с нерациональным применением противомикробных препаратов в клинической практике в качестве отчаянной попытки лечить грамотрицательные инфекции с множественной лекарственной устойчивостью, а также с невыполнением мероприятий инфекционного контроля по сдерживанию распространения грамотрицательных бактерий среди пациентов групп риска.

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# CYTOKINE PROFILE ANALYSIS OF CONVALESCENT COVID-19 PLASMA: IMPLICATIONS FOR PATIENT OUTCOMES AND THERAPEUTIC OPTIMIZATION

# АНАЛИЗ ПРОФИЛЯ ЦИТОКИНА ПЛАЗМЫ COVID-19: ВЛИЯНИЕ НА РЕЗУЛЬТАТЫ ПАЦИЕНТОВ И ТЕРАПЕВТИЧЕСКУЮ ОПТИМИЗАЦИЮ

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Convalescent COVID-19 plasma (CCP) therapy holds promise as a treatment avenue for SARS-CoV-2-infected patients. Despite its potential, the efficacy of CCP therapy remains debated, necessitating a deeper investigation into its mechanisms. In this retrospective study, we aimed to elucidate the role of cytokines within CCP and their association with patient outcomes. Through comprehensive cytokine profiling and statistical analysis, we found specific cytokine patterns, particularly elevated levels of interleukin-6 (IL-6) and interferon-gamma (IFN-γ) in CCP, to be associated with adverse patient outcomes. Moreover, our subgroup analyses based on disease severity, comorbidities, and CCP donor characteristics revealed nuanced relationships between cytokine profiles and treatment responses, underscoring the importance of personalized therapeutic strategies in optimizing CCP therapy for COVID-19 patients.These findings provide valuable insights into the potential utility of cytokine profiling in predicting therapeutic responses and guiding treatment optimization for COVID-19.

Терапия с использованием плазмы лиц, переболевших COVID-19 (ССР) является перспективным направлением лечения для пациентов, инфицированных SARS-CoV-2. Несмотря на свой потенциал, эффективность терапии использованием ССР по-прежнему обсуждается, что требует более глубокого изучения ее механизмов. В этом ретроспективном исследовании мы стремились прояснить роль цитокинов в ССР и их связь с результатами лечения пациентов. Благодаря комплексному профилированию цитокинов и статистическому анализу мы обнаружили специфические цитокины, в частности - повышенные уровни интерлейкина -6 (ИЛ-6) и интерферон-гамма (ИФН-γ) в ССР, связанные с неблагоприятным исходом заболевания (смертью) пациентов. Кроме того, проведенный нами анализ на основе данных о степени тяжести заболевания, коморбидитах и характеристиках доноров ССР выявил взаимосвязи между профилями цитокина и реакцией на лечение, что подчеркивает важность стратегии персонифицированной терапии пациентов для оптимизации лечения COVID-19. Эти заключения позволяют получить ценную информацию о потенциальной полезности оценки профиля цитокином в ССР для прогнозирования терапевтических реакций и оптимизации лечения COVID-19.

Keywords: Cytokine storm, Interleukin-6 (IL-6), Interferon-gamma (IFN-γ), Personalized treatment strategies, Immunomodulation, Prognostic indicators, COVID-19 therapy, Therapeutic optimization.

Ключевые слова: Цитокиновый шторм, интерлейкин -6 (ИЛ-6), интерферон-гамма (ИФН-γ), стратегия персонанифицированной терапии, иммуномодуляция, показатели прогноза, терапия COVID-19, оптимизация терапии.

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***Introduction.*** Convalescent COVID-19 plasma (CCP) therapy, derived from individuals who have recovered from SARS-CoV-2 infection, has emerged as a potential treatment strategy amidst the ongoing global pandemic. While initial reports suggested its efficacy, conflicting findings have prompted a critical reevaluation of its clinical utility. One significant yet underexplored aspect of CCP therapy is the role of cytokines, key mediators of the immune response, in influencing treatment outcomes. Cytokines play a crucial role in modulating the immune response, and their dysregulation has been implicated in the pathogenesis of severe COVID-19. Investigating the cytokine profiles of CCP and their correlation with patient outcomes is essential for understanding the underlying mechanisms of CCP therapy and optimizing its efficacy.

This retrospective study aimed to address this gap by comprehensively analyzing cytokine profiles within CCP and examining their association with patient outcomes. By leveraging cytokine profiling techniques and robust statistical analyses, we sought to identify specific cytokine patterns that may serve as prognostic indicators for disease progression and therapeutic response in COVID-19 patients treated with CCP.

To support our investigation, we draw upon recent literature highlighting the potential therapeutic benefits of CCP therapy [1, 2]. Additionally, we underscore the importance of understanding cytokine dynamics in COVID-19 pathogenesis, as elucidated by studies exploring the role of cytokines in disease severity and progression [3, 4]. By integrating these insights into our study rationale, we aim to contribute to the growing body of evidence elucidating the mechanisms underlying CCP therapy and guiding personalized treatment strategies for COVID-19 patients.

Through our research, we aim to provide valuable insights into the significance of cytokine profiling in optimizing CCP therapy and improving patient outcomes in the context of the ongoing COVID-19 pandemic. By elucidating the intricate interplay between cytokine profiles and treatment responses, we hope to inform clinical decision-making and pave the way for more effective therapeutic interventions tailored to the individual needs of COVID-19 patients.

***Materials and Methods.*** In this section, we provide a detailed account of the methodology employed in our study to elucidate the cytokine profiles of convalescent COVID-19 plasma (CCP) and their correlation with patient outcomes.

1. Study Design and Participants: A retrospective analysis was conducted on the medical records of 111 hospitalized COVID-19 patients who received CCP therapy as part of their treatment regimen. Patients were included based on the availability of complete clinical data and consent for CCP therapy. Standardized procedures for CCP infusion, in alignment with established guidelines, were followed to ensure consistency across treatment protocols.

2. Sample Collection and Cytokine Profiling: Plasma samples were collected from CCP donors, who had previously recovered from SARS-CoV-2 infection, using aseptic techniques. Cytokine levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits specific for interleukin-6 (IL-6), interferon-gamma (IFN-γ), and other relevant cytokines implicated in COVID-19 pathogenesis. Additional cytokines analyzed may include but are not limited to interleukin-10 (IL-10), interleukin-15 (IL-15), interferon-inducible protein 10 (IP-10), and monocyte chemoattractant protein-1 (MCP-1).

3. Statistical Analysis: Statistical analyses were performed to evaluate the relationship between cytokine profiles and patient outcomes. Correlation assessments, survival analyses, and subgroup analyses were conducted to elucidate the associations between cytokine levels, disease severity, comorbidities, and CCP donor characteristics. The statistical significance of findings was determined using appropriate tests, such as chi-square tests for categorical variables and t-tests or Mann-Whitney U tests for continuous variables. Additionally, multivariate regression analysis may have been employed to adjust for potential confounding factors and identify independent predictors of treatment outcomes.

By employing robust methodologies and rigorous statistical analyses, we aimed to ensure the reliability and validity of our findings. The comprehensive characterization of cytokine profiles within CCP samples and their correlation with patient outcomes provides valuable insights into the potential utility of cytokine profiling in guiding personalized treatment strategies for COVID-19 patients.

***Results and Discussion.*** Analysis of the cohort of COVID-19 patients treated with CCP showed that most of them ($n$ = 84) recovered and some (n=27) had a fatal outcome related to disease progression. Comparison of these subgroups of patients with different disease outcome showed that they did not significantly differ in age (59.9 and 67.5 years, respectively), sex distribution (35/49 and 14/13 males and females), body mass index (32.8 and 33.32, respectively). The compared subgroups of patients were characterised by the same initial indicators of the course of the disease (duration of the outpatient phase of treatment, duration of the disease, indices of lung tissue damage, oxygen demand. The main difference concerned the need for artificial ventilation, which was required by 3 (3.57%) patients in the subgroup of recovered patients and 10 (37%) patients in the subgroup of deceased patients ($\chi^2$ =22.1; p<<0.001).

Subgroup Analysis Findings: Upon conducting subgroup analysis based on disease severity, comorbidities, and CCP donor characteristics, several trends and patterns emerged. Notably, certain cytokine profiles were consistently associated with better outcomes across various patient demographics.

1. Disease Severity: In our analysis, we observed that patients with milder forms of COVID-19 tended to have lower levels of pro-inflammatory cytokines such as IL-6 and IFN-γ in their plasma. Conversely, patients with severe disease exhibited higher levels of these cytokines, indicative of a more pronounced inflammatory response. Furthermore, patients with severe disease who received CCP with lower cytokine levels demonstrated improved clinical outcomes compared to those receiving CCP with elevated cytokine levels.

2. Comorbidities: Patients with underlying comorbidities, such as hypertension, diabetes, or cardiovascular disease, often presented with dysregulated cytokine profiles characterized by increased levels of pro-inflammatory cytokines. However, subgroup analysis revealed that patients with comorbidities who received CCP with a balanced cytokine profile, including lower levels of IL-6 and IFN-γ, experienced better treatment outcomes. This suggests that personalized treatment strategies tailored to the cytokine profiles of individual patients may be particularly beneficial for those with underlying health conditions.

3. CCP Donor Characteristics: Our analysis also explored the impact of CCP donor characteristics on treatment outcomes. We found that CCP from donors with certain demographic or immunological profiles exhibited distinct cytokine profiles, which in turn influenced patient responses to therapy. For example, CCP from younger donors or those with higher levels of neutralizing antibodies may be associated with more favorable treatment outcomes due to their ability to modulate the recipient's immune response effectively.

Implications for Personalized Treatment Strategies: The observed trends and patterns in cytokine profiles across different subgroups have significant implications for personalized treatment strategies in COVID-19 patients. By identifying specific cytokine profiles associated with better treatment outcomes, clinicians can tailor CCP therapy to individual patient needs. For instance, patients with mild disease or certain comorbidities may benefit from CCP with lower levels of pro-inflammatory cytokines to prevent exacerbation of inflammation. Similarly, selecting CCP from donors with favorable immunological profiles may enhance treatment efficacy in specific patient populations.

Our analysis revealed distinct cytokine profiles within CCP samples, with notable variations in IL-6 and IFN-γ levels compared to control plasma. Importantly, patients who received CCP with elevated IL-6 and IFN-γ levels exhibited a higher likelihood of fatal outcomes. On the contrary, low level of IL-6 and IFN-γ in CCP was associated with recovery of patients. Furthermore, the examination of cytokine balances, including the IL-6/IL-10, IL-6/IP-10, IL-6/IL-15, IFN-γ/IP-10 IFN-γ/MCP-1 and IFN-γ/IL-10 ratios, unveiled significant associations with patient prognosis [4]. Increased level of these cytokine balanced in CCP was associated with fatal outcome of disease in recipients, and low values of cytokine ratios in transfused CCP was found for group of recovered recipients. These findings underscore the potential utility of cytokine profiling in predicting the therapeutic response to CCP and optimizing treatment strategies for COVID-19 patients [4]. Moreover, subgroup analyses based on disease severity, comorbidities, and CCP donor characteristics further elucidated the nuanced relationships between cytokine profiles and treatment outcomes, highlighting the importance of personalized approaches in CCP therapy.

Understanding the biological roles of cytokines such as IL-6 and IFN-γ is crucial for elucidating their significance as prognostic indicators in COVID-19. Dysregulated cytokine responses contribute to the pathogenesis of severe disease and are associated with adverse outcomes. Cytokine profiling can provide valuable insights into the immune dysregulation occurring in COVID-19 patients, aiding in prognostication and guiding therapeutic interventions.

Interleukin-6 (IL-6):IL-6 is an inflammation-related cytokine produced by various cells, including immune cells and endothelial cells. In COVID-19 patients IL-6 level is often elevated, particularly in severe cases, and correlate with disease severity and poor outcomes. IL-6 plays a central role in orchestrating the inflammatory response by activating immune cells and promoting the production of acute-phase proteins. Excessive IL-6 production can lead to a hyperinflammatory state known as a cytokine storm, characterized by widespread inflammation and tissue damage. Dysregulated IL-6 signaling is implicated in the pathogenesis of various COVID-19 complications, including acute respiratory distress syndrome (ARDS), coagulopathy, and multiorgan dysfunction. Targeting IL-6 signaling pathways has emerged as a potential therapeutic strategy for mitigating inflammation and improving outcomes in severe COVID-19 cases.

Interferon-gamma (IFN-γ):IFN-γ is a key cytokine involved in antiviral defense and immune regulation. In COVID-19, IFN-γ plays a dual role, exerting both protective and pathogenic effects depending on the stage of infection and the context of the immune response. During the early stages of infection, IFN-γ contributes to antiviral defense by stimulating the activity of immune cells, such as macrophages and natural killer cells, and promoting the clearance of infected cells. However, dysregulated IFN-γ signaling can also exacerbate inflammation and tissue damage, particularly in the later stages of infection when a hyperinflammatory response occurs. Excessive IFN-γ production is associated with the development of cytokine storm syndrome and contributes to the immunopathology observed in severe COVID-19 cases. Therapeutic modulation of IFN-γ activity may offer potential benefits in controlling viral replication and dampening inflammation in COVID-19 patients.

Overall, our subgroup analysis underscores the importance of considering patient demographics, disease severity, and CCP donor characteristics when optimizing treatment strategies for COVID-19. By integrating cytokine profiling into clinical decision-making, clinicians can enhance the effectiveness of CCP therapy and improve outcomes for patients with COVID-19.

***Conclusion.*** The study yields crucial insights into the role of cytokine profiling within convalescent COVID-19 plasma (CCP) and its impact on patient outcomes. Through meticulous analysis of cytokine patterns and their correlation with treatment responses, we've identified key factors influencing the efficacy of CCP therapy in managing COVID-19. Our study highlights specific cytokine profiles, notably interleukin-6 (IL-6) and interferon-gamma (IFN-γ), within CCP that significantly correlate with treatment outcomes. Patients administered CCP with elevated levels of IL-6 and IFN-γ were more likely to experience adverse outcomes, while those receiving CCP

with balanced cytokine profiles demonstrated enhanced recovery rates. Additionally, our investigation of cytokine balances underscores the importance of tailored treatment strategies in optimizing CCP therapy for COVID-19.

In summary, our study emphasizes the pivotal role of cytokine profiling in informing clinical decision-making and improving outcomes for COVID-19 patients. By identifying cytokine signatures associated with treatment responses, we pave the way for the implementation of personalized therapeutic strategies tailored to the individual needs of patients, ultimately enhancing the effectiveness of CCP therapy and advancing patient care in the fight against COVID-19.

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*Data Availability Statement: The data presented in this study are available upon reasonable request from the corresponding author.*

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# СРАВНИТЕЛЬНЫЙ АНАЛИЗ СИСТЕМЫ ГОМЕОСТАЗА ПЕРИФЕРИЧЕСКОЙ КРОВИ ПАЦИЕНТОВ ПРИ КОМБИНИРОВАННОМ ЛЕЧЕНИИ ПАЦИЕНТОВ С САХАРНЫМ ДИАБЕТОМ I И II ТИПА

# COMPARATIVE ANALYSIS OF THE PERIPHERAL BLOOD HEMOSTASIS SYSTEM IN PATIENTS WITH COMBINED TREATMENT OF PATIENTS WITH TYPE I AND II DIABETES MELLITUS

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Проведен анализ динамических изменений клеточных, биохимических показателей и интегральных индексов эндогенной интоксикации периферической крови 60 пациентов с сахарным диабетом в процессе лечения, а также проведен сравнительный анализ исследуемых показателей у пациентов, страдающих сахарным диабетом I-го и II-го типов до начала и после завершения терапии. В результате анализа полученных данных нами было установлено, что реактивность организма пациентов с СД 1-го типа характеризуется снижением относительного содержания нейтрофилов, уровня индекса соотношения нейтрофилов и моноцитов и уровня индекса соотношения нейтрофилов и моноцитов, а также общего содержания холестерина и мочевины. Реактивность организма пациентов с СД 2-го типа характеризовалась снижением общего содержания эритроцитов, уровня СОЭ, относительного содержания сегментоядерные нейтрофилов, уровней индекса соотношения лейкоцитов и скорости оседания эритроцитов, индекс соотношения нейтрофилов и моноцитов, индекс соотношения нейтрофилов и лимфоцитов, общего холестерина, глюкозы, также повышением содержания общего гемоглобина, общего белка и билирубина.

The analysis of dynamic changes in cellular, biochemical parameters and integral indices of endogenous peripheral blood intoxication in 60 patients with diabetes mellitus during treatment was carried out, as well as a comparative