

Original Research

Peripheral Blood Smears Distinguish Infective Fever after CAR-T Therapy

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Abstract

Background: Chimeric antigen receptor (CAR) T-cell therapy carries the risk of inducing severe and life-threatening toxicities such as cytokine release syndrome (CRS), neurotoxicity, and infection. Although CRS and infections have similar symptoms, their treatment strategies differ, and early diagnosis is very important. For CRS and infections, the fastest detection time currently takes more than 24 h, so a quick and simple method to identify a fever after CAR T-cell infusion is urgently needed. **Methods:** We enrolled 27 patients with recurrent fever treated with different types of CAR T-cells, including cluster of differentiation (CD) 7, CD19, CD22, and CD19-CD22 bicistronic CAR T-cells, and evaluated the infection events occurring in these patients. We detailed the morphology of CAR T-cells in peripheral blood smears (PBS) and reported the infection events, CAR transgene copy number, and inflammatory indicators within the first month after treatment. **Results:** Similar morphological characteristics were observed in the PBS of different CAR T-cells, namely, enlarged cell bodies, deep outside and shallow inside basophilic blue cytoplasm, and natural killer (NK) cell-like purplish red granules. There were ten infections in nine of the twenty-seven patients (33%). The percentage of atypical lymphocytes in PBS was significantly associated with CAR transgene copy number and absolute lymphocyte count in all patients. The atypical lymphocyte percentage was significantly higher in the non-infection group. **Conclusions:** In conclusion, the unique morphology of CAR T-cells in PBS can be used to evaluate CAR T-cell kinetics and provide reliable evidence for the rapid early identification of fever after CAR T-cell infusion. **Clinical Trial Registrations:** ChiCTR-OPN-16008526; ChiCTR-OPN-16009847; ChiCTR2000038641; NCT05618041; NCT05388695.

Keywords: chimeric antigen receptor T-cell therapy; peripheral blood smears; fever; infection; morphology; kinetics

1. Introduction

The emergence of chimeric antigen receptor (CAR) T-cell therapy has significantly revolutionized the current treatment paradigm, providing a highly promising alternative for individuals suffering from refractory or relapsed B-cell non-Hodgkin lymphoma and B-cell acute lymphoblastic leukemia (r/r B-ALL) [1–3]. However, despite the remarkable efficacy of CAR T-cell therapy, CAR T-cell recipients are highly susceptible to infection, which affects their prognosis due to many insults to immune function, including the malignancy, prior cytotoxic treatments, and the on-target-off-tumor effects of CAR T-cells [4–6]. Cytokine release syndrome (CRS) is the most common toxicity; the clinical presentations of CRS are typically characterized by fever, hypotension, and respiratory insufficiency, which are hard to distinguish from severe infection [7–9]. Clinically, rapid disease change is usually observed after CAR T-cell infusion and some of them develop seriously, especially grade 3–4 CRS, whereas the results of CAR transgene copy

number require 24–48 h, which is obviously not enough to meet urgent clinical needs. Although CRS can be successfully managed with interleukin-6 (IL-6) receptor monoclonal antibodies or corticosteroids, these drugs can also increase the incidence of infection [10–12]. Thus, the early identification between CRS and infections is important for providing appropriate management and improving the clinical outcome of these patients.

Current methods to diagnose infection complications in CAR T-cell recipients, including culture and non-culture-based methods such as nucleic acid detection/metagenomic next-generation sequencing (mNGS) and antigen detection by conventional serological antibody assays, are often time-consuming and expensive [13–15]. Analysis of peripheral blood smears (PBS) is a widely accepted and reliable diagnostic method in laboratory settings, enabling the rapid preliminary diagnosis of numerous hematological disorders [16–18]. By detecting the characteristic footprints left by various infections on blood cell morphology, analysis of



PBS can quickly provide valuable information on infection [19–21]. Recently, it was reported that morphology or CAR T-cells in PBS can provide important clues to assess their kinetics [22,23]. The morphology of CAR T-cells is very important, especially the atypical characteristics of the activation state, which are similar to the non-specifically activated T cells seen in some infections. However, few data are available on the morphological characteristics of CAR T-cells after infusion in humans, especially for distinguishing infections. In this study, we investigated the characteristics of CAR T-cells in PBS when the recipients had a fever after CAR T-cell infusion, preliminarily to distinguish CRS from infection, and combined a conventional pathogen detection method with mNGS to investigate the cause of fever.

2. Materials and Methods

2.1 Patient Cohort

From May 2019 through November 2022, 27 patients (t/r B-ALL, n = 6; t/r T-cell ALL (T-ALL), n = 1; and t/r diffuse large B cell lymphoma (t/r DLBCL), n = 20) with recurrent fever (defined as a body temperature above 38 °C at least twice in the first month after CAR T-cell infusion) following CAR T-cell therapy within 1 month were enrolled (**Supplementary Fig. 1**). The 27 patients were enrolled from the five ongoing clinical trials (NCT05388695, NCT05618041, ChiCTR-OPN-16009847, ChiCTR-OPN-16008526, ChiCTR2000038641). The patients were tested for potential infections and were classified into two groups according to whether there were infection events after CAR T-cell infusion. Detailed patient and laboratory characteristics are listed in Table 1.

The inclusion criteria of this study were as follows: (i) patients aged 18 years or older who had received CAR T-cell therapy in the past 31 days; (ii) patients without any unresolved infection for at least 1 month before starting treatment; and (iii) patients who experienced recurrent fever after CAR T-cell infusion. Prior to the infusion of CAR T-cells, all patients underwent disease evaluation through peripheral blood (PB) and bone marrow (BM) assessments. Immediately before administering CAR T-cell infusion, a disease assessment was performed in all patients using PB and BM evaluations. Antimicrobial prophylaxis was initiated alongside chemotherapy and maintained until blood count recovery or if a switch to an alternative therapeutic approach was required based on institutional guidelines. The five ongoing clinical trials in this study were approved by the institutional review board. This retrospective study was approved by the Medical Ethics Committee of the Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and Department of Hematology, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, Taiyuan, Shanxi. In strict accordance with the principles

outlined in the Declaration of Helsinki, written informed consent was obtained from each individual involved in the study.

2.2 Definition of Infection and CRS Grading

The criteria for infection were defined as patients of any age confirmed to carry a pathogen either by culture or non-culture-based method, including nucleic acid detection/mNGS or antigen detection by conventional serological antibody assays combined with relevant clinical signs and symptoms. The criteria for the positive detection of microbes by mNGS have been previously described [15]. A microbe detection was deemed positive if: (1) the read number ranked within the top for 10 bacteria/viruses/parasites and exhibited a fold change ≥ 10 times higher than any other bacterium/virus/parasite; or (2) the read number ranked within the top five for fungi and showed a fold change ≥ 5 times higher than any other fungus. In the case of *Mycobacterium tuberculosis*, it was considered positive if at least one read was mapped to the species or genus level. Non-tuberculous mycobacteria were defined as positive when the read number ranked within the top 10 in the bacteria. CRS was assessed using a 1 to 5 point scale proposed by Lee *et al.* [24], whereas immune effector cell-associated neurotoxicity syndrome (ICANS) was graded according to the CAR T-Cell Therapy-Associated Toxicity scale [24,25] and considered severe if the grade was ≥ 3 . The onset of CRS was determined by the emergence of CRS symptoms, such as a fever ≥ 38.0 °C. The resolution of CRS was defined as the disappearance of fever or other CRS symptoms. Tocilizumab and/or corticosteroids were administered to manage severe CRS.

2.3 PBS and Clinical Data Collection

Hematology laboratory data obtained from medical records following CAR T-cell infusion included age, sex, treatment history, CRS, therapeutic responses, complete blood count (CBC), CAR transgene copy number, flow cytometry data, and inflammatory indicators (ferritin, IL-6, C-reactive protein (CRP), procalcitonin (PCT)). Wright Giemsa-stained PBS was screened by a board-certified hematopathologist. In patients with recurrent fever, multiple PBS were performed.

2.4 Cellular Kinetic Parameters

Quantification of CAR T gene copy numbers was performed using droplet digital PCR (ddPCR; Bio-Rad, Hercules, CA, USA) before the infusion of CAR T-cells and at multiple time points afterward, in accordance with a standard protocol [26]. We utilized the Quantalife QX200 Droplet Digital PCR system (Bio-Rad) to analyze and amplify the reaction mixture consisting of 2X ddPCR Supermix (Bio-Rad), fluorescently labeled primers and probes, and DNA template. Following amplification, the plate was processed using the QX200 Droplet Reader (Bio-Rad, Her-

Table 1. Correlations between infection and peripheral blood parameters.

Baseline characteristic	Total (n = 27)	Any infections (n = 9)	No infection (n = 18)	p-value
Age [years, median (range)]	39 (7–66)	33 (24–66)	41 (7–52)	0.6581
Female [cases (%)]	9 (33%)	5 (56%)	4 (22%)	0.1085
Pathologic subtype [cases (%)]				
DLBCL	20 (74%)	7 (78%)	13 (72%)	>0.9999
B-ALL	6 (22%)	2 (22%)	4 (22%)	>0.9999
T-ALL	1 (4%)	0 (0%)	1 (6%)	>0.9999
Therapy				
CAR T-cell type				
CD19 CAR T-cell	10 (37%)	2 (22%)	8 (44%)	0.4059
CD22 CAR T-cell	1 (4%)	0 (0%)	1 (6%)	>0.9999
CAR19/22 ‘Cocktail’	11 (41%)	6 (67%)	5 (28%)	0.0969
CD19XCD22 Bicistronic CAR T-cell	4 (15%)	1 (11%)	3 (17%)	>0.9999
CD7 CAR T-cell	1 (4%)	0 (0%)	1 (6%)	>0.9999
Auto-HSCT + CAR T-cell				
Yes	20 (74%)	7 (78%)	13 (72%)	>0.9999
No	7 (26%)	2 (22%)	5 (28%)	
Post-CAR T-cell characteristic				
CRS grade [cases (%)]				
1–2	20 (74%)	7 (78%)	13 (67%)	>0.9999
3–5	7 (26%)	2 (22%)	5 (31%)	
ICANS grade [cases (%)]				
0	19 (70%)	6 (67%)	13 (67%)	>0.9999
1–2	6 (22%)	2 (22%)	4 (22%)	>0.9999
3–4	2 (7%)	1 (11%)	1 (6%)	>0.9999
Characteristic in PBS time points				
Days after CAR T-cell infusion (mean ± SD)	9.3 ± 2.7	10.7 ± 3.4	8.6 ± 2.0	0.0716
First fever days (mean ± SD)	3.8 ± 2.6	3.9 ± 2.4	3.7 ± 2.7	0.7296
Fever [#] (°C, mean ± SD)	38.5 ± 5.5	38.3 ± 0.4	38.6 ± 0.6	0.2661
White blood cell count (×10 ⁹ /L, mean ± SD)	1.8 ± 1.5	1.7 ± 1.5	1.9 ± 1.5	0.9799
Absolute lymphocyte count (×10 ⁹ /L, mean ± SD)	0.8 ± 0.8	0.8 ± 0.7	0.9 ± 0.8	0.9698
Atypical lymphocytes (%), mean ± SD)	22.5 ± 20.7	12.2 ± 10.7	27.6 ± 22.7	0.0247
Atypical lymphocytes count (×10 ⁹ /L, mean ± SD)	0.5 ± 0.7	0.2 ± 0.3	0.6 ± 0.8	0.2979
Peak CAR transgene copy number (Copies/μg DNA, mean ± SD)	39,448 ± 38,759	32,481 ± 42,529	43,735 ± 37,369	0.5321
Ferritin* (μg/mL, mean ± SD)	7771 ± 13,773	12,961 ± 21,029	5176 ± 7786	0.7334
IL-6* (pg/mL, mean ± SD)	73.3 ± 133.5	81.2 ± 51.3	32.0 ± 28.1	0.0155
IL-1β* (pg/mL, mean ± SD)	6.1 ± 2.5	6.1 ± 1.6	6.1 ± 2.8	0.5715
IL-2R* (U/mL, mean ± SD)	3404.6 ± 1911.5	2673.3 ± 1461.1	3745.9 ± 1999.2	0.2400
IL-8* (pg/mL, mean ± SD)	83.23 ± 88.9	120.6 ± 110.8	65.8 ± 70.0	0.1417
IL-10* (pg/mL, mean ± SD)	27.25 ± 31.0	36.8 ± 38.6	22.8 ± 25.6	0.2636
TNF-α* (pg/mL, mean ± SD)	61.3 ± 205.0	22.5 ± 6.8	79.5 ± 246.1	0.4016
CRP* (mg/L, mean ± SD)	60.4 ± 43.9	76.6 ± 60.2	52.3 ± 32.1	0.4327
PCT* (ng/mL, mean ± SD)	0.5 ± 0.6	0.4 ± 0.2	0.6 ± 0.8	0.7493

*: Fever ≥38.0 °C was detected; #: Temperature measured at the time of peripheral blood smear collection. CAR, chimeric antigen receptor; PBS, Peripheral blood smears; DLBCL, Diffuse large B cell lymphoma; B-ALL, B-cell acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia; HSCT, Hematopoietic stem cell transplant; ICANS, Immune effector cell-associated neurotoxicity syndrome; IL-6, Interleukin-6; IL-1β, Interleukin-1β; IL-2R, Interleukin-2R; IL-8, Interleukin-8; IL-10, Interleukin-10; TNF, Tumor necrosis factor; CRP, C-reactive protein; PCT, Procalcitonin.

cules, CA, USA), and the outcomes were evaluated using QuantaSoft software version 1.7.4 (Bio-Rad, Hercules, CA, USA). Each reaction was analyzed individually, and the findings were reported as copies/μL for CAR and the reference gene.

2.5 Statistical Analyses

For analyses of continuous variables, the Mann-Whitney U test or Student’s *t*-test was used. Categorical

variables were analyzed using Pearson’s chi-square test or Fisher’s exact test as appropriate. The association between two or more variables was assessed using Spearman’s rank correlation coefficient. Data analyses were conducted using GraphPad Prism 8.0.2 software (GraphPad Software, San Diego, CA, USA). Two-tailed *p*-values were shown for all analyses, and statistical significance was defined as *p* < 0.05.

3. Results

3.1 Patients' Characteristics

In this study, all 27 patients in the study experienced recurrent fever within 1 month after CAR T-cell infusion; the mean days of PBS during fever were 9.4 with a mean body temperature of 38.5 °C. Of the twenty-seven patients receiving CAR T-cell infusion, ten patients were on cluster of differentiation (CD19) CAR-T, one on CD22 CAR-T, ten on CAR19/22 'cocktail' therapy, five on CD19-CD22 bicistronic CAR-T, and one was on CD7 CAR-T therapy. Twenty of twenty-seven patients received CAR T-cell infusion following autologous stem cell transplantation (Table 1). Through the combined utilization of mNGS and conventional methods, positive pathogen detection was observed in nine of twenty-five patients, resulting in a total of ten infectious events. Among these events, five were attributed to viral infections, three were associated with fungal infections, and two were identified as bacterial infections (Supplementary Fig. 2). Notably, among the patients with infection, two experienced grade 4 CRS with a high CAR transgene copy number of 110,900 and 87,474, and were infected with *Aspergillus* and BK polyomavirus and human herpesvirus6-B (HHV6-B), respectively. While other infection group patients experienced mild CRS (grade 1–2) with a lower CAR transgene copy number ($10,245 \pm 10,203$).

3.2 Atypical Lymphocytosis on Fever after CAR T-cell Infusion

Before CAR T-cell treatment, a total of seven B/T-ALL patients, including two patients with extramedullary recurrence, had a median percentage of lymphoblasts in BM of 88.0%, ranging from 10.5% to 96.0% and a median percentage of lymphoblasts in PB of 46.0%, ranging from 0.0% to 85.0%. Only one patient with BM involvement DLBCL showed 69.0% lymphoblasts in BM and 5.0% lymphoblasts in PB. After CAR T-cell infusion, the lymphoblasts in all patients were decreased to 0% in their first PBS, with an increase of 2.0–85.0% atypical lymphocytes (Fig. 1). The percentage of atypical lymphocytes in PBS showed a positive association with the CAR transgene copy number and absolute lymphocyte count (Fig. 2), suggesting that the expanding lymphocytes were entirely composed of CAR-T cells.

3.3 Morphologic Features of CAR T-Cells

We observed similar morphological features of different CAR T-cells, including CD7, CD19, and CD22 CAR-T-cells, which showed enlarged cell bodies (about 4- to 5-fold higher than red blood cell) and an irregular and monocyte-like cell shape. The cell membrane of the CAR T-cells was smooth, the cytoplasm was abundant and bright, the outer cytoplasm was dark blue, the inner cytoplasm was light blue near the nucleus with strong basophilia, and the purplish-red particles similar to NK cells could be seen, but no vac-

uole was observed. Nuclei were mostly round or oval; indented; unfolded without pseudopodia; and had loose chromatin, an uneven distribution, small clumps, inconspicuous nucleoli, and visible pseudonucleolus. The nucleoplasm ratio was about 1:1 (Fig. 3). In addition, the dynamic changes of CD7 and CD19/22 CAR T-cells and the flow cytometry data or CAR transgene copy number in two patients within 1 month after therapy were also obtained (Fig. 4).

3.4 Higher Proportion of CAR T-Cells in PBS of Patients with CRS

Since the fever after CAR T-cell infusion often represents a state of CRS or infection, we compared the atypical lymphocyte percentage between the infection and non-infection groups. Significantly, among the 27 CAR T-cell recipients with fever, recipients without infection showed a notably higher percentage of atypical lymphocytes in PBS compared to recipients with infection (27.6% vs. 12.2%; $p < 0.05$). Furthermore, recipients with infection exhibited significantly elevated levels of IL-6 compared to recipients without infection (81.2 vs. 32.0; $p < 0.05$). These compelling findings strongly support the notion that the activation of systemic immune responses primarily stemmed from pathogenic microorganisms rather than CAR T-cells (Fig. 5).

4. Discussion

Despite the great successes of CAR T-cell therapy in hematological malignancy, infection remains a main obstacle in this field. Recipients are immunocompromised after CAR T-cell infusion, making them highly susceptible to opportunistic infections [26,27]. A previous study showed that in patients with infection after CAR T-cell infusion, significantly higher mortality within 90 days was noted [13,28]. Consequently, there is a justified need for studies focusing on enhancing infection prevention and treatment strategies for these high-risk patients.

In this study, we described the morphology of CD7, CD19 and CD22 CAR T-cells in detail and found that PBS morphology and atypical lymphocyte percentage were capable of reflecting the clonal kinetics of CAR T-cells. Lesesve *et al.* [29] examined the PBS of a patient with large cell B-cell lymphoma treated with CD19 CAR-T, and the morphological description of CAR T-cells detected under the microscope was similar to what we observed. In addition to CD19 CAR T-cells, we also observed two other types of CAR T-cells, and the results showed that different CAR T-cells had similar morphological characteristics. This finding supplements the morphological characteristics of CAR T-cells after infusion, which is conducive to the accurate and rapid identification of CAR T-cells. In a previous study, Faude *et al.* [30] established that the peripheral blood absolute lymphocyte count parameters can be used as a surrogate to assess CAR T-cell expansion, which was also found in our study. We further found that the percentage of

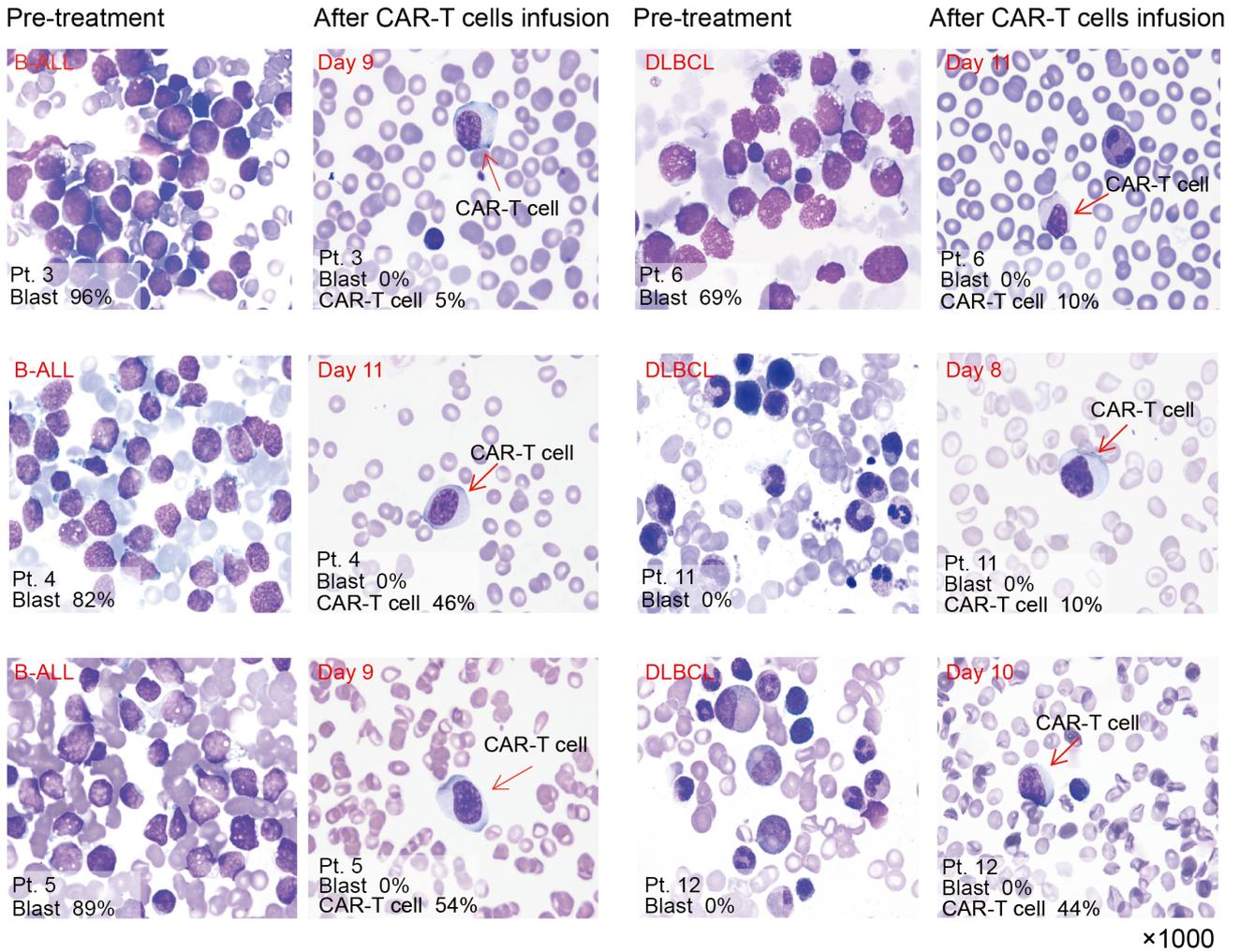


Fig. 1. PBS images before and after CAR T-cell infusion. Before CAR T-cell infusion, blasts could be seen in the PBS of three patients with B-ALL (left) and in one patient with DLBCL (right top). After CAR T-cell infusion, the blasts disappeared and instead were replaced with CAR T-cells (arrow). Pt, patient.

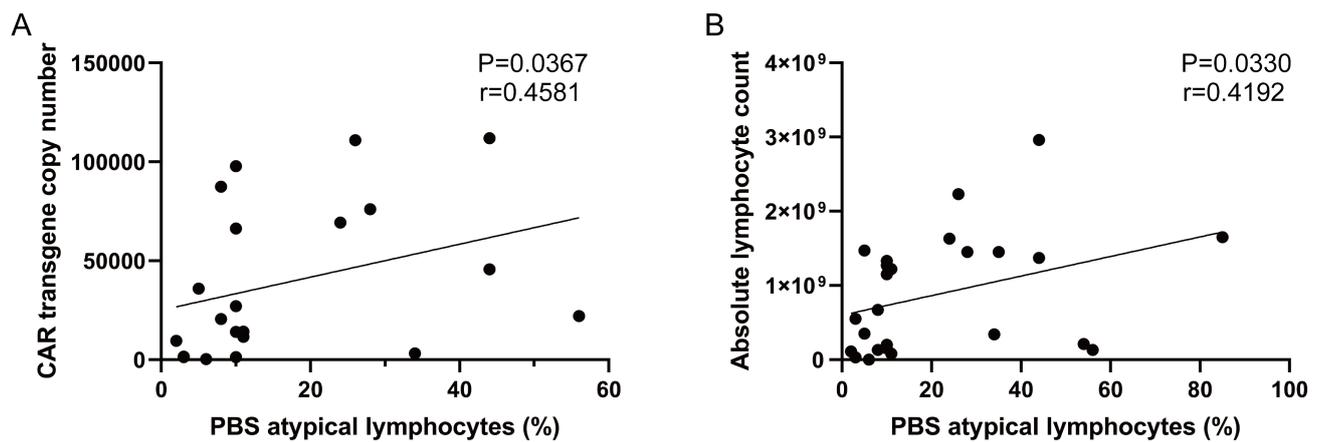


Fig. 2. Correlation between PBS atypical lymphocyte percentage, CAR transgene copy number, and absolute lymphocyte count. (A) Correlation between PBS atypical lymphocyte percentage and CAR transgene copy number. (B) Correlation between PBS atypical lymphocyte percentage and absolute lymphocyte count. Correlation analyses were performed with Spearman's rank test.

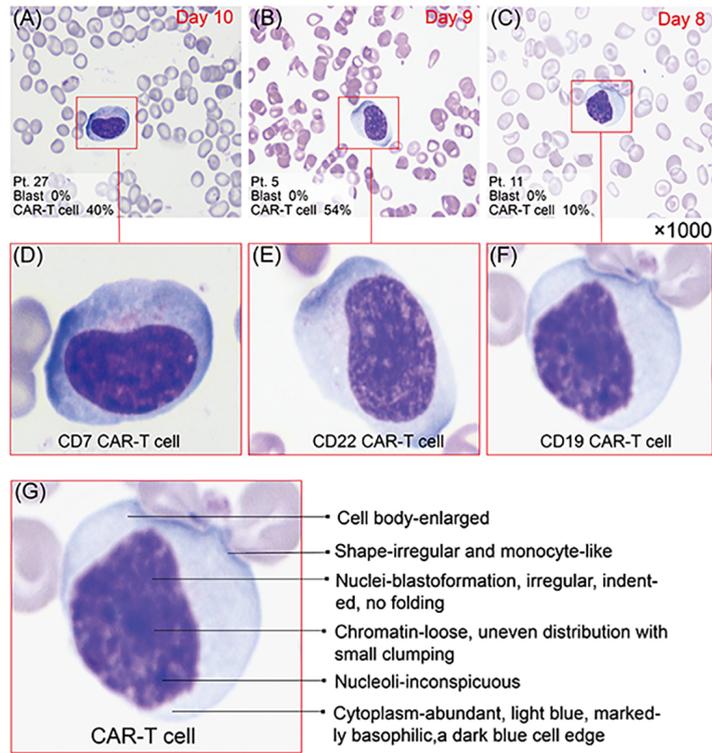


Fig. 3. PBS images of CD7, CD19, and CD22 CAR T-cells. (A–F) Similar morphological features were seen between these CAR T-cells, as detailed in (G), an enlarged picture of CD19 CAR T-cells. Pt, patient. Pt. 5 and Pt. 11 correspond to the images in Fig. 1, while Pt. 27 corresponds to the images in Fig. 4A.

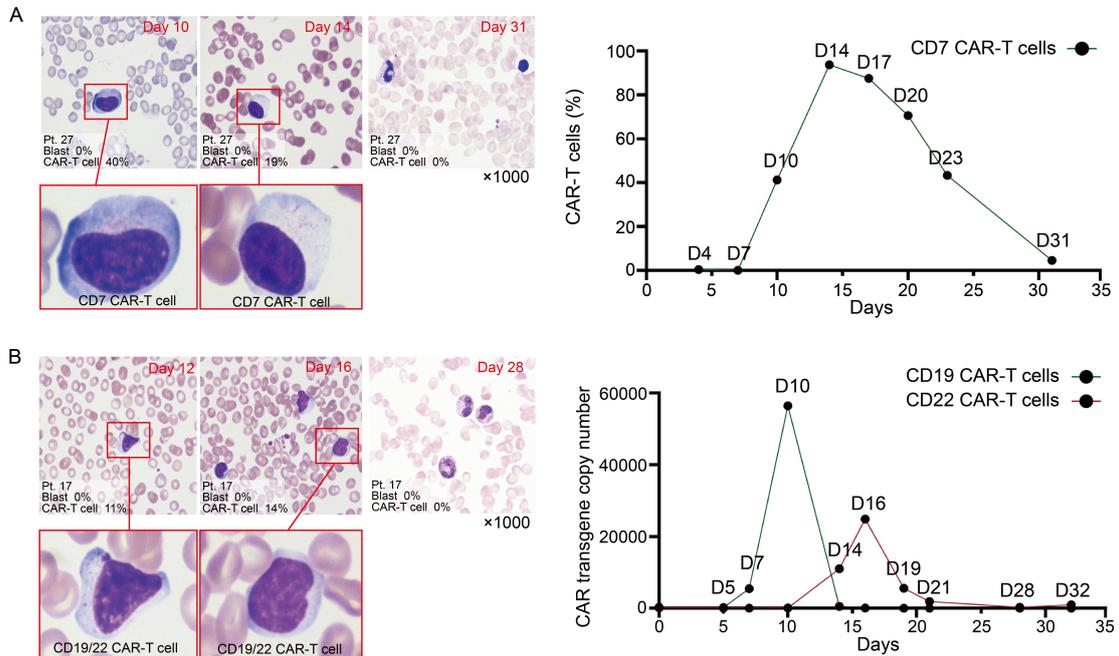


Fig. 4. Dynamic changes of CD7, CD19/CD22 CAR T-cells after CAR T-cell infusion with 1 month. (A) Flow cytometry data showed the CD7 CAR T-cells were increased within the first 14 days and declined and disappeared by day 31 in patient No. 27. (B) CD19/22 CAR T-cells were infused on days 0 and 1, and the CAR transgene copy number showed that the CD19 CAR T-cells were increased in the first 10 days, whereas CD22 CAR T-cells were increased by day 10. All CAR T-cells disappeared on day 28. Pt, patient.

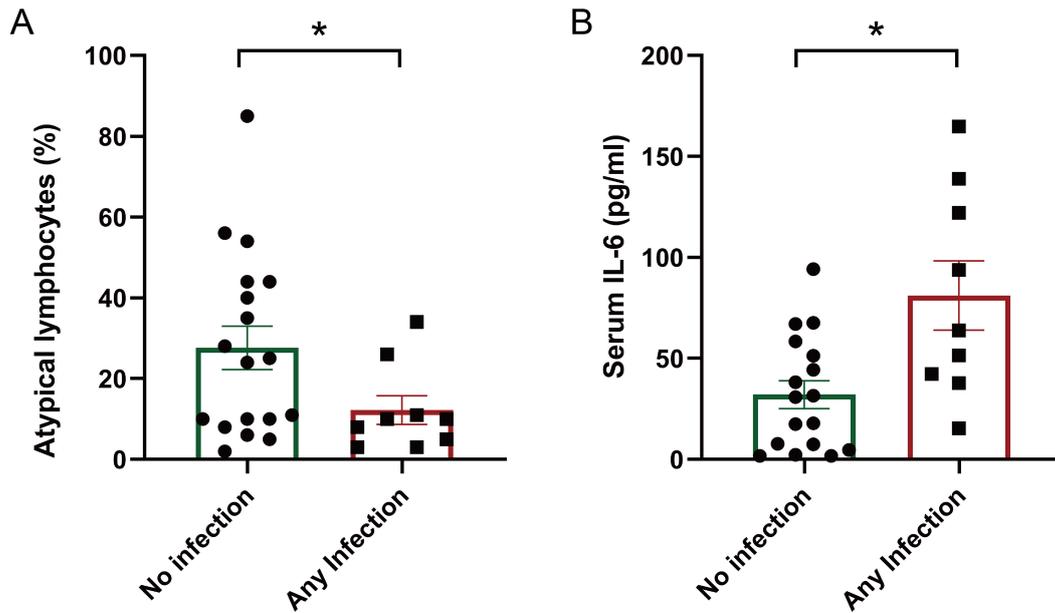


Fig. 5. Distribution of PBS atypical lymphocyte percentage (A) and IL-6 (B) levels in patients with no infection vs. any infection. *: $p < 0.05$.

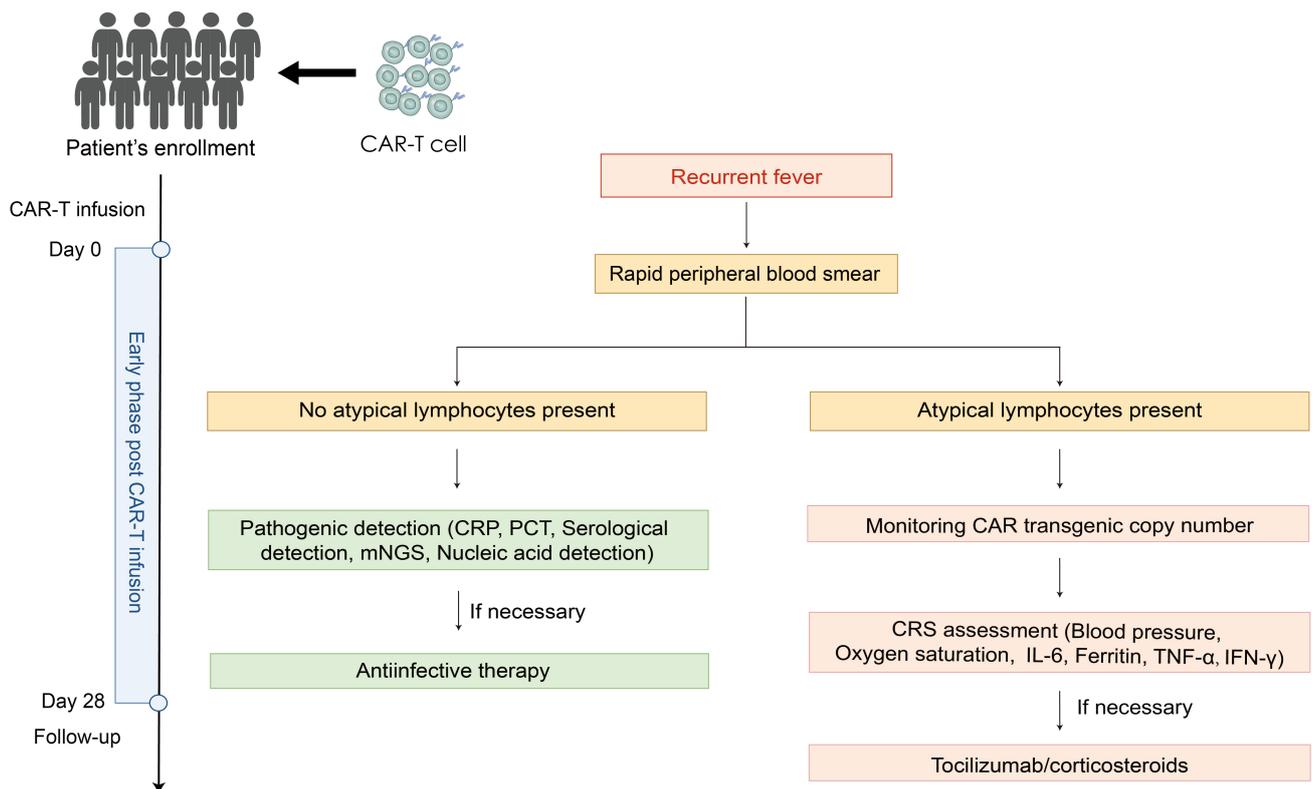


Fig. 6. Flow chart of quick diagnosis and management for recurrent fever patients post CAR T-cell infusion using PBS. CRP, C-reactive protein; PCT, procalcitonin; mNGS, metagenomic next-generation sequencing; CRS, cytokine release syndrome; IL-6, Interleukin-6; TNF- α , Tumor necrosis factor-alpha; IFN- γ , Interferon-gamma.

atypical lymphocytes in PBS was both positively correlated with CAR transgene copy number and absolute lymphocyte count ($p < 0.05$), suggesting that PBS could be a reliable method to assess the expansion of CAR T-cells in these re-

cipients. Furthermore, we grouped the patients by whether there were infection events and compared the PBS morphology of these two groups. Interestingly, although they were similar in many clinical parameters, including the grade of

CRS, ICANS, white blood cell count, absolute lymphocyte count, or some inflammatory markers (ferritin, CRP, PCT), the percentage of atypical lymphocytes was significantly higher in the non-infection group ($p < 0.05$), suggesting that the recurrent fever in this group was mainly due to CAR T-cell proliferation. Regarding the morphology observed in PBS samples, atypical lymphocytes exhibited deep outer and shallow inner cytoplasm, along with loosely and unevenly distributed chromatin, indicating the activated proliferative state of CAR T-cells, which aligned with an increased CAR transgene copy number. In our observations, CAR T-cells exhibited smooth cell membranes and abundant, bright cytoplasm with purplish-red granules showing cytotoxic effects as NK cells, which could be well distinguished from infection-associated lymphocytes (disordered dark blue cytoplasm) and infectious mononucleosis cells (bright light blue cytoplasm). Therefore, it should be noted that if PBS shows a large number of atypical lymphocytes, patients with post-infusion fever should be on high alert for CRS, which is an ideal time to immediately administer appropriate corticosteroids and IL-6 receptor antibodies rather than waiting for CAR transgene number results.

We observed a similar CAR transgene copy number between infection and non-infection groups. Further analyses revealed that in the infection group, two out of nine patients experienced grade 4 CRS and showed the highest CAR transgene copy number in this group (data not shown), suggesting that in patients with infection, the fever may be caused by both pathogenic microbes and CAR T-cells expansion. In addition, IL-6 is increased in critical illnesses such as sepsis and CRS, driven by the activity of antigen-presenting cells and CAR T-cells [31–33]. In this study, while a low percentage of atypical lymphocytes was noted in patients with infection, a high level of IL-6 was observed in these patients, suggesting that a higher level of IL-6 in CAR T-cell recipients may be due to an infection state rather than CAR T-cell activation only. In this study, a high IL-6 level accompanied by a low percentage of atypical lymphocytes in PBS may have been a potential indicator for the delayed administration of IL-6 receptor antibody treatment, as infections occurred in these fever CAR T-cell recipients.

Both infection and CRS could be involved in the pathogenesis of fever after CAR T-cell infusion [34,35]. Infection and CRS exhibit similar clinical symptoms [36]. CAR transgene copy number, flow cytometry analyses, mNGS, and conventional methods to investigate CAR T-cell proliferation and infection complications in recipients are expensive and time-consuming. PBS could be a more convenient and reliable method to assess CAR T-cell kinetics. While some patients with infection also experience severe CRS, identifying the cause of fever in such patients is complex, and examination at multiple time points to assess both CAR T-cell kinetics and microbiological tests is necessary, causing an appropriate temporal window of corticosteroids and IL-6 receptor antibody to break the CRS

inflammatory activation loop and avoiding the aggravation of infection, which is very important. Therefore, we suggest the diagnosis and management flowchart for patients with recurrent fever post-CAR T-cell infusion (Fig. 6).

5. Conclusions

In summary, our results showed that PBS could be an indicator of CAR T-cell expansion and a quick and reliable method to distinguish causes of fever in the early phase of post-CAR T-cell infusion.

Abbreviations

CD7, cluster of differentiation 7; PB, peripheral blood; BM, bone marrow; NK, natural killer; CAR, chimeric antigen receptor; PBS, peripheral blood smears; CRS, cytokine release syndrome; r/r B-ALL, refractory or relapsed B-cell acute lymphoblastic leukemia; r/r DLBCL, refractory or relapsed Diffuse large B cell lymphoma; HSCT, Hematopoietic stem cell transplant; ICANS, immune effector cell-associated neurotoxicity syndrome; HHV6-B, human herpesvirus6-B; CBCs, complete blood count; CRP, C-reactive protein; PCT, procalcitonin; mNGS, metagenomic next-generation sequencing; ddPCR, droplet digital PCR.

Availability of Data and Materials

The datasets are available upon request.

Author Contributions

JW and ZW designed this study, participated in the revision, and approved the final version to be released. LiaH, FM, ZG, and WT managed patients in clinical trials and provided patients' data. SH, JP, XY, JZ, and LifH analyzed and interpreted the data. SH, JP, and XY wrote the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The 5 ongoing clinical trials in this study were approved by the institutional review board. This retrospective study was approved by the Medical Ethics Committee of the Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and Department of Hematology, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, Taiyuan, Shanxi (Medical Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology: TJ-IRB20160314/ TJ-IRB20160310/ [2020] Lun Shen Zi (S205)/ [2022] Lun Shen Zi (S031); Academic Committee of Norman Bethune Hospital (Shanxi Academy of Medical Sciences): XS-2022-9-2).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2811299>.

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