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%...
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 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 1.10 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.
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: ChiCTR800018137, 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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These two authors contributed equally to this study.

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