrophomonas maltophilia 2 3 4.90%
Klebsiella pneumoniae 2 1 2.94%
Coagulase- staphylococcus 3 2.94%
Mycobacterium tuberculosis 1 2 2.94%
Enterobacter hormaechei 2 1.96%
Escherichia Coli 2 1.96%
Acinetobacter baumannii 2 1.96%
Pseudomonas aeruginosa 2 1.96%
Enterococcus faecium 1 1 1.96%
Staphylococcus aureus 1 1 1.96%
Elizabethkingia 1 0.98%
Leptotrichia 1 0.98%
Bacteroides 1 0.98%
Haemophilus parainfluenzae 1 0.98%
Acinetobacter minor 1 0.98%
Lactobacillus plantarum 1 0.98%
Corynebacterium nonfermentum 1 0.98%
Streptococcus salivarius 1 0.98%
Streptococcus oralis 1 0.98%
Streptococcus pseudopneumoniae 1 0.98%
Streptococcus mitis 1 0.98%
Clostridium difficile 1 0.98%
Staphylococcus capitis 1 0.98%
Corynebacterium casei 1 0.98%
Variococci 1 0.98%
Candida parapsilosis 3 1 3.92%
Aspergillus 2 1.96%
Hortaea werneckii 1 0.98%
Meyerozyma guilliermondii 1 0.98%
Pneumocystis jirovecii 1 0.98%
Candida tropicalis 1 0.98%
Human beta herpes virus 5 10 14 19 42.16%
Torque teno virus 28 27.45%
Human beta herpes virus 6B 13 12.75%
Human gamma herpes virus 4 1 11 11.76%
Human polyoma virus 1 11 10.78%
Human alpha herpes virus 1 7 6.86%
Hepatitis B virus 1 3 3.92%
Human polyoma virus 2 2 1 2.94%
Gallid alpha herpes virus 2 1 0.98%
Human alpha herpes virus α 1 0.98%
Human alpha herpes virus 2 1 0.98%
Bacteria
Fungi
Viruses
mNGS+
Double+
Conventional+
L
Patient's enrollment
Conditioning regimens
Peri-CAR-T period
Follow-up
CAR-T infusion Day 0
Onset of fever
Efficacy assessment Day 30
Microbiological diagnosis *
• Blood culture
• Nucleic acid detection
• mNGS
• Serological detection
CBC, Lung CT, etc. If indicated
Inflammation markers & cytokine profile
• IL-6
• IL-2R
• Ferritin
• hsCRP & PCT
Pathogen detected
Bacteria
S. maltophila, K. pneumoniae, Staphylococcus, TB, E. Coli
Antibiotics
Fungi
Candida species, Aspergillus and non-Aspergillus molds, Pneumocystis
Antifungals
Viruses
CMV, EBV, HHV-6B, BKV
Antivirals, γ-globulin
No pathogen & abnormal profile
Mild CRS
Supportive therapy, Oxygen therapy, etc.
Severe CRS
Anti-inflammatory treatments:
• Corticosteroids,
• Tocilizumab,
• Plasmapheresis.
Precautions
Viral infections
Precautions
• Bacteremia
• Invasive fungal infections

in the severe CRS group ($P < 0.05$). The infection densities for both fungi and bacteria were higher in the severe CRS group than in the mild CRS group (fungal infection: 0.23 vs. 0.05 type per patient, bacterial infection: 0.69 vs. 0.30 type per patient, Figure 1I). Of note, the *Stenotrophomonas maltophilia* was the most common bacterium (Figure 1K), accounting for 2.63% (2/76) in the mild CRS group and 11.54% (3/26) in the severe CRS group, which is consistent with the worse prognosis in patients with severe CRS [3].

The whole microbe spectrum in febrile CAR-T cell recipients is presented in Figure 1K. *Stenotrophomonas maltophilia* was the most common bacterium (detected in 5 patients [5/102, 4.90%]), followed by *Klebsiella pneumonia* (3/102, 2.94%), *Coagulase-negative staphylococcus* (3/102, 2.94%), *Mycobacterium tuberculosis* (3/102, 2.94%), *Escherichia Coli* (2/102, 1.96%), *Acinetobacter baumannii* (2/102, 1.96%), and other bacteria. The most common fungi were *Candida parapsilosis* (4/102, 3.92%) and *Aspergillus* (2/102, 1.96%). *Human beta herpes virus 5* (CMV) was the most common virus (43/102, 42.16%), followed by *Torque teno virus* (28/102, 27.45%), *Human beta herpes virus 6B* (13/102, 12.75%), *Human gamma herpes virus 4* (EBV, 12/102, 11.76%), and *Human polyoma virus 1* (BK virus, 11/102, 10.78%).

Combined mNGS and conventional methods showed positive pathogen detection in 79 infected patients. The conventional method alone showed positive pathogen detection in 51 patients (Figure 1J). Nine out of the 10 fungal infections were detected by mNGS. mNGS detected additional bacterial infections in 18 patients, fungal infections in 8 patients, and viral infections in 54 patients that would have been missed if only conventional methods were used. Thus, 35.44% (28/79) patients would not be properly diagnosed if only conventional methods were applied. On average, 1.05 mNGS tests and 16.10 conventional tests were conducted (Supplementary Figure S2).

In this study, the infection probability (77.45%, 79/102) was much higher than in gross patients who underwent CAR-T therapy with and without fever, as previously reported (22.6%, 30/133 [4]; and 40%, 33/83 [5]). Two factors led to the discrepancy of infection probability. First, the patients we enrolled were febrile CAR-T recipients. Febrile patients had a higher probability of infection than afebrile patients. Second, we combined mNGS with conventional methods, detecting more infection events compared to a single method.

Viruses were found to be the major source of infection in febrile CAR-T recipients, which was different from the previous findings that suggested bacteria as the main cause of infection in patients who underwent CAR-T therapy [4, 6]. The patients were free of CMV and HBV infection or were with resolved HBV infection at the time of enrollment.

FIGURE 1 Infection features in febrile chimeric antigen receptor (CAR)-T recipients during the peri-CAR-T cell treatment period. (**A-D**) Variations of cumulative infection events during the course of CAR-T cell infusion. Day 0 indicates the first day of CAR-T cell infusion. Conventional methods included microbe culture, immunological assay, and nucleic acid tests. **(E)** Infection probability of different microbes. Infection probability was defined as the percentage of infected patients over total patients. Of all patients, 77.45% patients had infection: 72.55% had viral infection, 26.47% bacterial infection, and 9.80% had fungal infection. **(F)** Infection densities of different microbes. Infection density was defined as the average type of microbe(s) per patient. The overall infection density was 1.72 microbes per patient, including 0.40 types of bacterium, 0.10 types of fungus, and 1.22 types of viruses. **(G)** Categorization of all infection events detected by mNGS and conventional methods alone or simultaneously. **(H)** Infection probability in different CRS groups. In the mild CRS group, the infection probability was 77.63% of total infection, 22.37% of bacterial infection, 5.26% of fungal infection, and 75% of viral infection. In the severe CRS group, the infection probability was 76.92% of total infection, 38.46% of bacterial infection, 23.08% of fungal infection, and 65.38% of viral infection. **(I)** Infection densities in different CRS groups. The average infection density per patient was 1.59 types of microbes in the mild CRS group and 2.08 types of microbes in the severe CRS group. **(J)** The number of infected patients detected by mNGS and conventional methods. **(K)** Distribution of microbe types among all febrile CAR-T recipients. A total of 42 microbe types were detected. **(L)** Flow-chart with suggested clinical decision-making for peri-treatment management of febrile CAR-T recipients. Upon commencement of the conditioning regimens, anti-bacterial/anti-fungal/anti-viral prophylaxis was used. When a CAR-T recipient experienced recurrent fever during the peri-CAR-T period, microbiological diagnosis and inflammation marker or cytokine profiles were obtained. Once pathogen(s) were detected, corresponding antibiotic/anti-fungal/anti-viral medication were used. If no infection was detected, supportive treatment and/or anti-inflammatory treatments were used for CRS control. In these situations, considerations were given towards potential viral infections, particularly in patients with mild CRS. In patients with severe CRS, there is a high likelihood of concurrent infection, especially bacterial and fungal infections that warrant particular caution. *Due to the difficulty of microbiological diagnosis, the pathogens might not be detected with a few tests. If the patient's symptoms do not improve and fever was not controlled after the aforementioned treatments, microbiological tests should be repeated. *Abbreviations*: mNGS, metagenomic next-generation sequencing; CBC, complete blood count; CT, computed tomography; HSV, *Herpes simplex virus*; VZV, *varicella-zoster virus*; HHV-6B, *Human beta herpes virus 6B*; BKV, *Human polyoma virus 1*; *S. maltophilia, Stenotrophomonas maltophilia*; *K. pneumoniae, Klebsiella pneumoniae*; TB, *Mycobacterium tuberculosis*; *E.Coli, Escherichia coli*; CMV, *Human beta herpes virus 5*; EBV, *Human gamma herpes virus 4*; CRS, cytokine release syndrome; IL-6, interleukin-6; hsCRP, high-sensitivity C-reactive protein; IL-2R, interleukin-2 receptor; PCT, procalcitonin

However, CMV and HBV infections were still detected in some patients during the peri-CAR-T period, suggesting the reactivation of CMV and HBV during CAR-T treatment [7].

Furthermore, we observed more fungal and bacterial infections in the severe CRS group, suggesting that CRS severity and infection were highly related and might affect each other. One possible explanation was that patients with severe CRS experienced more violent immune disturbance; the immune cells (macrophages, T cells, dendritic cells) could be damaged by the cytokine storm [8], which further worsened the immunity and exacerbated opportunistic infections. Another possibility could be patients infected with bacteria or fungi were more likely to develop severe CRS. Pathogens could directly activate monocytes and macrophages through pattern recognition receptors, and monocytes and macrophages are key mediators of CAR-T therapy-induced CRS [9, 10]. Moreover, the immunosuppressors used in severe CRS stages might also increase complex infections.

Proper management of infection and CRS is directly related to patient prognosis. Thus, it is vital to recognize possible infections. This study has significant clinical implications as it revealed the infection features in febrile CAR-T recipients during the critical peri-CAR-T cell treatment period. We also proposed a guiding strategy flow-chart for peri-treatment management of febrile CAR-T recipients (Figure 1L).

In conclusion, this study revealed that infection was very common among febrile CAR-T recipients during the peri-CAR-T cell treatment period. Viral infections were the most common cause of infection, followed by bacterial and fungal infections. Patients with severe CRS were at a high risk of developing fungal and bacterial infections. This study also highlighted the performance of mNGS as a powerful complement to conventional methods in standard clinical application due to its enhanced spectrum of microbiological diagnosis and efficiency.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The 5 trials in this study were approved by the institutional review board. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki. This study was approved by the Medical Ethics Committee of the Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20160310).

TRIAL REGISTRATION

The 102 patients were enrolled from the 5 ongoing clinical trials (CAR19/22 T cell cocktail therapy: ChiCTR-OPN-16008526, date of registration: 2016/05/24; CAR19/22 T cell cocktail therapy following ASCT: ChiCTR-OPN-16009847, date of registration: 2016/11/14; Humanized anti-CD19 scFv CAR-T, hCAR-T: NCT04888468 for patients aged from 3 to 21 years old and NCT04888442 for patients aged from 22 to 70 years old, date of registration: 2020/11/09 and 2019/11/25; CAR30 T cell therapy: ChiCTR-OPN-16009069, date of registration: 2016/08/23; BCMA T cell therapy: ChiCTR1800018137, date of registration: 2018/08/31).

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AUTHOR CONTRIBUTIONS

Study design: JLN, LY, LH, JW, MX and JFZ. Analysis and interpretation of data: JLN, LY, LLG, JW and MX. Drafting of the manuscript: JLN, LY, XCY, JW and MX. Critical revision of the manuscript: KHY, JMLG, JW and MX. Approval of the final manuscript: JLN, LY, LH, LLG, KHY, JMLG, XCY, JW, MX and JFZ.

CONSENT FOR PUBLICATION

Not required.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data supporting the findings of the article is available from the corresponding authors by request.

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