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blood advances

Diagnostic biomarkers to differentiate sepsis from cytokine release syndrome in critically ill children

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Key Points

- Cytokine profiling can distinguish cytokine release syndrome after chimeric antigen receptor T-cell (CAR-T) therapy from sepsis.
- Cytokine profiling establishes the immune response that occurs after CAR-T is distinct from the immune response with infection.

Chimeric antigen receptor (CAR) T-cells directed against CD19 have drastically altered outcomes for children with relapsed and refractory acute lymphoblastic leukemia (r/r ALL). Pediatric patients with r/r ALL treated with CAR-T are at increased risk of both cytokine release syndrome (CRS) and sepsis. We sought to investigate the biologic differences between CRS and sepsis and to develop predictive models which could accurately differentiate CRS from sepsis at the time of critical illness. We identified 23 different cytokines that were significantly different between patients with sepsis and CRS. Using elastic net prediction modeling and tree classification, we identified cytokines that were able to classify subjects as having CRS or sepsis accurately. A markedly elevated interferon γ (IFNγ) or a mildly elevated IFNγ in combination with a low IL1β were associated with CRS. A normal to mildly elevated IFNγ in combination with an elevated IL1β was associated with sepsis. This combination of IFNγ and IL1β was able to categorize subjects as having CRS or sepsis with 97% accuracy. As CAR-T therapies become more common, these data provide important novel information to better manage potential associated toxicities.

Introduction

Chimeric antigen receptor (CAR)–modified T cells with activity against $CD19^+$ B-cells have transformed outcomes for patients with relapsed and refractory (r/r) B-precursor acute lymphoblastic leukemia (B-ALL).[1] Historically, children with r/r B-ALL had poor survival.[2] A phase II, international, multicenter study of the CAR-T cell therapy tisagenlecleucel demonstrated a relapse-free survival of 66% at 18 months in pediatric patients with r/r B-ALL, similar to previous trials of tisagenlecleucel.[3-5] However, CAR-T cell treatment is associated with significant adverse events, predominantly cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS).[1,6] CRS is a systemic inflammatory response syndrome in which patients present with fever and signs of systemic inflammation. Although many cases of CRS are mild, children with CRS may develop shock with evolution to multiple organ dysfunction syndrome (MODS).[7]

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The full-text version of this article contains a data supplement.

In addition to CRS and ICANS, children treated with CAR-T cells are at risk for sepsis. Risk factors associated with sepsis in these patients include previous cytotoxic chemotherapy or hematopoietic stem cell transplant, recent lymphodepleting chemotherapy, neutropenia from leukemic infiltration of bone marrow, and the presence of indwelling central venous catheters. Since the clinical presentation and course of CRS and sepsis have significant overlap, most critically ill patients with suspected CRS are also treated empirically for sepsis. However, the pathophysiology and definitive treatments of these conditions are distinct. CRS is caused by the reciprocal activation of T cells and antigen presenting cells (such as macrophages) leading to hypercytokinemia.[8,9] CRS therapy is focused on modifying hypercytokinemia with cytokine blockade, particularly IL6 blockade, and suppressing the continued activation of T cells and macrophages if needed with corticosteroids.[7,10-12] CRS and CRS-like syndromes can be caused by immunotherapies such as CAR-T, and similar syndromes can also be caused by infectious syndromes, notably COVID-19.[1,13] In contrast, sepsis is caused by a dysregulated host response to infection, and therapy is focused on treating the underlying infection with broad-spectrum antibiotics.[14,15] Cytokine blockade has not been shown to be beneficial for most patients with sepsis.[16] The overlap of clinical and laboratory findings between CRS and sepsis currently precludes the ability to differentiate between these 2 life-threatening conditions at onset.

We and others have demonstrated a predictive pattern of cytokine expression in children and young adults treated with CAR-T cell therapy who go on to develop severe CRS compared with those with no, mild, or moderate CRS.[17,18] In this study, we sought to characterize differences in cytokines, chemokines, soluble receptors, and endothelial biomarkers between children with severe CRS and severe sepsis/septic shock. Our aims were to identify a parsimonious group of cytokines and biomarkers that reliably distinguish between CRS and sepsis at the time of pediatric intensive care unit (PICU) admission and to use our findings to generate novel hypotheses regarding differences in potential immune pathogenesis between CRS and sepsis. Based on aberrant macrophage activation in CRS, we hypothesized that cytokines associated with macrophage/monocyte activation, including IFNγ, sCD163, IL10, IL6, IP10 (CXCL10), MIG (CXCL9), MIP1α, MIP1β, and sCD30 would reliably distinguish CRS from sepsis at PICU admission in critically ill patients.

Methods

Study design and population

We prospectively collected cytokines, chemokines, soluble receptors, and endothelial biomarkers (hereafter referred to as cytokines) on patients at the Children's Hospital of Philadelphia (CHOP) under the auspices of 2 parent studies: a clinical trial of subjects <25 years old with B-ALL treated with CTL019 (tisagenlecleucel; NCT01626495) and an observational cohort of patients ≤18 years old admitted to the PICU for sepsis. Samples were collected between April 2012 and July 2015 for the CTL019 trial and between May 2014 and December 2016 for the sepsis study. Patients treated with CAR-T cell therapy were included in this analysis if they were admitted to the PICU for treatment of grade 3 or higher CRS per the rating scale devised by Porter et al[19] (Penn scale; supplemental Appendix 1) and had complete cytokine data. Severe CRS included patients with grade 4 CRS and some patients with grade 3 CRS. Grade 3 CRS is defined as severe when a patient required intensive care unit (ICU) admission and had hypotension requiring fluid boluses or low-dose vasoactive medications, coagulopathy requiring cryoprecipitate, or plasma transfusion, and/or hypoxemia requiring high-flow oxygen therapy or noninvasive mechanical ventilation. Grade 4 CRS is defined as life-threatening and is defined as a patient with hypotension requiring high-dose vasoactive medications and/or hypoxemia requiring invasive mechanical ventilation.[18-20] Grade 5 CRS (death) was not observed. Patients with sepsis were included if they met criteria for severe sepsis or septic shock as defined by the International Pediatric Sepsis Consensus Conferences,[15] had been enrolled in the previously mentioned sepsis study, and if blood was collected and stored. Please see supplemental Appendices 2 and 3 for detailed inclusion and exclusion criteria of both parent studies.

All patients with CRS and sepsis were enrolled in their primary studies with protocols approved by the CHOP Institutional Review Board, and after written informed consent was obtained from a legally authorized representative as per the Declaration of Helsinki. Only patients for whom specific informed consent had been obtained to use residual samples in future studies were included in the analysis of this study.

Data collection

For both CRS and sepsis patients, clinical data were abstracted from the medical record onto standardized case report forms. Data collected for CRS and sepsis patients were compared to identify shared clinical variables available for all patients, including demographics, comorbid conditions, sources of infection, use of vasoactive infusions, mechanical ventilation, treatment with corticosteroids, and laboratory indicators of organ dysfunction.

Blood collection and cytokine assays

For CRS patients, blood was collected within 1 day prior to CTL019 infusion and serially after infusion. The blood was processed as previously described, aliquoted, and frozen at −80°C for batched analysis.[17] The first available blood sample collected within 3 days of PICU admission was used for the primary analysis. For sepsis patients, blood was collected as soon as possible after PICU admission, but no later than 48 hours after sepsis recognition. Details of the analysis of samples have been published previously.[21] Forty-six cytokines were analyzed including ANG2, CD163, EGF, Eotaxin, FGF-Basic, GCSF, GM-CSF, HGF, ICAM1, IFNγ, IFNα, IL10, IL12, IL13, IL15, IL17, IL1β, IL1RA, IL2, IL4, IL5, IL6, IL7, IL8, IP10 (CXCL10), MCP1, MIG (CXCL9), MIP1α, MIP1β, RANTES, sCD30, sEGFR, sgp130, sIL_1RI, sIL1RII, sIL2Ra, sIL4R, sIL6R, sRAGE, sTNFRI, sTNFRII, sVEGFR1, sVEGFR2, sVEGFR3, TNFα, and VEGF.

Statistical analysis

Clinical characteristics at PICU admission were summarized using descriptive statistics. The analysis cohort was determined as the number of individuals for whom the complete panel of cytokine levels were available. For CRS patients, the window of blood collection of up to 3 days was allowed, with a median of 1 day and interquartile range [IQR] of 0 to 1 days. Cytokine levels were compared between the sepsis and CRS cohorts using an exact Wilcoxon rank-sum test. Cytokine levels within 3 days of PICU

admission were also compared with the preinfusion baseline value for the CRS cohort using the exact Wilcoxon signed-rank test. Statistical significance was determined using the Holm multiple comparison correction.[22]

Cytokine relationships were assessed with the Spearman rank correlation coefficient. Multivariable predictive modeling using 46 cytokines was employed to classify subjects as a member of the CRS cohort vs the sepsis cohort (yes/no). To prevent overfitting, the modeling was done using a regularized regression technique called elastic net, which uses a tuning parameter fit with cross validation to control the dimension of the predictors by controlling the overall size of the regression coefficients.[23] Elastic net tends to include or exclude correlated clusters of predictors. The predictive accuracy is assessed by successively fitting the model leaving 1 person out, and the percent of individuals who were correctly classified by the model fit without their data are the leave one out crossvalidation (LOOCV) predictive accuracy statistic. A sparse classification tree model was fit with the tree package in R using the default deviance split method.

The relative importance of the cytokines for prediction was also assessed by the feature importance, which first ranks the cytokines according to how much predictive accuracy would be lost by the elastic net procedure if the values of that variable were permuted; the incremental gains in accuracy for the model are then calculated as the variables are added 1 at a time according to this ranking (supplemental Appendix 4).[24] Modeling procedures were repeated by adding 10 clinical variables: baseline age (years), sex, race (White/other), and variables during ICU stay: vasoactive agent (yes/no), invasive mechanical ventilation (yes/no), corticosteroids use (yes/no), maximum measured lactate, heme dysfunction (yes/no), hepatic dysfunction, acute kidney injury (yes/no). Feature importance was reassessed with the larger set of potential predicters.

Analysis was performed using R 3.1.0 1 (R Development Core Team, Vienna, Austria), Scikit-learn Python library, and SAS 9.4 (Carey, NC).

Results

Of 54 patients treated with CAR-T cell therapy, 22 developed moderate/severe (grade 3/4) CRS and 26 patients developed mild grade 1/2 CRS. No patients had grade 5 CRS (death). Of the 22 with grade 3/4 CRS, 19 were admitted to the ICU. Sixteen had complete cytokine data and were included for analysis in this study (supplemental Figure 1A). A total of 108 patients were enrolled in the primary sepsis study, with 80 eligible for analysis in this study (supplemental Figure 1B).

The demographic characteristics of patients with severe CRS and sepsis are shown in Table 1. The CRS and sepsis populations differed in several ways. No patients in the sepsis population received tocilizumab. The CRS population was older than the sepsis population (median age, 14.5 years vs 6.7 years, respectively) and was more likely to be White (88% vs 49%, respectively. Two patients in the CRS population had documented infections, 1 patient with *Streptococcus mitis* bacteremia and 1 patient with a *Pseudomonas aeruginosa* tracheal aspirate specimen. The patient with *P aeruginosa* had colonization and this was thus felt to be a contaminant. The patient with *S mitis* had bacteremia occur and resolve before the development of CRS. The patient with *S mitis* developed fever without sepsis. Per institutional guidelines, all patients with CRS received antibiotics. No patients in the sepsis population had B-ALL; 9 patients had malignancies.

Table 1. Characteristics of included subjects in the sepsis and CRS groups

	CRS subjects (N = 16)	Sepsis subjects (N = 80)
Age*, median (IQR), y	14.5 (9.6-17.1)	6.7 (2.9-13.5)
Sex, n (%)		
Female	7 (44)	37 (46)
Male	9 (56)	43 (54)
Race, n (%)		
White	14 (88)	39 (49)
Black/African American	1 (6)	20 (25)
Asian	1 (6)	3 (4)
Indian	0	2 (3)
Other	0	15 (19)
Unknown	0	1 (1)
Any vasoactive agent, n (%)	12 (75)	63 (79)
Any invasive mechanical ventilation, n (%)	2 (13)	53 (66)
Any corticosteroids (sepsis) or tocilizumab or corticosteroids (CRS), n (%)	12 (75)	39 (49)
WBC count, median (IQR), $\times 10^9$/L	0.25 (0.1, 0.5)	9.60 (6.1, 16.3) (n = 72)
ALC, median (IQR), $\times 10^6$/L	37.5 (0, 157)	1262 (711, 2588) (n = 72)
Highest lactate, median (IQR), mmol/L	2.1 (1.4-3.4)	2.30 (1.5-3.7)
Hematologic dysfunction,† n (%)	5 (31)	21 (26)
Hepatic dysfunction, n (%)	0 (0)	16 (20)
Acute kidney injury, n (%)	3 (19)	7 (9)
Creatinine, median (IQR), mg/dL	0.7 (0.5-1.1)	0.4 (0.3-0.65)
Malignancy, n (%)	16 (100)	9 (12) (n = 12)
Infection, n (%)		
Bacterial	2‡ (100)	26 (32)
Bacterial‡ other§		10 (12)
Viral		19 (24)
None identified		25 (31)
UPENN CRS (1st) severity score/grade, n (%)		
3	5 (31)	
4	11 (69)	
5	0 (0)	
ASTCT CRS severity score/grade: n (%)		
2	2 (12)	
3	3 (19)	
4	11 (69)	

*At infusion (CRS subjects) or ICU admission (sepsis subjects).
†Defined as total bilirubin ≥4 mg/dL.
‡One patient with *S mitis* bacteremia, 1 patient with a *P aeruginosa* tracheal aspirate.
§Bacterial infections combined with other type of infection: 8 with viral, 1 parasitic, and 1 fungal.

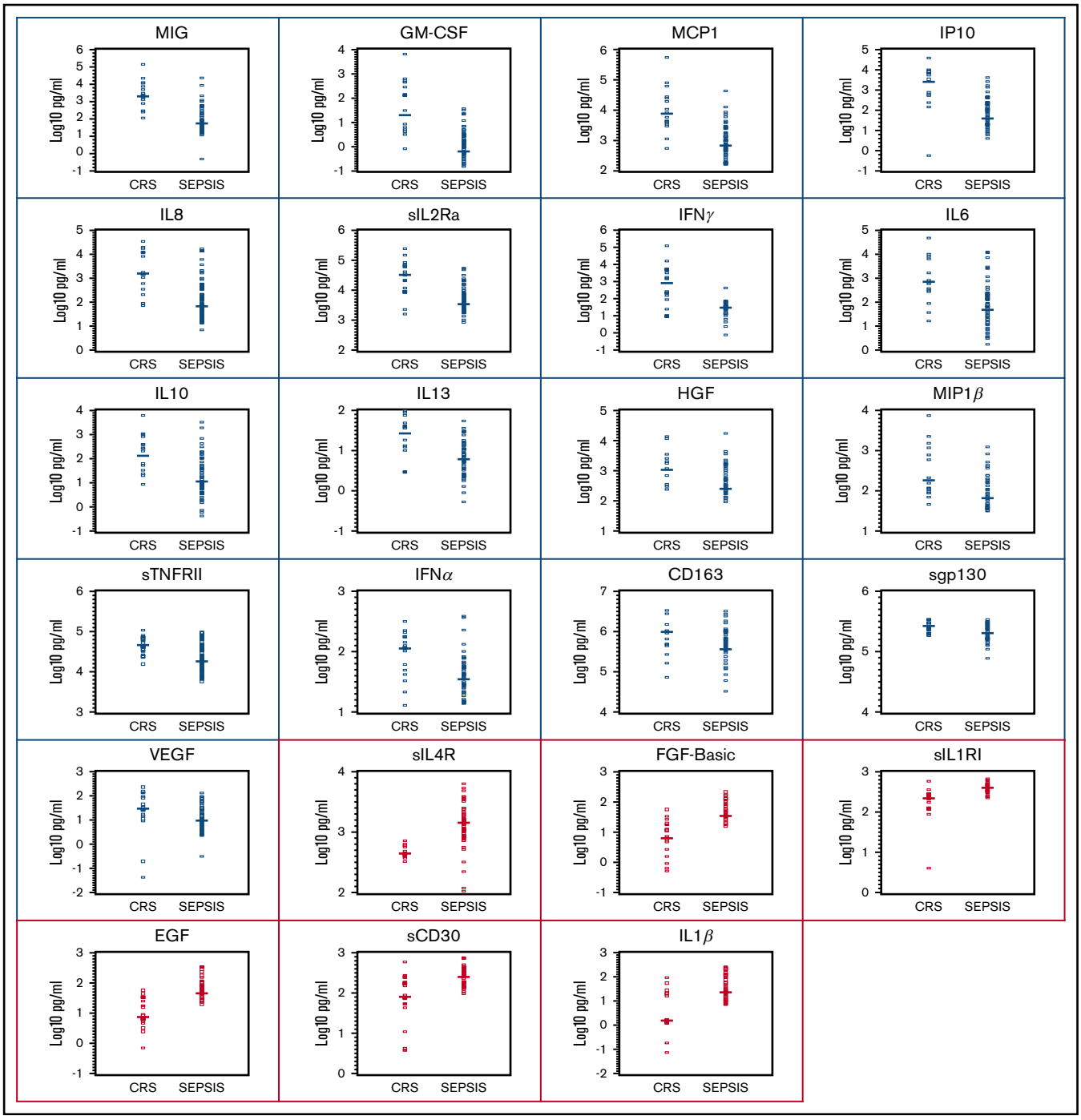

Figure 1. Cytokines with significant difference between CRS (N = 16) and sepsis (N = 80) at ICU admission. Cytokines that were significantly higher in CRS are shown first in black, followed by cytokines that were significantly higher in sepsis in red; graphs are ordered with respect to decreasing significance within each group. Group medians are noted with horizontal bars. Wilcoxon rank-sum test with Holm correction was used for multiple comparisons.

Twenty-three cytokines differed significantly between the CRS and sepsis cohorts (Figure 1). Patients with CRS had higher levels of markers associated with macrophage activation, such as MIG, GM-CSF, and MCP1, as compared with patients with sepsis. Conversely, patients with sepsis had highly elevated levels of the IL4 and IL13 receptor, sIL4R, and of the angiogenic growth factor, FGF-Basic, as compared with CRS patients. Table 2 briefly describes the biology associated with cytokines that differ between sepsis and CRS, those that are elevated in both, and those that are not elevated in either condition (23 cytokines; supplemental Figure 2).[25] A summary of the median and IQR for cytokines ranked by multiple-comparison–corrected *P* values for patients in the CRS and sepsis groups is available in supplemental Table 1. Four patients in the CRS group had cytokines drawn after

Table 2. Summary of the biological function of biomarkers that were significantly elevated in severe cytokine release syndrome, in sepsis, in both or in neither

Name	Cells that secrete; function	Name	Cells that secrete; function
Cytokines significantly more elevated in severe CRS as compared with sepsis			
IFNγ	T cells, NK cells; activates macrophages, induces MHC II expression	GM-CSF	Macrophages, T-cells, NK cells; promotes macrophage and eosinophil proliferation
IL6	T cells, B cells, monocytes; stimulates acute phase reactants	IL8	Macrophages, epithelial cells, endothelial cells; induces chemotaxis
IP10 (CXCL10)	Monocytes, endothelial cells, fibroblasts; Secreted in response to IFNγ	MCP1	Monocytes, macrophages; chemoattractant
sIL2Ra	Activated T and B cells; associated with mononuclear cell activation	MIG (CXCL9)	T-cells, NK cells, macrophages, NKT cells; chemotactic, induces differentiation of leukocytes, cell extravasation
IL10	Monocytes, T cells; anti-inflammatory cytokine	IL13	T-cells, mast cells, NKT cells; induce macrophage activation
HGF	Mesenchymal cells; growth and motility factor	MIP1β	Macrophages, monocytes; induce release of IL1, IL6, TNFα
sTNFRII	T cells, endothelial cells; pro-inflammatory cytokine	sCD163	Monocytes, macrophages; marker of macrophage activation
IFNα	NK-, NKT-, T-cells; activates macrophages	VEGF	Many cells; endothelial permeability mediator
sgp130	Present in sera; inhibits soluble IL6		
Cytokines significantly more elevated in sepsis as compared with severe CRS			
FGF-Basic	Basement membrane and blood vessels; growth factor involved in angiogenesis	EGF	Epithelial cells; stimulates cell growth and differentiation
sIL1RI	Activated mononuclear cells, neutrophils; involved in IL2 and IL6 production	sIL4R	T-cells, basophils, eosinophils; TH2 lymphocyte differentiation, blocks binding of IL4
sCD30	Activated T-cells and TH-cells; TH2 immune response, elevated in some malignancies	IL1β	Activated macrophages; pyrogenic, inflammatory response
Cytokines similarly elevated in sepsis and severe CRS			
IL2	T-cells; promotes differentiation	ICAM1	Endothelial cells, macrophages, lymphocytes; induced by IL1, TNFα, facilitates transmigration
sTNFRI	Multiple cell types; induces apoptosis in monocytes, antagonizes circulating TNFα	ANG2	Endothelial and smooth muscle cells; increased by TNFα and VEGF
IL15	Mononuclear cells; proliferation of NK cells	sVEGFR1	Multiple cell types; angiogenesis inhibitor
MIP1α	Macrophages; recruitment and activation of granulocytes	sVEGFR3	Multiple cell types; lymphangiogenesis inhibitor
sRAGE	Multiple cells; innate immune system activation	IL1Ra	Multiple cell types; mediates IL1 activation
Cytokines not elevated in sepsis or severe CRS			
IL17	TH17 cells; promotes inflammation	sVEGFR2	Multiple cell types; lymphangiogenesis inhibitor
Eotaxin	Endothelial cells; chemotaxis for eosinophils	IL12	Dendritic cells, macrophages; induces IFNγ
GCSF	Endothelial cells, macrophages; production of neutrophils and hematopoietic stem cells	IL5	TH2 cells, mast cells; stimulates IgA secretion
IL4	Basophils; differentiates TH cells to TH2 cells	RANTES	Platelets, fibroblasts; chemokine for T-cells, eosinophils, induces NK cells
IL7	Marrow stromal cells; stimulates production of lymphoid progenitors	sIL6R	Activated T-cells; buffer to block IL6 signaling
TNFα	Macrophages, monocytes; acute phase reactant	sIL1RII	Monocytes, neutrophils, macrophages, T-cells; decoy receptor, regulates IL1RI
sEGFR	Multiple cells; growth factor		

MHC, major histocompatibility complex; NK, natural killer; TH, T-helper.

tocilizumab administration. We performed the same analysis with those 4 patients removed, and this did not impact the overall results or conclusions (supplemental Table 2). Generally, the same cytokines remained elevated in the CRS cohort. All the cytokines that were more highly elevated in sepsis as compared with CRS remained more highly elevated. Some cytokines (such as VEGF) continued to be more highly elevated in CRS than in sepsis, but without statistical significance with Holm adjustment for multiple comparisons, likely because of the decreased sample size. Although the primary analysis compared cytokines from patients who developed severe CRS vs sepsis shortly after PICU admission, we also analyzed these cytokines kinetically at multiple time points over 1 month for patients who developed mild/moderate or severe CRS and sepsis and in normal healthy subjects (supplemental Tables 3 and 4; supplemental Figure 3). These figures demonstrate that not all cytokine levels remained static over time. For example, both IL1β and sIL4R were found to be higher in sepsis than in CRS; in some patients with CRS, IL1β increased over time to levels similar to those seen in sepsis, whereas sIL4R levels did not change. At the time of the cytokines drawn for the primary analysis, no patients had ICANS. We previously

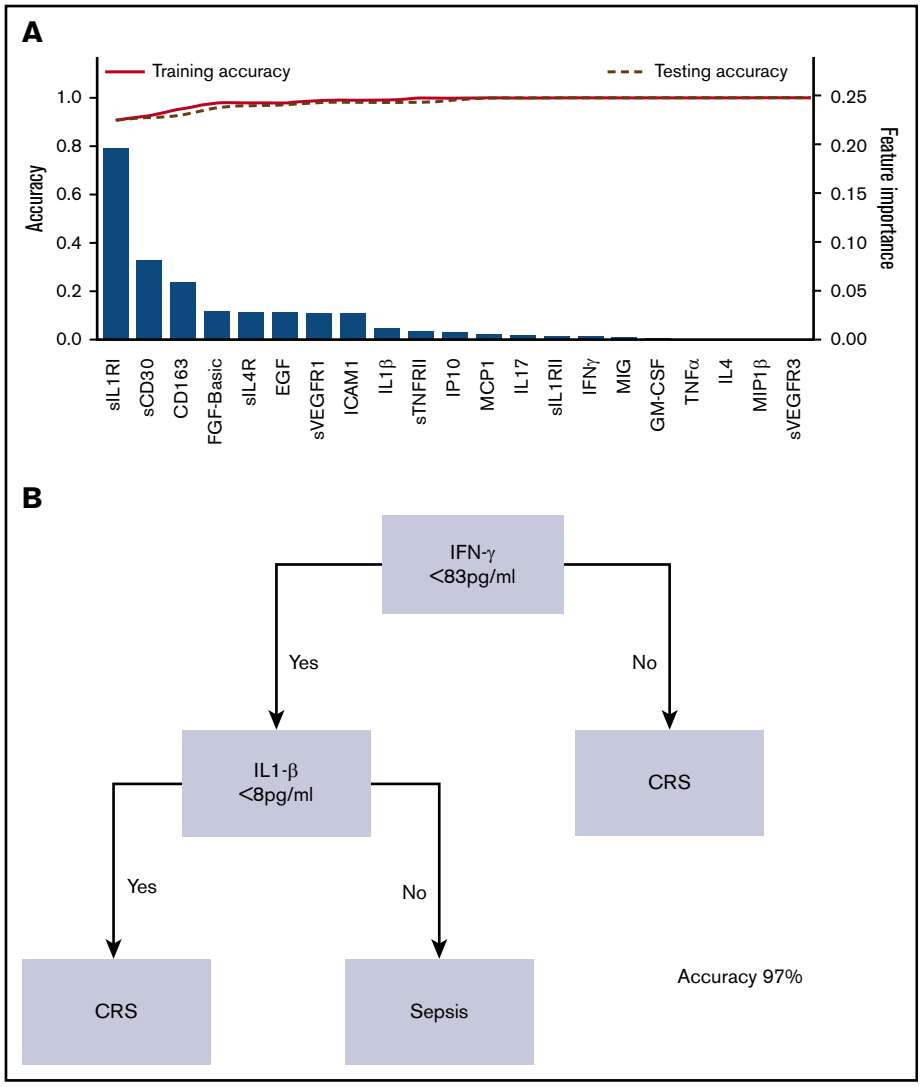

Figure 2. Training and testing accuracy and classification tree modeling of cytokines. (A) Feature importance for discrimination between sepsis (N = 80) and CRS (N = 16) cohorts. Cytokines are listed in terms of decreasing feature importance (right axis), and the performance of the model is shown (training and testing accuracy) when predictors are added to the model 1 at a time (left axis). (B) Classification tree model for discrimination between sepsis (N = 80) and CRS (N = 16) cohorts.

published the ICANS experience with these patients; the patients who developed ICANS did so after CRS.[26]

Elastic net prediction identified 24 cytokines that discriminated between CRS and sepsis (CD163, EGF, FGF-Basic, GM-CSF, HGF, IFNγ, IL13, IL17, IL1β, IL5, IL6, IL8, IP10, MCP1, MIG, MIP1α, MIP1β, sCD30, sIL1RI, sIL1RII, sIL2Ra, sIL4R, sIL6R, sVEGFR1) and perfectly classified the two cohorts in the training data. There was a 2% LOOCV error that corresponded to misclassifying 1 CRS and 1 sepsis subject when models were developed without those subjects.

As another picture of the relative importance of these cytokines, they were added to the model 1 at a time according to their feature importance ranking. Using this approach, we find that 90% of the accuracy is contributed by sIL1RI, and that the accuracy approached 100% with the addition of sCD30, sCD163, and FGF-Basic (Figure 2A). Because of the high degree of correlation between cytokines, high accuracy could be achieved with other models.

Supplemental Figure 4 shows a similar graph for the model fit using both cytokine and clinical variables. With the expanded predictors, the top 3 predictors (sIL1RI, sCD30, CD163) with respect to feature importance remained the same as in Figure 2A, with the 3-variable model having over 90% accuracy. When using classification trees, IFNγ and IL1β were selected for a sparse classification model such that patients with IFNγ $>$ 83 pg/mL or IFNγ $<$ 83 pg/mL and IL1β $<$ 8 pg/mL, were classified as CRS or sepsis (Figure 2B). This model had 97% accuracy, misclassifying 3 sepsis, but no CRS, patients.

As previously mentioned, there was a high degree of correlation between cytokines. Correlation heatmaps illustrating clustering of cytokines in CRS and sepsis are presented in supplemental Figure 5A and supplemental Figure 5B, respectively. Accordingly, multiple different cytokines may similarly distinguish between these different disease biologies. Nonoverlapping redundancy in clustering between the 2 conditions may allow for different cytokines to be used interchangeably to distinguish between CRS and sepsis.

Finally, white blood cell (WBC) count and absolute lymphocyte count (ALC) numbers at time of ICU admission are presented in supplemental Figure 6. As expected, patients in the CRS group tended to have lower WBC and ALC numbers. Of note, most cytokines were higher in the CRS group than in the sepsis group,

implying that a robust cytokine response is produced even in the setting of significant leukopenia and lymphopenia.

Discussion

We have demonstrated the cytokine profile in patients with sepsis differs consistently from those with CRS. We have identified key cytokines that can potentially be used to discriminate sepsis from CRS. We confirmed that cytokines associated with macrophage activation are more highly associated with CRS. In sepsis, we found elevations in endothelial biomarkers and proinflammatory cytokines made by numerous cell types. We have also shown that there are groups of correlated cytokines that are elevated in sepsis or CRS, but not the other condition. Importantly, the existence of significant biomarker clustering will allow for flexibility in development of clinical biomarker testing, because several cytokines may be used interchangeably. This will aid the development of rapid diagnostics to distinguish CRS from sepsis in critically ill children with cancer, thereby optimizing the management of these patients, while reducing potential harm.

Cytokines that were elevated in CRS, but not in sepsis, were primarily macrophage activation associated; these included IFNγ, CD163, IP10 (CXCL10), MCP1, GM-CSF, and others. This finding is congruent with previous observations made by our group and others about the fundamental role of macrophages in CRS following CAR-T cell therapy.[8,17,27] Severe CRS in some patients closely resembles hemophagocytic lymphohistiocytosis, with patients developing high fevers, hyperferritinemia, organomegaly, coagulopathy, and other associated stigmata of hemophagocytic lymphohistiocytosis.[7] In sepsis patients, however, it was the proinflammatory cytokines, including those associated with endothelial damage, that were most highly elevated. This is consistent with previous findings observed in patients with sepsis.[28-30]

We previously demonstrated that IFNγ, IL6, and sIL2Ra show a marked differential increase in patients with severe CRS compared with patients without severe CRS.[17] In the current analysis, we demonstrate that IFNγ, when used in combination with IL1β, can distinguish patients with severe CRS from those with sepsis with 97% accuracy. IFNγ is a powerful activator of macrophages.[31] Interestingly, IL1β, which is produced by activated macrophages, was higher in the sepsis cohort than in the CRS cohort at PICU admission. The discrepancy between IFNγ and IL1β levels between the cohorts implies a biological difference in macrophage activation in sepsis vs CRS, although the exact immune pathogenesis remains to be clarified. IL1β is an inflammasome-associated cytokine and may be secreted only in response to specific pathogens.[32] Importantly, IL1β levels are significantly lower in CRS than in sepsis at the time of PICU admission, but later in the course of CRS, the levels of IL1β in some patients rise to that of patients with sepsis. Cytokine profiles are dynamic in nature, and their interpretation changes with the course of an illness.

IL6 and sIL2Ra were also significantly different between patients with CRS and sepsis. IL6 blockade with tocilizumab has been established as the standard of care for the initial treatment of severe CRS in patients who have received CAR-T therapy.[6] There was a significant difference between median IL6 values in sepsis and CRS; however, high IL6 levels were also found in patients with sepsis. The utility of IL6 as an individual biomarker of CRS is, therefore, limited.

Cytokine profiling has the potential to guide clinical decision making in patients with critical illness. General markers, such as ferritin, D-dimer, and C-reactive protein may be elevated in patients with inflammatory states because of any cause, such as infection or underlying inflammatory disorders. Cytokine profiling may have particular utility in guiding inflammatory states that are caused by a dysregulated response to an infection, where immunomodulation must be used judiciously to temper the inflammatory response without impairing endogenous ability to fight the infection. This has been particularly important during the COVID-19 pandemic. In pediatric and young adult patients who decompensate rapidly, the use of cytokine profiling to identify patients with COVID-19–associated cytokine storm may be crucial in guiding therapy. Future work should examine the role of the modeling strategy described in this manuscript in other infectious and inflammatory conditions, including COVID-19. We have demonstrated that cytokine profiles can help differentiate between different disease phenotypes in patients with severe acute respiratory syndrome coronavirus 2 infection (SARS-CoV-2).[33] Whether or not cytokine blockade will be of benefit in patients with COVID-19–associated cytokine storm will need to be validated in prospective clinical trials.

One important limitation of our analysis is that there were no children with ALL in the sepsis cohort that was examined; however, there were 9 children with other malignancies included. We have previously demonstrated that the only cytokines that are significantly different at baseline between patients with and without ALL are sIL2Ra and MCP1.[17] MCP1 and sIL2Ra were both significantly more elevated in CRS than in sepsis. Notably, the cytokines selected according to feature importance and by the classification tree model did not use either of these biomarkers to differentiate between sepsis and CRS. As such, MCP1 and sIL2Ra should be interpreted with caution in children with ALL who are being evaluated for sepsis vs CRS. Although we would not anticipate a major difference, it is not known if patients with B-ALL who present with sepsis have a different baseline cytokine panel than those patients with B-ALL without sepsis or those patients with sepsis without B-ALL. Future work should directly compare the cytokine panels of patients with B-ALL and sepsis with those with CRS. We would anticipate that host factors such as B-ALL or other malignancy might lead to subtle difference in cytokine values either in sepsis or CRS, but not the marked differences seen in these cohorts.

Another important limitation is that there was no independent validation data and relatively modest sample sizes in both cohorts. To mitigate the risk of overfitting with small sample sizes, we used elastic net and LOOCV. However, further research should involve prospective validation of this panel in patients with CRS and sepsis, particularly in patients with B-ALL. We used a single time point (admission to PICU) to develop our model. As demonstrated in supplemental Figures 3A-L, cytokine levels are highly dynamic over time. We chose admission to PICU as the time point to include in the algorithm as this is the most clinically relevant time point; however, cytokine levels should be interpreted with caution during other time points.

A further limitation was the differences between age and race in the sepsis and CRS groups. Subjects in the CRS group tended to be older and were more likely to be White than those in the sepsis group. We would not expect large differences in the cytokine levels,

as seen between the groups reported in this study, on the basis of age or race alone. Future studies should include larger groups of children to better understand differences that may occur in cytokine response on the basis of age or race.

We present data from a single center experience using CTL019. Currently, the majority of experience in CRS has been drawn from T-cell effectors targeting CD19, CD20, and CD22, and the clinical response and cytokine profiling has been consistent so far.[6,17,34] We note that preliminary studies using B-cell maturation antigen–directed CAR also demonstrate a similar pattern of hypercytokinemia.[35] However, different CAR-T products may evoke a different cytokine response. We anticipate that the cytokine response seen with CTL019 would be similar to other CAR-T targeting CD19. However, the algorithm we present has only been tested in the setting of CTL019 and should be applied with caution to other CAR-T products. Furthermore, as new targets are identified and targeted, and different immune effector cells are used, the cytokine profiles remain to be determined, and will need to be evaluated in future studies. Of particular importance when applying these results to different CAR-T is the development of ICANS. We previously demonstrated that, with CTL019, ICANS occurs after CRS.[26] However, different CAR-T products may be associated with earlier onset of ICANS. ICANS has been shown to be associated with dysregulation of endothelial biomarkers, particularly ANG2.[36] In the setting of concomitant ICANS, ANG2 and other endothelial biomarkers may be less helpful in distinguishing CRS from sepsis.

We present a robust, clinically significant model that differentiates between the overlapping clinical entities of sepsis and CRS. We have characterized cytokines that are associated with sepsis, CRS, or both, and those that can differentiate between the 2 in pediatric patients. Validating these models in an independent cohort is important future work. As CAR-T therapies become more common, these data may lead to important discoveries to better manage potential associated toxicities.

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Authorship

Contribution: C.D., P.A.S., J.J.M., S.F.L., S.L.W., and D.T.T. designed the study, analyzed data and wrote the manuscript; C.H.J., D.M.B., and S.A.G. designed the study and analyzed data; E.P. and A.O. analyzed data and wrote the manuscript; and F.C., R.A., H.B., E.B., A.M.D., V.G., N.K., B.L.L., S.L.M., N.J.M., J.H.M., M.P., D.L.P., J.L.B., D.L.S., M.M.D., and D.Z. contributed to the study design, analyzed data, and edited the manuscript.

Conflict-of-interest disclosure: S.L.M. is a consultant for Novartis Pharmaceuticals and Kite Pharma and reports receiving commercial research support from Novartis Pharmaceuticals. S.A.G. reports personal fees and grants from Novartis and CRISPR/Vertex; personal fees from Allogene, Cellular Biomedicine Group (CBMG), Adaptimmune, TCR2 Therapeutics, Juno, Jazz, Eureka, Cellectis, La Roche, GlaxoSmithKline, Cure Genetics, Humanigen, Janssen, and Johnson & Johnson; and grants from Kite and Servier. C.H.J. has ownership interest (including patents) in Tmunity and Novartis. D.L.P., B.L.L., S.F.L., and J.J.M. hold patent applications (US 20180258149, US 20180140602, US 20150283178) related to CAR T cell therapy. S.F.L. and J.J.M. receive research funding from Novartis. S.F.L. also receives research funding from Tmunity Therapeutics. B.L.L. and C.H.J. are cofounders and equity holders in Tmunity Therapeutics. B.L.L. is a consultant for Novartis as well as CRC Oncology and is a member of the scientific advisory boards of Avectas, Brammer Bio, Cure Genetics, and Incysus. J.J.M. is a consultant for Shanghai Unicar-Therapy Bio-medicine Technology Co. Ltd, Simcere of America Inc., IASO Biotherapeutics, and Poseida Therapeutics, and is a member of the scientific advisory board of IASO Biotherapeutics. D.T.T., J.J.M., and S.F.L. hold patent application (US 20180252727) related to toxicities after CAR T cell therapy. D.T.T. serves on the advisory board for Amgen, Janssen, La Roche, and Humanigen. D.L.P. has employment or spouse employment with Roche and Genentech; holds stock in Genentech and Roche; acts as a consultant for Novartis, Kite/Gilead, Incyte, Gerson Lehrman Group, Glenmark, Janssen, and Adepcet Bio; holds patents with Novartis and Tmunity; receives research funding from Novartis; and serves on the National Marrow Donor Program Board of Directors, and the American Board of Internal Medicine (Former, Hematology board exam writing committee member through October 2019). H.B. holds stock in CSL Behring, and acts as a consultant for Kriya Therapeutics. D.L.P. is a patent inventor for use of CAR T cells in CD19+ malignancies. D.M.B. is a consultant and have received research funding from Tmunity Therapeutics, a consultant and member on scientific advisory board for Cellares Corporation, received royalties/milestones from patents from Tmunity Therapeutics and Novartis Institute for Biomedical Research. The remaining authors declare no competing financial interests.

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