UNIVERSITY OF MEDICINE AND PHARMACY

”VICTOR BABEŞ” TIMISOARA

FACULTY OF GENERAL MEDICINE

DEPARTMENT IV – BIOCHEMISTRY AND PHARMACOLOGY

TAMARA MIRELA POROSNICU

DOCTORAL THESIS

PLASMA CLEARANCE PARTICULARITIES IN THE CRITICALLY-ILL COVID-19 PATIENTS

Scientific Coordinator

PROF. UNIV. DR. SIRBU IOAN OVIDIU

T i m i s o a r a

2 0 2 4

TABLE OF CONTENTS

STUDY 1: THE IMPACT OF THERAPEUTIC PLASMA EXCHANGE ON INFLAMMATORY MARKERS AND ACUTE PHASE REACTANTS IN PATIENTS WITH SEVERE SARS-COV-2 INFECTION.

CONTEXT

The new coronavirus SARS-CoV-2, unlike the other two viruses, MERS (Middle East respiratory syndrome) and SARS (severe acute respiratory syndrome), was a real challenge for the entire global health system due to the many cases that required hospitalization in a short period of time, causing a global crisis in the health system. Regarding biological inflammatory markers, increased values of lactate dehydrogenase (LDH), ferritin, fibrinogen, interleukin-6 (IL-6), C reactive protein, D-dimers, and a reduced number of lymphocytes were described in multiple studies. Many of the abnormalities identified in hospitalized cases were caused by a “cytokine storm”, characterized by an exaggerated host response to the virus with similar characteristics to bacterial septic shock and negative fulminant evolution that is usually fatal. In other words, the leading causes of death in patients with COVID-19 infection are ARDS and cytokine storm syndrome, which lead to multisystemic organ failure.

Because a cytokine storm-mediated immune response is what causes organ damage, it stands to reason that removing damaging antibodies and cytokines might reduce the severity of the illness. The use of TPE in COVID-19 patients is based on the rationale that by removing the excess of proinflammatory cytokines, such as IL-6, tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β), TPE can attenuate the cytokine storm and prevent the subsequent multiorgan failure and acute respiratory distress syndrome (ARDS) that are often observed in severe cases. The elimination of other fibrin breakdown products, such as D-dimers, might also contribute to an improvement in the hemostatic equilibrium. In light of these considerations, TPE has lately been brought up as a potential supportive therapy option for severe SARS-CoV-2 infections.

However, despite the growing interest in TPE as a potential therapy for severe COVID-19, the impact of TPE on the inflammatory markers and acute phase reactants in patients with SARS-CoV-2 infection remains inadequately studied. Therefore, the objective of this systematic review was to present the role of plasma exchange therapy in lowering inflammation markers and acute phase reactants in critically ill COVID-19 patients and finding the optimal treatment protocol to improve patients’ survival.

RESULTS

After filtering the matching studies, we included in this systematic review 13 articles that used TPE as treatment in adult patients with severe COVID-19. The main outcomes extracted from the studied papers observed a total of 485 severely ill patients treated with TPE, with a mortality rate of 18.1% (72 patients). However, the mortality rate is arguably lower than in patients without TPE treatment because only six studies had a control group for comparison, and their results were not always statistically significant. However, the duration of ICU admission was reported as significantly lower than in control groups, averaging six days compared with fourteen days among controls

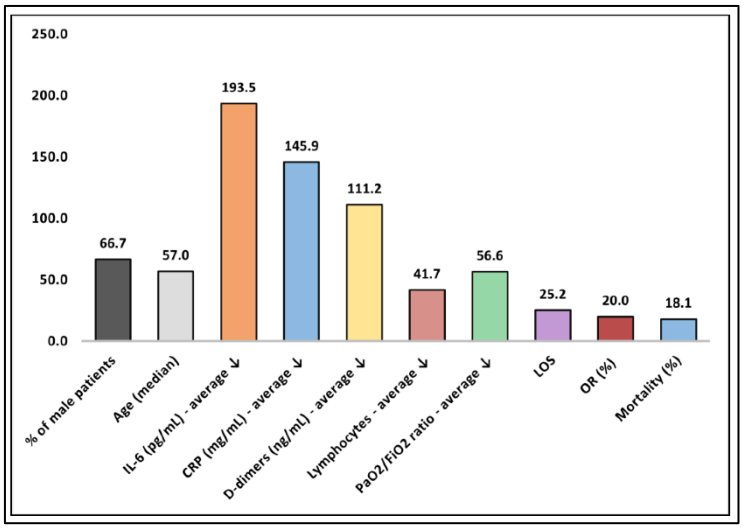
TPE was usually performed multiple times, averaging three times during hospitalization and ranging from one to seven times. The main component of replacement fluid was fresh frozen plasma in all 13 studies, or FFP with a combination of citrate dextrose solution, human albumin and normal saline, normal saline only, or 5% human albumin only. Regarding the safety of TPE, only one study reported one patient with associated hypotension, whereas another study reported femoral artery puncture in one patient and thrombophlebitis of the femoral vein in another patient.

Some key observations from the included studies comprise reduced inflammatory markers, reductions in SOFA scores, and the conclusion of better results in critically ill patients

when TPA is used early after disease onset. Another important finding is improved oxygenation after plasmapheresis, which was reported in five of the analyzed studies.

Regarding the change in inflammatory markers observed in patients with severe COVID-19, it was observed in all studies that reported a change in variation that inflammatory markers significantly decreased after undergoing TPE. The highest reduction of inflammatory markers was observed in a study by Khamis, which reported a 336 mg/mL decrease of CRP. One study reported a 574 pg/mL decrease in IL-6 levels post-TPE. However, data is inconsistent regarding the timing of serum marker measurement, or how long after TPE the measurements were taken. As seen in Table 2 and Figure 2, most of the patients were men (66.7%) who had an age range between 48–75 years and a median age of 57 years. The average IL-6 reduction after TPE was 193.5 pg/mL; CRP decreased by an average of 145.9 mg/L, whereas D-dimers decreased by 111.2 ng/mL. Lymphocyte count was also significantly lower after TPE with a 41.7 × 109/L decrease. The length of hospitalization, however, was very long; studies reported an average of 25.2 days of admission. The mortality rate varied significantly, which is probably due to the severity of patients involved, from 0.0% in some of the studies up to 60.0% in the study described by Matsushita et al. and an overall average of 18.1%. Lastly, only four studies reported the odds ratio for improvement after TPE compared with a control group, but data showed a significant improvement in clinical and inflammatory status of the patients from 17% to 32%.

Figure 1 – Summary of findings.



CONCLUSIONS

TPE could be regarded as an alternative therapy complementary to standard treatment in severely ill COVID-19 patients. Most studies describe a reduction in inflammatory mediators and improvement of coagulation function, as well as clinical status, compared with admission features after three to five TPE sessions. However, the described studies are not standardized, and the results show inconsistency between them; the majority report no significant improvement after TPE in comparison with a control group. In addition, some studies suggest that TPE improves survival rate by correcting the inflammatory status of the patients without significant side effects, although it is not yet statistically proven in trials with a higher number of patients to offer reliability. Thus, it is important to emphasize the need for more well-designed randomized controlled trials with larger sample sizes and standardized protocols to determine the true efficacy, safety, and optimal treatment protocol for TPE in the management of critically ill COVID-19 patients. This will help provide more robust and reliable evidence for the clinical application of TPE in this patient population.

STUDY 2: EFFICACY OF THERAPEUTIC PLASMA EXCHANGE IN SEVERE ACUTE RESPIRATORY DISTRESS SYNDROME IN COVID-19 PATIENTS FROM THE WESTERN PART OF ROMANIA.

CONTEXT

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has surprised the entire medical world with its devastating effects of severe ARDS and cytokine storm, but also by the scant therapeutic solutions which have proven to be effective against the disease. A number of antivirals, immunomodulatory, corticosteroid, and anticoagulant therapies have aroused interest in treating patients with COVID-19 disease who require intensive therapy, with or without mechanical ventilation. Among the plasma purification techniques, therapeutic plasma exchange (TPE) has been proposed from the very beginning as a possible adjuvant treatment in severe cases requiring admission to intensive care. The rationale behind using TPE for the treatment of COVID-19 patients includes the reduction in inflammatory cytokines levels, the stabilization of the endothelial membrane, treatment of hyperviscosity, reduction in antifibrinolytic mediators, and fibrin degradation products or the elimination of SARS-CoV-2 virus. Therapeutic plasma exchange can eliminate the mediators excessively released in the cytokine storm and improve the biomarkers related to a poor prognosis. These are large molecules (IL-6 = 28 kDa, ferritin = 475 kDa, LDH = 140 kDa, CRP = 25 kDa, D-dimers = 180 kDa, fibrinogen = 340kDa) that can be eliminated by a plasma-filter.

The main purpose of this study was to analyze the evolution of specific biological markers in COVID-19 disease before and one day after a TPE session and how the change in these parameters influences the patient's respiratory status, as well as the impact of TPE on survival rates. All the patients received antiviral, anticoagulant, and corticosteroid treatment.

RESULTS

The study involved a detailed analysis of data from 65 adult patients who underwent a total of 120 Therapeutic Plasma Exchange (TPE) procedures for COVID-19 treatment. This cohort consisted of 41 patients with a single session, 13 with two sessions, and 11 having three or more sessions. Clinical and laboratory data were critically assessed one day before and after TPE sessions using the Wilcoxon test paired sample. These patients primarily presented with moderate or severe Acute Respiratory Distress Syndrome (ARDS), as reflected by a median PaO2/FiO2 ratio of 98.25, and demonstrated increased inflammatory markers. Out of the 120 TPE sessions, only 110 were available for PaO2/FiO2 ratio evaluation post-TPE due to the exclusion of data from 10 sessions where patients were on Extracorporeal Membrane Oxygenation (ECMO).

Significant findings revealed that TPE effectively eliminated large molecules like IL-6, ferritin, D-dimers, C-reactive protein (CRP), lactate dehydrogenase (LDH), and fibrinogen in the majority of cases. The average concentrations of these inflammatory markers before the first TPE session were notably high. On the contrary, there was no observed significant improvement in various clinical features including pulmonary status, hemodynamic parameters, and organ functionality. However, it is noteworthy that patients with severe ARDS (PaO2/FiO2 < 100) did demonstrate a statistically significant improvement in oxygenation following the TPE sessions. On the other hand, those with mild to moderate ARDS showed no notable enhancement in oxygenation levels post-TPE. Among the total participants, 61% were mechanically ventilated during their first TPE. Interestingly, after 28 days, only 16 of the 65 patients (or 24.61%) survived, and out of the 40 mechanically ventilated patients, only seven pulled through.

Further comprehensive analysis highlighted that out of 535 patients admitted to the Intensive Care Unit (ICU) during the specified period, 401 underwent invasive mechanical ventilation. Tragically, a staggering 375 of these patients did not survive. However, among the

65 patients who had TPE, 56 were on invasive mechanical ventilation right from the outset. Encouragingly, 16 of these individuals survived, translating to a survival rate of 12.5% for intubated patients. This showcases a potential improvement in the survival rate for those who underwent invasive mechanical ventilation and received TPE, compared to those who only had invasive mechanical ventilation. This correlation suggests that TPE might offer a relative survival advantage for critical COVID-19 patients under specific circumstances.

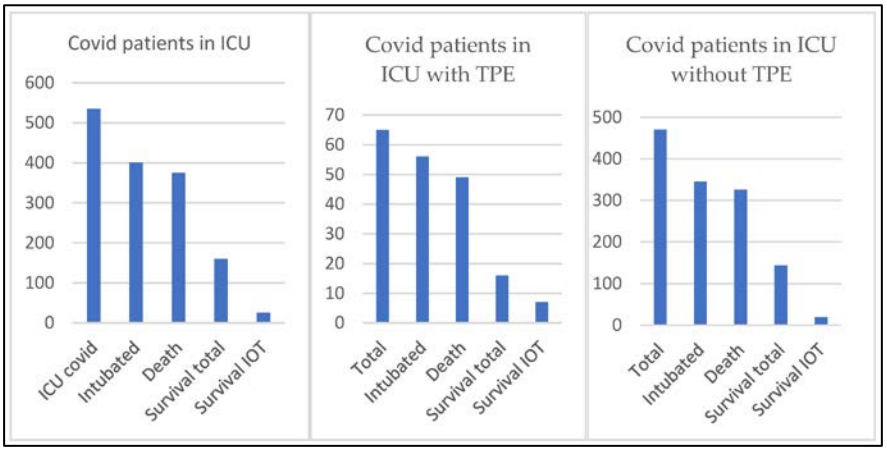


Figure 2 – Survival in ICU: total COVID patients versus COVID patients with/without TPE.

CONCLUSIONS

TPE can be used to reduce inflammation markers in COVID-19 critically ill patients and improve the PaO2/FiO2 ratio in patients with severe ARDS. This procedure also showed a minimum benefit in the survival of patients with extremely severe forms of COVID-19 disease. TPE should be used early with critically ill patients with ARDS. We consider that TPE and many other therapeutic approaches represent distinct pieces from the complicated puzzle of COVID-19 which still remains to be solved.

STUDY 3: ASSESSING THE OUTCOMES OF PATIENTS WITH SEVERE SARS-COV-2 INFECTION AFTER THERAPEUTIC PLASMA EXCHANGE BY NUMBER OF TPE SESSIONS.

CONTEXT

The global health system faced unprecedented challenges with the emergence of the SARS-CoV-2 virus, leading to a large influx of hospitalizations and triggering a worldwide health crisis. Over the past three years, extensive scientific and clinical efforts have sought solutions to prevent and treat COVID-19. Yet, gaps remain in understanding and treating severe forms of the disease. Severe COVID-19 often presents with heightened inflammation, causing acute respiratory distress syndrome (ARDS). Biomarkers such as LDH, ferritin, IL-6, C-reactive protein, and D-dimers have been identified as indicators of this inflammatory response. This overreaction, known as a cytokine storm, mirrors bacterial septic shock responses and has been a major cause of mortality due to multisystem organ failures in COVID-19 patients.

While vaccines have been widely distributed, challenges persist due to vaccine hesitancy and new outbreaks in 2023, even in regions with high vaccination rates. As a result, medical professionals continue to face severe COVID-19 cases, necessitating innovative treatments. In severe COVID-19 cases, the mortality rate remains concerning, and while many treatment approaches have been explored, only a handful have shown efficacy. One such approach is Therapeutic Plasma Exchange (TPE), or plasmapheresis. In TPE, the patient's plasma, containing harmful components, is replaced with healthier alternatives. TPE aims to remove pathological elements, such as antibodies and inflammatory proteins, and is commonly used to treat conditions like glomerulonephritis, myasthenia gravis, and Guillain-Barre syndrome.

Given that many COVID-19 complications stem from a cytokine storm, there is a hypothesis that removing harmful antibodies and cytokines could reduce disease severity. Consequently, TPE has been proposed as a potential treatment for severe COVID-19 cases, especially when other treatments have failed. This study's objective is to evaluate the impact of plasma exchange therapy on inflammation in critically ill COVID-19 patients, ascertain its efficacy in preventing complications, and determine the optimal treatment protocol based on the number of TPE sessions.

RESULTS

Of the 65 participants, 41 underwent one session of TPE, thirteen had 2 sessions, and eleven had more than two TPE sessions. There was no significant variation among the three groups concerning body mass index, age, gender, necessity for mechanical ventilation, and pre-existing comorbidities. The average age across the groups ranged from around 47.8 to 54.4 years. All groups exhibited a BMI indicative of class I obesity, and males were predominant, averaging 70.4% across the cohort. The majority of patients required invasive ventilation, with percentages of 58.5% in the first group, 76.9% in the second, and 63.6% in the third. The most frequent comorbidity was hypertension.

Upon ICU admission, blood analysis revealed IL-6 levels were notably higher in patients undergoing more than three TPE sessions, with average values significantly differing among the groups. CRP levels were also elevated in this group. Conversely, leucocyte median value and BUN were notably higher in patients who underwent 2 TPE sessions.

After the initial TPE session, IL-6 levels decreased most significantly in patients who underwent more than two sessions. LDH declined significantly across all groups, while CRP levels' difference remained significant among groups, but without a general significant change pre and post-TPE. The ESR decreased after TPE, with the highest levels in patients with over two TPE sessions. Notably, leucocyte levels rose after TPE across all groups.

In terms of patient outcomes, the mean arterial pressure (MAP) showed no notable difference among groups before TPE but post-TPE differences were statistically significant. Parameters like SOFA score, APACHE 2 score, oxygenation index, and PaO2/FiO2 ratio remained unchanged post-TPE. However, the ROX index was notably higher in the group with more than two TPE sessions. Regarding intubation, the proportions were 43.9% in group 1, 38.5% in group 2, and 45.5% in group 3. Out of all patients, only 18 survived, but the mortality rate did not vary significantly between groups based on TPE sessions. Similarly, survival probability, based on TPE sessions, showed no significant difference among the groups.

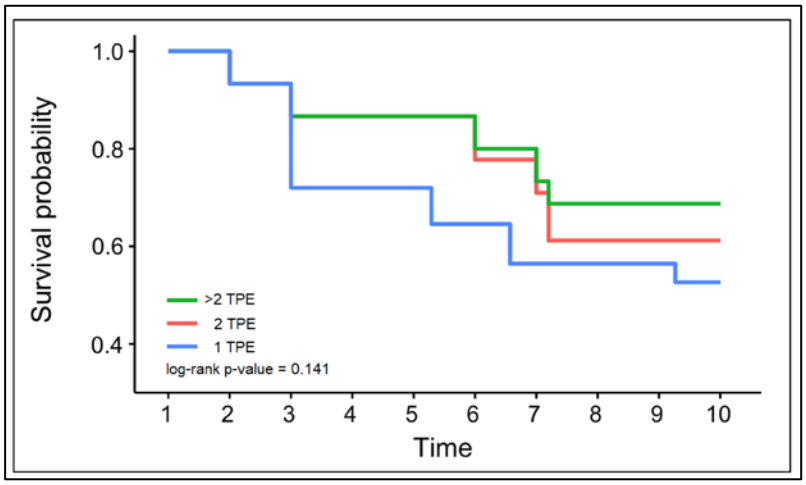


Figure 3 – Kaplan–Meier survival analysis by number of TPE sessions.

CONCLUSIONS

Almost three years have passed since the onset of the pandemic, and the complex mechanism of the disease is not fully known, and the race to find an efficient treatment is still not over. From the available data, we found that TPE might be considered a complementary alternative therapy for COVID-19 patients with severe illness. Even after one TPE session it was observed that there was a substantial decrease in inflammatory mediators, improvement in coagulation function, and improvement in clinical status, in a non-inferior way compared to patients who underwent two or more sessions of plasmapheresis. The respiratory function was however significantly improved after two or more TPE sessions compared to only one session, but overall, the survival was not significantly influenced. However, the available literature and their outcomes are very variable, with the majority of them failing to indicate an improvement compared to a control group. Nevertheless, this method has not yet been statistically verified in trials with a larger number of patients in order to be considered reliable in terms of a proper study protocol and a significant improvement in patients' survival.