

# Infectious Complications Following CD19 Chimeric Antigen Receptor T-cell Therapy for Children, Adolescents, and Young Adults

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**Background.** Infectious complications of chimeric antigen receptor (CAR) T-cell immunotherapy in children and young adults have not been well described.

**Methods.** Medical records of patients ≤26 years old receiving CD19 CAR T-cell infusion (CTI) at a single institution between 2014 and 2017 were reviewed. The number of infections per 100 days-at-risk (infection density) in the 90 days preceding and 0–28 and 29–90 days after CTI was calculated. Poisson regression and Cox analyses were utilized to identify risk factors for infections.

**Results.** Eighty-three patients received CTI during the study period. Most patients (98%) had refractory or relapsed acute lymphoblastic leukemia (ALL). Infections occurred in 54% of patients in the 90 days before CTI (infection density, 1.23) and in 40% of patients in the first 28 days following CTI (infection density, 2.89). Infection density decreased to 0.55 in the 29–90 days post-CTI. Most infections were bacteremias (39%) or respiratory viral infections (43%). Pre-CTI risk factors associated with infection included prior hematopoietic cell transplantation (HCT), immunoglobulin G (IgG) level <400 mg/dL, and lymphodepletion other than cyclophosphamide plus fludarabine; post-CTI risk factors included higher-severity CRS and IgG <400 mg/dL.

**Conclusions.** Infection rates in children and young adults receiving CD19 CAR T-cell therapy increase in the first month and then decline. Understanding types and timing of infections and contributing risk factors may help inform prophylactic and monitoring strategies. Specific attention should be given to patients with prior HCT, severe hypogammaglobulinemia, and severe CRS.

**Keywords.** ALL; CAR T-cell; immunotherapy; pediatric; infection.

Immunotherapy using chimeric antigen receptor (CAR) T-cells for hematologic malignancies and other diagnoses is increasing rapidly [1]. This approach has resulted in high response rates in children with refractory or relapsed (R/R) B-cell acute lymphoblastic leukemia (B-ALL) [2–4]. The first commercially available CD19 CAR T-cell therapy, tisagenlecleucel (Kymriah), was approved by the Food and Drug Administration in 2017 [5].

Patients under consideration for CAR T-cell therapy are at high risk for infections due to extensive prior antitumor therapies. Before CAR T-cell infusion (CTI), patients receive lymphodepleting chemotherapy that leads to additional

cytopenias. Furthermore, CD19 CAR-T cells may deplete normal CD19 B cells in the host, potentially causing prolonged B-cell aplasia and low immunoglobulin G (IgG) levels [6, 7]. Finally, these patients often develop cytokine release syndrome (CRS) following CTI, which may require treatment with anti-interleukin 6 (IL-6) therapies (eg, tocilizumab), steroids, and admission to the intensive care unit (ICU), with associated increased risk for immunosuppression and nosocomial infections [8–10].

The infectious complications of CAR T-cell therapy have not been well studied in children and young adults. Little is known about optimal prophylactic strategies in this setting. Two studies have demonstrated a relatively high rate of infections in adult patients after CD19 CTI, especially in the first 28 days [11, 12]. Patient factors such as underlying ALL, increased number of prior chemotherapy courses, higher CAR T-cell doses, and CRS were associated with higher infection rates. The majority of early infections were bacterial, whereas respiratory virus infections predominated at later time points.

In this study, we examine the epidemiology of and risk factors for infections occurring in the first 90 days after CD19 CTI in children and young adults.

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## METHODS

### Patients

This study was approved by the Institutional Review Board at Seattle Children's Hospital. We reviewed medical records of patients aged 1–26 years receiving CD19 CAR T cells [2] at our institution between 2014 and 2017. The majority of patients remain under our center's care for a minimum of 28 days after therapy, as this is the time frame when the majority of complications are expected [11, 12]. Each patient's lymphodepletion regimen was determined by the treating physician. Bacterial prophylaxis was not routinely prescribed. Patients with a history of prior hematopoietic cell transplantation (HCT) were routinely started on acyclovir prophylaxis if not already receiving it. The type and duration of fungal prophylaxis were prescribed at the discretion of the treating physician. All patients were prescribed prophylaxis for *Pneumocystis jirovecii* with trimethoprim-sulfamethoxazole or inhaled pentamidine. Intravenous immunoglobulin G (IVIG) supplementation was recommended for patients with IgG levels <400 mg/dL. Empiric antimicrobial therapy was prescribed for fever and neutropenia. Further details of the lymphodepleting regimens and supportive care approaches for patients who developed CRS are in the Supplementary Data.

### Data Collection

We reviewed medical records for infections occurring in the 90 days before and after CTI. Patients contributed data up to 90 days post-CTI; the date of death; the date of loss to follow-up; or the date of receipt of additional antitumor therapies for relapse, repeat CTI, or as conditioning for HCT, whichever occurred first. Absolute neutrophil (ANC) and lymphocyte (ALC) counts were collected for each patient before lymphodepletion, from all available laboratory data between days 0 and 28 post-CTI, and on day 63 (per study protocols) post-CTI. IgG levels were collected before lymphodepletion and on days 21 and 63 (per study protocols) post-CTI. Treatment for CRS, including use of tocilizumab and/or steroids, was recorded.

### Definitions

The primary outcome of interest was infection in the 90 days post-CTI. An infection episode was defined as the presence of clinical findings plus corroborating laboratory, radiographic, histopathologic, and/or microbiologic evidence. Infections were classified as bacterial (bacteremia or site infection), viral (respiratory or other), or fungal (proven or probable) [13]. Infection onset was defined as the day of initial positive diagnostic test or clinical diagnosis. Infections were excluded if they were already present at the time of CTI. Infection severity was categorized as 1 (mild) if no treatment was required, 2 (moderate) if only oral treatment or minimal supportive care was required, 3 (severe) if intravenous therapy or hospitalization was required, 4 if life threatening, or 5 if fatal [14]. Testing for infection was

sent based on provider discretion guided by symptoms, further outlined in the Supplementary Data. Further details pertaining to the assessment of the timing, type, and severity of each infection are also described in the Supplementary Data.

The severity of CRS was graded from 1–5 as previously described [15], and definitions of each grade are shown in [Supplementary Table 1](#).

### Statistical Analysis

Descriptive statistics (median, range, percentage) are reported for key variables. We calculated the infection density per 100 days-at-risk based on the number of infections per person-days-at-risk and multiplied by 100. This was calculated for the 90 days before CTI and the 0–28- and 29–90-day periods after CTI. The estimates for infection densities are only based on data obtained during the person-days-at risk contributed per patient. We also demonstrate the cumulative incidence of any and each type of infection in the first 28 days after CTI, treating death and loss to follow-up as competing risk events.

Poisson regression was utilized to identify associations between pre-CTI factors and infection density within the first 28 days after CTI. Time-dependent Cox proportional hazards models were used to assess the impact of pre- and post-CTI factors on the time to first infection within 28 days after CTI. For all factors, only data occurring before an infection event were included. For each infection event, the ANC and ALC values from the day of the event were considered as dichotomous data points (ANC < 500 or no, ALC < 300 or no). When a value for that day was not available, the most recently available value was used instead. For both Poisson and Cox regression, variables with a *P* value of <.25 in univariate models were considered for multivariate adjusted models. Poisson and Cox models were evaluated for overfitting or underfitting and Schoenfeld residuals, respectively. Patients were censored from the study before day 90 post-CTI if they died, were lost to close follow-up, or received further lymphodepleting chemotherapy in the setting of relapse, repeat CTI, or as conditioning for HCT.

## RESULTS

### Patient Characteristics

Eighty-three patients received CTI during the study period. The median duration of follow-up (range) was 45 (9–90) days. Patient and treatment characteristics are described in [Table 1](#). All patients had underlying B-ALL except for 1 patient with mixed leukemia and 1 with B-cell lymphoma. All patients had relapsed or refractory disease. The median age (range) was 12 (1–25) years. Forty-six patients (55%) had previously undergone allogeneic HCT, and none were on immunosuppressive therapy or had graft-vs-host disease for at least 4 weeks before CTI. Before receipt of lymphodepleting chemotherapy, 18%

**Table 1. Demographic, Laboratory, and Clinical Characteristics**

Baseline Characteristics	No. (% Unless Indicated), n = 83 Unless Specified
Median age (range), y	12 (1–25)
Female sex	33 (40)
Race	
Asian	9 (11)
White	48 (57)
Hispanic/Latino	14 (17)
Black or Pacific Islander	2 (2)
Other	4 (5)
Unknown	6 (7)
Underlying disease	81 (98)
B-ALL	1 (1)
B-cell lymphoma	1 (1)
Mixed phenotype leukemia	
Prior allogeneic HCT	46 (55)
Disease status	
Refractory	8 (10)
Relapsed	
1	29 (35)
2	31 (38)
3	10 (12)
4	5 (6)
Hematologic parameters before lymphodepletion, No. (%), median (range)	
ALC < 300 cells/mm <sup>3</sup>	15 (18), 826 (0–8476)
ANC < 500 cells/mm <sup>3</sup>	28 (34), 1071 (0–11 284)
IgG < 400 mg/dL	13 (16), 617 (143–1480)
Pre-CAR T-cell infections (days –90 to –1), No. (%)	45 (54)
Bacterial	29 (35)
Fungal	15(18)
Viral	20 (24)
Antimicrobial prophylaxis, No. (%)	
Anti-PJP	83 (100)
Antiviral	29 (35)
Antifungal	42 (51)
Fluconazole	12 (14)
Antimold azole	21 (25)
Echinocandin	2 (2)
Cyclophosphamide/fludarabine lymphodepletion <sup>a</sup>	54 (65)
Cyclophosphamide only (3 g/m <sup>2</sup> )	23 (28)
Other <sup>b</sup>	6 (7)
Post-CAR T-cell infusion characteristics	
Hematologic parameters, No. (%), median (range)	
ANC < 500 cells/mm <sup>3</sup>	
D +7 (n = 82)	62 (76), 32 (0–3043)
D +21 (n = 79)	38 (48), 624 (0–6636)
D +63 (n = 40)	5 (13), 2061 (0–7730)
ALC < 300 cells/mm <sup>3</sup>	
D +7 (n = 82)	57 (70), 129 (0–1606)
D +21 (n = 79)	28 (36), 429 (0–3515)
D +63 (n = 40)	8 (20), 567 (0–4183)
IgG level < 400 mg/dL	
D +21 (n = 78)	11 (14), 584 (168–1210)
D +63 (n = 56)	16 (29), 455 (190–760)
Cytokine release syndrome, any	74 (89)
Grade 1–2	66 (79)

**Table 1. Continued**

Baseline Characteristics	No. (% Unless Indicated), n = 83 Unless Specified
Grade 3–5	8 (10)
Corticosteroids and/or tocilizumab	43 (52)
ICU admission by day 28	48 (58)
Death by day 28	3 (4)
Infection related	0
Death by day 90	6 (8)
Infection related	1 (1)

Abbreviations: ALC, absolute lymphocyte count; ALL, acute lymphoblastic leukemia; ANC, absolute neutrophil count; CAR, chimeric antigen receptor; HCT, hematopoietic cell transplant; IgG, immunoglobulin G; PJP, *Pneumocystis jirovecii*.

<sup>a</sup>Fludarabine 30 mg/m<sup>2</sup> × 4 days plus cyclophosphamide 500 mg/m<sup>2</sup> × 2 days (days 1, 2).

<sup>b</sup>Fludarabine only (2%), cyclophosphamide only (1.5 g/m<sup>2</sup>, 4%), or cyclophosphamide and etoposide (1%).

had an ALC <300 cells/mm<sup>3</sup>, 35% had an ANC <500 cells/mm<sup>3</sup>, and 16% had a serum IgG <400 mg/dL. The most common lymphodepletion regimen consisted of fludarabine plus cyclophosphamide (65%). Doses of CAR T cells ranged from 0.5 × 10<sup>6</sup> to 10 × 10<sup>6</sup> CAR T cells/kg.

Details of antimicrobial prophylaxis are outlined in [Table 1](#). At the time of CTI, no patient had a positive blood culture within 48 hours or had fever with clinical signs of infection.

**Noninfectious Complications After Lymphodepleting Chemotherapy and CTI**

The majority of patients (96%) experienced neutropenia (ANC < 500 cells/mm<sup>3</sup>) at the time of, or within 7 days of, CTI. Of these, 42% for whom complete data were available did not recover from neutropenia (defined as 2 measurements of ANC >500 cells/mm<sup>3</sup>) by day 28 post-CTI. Three patients died while still neutropenic in this time period. For the patients who were neutropenic and did recover by day 28 (58%), the median time to recovery was 15 days. Severe hypogammaglobulinemia (IgG <400 mg/dL) was identified in 14% of patients at day 21 ([Table 1](#)). By day 63 post-CTI, 13% of 40 patients with available data remained neutropenic and 20% lymphopenic. Of 56 patients with IgG levels available, 29% remained hypogammaglobulinemic with a median IgG level of 455 mg/dL (irrespective of IgG supplementation).

Forty-eight (58%) patients had an ICU admission in the 28 days post-CTI. CRS occurred in 89% of patients at a median (range) of 5 (1–11) days after infusion ([Table 1](#)). The majority of these cases were categorized as grade 1 or 2 (79%), with CRS grade 3 or higher occurring in 8 patients (10%). Forty-three (52%) patients received treatment for CRS with either tocilizumab and/or steroids.

**Infections Pre-CTI**

Forty-five (54%) patients had at least 1 infection in the 90 days before CTI, as detailed in [Supplementary Table 2](#). Forty-eight percent of the infections in this time period were bacterial,

and 34% were viral. Nine patients (11%) were being treated for proven or probable fungal infections. The infection density for this time period was 1.23 infections per 100 patient-days-at-risk (Figure 1).

#### Infections in the First 28 Days Post-CTI

Thirty-three patients (40%) had 37 incident infections in the first 28 days post-CTI (Table 2). Twenty infections (54%) were bacterial, including 16 instances of bacteremia. Of these episodes of bacteremia, 6 were caused by gram-negative organisms, 10 by gram-positive bacteria, and all occurred within the first 15 days post-CTI (Figure 1). Three of the 11 patients who developed bacteremia had infection with an organism that was identified in the pre-CTI period. Sixteen patients had viral infections, the majority of which were due to respiratory viruses (38% of total infections). Two patients developed herpes zoster, with skin manifestations in both; neither patient was taking acyclovir prophylaxis. One patient with pre-CTI probable pulmonary invasive mold infection on mold-active therapy with voriconazole was found to have proven pulmonary infection with *Cunninghamella* on day 21 post-CTI. It is unclear whether this represented worsening of previous infection or new infection. The cumulative incidences of infections overall and by infection type are in Figure 1. The infection density during the first 28 days was 2.89 infections per 100 days-at-risk (Figure 2).

#### Infections in Days 29–90 Post-CTI

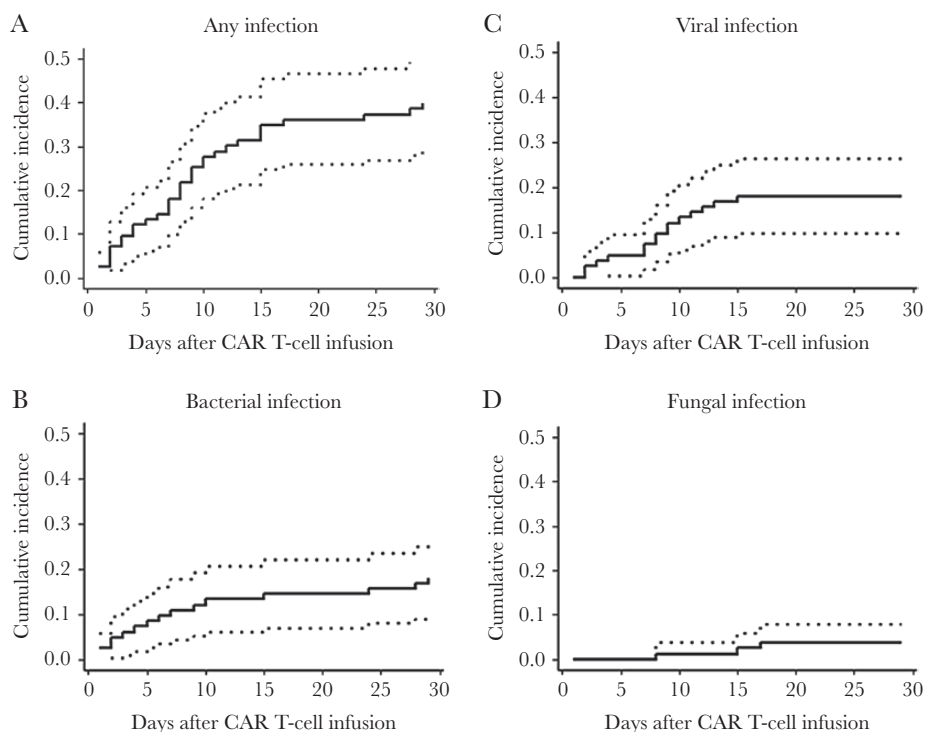
Forty-eight patients contributed data during this time period, with a median duration of follow-up (range) of 51 (29–90) days. There were 7 viral infections in this time period, which comprised the majority of infections (58%). All were due to respiratory viruses (Table 2). Five patients had bacterial infections, and there were no fungal infections. The infection density in the 29–90 days post-CTI decreased to 0.55 per 100 days-at-risk (Figure 2).

#### Infection Severity

Table 2 demonstrates the severity of infections. Between 0 and 90 days after CTI, mild or moderate infections accounted for 21 of 49 total episodes (43%), most of which were viral infections. There were 22 severe infections (45%) and 6 (13%) life-threatening infections, most of which were bacterial. There were no fatal infections in the first 28 days post-CTI. One death occurred on day 51 post-CTI due to septic shock with *Aeromonas hydrophila*.

#### Risk Factors for Infections in the First 28 Days Post-CTI

The proportion of patients with any infection in the 28 days post-CTI stratified by CRS grade is shown in Figure 3A. A higher proportion of patients with higher-severity CRS had infections; 30% (95% confidence interval [CI], 7%–65%) had no CRS, 46% had grade 1 (95% CI, 28%–66%), 32% had grade



**Figure 1.** Cumulative incidence curves of time to first infections in the first 28 days after CAR T-cell therapy. A–D, Cumulative incidences among all patients (n = 83) of any (A), bacterial (B), viral (C), and fungal (D) infections within 28 days after CTI. Dotted lines represent 95% confidence intervals. Death and loss to follow-up were treated as competing risks. Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome.

**Table 2. Incidence and Severity of Infections After CAR-T-cell Infusion**

Type of Infection	Infection Days 0–28		Infection Days 29–90		Severity		
	Events	No. of Patients (%)	Events	No. of Patients (%)	Mild–Moderate	Severe	Life-Threatening/Fatal
Any infection	37	33 (40)	12	11 (17)	21	22	6
Bacterial infections	20	15 (18)	5	5 (6)	3	16	6
Bacteremia <sup>a</sup>	16	11 (13)	3	3 (5)	0	13	6
Site infection <sup>b</sup>	4	4 (5)	2	2 (2)	3	3	0
Viral infections	16	16 (19)	7	7 (11)	19	4	0
Respiratory virus <sup>c</sup>	14	14 (17)	7	7 (11)	18	3	0
Other virus (herpes zoster)	2	2 (2)	0	0	1	1	0
Fungal infections <sup>d</sup>	1	1 (1)	0	0	0	0	1

<sup>a</sup>Six gram-negative infections: *Escherichia coli* (3), *Klebsiella oxytoca* (1), *Pseudomonas aeruginosa* (1), *Enterobacter cloacae* (1). Thirteen gram-positive infections: *Enterococcus faecalis* (5), *Streptococcus viridans* (2), coagulase-negative staphylococci (3), *Gordonia* species (1), *Microbacterium* species (1), *Clostridium tertium* (1).

<sup>b</sup>Skin and soft tissue infection (3), pneumonia (1), central line site infection (1), proctitis (1).

<sup>c</sup>Rhinovirus/enterovirus (10), parainfluenza (5), respiratory syncytial virus (3), influenza A (1), human metapneumovirus (1), coronavirus (1).

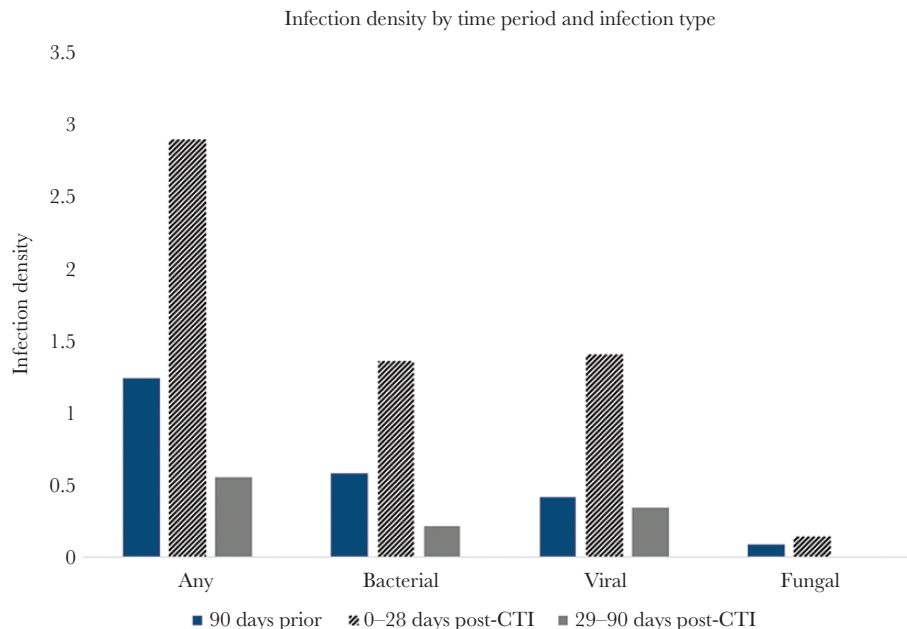
<sup>d</sup>*Cunninghamella* pulmonary infection.

2 (95% CI, 18%–50%), and 62% had grade  $\geq 3$  CRS (95% CI, 24%–91%). Of note, no patients had grade 4 CRS, and only 1 patient had grade 5 CRS and died on day 11 post-CTI. Thus, our understanding of the true impact of higher-grade CRS on infection risk is limited from this cohort. The proportion of patients with infection who were neutropenic at the time of infection is depicted in Figure 3B. Most patients with bacteremia (90%) were neutropenic, whereas viral infections occurred in similar numbers of patients with and without neutropenia.

The results of Poisson regression analysis of the impact of baseline patient and clinical variables on infection density within 28 days after CTI are shown in Table 3. Prior HCT and

IgG <400 mg/dL were associated with increased infection density but did not reach statistical significance in the adjusted model. Lymphodepletion with combination cyclophosphamide and fludarabine was associated with significantly decreased infection density (rate ratio, 0.46; 95% CI, 0.27–0.77) compared with other regimens in the adjusted model. Age, sex, refractory vs relapsed B-ALL status, CART-cell dose, pre-CART-cell infections, and prelymphodepletion neutropenia and lymphopenia were not associated with increased infection density.

In a Cox proportional hazards model of the impact of pre- and post-CTI variables on time to first infection, prior HCT (hazard ratio [HR], 2.15; 95% CI, 0.98–4.73) and post-CTI



**Figure 2.** Infection density before and after CAR T-cell therapy. Infection density was calculated as the total number of infection events per patient-days-at-risk multiplied by 100. Only data obtained during person-days-at risk contributed to these estimates. Infection densities are shown for any infection and by pathogen category for the time periods 90 days before CTI and 0–28 and 29–90 days after CTI. Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome.



hypogammaglobulinemia (HR, 2.41; 95% CI, 1.02–5.69) were associated with increased infection risk in the first 28 days in the adjusted model (Table 4). This analysis also confirmed the decrease in infection risk with cyclophosphamide and fludarabine lymphodepletion. Severe CRS was associated with an increased risk for infection but did not reach statistical significance in the adjusted model.

## DISCUSSION

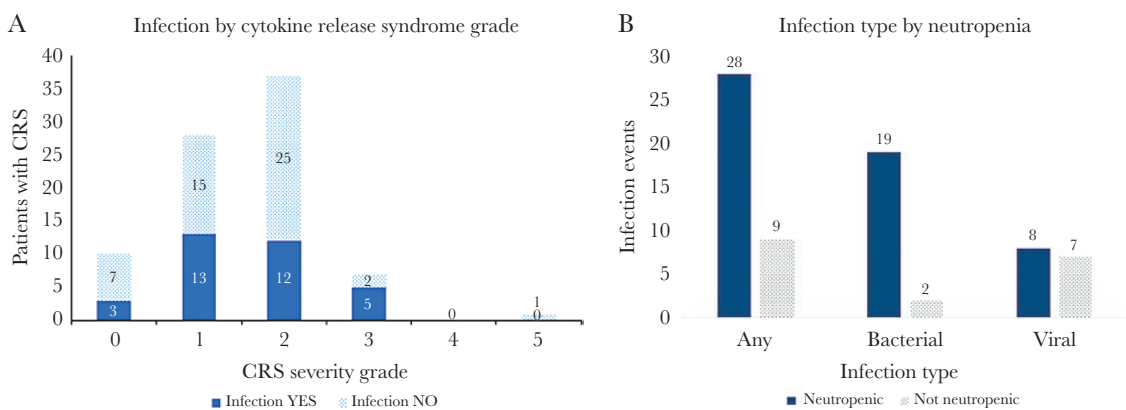
This study is the first in-depth description of the frequency, type, severity, and risk factors for infections following CD-19 CAR T-cell therapy in children, adolescents, and young adults. Overall, 33 of 83 patients (40%) experienced 1 or more infections in the first 28 days after CTI. Infection density more than doubled in the first 28 days post-CTI when compared with the 90 days pre-CTI and then decreased in the subsequent 29–90 days post-CTI. Pre-CTI HCT, lymphodepletion with a regimen other than cyclophosphamide plus fludarabine, and post-CTI hypogammaglobulinemia were associated with increased infection.

Although the overall incidence of any infection in the first month is similar to that found in adults with ALL following CTI [12], the infection density of 2.89 infections per 100 days-at-risk was higher in our cohort [11]. As in the adult studies, our patients experienced a predominance of bacterial infections, specifically bacteremias, especially within the first 2 weeks post-CTI. We observed a higher incidence of respiratory viral infections compared with findings in adult CTI recipients, which may be due to the younger age of our patients. Respiratory viral infections are common in pediatric immunocompromised populations [16, 17]. One patient (1%) was diagnosed with a proven invasive mold infection on day 21 post-CTI while receiving voriconazole for previously

diagnosed probable invasive mold infection. This individual had concomitant severe neutropenia and mild CRS treated with tocilizumab. It is unclear whether this infection represented new fungal disease or worsening of the previously diagnosed infection.

Our data suggest that prior HCT and hypogammaglobulinemia may be risk factors for infections in the first 28 days post-CTI. Interestingly, preexisting infection and prelymphodepletion lymphopenia and neutropenia did not significantly influence risk of infection. Lymphodepletion with the combination of cyclophosphamide and fludarabine was associated with a decreased incidence of infection in the short term. Although this combination regimen has been associated with improved cancer outcomes after CD19 CAR T-cell therapy in ALL [18], a relationship with decreased infection has not been previously demonstrated. In our cohort, those patients who received CAR T-cell therapy more recently were more likely to have received this combination lymphodepletion. These patients may also have been more likely to have received antibacterial prophylaxis or have received CAR T-cell therapy earlier in their ALL disease process. In addition, cyclophosphamide dosing of 3 g/m<sup>2</sup> may have caused more bone marrow suppression than the combination conditioning regimen with a lower cyclophosphamide dose (0.5 g/m<sup>2</sup>). Unlike previous studies, we did not demonstrate a link between higher CAR T-cell doses and infection. However, our dose range was narrower than those used in previous studies in adult patients.

Prior studies in adult CAR T-cell recipients with lymphoma, chronic lymphocytic leukemia (CLL), and ALL demonstrated that severe CRS (grade 3 or higher) was associated with increased infections [11, 12]. In our patients with ALL, we found a similar association, but this did not reach statistical significance. However, we had fewer patients with severe CRS than in other studies, which may have limited our ability to identify



**Figure 3.** Infection events in the first 28 days after CAR T-cell therapy stratified by CRS and the presence of neutropenia. A, Proportion of patients with infection by grade: 0 (no CRS): 30% (95% CI, 7%–65%); 1: 46% (95% CI, 28%–66%); 2: 32% (95% CI, 18%–50%); ≥3: 62% (95% CI, 24%–91%). Of note, the infection episode may have occurred before, during, or after CRS during this time period. B, The number of patients with each infection type stratified by the presence or absence of neutropenia (ANC < 500 cells/μL) at the time of infection. Abbreviations: CAR, chimeric antigen receptor; CI, confidence interval; CRS, cytokine release syndrome.

**Table 3. Pre-CAR T-cell Infusion Factors and Association With Infection Density Within 28 Days of Infusion**

Pre-CAR T Variables	Univariate Ratio of Infection Densities (95% CI)	P Value	Adjusted Ratio of Infection Densities (95% CI) <sup>a</sup>	P Value
Age	0.99 (0.96–1.03)	.66		
Sex (male)	0.93 (0.57–1.54)	.78		
Prior HCT	1.69 (1.01–2.92)	.05	1.54 (0.92–2.69)	.11
Pre-CAR T infections (day –90 to day 0)	0.83 (0.51–1.37)	.46		
Relapsed (vs refractory) ALL	1.6 (0.66–5.28)	.36		
Cyclophosphamide/fludarabine (vs other lymphodepletion)	0.5 (0.30–0.82)	.01	0.46 (0.27–0.77)	<.01
Prelymphodepletion parameters				
IgG < 400 mg/dL	1.44 (0.77–2.53)	.23	1.69 (0.88–3.06)	.09
ALC < 300 cells/mm <sup>3</sup>	1.05 (0.53–1.90)	.88		
ANC < 500 cells/mm <sup>3</sup>	1.13 (0.68–1.87)	.62		
CAR T dose (≥5 × 10 <sup>6</sup> cells/kg vs other)	1.02 (0.49–1.92)	.95		

Abbreviations: ALC, absolute lymphocyte count; ALL, acute lymphoblastic leukemia; ANC, absolute neutrophil count; CAR, chimeric antigen receptor; HCT, hematopoietic cell transplant; IgG, immunoglobulin G.

<sup>a</sup>Multivariate analysis includes variables with *P* < .25 in univariate analysis.

a clear link with infection. The low rates of CRS in this cohort may be related to the different CAR T-cell product and/or an early intervention strategy for CRS [19].

Neutropenia is well known to be associated with increased risk of bacterial infections [20]. In a study of adult CTI recipients, the median time to neutrophil recovery was 6 days, but prolonged cytopenias have been demonstrated previously, even in patients with MRD-negative remission after CAR T-cell therapy [3, 7, 11, 21]. In our study, almost half of the pediatric patients remained neutropenic at day 21, with persistent neutropenia through day 63 in 13% of patients. Given that 90% of bacterial infections in the first 28 days occurred during neutropenia [7], this has important implications for the longer-term management of patients with persistent cytopenias.

For patients in whom records were available for the 29–90-day period post-CTI, infection rates dropped to a rate even lower than in the pre-CTI period. This is despite the fact that sicker patients were less likely to return to their home centers after the first month and therefore were more likely to be included in our analysis. One infection-related death occurred on day 51 due to multiorgan failure attributed to bacterial sepsis. The infection density in the 29–90-day time period in this study was similar to that found beyond 90 days in adult CTI recipients [7].

Persistent B-cell aplasia is a known effect of CD19 CAR T-cell therapy and indeed can serve as a proxy of persistence of CAR T cells themselves [3]. We found that 29% of patients had IgG levels of <400 mg/dL by 63 days post-CTI, and severe

**Table 4. Risk Factors for Time to First Infection Within 28 Days of CAR-T-cell Infusion**

	Univariate Hazard Ratio (95% CI)	P Value	Multivariate Hazard Ratio (95% CI) <sup>b</sup>	P Value
Pre-CTI variables <sup>a</sup>				
Prior HCT	2.23 (1.06–4.70)	.04	2.15 (0.98–4.73)	.05
Lymphodepletion with cyclophosphamide plus fludarabine	0.57 (0.28–1.02)	.05	0.45 (0.21–0.96)	.04
Post-CTI variables				
CRS grade				
1–2 (n = 66)	0.57 (0.22–1.51)	.26	0.44 (0.16–1.23)	.11
3–5 (n = 8)	2.47 (0.74–8.32)	.14	1.81 (0.53–6.15)	.34
Tocilizumab use	1.15 (0.48–2.77)	.75		
Steroid use	0.55 (0.16–1.87)	.34		
ANC < 500 cells/μL <sup>c</sup>	1.14 (0.49–2.65)	.75		
ALC < 300 cells/μL <sup>c</sup>	0.92 (0.37–2.26)	.85		
IgG < 400 mg/dL	1.99 (0.99–4.42)	.09	2.41 (1.02–5.69)	.04

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; CTI, CAR-T-cell infusion; HCT, hematopoietic cell transplant; IgG, immunoglobulin G.

<sup>a</sup>All pre-CTI variables included in the Poisson model (Table 3) were included in this analysis, except for pre-CTI IgG, which showed significant collinearity with post-CTI IgG. Only variables meeting the *P* value criterion for inclusion in the multivariate model (see below) are shown.

<sup>b</sup>Multivariate model includes variables with *P* < .25 in the univariate model only.

<sup>c</sup>ANC and ALC data from the day of infection event were utilized when available; otherwise the most recent value was utilized. For each event, each value was considered as a dichotomous variable.

hypogammaglobulinemia was associated with increased infection risk. Persistent hypogammaglobulinemia is also a common feature after CTI in adults [6, 11, 22]. Further study of the duration and extent of these deficits could influence infection prevention strategies such as vaccination and more standardized administration of IVIG supplementation.

Standardization of antimicrobial prophylaxis in patients receiving CTI may help prevent infections. Specifically, we identified 2 patients who developed herpes zoster in the first month after CTI, both of whom had not received antiviral prophylaxis. We have now instituted a policy of antiviral prophylaxis with acyclovir for all patients starting at lymphodepletion and continuing for at least 100 days. We are also instituting standard approaches to antibiotic prophylaxis during neutropenia in patients who are expected to have a prolonged duration (>7 days) of ANC  $\leq 200/\text{mm}^3$ . Ensuring that prophylaxis covers previously identified infections is an important consideration. Indeed, 3 of the 11 patients with bacteremia in the 0–28-day post-CTI period had preceding bacteremia with the same organism. Although there was only 1 episode of invasive fungal disease, our standard approach to antifungal prophylaxis has also evolved. Fluconazole is given to all patients during neutropenia; for patients with a history of mold infection, we consider starting or continuing a mold-active azole. The optimal type and duration of prophylactic regimens need further study [21].

This study has limitations given the retrospective design and use of data from a single center. Analyses of infection events beyond 28 days after CTI included fewer patients due to loss to follow-up after patients returned to their primary oncologists. This may have resulted in biasing analyses during this time period toward sicker patients. Our patients received variable antimicrobial prophylaxis regimens, and our analysis was not powered to perform risk factor analysis for each type of infection individually; risks may vary for viral, bacterial, and fungal infections. It is possible that patients with lymphopenia and neutropenia were more likely to receive antimicrobial prophylaxis, which may have altered our ability to detect an association between these factors and infection outcomes.

Assessing causality for the infections in the early period after CTI is difficult. Many of the infections may not be directly attributable to the product itself given the underlying risk factors, the conditioning lymphodepleting chemotherapy, and the development and treatment of CRS. In fact, the infection characteristics in adult patients were found to be similar to ALL patients receiving salvage chemotherapy [11]. In addition, these data may not be generalizable to CAR T-cell treatments for other hematologic or solid malignancies. Finally, the use of different CAR T-cell constructs or concurrent antitumor therapies could likely affect infection risk as well.

Increasing use of CAR T-cell therapy for the treatment of malignancies is leading to improvements in survival for high-risk children with ALL, while also posing risks for

infections, particularly during the early period after CTI. Our results suggest that viral, bacterial, and fungal prophylaxis and lymphodepletion regimens need to be carefully considered in the setting of CAR T-cell therapy. Patients with previous HCT, hypogammaglobulinemia, and severe CRS may be at highest risk. Further study of patients with standardized prophylactic regimens and long-term follow-up will be useful for optimizing treatment guidelines for these high-risk children.

### Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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