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CONTENTS **СОДЕРЖАНИЕ**

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Secondary Infections in Patients with Extremely Severe COVID-19 During ECMO Therapy

Sergey S. Andreev[1]*, Mikhail V. Ketskalo[1,2], Polina O. Narusova[1], Maryana A. Lysenko[1,3]

[1] City Clinical Hospital № 52, Moscow City Health Department
3 Pekhotnaya Str., 123182 Moscow, Russia
[2] V. I. Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Ministry of Health of Russia,
4 Academic Oparin Str., 117997 Moscow, Russia
[3] N. I. Pirogov Russian National Medical Research University, Ministry of Health of Russia,
1 Ostrovityanov Str., 117997 Moscow, Russia

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***Correspondence to:** Sergey S. Andreev, e-mail: nerowolf@mail.ru

Summary

Up to 70% of patients hospitalized with COVID-19 need respiratory support, up to 10% need high–flow oxygen therapy, non-invasive and invasive ventilation. However, standard methods of respiratory support are ineffective in 0.4–0.5% of patients. In case of potentially reversible critical refractory respiratory failure those patients may require ECMO. Management of patients with extremely severe COVID-19 associates with numerous clinical challenges, including critical illness, multiple organ dysfunction, blood coagulation disorders, requiring prolonged ICU stay and care, use of multiple pharmacotherapies including immunosuppressive drugs. Pharmacological suppression of immunity is associated with a significant increase in the risk of secondary bacterial and fungal infections. Currently, data on epidemiology of secondary infections in patients with COVID-19 undergoing ECMO is limited.

Aim. To study the prevalence and etiology of secondary infections associated with positive blood cultures in patients with extremely severe COVID-19 requiring ECMO.

Materials and methods. A single-center retrospective non-interventional epidemiological study including 125 patients with extremely severe COVID-19 treated with ECMO in April 2020 to December 2021.

Results. Out of 700 blood culture tests performed in 125 patients during the study, 250 tests were positive confirming bacteremia/fungemia. Isolated pathogens varied depending on the duration of ECMO: gram-positive bacteria (primarily coagulase-negative staphylococci) dominated from the initiation of ECMO support; increased duration of ECMO associated with an increasing proportion of pathogens common in ICU (*Klebsiella pneumoniae* and/or *Acinetobacter baumannii* with extensively drug resistant and pan-drug resistant phenotypes, and vancomycin-resistant *Enterococcus faecium*). When ECMO lasted more than 7–14 days, opportunistic pathogens (*Candida species, Stenotrophomonas maltophilia, Providencia stuartii*, non-diphtheria corynebacteria, *Burkholderia species* and others) prevailed as etiological agents.

Conclusion. Longer duration of ECMO resulted in increasing rates of infectious complications. In patients undergoing ECMO for more than 14 days, the microbiological landscape becomes extremely diverse, which hampers choosing an empirical antimicrobial therapy. Since potential pathogens causing secondary infections in patients during ECMO are difficult to predict, rapid identification of rare opportunistic pathogens and their sensitivity profile, followed by targeted administration of antimicrobials, seems most beneficial.

Keywords: COVID-19; ECMO; secondary infections; multi-drug resistant pathogens; K. pneumoniae; antimicrobial therapy; opportunistic pathogens

Conflict of interest. The authors have no conflicts of interest to declare that are relevant to the content of this article.

Introduction

In 2019, the first cases of respiratory viral infection caused by a novel coronavirus named SARS-CoV-2 were described in the People's Republic of China [1]. In March 2020, the World Health Organization declared the disease caused by SARS-CoV-2, COVID-19 (COronaVIrus Disease 2019), a pandemic [2, 3]. COVID-19 has become not only a medical problem, but also a major social issue, leading to lockdowns and economic crises. By February 2022, the number of confirmed cases had reached 106 million and the number of deaths had exceeded 2.3 million [4].

According to epidemiologic studies, the severity of COVID-19 varied depending on the strain of SARS-CoV-2 infection. The number of patients with an extremely severe course of COVID-19 (requiring lung ventilation) reached 8.1% of the total number when infected with the «delta» strain B.1.617.2 [5].

COVID-19 is characterized by multi-organ involvement and leads to several complications, including respiratory failure, immune response hyperactivation syndrome («cytokine storm»), and coagulopathy [6]. Another concern is drug toxicity and drug interactions, especially in the setting of

polypharmacy in the ICU [7–9]. Patients with milder COVID-19 rarely have co-infections and secondary infectious complications. Routine administration of antibiotics to patients with viral infections, including COVID-19, does not reduce the risk of secondary infections or the likelihood of progression of viral lung injury [10, 11]. In patients with severe and extremely severe COVID-19, secondary infections (bacterial, fungal) may play a critical role in poor outcome [12].

To date, the WHO and national societies of different countries have adopted protocols for the management of patients with COVID-19 [6, 13, 14]. Some issues, especially in drug therapy, remain controversial, but most recommendations outline the main directions of management of patients with novel coronavirus infection: 1) antiviral therapy; 2) respiratory support; 3) anticoagulation and coagulation control; 4) immunosuppressive therapy to control the «cytokine storm»; 5) prevention and treatment of complications [15–17].

Despite a wide range of approved drugs with antiviral activity against SARS-CoV-2 (remdesivir, molnupiravir, nirmatrelvir/ritonavir, viral neutralizing monoclonal antibodies), none of them is intended to treat patients with extremely severe respiratory failure (requiring mechanical ventilation and/or extracorporeal membrane oxygenation) [6, 18]. Anticoagulant therapy is indicated in all patients with severe COVID-19 unless there are absolute contraindications [19]. Immunosuppressive therapy, including systemic steroids, genetically engineered biological agents that block effects of the key proinflammatory cytokine IL-6 (tocilizumab, sarilumab, levilimab), and extracorporeal therapies (plasmapheresis, therapeutic plasma exchange), is used in most patients with severe/extremely severe COVID-19 [6].

Respiratory therapy is the mainstay of severe and extremely severe COVID-19 treatment. Up to 70% of hospitalized patients require respiratory support, up to 10% require high flow oxygen therapy, non-invasive and invasive ventilation, and in 0.4–0.5% of patients conventional respiratory support proves ineffective. Extracorporeal membrane oxygenation (ECMO) is suggested for potentially reversible critical and refractory respiratory failure.

According to international consensus, it is not recommended to open new ECMO centers during a pandemic because of the high cost of material, human and organizational resources [20]. Nevertheless, ECMO often remains the only way to help patients with COVID-19 with critical respiratory failure (PaO_2/FiO_2 index reduction below 80 for more than 6 hours on protective parameters of respiratory support) [21, 22].

The management of patients in the ECMO center is associated with numerous clinical challenges, including critical illness, extremely severe damage to the lungs, other organs and systems, coagulation disorders, the need for prolonged stay in the intensive care unit, concomitant immunosuppressive and antimicrobial therapy. Antiviral therapy is irrelevant for patients in ECMO centers both because of the lack of sufficient scientific evidence (patients with COVID-19 requiring mechanical ventilation and/or ECMO are excluded from clinical trials) and because of the duration of the disease (acute respiratory distress syndrome develops 7–8 days after the onset of the disease, when there is no rationale for the use of antivirals). The main methods of COVID-19 drug therapy during ECMO remain anticoagulant and immunosuppressive, including systemic steroids and genetically engineered biological drugs (blockers of IL-6 receptors, IL-6 and IL-1) [6, 22]. However, medical immunosuppression is also associated with a significant increase in the risk of secondary infectious complications, primarily bacterial and fungal [24, 25].

Pathogens characterized by XDR (extensively drug resistant, meaning resistance to almost all but one or two classes of antibiotics) and PDR (pan-drug-resistant, meaning resistance to all classes of antimicrobials studied) phenotypes are typical of critically ill patients who stay in the ICU for long periods of time [26–28].

Study aim was to examine the prevalence and etiology of secondary blood-borne pathogen infections in patients with severe COVID-19 requiring ECMO.

Materials and Methods

We conducted a single-center, retrospective, noninterventional epidemiologic study including 125 patients with extremely severe COVID-19 treated at the ECMO Center of the Moscow City Clinical Hospital 52 of the Department of Healthcare from April 2020 to December 2021, who required extracorporeal membrane oxygenation (ECMO). The mean age of the patients was 48.7±10 years (18 to 72 years), 91 (72.8%) of them were men. 109 patients (87.2%) underwent veno-venous (VV) ECMO, 2 patients (1.6%) veno-arterial ECMO, and 14 patients (11.2%) had other ECMO circuit variations (including veno-veno-venous, veno-arterio-venous). The mean duration of ECMO was 18.5 days (ranging from 1 to 141 days).

Baseline patient characteristics were similar to those of the European ECMO Registry, but the proportion of patients with VV ECMO was significantly higher in the EuroELSO Registry patient cohort (92.5% vs. 87.2%, Fisher's exact test $P= 0.038$). Outcomes such as weaning from ECMO (χ^2 test $P<0.001$) and discharge from hospital (χ^2 test $P<0.001$) were significantly better in patients from the EuroELSO registry (Table) [22]. In the cohort of

Table. Characteristics of patients from the ECMO Center and the EuroELSO Registry (completed cases as of October, 3 2022).

Parameters	Registry	
	ECMO Center Registry (Hospital 52)	EuroELSO Registry
Number of patients	125	6.112
Mean age, years	48.7±10 [18;72]	51.6* [16; 84]
Men, %	72.8	72.6
Women, %	27.2	27.4
ECMO circuit type, %	87.2 — veno-venous 12.8 — other	92.5 — veno-venous 7.5 — other
Mean duration, days	18.5	26.3
Successful ECMO-weaning, *n* (%)	22 (17.6)	3,440 (56.3)
Discharged from the hospital, *n* (%)	12 (9.8)	3,259 (53.3)

Note. * — EuroELSO registry does not provide data on the standard deviation of age.

patients from the ECMO Center of Hospital 52, twenty-two patients (17.6%) were successfully weaned from ECMO and 12 patients (9.6%) were discharged from the hospital. In our opinion, one of the factors negatively influencing ECMO weaning and hospital mortality could be secondary infections, including those associated with bacteremia.

During hospitalization, 82.4% (103) of patients received renal replacement therapy. Drug therapy was administered according to the current version of the provisional «Guidelines of the Russian Ministry of Health for Prevention, Diagnosis and Treatment of Novel Coronavirus Infections (COVID-19)». All patients received IL-6 antagonists (tocilizumab, sarilumab, levilimab, olokizumab), while 79 patients (63.2%) received systemic steroids.

Blood cultures were obtained when a bacteremia-associated or fungemia-associated infection was suspected by the treating physician or as recommended by the clinical pharmacologist. Pathogen identification was performed by matrix-associated laser desorption/ionization time-of-flight mass spectrometry MALDI-TOF MS (Bruker), antibacterial susceptibility testing was performed by the Phoenix 100 automated system (BD), and beta-lactamase genes were detected by real-time PCR using the BacResist GLA reagent kit or the GeneXpert analyzer (Cepheid).

Statistical analysis of data. No prespecified power calculation was performed. All patients treated at the center during the study period were included in the study. Means and standard deviations were used for descriptive statistics. Data were analyzed using the IBM SPSS STATISTICS V22.0 statistical software package.

Results

A total of 700 blood tests were performed in 125 patients, of which bacteremia/fungemia was detected in 250 cases. The frequency of positive blood cultures increased significantly with the duration of ECMO: from 18.4% (in 23 of 125 patients) in the first 48 hours after the start of the procedure to 68.4% (in 39 of 57 patients) 14 days or more after the start of ECMO (fig. 1).

We observed a change in the pattern of pathogens isolated from blood culture as a function of time after ECMO initiation: initially, Gram-positive pathogens (primarily coagulase-negative staphylococci) were predominant. When the duration of ECMO exceeded 7 days, the most common pathogens were those typical of intensive care units (*Klebsiella pneumoniae, Acinetobacter baumannii* with XDR/PDR phenotype, vancomycin-resistant Enterococcus faecium) and opportunistic agents (*Candida species, Stenotrophomonas maltophilia, Providencia stuartii*, non-diphtherial *Corynebacterium species*, *Burkholderia species*, and others).

Gram-positive pathogens. The percentage of gram-positive pathogens was maximal in the first 48 hours after ECMO initiation: they were isolated in 17 of 125 patients (13.6%) (Fig. 1).

The pathogen profile changed over time (Fig. 2). Coagulase-negative staphylococci (CoNS), including oxacillin-resistant, were dominant, accounting for 14 of 28 (50%) of all positive blood cultures in the first 2 days of ECMO. With increasing duration of

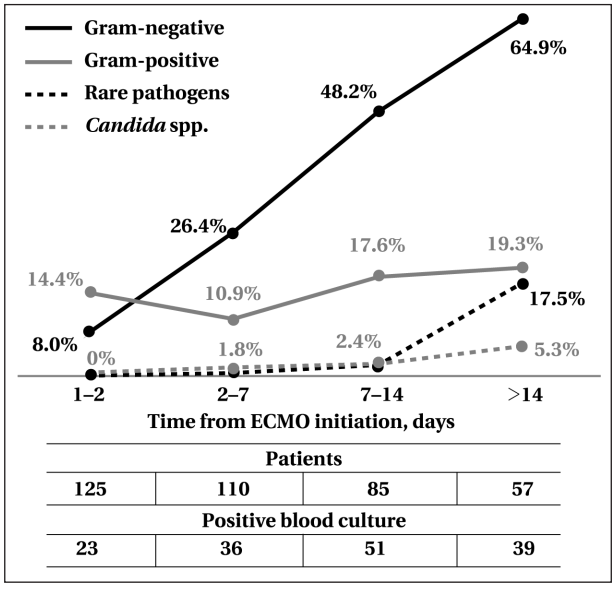

Fig. 1. Frequency of pathogen isolation from blood culture in patients during ECMO.

ECMO, the percentage of bacteremia due to *E. faecium*, including vancomycin-resistant, increased from 6.5% (1 of 14) in the first 2 days of ECMO to 12.8% (in 5 of 39 blood cultures) after 14 or more days of ECMO. *S. aureus* was not isolated in patients during ECMO.

Gram-negative pathogens. The proportion of gram-negative pathogens increased with increasing duration of ECMO: from 8.8% (in 11 of 125 patients) during the first 48 hours to 64.9% (in 37 of 57 patients) when ECMO was performed for more than 14 days (Fig. 3).

The most common pathogen was *K. pneumoniae* with XDR phenotype and resistance to carbapenems in 100% and PDR phenotype in 9 cases. The incidence of *K. pneumoniae* bacteremia during ECMO increased from 4% (5 cases in 125 patients) at day 2 to 37.6–41.2% (in 32 of 85 and 24 of 57 patients receiving ECMO for 7–14 and more than 14 days, respectively). Other *Enterobacterales* were less common and were mostly isolated during prolonged ECMO. *Escherichia coli, Proteus mirabilis,* and *Serratia marcescens* were isolated from blood cultures in some patients.

Acinetobacter baumannii was isolated in patients from the first 48 hours of ECMO initiation. The incidence of *A.baumannii* bacteremia increased from 5 cases (4% of all patients) in the early period to 8.8% (in 5 of 57 patients) with long duration of ECMO. All isolated strains were characterized by the XDR phenotype and remained susceptible only to polymyxins.

Pseudomonas aeruginosa was isolated from blood cultures in 2 patients after 7 days of ECMO and in 4 patients with ECMO duration longer than 14 days. All *P. aeruginosa* strains isolated were resistant to carbapenems.

Resistance genes were detected in 17 patients (13.6% of all patients included in the study). The isolated bacteria were characterized by a high diversity of beta-lactamase encoding genes: *Enterobacterales* (mainly *K. pneumoniae*) had class A (CTX-M, TEM and SHV; KRS), D (OXA-48-like) and B (NDM) beta-lactamase genes, *A. baumannii* had class D carbapenemases (OXA-23-, OXA-40-, OXA-51-like), while *P. aeruginosa* had class A and B (IMP, NDM and VIM). In one patient, the mechanism of resistance could not be verified in *K. pneumoniae* with the panresistant phenotype (PCR did not identify the genes encoding the most common class A, B and D beta-lactamases).

Invasive candidiasis. Invasive candidiasis with candidemia was detected in 7 patients (5.6% of the total study population). The incidence of candidemia increased with prolonged ECMO duration, from 0% at baseline to 8.8% (in 5 of 57 patients) at 14 days. Different *Candida species* were identified: *C. albicans* — in 5 samples, *C. auris* — in 3 samples,

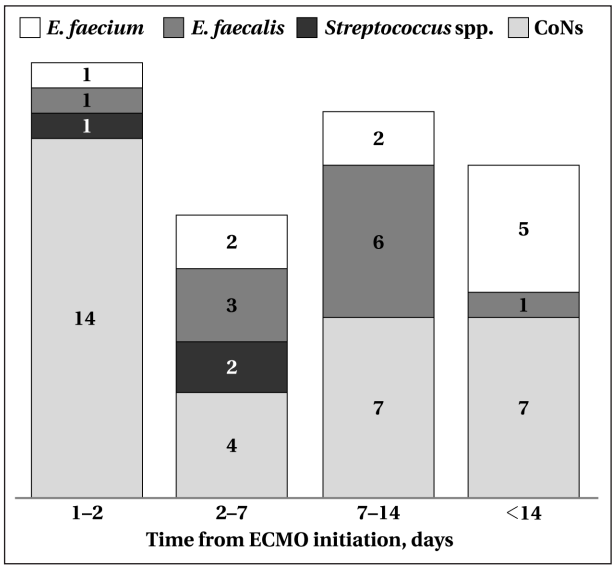

Fig. 2. Isolation of Gram-positive pathogens from blood cultures at different durations of ECMO (absolute number of pathogens isolated).

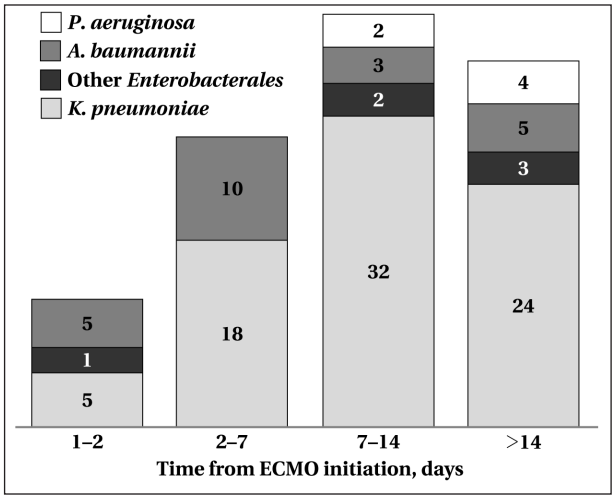

Fig. 3. Isolation of Gram-negative pathogens from blood cultures at different durations of ECMO. Absolute number of pathogens isolated shown.

C. parapsilosis — in 1 case. In one patient with *C. auris* candidemia, the pathogen was isolated three times and blood culture sterility could not be achieved.

Infections caused by rare opportunistic pathogens. During prolonged ECMO, a large number of non-ICU pathogens were isolated from patients' blood cultures: in 3 of 85 patients (3.5%) after 7 days of ECMO and in 8 of 57 (14%) when ECMO lasted longer than 14 days. The spectrum of pathogens included gram-negative (*Providencia stuartii, Stenotrophomonas maltophilia, Burkholderia cepacia* and *B. multivorans, Delftia acidovorans, Achromobacter xylosoxidans*) and Gram-positive

bacteria (*Corynebacterium striatum* and other non-diphtheria *Corynebacterium species*).

Discussion

Patients in ECMO centers are characterized by a combination of risk factors for secondary infections, including those caused by extremely resistant Gram-positive (oxacillin-resistant *Staphylococcus*, vancomycin-resistant *Enterococcus*), Gram-negative (carbapenem-resistant *Enterobacterales* and non-fermenting Gram-negative bacteria), and opportunistic pathogens (fungi, rare pathogens). Several risk factors for an infection directly relate to ECMO: cannulae that cannot be removed if a bloodstream infection develops, changes in the pharmacokinetics of antimicrobial drugs that prevent them from reaching adequate concentrations at the site of infection. Other infection promoting factors do not relate to ECMO: critical illness, prolonged ICU stay, massive lung tissue injury, prolonged lung ventilation, leukopenia, lymphocytopenia, secondary hypogammaglobulinemia, concomitant drug therapy and drug interactions, renal replacement therapy, need for repeated blood component transfusions [26, 29]. According to epidemiologic studies, the SARS-CoV-2 virus itself serves as a risk factor for the development of some secondary infections in patients with severe COVID-19, mainly mycoses [30, 31].

The results of the study (successful weaning from ECMO, discharge from hospital) were worse than those presented in the European and global ECMO registry [22], with a higher frequency of secondary infections complicated by bacteremia, which in our opinion contributed significantly to the attributable mortality of the patients.

The spectrum of isolated Gram-negative pathogens was consistent with data from regional and local microbiological studies, but there was an early development of severe secondary infections complicated by bacteremia on days 2–5 of ECMO [32, 33]. This was probably due to both the initial severity of the patients' illness and the combination of infection risk factors in patients with critical lung injury. With a relatively high frequency of Co-NS isolation, a low frequency of bacteremia due to *Staphylococcus aureus*, both MSSA and MRSA, was observed, although this pathogen was isolated in 8 patients from other sites (respiratory tract, pleural fluid, urine).

The widespread use of polymyxins as the only effective antimicrobial therapy for infections caused by XDR Gram-negative pathogens has led to the selection of pathogens naturally resistant to colistin and an increase in bacteremia caused by Gram-positive bacteria, fungi, *Enterobacterales* (*Proteus mirabilis, Serratia marcescens*), non-fermenting Gram-negative bacteria (*Providencia stuartii, Burkholderia cepacia*).

Based on the results of this study, a modification of the perioperative prophylaxis and empirical antimicrobial therapy regimen in the first 48 hours after ECMO initiation is recommended: the spectrum of antimicrobial agents should cover Gram-positive pathogens, primarily coagulase-negative staphylococci, with separate consideration of MR-CoNS risk factors.

When prescribing empirical antimicrobial therapy for prolonged ECMO, the microbiologic profile of the patient's ward and previous medications are considered. The predominant pathogens are *K. pneumoniae* and *A. baumannii*, which are carbapenem-resistant and produce a wide range of class A, D, and B beta-lactamases. Due to the prolonged stay of ECMO cannulas and the impossibility of their removal, the prescribed drugs must be active against pathogens with a high potential for biofilm formation (e.g. *Burkholderia cepacia, Candida species*).

Predicting which pathogen will cause a «new wave» of infection in a patient on ECMO for more than 7 days and receiving broad-spectrum and extra-broad-spectrum antimicrobial therapy is challenging. A significant increase in both typical pathogens characterized by extreme and pan-resistance (e. g., Gram-negative bacteria with high frequency of clinical and/or microbiological resistance to polymyxins, high frequency of infections caused by vancomycin-resistant *E. faecium*) has been observed. During prolonged ECMO, a large number of atypical ICU pathogens have been isolated from blood cultures. Rare pathogens with natural resistance to most antibiotics (non-fermenting Gram-negative bacteria, non-diphtheria *Corynebacterium*) were isolated in 17.5% of patients 14 days after the start of ECMO. Most of the isolated pathogens have been described as extremely rare infectious agents in immunocompromised patients. They are characterized by multiple drug resistance and the ability to cause nosocomial infections of the bloodstream, respiratory tract, and urinary tract [34–36].

The increase in infections due to *E. faecium*, especially vancomycin-resistant (VRE) bacteria, was probably due to the combination of typical risk factors including the prescription of a wide range of antibacterial drugs targeting Gram-negative pathogens and the high frequency of empirical oral vancomycin administration in patients with diarrhea.

The increased incidence of invasive candidiasis was probably related to an «accumulation» of risk factors for opportunistic infections such as critical illness, prolonged mechanical ventilation, prolonged placement of invasive lines (ECMO cannulas, central venous catheters, arterial catheters), renal replacement therapy, critical illness-related and medical immunosuppression, antibiotic therapy, repeated

transfusions of blood components, and prolonged lymphopenia due to viral infection. The choice of antifungal agents for invasive candidiasis in patients on ECMO is limited. This is due to both patient characteristics (changes in drug pharmacokinetics due to critical illness and the presence of an ECMO circuit) and microbial characteristics (high prevalence of non-albicans *Candida species*, including *C. auris*). The inability to completely eradicate the pathogen from the bloodstream in *C. auris* candidemia was probably due to its properties such as high rate of biofilm formation and multiple resistance to antifungal drugs, as well as the impossibility of removing the ECMO cannulae. Significant variability of pharmacokinetics in critical illness, high risk of adverse drug interactions, inability to remove invasive lines (primarily ECMO cannulae), spectrum of pathogens make echinocandins the preferred option of antifungal therapy, while the use of triazoles is ineffective both due to resistance of microfungi and suboptimal pharmacokinetic parameters.

The results suggest that routine administration of many antimicrobial classes, such as third-generation cephalosporins, fluoroquinolones, and fluconazole, is ineffective in patients with extremely severe COVID-19. Most isolated pathogens are resistant to these classes of antimicrobials, and their administration carries a high risk of selection of multidrug-resistant strains (the concept of «collateral damage») and is associated with a high potential for drug toxicity and drug interactions [37, 38].

Study limitations. The study was retrospective and non-comparative and did not take into account the characteristics of different viral strains (patients were studied during «waves» caused by SARS-CoV-2 strains from «alpha» B.1.1.7 to «delta» B.1.617.2). Resistance mechanisms were detected in only a small fraction of Gram-negative bacteria.

The isolation of some bacteria (coagulase-negative staphylococci, rare non-fermenting Gram-negative bacteria such as *Burkholderia* spp., *Delftia acidovorans*, etc.) from blood culture was not considered contamination. The isolation of pathogens from the sterile site (blood) in critically ill patients with systemic inflammatory reaction was always considered clinically significant. These data were confirmed by repeated isolation of these rare and atypical pathogens from blood culture and/or other sites, clinical efficacy of antimicrobial therapy targeting isolated pathogens.

The frequency of invasive candidiasis was lower than that reported by other centers, which may be due to diagnostic problems (clinical inability to collect large daily blood samples for microbiological examination; unavailability of routine determination of serum mannan/antimannan antibody levels).

Conclusion

Patients with severe COVID-19 requiring ECMO are at high risk for secondary infections. The incidence of infectious complications, including those associated with bacteremia/fungemia, increases progressively during ECMO and reaches 68.4% at a duration of more than 14 days. During the first 48 hours, coagulase-negative staphylococci, including those resistant to oxacillin, play a leading role. With increasing duration of ECMO, the incidence of bacteremia caused by Gram-negative bacteria with extreme drug resistance and panresistance phenotypes increases. When the duration of ECMO is longer than 14 days, the patient's microflora becomes extremely diverse, with the most common pathogens being Gram-negative bacteria with XDR and PDR resistance phenotypes, vancomycin-resistant enterococci, *Candida species*, and rare opportunistic agents.

As the spectrum of pathogens causing secondary infections in ECMO patients becomes more diverse and difficult to predict, rapid identification of rare opportunistic pathogens and their susceptibility profile (MALDI-TOF MS, antigenic studies, staining with special methods, PCR, etc.) as well as targeted antimicrobial therapy are crucial.

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The Predictive Value of Cystatin C for AKI in Patients with COVID-19

Magomedali O. Magomedaliev[1], Daniil I. Korabelnikov[1,2], Sergey E. Khoroshilov[3]

[1] Military Clinical Hospital 1586, Ministry of Defense of Russia, Russia
[2] Haass Moscow Medical and Social Institute,
5 Brestskaya 2nd Str., 123056 Moscow, Russia
[3] Academician N. N. Burdenko Main Military Clinical Hospital,
Ministry of Defense of Russia, Russia

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***Correspondence to:** Magomedali O. Magomedaliev, magomedalim@mail.ru

Summary

Objective. To evaluate a potential of cystatin C blood concentration to predict acute kidney injury (AKI) in patients with severe and extremely severe pneumonia associated with COVID-19.

Materials and methods. An observational prospective study of 117 patients with severe and extremely severe pneumonia associated with a COVID-19 in an ICU setting was conducted in 2020–2022 (site: multifunctional Medical Center, 1586 Military Clinical Hospital of the Ministry of Defense of Russia, Moscow Region, Russia). Routine laboratory tests and instrumental examinations were performed according to generally accepted protocols. Cystatin C concentrations in blood (s-CysC) and urine (u-CysC) were measured by immunoturbidimetric method.

Results. AKI was diagnosed in 21 (17.9%) patients, kidney dysfunction without AKI was found in 22 (18.8%) patients with severe and extremely severe pneumonia associated with COVID-19. s-CysC and u-CysC levels in the group of patients with AKI were statistically significantly higher compared to the levels in the group of patients without AKI. The levels of s-CysC obtained within Day 1 — T (-1), and Day 2 — T (-2) prior to AKI onset turned out to be the independent factors for AKI development in patients with severe and extremely severe pneumonia associated with COVID-19: OR 5.37, Wald chi-square 5.534 (CI: 1.324; 21.788); P=0.019 and OR 3.225, Wald chi-square 4.121 (CI: 1.041; 9.989); P=0.042, respectively. s-CysC T (-2) value is informative, and s-CysC T (-1) is a highly informative predictor of AKI development in severe and extremely severe pneumonia associated with COVID-19: ROC AUC 0.853 (95% CI, 0.74–0.966), P<0.001) with 90% sensitivity and 73% specificity at a cut-off of 1.67 mg/L, and ROC AUC 0.905 (95% CI, 0.837–0.973), P<0.001) with 90% sensitivity and 73% specificity at a cut-off of 1.69 mg/l, respectively. Serum CysC levels started increasing 3 days prior to AKI onset, outpacing the increase of SCr levels. The u-CysC levels were not predictive of AKI development. Impaired renal function probability was increasing with patients' age (P<0.0001).

Conclusions. Serum CysC seems to be a statistically significant predictor of AKI. s-CysC levels started increasing 3 days prior to AKI onset, surpassing the increase of SCr levels in patients with severe and extremely severe pneumonia associated with COVID-19. Urine CysC did not achieve statistical significance as a predictor for AKI, although u-CysC concentrations were significantly higher on days 3, 2, 1 prior to AKI onset and on the day of AKI onset in the group of patients with AKI.

Keywords: acute kidney injury; AKI; cystatin C; s-CysC; u-CysC; COVID-19; pneumonia development

Conflict of Interest Disclosures. The authors declare no conflict of interest.

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Introduction

COVID-19 is an infectious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [1]. The first outbreak of COVID-19 was reported in late 2019 in Wuhan City, Hubei Province, People's Republic of China [2]. According to the World Health Organization, as of June 10, 2022, there were more than 532,201,219 cases and 6,305,358 deaths from COVID-19 worldwide [3].

SARS-CoV-2 enters the human body through the epithelium of the upper respiratory tract [4], stomach and intestines [5]. Approximately 81% of people have a mild illness, while 14% have severe manifestations and 5% progress to an extremely severe illness [6].

Due to the severity of the disease, about 10.2% of patients with COVID-19 are treated in the intensive care unit (ICU) [7].

According to Li X. et al. (2020), 11% of patients treated in the ICU develop multiple organ failure [8], including acute kidney injury (AKI) with pulmonary-renal syndrome [9].

The combination of acute respiratory failure and renal injury may worsen the patient's condition, significantly reduce the efficacy of treatment, and complicate the outcome of the disease [10].

Clinical manifestations of renal dysfunction range from isolated proteinuria (43.9%) and hematuria (26.7%) [11] to AKI requiring renal replacement therapy (RRT). When renal dysfunction occurs, hospital mortality increases from 13.2% with normal blood creatinine levels to 33.7% with elevated blood creatinine levels [12]. According to American researchers (Richardson S. et al., 2020), among 5.700 patients hospitalized with COVID-19, AKI requiring RRT was diagnosed in 3.2% of cases, while in patients receiving ICU therapy it was found in 22% of cases [13]. AKI was more likely to develop in patients with elevated serum creatinine (SCr) at baseline compared with those in the reference range [11].

Chinese researchers (Cheng Y. et al., 2020) diagnosed AKI in 5.1% of 701 hospitalized patients with COVID-19 [11].

A similar incidence of AKI in COVID-19 was reported by other investigators (Yildirim C. et al., 2021), who identified AKI in 4.9% of patients with COVID-19 (17 cases out of 348) [14].

A significantly higher incidence of AKI was described in a retrospective study (Diao B. et al., 2021), where it was observed in 27.06% of cases (in 23 of 85 patients). Dependency analysis of factors in the groups of patients with AKI showed that the prevalence of AKI among patients was associated with age (65.22% if ⩾60 years vs. 24.19% if <60 years, $P<0.001$), comorbidities (69.57% if present, 11.29% if absent, $P<0.001$), hypertension (39.13% present, 2.90% absent, $P=0.0007$), and coronary artery disease (21.74% present, 4.84% absent, $P=0.018$) [15].

A meta-analysis by Chen Y.-T. et al. (2020), which included 20 studies (China, Italy, UK, USA), found that AKI occurred in 8.9% of 6,945 patients with COVID-19 (95% CI, 4.6–14.5) [16].

Currently, there is a need to identify a reliable, highly sensitive and specific biomarker for the prognosis and early diagnosis of AKI.

Literature review has shown that CysC is a reliable diagnostic and prognostic biomarker of AKI, and its concentration directly correlates with the severity of kidney damage [17]. The more renal function is impaired, the higher the concentration of cystatin-C in blood (s-CysC) and urine (u-CysC) is determined [18,19].

Literature data suggest both an anticipatory increase in s-CysC during AKI and an earlier decrease in its level compared to SCr when AKI resolves with treatment ($P<0.001$) [20].

CysC is a polypeptide that blocks the destruction of the extracellular protein matrix by inhibiting cysteine proteases. It is produced by nucleated cells, does not enter the systemic circulation, and is 99% metabolized in the kidneys [21], while the remaining insignificant amount is excreted unchanged in the urine [22].

The above kinetics and other characteristics provide a rationale for considering CysC as an almost ideal endogenous biomarker that allows an objective assessment of renal function [23].

Aim: To investigate the prognostic value of cystatin C levels in blood serum and urine for confirmation of acute kidney injury in patients with severe and extremely severe pneumonia associated with COVID-19.

Materials and Methods

An observational study with the participation of patients with severe and extremely severe (critical) COVID-19 treated in 2020–2022 in the ICU of the Multidisciplinary Medical Center, 1586 Military Clinical Hospital of the Ministry of Defense of Russia (1586 MCH) was conducted. The study included 117 patients with severe and critical COVID-19-associated pneumonia, of whom 75 were men and 42 were women.

Inclusion criteria:

— Age 18 to 80 years;

— COVID-19 confirmed by polymerase chain reaction (PCR) identification of viral genetic material in nasopharyngeal swabs; and/or antibodies detected by enzyme-linked immunoassay in blood; typical clinical and laboratory presentation, lung involvement documented by computed tomography;

— Signs of severe pneumonia, i.e, at least one of the following: dyspnea (respiratory rate >30/min), SpO_2⩽93%, oxygenation index ⩽300 mm Hg, agitation, impaired consciousness, hemodynamic instability (systolic blood pressure less than 90 mm Hg and/or diastolic blood pressure less than 60 mm Hg), oligo- or anuria, CT evidence of viral-induced lung injury (grade 3–4 on the semi-quantitative scale used in Russia), arterial blood lactate >2 mmol/L, two or more qSOFA scores, acute respiratory distress syndrome, respiratory failure requiring respiratory support, including high-flow oxygen therapy and noninvasive ventilation, septic shock, multiple organ failure.

Exclusion criteria were chronic kidney disease or suspected chronic kidney disease; proteinuria and hematuria in the previous 3 months; history of renal transplantation; iatrogenic complications (pneumothorax, hemothorax, chylothorax, aspiration pneumonia, allergic reactions to medications).

Patients received intensive multimodal intensive therapy according to the current provisional guidelines for prevention, diagnosis and treatment of novel coronavirus infection (COVID-19).

According to the results of the studies performed, patients were divided into two groups: group 1 ($N=96$) including patients without AKI and group 2 ($N=21$) of patients with AKI.

The clinical, laboratory and instrumental characteristics of the groups are shown in Table 1.

Table 1. Clinical and laboratory characteristics of patients.

Parameters	Values of parameters in groups				
	Patients, total, *n*=117	Without AKI, n=96	With AKI, n=21	Mann–Whitney *U*-test	*P*-value
Age, years	49 (43; 62)	47,5 (41; 55)	65 (58; 71)	*U*=157.5; *Z*=3.755	0.0002
Male/female, *n*/*n*	75/42	66/30	9/12	—	—
Mortality, *n* (%)	26 (22.2)	15 (15.62)	11 (52,4)	—	—
Severity of disease according to the NEWS scale, points, max	10 (8; 11)	9 (8; 11)	12.5 (10; 13)	*U*=202.5; *Z*=–3.219	0.001
Urea, mmol/L, max	8.55 (6.7; 12.1)	7.7 (6.6; 10.4)	17.25 (11,6; 20.2)	*U*=103; *Z*=–4.486	0.00001
Creatinine, μmol/L, max	104 (94; 129)	99.5 (94; 104)	174.5 (156; 309)	*U*=15; *Z*=–5.632	<0.001
C-reactive protein, mg/L, max	134.25 (62; 1759)	118.1 (58.65; 166.1)	173.8 (63.1; 203.5)	*U*=303; *Z*=–1.881	0.0599
Ferritin, μg/L, max	560.9 (102; 708.3)	596.35 (102; 711)	102 (102; 579.5)	*U*=298; *Z*=1.971	0.0486
Leukocyte count, 1,000 cells/μL, min	7.05 (5.76; 8.92)	6.65 (5.38; 8.41)	8.45 (6.95; 10.7)	*U*=285.5; *Z*=–1.983	0.0473
Lymphocytes, %, min	3.5 (2; 7)	4 (2; 8)	2.5 (1; 4)	*U*=344.5; *Z*=1.352	0.1763
Platelets, 1,000 cells/μL, min	165 (120; 220)	178 (147; 224)	99 (51; 123)	*U*=168.5; *Z*=3.231	0.0012
D-dimer, mg/L, max	2.19 (0.66;7. 67)	1.55 (0.61; 4.53)	9.995 (2.78; 10)	*U*=229.5; *Z*=–2.841	0.0045

Note. For rows 1, 4–12, results given as *Me* (*Q1*; *Q3*). Max represents the maximum value for all days of stay in the ICU, min represents the minimum value for all days of stay in the ICU.

The study was approved by the local ethics committee of the Moscow Haass Medical and Social Institute and was conducted in accordance with the principles of good clinical practice (GCP) and national standards of medical care to ensure the safety and well-being of study participants.

Laboratory Methods. All instrumental and laboratory tests were performed at 1586 MCH according to existing standards and protocols, and the results were documented prospectively from the time of patient admission to the ICU until discharge from the hospital. Venous blood and urine samples were collected simultaneously during the first 24 hours of ICU admission and then once daily from 6:00 am to 7:00 am, and delivered to the laboratory within 10–20 minutes. In some cases, samples were frozen at –20°C and brought to the laboratory as they accumulated, followed by a single thaw.

Levels of s-CysC and u-CysC were determined by immunoturbidimetric method on an automated biochemical analyzer AU 480 (Beckman Coulter, Inc., Brea, CA, USA), using reagents from DiaSys Diagnostic Systems GmbH, Holzheim, Germany.

The following abbreviations were used to indicate the stages of the study: T (-3) — 3 days before development of AKI; T (-2) — 2 days before development of AKI; T (-1) — 1 day before development of AKI; T (0) — day of development of AKI; T — first day of ICU admission.

Statistical Analysis. After the initial assessment of clinical and laboratory data, differences between the groups were determined. Statistical analysis was performed with Excel 2013 (Microsoft Office 2013, Microsoft, USA) and SPSS Statistics (IBM, USA) software packages. Significance of intergroup differences was determined by the nonparametric Mann–Whitney *U* test. Logistic regression was used to predict the likelihood of AKI using multiple s-CysC and u-CysC values. The optimal threshold for predicting AKI with relevant sensitivity and specificity was determined using the ROC curve. Data were presented as median (*Me*) and interquartile range (*Q1* and *Q3*).

The estimated power of the sample was determined according to the method (formula) of Peduzzi P. et al. (1996), developed to determine the minimum sample size in logistic regressions [24], and was 55 patients. Considering that the estimated sample size was less than 100 patients, the power was increased using the method of J. Scott Long [25].

Differences at $P<0.05$ were considered significant.

Results

Of the 117 patients enrolled in the study, 17.9% (*N*=21) were diagnosed with AKI according to KDIGO (Kidney Disease: Improving Global Outcomes) criteria, including 10 patients with stage 1, 4 patients with stage 2 and 7 patients with stage 3.

Increased SCr was observed in all cases, while urine volume decreased in only three patients. Renal dysfunction (increased SCr above the reference range but not meeting KDIGO diagnostic criteria) was observed in 18.8% (*N*=22) of cases, suggesting that renal function was impaired in at least 36.8% (*N*=43) of patients overall. RRT (sessions of prolonged veno-venous hemodiafiltration) was performed in four patients.

22.2% of patients (*N*=26) died, including 52.4% (*N*=11) in the group with AKI and 15.62% (*N*=15) in the group without AKI (χ^2 test 13.468, $P<0.001$).

The immediate cause of death was acute respiratory failure in 19 patients, sepsis in 2, and heart failure in 5.

CysC concentrations in the groups are shown in Table 2. The analysis showed significant intergroup differences in s-CysC at different time points: T (*P*=0.0270), T (0) (*P*<0.001), T (-1) (*P*<0.001), T (-2) (*P*=0.0002), T (-3) (*P*=0.0218).

We also observed significant intergroup differences in u-CysC levels, except for the first day of hospitalization in ICU: T (*P*=0.1299), T (0) (*P*=0.0396),

Table 2. Intergroup differences in the levels of CysC, *Me* (*Q1*; *Q3*).

Material	CysC concentration. mg/L			Mann-Whitney *U*-test	*P-value*
	Patients, total, *n*=117	Without AKI, *n*=96	With AKI, *n*=21		
		Blood			
During all days in the ICU, max	1.64 (1.54; 1.98)	1.52 (1.22; 1.69) max	2.3 (1.86; 3.25)	*U*=61; *Z*=5.033	<0.001
T	1.37 (1.09; 1.69)	1.325 (1.055; 1.625)	1.67 (1.37; 1.79)	*U*=268.5; *Z*=–2.211	0.0270
T (0)	1.59 (1.31; 1.83)	1.52 (1.22; 1.69) max	2.155 (1.9; 2.6)	*U*=57; *Z*=–5.085	<0.001
T (-1)	1.56 (1.285; 1.82), *n*=76		1.98 (1.82; 2.3)	*U*=78; *Z*=–4.566	<0.001
T (-2)	1.555 (1.26; 1.79), *n*=74		1.9 (1.79; 2.1), *n*=16	*U*=102; *Z*=–3.708	0.0002
T (-3)	1.55 (1.26; 1.7), *n*=73		1.705 (1.58; 1.91), *n*=15	*U*=171.5; *Z*=–2.294	0.0218
		Urine			
During all days in the ICU, max	0.465 (0.19; 1.87)	0.36 (0.17; 1.55)	1.835 (0.9; 5.53)	*U*=219; *Z*=–2.969	0.0030
T	0.35 (0.15; 0.685)	0.25 (0.15; 0.51)	0.57 (0.32; 1.42)	*U*=320.5; *Z*=–1.514	0.1299
T (0)	0.42 (0.19; 1.7)	0.36 (0.17; 1.55) max	1.055 (0.41; 4.59)	*U*=289.5; *Z*=2.057	0.0396
T (-1)	0.45 (0.19; 1.75)		1.2 (0.6; 3.27)	*U*=175.5; *Z*=2.636	0.0084
T (-2)	0.42 (0.18; 1.73)		1.315 (0.63; 2.7)	*U*=214; *Z*=1.668	0.0452
T (-3)	0.42 (0.19; 1.54)		1.2 (0.57; 1.5)	*U*=159; *Z*=2.156	0.0311

Note. For tables 2–4: T — first day of the hospitalization in the ICU; T (0) — the day of AKI development; T (-1) — 1 day prior to AKI development; T (-2) — 2 days prior to AKI development; T (-3) — 3 days prior to AKI development. max — the maximum value for all days of ICU stay.

T (-1) (*P*=0.0084), T (-2) (*P*=0.0452), T (-3) (*P*=0.0311).

In the control patients (without AKI), cystatin-C was not measured daily but every 48–72 hours, so the maximum cystatin-C concentrations during all days in the ICU were used to calculate intergroup differences, logistic regression and ROC analyses.

Regression analysis (Table 3) showed a significant relationship between s-CysC level on the day of AKI development (s-CysC, T (0) mg/L: B=2.175; Wald χ^2=8.184; exponent=8.805 [95% CI, 1.984; 39.081]; *P*=0.004), 1 day earlier (s-CysC, T (-1) mg/L: B=1. 681; Wald χ^2=5.534; OR 5.37; [95% CI, 1.324; 21.788]; *P*=0.019) and 2 days earlier (s-CysC, T (-2) mg/L: B=1.171; Wald χ^2=4.121; OR 3.225 [95% CI, 1.041; 9.989]; *P*=0.042), and no significant association between u-CysC and development of AKI.

The s-CysC, T (0), s-CysC, T (-1), and s-CysC, T (-2) were the prognostically significant models, and ROC analysis was performed to evaluate their quality and determine the area under the ROC curve and the optimal CysC threshold for predicting the development of AKI (Table 4): (s-CysC, mg/L, T (0) ROC AUC 0.936 (95% CI, 0.883-0.99; *P*<0.001), sensitivity 92%, specificity 84%; s-CysC, threshold value 1.79 mg/L, T (-1) ROC AUC 0.905 (95% CI, 0.837–0.973; *P*<0.001), sensitivity 92%, specificity 78%; s-CysC, 1.69 mg/L, T (-2) ROC AUC 0.853 (95% CI, 0.74–0.966; *P*<0.001), sensitivity 90%, specificity 73%; CysC, mg/L, threshold value 1.79 mg/L (Fig.).

Discussion

The search for promising laboratory markers to assess the COVID-19 patients and predict potential complications, including AKI, continues.

Studies have been published showing an increase in s-CysC concentration prior to the development of AKI and progression of lung infiltration in COVID-19. It has also been reported that s-CysC levels are higher in patients who later died.

In the present study, 36.8% (*N*=43) of the patients had impaired renal function. AKI developed in 17.9% (*N*=21) and renal dysfunction without progression to AKI was revealed in 18.3% patients (*N*=22), indicating a significant prevalence of renal impairment.

The incidence of renal impairment in COVID-19 varies widely from 0.5 to 36.6% [26] and may depend on the clinical manifestations of COVID-19, direct toxic effects of the virus, hypoxia and development of shock [27]. Proteinuria has been reported in

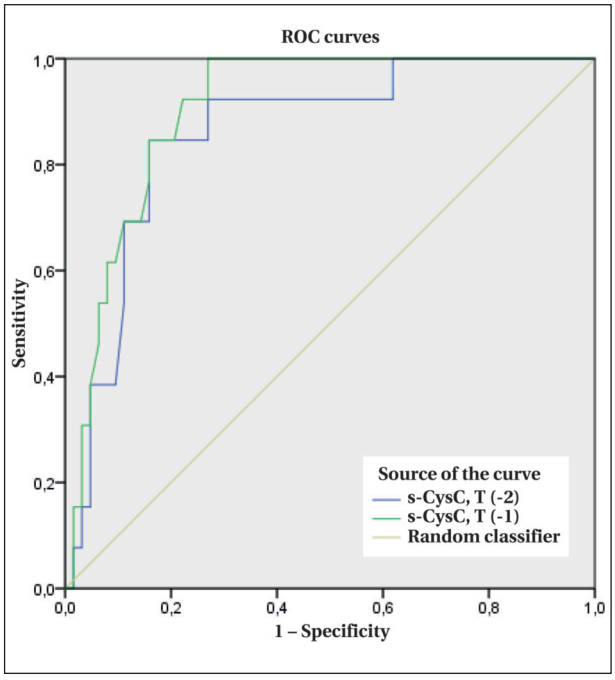

Fig. AUC ROC value of s-CysC T (-2), s-CysC T (-1) for the prediction of AKI development.

Table 3. Logistic regression for predicting AKI development in patients with severe and extremely severe pneumonia associated with COVID-19.

Time of CysC measurement	B	SE	Wald χ^2	*P*-value	OR	95% CI	
						Lower limit	Upper limit
Blood							
T	1.238	0.852	2.109	0.146	3.448	0.649	18.327
T (0)	2.175	0.76	8.184	0.004	8.805	1.984	39.081
T (-1)	1.681	0.715	5.534	0.019	5.37	1.324	21.788
T (-2)	1.171	0.577	4.121	0.042	3.225	1.041	9.989
T (-3)	0.585	0.495	1.398	0.237	1.794	0.681	4.73
Urine							
T	0.072	0.136	0.279	0.597	1.074	0.823	1.402
T (0)	0.119	0.97	1.513	0.219	1.127	0.932	1.363
T (-1)	0.089	0.113	0.618	0.432	1.093	0.876	1.363
T (-2)	0.057	0.123	0.212	0.645	1.058	0.832	1.346
T (-3)	0.061	0.126	0.235	0.628	1.063	0.831	1.359

Note. B — coefficient; SE — standard error; OR — odds ratio.

Table 4. ROC analysis for predicting the development of AKI in patients with severe and extremely severe pneumonia associated with COVID-19.

Time of measurement of CysC	AUC of the ROC-curve	*P*-value	SE	95% CI		Cut-off value of CysC, mg/L	Sensitivity, %	Specificity, %
				Lower limit	Upper limit			
T (0)	0.936	<0.001	0.027	0.883	0.99	1.79	92	84
T (-1)	0.905	<0.001	0.035	0.837	0.973	1.69	92	78
T (-2)	0.853	<0.001	0.058	0.74	0.966	1.67	90	73

43.9% and hematuria in 26.7% of COVID-19 patients [11].

A group of researchers (Richardson S. et al., 2020) analyzed the outcomes of 5.700 patients admitted for COVID-19 and reported that RRT was performed in 3.2% (*N*=81) of general ward patients and in 22% of ICU patients [13].

In a meta-analysis (Silver S. A. et al., 2021) based on MEDLINE, Embase, and Cochrane databases, 54 research papers with 30,639 patients were reviewed, of which 2,525 patients receiving inpatient therapy for COVID-19 in 48 studies were analyzed for the need for RRT. The overall prevalence of AKI was 28% (95% CI, 22–34%; I^2=99%), with 9% of patients receiving RRT (95% CI, 7–11%; I^2=97). Among patients treated in the ICU, AKI occurred in 46% (95% CI, 35–57%; I^2=99%) of cases and RRT was initiated in 19% (95% CI, 15–22%; I^2=88%) of patients [28].

In a retrospective study (Kanbay M. et al., 2022) (*N*=770), AKI was detected in 11.9% (*N*=92) of patients hospitalized with COVID-19. We also found that the duration of treatment in the ICU (16 days vs. 9.9 days, *P*<0.001), the rate of ICU admission (63% vs. 20.7%, *P*<0.001), the development of cytokine storm (25.9% vs. 14%, *P*=0.009) and mortality (47.2% vs. 4.7%, *P*<0.001) were significantly higher in the AKI group.

In the same study, data on the management of adult patients (*N*=100) with severe COVID-19 treated in the ICU were summarized. AKI (according to KDIGO criteria) was diagnosed in 81% of patients (*N*=81), including 44, 10, and 27 patients with stage 1, 2, and 3 AKI, respectively [29].

Chan L. et al. (2021) found that of 3,993 patients hospitalized for COVID-19, AKI occurred in 46% of patients (*N*=1835), with 19% (*N*=347) receiving RRT. Stage 1 AKI occurred in 39%, stage 2 in 19% and stage 3 in 42% of patients. 24% of patients (*N*=976) were admitted to the ICU, and AKI was diagnosed in 76% of cases (*N*=754). Proteinuria was detected in 84% of the 435 patients with AKI, hematuria in 81%, and leukocyturia in 60%. The mortality rate was 50% in the group with AKI and 8% in the group without AKI (OR 9.2; 95% CI, 7.5–11.3). At hospital discharge, 35% of patients in the AKI group had not recovered renal function [30].

Fisher M. et al (2022) investigated the prevalence of AKI in patients with COVID-19 in a retrospective observational study. Of 3,345 patients, 56.9% (*N*=1,903) developed AKI. Male sex, black race, and age over 50 years were found to be independent risk factors for the development of AKI [31].

The relatively lower incidence of AKI in COVID-19 (18.3%) in the patients we observed may be explained by the fact that the infectious hospital with ICU was located in a recently built building, which largely determined the low frequency of hospital-acquired infections.

The overall mortality of patients was 22.2% (*N*=26), and it was significantly higher in the group with AKI (52.4%, *N*=11) compared with the group

without AKI (15.62%, *N*=15) (χ^2 test=13.468, $P \leqslant 0.001$), suggesting an adverse effect of AKI on mortality.

Pei G. et al. (Wuhan, China, 2020) showed in a retrospective single-center study that the incidence of AKI (according to KDIGO criteria) in the cohort of hospitalized patients was 4.7% (22 of 467 patients). At the same time, proteinuria was observed in 65.8% and hematuria in 41.7%, indicating a high incidence of renal injury in patients with COVID-19. The latter patients had a higher mortality compared to patients without renal impairment: 11.2% (28 of 251) vs. 1.2% (1 of 82) [32].

Intergroup differences in s-CysC levels at time points T (*P*=0.0270), T (0) (*P*<0.001), T (-1) (*P*<0.001), T (-2) (*P*=0.0002), T (-3) (*P*=0.0218) and in u-CysC at time points T (*P*=0.1299), T (0) u-max (*P*=0. 0396), T (-1) u-max (*P*=0.0083), u-max (*P*=0.0452), T (-3) u-max (*P*=0.0310), which cannot be explained by impaired urinary filtration and reabsorption or CysC metabolism alone. Hyperproduction of CysC with the underlying severe systemic inflammation may play a role in the increase of CysC concentration in severe and critical pneumonia associated with COVID-19.

Analysis of the association of s-CysC with the development of AKI showed that s-CysC concentration 2 days (OR 3.225, Wald χ^2=4.121 (CI, 1.041; 9.989); *P*=0.042) and 1 day (OR 5.37, Wald χ^2=5.534 (CI, 1.324; 21.788); *P*=0.019) before the development of AKI was a significant predictor of AKI. The level of s-CysC started to increase three days before the development of AKI (intergroup difference, *P*=0.021753) before the increase in SCr levels, demonstrating the validity of these models for predicting AKI.

Analysis of u-CysC changes showed that it was not a significant predictor of AKI development.

We could not find any published studies addressing u-CysC evolution during COVID-19.

ROC analysis (Table 4) of significant predictors of AKI showed excellent performance of the s-CysC T(-1) model (ROC AUC 0.905 (95% CI, 0.837–0.973), *P*<0.001) and good performance of the s-CysC T(-2) model (ROC AUC 0.853 (95% CI, 0.74–0.966), *P*<0.001) in predicting AKI development at thresholds of 1.69 mg/L and 1.67 mg/L, respectively.

A single-center observational retrospective study by Yildirim C. et al (2021) evaluated the diagnostic and prognostic value of s-Cys C for the control of COVID-19-induced AKI. Among 348 patients with COVID-19, 17 (4.9%) cases developed AKI (including stage 1 in 1.3% (*N*=4), stage 2 in 9.0% (*N*=3), and stage 3 in 76.9% (*N*=10)). ROC analysis demonstrated the feasibility of using s-Cys C to predict COVID-19-induced AKI (AUC 0.96 (0.90–1.0) with sensitivity 90.0 (55.5–99.75), specificity 88.5 (84.6–91.7) [14].

Pode Shakked N. et al. (2022) also published a paper showing that s-CysC is an excellent predictor of COVID-19-associated AKI (ROC AUC 0.87) and the need for RRT (ROC AUC 0.95). Fifty-two patients with COVID-19 treated in the emergency department of the University of Cincinnati Medical Center (USA) were followed up. Of these, 42.3% (*N*=22) developed AKI and 36.4% (8 of 22) required RRT [33].

This is consistent with data from another study (Chen S. et al. 2021) showing that s-Cys C level increased earlier than that of SCr in patients with COVID-19 with renal impairment and was also more valuable in predicting disease severity [34].

The direct correlation between s-Cys C level and COVID-19 severity was also confirmed in our study: s-CysC and u-CysC had high prognostic significance for poor outcome (death) in pneumonia associated with severe and critical COVID-19. Elevated levels of s-CysC (1.44 mg/L and above) and u-CysC (0.86 mg/L and above) were associated with a fatal outcome [35, 36].

Ramos-Santos K. et al. (2022) confirmed the association between an increase in s-CysC and the development of AKI. In the group with AKI, the level of s-CysC was higher than in the group without AKI (*P*=0.001), and it increased earlier than SCr. An increase in s-CysC above 0.84 ng/mL increased the risk of developing AKI by a factor of 23 (OR, 23.7, 95% CI, 2.59–217.00, *P*=0.005) [37].

Conclusion

s-CysC is a significant predictor of AKI: its level starts to increase 3 days before the development of AKI and outpaces the increase in SCr concentration in patients with COVID-19-associated severe and critical pneumonia. u-CysC has only moderate relative value in predicting the development of AKI.

The use of s-CysC as a novel AKI biomarker in the management of patients with COVID-19 may contribute to the early detection of renal dysfunction enabling the prevention of AKI through initiation of preventive renal protection.

Authors' contribution.

Magomedali O. Magomedaliev: idea of the study, study design; review of publications on the subject of the paper; statistical processing of the material; obtaining data; database storage and processing; data analysis; discussion of the results; discussion of the paper format; drafting the manuscript, design of illustrations.

Daniil I. Korabelnikov: idea of the study, study design; review of publications on the subject of the paper; data analysis; discussion of the results; discussion of the paper format; drafting the manuscript; editing the manuscript; translation into English.

Sergey E. Khoroshilov: idea of the study; discussion of the results.

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Mortality Risk Factors in Neonates Requiring Interhospital Transport

Rustam F. Mukhametshin[1,2]*, Olga P. Kovtun[1], Nadezhda S. Davydova[1], Andrew A. Kurganski[3]

[1] Ural State Medical University, Ministry of Health of Russia,
3 Repin Str., 620028 Yekaterinburg, Sverdlovsk region, Russia
[2] Region Clinical Hospital for Children
32 S. Deryabinoy Str. 620149 Yekaterinburg, Russia
[3] The First President of Russia B.N. Yeltsin Ural Federal University
19 Mira Str., 620002 Ekaterinburg, Russia

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***Correspondence to:** Rustam F. Mukhametshin, rustamFM@yandex.ru

Summary

Objective. To identify predictors of newborn infants mortality before medical evacuation.

Materials and methods. The observational, cohort, retrospective study included 564 newborns: 526 patients survived and 38 died after 604 visits of the resuscitation-consultation Center transport team (critical care transport — CCT team). Patient's anamnesis, objective data of a patient at the time of examination by CCT team, the volume of intensive care provided and treatment adjustments during preparation for the transfer, records of patient's monitored parameters and indicators of prognosis were analyzed.

Results. Compared to survivors, non-survivors neonates exhibited significant increases in premature newborns (gestation period <29 weeks in 55.26% vs 10.27% in survivors, $P<0.001$) and significantly increased need in a high-frequency ventilation (7.89% [1.66–21.38] vs 0.57% [0.12–1.66] in survivors, $P=0.005$), and in catecholamines support (use of adrenaline was 13.51% [4.54–28.77] in non-survivors vs 0.76% [0.21–1.94] in survivors, $P<0.001$). Both early and late neonatal infections predominated in non-survivors: ([26.32% [13.40–43.10] vs 8,75% [6,47–11,49, early infection, non-survivors vs. survivors, respectively, $P=0.002$) and (23.6% 8 [11.44–40.24] vs 10.46% [7.97–13.39], late infection, non-survivors vs. survivors, respectively, $P=0.028$). Significant differences in the fraction of inspired oxygen (30% [30–30] vs 45% [30–60], $P<0.001$), oxygenation saturation index (2.71 [2.54–3.03] vs 4.48 [2.55–7.67], $P<0.001$), and SpO_2/FiO_2 ratio (316.67 [313.33–320] vs 207.25 [151.67–313.33] $P<0.001$) were found between the groups of survived vs. non-survived neonates, respectively. Logistic regression model revealed following markers of neonatal mortality: birth weight, development of early and late neonatal infection, and the oxygenation saturation index.

Conclusion. Low birth weight, development of early or late neonatal infection and an increase in the oxygenation saturation index are the risk factors of death in newborns requiring medical evacuation.

Keywords: newborn transportation; threat-metric scale; neonatal intensive care; risk of death; oxygenation index

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Introduction

Reducing neonatal and infant mortality remains a priority task of the health care system and an integral indicator of its effectiveness [1]. The system of perinatal regionalization provides an effective way to reduce the mortality of preterm infants by referring them to medical facilities that have the required level of care and sufficient patient flow to provide optimal intensive care [2–4]. Postnatal referral aims to transfer the newborn to an institution with the required level of medical care to reduce complications [5]. At the same time, transportation of newborns with a gestational age of less than 32 weeks and a birth weight of less than 1500 grams has a significant effect on neonatal mortality after adjustment for other risk factors (OR=3.3) [6]. Determining the severity of the neonate's condition prior to transportation remains one of the most important tools for predicting future risks of morbidity and mortality based on available baseline data, which allows the best possible decision to be made for the benefit of the patient [6]. The current federal documents related to the activities of the outreach resuscitation team of the neonatal intensive care center regulate only the general organizational principles of care (Order 921n of the Russian Ministry of Health dated November 15, 2012 «On Approval of the Procedure of Medical Care in Neonatology»), and technical equipment (Order 388n of the Russian Ministry of Health dated June 20, 2013 «On Approval of the Procedure of Emergency (including Emergency Specialized) Medical Care») without precise definition

of approaches to severity and prognosis assessment, algorithms and management rationale. While there are a variety of scales for predicting outcomes in neonatal patients, no consensus exists on the choice of tool for assessing a newborn requiring medical transfer to a higher level of care [7].

The aim of the study was to identify predictors of fatal outcome in newborn patients before medical transportation.

Materials and Methods

An observational, cohort, retrospective study included data from all visits of the transport team of the Neonatal Intensive Care and Consultation Center (NICCC) of the Ekaterinburg Regional Children's Clinical Hospital (RCCH) during the period from August 1, 2017 to December 31, 2018. After excluding patients with congenital anomalies requiring emergency surgical intervention (*N*=34), the number of cases was 640. Complete data or outcomes were not available for 36 cases. The final sample consisted of 604 cases of transport team visits to 564 newborns hospitalized in medical institutions of the Sverdlovsk region and remotely monitored by the NICCC due to their severity. The decision on the possibility of transport was made jointly by the head of the neonatal department of the obstetric care organization and the responsible physician of the outreach resuscitation team on the basis of the current regional regulation (Order No. 1687p of the Ministry of Health of the Sverdlovsk Region dated October 4, 2017) after assessing the severity and possible risks.

Data on hospital outcomes were obtained from primary medical records. In the study sample, two groups were distinguished according to the outcome: survivors (*N*=526) and non-survivors (*N*=38) (Fig.). We evaluated medical history, status at the time of examination by the transport team intensivist, level of intensive care and its modification before transport, monitored parameters (heart rate and SpO_2, noninvasive blood pressure, body temperature), and neonatal assessment using three scales, including the original Clinical Assessment Scale for the Premature Newborn (CASPN) [8], the Neonatal Therapeutic Intervention Scoring System (NTISS) [9], and the Transport Risk Index of Physiologic Stability for Newborn Infants (TRIPS) [10]. The oxygen saturation index was calculated using the formula $(FiO_2 \times MAP)/SpO_2$. The umbilical venous catheter was used as the standard initial vascular access in neonates during the first day of life. If venous access was established after the first day of life, peripherally inserted central catheters or peripheral Venflon-type needle catheters were placed. Fluid therapy and parenteral nutrition were planned and administered according to the clinical guidelines for parenteral nutrition in neonates of the Russian Association of Perinatologists and Association of Neonatologists (2015). The transport equipment consisted of transport incubator ITN-1 (UOMZ, Ekaterinburg, Russia), transport ventilator Stephan F120 Mobile (Stephan, Germany), syringe dispenser B. Braun Perfusor Compact S (B. Braun, Germany), patient monitor Philips MP 40 (Philips Medizin Systeme Boblingen GmbH, Germany). During the preparation phase, the transport team equipment was used for monitoring and respiratory support.

For descriptive statistics, we used median and interquartile range, percentage, 95% CI of the per-

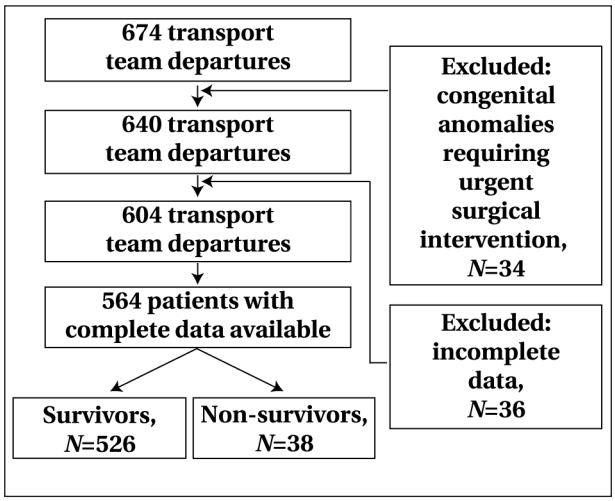

Fig. Study Design Flowchart.

Table 1. History of patients, *Me* [IQR].

Parameter	Values in groups		*P*-value
	Survivors, *n*=526	Non-survivors, *n*=38	
Age of patients on admission to the NICCC, hours	24 [4; 51]	17.5 [5; 49]	0.999
Age of patients at the time of examination by the transport team intensivist, hours	38 [24; 90]	33 [16; 110]	0.595
Age of patients at the time of transfer, hours	38 [25; 86]	30 [14.5; 83.5]	0.817
Birth weight, g	2.555 [1.730; 3.280]	1.050 [630; 2.360]	<0.001*
Gestational age, weeks	36 [33; 38]	28 [25; 37]	<0.001*
Apgar score 1, points	6 [4; 7]	4 [2; 5]	<0.001*
Apgar score 5, points	7 [6; 8]	5 [4; 6]	<0.001*

Note. For Tables 1 and 4: *Me* — median; IQR — interquartile range. For Tables 1–4: *n* — number of cases in the group; NICCC — neonatal intensive care and consultation center. * — significant differences.

centage, and standard error. Sample normality was tested using the Shapiro–Wilk method. When analyzing quantitative data with non-normal distribution of two independent samples, the Mann–Whitney test was used. Fisher's exact test was used to analyze binary data of two independent samples. Logistic regression was analyzed using BioStat Pro 7.0.1.0 and Mathlab R2017a software.

Comparison of the groups of survivors and non-survivors revealed significant differences in birth weight, gestational age, and Apgar scores at 1 and 5 minutes (Table 1).

When analyzing the level of referral, we found significant differences: referrals from level 2 facilities without an ICU occurred in 31.75% of cases [27.79–35.92] in the survivor group and in 13. 16% [4.41–28.09] in the non-survivor group, P=0.017; referrals from level 3 facilities occurred in 8.94% [6.64–11.71] in the survivor group and in 28.95% [15.42–45.90] in the non-survivor group, P<0.001.

When analyzing the underlying diseases in the study groups, we found a significant difference in the frequency of early neonatal infection (8.75% [6.47–11.49] vs. 26.32% [13.40–43.10], survivors vs. non-survivors, respectively P=0.002) and late neonatal infection (10.46% [7.97–13.39] vs. 23.68% [11.44–40.24], survivors vs. non-survivors, respectively, P=0.028) (Table 2).

The median age of fatal outcome was 6.5 [2; 17] days. Two deaths were recorded within the first 24 hours after birth. Both patients were not transferred because they were deemed non-transportable by the transport team specialists.

Results

Analysis of the distribution of patients by birth weight showed significant differences between survivors and non-survivors. More than 34% of the non-surviving neonates had a birth weight of less than 750 grams, whereas only 1.52% of the surviving patients had a birth weight of less than 750 grams. In the surviving group, there was a significant predominance of patients with a birth weight greater than 1500 grams: 81.56% [77.97 to 84.78] vs. 34.21% [19.63 to 51.35] in the non-surviving group, P<0.001. Similar patterns were observed when analyzing the distribution by gestational age: infants with a gestational age of less than 29 weeks were significantly more common in the non-surviving group (55.26% vs. 10.27%, P<0.001), while infants with a gestational age of more than 32 weeks were significantly more common in the surviving group. Patients with NEC who showed signs of perforation (3.01% [1.77–4.78] of all patients) underwent emergency laparotomy with bowel resection and stoma creation within 12 hours of admission to the tertiary hospital. The same procedure was performed in patients diagnosed with lower bowel obstruction (2.13% [1.1–3.69] of all patients). The need for emergency surgery was significantly more frequent in the non-survivors (13.16% [4.41–28.09] vs. 4.75% [3.10–6.94] in the survivors, P=0.043).

When analyzing the management strategies of the transport team, we found a significant difference in the percentage of patients deemed non-transportable, 1.71% [0.79–3.22] in the survivor group vs 28.95% [15.42–45.90] in the non-survivors (P<0.001). Successful evacuation on the first attempt was significantly more common in the survivor group (93.22% [90.56–95.32] vs 76.00 [54.87–90.64], P=0.008).

Respiratory treatment. At the time of evaluation by the transport team intensivist, there were significant differences in ventilatory support parameters

Table 2. Diseases in the groups, % [95% CI].

Diagnosis	Values in groups		*P*-value
	Survivors, *n*=526	Non-survivors, *n*=38	
Hematologic abnormalities	0.57 [0.12–1.66]	2.63 [0.07–13.81]	0.244
Coagulation abnormalities	2.28 [1.18–3.95]	0,00 [0.00–9.25]	1.000
Perinatal asphyxia	8.94 [6.64–11.71]	2.63 [0.07–13.81]	0.238
Congenital anomalies not requiring urgent surgical intervention	1.71 [0.79–3.22]	2.63 [0.07–13.81]	0.505
Chronic lung disease	1.14 [0.42–2.47]	0.00 [0.00–9.25]	1.000
Hemolytic disease of the newborn	3.04 [1.75–4.89]	0.00 [0.00–9.25]	0.616
Metabolic disorders	1.33 [0.54–2.72]	0.00 [0.00–9.25]	1.000
Nonimmune hydrops fetalis	0.19 [0.00–1.05]	2.63 [0.07–13.81]	0.130
Early neonatal infection	8.75 [6.47–11.49]	26.32 [13.40–43.10]	0.002*
Late neonatal infection	10.46 [7.97–13.39]	23.68 [11.44–40.24]	0.028*
Neonatal respiratory distress syndrome	32.51 [28.52–36.70]	34.21 [19.63–51.35]	0.859
Transient tachypnea of the newborn	11.03 [8.48–14.02]	2.63 [0,07–13.81]	0.164
Meconium aspiration syndrome	2.85 [1.60–4.66]	2.63 [0.07–13.81]	1.000
Perinatal brain injury	4.94 [3.25–7.16]	0.00 [0.00–9.25]	0.246
Prematurity	5.32 [3.57–7.60]	0.00 [0.00–9.25]	0.246
Diabetic fetal macrosomia	0.76 [0.21–1.94]	0.00 [0.00–9.25]	1.000
Rhythm and conduction disturbances	1.90 [0.92–3.47]	0.00 [0.00–9.25]	1.000
Lower intestinal obstruction	2.28 [1.18–3.95]	0.00 [0.00–9.25]	1.000

Note. For Tables 2 and 3: CI — confidence interval. * — significant differences.

Table 3. Treatment in the groups, % [95% CI].

Treatment	Values in the groups		*P*-value
	Survivors, *n*=526	Non-survivors, *n*=38	
Nasal CPAP	11.22 [8.65–14.23]	7.89 [1.66–21.38]	0.787
Lung ventilation	47.53 [43.19–51.90]	78.95 [62.68–90.45]	<0.001*
High frequency lung ventilation	0.57 [0.12–1.66]	7.89 [1.66–21.38]	0.005
Dopamine	6.65 [4.68–9.13]	29.73 [15.87–46.98]	<0.001*
Epinephrine	0.76 [0.21–1.94]	13.51 [4.54–28.77]	<0.001*
Dobutamine	0.19 [0.00–1.05]	0.00 [0.00–9.49]	1
Prostaglandins	3.04 [1.75–4.89]	5,26 [0.64–17.75]	0.345
Sedation	3.99 [2.49–6.04]	13,16 [4.41–28.09]	0.025
Myoplegia	0.19 [0.00–1.05]	2.63 [0.07–13.81]	0.13

Note. CI — confidence interval; CPAP — continuous positive airway pressure; * — significant differences.

between the study groups. Non-survivors were more likely to receive ventilatory support (78.95% [62.68 to 90.45] vs. 47.53% [43.19 to 51.90] in survivors, *P*<0.001), including high-frequency ventilation (7.89% [1.66 to 21.38] vs. 0.57% [0.12 to 1.66], *P*=0.005). Medical sedation for synchronization with a ventilator was used significantly more often in the non-surviving group (13.16% [4.41–28.09] vs 3.99% [2.49–6.04], *P*=0.025). Mechanical ventilation was performed in time-cycled pressure-limited mode. Comparison of respiratory support parameters revealed significant differences in inspiratory time (0.34 [0.33–0.35] vs. 0.28 [0.27–0.31], *P*=0.001), due to the predominance of extremely premature infants among the non-survivors, inhaled oxygen fraction (30% [30–30] vs. 45% [30–60], *P*<0.001), oxygen saturation index (2.71 [2.54–3.03] vs. 4.48 [2.55–7.67], *P*<0.001), and SpO_2/FiO_2 ratio (316.67 [313.33 to 320] vs. 207.25 [151.67 to 313.33] in the survivors and non-survivors groups, respectively (*P*<0.001, Table 4). The need to modify ventilatory parameters during the preparation for transportation was significantly more frequent in the non-survivors (31.58% [17.50–48.65] vs. 14.83% [11.90–18.16], *P*=0.012), while the difference in the percentage of patients requiring tracheal intubation or reintubation was not significant (2.85% [1.60–4.66] vs. 5.26 [0.64–17.75] in survivors and non-survivors, respectively, *P*=0.319). The difference in the frequency of tension pneumothorax drainage was also not significant between the groups (0.19% [0.00–1.05] and 0.00% [0.00–9.25] in the survivors and non-survivors, respectively, *P*=1.000).

Catecholamines were used more frequently in the non-surviving group: dopamine use was 29.73% [15.87 to 46.98] vs. 6.65% [4.68 to 9.13] in the survivors (*P*<0.001), and epinephrine use was 13.51% [4.54 to 28.77] vs. 0.76% [0.21 to 1.94] in the survivors (*P*<0.001) (Table 3). At the same time, the difference between the groups in dopamine and epinephrine dosage during continuous intravenous administration was insignificant: dopamine 5 [5–7] μg/kg/min vs 5 [5–8] μg/kg/min in survivors and non-survivors, respectively (*P*=0.8970), epinephrine 0.4 [0.2–1] μg/kg/min vs 0.25 [0.1– 0.3] μg/kg/min in survivors and non-survivors, respectively (*P*>0.05). No intergroup differences were found in inotropic index values: 5 [5–8.5] vs 7 [5–10] in survivors and non-survivors, respectively, *P*=0.379. Fluid therapy was administered at 68.97 [55.38–88.89] mL/kg/day in the survivors and 98.78 [72.73–155.84] mL/kg/day in the non-survivors (*P*=0.001), due to significant differences in weight and gestational age. Surviving patients were less likely to require vascular access (0.19% [0.00–1.05] vs. 5.26% [0.64–17.75], *P*=0.012), administration of fluid therapy or volume loading (0.57% [0. 12 to 1.66] vs 10.53% [2.94 to 24.80], *P*=0.001), catecholamine administration or dose increase (0.38% [0.05 to 1.37] vs 15.79% [6.02 to 31.25], *P*<0.001).

Significant intergroup differences were observed in the number of manipulations performed by the transport team during preparation. The survivors had an average of 0.21 [0.41] manipulations per patient, while the non-survivors had an average of 0.71 [0.46] manipulations per patient (*P*<0.001). During interhospital transportation, the frequency of intensive care modifications did not differ between groups.

When comparing the monitoring parameters, we observed significant differences in heart rate, SpO_2, systolic and diastolic blood pressure (Table–4).

The non-survivors had higher scores on all scales, including CASPN (6 [5–8] vs 4 [2–5], *P*<0.001), NTISS (19.5 [18–25] vs 15 [11–17], *P*<0.001), and TRIPS (31 [20–47] vs 14 [1–20], *P*<0.001).

Four significant predictors of fatal outcome were identified in the study sample using a logistic regression model: birth weight, early or late neonatal infection, and oxygen saturation index (Table 5).

Discussion

Analysis of putative predictors of mortality in the neonatal population points primarily to birth weight and gestational age. Preterm infants are particularly vulnerable to various complications, additional morbidity and mortality associated with respiratory disorders, feeding difficulties, susceptibility to hypothermia, and high infectious risks [11]. Complications of preterm birth are the leading

Table 4. Parameters of respiratory support and monitoring, *Me* [IQR].

Parameter	Values in groups		*P*-value
	Survivors, *n*=526	Non-survivors, *n*=38	
Respiratory rate, per minute	50 [50; 50]	50 [45; 50]	0.119
Inspiratory pressure (P_{insp}), cm H_2O	18 [18; 20]	20 [18; 21.5]	0.08
Positive end expiratory pressure (PEEP), cm H_2O	5 [5; 5]	5 [5; 5]	0.908
Inspiratory time, sec	0.34 [0.33; 0.35]	0.28 [0.27; 0.31]	0.001*
Inhaled oxygen fraction (FiO_2), %	30 [30; 30]	45 [30; 60]	<0.001*
Mean airway pressure (MAP), cm H_2O	8.75 [8.4; 9]	8,89 [7.89; 10.87]	0.357
Oxygen saturation index	2.71 [2.54; 3.03]	4.48 [2.55; 7.67]	<0.001*
SpO_2/FiO_2	316.67 [313.33; 320]	207.25 [151.67; 313.33]	<0.001*
Heart rate, per minute	142 [140; 142]	142 [130; 149]	0.282
Systolic blood pressure, mm Hg	64.5 [62; 65]	55 [40; 60]	<0.001*
Diastolic blood pressure, mm Hg	39 [38; 40]	33 [22; 39.5]	<0.001*
Body temperature, °C	36.6 [36.6; 36.6]	36.6 [36.5; 36.6]	0.157
SpO_2, %	95 [95; 95]	92.5 [91; 95]	<0.001*

Note. Me — median; IQR — interquartile range; * — significant differences.

cause of mortality in children under 5 years of age worldwide, accounting for approximately 1 million deaths in 2015 [12]. A significant prevalence of prematurity was found in the non-surviving neonates, whereas gestational age analysis showed a predominance of infants at 28 weeks' gestation or less in the non-surviving group. A lower Apgar score in the non-survivors is reasonable due to the prevalence of prematurity in this group [13]. Furthermore, a low Apgar score (5 or less at 10 minutes) is associated with an additional risk of neonatal death in both preterm and term infants [14]. However, our logistic regression analysis showed that birth weight was one of the four significant predictors of fatal outcome, while Apgar score data were not significant in the constructed model.

Another important determinant of NICU outcomes, according to the literature, is the level of medical organization providing care to the newborn. Obladen M. reported worse outcomes in NICUs with low patient volume and bed capacity [15]. Poets C. F. et al. in their review indicate a 2–3-fold increase in perinatal mortality among preterm infants in facilities with less than 500 deliveries per year and a 40–80% increase in this parameter in facilities with less than 1000 deliveries per year compared to large hospitals. For preterm infants, the risk of death was also twice as high in low volume facilities as in tertiary care facilities. In addition, the risk of death was increased by up to 56% for infants born in a birth center with fewer than 36 (or 50 very low birth weight) births per year compared with a facility with a large NICU [16]. Lasswell S. M. et al. observed an increased odds of death for very low birth weight infants (38% vs 23%, odds ratio 1.62, 95% CI, 1.44–1.83) and extremely preterm infants (15% vs 17%, odds ratio 1.55, 95% CI, 1.21–1.98) born in non-tertiary care facilities. The observed outcome did not change over time (*P*=0.87) [17]. The effect of level of care was even greater with decreasing gestational age [2].

Recently, Hentschel R. et al. confirmed the above patterns. Infants in small NICUs had an increased risk of mortality after risk adjustment using CRIB (Clinical Risk Index for Babies) (OR 1.48, 95% CI, 1.16–1.90, *P*=0.002) and PREM (Prematurity Risk Evaluation Measure) (OR 1.39, 95% CI, 1.11–1.76, *P*=0.005) scores. In a subgroup analysis, mortality was significantly higher in small NICUs in the moderate risk group (OR 1.49, 95% CI, 1.02–2.17, *P*=0.037 with CRIB) and in the high risk group (OR 1.70, 95% CI, 1.16–1.90, *P*=0.002 with CRIB and OR 1.39, 95% CI, 1.11–1.76, *P*=0.005), but not in the low and very high risk subgroups [4]. The observed differences in mortality during hospitalization in different levels of care were considered to be the result of antenatal

Table 5. Logistic regression model for death predictors.

Parameter	Estimate	SE	*P*
Intercept	–0.51	1.06	0.633
Birth in a medical institution without a NICU	0.32	0.558	0.569
Body weight at birth	–0.0016	0.000448	<0.001*
Apgar 1	–0.14	0.3	0.654
Apgar 5	0.09	0.35	0.779
Emergency surgery	–0.09	0.97	0.889
Oxygen saturation index	0.32	0.08	<0.001*
Catecholamine infusion	0.87	0.57	0.126
Intensive care modification	–0.44	0.61	0.467
Early neonatal infections	2.13	0.77	0.006*
Late neonatal infections	1.84	0.87	0.034*

Note. SE — standard error; * — significant differences.

routing. Patients in the high perinatal risk group were hospitalized in level 2 and 3 institutions with the possibility of neonatal intensive care. At the same time, level 1 and 2 institutions follow the rule of continuous observation, i. e., any patient requiring intensive care is referred to the NICCC, while level 3 institutions seek consultative care only for the most severe patients, including surgical patients. For this reason, only complicated cases from level 3 facilities came to the attention of the transport team, resulting in a high proportion of fatal outcomes. This is probably related to the lack of a significant effect of delivery in a medical facility without neonatal intensive care on the risk of death. The exclusion of patients of tertiary medical institutions from the analysis will probably allow to compensate for the sampling bias associated with selective referrals from these institutions.

Procedures to stabilize hemodynamics during pretransport preparation and transport are not uncommon in neonatal intensive care. Kumar P. P. et al. indicate that 29.8% of patients required additional volume loading and 10.6% required continuous catecholamine infusion during transport [18]. Leung K. K. Y. et al. reported that inotropes were used in 14.5% of neonatal transport cases. This is associated with a higher relative risk of complications during transport and within one hour of arrival, which reaches 2.51 (1.11 to 5.67) after adjustment for other variables [19]. The differences in blood pressure observed between the groups were consistent with normal values adjusted for gestational age. Catecholamines to stabilize hemodynamic parameters were used more frequently in non-survivors. However, logistic regression did not show an effect of the frequency of intensive care modification and catecholamine use on the risk of mortality. This may be due to the difficulty in determining the need for medical hemodynamic support in neonates. In contrast to the adult patient, maintaining a normal blood pressure early in neonates does not guarantee adequate organ perfusion [20]. The reference, albeit indirect, method of perfusion assessment in neonatology is functional echocardiography with determination of volumetric blood flow in the superior vena cava [21]. Literature data confirm that low blood flow in the superior vena cava is closely associated with subsequent intraventricular hemorrhage or neurodevelopmental disorders [22, 23]. However, even this method is not considered to be sufficiently accurate in describing hemodynamic disturbances [24]. Thus, significant difficulties remain in determining the indications for catecholamines. Level 1–2 institutions do not have routine access to perfusion assessment techniques used in neonatal intensive care, and medical management of hemodynamics is often not based on strict indications [20], which does not allow hemodynamic parameters and treatment modalities to be considered as predictors of mortality.

The higher frequency of intensive care modifications in the non-surviving group could indicate both the initial severity of the patient and the insufficient therapeutic activity of the referring medical organization. As a result, we observed a discrepancy between patient severity and the level of care available in the medical organization, which was «compensated» by the transport team. At the same time, the earliest possible provision of appropriate care is known to be associated with better clinical outcomes [25]. Inadequate pretransport preparation at the referring institution increases the need for intensive care en route [26]. Significant differences in scores on all three scales between survivors and non-survivors indicate a significantly greater severity of illness in the non-survivor group.

Adjustment of respiratory support parameters was the most common procedure performed during pre-transport preparation, especially in non-survivors. High-frequency lung ventilation is the most commonly used method of respiratory support in patients with critical respiratory illness. However, all strategies of its use lack sufficient evidence base. Furthermore, this method is only available in a small number of medical institutions, and the decision to use it is often made empirically rather than on the basis of evidence-based recommendations [27–29]. A higher incidence of HF lung ventilation was observed in the non-surviving group, but no differences in mean airway pressure were found. Therefore, the frequency of HF lung ventilation cannot be unambiguously interpreted as a marker of the severity of respiratory failure in the groups.

A greater dependence on oxygen during lung ventilation, a higher oxygen saturation index, and a lower SpO_2/FiO_2 were found in the non-survivor group. The oxygen saturation index correlates well with the Respiratory Severity Score (RSS) [30] and was, together with birth weight, a significant predictor of mortality in the logistic regression model, which is in agreement with the literature [31, 32].

The incidence of sepsis ranges from 4 to 22 cases per 1000 live births [33]. Neonatal sepsis remains an important cause of neonatal death [34]. Early neonatal sepsis, which develops in the first 72 hours after birth, is fatal in 7.0–23.1% of cases, depending on the pathogen [35]. The prevalence of late neonatal sepsis ranges from 0.61% to 14.2% of hospitalized neonates, depending on gestational age [36]. Mortality may be as high as 26.7% [37]. Logistic regression method confirmed the role of neonatal infection in the risk of mortality.

Limitations. First, because the aim of the study was to analyze the death risk predictors in patients requiring interhospital transport, we included only

data available at the time of examination by the intensivist of the transport team and relevant to medical transfer. We did not examine detailed data on obstetric history, because its influence on the probability of neonatal death has been extensively studied in the literature. Second, the observation of neonates by the intensive care and consultation center in the medical organizations of the service area was not «continuous», which created a bias in the initial sample and affected the accuracy of the logistic regression model. Third, the end point for recording outcomes in each case of medical care was completion of hospitalization. Repeated and further hospitalizations, illnesses, and fatal outcomes that occurred later were not included in the study.

Conclusion

Predictors of neonatal mortality before the medical transportation include low birth weight, early or late neonatal infection, and oxygen saturation index.

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Inter-Alpha Inhibitor Proteins as a Predictor of Necrotizing Enterocolitis in Newborn Infants

Safaa A. ELMeneza*, Neveen M. Arafat, Iman M. El-Bagoury, Amal Gaber

Faculty of Medicine for Girls, AL-Azhar University
383C+QMC Youssef Abbas Str., Nasr city, 11751 Cairo, Egypt

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***Correspondence to:** Safaa A. ELMeneza, safaa5@hotmail.com

Summary

Necrotizing enterocolitis is a devastating emergency, multifactorial disease. Inter-alpha inhibitor proteins are serine protease inhibitors involved in many physiological and pathological activities.

Aim: this study was designed in order to assess the value of inter-alpha inhibitor proteins in predicting and improving accuracy of diagnosis of NEC in newborn infants with non- precise abdominal and intestinal manifestations.

Materials and Methods. This study was prospective longitudinal research that included 80 newborn infants presented with non-specific abdominal manifestations. Infants were divided into two groups. Group A; infants who developed necrotizing enterocolitis, they had stage II or III necrotizing enterocolitis according to modified Bell's criteria. Group B; included infants who did not develop necrotizing enterocolitis. Serum inter alpha inhibitor proteins level was measured by ELISA.

Results. In necrotizing enterocolitis group, the median inter-alpha inhibitor protein level was (9.38 mg/L), this was significantly lower than non-necrotizing enterocolitis group (44.40 mg/L), $P<0.01$. Inter-alpha inhibitor protein was reduced in stage IA than stage IIIB. Inter-alpha inhibitor protein values were decreased in preterm and full term infants with sensitivity of 98 % and specificity of 96% at cutoff <19.42 and <19.96 mg/L. The cut off in non-survival cases was >13.29 mg/L with sensitivity of 53.33 % and specificity of 92.31%.

Conclusion. Inter-alpha inhibitor protein levels were reduced in full term and preterm infants with necrotizing enterocolitis, consequently it may improve diagnosis of necrotizing enterocolitis in newborn infants. It has prognostic value and correlate with severity of necrotizing enterocolitis. It might predict non- survival cases.

Keywords: necrotizing enterocolitis; newborn infants; inter-alpha inhibitor proteins; surgical neonatal emergencies; newborn infants; preterm infants

Conflict of interest. The authors declare no conflict of interest. There are no financial or nonfinancial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

Introduction

Necrotizing enterocolitis (NEC) is a multifactorial overwhelming disease typically occurs without clinical warning [1]. Diagnosis of NEC can be difficult, because symptoms and signs may be nonspecific especially in preterm and sick full term infants who have feeding intolerance and non-specific abdominal disorders. A presumption of NEC will be followed by restraint of enteral feeding for 3 days at least. It is important to detect useful biomarkers to confirm the diagnose and establish the seriousness of NEC. Early diagnosis will promote early intervention, safe care and better outcome of newborn infants with inconclusive diagnosis of NEC and prevent unnecessary discontinue of enteral feeding and use of parenteral nutrition and antibiotics especially in developing countries with limited resources [2].

Inter-alpha inhibitor proteins (IαIp) molecules are part of innate immunity and play a critical role during inflammation. IαIp molecules have unique immunomodulatory effects by reducing TNF-α during systemic inflammation and augmenting anti-inflammatory IL-10 during sepsis in neonatal rats [3–5], but a few researches confirm its role in diagnosis of NEC.

Our research question was can IαIp predict the diagnosis of NEC in newborn infants? Hence, this study was designed in order to assess the value of Inter-alpha inhibitor proteins in predicting and improving the accuracy of diagnosis of NEC in newborn infants with non- precise abdominal and intestinal manifestations.

Materials and Methods

This was prospective longitudinal study carried out in NICU. It involved 80 newborn infants admitted to the NICU.

Ethical approval. The study protocol was approved by the ethics committee of Faculty of Medicine for Girls, AL-Azhar University. Approval number is 202010422 on 6/10/2020.

Informed consent was obtained from the parents after explaining the aim of the study. The aim, steps of the study, were discussed with the parents. Confidentiality of all data was ensured. The researchers

explained to the parents the nature of the study, the possible benefits to understand the nature of the disease. There was no additional risk/pain or invasive procedures as the extra test will be performed with the routine investigations. Also we informed parents that they can withdraw at any time.

The inclusion criteria included newborn infants presented with non-precise abdominal and intestinal manifestations as feeding intolerance, increased gastric aspirates, abdominal distention, and abdominal tenderness.

The exclusion criteria included newborn infants with congenital anomalies and symptoms suggestive errors of metabolism.

According to the results of investigations, the newborn infants were divided into two groups: Group A; infants who developed NEC and group B; infants who did not develop NEC. Modified Bell's staging criteria was used to estimate NEC stages [6].

All studied neonates were subjected to complete prenatal and natal history taking, thorough clinical examination, radiological and laboratory investigations including the serum inter alpha inhibitor proteins at the time of initial presentation.

Plasma IαIp levels were measured quantitatively using a competitive enzyme-linked immunosorbent assay with a monoclonal antibody against human IαIp.

The neonates were evaluated for the increase in abdominal girth, abdominal tenderness or redness, absence of intestinal sound as well as amount and colour of gastric residuals. Sign of respiratory distress or circulatory failure and hematological disorders as DIC were assessed. Also cases were assessed for temperature instability, apnea, bradycardia, lethargy, hypotension, emesis or blood in stool. Results of laboratory investigation as metabolic acidosis, and thrombocytopenia, and radiological findings determine the plane of treatment.

The newborn infants who proved to be NEC were assessed for need of ventilatory support when there was frequent apnea or respiratory failure, or need of vasopressors/inotropes as well as surgical consultation.

Statistical Analysis. Data were collected, coded, revised and entered to the Statistical Package for Social Science (SPSS) version 20. The statistical significance criterion was $P \leqslant 0.05$.

The criterion for assessing the normality of the parameters distribution, descriptive statistics was assessed using the kolmogorov smirnov test and Shapiro wilk test. To measure the diagnostic

Table 1. Comparison between infants who developed NEC and those who did not developed NEC/suspected group.

Parameters	Value in groups		Test	
	Not developed NEC	Developed NEC	χ^2/t*	*p*
Gender, *n* (%)				
Male	30 (57.70)	15 (53.60)	0.126	0.723
Female	22 (42.30)	13 (46.40)		
Gestational age, weeks				
Mean ±SD	33.96±3.85	34.39±3.56	–0.490*	0.625
Maturity, *n* (%)				
Pre term	33 (63.5)	17 (60.7)	0.059	0.809
Term	19 (36.5)	11 (39.3)		
Type of delivery, n (%)				
NVD	15 (28.8)	8 (28.6)	0.001	0.979
Postnatal age, days				
Mean ±SD	1.84±1.45	1.82±1.44	0.064*	0.949
Consanguinity, n (%)				
Negative	41 (78.8)	19 (67.9)	1.172	0.279
Positive	11 (21.2)	9 (32.1)		
Outcome, n (%)				
Non-survival	10 (19.2)	18 (64.8)	16.24	<0.001
Survival	42 (80.8)	10 (35.2)		

Note. * — Independent *t*-test; χ^2 = Chi square test.

Table 2. Comparison between infants who developed NEC and those who did not develop NEC as regard to abdominal examination.

Indicators	Value in groups, *n* (%)		Chi square test	
	Not developed NEC	Developed NEC	χ^2	*P*-value
Abdominal distension	38 (73.1)	23 (82.1)	0.826	0.363
Abdominal tenderness	14 (26.9)			
Occult blood in stool				
Negative	47 (90.4)	0 (0.0)	21.945	<0.001
Positive	5 (9.6)	28 (100.0)		
Gastric aspirate				
Bloody	2 (3.8)	26 (92.86)	27.619	<.001
Bilious	0 (0.0)	2 (7.14)		
Negative	50 (96.2)	0 (0.0)		

ability of the IaIp, the ROC curve was used for mapping of the sensitivity versus for all possible values of the cut-off point between cases and controls.

The cut-off point chosen was the best point for balancing the sensitivity and specificity on the curve. The cut-off point corresponding to these sensitivity and specificity values is the one closest to the (0, 1) point and was taken to be the cut-off point that best differentiates between the NEC cases and non NEC cases.

Results

Description of the study population

The study population included 80 newborn infants. Thirty cases were full term infants and fifty cases were preterm infants. Twenty-eight cases developed NEC of stage II and III according to Bell's classification, while 52 cases did not develop NEC.

There was no significant difference regarding gestational age, gender, mode of delivery, postnatal age, and anthropometric measurements between infants who developed NEC and those who did not develop NEC, $P>0.05$.

In the NEC group, 17 (60.7%) newborn infants were preterm and 11 (39.3%) newborn infants were full-term (Table 1).

Clinical presentation

There was non-significant difference between the NEC group and non NEC group regarding abdominal distension; 82.1% of newborn infants of NEC group had abdominal distension, while 73.1% of the non NEC group had abdominal distension, $P>0.05$. Our study showed that gastric residuals is an indicator for newborn infants who developed NEC. All the patients in NEC group had gastric aspirates; 92.86. % of cases had bloody brownish residual and 7.10% had bilious residual (Table 2).

Laboratory findings

In the NEC group, the median inter-alpha inhibitor protein level was 9.38 mg/L which was significantly lower than the non NEC group (44.40 mg/L), $P<0.01$. Inter-alpha inhibitor protein level was reduced in stage IIIB than IA stage (Table 3, 4).

The total leukocytic count was significantly increased in NEC group, $P<0.001$, there was insignificant difference in hemoglobin level and red blood cells count or platelets.

The Table 2 shows that there was no statistically significant difference between the two groups as regard to abdominal examination with $P>0.05$ while

Table 3. Comparison between infants who developed NEC and those who did not develop NEC as regard to inter-alpha inhibitor protein level, *Median* (*IQR*).

Indicators	Inter-alpha inhibitor protein, mg/L	Mann-Whitney test	
		U	*P*-value
All cases			
No NEC	44.40 (28.8–62.02)	–7.344	<0.001
NEC	9.38 (4.45–14.64)		
Preterm			
No NEC	29.04 (13.04–57.73)	–5.745	<0.001
NEC	7.75 (3.17–13.04)		
Full term			
No NEC	25.96 (15.33–44.38)	–4.497	<0.001
NEC	13.52 (9.10–16.79)		
No NEC			
Preterm	29.04 (13.04–57.73)	–0.353	0.724
Full Term	25.96 (15.33–44.38)		
NEC			
Preterm	7.75 (3.17–13.04)	–1.717	0.086
Full Term	13.52 (9.10–16.79)		
Outcome			
All non-survival	21.22 (13.73–47.92)	1.242	0.214
All survival	34.32 (13.2–54.42)		
Blood culture			
Positive	21.54 (11.21–35.23)	–4.150	<0.001
Negative	54.81 (44.38–67.90)		

Table 4. Inter-alpha inhibitor protein levels in relation to stage of NEC, *Median* (*IQR*).

Staging	*n*	Inter-alpha inhibitor protein,+ mg/L	Kruskall–Wallis test	
			K	*P*-value
IA	35	57.73 (44.38–67.9)	44.045	0.001
IB	17	25.1 (21.56–28.4)		
IIA	6	17.98 (17.35–19.42)		
IIB	10	13.07 (9.65–13.52)		
IIIA	6	6.75 (4.5–7.75)		
IIIB	6	1.03 (0.73–1.92)		

occult blood in stool and gastric aspirate showed highly statistically significant difference between the two groups with $P>0.001$.

Blood culture was positive in 38.5% of NEC group and in 3.6% of non NEC group, $P<0.01$ (Table 5, 6).

The figure shows the cut off point for the inter-alpha inhibitor protein in relation to gestational age as well as for prediction of mortality among the studied groups.

Discussion

Necrotizing enterocolitis is the most serious gastrointestinal disorder to occur in the newborn infant population. Mortality approaches 100% in the most severe cases with perforation, peritonitis, and sepsis. NEC mainly affect premature infants, but it is recognized in full-term infants too [7]. There are new markers that may help diagnosis of NEC, but with variable significance [8, 9]. IαIp is a fairly new marker with high sensitivity and specificity in detection of neonatal sepsis [10], but a few researches confirm its role in diagnosis of NEC. This study was designed in order to assess the value of inter-alpha inhibitor proteins in predicting and improving the accuracy of diagnosis of NEC in newborn infants with non-precise abdominal and intestinal manifestations.

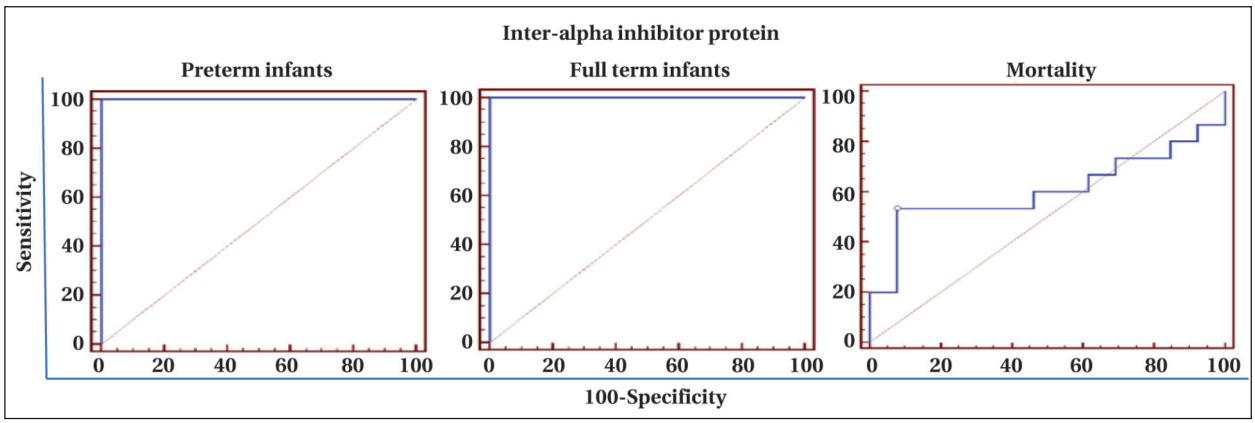
In this study, 60.7% of newborn infants who developed NEC were preterm and 39.3% were full term infants. Premature babies are prone to develop NEC due to multiple risk factors including immature mucosal barrier, gastrointestinal dysmotility and stasis which lead to malabsorption, bacterial growth and microbial dysbiosis that lead to mucosal injury [11].

Table 5. Comparison between infants who developed NEC and those who did not develop NEC as regard to hematological investigation.

Parameter	Value in groups		Independent *t*-test	
	Not developed NEC	Developed NEC	*t*	*P*-value
	Mean ± SD			
RBCs/µL	4.05±1.00	4.08±0.76	–0.158	0.875
PLT/µL	83.23±34.89	81.68±32.97	0.194	0.847
Hb(g/dL)	14.19±3.09	14.15±3.36	0.059	0.953
			Mann–Whitney test	
			Z	*P*-value
	Median (IQR)			
WBCs/µL	19.25 (12.15–27.5)	31.2 (21.8–66.5)	–3.657	<0.001
Lympho (%)	28.45 (14.5–46.1)	25.55 (15–35.8)	–0.042	0.967
Mono (%)	4.3 (1.8–10.6)	10.45 (6.75–13.2)	–1.825	0.068
Eosino (%)	0 (0–0)	0 (0–0)	–0.126	0.900

Table 6. Comparison between infants who developed NEC and those who did not develop NEC as regard to blood culture.

Blood culture	Value in groups, *n* (%)		Chi square test	
	Developed NEC	not developed NEC	χ^2	*P*-value
Negative	32 (61.5)	27 (96.4)	11.444	0.001
Positive	20 (38.5)	1 (3.6)		



Indicators	Cut-off point	AUC	Sensitivity	Specificity	PPV	NPV
Preterm	<19.42 mg/L	0.996	98	96	96.1	98
Full term	<19.96 mg/L	0.993	98	96	96	98
Mortality	>13.29 mg/L	0.70	53.33	92.31	88.9	63.2

Fig. Receiver operating curve of inter-alpha inhibitor protein.

The current study showed that mean levels of inter-alpha inhibitor protein were significantly decreased in the NEC group (9.38 mg/L) than the non NEC group (44.40 mg/L), $P<0.01$. This was in agreement with previous study done by Chaaban et al. who reported that the mean IαIp level in the confirmed NEC group was significantly lower than the control group [12] and with study of Shah et al. who found that IαIp levels were significantly decreased in infants with NEC compared to newborn with spontaneous intestinal perforation and matched controls. The diagnostic accuracy of IαIp for NEC was superior to that of CRP [13].

In this study, further analysis of the IαIp mean values in relation to gestational age, showed that IαIp can predict NEC in preterm and full term infants; the mean values were decreased in preterm infants and full term infants with NEC than in preterm and full term infants who did not develop NEC, $P<0.01$. Furthermore, there was no significant differences in IαIp levels between full term infants with NEC and preterm infant with NEC.The cutoff point for diagnosis of NEC was <19.42 mg/L in preterm infants and <19.96 mg/L in full term infants. The sensitivity was 98% and specificity was 96% in preterm and full term infants. These data showed that IαIp can be used to predict NEC in preterm as well as full term infants with suspected abdominal manifestations, up to our knowledge, this is first study to show the cutoff value in preterm and full term infants.

A probable justification for low values of IαIp among NEC cases may be due to down-regulation of IαIp synthesis, it is considered as a negative acute-phase reactant [14]. Also IαIp is very susceptible to proteolysis by numerous proteinases implicated in inflammation — namely plasmin, thrombin and kallikrein. Plasma IαIp is particularly sensitive to cleavage by neutrophil elastase, and the light chain bikunin released from the IαIp complex exerts its inhibitory activity on serine proteases [15].

Our study showed association between IαIp and severity of NEC as IαIp level was reduced among neonates with stage IIIB than those with stage IA, also there was significant decrease in IαIp among cases who showed ascites and pneumoperitoneum. In severe sepsis there is significant consumption of systemic IαIp and extended secretion of elastase that decompose IαIp [16]. Hepatic IαIp biosynthesis is also down regulated throughout severe inflammation like advanced NEC stages.

Also we found significant decrease of IαIp in cases with positive blood culture than those with negative blood culture, this finding is not matched with the previous study. 12 It is reported that the defending impacts of IαIp may be due to its effect as potent inhibitors of furin which is endogenous cell membrane-associated serine endoprotease, that has role in incomplete initiation of proteolysis of bacterial toxins [17].

Also, IαIp values insignificantly decreased among non-survival cases. The cutoff point for mortality was >13.29 mg/L with sensitivity of 53.33 % and specificity of 92.31%. As far as we know this is first study to look at the differences between IαIp in survival and dead cases with NEC. IαIp values were inversely related with mortality in adult with sepsis. Failure of recovery of IαIp levels over the course of sepsis is associated with an unfavorable outcome [18].

There is shortage of precise clinical and laboratory findings that constrains early diagnosis of NEC and results in over diagnosis with subsequent vigorous treatment. The typical clinical diagnostic features of necrotizing enterocolitis may be delayed, while early diagnostic symptoms and signs as feeding intolerance are not specific and may be related to prematurity or other illness as sepsis. There are no single conclusive laboratory tests to diagnose necrotizing enterocolitis; abnormal leukocytes count or platelets could be related to infection. The early imaging may be normal or shows mild ileus as fixed dilated loops of bowel, that may need to be repeated to confirm the diagnosis, this carry risk of exposure to radiation. Extraluminal air outside the intestine or pneumatosis intestinalis are sign of advanced necrotizing enterocolitis. IaIp can be added to diagnostic tool of NEC due to its high sensitivity, specificity in predicting NEC and its prognostic value.

Biomarker research has remarkable capacity to expand our realizing of the pathogenesis of NEC and consequently progress in early diagnosis and management [19]. Although there is emerging role for proteomic or a metabolomic studies, but it is expensive and needs multidisciplinary studies and efforts by researchers with diverse expertise [20].

Conclusion

From these findings we conclude that inter-alpha inhibitor protein levels were reduced in full term and preterm newborn infants with NEC, consequently use of inter-alpha inhibitor protein as potential marker may improve the diagnosis of NEC in neonates with nonspecific and suspected abdominal disorders. Inter-alpha inhibitor protein had prognostic values and its level is associated with the severity of NEC and might predict mortality.

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Tonic Eye-Opening Associated with the «Burst-Suppression» Pattern in Patients with Acute Anoxic Brain Injury (Case Series)

Mikhail V. Sinkin[1,2]*, Amayak G. Broutian[3], Ekaterina G. Seliverstova[1], Kirill A. Salimov[4], Elena A. Baranova[5,6], Konstantin A. Popugaev[1,7]

[1] N. V. Sklifosovsky Research Institute of Emergency Medicine, Moscow Department of Health, 3 Bolshaya Sukharevskaya Square, Bldg. 1, 129090 Moscow, Russia
[2] A. I. Evdokimov Moscow State University of medicine and dentistry, Ministry of Health of Russia 20 Delegatskaya Str., Build 1, 127473 Moscow, Russia
[3] Research Center for Neurology 80 Volokolamskoe Highway,125367 Moscow, Russia
[4] Eramishantsev City Clinical Hospital, Moscow City Health Department, 15 Lenskaya Str., 129327 Moscow, Russia
[5] Federal Center for Brain Research and Neurotechnologies, Federal Medical-Biological Agency of Russia, 10 Ostrovityanova Str., 1 bldg, 117513 Moscow, Obruchevsky district, Russia
[6] Kazan State Medical Academy, branch of the Russian Medical Academy of Continuous Post-Graduate Education, Ministry of Health of Russia, 36 Butlerova Str., 420012 Kazan, Russia
[7] A. I. Burnazyan Federal Medical Biophysical Center, 46 Zhivopisnaya Str., 123182 Moscow, Russia

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***Correspondence to:** Mikhail V. Sinkin, mvsinkin@gmail.com

Summary

Massive anoxic brain injury caused by cardiac arrest leads to wakefulness suppression up to coma. The prediction of outcome is based on the analysis of the clinical features and the results of instrumental tests. One of the well-known signs of an unfavorable prognosis is involuntary motor activity, which is most commonly represented by myoclonus. In case of their cortical origin, they are accompanied by epileptiform activity in the electroencephalogram (EEG).

Material and methods. We present a case series and literature review concerning a very rare fatal sign, nonrhythmic spontaneous eye opening accompanied by a «burst-suppression» pattern (BS) in the EEG. All patients suffered from transient acute hypotension or arrhythmia that required cardiopulmonary resuscitation (CPR) in three cases. A literature search found only 11 publications describing post-anoxic tonic eye-opening (PATEO).

Results. The PATEO with BS was observed for less than a day followed by cessation of brain bioelectric activity in all patients. Only two patients exhibited isolated eye-opening and closing, while the rest had axial and limbs myoclonus just after CPR. In one case, eyelid opening was followed by a clonic movement of the head to the right, the EEG bursts were prolonged and had spike-like morphology. Three patients received antiepileptic and sedative therapy. All patients died in 3–43 days after the fatal cardiovascular event.

Visual superposition of bursts in EEG and myogram of m. orbicularis oculi demonstrating identical morphology for EEG and myographic bursts was described for the first time. Our cases and literature review confirm that, regardless of the intensive treatment, patients with PATEO have fatal outcomes.

Conclusion. The clinical and electrographic PATEO with BS phenomenon always indicates a lethal prognosis. The origin of PATEO is still under discussion. We suggest that it could be caused by disinhibition of subcortical and stem structures during extensive death of cerebral cortical neurons.

Key words: EEG; burst-supression; myoclonus; anoxia

Conflict of interest. The authors declare no conflict of interest.

Introduction

Prognostication in coma is essential for selecting an intensive care strategy [1]. For this purpose, both clinical signs and instrumental methods of central nervous system investigation such as computed tomography (CT), electroencephalography (EEG), somatosensory evoked potentials (SSEPs) are used. Beyond pharmacologic sedation, complete cessation of any motor activity or myoclonus indicates severe brain damage and a poor prognosis for recovery of consciousness [2]. In contrast, motor seizures and other manifestations of motor activity are not associated with a fatal outcome and are a positive prognostic factor, reflecting preserved cerebral function [3]. Spontaneous and reflex movements observed in brain death are distinct, but the location of active myotomes below the C2 spinal segment is their obligatory feature [4].

We observed five patients in deep coma after acute anoxic brain injury with a rare and unusual sign, isolated slow eyelid opening and closing, which lasted for several dozen hours consecutively and was followed

by a fatal outcome regardless of the chosen intensive therapy approach. Simultaneously, the EEG recorded a burst suppression pattern (BSP), in which the bursts occurred synchronously with the onset of upper eyelid movement. We present a summary of patient histories and a review of publications confirming that postanoxic tonic eyelid opening (PATEO) is a poor clinical and EEG prognostic sign indicating an extremely high probability of fatal outcome. The methodology of data analysis and presentation was approved by the local ethics committee of the N.V. Sklifosovsky Research Institute for Emergency Medicine.

Materials and Methods

Case 1. Patient D., 57 years old, was admitted to the clinic of the Federal Medical and Biological Center named after Burnazyan of the Russian Federal Medical and Biological Agency for surgical treatment of gastric tumor. On the first day after the surgery, which included subtotal distal gastric resection with creation of gastroenteroanastomosis, the patient developed massive intra-abdominal bleeding, which was controlled by repeated surgical intervention. Due to severe hypotension caused by hemorrhagic shock, coma developed along with respiratory failure requiring mechanical ventilation, while no cardiac dysfunction or arrhythmias were noted. The patient remained in critical condition the next day. Neurological status: muscular atonia, total areflexia, GCS score of 3, FOUR score of 1. Severe generalized myoclonus of the body and limb muscles accompanied by periodic opening/closing of the eyelids with a frequency of about once every 8–10 seconds. This was considered a manifestation of myoclonic epileptic status, and drug therapy with sodium thiopental was initiated, during which the myoclonus completely resolved, but the slow spontaneous opening/closing of the eyelids persisted for 24 hours, and the eyeballs remained immobile. Video-EEG monitoring, started simultaneously with thiopental infusion, showed that bioelectrical activity of the brain was represented by BSP with the same morphology of bursts with amplitude reaching 200–300 μV, duration varying in the range of 2–10 seconds, and intervals between bursts ranging from 8 to 10 seconds. All EEG bursts were accompanied by eyelid opening and closing (Fig. 1).

This EEG pattern persisted until death, which occurred on the third day after the initial onset of signs and symptoms.

Case 2. Patient N., female, 33 years old, was admitted to the emergency department of A. K. Yeramishantsev City Clinical Hospital with suspicion of ectopic pregnancy. Laparoscopic tubectomy with abdominal drainage was performed. Total blood loss was about 25% of circulating blood volume, blood transfusion was performed with stabilized systemic hemodynamics. The sedated and ventilated patient was transferred from the operating room to the intensive care unit and later extubated. Ten hours after surgery, the patient suddenly developed asystole and cardiopulmonary resuscitation (CPR) was immediately initiated. Spontaneous sinus rhythm was restored after 11 minutes of resuscitation, with no evidence of myocardial ischemic injury on ECG. The patient's condition remained critical, she was in deep coma with stereotyped brief eyelid opening/closing with upward deviation of the eyeballs, repeated every 20 seconds. The patient was started on valproic acid 900 mg daily, and considering the resistance to increasing doses of propofol, sodium thiopental 40 mg/h was added with gradual increase up to 100 mg/h. After initiation of anticonvulsant therapy, EEG monitoring was initiated. The burst suppression pattern was recorded with paired bursts occurring with eyelid opening and partial lowering (the first component of the burst) and repeated eyelid opening at the time of the second component. Both lid openings were accompanied by upward eye deviation, and all bursts had the same morphology. To rule out artifactual origin of EEG bursts due to the patient's eyelid opening, a muscle relaxant trial was performed. Administration of rocuronium abolished the clinical manifestations but did not alter the EEG picture. When the dose of sodium thiopental was decreased, the duration of bursts increased to 20–30 seconds and their amplitude reached 400 μV, and when the dose was increased, paired bursts were transformed into single bursts of about 1 second duration. At the end of the second day after CPR, the bioelectrical activity of the brain was reduced to a minimum, with an amplitude of 2–4 μV. Sedation was discontinued, but PATEO did not resume.

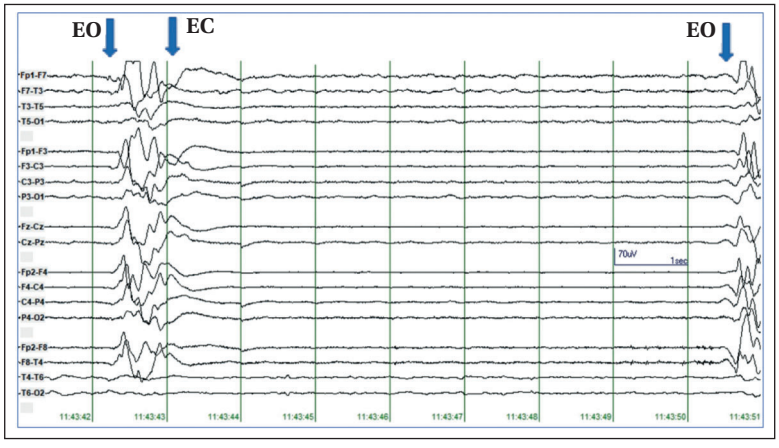

Fig. 1. EEG of patient D. Spontaneous eyelid opening during EEG bursts.
Note. Vertical arrows indicate eyelid opening (EO) and closing (EC).

The disease was later complicated by infection with progressive multiple organ failure, and death occurred on the 43rd day of hospitalization.

When analyzing the EEG of patient N. we first used the EegRev viewer (developed by A.G. Brutyan) to superimpose several bursts in the BSP. The result of processing, demonstrating complete similarity of morphology of EEG and EMG elements at the moment of PATEO, is shown in Fig. 2.

Case 3. Patient B., female, 52 years old, was admitted to the Interregional Clinical and Diagnostic Center, Kazan, with the diagnosis of dissecting aneurysm of the ascending aorta. Prosthetic repair of the ascending aortic hemi-arch was performed under cardiopulmonary bypass. Respiratory failure with low oxygenation requiring prolonged mechanical ventilation developed after surgery. On postoperative day 12, in addition to respiratory failure, acute hypotension and ventricular fibrillation occurred, requiring cardiopulmonary resuscitation, which resulted in cardiac rhythm recovery and hemodynamic stabilization. Impaired consciousness progressed to deep coma and the patient had myoclonic contractions of upper and lower limb and trunk muscles with EEG showing generalized spike-like bursts followed by polymorphic slower impulses lasting 2.5–3 seconds. The patient's condition was diagnosed as nonconvulsive status epilepticus and treatment with sodium thiopental 340 mg/h was initiated. After 4 hours, with continued thiopental infusion, the burst-suppression pattern appeared, with tonic eyelid opening and closing lasting up to 3 seconds between bursts, with episodes of suppression on the EEG lasting up to 10 seconds (Fig. 3). The myoclonus ceased. The PATEO phenomenon was observed continuously for 4 hours, then the eyelid opening/closing gradually stopped and the morphology of the bursts changed from sharper waves to slow-wave theta-band activity. Twenty-four hours after CPR, the last burst of theta waves with an amplitude of up to 20 μV was recorded, and for the next 20 hours there was no electrical activity of the brain. Asystole occurred at 44 hours after CPR.

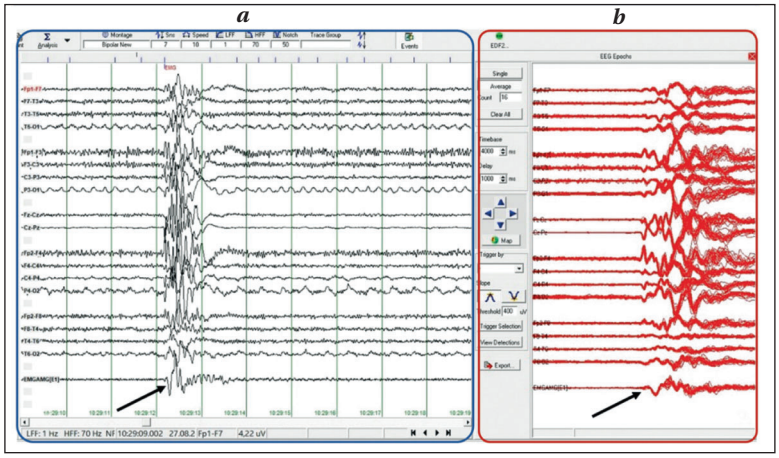

Fig. 2. Electroencephalogram of patient N. as seen in the EEG viewer EegRev. Longitudinal bipolar double banana montage, with myogram registration from the circular muscle of the left eye.
Note. *a* — Native recording, BSP; *b* — Superimposition of 16 bursts showing the same morphology of EEG and muscle contractions (indicated by an arrow).

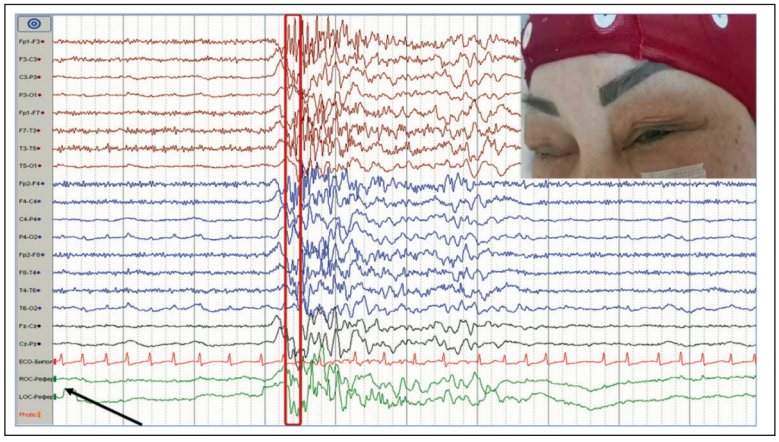

Fig. 3. EEG of patient B. Longitudinal bipolar double banana montage, with myogram registration from the circular muscle of both eyes.
Note. The oculographic channels are indicated by an arrow. The inset shows the beginning of the burst accompanied by the opening of the eyelids. The right eyelid opened less due to edema.

Case 4. Patient M, 89 years old, was admitted to the Sklifosovsky Research Institute for Emergency Medicine after a cerebral infarction of the left middle cerebral artery, and due to a coronavirus infection she was admitted to the ICU of the Infectious Diseases Department of the Institute. She received antihypertensive, antibacterial, and fluid therapy and prophylaxis of thromboembolic and infectious complications. A chest CT scan revealed progressive lung involvement with increasing respiratory failure. Ventilation was started and an inferior tracheostomy was performed. After 6 days, the patient developed right-sided pneumothorax and hydrothorax requiring pleural drainage. The patient's condition progressively worsened, and on the 20th day of hospitalization, cardiac arrest occurred, and cardiopulmonary resuscitation (CPR) was started, which resulted in rhythm restoration and hemodynamic stabilization. On the first day after CPR, the patient was in a deep coma (GCS 3, FOUR 0), muscle tone was globally reduced. There was no spontaneous motor activity. Twenty hours after CPR, periodic spontaneous open-

ing and closing of the eyes occurred, accompanied by a brief myoclonic movement of the head to the left. During video-EEG, BSP was recorded with the duration of uniform bursts up to 6 seconds. The moment of slow eyelid opening coincided with the high-amplitude onset of the burst, and the clonic head movement coincided with its low-amplitude segment containing rhythmic sharp waves (Fig. 4).

To rule out ictal origin of the movements, 700 mg of valproic acid was administered intravenously. Since there was no change, the situation was considered a clinical and electrographic manifestation of severe encephalopathy. The next day, spontaneous eye movements and head jerking stopped, and EEG recorded BSP with short monomorphic bursts and interspike intervals of up to 20 seconds. From the next day onward, EEG showed no bioelectrical activity of the brain, deep coma persisted, brainstem reflexes were absent, muscle atonia was seen, and spontaneous breathing stopped. The patient died on the 7th day after CPR.

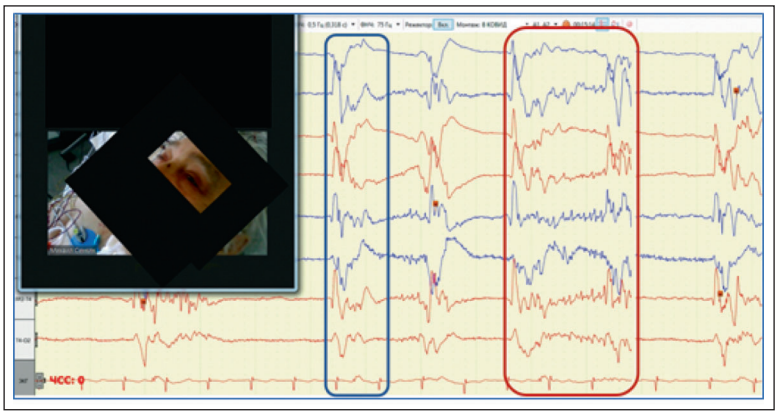

Fig. 4. EEG video window via Zoom application used to communicate with the computer to record the EEG in the infectious ward [5].
Note. The blue frame marks the fragment of the onset of BSP, which coincided with the beginning of eyelid opening, and the red frame marks the clonic rotation of the head to the left.

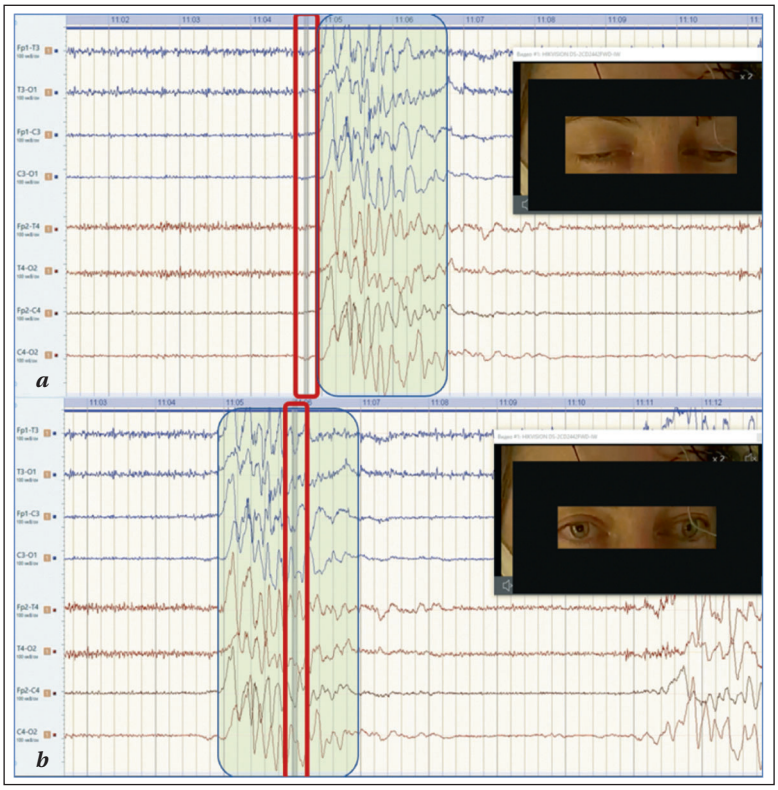

Fig. 5. Video-EEG of patient R. Longitudinal bipolar montage with double interelectrode distance.
Note. *a* — synchronous video recording marker (in the red frame) is set at the interspace, at this moment the patient's eyes are closed; *b* — synchronous video recording marker is set at the center of the flash (highlighted by the green field). The video recording shows a wide opening of the eyes.

Case 5. Patient R., female, 41 years old, with multiple metastatic lesions of the brain and spinal cord, was admitted to the Sklifosovsky Research Institute for Emergency Medicine with a diagnosis of clinical death after a sudden development of coma. Cardiopulmonary resuscitation (CPR) allowed to restore the cardiac rhythm, but the patient's condition remained critical the next day after admission. There was complete areflexia and no muscle tone in the extremities was present. The GCS score was 3 and the FOUR score was 1. Approximately 24 hours after CPR, periodic spontaneous eye opening and closing was noted, which was considered a manifestation of status epilepticus. Continuous EEG recording showed BSP with polymorphic high-amplitude uniform arrhythmic bursts lasting approximately 1.5–2.0 seconds with an interspike interval of 3 to 10 seconds, synchronous with tonic eyelid opening and closing not accompanied by other motor manifestations (Fig. 5).

In the study of somatosensory EPs during median nerve stimulation, no component from the cortical evoked potential response generator (N20–P23 complex) was recorded (Fig. 6).

This was considered as clinical and EEG manifestation of anoxic encephalopathy, therefore anticonvulsants and sedatives were not administered, EEG monitoring was continued. The clinical and electrographic phenomenon lasted for about 20 hours, then the bioelectrical activity of the brain disappeared. There was no subsequent improvement, and death occurred 12 days later due to multiple organ failure.

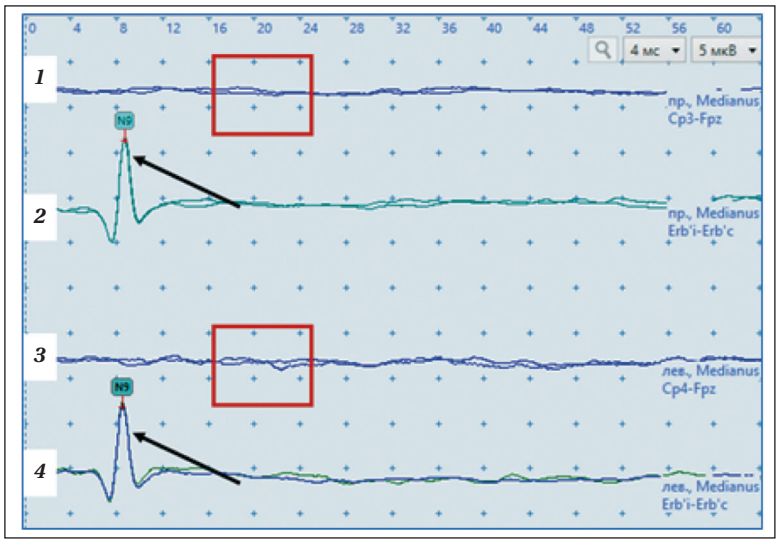

Fig. 6. Somatosensory evoked potentials during median nerve stimulation in patient R.
Note. Traces 1, 3 reflect cerebral leads in the projection of the postcentral gyrus, traces 2, 4 are from Erb points in the projection of the cervical plexus. The arrows indicate the preserved components of the peripheral response 9, the red boxes indicate the presumed location of the missing components N20-P23, reflecting the functional state of the primary sensory cortex.

Thus, all reported cases have in common an unusual clinical sign of tonic eyelid opening and closing in patients with deep areactive coma, which occurred several hours after acute diffuse anoxic brain injury. In all patients, the PATEO phenomenon occurred several hours after CPR, but always within the first day, and its duration did not exceed 24 hours. In one case, eyelid elevation was accompanied by upward deviation of the eyes, and in the other two cases there were synchronous myoclonic jerks of the axial neck muscles leading to head rotation.

All patients showed a specific EEG phenomenon, i. e., BSP with burst onset coinciding with eyelid opening (Table 1).

Discussion

Since verbal contact with the patient in coma is impossible, clinical evaluation is based on the analysis of reflexes and movements, either spontaneous or stimulation-induced. Such motor activity may be a sign of epileptic seizure, delirium, unspecific behavioral changes, disinhibited spinal reflexes in brain death, brainstem herniation, and may indicate an unfavorable prognosis of the disease [6]. EEG recording and analysis of time-related bioelectrical and clinical phenomena is one of the main ways to determine the origin of such movements [7].

We report a well-documented series of cases demonstrating an unusual clinical sign — periodic tonic spontaneous eyelid opening and closing in patients in deep coma caused by acute anoxic brain injury, always accompanied by a burst-suppression pattern on the EEG. The extreme rarity of this phenomenon led to difficulties in the choice of intensive treatment.

A search of the MEDLINE and RSCI databases using the queries «burst suppression,» «postanoxic myoclonus,» «postanoxic myoclonus,» «postanoxic seizures,» «postanoxic movements,» «postanoxic burst suppression,» «postanoxic eye opening,» and «periodic eyelid opening,» and selecting papers describing «eye opening» or «tonic eyelid opening,» yielded 11 publications reporting data from 37 patients with similar clinical and EEG manifestations (Table 2).

The combination of BSP with periodic eyelid opening and closing was first described by P. Wolf in 1977 in a case series of 5 patients with postanoxic encephalopathy who had various spontaneous movements considered to be myoclonus that occurred synchronously with the onset of a burst on the suppressed EEG [8]. In one patient, on day 6 after cardiac arrest and successful cardiopulmonary resuscitation, a nonrhythmic, intermittent, slow raising and lowering of the eyelids lasting approximately 1.5 seconds was observed. This sign was preceded by myoclonus in the axial muscles and limb flexors. The author pointed out that not all bursts in BSP were accompanied by eyelid opening, but when they occurred, they always coincided with a burst of electrographic activity. Two days after the onset of spontaneous eye opening, the patient died.

Table 1. Characteristics of clinical cases.

Clinical case	Age, years	Sex	Specifics	AEDs	Time to death, days
1	57	m	IEO	VA, propofol, thiopental	3
2	33	f	IEO + upward eye deviation	VA, thiopental	43
3	52	f	IEO + myoclonus of limbs and axial muscles	Thiopental	2
4	89	f	IEO + axial neck myoclonus	No	7
5	41	f	IEO	No	6

Note. IEO — isolated eyelid opening; AED — antiepileptic drug; VA — valproic acid.

Table 2. Publications on PATEO with BSP.

No	Author(s)	Number of observations (m/f)	Age, years	Movements other than PATEO	EEG pattern	Time from CPR to death
1	Wolf P. (1977)	1 (0/1)	62	LM, phrenic, mimic, masticatory, tongue AM		2 days
2	McCarty G. E. et al. (1981)	4 (1/3)	4–68	UED, mild decerebrate postural movements in arms of 1 patient		12–24 hours
3	Jordan J. et al. (1982)	1 (1/0)	54	UED, LM in legs not related to BSP	Diffuse slowing after cessation of BSP	28 days
4	Mori E. et al. (1983)	1 (0/1)		GTCSE, AM, UED	Transformation of BSP into GPD after AED administration	Akinetic mutism 1 month after CPR
5	Reeves A. L. et al. (1997)	12 (7/5)	35–90	AM, MK, UED, chewing, tongue protrusions, bobbing		During acute phase of brain injury
6	Fernández-Torre J. et al. (2008)	1 (0/1)	50	Swallowing		5 days
7	Ferrara J. et al. (2012)	4 (3/1)	34–62			Up to 8 days
8	Crawford J. et al. (2015)	1 (1/0)	12			48 hours
9	Dericioglu N. et al. (2015)	1 (0/1)	72	UED	Transformation of BSP into BiIPD	75 days
10	Afra P. et al. (2019)	1 (0/1)	46		Bursts up to 2 s, transformation of BSP into GPD	3 days followed by discontinuation of CPR
11	Alsallom F. et al. (2021)	10 (5/5)	33–74	LM in 4 patients		Up to 7 days

Note. LM — limb myoclonus; AM — axial myoclonus; UED — upward eye deviation; GTCSE — generalized tonic-clonic status epilepticus; AED — antiepileptic drugs; BiIPD — bilateral independent periodic discharges; GPD — generalized periodic discharges.

The first detailed description of slow eye opening and closing synchronous with BSP was published in 1981 by McCarty G. E. et al., who reported 4 patients after cardiopulmonary resuscitation with such isolated stereotypic movements. In all patients, the episode of BSP with eye opening lasted several hours and gradually subsided, followed by cessation of bioelectrical activity on the EEG and death within the next 24 hours. Only one patient had synchronous upward deviation of the eyeballs and mild decerebrate movements that occurred independently of EEG activity. The publication did not indicate whether the patients had received anticonvulsant therapy, but the authors compared the finding to postanoxic myoclonus and noted that it was unclear whether the symptom was a manifestation of the epileptic status or a disinhibition phenomenon due to the termination of central inhibitory influences caused by cortical damage [9].

In a series of 12 cases, Reeves A. L et al. observed more than one type of movement in most patients (92%). Most commonly, there was a combination of PATEO with movements of the face, mouth, and tongue muscles followed by myoclonus, and in two patients more than 4 different types of such movements were observed. In the discussion section, the authors suggested that PATEO with BSP was a manifestation of an epileptic seizure, since the bursts on the EEG reflected cortical activity, with neurons exciting subcortical structures. At the same time, despite antiepileptic treatment administered to half of the patients, all patients reported in the paper died [10].

Among other publications, the description of various movements accompanying the phenomenon of eye opening and closing is quite interesting. For example, Fernández-Torre J. L. et al. described a 50-year-old patient who underwent CPR with rhythm recovery and, in addition to BSP with eyelid opening, had swallowing movements and generalized myoclonus, not always coinciding with the burst in BSP. Rhythmic sharp waves were observed in BSP, and the authors considered this to be an ictal state and administered propofol sedation and anticonvulsants. Despite intensive therapy, the patient died on day 5 after CPR [11].

In a series of 4 observations of BSP with eye opening published by Ferrara J. M. et al, a slight upward deviation of the eyes was recorded in two patients synchronous with their opening. The authors also noted that eye opening ceased in all 4 patients 12 hours after onset, while one of them still had BSP on the EEG, but with a reduced amplitude and frequency of bursts [12].

Eye opening in BSP has not only been observed in adults. For example, Crawford J. R. et al. described this phenomenon in a 12-year-old boy

with anoxic brain damage due to combustion product poisoning [13].

The largest series of observations in patients with PATEO and BSP was reported by Alsallom F. et al. who analyzed video EEG monitoring recordings obtained to predict the progression of coma developing after cardiac arrest. In the series presented, three patients underwent magnetic resonance imaging (MRI) of the brain, which showed relative preservation of the brainstem with significant cortical damage. Because the authors believed that PATEO was an ictal phenomenon, 8 patients were treated with anticonvulsants. Despite this approach, all patients died with a maximum follow-up of 7 days after the onset of PATEO [14].

PATEO with BSP and less fulminant progression. Similar to our observations, all previously published cases of PATEO with BSP were fatal. However, a few publications have described patients with a less pernicious course of postanoxic encephalopathy or with a different electrographic pattern.

In response to the first publication describing PATEO [9], Jordan E. et al. reported a 54-year-old man who underwent CPR followed by generalized myoclonus and then BSP associated with PATEO. Contrary to other observations, the authors pointed out that the day after cessation of PVP, the EEG retained suppressed activity manifested as diffuse slowing, although death occurred on the 28th day after CPR [15].

Dericioglu N. et al. observed a 72-year-old patient with amyotrophic lateral sclerosis who had PATEO with BSP and upward eye deviation on the following day after CPR. It was considered a manifestation of status epilepticus and anticonvulsant therapy was started. Twelve hours later, PATEO ceased and BSP on EEG changed to bilateral independent periodic discharges, which continued with gradual attenuation for over 18 hours. Subsequently, suppressed activity persisted on the EEG, and MRI performed 1 week after CPR showed diffuse damage to the cortex and basal ganglia. Death occurred on the 75th day after CPR [16].

A similar case of «ictal PATEO» was described by Afra P. et al. They reported a 46-year-old female patient with BSP bursts accompanied by EEG-documented epileptic seizures of up to 25 seconds duration. Initial propofol sedation with gradual dose escalation resulted in conversion to BSP with the same burst duration, but PATEO ceased. Decreased sedation with the addition of anticonvulsants resulted in the appearance of the generalized periodic discharge pattern with the same morphology on the EEG. An MRI performed on day 3 showed severe diffuse brain damage, including the basal ganglia, and treatment was discontinued due to the unfavorable prognosis [17].

The only known case of PATEO with BSP in a patient who developed akinetic mutism was published in 1983 by Mori E. et al. A patient with heart failure and atrial fibrillation after cardiopulmonary resuscitation developed a state of generalized tonic-clonic convulsions lasting more than 24 hours, after which rapid eyelid elevation with eye deviation, neck extension, and pupillary hippus remained among the motor manifestations that persisted with anticonvulsant therapy. The movements occurred synchronously with bursts of BSP on the EEG. Over the next 24 hours, the movements ceased and generalized periodic discharges were recorded on the EEG. At the time of publication, the patient was in a state of «akinetic mutism» (corresponding to the modern concept of unresponsive wakefulness syndrome), and there were episodes of alpha rhythm on the EEG [18].

For the first time in the national literature, we described a series of clinical cases of PATEO with BSP. Our observations were in agreement with previously published reports. An isolated oculopalpebral subtype of PATEO was also found in two patients. In one patient, eyelid elevation was accompanied by upward deviation of the eyeballs, and two consecutive bursts corresponding to these movements were registered on the EEG. In another case, PATEO was accompanied by a myoclonic movement of the head to the right, also consistent with published cases. PATEO was observed for a relatively short period of time in all patients, regardless of the life span after CPR, the eyelid movements always stopped, and further observation never showed a recovery of clinical or bioelectrical activity of the brain.

To date, the mechanism and functional topography of the PATEO phenomenon with BSP remain undetermined. The lethality of this symptom is undisputed and has been confirmed in the cases presented. However, its ictal origin remains the main subject of discussion. Despite its small surface area, the upper eyelid has a complex neuromuscular apparatus and central innervation pathways. Two transverse striated muscles, the orbicularis oculi and the levator palpebrae (LP), are responsible for eyelid opening. The upper tarsal muscle, which is composed of smooth muscle tissue and is innervated by fibers from the upper cervical sympathetic node, adjoins the anterior surface of the LP. The motor neurons of the LP are located in the central parts of the caudate nucleus and are controlled by the premotor parts of the cerebral cortex, while the surrounding gray matter is involved in maintaining the tonic activity of this muscle [19].

The first publications describing PATEO suggested that this sign was a special type of postanoxic myoclonus with the location of the abnormal source of activity in the brainstem. Neurophysiological

Table 3. Neurophysiological characteristics of myoclonus depending on the anatomical localization of the source of abnormal activity.

Neurophysiological characteristics	Localization		
	Cortical	Subcortical	Segmental and peripheral
EEG	Epileptiform activity — generalized spike waves	No specific signs, may be totally absent	No specific signs, may be totally absent
Duration of muscle contraction	20–70 ms	75–300 ms	>100 ms
Inverse averaging of EEG by myogram	Characteristic activity 30-40 ms before muscle contraction	No activity	No activity

characteristics of myoclonus depending on the anatomical location of the source of abnormal activity are presented in Table 3, but all are characterized by a muscle contraction duration of less than 300 ms [20].

The presence of epileptiform impulses in the bursts, the long duration of the eye opening/closing phase, which significantly exceeds the duration of the myoclonus, indicates the failure of the hypothesis that PATEO with BSP represents subcortical myoclonus.

Electroencephalographic BSP reflects the functional dissociation of the brain, which can occur both under the influence of sedatives and in its diffuse damage [21].

Depending on the etiology of the disease, the predictive value of BSP may vary, but in anoxic injury it always indicates an unfavorable prognosis [22, 23]. The same morphology of bursts in BSP is a consistent ominous sign. The analysis of the EEG of 101 patients with BSP by Hofmeijer J. et al. showed that this particular type of bursts can only be observed in patients with diffuse cortical lesions, most often due to acute anoxia [24].

For the first time, we performed a software superimposition of bursts in BSP in patients with PATEO (Fig. 2), which showed their complete identity, including the morphology of muscle oscillations registered by the myographic channel. This finding supports the hypothesis that PATEO is a phenomenon of periodic disinhibition of nuclei located in the medulla oblongata due to acute extensive cortical and subcortical injury and is similar in origin to BSP with the same morphology of bursts. This is also supported by the obligatory occurrence of the sign in the acute phase of brain damage, its rapid exhaustion without recurrence, and its absolute fatality with any intensive care strategy. The only «less ominous» case of PATEO with BSP described in the literature [18] may have been a non-convulsive status epilepticus with minimal motor manifestations due to the underlying drug sedation.

The hypothesis of ictal origin of PATEO is based on the evaluation of the «epileptiform» morphology of the bursts and their relationship with the corresponding motor manifestations. In a number of patients presented in the literature review above [10, 14], PATEO had a different morphology and was also accompanied by epileptiform activity during which the eyes remained open. It is likely that the reported patients had a combination of PATEO and non-convulsive status epilepticus, which is common in patients after CPR [25]. Cases of similar tonic eye opening in response to painful nipple stimulation in patients who were declared brain dead further support our assumption of the non-ictal nature of PATEO [26].

Conclusion

The reported cases of the combination of BSP and periodic nonrhythmic tonic eyelid opening and the review of the literature indicate a very ominous character of this sign. The origin of PATEO remains controversial, but in our opinion it could be due to the disinhibition resulting from the cessation of control of dead cortical neurons over the nuclei of subcortical and stem structures, which still retain partial bioelectrical activity.

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Methodical Approach to fMRI Assessment of Motor Connectome in Patients After Severe Traumatic Brain Injury

Marina V. Stern[1,2]*, Elena V. Sharova[2], Ludmila A. Zhavoronkova[2], Vladimir T. Dolgikh[1], Artem N. Kuzovlev[1], Igor N. Pronin[3]

[1] Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, 25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia
[2] Institute of Higher Nervous Activity and Neurophysiology, RAS, 54 Butlerova Str., Moscow 117485, Russia
[3] N. N. Burdenko National Medical Research Center of Neurosurgery, Ministry of Health of Russia 16 Tverskaya-Yamskaya 4th Str., 125047 Moscow, Russia

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***Correspondence to:** Marina V. Stern, mstern@fnkcrr.ru

Summary

The aim of the study. To identify alterations of motor connectome in patients with varying degrees of hemiparesis after severe traumatic brain injury (TBI) versus healthy volunteers.

Material and methods. The study included 29 patients with TBI aged 18 to 35 years and 23 healthy volunteers aged 20 to 32 years. Participants underwent a comprehensive clinical and neuroimaging study. Motor impairment was evaluated via muscle strength assessment using a five-score scale. The fMRI data were processed using a dedicated CONN software package. Anatomical 3-D connection masks of the whole brain motor functional system in the predetermined regions of interest (ROIs) were used for the assessment. Then the group indicators of functional connectivity (statistical significance of the connection) were computed.

Results. It was established that the structure of connections in healthy individuals performing active movement with the right (leading) hand is determined by formation of focus in the cortical and subcortical ROIs in the contralateral hemisphere. With passive movement of the right hand the pale ball becomes functionally active in addition to the activated areas. The striopallidar system structures became active on both sides, and connectivity with the additional motor cortex and the motor cortex of the ipsilateral hemisphere emerged as the paresis increased during active movement. The focus of motor activity during passive movement was determined in the motor cortex and putamen, which makes it possible to use a passive test in patients with gross motor disorders or unconsciousness for a full assessment of the entire structural and functional brain connectome.

Conclusion. As hemiparesis increased in patients after severe traumatic brain injury, a decrease in the total number of connection appeared; simultaneous engagement of ancient primordial structures, such as bilateral activation of pale globes, demonstrated neuroplasticity.

Keywords: traumatic brain injury; chronic critical illness; pathogenesis of motor connectome impairment; neuroplasticity

Conflict of interest. The authors declare that there is no conflict of interest.

Founding. The study had no sponsorship.

Introduction

Advances in critical care medicine improve patient survival even after severe traumatic brain injury (TBI) [1–4]. However, TBI is associated with severe impairment of movement, cognitive function and memory, alterations in the immune system, prolonged disorders of consciousness and the autonomic nervous system [1, 5–7]. The study of the function of cerebral systems is important both for the exploration of the pathogenesis of motor dysfunction and compensatory mechanisms [8, 9] and for the development of neurorehabilitation methods for patients after severe TBI [10, 11], including those in chronic critical illness with prolonged reduced consciousness. Functional magnetic resonance imaging with assessment of functional connectivity between specific regions is a contemporary diagnostic method for motor disorders [12, 13]. The advantages of this approach include the standardization of the «regions of interest» and the quantification of the connectivity parameters, which can be measured in the range from –1 to +1 [12]. This allows for a wide range of comparisons of group data regardless of the morphological characteristics of the brain. According to the literature, the parameters obtained from fMRI data can serve as markers of neuronal activity [14, 15]. Therefore, studying the connectivity of the motor functional system is a promising way to assess the survival of components of the human brain motor system after TBI.

The aim of the study was to identify changes in the patterns of the motor function system in healthy subjects and patients with varying degrees of hemiparesis after severe traumatic brain injury (TBI).

Materials and Methods

Fifty-two subjects who met the inclusion criteria and had no exclusion criteria were included in the study (Fig. 1). The study was retrospective and observational.

Inclusion criteria:

— traumatic brain injury occurring 1-6 months earlier;

— right-sided hemiparesis;

— ability to follow instructions;

— right-handedness according to the Annette questionnaire [11].

Exclusion criteria:

— low level of consciousness;

— infectious complications and signs of acute infection;

— any contraindication to functional MRI and EEG;

— metal elements in the examined area (prostheses, clips, splinters);

— inappropriate patient behavior, such as panic attack, psychomotor agitation;

— inability to remain motionless during the examination;

— the need for continuous intensive care;

— the need for continuous monitoring of parameters such as ECG, blood pressure, respiratory rate.

Table 1 shows the general characteristics of the patients studied.

Patients were treated at the Neurotrauma Department of the N. N. Burdenko Scientific and Research Center for Neurosurgery. An fMRI study was performed simultaneously with the clinical examination.

The clinical observation included a comprehensive neurological examination. The level of consciousness was determined according to the Glasgow Coma Scale [16], using a five-point scale to assess movement disorders [17]. Right-sided hemiparesis in right-handed patients was chosen as a model because the majority of the population is right-handed and the function of the dominant and non-dominant hand differs in functional MRI characteristics in healthy subjects [19]. The focus was on the function and compensation mechanisms of the dominant hemisphere.

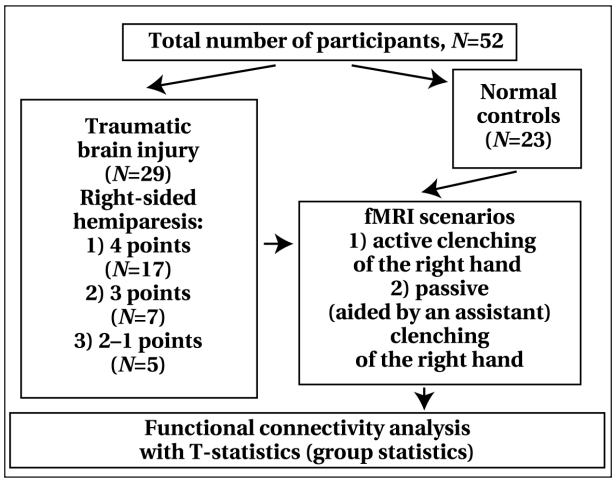

Fig. 1. Flowchart of the study.

Each participant underwent structural magnetic resonance imaging (MRI in T1 and T2 modes) and fMRI on a 3.0 Tesla GE Healthcare (General Electric, USA) magnetic resonance tomograph at the Department of X-ray and Nuclear Imaging of the Burdenko Scientific Research Center. The 3D FSPGR pulse sequence (BRAVO) was used to obtain structural data in the whole brain volume. The following parameters were used: TR=8.8 ms, TE=3.5 ms, slice thickness = 1 mm, FOV=250 mm, image matrix 256×256, voxel size 0.97×0.97×1.0 mm, while an echoplanar sequence was used to obtain functional data. Spin echo (BOLD T2) had the following characteristics: TR=2000 ms, TE=30 ms, slice thickness = 3 mm, FOV 250 mm, image matrix 128×128, voxel size 1.95×1.95×3 mm. In each time series, 300 sets of functional volumes were acquired, each containing 24–40 axial sections covering the entire brain. The scan time per functional volume was 2 seconds. The total number of slices in a functional series was 7000–12000. The signal-to-noise ratio was 1.0.

The fMRI with motor tests was performed with the subject's eyes closed using a block paradigm consisting of alternating periods of rest and movement, each lasting 30 seconds. The results of five repetitions of each test were averaged. Motor artifacts were corrected using a generalized linear model (GLM). The fMRI data (+BOLD response) were

Table 1. Patient characteristics.

Parameter	Right-sided hemiparesis				Healthy subjects	*P*
	Total patient cohort	4 points	3 points	2–1 points		
Number of patients	29	17	7	5	23	
Age, years	33±5.6	29±5	35±7	30±3	23.5±8	0.09
Sex	M — 19, F — 10	M — 10, F — 7	M — 5, F — 2	M — 3, F — 2	M — 14, F — 9	0.15
Consciousness level on CRS scale	Full 100%	Full 100%	Full 100%	Full 100%	Full 100%	
Average time after traumatic brain injury (days)	46±13	35±7	52±8	32±10	n/a	0.07
Right-handedness	100%	100%	100%	100%	100%	

processed according to a uniform protocol using SPM8 software in Mathlab 7.0 and Brainwave.

Two experimental situations were considered: an active test in which the fingers of the right hand were independently clenched/unclenched into a fist on command, and the performance of this movement with the help of an assistant. Each subject was instructed to remain in a quiet position. A structural MRI was also performed during the study to determine the comparability of the study groups. The study lasted 10 minutes and 12 seconds. A command was given (to the patient or assistant) to clench the hand into a fist for 30 seconds with a 30-second pause, and the subject performed 10 such series during the study.

Statistical analysis and connectivity construction were performed between the regions of interest (ROI) defined by the researcher. All obtained and saved ROIs in NIFTI format were transferred to MATLAB\toolbox\SPM\toolbox\CONN\rois system disk. The construction of functional relations was carried out in the CONN (Connectivity Toolbox) software based on MATLAB. This software allows to build graphical, 3D and 2D models of brain connectivity, as well as to estimate the strength, polarity and significance of connections. To determine the significant level of functional interaction between each pair of ROIs, we used Pearson correlation analysis followed by application of Fisher's bivariate transformation. Two-sample Student's t-test was used for intergroup analysis. The threshold of statistical significance was $P<0.05$ with correction for multiple comparisons.

The «mask» was designed based on literature data on subcortical and cortical support of voluntary movement [18], taking into account the multifaceted and multidirectional relationship of subcortical structures, as well as the density of the location of these entities. It combined all structures of interest: putamen, caudate nucleus, globus pallidus, precentral gyrus, amygdala, inferior frontal gyrus, supplementary motor cortex and cerebellum, thalamus, hippocampus. The connectivity of this mask was assessed in healthy subjects and patients with severe traumatic brain injury while performing active and passive right hand movements during an fMRI examination. Figure 2 shows the localization of the regions of interest between which functional connectivity was examined.

Given the multicomponent nature of this mask, all ROIs were grouped into networks reflecting their specific contribution to motor activity.

Network 1 included the caudate nucleus, putamen, globus pallidus and hypothalamus. In addition to memory, it also performs encoding and perception of external space. The interaction of the neostriatum

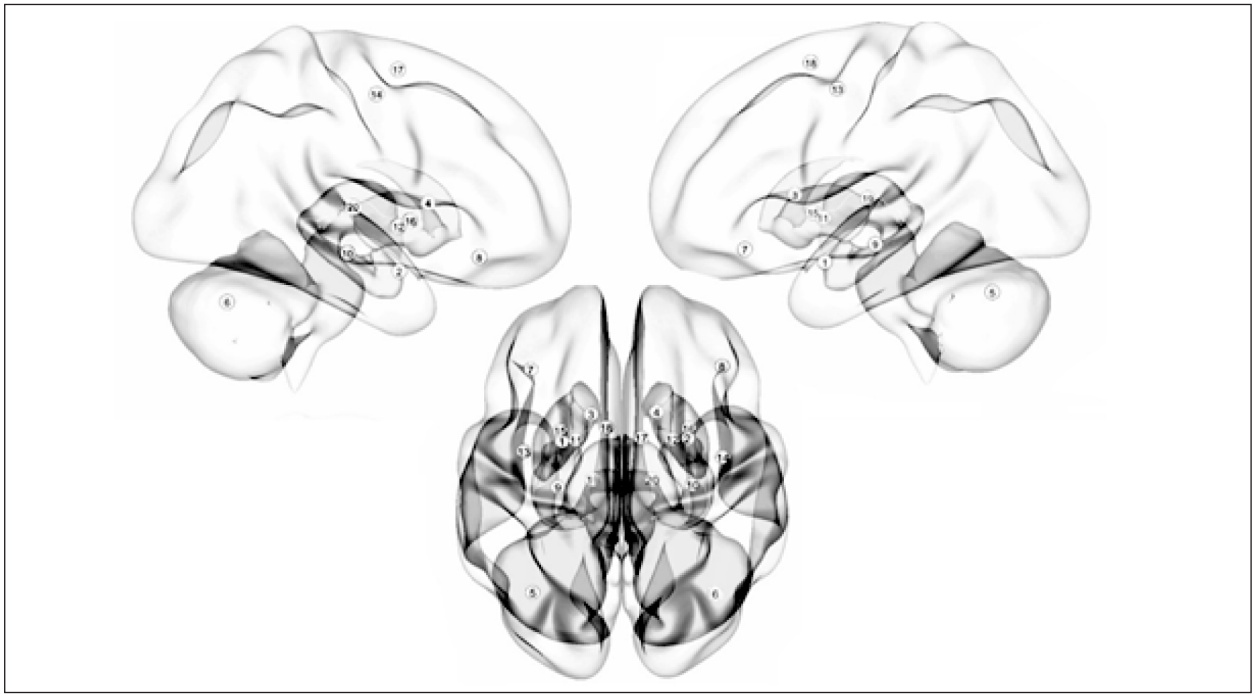

Fig. 2. Scheme of «regions of interest» for the evaluation of fMRI connectivity in the system of subcortical support of voluntary movement.

Note. «Regions of interest» according to the coordinates of the AAL atlas: 1 — left amygdala; 2 — right amygdala; 3 — left caudate nucleus; 4 — right caudate nucleus; 5 — left cerebellar hemisphere; 6 — right cerebellar hemisphere; 7 — left inferior frontal gyrus; 8 — right inferior frontal gyrus; 9 — left hippocampus; 10 — right hippocampus; 11 — left globus pallidus; 12 — right globus pallidus; 13 — left precentral gyrus; 14 — right precentral gyrus; 15 — left septum; 16 — right septum; 17 — right supplementary motor cortex; 18 — left supplementary motor cortex; 19 — left thalamus; 20 — right thalamus. Original drawing by the author.

Table 2. Severity of paresis in traumatic brain injuries of various brain regions (according to structural MRI).

Damaged structure	Frequency of paresis development in structure damage, %			*P*
	Paresis 4 points	Paresis 3 points	Paresis 2 points	
Brainstem	0	0	62.5	>0.05
Pons	17	0	50	>0.05
Left cerebral peduncle	0	0	25	>0.05
Right cerebral peduncle	17	20	25	>0.05
Both peduncles	0	0	25	>0.05
Corpus callosum	33	20	50	>0.05
Right thalamus	0	20	12.5	>0.05
Left thalamus	0	0	12.5	>0.05
Right subcortical nuclei	33	0	37.5	>0.05
Left subcortical nuclei	33	0	37.5	>0.05
Basal areas	0	20	50.0	>0.05
Right frontal lobe	50	60	87.5	>0.05
Left frontal lobe	50	60	87.5	>0.05
Right parietal lobe	33	80	62.5	>0.05
Left parietal lobe	33	40	62.5	>0.05
Right temporal lobe	50	60	62.5	>0.05
Left temporal lobe	33	60	87.5	>0.05
Right occipital lobe	17	20	37.5	>0.05
Left occipital lobe	0	0	50	>0.05
Diffuse axonal injury	33	20	87.5	>0.05

(caudate nucleus and putamen) and the paleostriatum (globus pallidus) allows the maintenance of position at rest. Thus, this network integrates subcortical structures of the motor functional system (MFS), which are functionally related to the maintenance of a certain posture in space.

Network 2 included thalamus, hippocampus, inferior frontal gyrus — structures of mesolimbic part of dopaminergic system and sites of its projection to cortex. In the motor system they form the afferent cluster.

Network 3 included precentral gyrus, caudate nucleus, putamen, amygdala, globus pallidus, which are part of extrapyramidal and motivational systems. Thus, this network contains MFS components responsible for voluntary state of rest or coordinated movement and motivation.

Network 4 included the precentral gyrus, supplementary motor cortex, amygdala and cerebellum, which are the components of the MFS that directly provide the precise motor act.

Paired *t*-statistics based on the selection of appropriate covariance matrices were used to analyze functional connectivity. A color scale was used to conveniently display the direction of connectivity. The color scale corresponded to the effect size (*T*-value). That is, the color indicated the highest significance, and deviations from red or blue indicated the «direction» of activation. Red was positive and blue was negative.

The studies were conducted in accordance with the principles of the Declaration of Helsinki, after obtaining informed consent from the subjects and approval from the ethics committees of the relevant institutions and the Scientific Research Center of Neurosurgery.

Results and Discussion

First, we analyzed the fMRI connectivity of the motor functional system of healthy subjects during active and passive movements of the right hand in healthy subjects. In Fig. 3 they are shown as schematic diagrams reflecting the level of significant connections (p-FDR corr <0.05) between the specified regions of interest. Obviously, the performance of active movement (Fig. 3, *I*) is associated with the formation of a «focus» of functional activity that includes both cortical and subcortical structures. The correlation between the structures of the subcortical (caudate nucleus, septum, globus pallidus) and cortical (motor, supplementary motor cortex and inferior frontal gyrus) levels of the motor functional system was recorded.

The subcortical nuclei (caudate, septum) form most of the interhemispheric as well as intrahemispheric connections. The lack of symmetrical frontal interaction indicates that this movement is automated. At the same time, the large number of bilateral connections of the amygdala, whose main function is to induce action, attracted attention.

Passive movement in normal subjects (Fig. 3, *II*) is characterized by a greater number of connections of subcortical structures than of cortical ones in network 3 (Fig. 3, *II, b*). This fact indicates the crucial role of subcortical structures (caudate nucleus and globus pallidus) in the regulation of muscle tone. The activity of the cortical regions and the activation of the structures of the corticospinal pathway (motor cortex and supplementary motor cortex) confirm the assumption that this test can be used to verify the functional integrity of this pathway.

Table 3 shows the matching pairs of subcortical connections and their significance (*T*-value) for

both studied scenarios of right hand movements, reflecting specific behavior of subcortical structures of the functional motor system. Of the 6 pairs, three (involving the caudate nucleus) showed a decrease in significance from the active to the passive test. This is most likely due to the fact that the passive test requires maximum relaxation, i. e., a conscious reduction of postural control. However, two amygdala connections had maximum significance during the active movement compared to the passive movement, reflecting the importance of motivation to perform the active movement. The largest number of connections was registered from the left globus pallidus area and the left motor cortex.

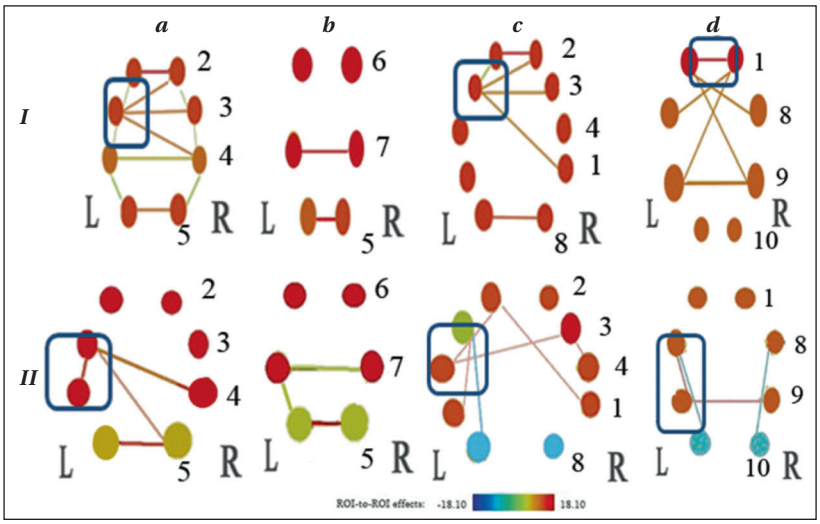

Fig. 3. Functional connections of subcortical and cortical-subcortical segments of the motor functional system according to fMRI data.
Note. *I* — with active right hand movement (*N*=23); *II* — with passive right hand movement (*N*=23). *a* — network 1; *b* — network 2; *c* — network 3; *d* — network 4. 1 — amygdala; 2 — caudate nucleus; 3 — cortex; 4 — globus pallidus; 5 — hippocampus; 6 — lower frontal gyrus; 7 — thalamus; 8 — motor cortex; 9 — supplementary motor cortex; 10 — cerebellum. Original drawing by the author.

In patients with TBI, the number of significant connectivity components of the MFS and changes in connectivity structure were found to decrease with increasing severity of hemiparesis compared to normal subjects during active movement of the right hand (Fig. 4). Thus, in patients with mild right hemiparesis during active movement of the paretic arm (Fig. 4, *II*), the putamen nuclei of both hemispheres were the foci of predominant subcortical activity, with a greater number of interactions on the right side (network 4, Fig. 4, *b*). The latter fact can be regarded as evidence for the involvement of the right subcortical nuclei in the compensatory activity. The interaction of the symmetric areas of the motor and supplementary motor cortices, as well as their bilateral connections, which are not normally seen, can also be attributed to this fact. In the group of patients with severe (3 points) right-sided hemiparesis who were able to move independently, the pattern of MFS connectivity (Fig. 4, *III*) showed a reduction of connections between the putamen and the caudate nucleus as well as motor cortical regions, predominantly left hemispheric ones (Fig. 4, *a, b, d*). Meanwhile, we detected an increase in connectivity, mainly of the globus pallidus (an older subcortical structure), both unilaterally to the left and diagonally with the subcortical nuclei of the right hemisphere. The connectivity of the left motor cortex and cerebellum, uncharacteristic of other groups, appeared (Fig. 4, *d*). We also tend to consider the above qualitative changes in the pattern of MFS connections as compensatory cerebral rearrangements [4].

As TBI is often associated with severe hemiparesis (2-1-0), as well as speech disturbances or reduced consciousness [1], the use of an active motor test to investigate the functional connectivity of the DFS is not feasible. However, a passive motor test is possible in all categories of patients. Therefore, we performed a comparative analysis of fMRI connectivity in groups of healthy subjects as well as in

Table 3. *T*-statistics of matched pairs of connections at rest, during active and passive movement of subcortical connections in healthy subjects.

Area of analysis	Right hand movement			
	active		passive	
	T-value	*P*-unc	*T*-value	*P*-unc
Left caudate nucleus — left globus pallidus	5.51	0.0003	3.44	0.0003
Left caudate nucleus — left putamen	3.27	0.0085	2.56	0.0005
Left amygdala — left putamen	9.17	0.0002	8.17	0.0001
Right caudate nucleus — right putamen	4.35	0.0007	3.16	0.0003
Right caudate nucleus — right globus pallidus	2.22	0.0048	1.18	0.0032
Right amygdala — right putamen	5.59	0.0079	4.39	0.0029

Note. *T*-value is the cut-off point of *t*-distribution, a measure of significance recommended for use with samples of less than 30 subjects with an unknown standard deviation.

patients with mild, severe and gross right-sided posttraumatic hemiparesis using the system described above (Fig. 5).

Analysis of MFS networks during passive movement (Fig. 5) showed that connectivity in all groups was characterized by a slightly lower number of significant connections, especially cortical ones (Fig. 4, *d*), compared to active movement.

Furthermore, the pattern of rearrangements was similar to that of the active test, with denser connections (foci of activity) in the motor cortex and putamen. We also found specific changes in MFS connectivity that are characteristic of passive movement in patients with hemiparesis. For example, the role of the ipsilateral right motor cortex in the formation of cortical-subcortical connections was prominent in all hemiparesis groups (Fig. 5, *b*). In addition, we observed an increased importance of the left globus pallidus (paleostriatum), contralateral to the movement, in the formation of subcortical connectivity with increasing hemiparesis (Fig. 5, *a*). These features could also be considered a manifestation of compensatory neuroplastic rearrangements.

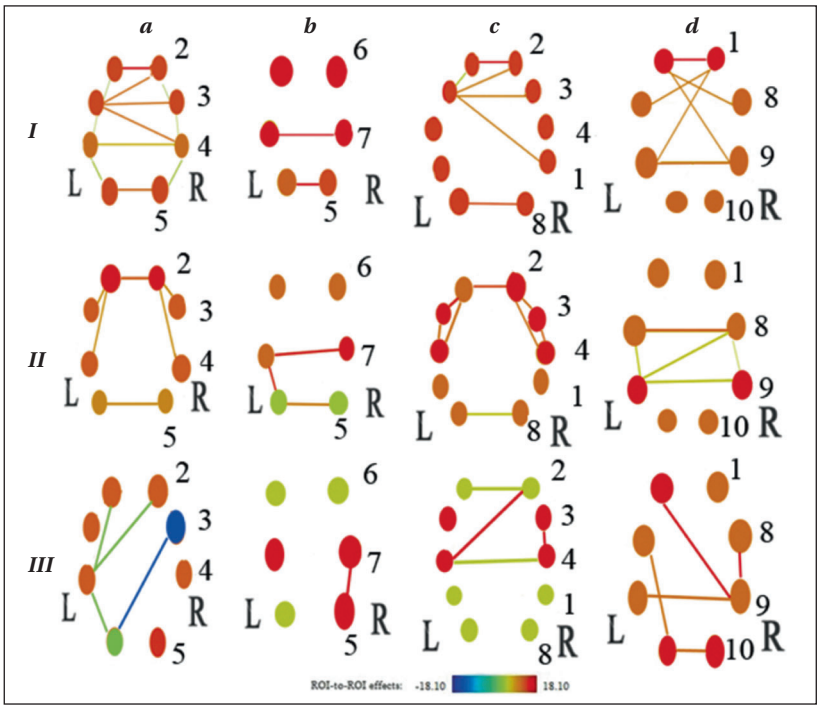

Fig. 4. Important functional connections of subcortical and cortical-subcortical segments of the motor functional system during active movement of the right hand in healthy subjects and patients with TBI according to fMRI data.
Note. *I* — healthy subjects (*N*=23); *II* — patients with mild right-sided posttraumatic hemiparesis, 4 points (*N*=18); *III* — patients with severe right-sided posttraumatic hemiparesis, 3 points (*N*=7). *a* — network 1; *b* — network 2; *c* — network 3; *d* — network 4. 1 — amygdala; 2 — caudate nucleus; 3 — cortex; 4 — globus pallidus; 5 — hippocampus; 6 — lower frontal gyrus; 7 — thalamus; 8 — motor cortex; 9 — supplementary motor cortex; 10 — cerebellum. Original drawing by the author.

In patients with mild hemiparesis, intrahemispheric lateralized interactions were found to be more significant than in normal subjects. Analyzing passive movements of the paretic right hand in the same group revealed a similar pattern of connectivity. However, connectivity between caudate nuclei and symmetric cortical areas (supplementary motor cortex, precentral gyrus) was more significant than during independent fist clenching (Table 4). With increasing paresis, a progressive reduction in the level of connectivity during active movement was observed. Meanwhile, a comparison of active and passive motor tests showed higher connectivity values during passive movement, which may reflect the functional capabilities of the motor system and, accordingly, functional integrity.

Our data confirm the informative value of passive motor testing in patients with gross motor impairment to assess functional motor integrity at any level [19, 20]. The results obtained are in

Table 4. *T*-statistics of matched pairs of connections at rest, during active and passive movement of subcortical connections in the group of post-TBI patients.

	4 points				3 points				2–1 points			
	Active		Passive		Active		Passive		Active		Passive	
Area of analysis	***T*-value**	***P*-unc**	***T*-value**	***P*-unc**	***T*-value**	***P*-unc**	***T*-value**	***P*-unc**	***T*-value**	***P*-unc**	***T*-value**	***P*-unc**
Lest septum — left thalamus	6.44	0.0015	5.11	0.015	5.25	0.0045	12.50	0.0063	3.73	0.016	6.87	0.005
Left septum — left caudate nucleus	4.20	0.0015	3.90	0.0437	6.54	0.0028	12.59	0.0063	4.31	0.049	10.49	0.006
Left caudate nucleus — right caudate nucleus	8.54	0.0028	6.60	0.0066	5.19	0.052	12.15	0.006	6.51	0.005	13.87	0.033
Left septum — left globus pallidus	4.64	0.0049	3.59	0.0348	4.25	0.0025	10.70	0.0033	7.17	0.024	9.18	0.010
Left supplementary motor cortex — right supplementary motor cortex	12.83	0.004	10.15	0.006	6.74	0.002	14.5	0.006	10.3	0.004	16.22	0.024
Right globus pallidus — right septum	2.90	0.0518	1.19	0.0345	3.19	0.005	11.35	0.008	9.40	0.003	12.26	0.025

Note. The names of the structures are labeled according to the AAL atlas. *T*-value is the cut-off point of *t*-distribution, a measure of significance recommended for use with samples of less than 30 subjects with an unknown standard deviation.

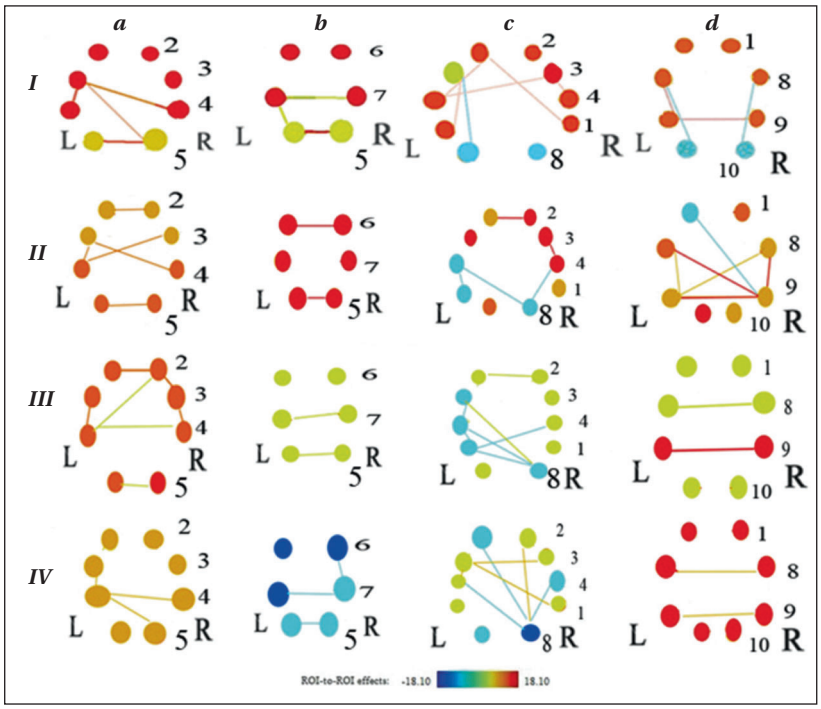

Fig. 5. Significant functional connections of subcortical and cortical-subcortical segments of the motor functional system during passive movement of the right hand in healthy subjects and patients with CHMT according to fMRI data.
Note. *I* — healthy subjects (*n*=23); *II* — patients with mild right-sided posttraumatic hemiparesis, 4 points (*n*=18); *III* — patients with severe right-sided posttraumatic hemiparesis, 3 points (*n*=7); *IV* — patients with severe posttraumatic hemiparesis, 1–2 points (*n*=5). *a* — network 1; *b* — network 2; *c* — network 3; *d* — network 4. 1 — amygdala; 2 — caudate nucleus; 3 — cortex; 4 — globus pallidus; 5 — hippocampus; 6 — lower frontal gyrus; 7 — thalamus; 8 — motor cortex; 9 — supplementary motor cortex; 10 — cerebellum. Original drawing by the author.

agreement with those of Hallett M. L. et al. [12], who showed that as the motor deficit worsens in patients with cerebral circulation impairment, the number of interhemispheric functional connections and intrahemispheric connectivity decreases, while the activity of bilateral areas of supplementary motor cortex increases. Our findings add to the current understanding of the main cortical response to the performance of active and passive tests, which is seen in the sensorimotor area of the contralateral hemisphere, the supplementary motor cortex, and the cerebellum [21–23]. An important feature of motor fMRI responses in patients with severe TBI, noted in many publications, is an increase in diffuse hemodynamic changes with activation of brain regions uncharacteristic of healthy individuals [24–27], whereas connectivity data suggest that this may be due to activation of subcortical structures.

Conclusion

The obtained data on rearrangements of fMRI connectivity of the motor functional system significantly extend our knowledge of the pathogenetic relevance of movement disorders in traumatic brain disease. Patterns of motor system connectivity during right hand movement in normal subjects usually reflect the specific type of motor activity. With increasing hemiparesis in post-TBI patients, multidirectional changes in MFS connectivity were observed. On the one hand, the total number of functional connections decreased, and on the other hand, the involvement of older structures (globus pallidus) and ipsilateral regions was observed in response to the reduction of significant interhemispheric interactions. In our opinion, the revealed patterns represent compensatory pathways of neuroplasticity in patients after severe traumatic brain injury.

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The Effect of Xenon on the Activity of Glycogen Synthase Kinase-3β in the Perifocal Zone of Ischemic Cerebral Infarction (Experimental Study)

Anton V. Ershov[1,2], Ivan A. Krukov[1,3], Victoria V. Antonova[1], Anastasia A. Baeva[1]

[1] V. A. Negovsky Research Institute of General Reanimatology,
Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology,
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia
[2] I. M. Sechenov First Moscow State Medical University, Ministry of Health of Russia,
3 Rakhmanovsky Lane, GSP-4, 127994 Moscow, Russia
[3] D. Rogachev National Medical Research Center for Pediatric Hematology,
Oncology and Immunology, Ministry of Health of Russia,
1 Samora Mashela Str., GSP-7, 117997 Moscow, Russia

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***Correspondence to:** Anton V. Ershov, salavatprof@mail.ru

Summary

Aim of the study. To determine the effects of xenon exposure at a dose of 0.5 MAC of different duration on the content and enzyme-inactivating phosphorylation of the glycogen synthase kinase-3β (GSK3β) in the perifocal zone of ischemic cerebral infarction in an experimental setting.

Materials and methods. The Long method was used for modelling brain ischemia/reperfusion in 39 rats weighing 300–350 g. Study group animals was exposed to xenon at a dose of 0.5 MAC during 30, 60 and 120 minutes whereas control group animals received an oxygen-air mixture. Sham-operated animals served as a comparison group. The levels of GSK3β and phospho-GSK3β in brain homogenates were determined by blotting using specific antibodies.

Results. In ischemic stroke model, the content of GSK3β did not significantly change in control animals compared to comparison group. However, control group animals exhibited significant (2.7-fold, $P<0.001$) decrease in the content of its phospho-GSK3β in the perifocal zone of ischemic cerebral infarction. Inhalation of 0.5 MAC xenon during 30 minutes did not lead to an increase in phosphorylation of the GSK3β enzyme ($P=0.9$), however, 60 and 120 minutes of 0.5 MAC xenon exposures resulted in the increase in phosphorylated form of the enzyme by a factor of 2.1 ($P=0.005$) and 2.3 ($P=0.001$), respectively, compared to the control group.

Conclusion. The results reveal a possible molecular mechanism (i. e., execution of neuroprotective and anti-inflammatory effects of xenon due to GSK-3β inactivation) and show the prospects for using 60 and 120 minutes of 0.5 MAC xenon exposures in ischemic brain damage after a stroke, traumatic brain injury and other brain lesions.

Key words: glycogen synthase; GSK3ß; brain ischemia model; ischemic stroke; xenon; neuroprotection

Conflict of interest. The authors declare no conflict of interest.

Introduction

According to the World Health Organization, brain diseases account for one-third of all diseases in developed countries, and cerebrovascular disorders are the second leading cause of death in a group of cardiovascular diseases [1]. The brain mostly depends on energy metabolism, which is driven by an adequate supply of glucose and oxygen in the bloodstream and follows mainly aerobic glycolysis pathways. Disturbances in gas exchange or blood supply to the brain trigger neurometabolic and neurotransmitter processes that lead to ischemic damage of neuronal tissue. Similar pathophysiological responses can occur as a result of traumatic brain injury, tumor-associated brain compression, cardiac arrest, or surgical intervention [2, 3]. Ischemic brain injury is multi-component, with excitotoxicity mediated by upregulation of NMDA receptors being a key factor [4, 5]. The cascade of subsequent responses is complex and not fully understood, with glycogen synthase kinase-3 (GSK-3) playing a key role [6]. Its uniqueness in regulating cellular functions is due to its effect on the activity of about fifty proteins, whereas activity of the enzyme is mediated by numerous extracellular stimuli [7].

GSK-3 enzyme is a serine/threonine protease with multiple functions, including participation in cell division, proliferation, differentiation and adhesion [8]. Impaired function of GSK-3 has been found in cancer, diabetes mellitus, Alzheimer's disease and several other diseases. For example, GSK-3, together with phosphodiesterase type 4 (PDE-4), was found to regulate the activity of type 2 dopamine receptors, which play a role in the pathogenesis of schizophrenia. This kinase has also been implicated in mood regulation and in the mechanisms of affective disorders [9]. In mammals, GSK-3 exists in two isoforms, alpha (α) and beta (β), which are encoded

by different genes. In the brain, GSK-3β is found in both neurons and glial cells in almost all regions. In the cell, GSK-3 is mainly found in the cytoplasm, predominantly in the active form, but is also present in nuclei and mitochondria, where its activity is higher. Prolonged kinase activation leads to neurodegeneration [7]. GSK-3 inactivation occurs through its phosphorylation under the influence of various stimuli (e. g., neurotransmitters, growth factors, cytokines, etc.) [7, 8]. The basal activity of GSK-3β depends on the phosphorylation at tyrosine 216 [10]. Phosphorylation at serine 9 inactivates GSK-3β representing the main mechanism of regulation [11]. In addition, its phosphorylation at serine 389 by the protein kinase p38 is an important pathway of GSK-3β inhibition in the brain [12].

The GSK-3β enzyme plays a fundamental role in neuroplasticity and neurodegeneration. Active GSK-3β has been shown to inhibit axonal growth. Exposure to growth factors inhibits this kinase, which enables the synthesis of cytoskeletal proteins and promotes axon growth and branching [13]. Activation and blockade of GSK-3β in glutamatergic synapses plays an important role in synaptic plasticity underlying sleep, learning and memory processes [14]. Inhibition of this kinase has also been shown to stimulate the production of neuroprotective chaperones and block pro-apoptotic caspase-3. These effects are generally considered to be neuroprotective [15].

In addition, GSK-3β is a key enzyme that regulates the permeability of the mitochondrial pore, the opening of which during ischemia leads to the entry of water and solutes, contributing to matrix swelling and rupture of the outer mitochondrial membrane. Meanwhile, cytochrome C is released from the intermembrane space, triggering the process of apoptosis and ferroptosis. GSK-3β phosphorylation decreases its activity, which prevents pore opening and protects the cell from ischemic damage [16, 17].

In addition to neurons, high levels of GSK-3 have been found in neutrophils, and high levels of phosphorylated GSK-3 indicate their activation and participation in the inflammatory response through increased production of proinflammatory interleukins (IL-1β, IL-6, IL-12) and decreased anti-inflammatory IL-10 [19-20].

Given the important role of GSK-3β in cell and tissue function, it can be considered a promising biological target for pharmacotherapy [7]. Synthetic inhibitors of GSK-3β have been developed that exhibit antidepressant, mood stabilizing, and neuroprotective effects in experimental studies [21, 22].

According to literature data, halogenated anesthetics also inactivate GSK-3 through its phosphorylation, which results in reduction of systemic inflammatory response due to inhibition of proinflammatory cytokine production, leukocyte activity and tissue infiltration, maintenance of intercellular endothelial contacts and endothelial barrier integrity, and also due to normalization of cellular antioxidant enzyme levels, which reduces cellular damage [23].

The use of inert xenon gas to protect the brain during ischemia from stroke, traumatic brain injury, and other causes is a promising avenue of research. Recent studies have shown that 30 min exposure to 0.5 MAC xenon increases the levels of phosphorylated GSK-3β and antioxidant protective enzymes such as catalase, superoxide dismutase, heme oxygenase in rat brain homogenates, suggesting a novel molecular mechanism of its neuroprotective activity [24]. Thoresen M. et al. experimentally demonstrated a reduction in the extent of damage and an improvement in neurological outcome after xenon inhalation following traumatic brain injury [25]. Campos-Pires R. et al. found that 50% xenon can reduce the severity of secondary brain damage during ischemia [26]. In addition, this concentration of xenon has a marked analgesic effect and has been successfully used in inhalation for pain after injury and burns [27], as well as for post-traumatic stress disorder [28]. Xenon has been shown to increase the neutrophil apoptosis potential. This confirms the anti-inflammatory properties of this gas [29].

The pathological cascade of glutamate excitotoxicity is implemented in the first minutes and hours after ischemic brain injury. During the first three days, neuronal and glial apoptosis occurs in the penumbra zone, often leading to the subsequent development of postischemic encephalopathy [30], so the study of the molecular mechanisms of neuroprotection during this period and the search for its pharmaceutical correction are essential for intensive care medicine.

Aim of the study: To determine the effect of different times of exposure to 0.5 MAC xenon on the level and phosphorylation (inactivation) of the enzyme glycogen synthase kinase-3β in the perifocal area of experimental ischemic stroke.

Materials and Methods

Experiments were performed on male Wistar rats weighing 300–350 g (*N*=49). The animals were deprived of food on the eve of the experiment, but had free access to water. The study protocol was approved by the local ethics committee. The experiments were performed in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes.

Under intraperitoneal anesthesia with 12% chloral hydrate 300 mg/kg, focal ischemia was simulated using the Long method. After a midline cervical incision and separation of the common carotid

artery from the right side, it was clamped with a vascular clip and a Vicryl 3–0 ligature was applied to the internal carotid artery. A 0.25-mm diameter silicone-coated nylon thread was inserted through a section of the external carotid artery into the internal carotid artery to a depth of 19–21 mm until it was occluded and fixed to the internal carotid artery with a vascular clip. Blood flow was occluded for 60 minutes, after which the suture was removed from the vessel, restoring blood supply to the area of the middle cerebral artery. Immediately after suture removal, the animals were randomly divided into 4 groups based on postoperative inhalation therapy:

— control group (*N*=10) with ischemic stroke received the oxygen-air mixture;

— study groups — 0.5 MAC xenon with exposures of 30 (*N*=10), 60 (*N*=9), and 120 (*N*=10) minutes.

The control group also consisted of sham-operated animals (*N*=10) that were anesthetized and underwent all phases of surgery except for blood flow occlusion and inhalation therapy.

On day 7 after ischemia, euthanasia was performed (decapitation under chloral hydrate anesthesia), brains were extracted and lysed in hot buffer (62.5 mM Tris-HCl, pH 6.8; 2% SDS; 10% glycerol; 50 mM DTT, 0.01% bromophenol blue) at 94°C for 4 minutes. Proteins were separated on a 12% polyacrylamide gel and transferred to PVDF membranes (Amersham, USA). Subsequently, 5% BSA in Tris buffer (25 mM Tris pH 7.4, 0.15M NaCl, 0.1% Tween20) blocked the nonspecific binding sites. The membranes were then incubated for 12 hours at +4°C with antibodies in 5% BSA/tris-buffer solution (antibodies against GSK-3β and phospho-GSK-3β (Cell Signaling, USA)). The membranes were incubated for 1 hour with the second antibodies (anti-mouse or rabbit immunoglobulins conjugated to horseradish peroxidase and diluted in 5% BSA/tris-buffer solution). Visualization was performed using a SuperSignal West Pico blotting panel (ThermoFisher, USA) on a Hitachi-557 spectrophotometer (Hitachi Ltd., Japan). ImageJ software was used for densitometric analysis. Levels of GSK-3β and the phosphorylated form of GSK-3β were expressed in arbitrary units (AU).

Statistical analysis was performed using Statistica 10.0 (StatSoft, Inc.) and MedCalc 12.5.0.0 (MedCalc Software bvba). The Shapiro-Wilk criterion was used to determine the type of distribution of the variables. Considering the non-normal distribution, the median with interquartile range was used for descriptive statistics. Intergroup differences in independent groups were assessed by the Kruskal-Wallis H-criterion with Dunn's a posteriori test (to address multiple hypothesis testing), and to compare GSK-3β and phospho-GSK-3β in related groups, the Wilcoxon test was used. Differences were considered significant at $P<0.05$.

Results and Discussion

Thrombolysis is the most effective treatment for ischemic stroke according to controlled trials; however, reperfusion therapy is performed in only 10% of cases due to potential contraindications [31]. Therefore, the search for neuroprotective therapies aimed at preventing, slowing down or interrupting molecular and biochemical processes during ischemic damage, such as mitochondrial dysfunction, overproduction of reactive oxygen species, production of pro-apoptotic proteins, apoptosis or neuronal necrosis, becomes important [32–36].

Inhalational anesthetics are widely used in medical practice, but the mechanisms of their anesthetic action, as well as their neuroprotective and neurotoxic effects when acting on the central nervous system remain incompletely studied and are the subject of active research [37, 38]. The neuroprotective effect of xenon has been demonstrated in a number of experimental works, but the molecular mechanisms of this phenomenon are still under investigation [3]. The protective properties of xenon have been shown to occur in ischemia even at preconditioning doses [39, 40], as demonstrated in an experiment in neonatal rats that had a reduced area of ischemic damage after gas inhalation [41], as well as in clinical studies of subanesthetic doses of 50% xenon in perinatal hypoxia-ischemia [42]. One of the key enzymes involved in neuroprotection may be GSK-3β [7, 8]. The Kruskal–Wallis test showed no significant differences in GSK-3β levels between all groups of animals studied (*P*=0.765). Thus, we found that ischemic stroke had no significant effect on GSK-3β levels, which increased by only 2.3% in the perifocal area compared to sham-operated animals (*P*=0.765). The inhalation of 0.5 MAK xenon with an exposure time of 30 minutes after restoration of brain blood flow also had no effect on GSK-3β levels, which were 0.7% higher than the control and 3% higher than the same parameter in the sham-operated animals (*P*=0.765).

Inhalation of 0.5 MAK xenon at 60 min exposure after restoration of brain blood flow did not result in a decrease in GSK-3β compared to the 30 min exposure: GSK-3β levels remained only 0.4% higher compared to control and 1.9% higher compared to sham-operated animals (*P*=0.765). Inhalation of 0.5 MAK xenon at 120 min exposure did not result in a decrease of GSK-3β compared to 60 min exposure, control and sham-operated animals (*P*=0.765). Thus, no significant differences were found between all groups studied, indicating the lack of effect of 0.5 MAK xenon on GSK-3β levels.

Phosphorylation of GSK-3β through a cascade of sophisticated reactions is known to limit inflammation and reduce neuronal apoptosis in the ischemic zone [19, 20].

Table. Densitometric analysis of Western blots for GSK-3β and phospho-GSK-3β enzyme levels in the perifocal zone of ischemic stroke on exposure to 0.5 MAC xenon, *Me* (*LQ*; *HQ*).

Animal groups (*n*) and exposure time	Levels, arbitrary units	
	GSK-3β	Phospho-GSK-3β
Sham-operated (*n*=10)	2058917 (1887323; 2587112)	1458767 (1287333; 1785132)#
Control (*n*=10)	2105765 (1907123; 2754439)	540277* (487337; 685111)
30 min exposure (*n*=10)	2121112 (1888543; 2659531)	752112 (598344; 878444)
60 min exposure (*n*=9)	2098155 (1785548; 2444768)	1109375* (998376; 1289335)
120 min exposure (*n*=10)	2020334 (1831546; 2567731)	1239325* (989444; 1315128)
Kruskal–Wallis H test	#*P*=0.765	**P*<0.001

Note. *P* — when comparing GSK-3β and Phospho-GSK-3β within groups.

We found significant differences in the levels of phosphorylated GSK-3β in the groups of animals studied ($P<0.001$, Kruskal-Wallis test).

The level of phosphorylated GSK-3β was 29.1% lower in sham-operated animals compared to its active form (Wilcoxon test, $P=0.005$). Ischemic stroke in control animals resulted in a marked decrease in phosphorylated GSK-3β in the perifocal area compared to sham-operated animals (2.7-fold, Dunn's test, $P<0.001$), with the level of phosphorylated GSK-3β being 74.3% lower compared to GSK-3β (Wilcoxon test, $P=0.005$), all indicating activation of the enzyme in the penumbral area.

Inhalation of 0.5 MAC xenon for 30 minutes after restoration of cerebral blood flow did not increase phosphorylated GSK-3β in the penumbra compared to controls (Dunn's test, $P=0.9$), but it remained 48.4% lower than in sham-operated animals (Dunn's test, $P<0.001$). The level of phosphorylated GSK-3β was 64.5% lower compared to its active form (Wilcoxon test, $P=0.003$). Sixty minutes of inhalation of 0.5 MAC xenon after restoration of cerebral blood flow resulted in a 2.1-fold increase in the level of phosphorylated GSK-3β in the penumbral area compared with controls (Dunn's test, $P=0.005$), but its level did not differ from that observed after 30 minutes of exposure to 0.5 MAC xenon (Dunn's test, $P=0.177$) or from that in sham-operated animals (Dunn's test, $P=0.461$). The level of phosphorylated GSK-3β was 47.1% lower than that of the active form (Wilcoxon test, $P=0.008$).

Inhalation of 0.5 MAC xenon after 120 minutes of exposure resulted in a 2.3-fold increase in phosphorylated GSK-3β in the perifocal area of ischemic stroke compared to controls (Dunn's test, $P=0.001$), but no increase in phosphorylated GSK-3β compared to 60 minutes of exposure (Dunn's test, $P=0.9$) and compared to sham-operated animals (Dunn's test, $P=0.9$). The level of phosphorylated GSK-3β in this group was 38.7% lower than its active form (Wilcoxon test, $P=0.005$).

The results showed that ischemic stroke in control animals led to a remarkable (2.7-fold, Dunn's test, $P<0.001$) decrease in the level of phosphorylated GSK-3β in the perifocal area of ischemic stroke, indicating activation of the enzyme in the penumbra and activation of neuronal apoptosis. The level of phosphorylated GSK-3β increased with prolonged exposure, whereas the level of the major form of GSK-3β did not change significantly. Inhalation of 0.5 MAC xenon for 30 minutes had no effect on phosphorylation (inactivation) of GSK-3β enzyme, whereas inhalation of xenon for 60 and 120 minutes resulted in increased phosphorylation of GSK-3β enzyme, decreased ratio of phosphorylated and active forms, and probably inhibition of neuronal apoptosis in the perifocal area of ischemic stroke.

Cytokines, NO derivatives, hormones, and oxidation products of nucleic acids and proteins are known to be involved in the initiation of apoptosis [34, 43]. Resolution of excitotoxicity plays a key role in primary neuroprotection in the acute phase of stroke [44]. Secondary neuroprotection includes reduction of delayed neuronal death and the severity of remote sequelae of ischemia, such as inactivation of NO synthase, blockade of oxidative stress, inhibition of proinflammatory cytokine production, mitochondrial protection, normalization of protein synthesis, and interruption of neuroapoptosis pathways [45]. In general, neuroprotective therapy should aim to both preserve and restore affected neurons as well as other cell populations that may have suffered ischemia, such as macro- and microglia, endothelial cells, and neutrophils [44, 46]. According to Smith W.S., therapeutic exposure to xenon reduces the severity of perivascular inflammation and decreases infarct volume, leading to improved neurological outcome [43]. Xenon inhalation has been found to disrupt abnormal functional connections between neurons and alter their metabolism by improving microcirculation and oxygen delivery [48, 49].

The observed increase in the level of phosphorylated GSK-3β confirms the possibility of its inactivation upon exposure to xenon. Kuzovlev A. N. et al. found that inhalation of 50% xenon for 30 minutes did not affect the level of GSK-3β enzyme, but caused an almost twofold increase in its phosphorylated form [24]. Likhvantsev V. V. et al. showed that the increase of phosphorylated GSK-3β can be observed after administration of sevoflurane and desflurane after experimental ischemia/reperfusion

simulation [23, 37]. Grebenchikov O. A. et al. demonstrated that phosphorylation of GSK-3β in neutrophils downregulates the expression of CD66b and CD11b degranulation markers on their surface [29, 50, 51].

Conclusion

Our results reveal a possible molecular mechanism of the neuroprotective and anti-inflammatory effect of xenon during brain ischemia/reperfusion through the inactivation of GSK-3β, which leads to the inhibition of neuroapoptosis in the perifocal area of ischemic stroke by reducing the ratio of phosphorylated to active forms of the enzyme. The results of the study show the prospects for clinical use of 0.5 MAC xenon inhaled for 60 and 120 minutes in ischemic and reperfusion brain damage due to stroke, traumatic brain injury and other causes.

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Methods of Extracorporeal Hemocorrection in Sepsis (Review)

Victor A. Kovzel[1], Lyubov A. Davydova[1], Alexey V. Karzin[2], Sergey V. Tsarenko[1], Vera Yu. Baturova[3], Alexander A. Polupan[1], Alexey I. Gutnikov[1]*

[1] M. V. Lomonosov Moscow State University,
1 Leninskiye gory Str., 119234 Moscow, Russia
[2] National Medical Research Center, Center for Treatment and Rehabilitation
3 Ivankovskoe highway, 125367 Moscow, Russia
[3] N. I. Pirogov City Clinical Hospital № 1, Moscow Department of Health,
8 Leninsky Ave., 119049 Moscow, Russia

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***Correspondence to:** Alexey I. Gutnikov, agutnik@mail.ru

Summary

Sepsis and septic shock remain a major problem in critical care medicine being the most common causes of death in the intensive care unit. Currently, such methods of extracorporeal blood purification as hemodiafiltration, high-volume hemofiltration, high cut-off (HCO) membrane hemofiltration are among preferable options for treatment of severe systemic disorders and pathological conditions including sepsis.

The purpose of the review is to show the potentialities and prospects of the use of various extracorporeal hemocorrection methods, including those that are commonly employed in medical practice, and novel ones, either recently developed, or still under the development in experimental settings according to sepsis pathophysiology. The selected 82 papers represent comprehensible clinical and experimental data from the literature of the last five years and several earlier publications remained of current interest in a medical practice.

The review presents current methods of extracorporeal hemocorrection (EHC) in patients with sepsis. The clinical pathophysiology of sepsis is described in relation to treatment options that target endotoxemia and «cytokine storm». We consider commonly used EHC methods (hemodiafiltration, high-volume hemofiltration, high cut-off membrane hemofiltration and others) and novel promising technologies that include extracorporeal kidney support device, immune support system, leukocyte inhibition module, and artificial spleen, which have been recently developed and are still under investigation in the intensive care.

Conclusion. Currently, EHC methods are increasingly used not only to support renal function, but also as pathogenetic therapy option for multiple organ support and immunomodulation by reducing the level of circulating inflammatory mediators. Exploration of novel extracorporeal blood purification techniques for the pathogenetic treatment of patients with sepsis seems encouraging and promising.

Keywords: sepsis; extracorporeal hemocorrection; endotoxin

Conflict of interest. The authors declare no conflict of interest.

Introduction

Currently, extracorporeal blood purification (EBP) plays an important role in the management of sepsis. EBP is defined as the targeted quantitative and qualitative modification of the cellular, protein, water-electrolyte, enzyme, and gas composition of blood by processing it outside the body's vascular system [1].

Modern methods of EBP are based on centrifugation, precipitation, membrane, adsorption, electrochemical, photochemical, electromagnetic and immunomagnetic technologies. Centrifugation, membrane and adsorption EBP methods are the most widely used in sepsis [1]. It should be noted that centrifugation technology and apheresis are separation-type methods based on blood fractionation and removal of one or another component.

Centrifugation technology uses the principle of weight fractionation of whole blood components. This principle is implemented as follows: the centrifugal force generated by the centrifuge separates the blood cells according to their mass, forming fractions. This technology makes it possible to separate plasma and the main cellular components from the blood, which is the core of plasmapheresis and various types of cytapheresis.

Membrane technology, in which diffusion, ultrafiltration (filtration), convection and osmosis play a leading role, allowing the transfer of proteins, electrolytes and gases, is based on transmembrane mass transfer, depending on the type of membrane, the size and number of pores and the surface area. A semipermeable membrane is a selectively permeable barrier between two phases. Mass transfer across a membrane is also called permeability because it occurs only when there is a driving force or potential gradient of some effect on the system on either side of the membrane [2].

Adsorption technology is based on the absorption of substances from biological fluids by forming bonds with active centers on the surface of the adsorbent. It is based on specific and non-

specific mechanisms (adsorption, absorption, chemisorption, ion exchange and complexation). The technology is implemented through a series of processing steps of both whole blood and its components, using activated carbon, ion exchange resins, and selective (immune, affinity, and receptor) compounds as sorbents [2–4].

Exchange transfusion, hemoadsorption, plasmapheresis, non-selective plasma adsorption are the least selective technologies. The most specific in the removal of certain substances are methods of immunoadsorption, affinity adsorption and biospecific adsorption of blood and its components [2]. In the efferent therapy of sepsis, in addition to all the above technologies, membrane and adsorption methods are used. Further discussion will focus on these methods of EBP.

According to the international multicenter study Sepsis Occurrence In Acutely Ill Patients (SOAP) (results from 198 European medical centers), the average in-hospital mortality from hospital-acquired sepsis was 24.1% (ranging from 14% in Switzerland to 41% in Portugal) [5]. Results from another multicenter study, Promoting Global Research Excellence in Severe Sepsis (PROGRESS), showed a nosocomial mortality rate of 49.6% [6]. The prognosis in sepsis is often unpredictable: mortality in leading clinics in developed countries reaches 40%, while in septic shock it can be as high as 80–90% [7]. Therefore, sepsis and septic shock remain a major challenge in clinical medicine, as they are the leading causes of death in the intensive care unit (ICU). Sepsis and the resulting inflammatory response can lead to multiple organ dysfunction [8].

Sepsis is a life-threatening acute organ dysfunction resulting from an impaired host response to infection. In essence, it is an impaired inflammatory homeostasis triggered by infection with a multifactorial progression. Hyperreactive pro- and anti-inflammatory mechanisms can interfere with each other, creating a destructive immunological dissociation that increases the risk of death. Inflammatory signals inhibit homeostatic signals, which have a higher priority for organism survival, whereas pro- and anti-inflammatory cytokines act simultaneously in sepsis. Such redundant and multifaceted simultaneous signaling, with multiple effects on effector tissues, ultimately leads to immune imbalance.

After infection, the pathogen encounters the body's innate immune system, including leukocytes, epithelial and endothelial cells. The innate immune cells «recognize» pathogens through PAMPs (pathogen-associated molecular patterns), such as lipopolysaccharide (LPS) activating intracellular cascades of inflammatory mediator production. Proinflammatory cytokines involved in the pathogenesis of sepsis include tumor necrosis factor (TNF), interleukin-1β (IL-1β), IL-12, and IL-18 [9]. Blocking or eliminating these cytokines was found to be protective in animal models of acute fulminant infection [10]. Importantly, the anti-inflammatory response can also lead to critical multi-organ failure through a diminished immune response, also referred to as «immune paralysis». Thus, the other side of the immune response imbalance in sepsis is immune suppression, affecting both the innate and adaptive immune systems. The understanding that LPS and other PAMPs can induce the production of cytokines, which in turn, when released into the systemic circulation, exert their deleterious effects on effector cells and tissues (primarily on the endothelium of organ capillaries and other immune defense participants, leading to a state of septic multiple organ failure or immunological anergy, respectively) provides a rationale for the routine use of various EGC methods in sepsis. The aim of this paper is to expand the knowledge of a broad medical audience about the role of current extracorporeal blood purification in sepsis.

Extracorporeal Blood Purification in Sepsis

The basic technologies used in EBP include separation, diffusion, convection, and adsorption, with the possibility of using any combination of these, depending on the clinical context and the specific needs of the patient. Table 1 shows the main modern methods of EBP used in sepsis and summarizes their principles.

Earlier EBP methods were used in patients with sepsis primarily to replace renal clearance and reduce uremic complications. In recent years, there has been a paradigm shift from renal replacement alone to more comprehensive multiple organ support therapy (MOST). Extracorporeal blood purification (EBP) methods are theoretically well suited for the MOST concept. EBP methods only have access to the intravascular compartment and can therefore partially influence its composition by removing unwanted components from the blood, such as inflammatory mediators and LPS, or by adding necessary components, such as bicarbonate, which replenishes the buffering capacity of the blood [11]. Support in cardiovascular failure can be achieved by controlling fluid balance, optimizing preload and afterload (using transmembrane fluid transfer along a hydrostatic gradient). Removal of extravascular pulmonary fluid is also possible [12, 13]. Elimination of uremic toxins and correction of blood acid-base balance (ABB) can potentially alleviate septic encephalopathy (ABB and water-electrolyte disturbances can be effectively addressed with dialysis technologies). Moreover, continuous renal replacement therapy (RRT) offers the added benefit of minimizing both osmotic shifts and hemodynamic

Table 1. Extracorporeal blood purification methods used in sepsis.

Method	Aim	Principle
Separation — plasmapheresis — plasma exchange	Separation of plasma and blood cells, control of hemostasis (in case of freshly frozen plasma replacement)	Centrifugal separation of blood components based on differences in their specific gravity
hemodialysis/filtration (CVVHDF) Continuous veno-venous	Multiple organ support therapy (MOST) + non-selective elimination of inflammatory mediators	Combination of diffusion and convection mass transfer
High volume hemofiltration (HVHF) гемофильтрация (HVHF)		Convective elimination of toxins
ГHigh cut-off membrane (HCOM) hemofiltration	Non-selective elimination of inflammatory mediators	Convective elimination of toxins
Coupled plasma filtration-adsorption (CPFA)	Non-selective elimination of inflammatory mediators	Adsorption of toxins from plasma
Molecular Adsorbent Recirculating System (MARS)	Elimination of water, low- and medium molecular weight substances, hydrophobic albumin-bound components of blood plasma	Low flux hemodialysis and semiselective adsorption with albumin regeneration
Fractionated Plasma Separation and Adsorption (FPSA)	Elimination of water, low- and medium molecular weight substances, hydrophobic albumin-bound components of blood plasma	High flux hemodialysis and selective plasma filtration
Endotoxin adsorption	Selective elimination of endotoxins	Endotoxin adsorption
Cytokin adsorption	Selective elimination of inflammatory mediators	Adsorption of inflammatory mediators
oXiris membrane	Non-selective adsorption of endotoxins and inflammatory mediators	By modifying the positively charged polyimine-ethylene layer, the AN69 membrane adsorbs negatively charged endotoxins and cytokine molecules

disturbances that can potentially impair brain perfusion [14]. In liver failure, the albumin-regenerative dialysis system promotes the elimination of albumin-related toxins, such as bilirubin, to provide partial liver support [15]. The extracorporeal circuit can control body temperature by changing the line length, i.e., the heat exchange area, and the temperature of the dialysis and/or replacement solution, which is used in hyperthermia or severe hypothermia. Thus, it is possible to beneficially affect multiple target organs in sepsis using EBP methods.

The reduction of blood cytokines is believed to result in decreased mortality in sepsis [16]. Over the years, several extracorporeal methods have been developed to target circulating blood substances such as LPS, inflammatory mediators, and coagulation factors. In addition, new experimental systems using phagocytic cells, immobilized antibodies for targeted immunomodulation, and magnetic nanoparticles coated with artificial human opsonin have emerged. A brief review of the main methods currently used in clinical practice for the purpose of extracorporeal blood purification in sepsis is given below.

Hemodiafiltration (HDF, CVVHF) and High-Volume Hemofiltration (HVHF) in Sepsis

Hemodiafiltration is a combination of diffusive and convective mass transfer that efficiently removes both small and medium-sized molecules. This method transports toxins dissolved in a hydrostatic pressure gradient filtered fluid through a semipermeable membrane. In the ICU, continuous veno-venous hemodiafiltration (CVVHDF) is preferred [17, 18]. Replacement of the removed fluid is performed in the pre- or postdilution mode, i. e., before or after the filter.

Several factors influence the choice of HDF and dilution method [19].

- The clearance of low molecular weight substances in hemodiafiltration is identical to that in hemodialysis.
- The clearance of medium molecular weight substances in hemodiafiltration is significantly higher than in hemodialysis and increases with increasing ultrafiltration rate.
- Predilution is preferable for elimination of medium molecular weight substances with insignificant reduction in urea and creatinine clearance.
- Postdilution provides adequate (compared to hemodialysis) elimination of low-molecular-weight substances with a slight reduction in clearance of medium-molecular-weight substances compared to predilution.

It has long been thought that decreased blood cytokine levels during EBP may result in reduced mortality in patients with sepsis. Three theories have been proposed to explain the potential benefit of high-volume hemofiltration (HVHF) in sepsis.

Ronco et al. proposed the «peak concentration hypothesis», suggesting that reducing both pro- and anti-inflammatory mediators («cutting off»

peak concentrations) may limit the associated target organ damage. Nevertheless, some studies have demonstrated clinical improvement and improved survival in septic patients with HVHF without significant reduction in blood levels of mediators [16].

Honore et al. proposed a more dynamic view to potentially explain these results, which they called the «immunomodulation threshold hypothesis». It is suggested that after removal of inflammatory mediators from the bloodstream in HVHF, these mediators are subsequently removed at the tissue level. Removal of inflammatory mediators at the tissue level leads to a clinical effect even without a significant change in their blood levels [20, 21].

Di Carlo and Alexander, with the «mediator delivery hypothesis» [22], suggested that HVHF not only removes solutes, but also increases lymphatic transport between the interstitial and intravascular compartments. This increase in lymphatic transport has been demonstrated in several studies showing a 20–40-fold increase in lymphatic flow with HVHF [23, 24]. According to the authors, this lymphatic mechanism enhances endogenous clearance of inflammatory mediators, particularly at the tissue level.

Ratanarat et al. also showed that HVHF reduced the levels of endotoxin and apoptosis mediators [25]. Regarding the recovery of renal function, the study by Boussekey et al. showed a significant increase in urine output with HVHF [26]. In addition, other studies in severe sepsis and septic shock have shown a reduced need for vasopressors with HVHF [27–29].

Potential side effects of HVHF include loss of vitamins, micronutrients, and some medications. An important issue in HVHF is appropriate antibiotic dosing. There are guidelines for antimicrobial dosing in critically ill patients undergoing renal replacement therapy. Therefore, monitoring of antibiotic concentrations and adequate dose adjustments are critical to prevent inappropriate dosing [30]. Due to the high clearance of small molecules in HVHF, strict electrolyte monitoring is also necessary to avoid hypokalemia and hypophosphatemia observed in some studies [31, 32].

In a prospective, randomized, multicenter IVOIRE trial [33] comparing «standard dose» hemofiltration (35 mL/kg per hour) with HVHF (70 mL/kg per hour), the authors found no reduction in 28-day mortality and no improvement in hemodynamic and organ function parameters in patients with sepsis.

Hemofiltration Through a High Cut-off Membrane

Most commercially available hemofilters do not provide effective removal of cytokines and endotoxins due to the relatively low cutoff of the membranes. High cut-off membranes (HCOM) have a larger pore diameter. This technical feature allows to increase the permeability up to a cut-off of 100 kDa [34]. HCOMs used in RRT have demonstrated higher clearance rates of some inflammatory mediators compared to standard membranes [35]. In a pilot RCT, 30 patients were assigned to HCOM-CVVHF or standard continuous venovenous hemofiltration (CVVHF) with an average continuous RRT rate of 31 ml/min. Compared to standard CVVHF, HCOM-CVVHF reduced the need for norepinephrine infusion and demonstrated better clearance of IL-6 and IL-1 [36]. HCOM improves peripheral blood monocyte proliferation and polymorphonuclear cell phagocytosis and decreases lymphocyte proliferative activity, but, as with HVHF, is associated with loss of vitamins, micronutrients, and antibiotics, making accurate dosing of the latter difficult. Protein and albumin losses were higher with convection (compared to diffusion) and at higher flow rates [37].

Combination EBP Methods

Combined EBP methods, such as CPFA, MARS, and FPSA, have been developed to overcome the disadvantages and enhance the advantages of membrane and adsorption extracorporeal methods. However, these methods have not been widely used in routine sepsis care, and few studies of these methods in the context of multi-organ support in septic shock speak more about the benefit of abandoning these methods in the treatment of sepsis. In particular, a second clinical trial (COMPACT 2) investigating the effect of high-dose CPFA was stopped early due to higher mortality in the CPFA group compared to the control group, especially in the first days of treatment, as observed in the interim analysis. It was also mentioned that CPFA is no longer indicated for the treatment of septic shock [38].

LPS Adsorption

Endotoxin is incorporated into the outer membrane of gram-negative bacteria and is considered one of the major biological agents causing sepsis. Lipopolysaccharide (LPS)-circulating endotoxin (ET) activates the coagulation system, complement, blood cells (monocytes, macrophages, neutrophils, eosinophils), and endothelial cells with initiation of multiple mediator release, which clinically manifests as a severe systemic inflammatory response with development of multiple organ failure [39, 40].

Removal of endotoxins from the blood of patients with sepsis and septic shock can reduce the severity of multiple organ failure and associated mortality. To implement this idea, specific methods of endotoxin adsorption have been developed.

Among the adsorbents currently used in clinical practice are immobilized polymyxin B (PMX-B) cartridges (Toraymyxin-20R, Japan), LPS adsorber (Alteco Medical AB, Sweden), MATISSE-Fresenius sys-

tem (Fresenius SE, Germany), Toxipac (NPF «Pokard», Russia), Efferon-LPS (OOO «Efferon», Russia).

The most studied and widely used in patients with sepsis and septic shock is the PMX-B immobilized cartridge. PMX-B is a cationic polypeptide antibiotic with high activity against Gram-negative bacteria and affinity for endotoxins. Intravenous use of PMX-B is limited due to its high nephro- and neurotoxicity. The ability to fix PMX-B to the polystyrene fiber of the cartridge allows the removal of ET without the risk of side effects. The PMX-B (Toraymyxin-20R) cartridge was developed and approved for clinical use in Japan in 1993. The results of one of the largest open-label, controlled studies conducted in Japan were published in 2003. 314 patients with sepsis were followed, of which 206 (the main group) were treated with Toraymyxin-20R. The study showed that the 28-day mortality rate in the main group was reduced to 32% [41]. It should be noted that Toraymyxin-20R is currently the most studied of all endotoxin adsorber cartridges [42–44].

The LPS adsorber consists of a series of porous polyethylene plates coated with an ET-specific peptide. This adsorbent is designed to adsorb endotoxins from blood. Currently, there are few publications in the world literature on the use of LPS Adsorber in patients with sepsis. Russian authors reported the results of using LPS Adsorber in the treatment of patients with sepsis and septic shock [45]. They have shown that the addition of the adsorbent to the treatment leads to a decrease in the levels of ET and inflammatory mediators and clinical improvement manifested by restoration of respiratory and hemodynamic parameters. A comparative analysis of the efficacy of Toraymyxin 20R and LPS Adsorber in patients with Gram-negative sepsis showed no significant differences in disease outcome [46, 47].

The MATISSE-Fresenius system is an ET sorption system based on the ability of serum albumin to covalently bind to macroporous acrylic polymer beads. The results of a randomized trial showed no significant effect compared to standard therapy in patients with sepsis [48].

Toxipack sorption column (NPF «PCARD», Russia) is designed for selective adsorption of ET. It consists of an adsorbent containing a polysaccharide granular matrix and a chemical ligand specific for gram-negative bacteria. Clinical experience with this adsorbent is limited. Sokolov A. A. and Handel L. L. reported improvement of clinical and laboratory parameters and reduction of organ dysfunction in patients with sepsis and septic shock using the Toxipack column [49, 50].

The Efferon LPS adsorption column (ZAO Efferon, Russia), which contains a multimodal hemoadsorbent based on a super cross-linked styrene-divinyl-benzene copolymer with immobilized LPS-selective ligand, is also intended for endotoxin adsorption and is currently one of the most studied columns for lipopolysaccharide adsorption. In an in vitro and ex vivo experimental study, data were obtained demonstrating the efficacy and safety of this device for LPS adsorption [51]. In a clinical trial, patients (*N*=9) with confirmed Gram-negative bacterial infection and septic shock (SEPSIS-3, 2016) underwent LPS-selective hemoperfusion. LPS adsorption using the Efferon-LPS column was found to result in a rapid reduction in endotoxin activity (EAA test), a more than twofold reduction in plasma interleukin-1 levels (immunoassay), as well as a significant clinical improvement in seven out of nine patients with septic shock, suggesting the need for further expanded clinical studies to evaluate the efficacy of this adsorbent and its contribution to reducing mortality in patients with septic shock [52].

In recent years, publications have appeared indicating the ineffectiveness of standard regimens for the use of LPS adsorption cartridges with PMX-B. A prospective, randomized, multicenter, controlled trial of 243 patients with septic shock found a non-significant increase in mortality in the treatment group [53]. In addition, the use of PMX-B had no effect on the severity of multiple organ failure. The inefficacy of PMX-B can be explained by possible adsorption of antibiotics during the EBP procedure, therefore studies adjusting the dose of antibiotics according to their concentration during hemoperfusion could influence the final results [54].

Cytokine Adsorption

Another option for non-selective adsorption is the use of cytokine adsorbing columns (cartridges) such as CytoSorb, PMMA, CYT-860-DHP, Lixelle, CTR-001, HA330, and MPCF-X [55]. Binding to various cytokines and mediators results from specific hydrophobic interactions, electrostatic attraction, hydrogen bonding, and van der Waals forces. In this regard, the structures of these columns vary considerably.

One of the most widely used and studied cytokine adsorption cartridges, Cytosorb, and a relatively novel, well-studied polymethyl methacrylate (PMMA) membrane were selected for a detailed review of this EBP method.

Animal models of sepsis have demonstrated the ability of CytoSorb to remove pro- and anti-inflammatory cytokines, other inflammatory mediators, and metabolites from the blood and to improve survival in sepsis [56, 57]. However, only one multicenter RCT has evaluated its efficacy in human sepsis [58]. In this study, 97 patients with septic shock and acute lung injury or acute respiratory distress syndrome were randomized to receive standard therapy or hemoadsorption with CytoSorb for

6 hours per day for up to 7 consecutive days. The authors found no differences in IL-6 levels (primary endpoint) or plasma levels of other key cytokines between the two groups. There was no evidence of improvement in multiple organ dysfunction in the CytoSorb group.

In addition to the small sample size, this study has several limitations. For example, according to the study design, therapy was administered in short (6-hour) daily sessions. This strategy may be inappropriate, as 24 hours of exposure is thought to be necessary for complete saturation of the adsorbent, and treatment-free intervals may lead to recovery of cytokine levels [56]. In addition, the mean baseline IL-6 levels were 552 (162 to 874) pg/mL (in the CytoSorb group) and 590 (125 to 2147) pg/mL (in the control group), which is considered low in sepsis. Since cytokine removal with CytoSorb is dependent on the level of inflammatory mediators, the combination of short duration of therapy and low IL-6 levels likely prevented the detection of potential adsorption efficacy. All other studies have been observational. The largest cohort is based on data from an international registry of 198 patients (68% with sepsis) [59]. In these patients, CytoSorb use was associated with lower IL-6 levels and lower than expected hospital mortality. Two case series reported reduced norepinephrine requirements and lactate levels in 20 and 26 patients with sepsis, respectively, who received efferent therapy with CytoSorb in combination with prolonged RRT [60–62]. The validity of these observational studies is largely limited by the lack of a control group.

Thus, experimental models and observational studies have shown significant clinical improvement, while RCTs have not yet demonstrated clinical benefit. However, their limited number and size, as well as the relatively low severity of the patients included, do not allow a definitive conclusion. Therefore, further studies should focus on populations with high blood cytokine levels, including pre-cytokine adsorption assays. Adequate definition of the target population is essential for future evaluation of cartridges and devices (not only Cytosorb) to prevent both misuse and unwarranted abandonment of this method.

The polymethylmethacrylate (PMMA) membrane is a synthetic polymeric membrane with a symmetrical microporous structure. This membrane, like CytoSorb, is capable of adsorbing low and medium molecular weight molecules such as cytokines, beta-2-microglobulin and immunoglobulin light chains [63]. Due to its extremely high adsorption properties, a PMMA membrane has been proposed for EBP in sepsis. Continuous veno-venous HDF with a PMMA blood filter has been reported to improve 28-day survival in patients with septic shock [64]. However, the PMMA membrane is characterized by a high degree of clogging due to non-selective adsorption of macromolecular substances, mainly proteins, on the membrane pores, as evidenced by the increase in transmembrane pressure in the filter over time [65]. The high thrombotic potential can also be explained by structural changes in the adsorbed proteins, causing activation and adhesion of platelets to the membrane surface. To solve these problems, a new PMMA-based membrane was developed to limit the structural changes of the adsorbed proteins, thereby improving permeability and preserving adsorption properties [66].

oXiris Membrane

The AN69-based oXiris membrane is modified with a positively charged polyimine ethylene layer capable of adsorbing negatively charged endotoxin molecules. In addition to its adsorption capacity, the oXiris filter is a conventional dialysis filter capable of full dialysis purification. In contrast to the 4 recent developments in extracorporeal blood purification described above, the oXiris membrane was evaluated in a RCT [67].

The aim of this study was to evaluate the ability of the oXiris dialysis filter membrane to reduce endotoxin and cytokine levels during a 24-hour treatment period in patients with septic shock and to compare the results with those obtained using a standard filter. Endotoxin levels were significantly reduced with the oXiris filter compared to the standard filter. In in vitro studies, the oXiris filter was the only hemoperfusion device tested that demonstrated removal of both endotoxins and cytokines [68]. Importantly, indiscriminate removal of all cytokines can disrupt immune regulation, but when one or more cytokines are in excess, as in sepsis, the proportion of the latter removed by adsorption will be greater than that of cytokines present at lower concentrations, theoretically helping to restore cytokine balance [69]. Circulating levels of TNF-α, IL-6, IL-8 and IFNγ were significantly reduced with both filters, but to a greater extent with oXiris than with the standard filter. The other cytokines analyzed (IL-1β, IL-2, IL-4, IL-10 and GM-CSF) were present at very low levels and were not compared between groups [67]. Blood lactate levels were significantly reduced in the oXiris group during the first 24 hours of treatment, and norepinephrine doses were also reduced after only 4 hours in the oXiris group. These observations suggest that the ability of the oXiris filter to remove endotoxins and cytokines may improve hemodynamic status. The main limitation of this study was the small cohort size of only 16 patients [67]. A recent study (2022) in a sample of 30 patients with septic shock clearly demonstrated that the use of oXiris-CVVH was associated with lower mortality, lower norepinephrine dose, lower lactate, procalcitonin

Table 2. The latest EBP methods.

Method	Aim	Principle
Renal assist device (RAD)	Replacement of filtration, transport, metabolic, endocrine and immunological function of the kidneys	Cell technology: hemofiltration through a membrane coated with renal cells
Extracorporeal immune support system (EISS)	Direct immunomodulation and phagocytosis	Cell technologies: plasma perfusion through a chamber with phagocytic mononuclear cells
Leukocyte inhibition module (LIM)	Direct immunomodulation and leukocyte apoptosis	Extracorporeal immunotherapy: blood perfusion through a polyurethane matrix with covalently bound Fas-stimulating antibodies
Magnetic opsonin and biospleen device (MOBD)	Removal of microorganisms and cellular debris from blood	Magnets remove opsonin-related toxins from the blood
Modified reduced graphene oxide pellets	Selective endotoxin removal	PMX-B applied to reduced graphene oxide pellets binds and removes endotoxins.

levels and leukocyte count compared to AN69-CVVH [70]. A pilot RCT showed that use of the oXiris filter may improve hemodynamics during initial continuous RRT in severe surgical septic shock with AKI [71]. Further large multicenter RCTs are needed to determine the effect of the oXiris filter on patient outcomes.

Promising Methods of Extracorporeal Blood Purification

New methods of EBP based on cell technologies are able to directly regulate the immune system and may become valuable tools in the armamentarium of future sepsis treatments (Table 2).

Renal Assist Device

The Renal Assist Device (RAD) is an extracorporeal device that uses a standard hemofiltration cartridge containing approximately 109 proximal renal tubular cells grown as a fusion monolayer along the inner surface of the fibers. The cells used in this device are isolated from donor kidneys for cadaveric transplants deemed unsuitable for transplantation. The non-biodegradability and pore size of the hollow fibers allow the membrane to act as a scaffold for the cells and a protective immune barrier. The RAD can be placed in series with the hemofilter in the extracorporeal circuit. In this scheme, the RAD is placed in series with the continuous venous hemofiltration (CVVH) circuit. The blood after the CVVH filter is pumped into the RAD cartridge and the processed blood is then returned to the patient. Ultrafiltrate (UF) from the CVVH is reabsorbed by the cell-coated hollow fibers of the RAD, and the treated UF exiting the RAD lumen is removed from the blood. The above scheme allows simultaneous replacement of filtration, transport, metabolic and endocrine functions of the kidney [72].

Extracorporeal Immune Support

Neutrophils and monocyte-derived macrophages play an important role in phagocytosis and antibacterial defense. Immune phagocytosis results in the efficient removal of live and dead pathogens such as bacteria, cell debris, endotoxins and exotoxins. In the absence of this specific neutrophil function, immune paralysis can occur. The Extracorporeal Immune Support System (EISS) is a promising experimental immunomodulatory therapy for sepsis.

It is based on the hypothesis that temporary replacement of phagocytic mononuclear functions by an extracorporeal cellular reactor can help the patient overcome the critical phase of immunosuppression in sepsis. First, the blood passes through a hemofilter. Next, the patient's separated plasma is perfused through the cellular compartment of the extracorporeal bioreactor, where phagocytes remove antigenic and apoptotic material that has escaped the patient's own neutrophils and macrophages. The circuit then ensures that the purified plasma passes through the cell filter and is returned to the patient [73]. Using a model of Gram-positive sepsis in pigs, Sauer et al. observed lower lactate concentrations and higher arterial blood oxygen pressure (PaO_2) in the group of animals that underwent the EISS procedure, indicating significantly better oxygen delivery in these animals. No adverse effects on the lungs or other organs were observed [74]. The first human study was performed in 10 patients with septic shock [75]. All patients tolerated the treatment well. There was a significant reduction in bacterial endotoxin, C-reactive protein and procalcitonin levels. Vasopressor doses were also reduced. Thus, the first studies of EISS demonstrated the safety of the procedure and yielded interesting and promising results.

Leukocyte inhibition module. The leukocyte inhibition module (LIM) is an experimental extracorporeal therapy aimed at reducing leukocyte activity [76]. In sepsis, unwanted overactivation of leukocytes occurs. To prevent or interrupt the inflammatory cascade, activated leukocytes, especially neutrophils, should be immediately inactivated and/or removed from the bloodstream. Adequate cross-binding of the appropriate ligands to the Fas

receptors on the surface membrane of neutrophils is known to stimulate pro-apoptotic signaling pathways [77]. Systemic administration of immunomodulatory antibodies can reduce neutrophil hyperactivity; however, this approach is expensive and can be associated with serious side effects. LIM is a biofunctional medical device for extracorporeal circulation that contains a polyurethane matrix with covalently bound Fas-stimulating antibodies. When neutrophils in the circulating blood come into contact with immobilized Fas antibodies, they rapidly become inactive and begin to undergo apoptosis [76]. Inactivated neutrophils can then be removed from the blood by phagocytosis or sequestration in the spleen. In this case, antibodies against Fas remain in the cassette and are cleared from the blood.

In an animal study, IL-8-mediated leukocyte chemotactic migration activity was completely abolished in animals treated with LIM and increased in animals not treated with this method. In addition, serum levels of TNF-α remained stable in the LIM group but increased in the other groups. As for human studies, the authors of the Leukocyte Inhibition Module Frankfurt (LIMFRA) single-center RCT concluded that LIM is safe and effective in limiting neutrophil-mediated perioperative inflammation [76]. These initial results with the LIM device demonstrate the potential for immunomodulation primarily in the cardiopulmonary bypass setting. It has not yet been studied in specific sepsis conditions in either animals or humans. The LIM technology has been applied at a very early stage of the inflammatory cascade. In cardiac surgery practice, the LIM cartridge has been integrated into the circulatory circuit, i. e., LIM has been used simultaneously with the event that would be expected to cause neutrophil activation. The future of this method is unclear and requires further study.

Biospleen Device

In 2014, Nature Medicine published a technical letter by Kang et al. on the possibilities of a new device for extracorporeal blood purification [78]. The authors named this device the «Magnetic Opsonin and Biospleen Device» (MOBD). Blood flowing through the MOBD circuit is mixed with magnetic nanoparticles coated with an engineered human opsonin, mannan-binding lectin (MBL), which captures a wide range of pathogens and toxins without activating complement and coagulation factors. Magnets remove opsonin-related toxins from the blood, and the purified blood is then returned. MOBD effectively removes many gram-negative and gram-positive bacteria, fungi, and endotoxins from human whole blood flowing through a single MOBD unit at up to 1.25 L/h *in vitro* [78].

To develop a broad-spectrum opsonin that could be used to easily purify whole blood flowing through the extracorporeal circuit, a large (650 kDa) native mannan-binding lectin (MBL) protein was engineered and then the collagen helix was removed. The remaining MBL carbohydrate recognition domain (MBL-CRD) was fused to an Fc fragment of human IgG1, allowing for high expression and secretion, as well as efficient, rapid and inexpensive purification of the smaller (90 kDa, compared to the original 650 kDa) recombinant protein. The resulting FcMBL was then attached to nanoparticles.

MOBD also allows researchers and clinicians to overcome two important issues, according to the authors of the paper [78]. First, the use of broad-spectrum FcMBL opsonin allows rapid treatment of systemic blood infections and prevention of sepsis progression without prior identification of the causative agent. Second, it is possible to rapidly process the entire volume of a patient's blood and perform multiple cycles of blood purification without significant coagulation of the blood or significant changes in blood composition.

Since FcMBL binds to several clinical isolates of antibiotic-resistant microorganisms, MOBD may also provide an effective therapeutic strategy for patients who have failed previous drug treatments. This dialysis-like blood purification system allows the entire volume of blood to be passed through the device repeatedly during a single procedure. Thus, even if only a fraction of pathogens are removed in a single pass, the number of pathogens in the bloodstream can be significantly reduced by circulating the blood multiple times over a 24-hour period. Although this procedure does not remove pathogens present in organs or abscesses, the results of the present study show that it can significantly reduce the spread of infectious agents to distant sites and reduce circulating endotoxin and inflammatory cytokine levels, thereby extending the time for exposure to other treatments, including antibiotic therapy. In fact, broad-spectrum antibiotic therapy or targeted therapy can be used in conjunction with the procedure because FcMBL magnetic opsonins bind to both dead and live pathogens. The ability of the MOBD to effectively trap pathogens also provides a potential way to rapidly capture large numbers of live infectious agents, helping to expedite pathogen identification and antibiotic susceptibility testing. Finally, this blood purification device is a versatile technology because it can be used to remove proteins (such as cytokines or autoantibodies) as well as other cell types (such as circulating cancer cells, stem cells, fetal cells in the maternal bloodstream) from whole patient blood volumes by coating magnetic beads with appropriate ligands specific for particular cells or proteins [78].

Modified Reduced Graphene Oxide Pellets

Reduced graphene oxide (rGO) is a promising endotoxin adsorbent for hemoperfusion due to its excellent adsorption capacity, but has the side effect of non-specific adsorption and low blood compatibility. Polymyxin B (PMX-B) acts as an organic affinity ligand that can specifically bind endotoxins. As an anticoagulant, heparin (Hep) can reduce the risk of blood clotting and improve the blood compatibility of materials. In the study by Li et al., the adsorbent used was reduced graphene oxide (rGO) with PMX-B and Hep on pellets, with polydophamine (pDA) as the active coating to immobilize PMX-B and further bind to Hep. These features were expected to successfully immobilize PMX-B and Hep on the rGO pellets. PMX-B endowed rGO pellets with higher adsorption capacity (143.84±3.28 EU/mg) and good selectivity for endotoxin. Hep significantly improved the compatibility of rGO pellets with blood. These modified rGO pellets also achieved good adsorption capacity and endotoxin adsorption selectivity in plasma, serum, and blood. Thus, rGO/pDA/PMB/Hep pellets appear to be promising adsorbents for endotoxin removal during EBP [79].

Trends in the Use of Extracorporeal Blood Purification

In recent decades, there has been a paradigm shift in the use of EBP. Until recently, EBP was used exclusively as renal replacement therapy to correct complications associated with acute renal failure. Currently, these methods are increasingly being used not only to support renal function, but also as a pathogenetic therapy for multi-organ support and immunomodulation by reducing levels of circulating inflammatory mediators [80–82].

A particular area of research is the clearance of specific substances such as antibiotics and other drugs, components of parenteral nutrition formulas, micronutrients and albumin through new cartridges. There is limited data on this topic, and consensus guidelines for device-specific antibiotic dosing have not yet been developed. These issues have been addressed in the international Surviving Sepsis Campaign guidelines [83].

Conclusion

We have briefly reviewed some of the extracorporeal treatment methods, including hemodiafiltration and high-volume hemofiltration, high cut-off membrane hemofiltration, plasmosorption combined with hemofiltration, albumin dialysis with albumin regeneration, hemodialysis with selective plasma filtration and adsorption, various techniques for adsorption of endotoxin and cytokines. We have also highlighted new experimental systems using human phagocytes and immobilized antibodies for targeted immunomodulation, as well as magnetic nanoparticles in addition to conventional membranes and adsorbents.

Importantly, of all the EBP methods listed, only a few have evidence from randomized clinical trials for their use in specific diseases. The study of novel variants of EBP as a method of pathogenetic treatment in patients with sepsis seems both intriguing and promising.

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XIII Международная конференция 6-7 октября 2023 года

"АКТУАЛЬНЫЕ АСПЕКТЫ ЭКСТРАКОРПОРАЛЬНОГО ОЧИЩЕНИЯ КРОВИ В ИНТЕНСИВНОЙ ТЕРАПИИ"

Глубокоуважаемые коллеги!

Приглашаем Вас принять участие в работе XIII Международной конференции **«Актуальные аспекты экстракорпорального очищения крови в интенсивной терапии»**

Конференция состоится в гибридном формате, 6-7 октября 2023 года

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3. *Экстракорпоральная поддержка для профилактики развития СПОН (механическая поддержка кровообращения, включая ЭКМО при ОСН; применение ECCO2 R; высокообъёмный плазмообмен и др.)*
4. *Патофизиологические обоснования применения сорбционных экстракорпоральных методик в комплексной ИТ сепсиса и СШ.*
5. *Комбинированные/гибридные методы экстракорпоральной терапии*
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В программе конференции предполагаются онлайн лекции ведущих специалистов из Европы, Северной Америки и Китая: **Alex Zarbock** (Германия) **Faouzi Saliba** (Франция), **John Kellum** (США), **Sean Bagshaw** (Канада), **Zhiyong Peng** (Китай) и другие.

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121552, Россия, Москва, Рублевское шоссе, 135
ФГБУ "НМИЦ ССХ им. А.Н. Бакулева" МЗ РФ
E-mail: org_ncssh@bakulev.ru
Телефон: (495) 414-77-34