Efficacy of Therapeutic Plasma Exchange Alone or in Combination with Ruxolitinib for the Treatment of Penn Class 3 and 4 Cytokine Release Syndrome Complicating COVID-19

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Abstract

This study was conducted to validate the efficacy of therapeutic plasma exchange (TPE) in reducing the excessive cytokine load complicating a subset of patients with severe COVID-19 and respiratory compromise. Additionally, this trial explored molecular signals of potential benefit by the addition of JAK inhibition to TPE. Findings included an improvement in cytokine excess and oxygenation along with incremental benefit in cytokine reduction with the addition of ruxolitinib. While exploratory in nature, this study adds momentum to the pursuit of larger randomized trials.

Introduction

Observations early in the viral pandemic of 2020 noted the resemblance between severe coronavirus disease 2019 (COVID-19) infection and the hypercytokinemic state of secondary hemophagocytic lymphohistiocytosis (sHLH) [1]. Conti and colleagues [2] have outlined the binding of COVID-19 to the Toll-like receptor with subsequent cytokine driven fever and pneumonitis, while in Kenderian's [3] murine model of CRS, ruxolitinib abrogated the development of cytokine excess. Cao [4] reported faster clinical recovery with JAK inhibition and Capochiani's RESPIRE trial reported an 89% overall response rate (ORR) with ruxolitinib therapy [5]. Discordant findings regarding JAK inhibition have been reported on the superiority of baricitinib plus remdesivir over remdesivir alone in shortening recovery times in hospitalized patients [6] while the randomized RUXCOVID trial failed to impact severe complications of the disease by adding ruxolitinib

to standard of care [7]. Other groups [8,9] have reported inflammatory cytokine profiles along with evidence that elevated IL-6 and TNFα are strong predictors of disease severity and survival. While cytokine excess could be an epiphenomenal surrogate for another process, therapeutic strategies have evolved around addressing the excessive cytokine levels complicating a significant fraction of COVID-19 patients.

Several case reports [10,11] coupled with a small case series [12] reported beneficial intervention with therapeutic plasma exchange (TPE) in COVID-19 patients cytokine release syndrome (CRS). Gluck et al. [8] reported a formal prospective trial of 10 patients treated early in the pandemic before glucocorticoids, antiviral agents or convalescent plasma usage became commonplace. That study mapped daily cytokine levels and demonstrated marked reduction in cytokine load, improvement in oxygenation, and enhanced clinical outcomes with TPE.

The FDA approved trial (NCT04374149) reported herein was conducted to validate the previously reported efficacy of TPE in reducing cytokine excess in severely ill COVID-19 patients, to expand the mapping of cytokine signatures throughout the illness, and to evaluate the impact of the addition of ruxolitinib treatment in ameliorating cytokine excess and clinical outcomes.

Materials and Methods

Study population

Eligible patients were COVID-19 positive by polymerase chain reaction (PCR), met criteria for Penn class 3 or 4 CRS, were aged 12 to 80 and required supplemental oxygen or mechanical ventilation. Patients were excluded if pregnant, breastfeeding, categorized as Class 3 or 4 New York Heart Association heart failure, stage 4 obstructive lung disease or interstitial lung disease with chronic hypoxic respiratory failure requiring supplemental oxygen at baseline. Patients were also excluded for current use of disease-modifying anti-rheumatic drugs (with the exception of hydroxychloroquine or IL-6 inhibitors), chronic corticosteroids if in excess of prednisone 10 mg/day or equivalent, suspected or confirmed clinically significant bacterial infection, history of tuberculosis, HIV, or irritable bowel disease, creatinine clearance of less than 15 mL/min, absolute neutrophil count less than 1000/µL, platelet count less than 50,000/µL and AST or ALT greater than five times the institutional upper limit of normal.

Trial design

The trial, as approved by the FDA and the health system's Institutional Review Board (IRB), included two single arm cohorts of ten patients each. All patients provided informed consent. Cohort 1A underwent TPE with one plasma volume exchange daily for two consecutive days then every other day three times for a total of five exchanges using the Spectra Optia Apheresis System (Terumo BCT Inc., Lakewood, Colorado, USA) employing centrifugal blood component separation. Patients received isovolemic replacement with 5% albumin. Cohort 1B utilized the same protocol of TPE with the addition of a 14-day course of ruxolitinib at 5 mg po twice daily beginning the day prior to first TPE. These cohorts functioned as two consecutive phase 1, 2 trials performed to explore the potential value of further expansion into subsequent randomized studies. In the absence of a validated COVID-19 specific grading system for CRS, the Penn classification [13] was employed by extrapolation. Patients had to complete at least two procedures of TPE to be considered evaluable.

Study endpoints

The primary endpoint of the trial was to document the efficacy of TPE alone or TPE plus ruxolitinib in decreasing the CRP and cytokine load (IL-6, IL-8, IL-10, TNFα, IFNγ, GM-CSF) by measuring these levels daily and immediately post TPE over a 14-day period. Secondary endpoints included improvement in oxygenation and median time of mechanical ventilation. Supplemental oxygen requirements were tracked daily for non-ventilated patients. The oxygenation index (OI) was calculated daily for ventilated patients.

Laboratory assessments

CRP and fibrinogen levels were analyzed by the hospital laboratory. Cytokine assays were performed in the cancer institute research laboratory. Serum levels of IL-6, IL-8, IL-10, TNFα, GM-CSF, and IFNγ were measured using a Magnetic Luminex Performance Assay (R&D Systems) according to the manufacturer's protocol [14]. Data were analyzed using Bio-Plex Manager Software and GraphPad Prism. COVID-19 serology was assayed at baseline, day 7 and day 14 utilizing Bio-Rad's Platelia SARS-CoV-2 total antibody assay.

Safety

Study related adverse events were graded using NCI Common terminology criteria for adverse events (CTCAE Version 5.0). The safety analysis included all patients who underwent at least one TPE. All patients were reviewed by the study Data Safety and Monitoring Board.

Statistical analysis plans

Two-sample median comparisons of CRP, IL-6, IL-10, and TNFα levels at baseline, following the second and fifth (final) plasma exchange and at day 14 for all 20 patients were performed using the Wilcoxon Signed Rank test (Figure 1). In addition, the median of CRP, IL-6, IL-10, and TNFα levels at baseline, following the second and fifth (final) plasma exchange and at day 14 were compared between cohorts 1A and 1B (Figure 2).

Results

Eligible patients were enrolled consecutively as referred from critical care services. Patient demographics and clinical characteristics are outlined in Table 1. There was one death in cohort 1A and two deaths in cohort 1B within the day 28 study window. The cohorts were remarkable for Hispanic female dominance in cohort 1A and Caucasian male dominance in cohort 1B. Additionally, the imbalance between cohorts with respect to previous or concomitant therapy reflects the greater adoption of convalescent plasma and antiviral medication over the time span of the trial. Baseline respiratory status and comorbidities were similar.
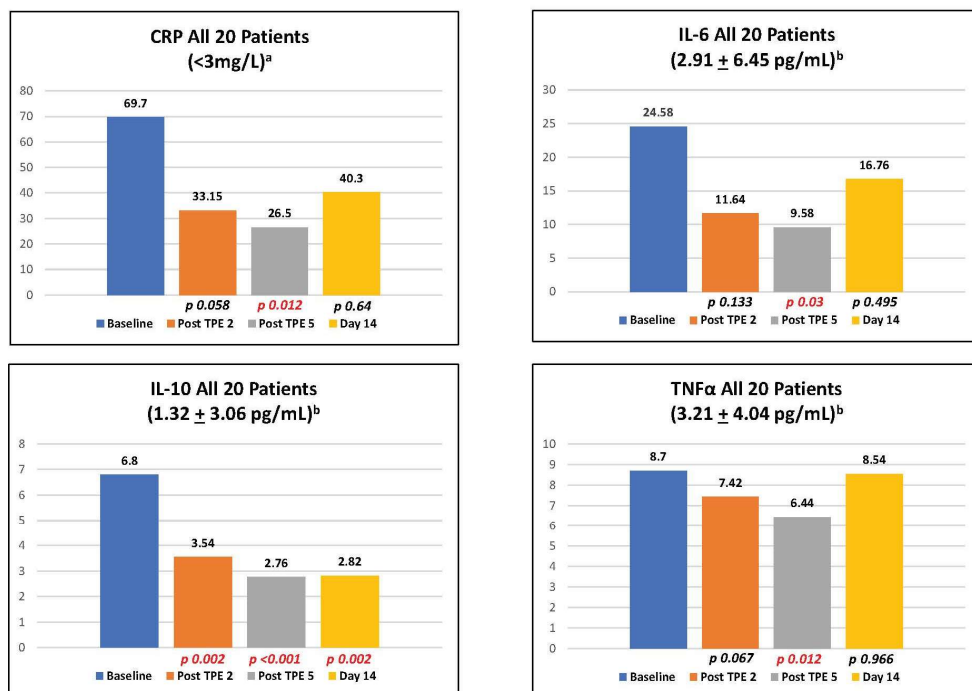
Figure 1: Two-sample Median Comparisons for CRP and Cytokine Levels. The median of CRP and cytokine levels was compared in all 20 patients following the second and fifth (final) plasma exchange and at day 14. [a]Non-cardiac (inflammatory) CRP reference range. [b]Cytokine levels represent average concentrations + standard deviation (Kim et al. [18]).
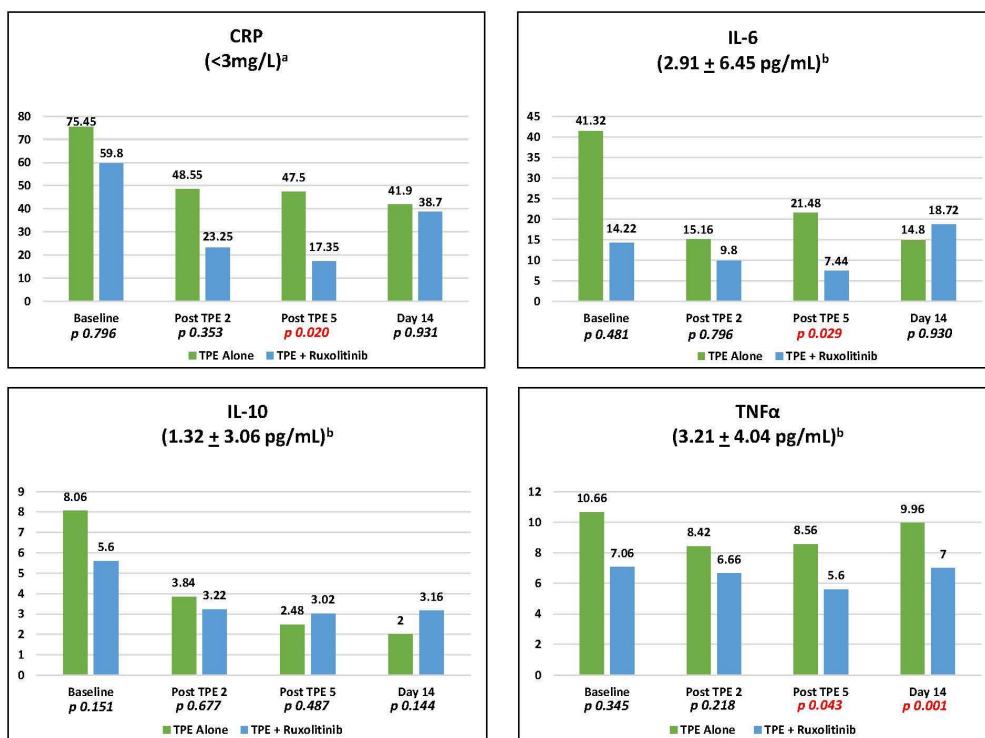

Figure 2: CRP and Cytokine Comparisons between Cohort 1A and Cohort 1B. The median of CRP and cytokine levels was compared between Cohort 1A (TPE alone) and Cohort 1B (TPE + Ruxolitinib) at baseline, following the second and fifth (final) plasma exchange, and on day 14.

Table 1: Demographic and Clinical Characteristics of Patients.

	Cohort 1A (TPE Alone)	Cohort 1B (TPE + Rux)
N	10	10
Age, Mean ± SD	51.8 ± 12.6	57.4 ± 8.8
Body Mass Index (kg/m²), Mean ± SD	34.4 ± 10.2	36.0 ± 5.6
Gender, N (%)		
Female	7 (70)	1 (10)
Male	3 (30)	9 (90)
Race/Ethnicity, N (%)		
Hispanic	6 (60)	2 (20)
White	2 (20)	6 (60)
Asian	1 (10)	0
Black	1 (10)	2 (20)
Days from COVID+ test to 1st TPE, Median (IQR)	4.5 (3, 6)	9 (4,12)
ABO Blood Group, N (%)		
A-	1 (10)	1 (10)
A+	2 (20)	2 (20)
B+	2 (20)	1 (10)
O+	5 (50)	6 (60)
Respiratory Status, N (%)		
Penn Class 3 Nasal Cannula or High Flow Nasal Cannula	4 (40)	4 (40)
Penn Class 4 Invasive Mechanical Ventilation	6 (60)	6 (60)
Previous or Concomitant Therapy		
Glucocorticoids	9 (90)	10 (100)
Convalescent Plasma	2 (20)	7 (70)
Remdesivir	3 (30)	8 (80)
Comorbidities, N (%)		
Diabetes	3 (30)	3 (30)
Hypertension	5 (50)	5 (50)
Obesity	6 (60)	8 (80)

TPE resulted in immediate reductions in CRP, IL-6, IL-10, and TNFα. Ruxolitinib treatment incrementally reduced CRP ($p = 0.020$), IL-6 ($p = 0.029$), and TNFα ($p = 0.043$) levels following the fifth TPE compared to TPE alone (Figure 1). Additionally, ruxolitinib substantially reduced day 14 TNFα ($p = 0.001$) compared to TPE alone (Figure 2). The oxygenation index was improved by 35.3% by day 3 with TPE alone and 30% with TPE plus ruxolitinib reflecting no incremental benefit from the addition of ruxolitinib. The median time on mechanical ventilation was six days for both cohorts. Fifteen of 20 patients were either extubated or returned to room air (7 in cohort 1A, 8 in cohort 1B) within the 28-day study window. COVID-19 seropositivity was present in all 20 patients at baseline with no conversion to seronegativity through study day 14. There were no serious adverse events related to TPE or ruxolitinib treatment. All patients received 5% human albumin as replacement therapy without the need for fresh frozen plasma (FFP).

Discussion

This study is the first to report the combination of two complementary treatments to address the hypercytokinemic state of CRS complicating COVID-19 infection. The improvement in cytokine levels with TPE reproduces the findings of our earlier pilot study [8] as well as findings of others [10-12]. Noted were significant reductions of CRP, IL-6, IL-10, and TNFα with no baseline signal of excess IFNγ or GM-CSF. The addition of ruxolitinib resulted in statistically significant reductions in CRP, IL-6, and TNFα following the fifth TPE as well as day 14 TNFα levels. When this study was initiated in June 2020, it was notably within a different clinical context compared to our pilot trial and other case reports. As previously mentioned, the majority of patients received antecedent and concomitant therapies following a diagnosis of CRS. Some of the enrolled patients had been exposed to these interventions for several days. Therefore, in contrast to our pilot study, which was free of concomitant therapies, patients in this study had decreased baseline cytokine levels as reflected in CRP values which were 56% lower at study entry. This observation likely reflects the immediate impact of glucocorticoids and other therapies. Furthermore, the utilization of high flow oxygen skewed comparisons with previous studies wherein many of these patients would have been mechanically ventilated. This FDA approved trial required the inclusion of emerging therapies deemed of possible merit and thus introduced uncontrolled complexity in the interpretation of the data.

The favorable impact of ruxolitinib on IL-6 and TNFα levels is particularly noteworthy as these cytokines may play a key role in COVID-19 induced CRS. IL-6 has been consistently reported at excessive levels and many have suggested that it is "the kingpin" of the process [15]. The literature also reflects an increasing awareness of the role of TNFα in what is likely a highly multifactorial cytokine load. Del Valle et al. [8] noted that elevated "IL-6 and TNFα are significantly associated with a worse prognosis (page 1637)" and are "independently predictive of patient outcomes in terms of both disease severity and survival (page 1640)." Our findings of incremental benefit of ruxolitinib treatment on IL-6 and TNFα levels post apheresis ($p = 0.029$ and $p = 0.043$ respectively) as well as day 14 TNFα ($p = 0.001$) underscore the need for further evaluation of ruxolitinib in the treatment of CRS. Dose escalation of ruxolitinib is warranted as the 5 mg twice daily dose demonstrated a positive signal of impact without any evidence of toxicity. A year into the global pandemic of 2020, a brisk death toll persists in the face of a paucity of predictably effective therapies. Just reported was a randomized trial noting the failure of convalescent plasma in COVID-19 severe pneumonia to improve clinical status or overall mortality [16] and the failure of tocilizumab to improve clinical outcomes in hospitalized patients [17]. The results of the FDA approved trial presented here lend support to the pursuit of larger, prospective, randomized trials to better define the risks of clinical benefit of TPE alone and in conjunction with JAK inhibitors and other novel agents.

Declaration of Competing Interest

W. Jeffery Edenfield serves as a consultant for Chimerix, Inc., unrelated to the submitted work. The remaining authors declare that there are no competing interests.

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W. Larry Gluck: Conceptualization, Methodology, Formal analysis, Data curation, Writing – Original draft, Funding acquisition. **Sean Callahan:** Investigation, Supervision, Writing – Review and Editing. **Robert Brevetta:** Investigation, Supervision, Writing – Review and Editing. **Antine Stenbit:** Investigation, Supervision, Writing – Review and Editing. **Wesley Smith:** Investigation, Visualization, Data curation, Writing – Original draft, Formal analysis. **Julie Martin:** Project administration, Visualization, Data curation, Writing – Original draft, Formal analysis. **Anna Blenda:** Data curation, Validation, Writing – Review and Editing. **Sergio Arce:** Data curation, Validation, Writing – Review and Editing. **W. Jeffery Edenfield:** Visualization, Funding acquisition, Writing – Review and Editing.

Acknowledgments

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