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INTENSIVE CARE UNIT: FROM SEPSIS TO COVID-19

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CONTENTS

Introduction

Background and outline of the thesis

1. Intensive care medicine and challenges for the intensivist.

Intensive care medicine has changed over the years. Starting from the first intensive care units, born in Copenhagen during the polio epidemic, until today, the approach to critical illness has evolved a lot. In intensive care medicine, the ability to temporarily support or replace organ function has remained crucial, but was has changed is the way this has been performed. Over the past ten years, a more conservative approach in many intensive care interventions has been progressively applied, making more attention to avoiding iatrogenic harm.[1] Lung protective mechanical ventilation strategies as well as lighter sedation or fewer transfusions are just some of the measures taken in recent years.[1] Quality improvement has become standard with more reliable care and the use of care bundles to make patient management safer.[1] At the same time, there has been an attempt to personalize the approach to the critically ill patient. This probably has represented the greatest innovation, but also the greatest challenge, of the last few years. Traditionally, the tendency was to label patients according to their disease or condition and to treat them using similar interventions or therapies for all patients with the same diagnosis.[2] Now, the intensivists have moved into an era where they try to personalize treatments for individual patients.[2] The same critical illness can develop in patients with different histories, comorbidities, chronic therapies, and phenotypes. Moreover, the aging population has meant that even the intensivist is faced with increasingly older and more frail patients. Hence, the need to individualize the approach and the treatments. This is particularly true for a complex syndrome such as sepsis, a heterogeneous disease affecting many patients with different ages and characteristics. But the same is also true for Acute Respiratory Distress Syndrome (ARDS), which intensivists have had to deal with very closely in the last two years with the COVID-19 pandemic. The COVID-19 pandemic itself has represented the last, unprecedented, challenge for intensivists, who had to manage a new disease, with a lack of pathophysiological knowledge, affecting

thousands of patients with different ages and histories, trying to provide the best care for all, even with limited resources.

2. From sepsis to COVID-19

Sepsis definition, recognition, and management have undergone a profound evolution over the last three decades.[3] The progressive knowledge, over the years, of the pathophysiological mechanisms underlying this disease, has led to the actual definition of sepsis which is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection.[4] It was possible to reach this definition after comprehension of the complexity of this syndrome, which involves both pro- and anti-inflammatory responses[5], affecting individuals heterogeneous in age, underlying comorbidities, concurrent injuries, medications, and sources of infection.[4] The understanding of the complex pathophysiology has also led to the need to recognize different clinical phenotypes in order to identify groups of patients who may benefit from certain interventions.[6] Over the past decades, many trials on sepsis or septic shock failed due to the heterogeneity of the populations enrolled, explaining why we still have no new therapies.[2] The negative results of many of these trials do not mean that some of the treatments tested may not be effective in selected subgroups of patients. Hence, the importance of individuating the clinical, biomolecular, and immune phenotype and applying a targeted treatment.[6] A personalized approach enables to choice of the best treatment for each patient, thus maximizing the chance of survival. The need to subclassify the disease, allowing for more targeted treatments, is shared with ARDS.[7] ARDS is a complex syndrome, that can be triggered by a wide variety of causes, among which are pulmonary or extrapulmonary sepsis, aspiration, trauma, blood product transfusions, and pancreatitis.[7] Despite the Berlin consensus definition enabling the identification of ARDS, it remains an under-recognized disease, with limited therapies and a high mortality

rate.[8] Under-recognition may arise from the clinical heterogeneity observed among patients who meet standard ARDS criteria.[7] Identifying subphenotypes, based on physiologic, clinical, and biological characteristics, may be useful to distinguish patients at risk of poor outcome and to apply a tailored approach.[7] This was particularly true also for COVID-19-related ARDS. Since the very early stage of the pandemic, it was evident that COVID-19-related ARDS had some peculiar characteristics.[9] Two patient phenotypes with different clinical and radiological presentations were proposed, thus allowing the application of different treatment strategy.[9] This approach and this passage from "poorly characterized" to "personalized" medicine [2] in various clinical settings of intensive care medicine, from the well-known pathologies to those recently emerged, has been made possible by a profound revolution in the approach to clinical research in intensive care over the years.

3. Outline of the thesis.

This background is functional for introducing the topics that are covered in the articles presented below and for creating a common thread between them. This thesis is the result of three years of work in an intensive care unit (Clinica di Anestesia e Rianimazione Generale, Respiratoria e del Trauma Maggiore of "Azienda Ospedaliero Universitaria delle Marche" - Ancona), which at a certain moment saw its clinical routinary activity disrupted by an unprecedented pandemic. In the same way, the research activity came to a sudden halt. For this reason, the first articles, based on studies on sepsis conducted before the pandemic, are followed by those conducted during the pandemic, during which we tried to implement known knowledge and approaches, also in the context of research, to understand something more about a pathology that presented from the beginning peculiar characteristics.

Chapter 1 presents a prospective observational study on critically ill adult patients with sepsis/septic shock who underwent renal replacement therapy for acute renal failure and haemoadsorption with Cytosorb as adjunctive therapy for 24 hours. This study aimed to evaluate changes in cytokines, haemodynamics, and microcirculation during blood purification with Cytosorb adsorber in septic patients.

Chapter 2 presents a single-center, randomized, double-blind, placebo-controlled phase II trial including adult patients with a diagnosis of sepsis or septic shock for less than 24 hours, which aimed to test whether the infusion of IgM-enriched immunoglobulins improves microvascular perfusion during sepsis.

Chapter 3 presents a prospective observational pilot study including consecutive adult patients admitted to the intensive care unit (ICU) for or with infection-related illness. This study aimed to assess the correlation between MR-proADM and microvascular flow index (MFI) in a small cohort of 20 adult critical care patients diagnosed with an infection, sepsis, or septic shock.

Chapter 4 presents a single-center retrospective observational study including 100 consecutive septic patients (≥ 16 years) requiring norepinephrine infusion, admitted to our ICU between 2018 and 2019 after the institution of a *patient-tailored therapy* protocol, compared with a historical control group of 100 patients admitted between 2010 and 2013 (*historical controls*). This study aimed to evaluate the outcome of patients with septic shock after the institution of a *patient tailored therapy* protocol in our ICU.

Chapter 5 presents the first study conducted after the outbreak of the pandemic. This was an observational retrospective study in which we reviewed data from mechanically ventilated patients with SARS-CoV-2 severe pneumonia admitted to our ICU in March 2020, who underwent an evaluation of the sublingual microcirculation through incident dark field video microscopy (Cytocam, Braedius Medical, Amsterdam, NL).

Chapter 6 presents a case series carried out in collaboration with the "Bambin Gesù" hospital in Rome. In this case series, we reported microvascular dysfunctions in three pediatric clinical cases who developed COVID-19 infections with the need for pediatric critical care.

Chapter 7 presents an observational retrospective study, in which we examined the alterations of sublingual microcirculation in patients with SARS-CoV-2 during VV-ECMO treatment and assessed the relationship between microvascular parameters and ventilation, haemodynamics, and laboratory tests.

Chapter 8 presents a retrospective single-center study on adult patients with SARS-CoV-2 pneumonia requiring invasive mechanical ventilation for ≥ 48 hours. This study aimed to describe the prevalence of hyperoxemia and excessive O_2 administration in mechanically ventilated patients with SARS-CoV-2 pneumonia and determine whether hyperoxemia is associated with mortality in the ICU or the onset of ventilator-associated pneumonia (VAP).

Chapter 9 presents a single-center observational retrospective study, including patients admitted to our ICU from March 2020 to May 2021 for acute respiratory failure from SARS-CoV-2 infection who developed *PDR Acinetobacter baumannii* superinfection. This study aimed to describe the evolution of the superinfection from *PDR Acinetobacter baumannii* in patients with acute respiratory failure from SARS-CoV-2 infection admitted to ICU and compare the impact of two different antibiotic strategies on microbiological negativization.

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Chapter 1

Changes in cytokines, haemodynamics and microcirculation in patients with sepsis/septic shock undergoing continuous renal replacement therapy and blood purification with Cytosorb

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Abstract

Background: Extracorporeal blood purification therapies have been proposed as a strategy to remove inflammatory mediators during sepsis, thus improving outcome.

Objectives: We aimed to evaluate changes in cytokines, haemodynamics and microcirculation during blood purification with Cytosorb adsorber in septic patients.

Methods: Prospective observational study on critically ill adult patients with sepsis/septic shock underwent renal replacement therapy (RRT) for acute renal failure and haemoadsorption with Cytosorb as adjunctive therapy for 24 h. Measurements were taken at baseline, after 6 and 24 h: haemodynamic parameters, arterial and central venous blood gases, plasma levels of tumour necrosis factor alpha, interleukin (IL) 1-beta, IL-6, IL-8 and IL-10. The sublingual microcirculation was assessed with sidestream dark field videomicroscopy to evaluate the perfused vessel density (PVD) and microvascular flow quality. Tissue oxygenation and microvascular reactivity were assessed with thenar near infrared spectroscopy (NIRS) with a vascular occlusion test.

Results: Nine patients; plasma levels of IL-8 decreased at 24 h ($p < 0.05$ versus 6 h); no significant variation was found for other cytokines. Haemodynamic remained stable throughout the observation. Microvascular perfusion improved over time, with an increase in PVDs at 6 and 24 h (from 13.9 [13.3-16.4] to 15.7 [15-17.3] and 17 [14.8-18.6] mm/mm2 respectively, $p = 0.003$) and total vessel densities at 24 h (14.9 [13.9-16.9] vs. 17.9 [15.3-20], $p = 0.0015$). No significant variation was detected in NIRS-derived parameters. The Sequential Organ Failure Assessment score decreased from 12 ± 3 to 10 ± 1 at 24 h ($p = 0.039$).

Conclusions: In septic patients undergoing RRT, haemoadsorption with Cytosorb seems to determine a decreasing in plasma levels of IL-8, although levels of other cytokines did not vary

significantly, and an improvement of microcirculation despite no significant variation in macro-haemodynamics.

Keywords: Blood purification; Cytokines; Haemoadsorption; Sepsis; Septic shock; Sorbents; Sublingual microcirculation.

Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. This involves an early activation of pro- and anti-inflammatory pathways, with a dual mechanism of inducing both cytokine-mediated cell damage and a state of severely impaired immunity (immunoparalysis) [2]. Systemic inflammation is the leading cause of microcirculatory alterations observed during sepsis, which play a key role in the pathogenesis of tissue hypoperfusion and organ dysfunction [3]. Pro-inflammatory cytokines are the main factors responsible for leukocyte activation, oxidative stress, endothelial glycocalyx dysfunction and impaired nitric oxide pathway, resulting in reduced red blood cell deformability and impaired hemorheology, loss of microvascular tone, microcirculatory shunting, tissue oedema and oxygen extraction deficit [4, 5].

Extracorporeal blood purification therapies have been proposed as a strategy to improve outcome of septic patients, attenuating the systemic expression of pro- and anti-inflammatory mediators and restoring the immune homeostasis [6]. Among these techniques, haemoadsorption places sorbents in direct contact with blood in an extracorporeal circuit: solutes are attracted by the sorbent through hydrophobic interactions, ionic attractions, hydrogen bonding and Van der Waals interactions [6]. Haemoadsorption acts through a non-specific removal of a broad spectrum of inflammatory mediators, which can also include microbial toxins [7]. The CytoSorb® technology (CytoSorbents Corporation, Monmouth Junction, NJ, USA) is based on cartridges containing biocompatible polystyrene divinyl benzene copolymer beads that can remove several cytokines both in vitro and in vivo [8]. Preclinical data in rat models of sepsis is encouraging, showing a reduction in circulating cytokines, increased blood pressure and improved short-term survival with CytoSorb haemoadsorption [9]. We hypothesize that haemoadsorption with CytoSorb used as an adjunctive therapy during sepsis, by removing circulating inflammatory mediators, may contribute to restore microvascular perfusion.

Materials and Methods

This prospective observational study was approved by the local Ethics Review Committee (Comitato Etico Regione Marche, reference number CE150164; NCT03456180, www.clinicaltrials.gov). Written informed consent was obtained from the patients or their next of kin. All the adult patients admitted to the 14-bed medicalsurgical Intensive Care Unit of Azienda Ospedaliera Ospedali Riuniti of Ancona (Italy) between December 2016 and 2017 with diagnosis of sepsis or septic shock following the Sepsis-3 definition [1] and requiring continuous renal replacement therapy (CRRT) for acute kidney failure were eligible to participate. Exclusion criteria were as follows: hypersensitivity or allergy to any component of the CytoSorb adsorber; pregnancy; factors impeding the evaluation of the sublingual microcirculation (oral surgery or maxillofacial trauma); indication to undergo a blood purification technique other than haemoadsorption with CytoSorb; enrolment in another trial evaluating the microcirculation and lack of informed consent. Patients were managed according to the 2016 Surviving Sepsis Campaign guidelines [10]. Acute renal failure requiring CRRT had to be present despite maximum standard therapy including adequate fluid resuscitation and norepinephrine administration targeted to achieve a mean arterial pressure (MAP) > 65 mm Hg [10]. CRRT was performed in continuous veno-venous haemodialysis (CVVHD) mode with citrate-based anticoagulation. The CytoSorb adsorber was integrated in the CVVHD circuit. Blood flow rates (100–150 mL/min) and dialysis doses (20–30 mL/kg/h) were applied according to standard care. All patients had a hemodynamic monitoring performed with transpulmonary thermodilution technique with a PICCO2 monitoring device (Pulsion Medical Systems, Munich, Germany).

Measurements

Measurements were taken at baseline and after 6 and 24 h after the beginning of treatment with CytoSorb. These included heart rate, MAP, arterial and central venous blood gases, haemodynamic variables, vasoactive drugs doses. Arterial blood samples were collected at each time point in order to assess plasma levels of interleukin (IL) 1-beta, IL-6, IL-8, IL-10 and tumour necrosis factor

alpha (TNF-alpha). Procalcitonin levels were measured at baseline and after 24 h. The Sequential Organ Failure Assessment (SOFA) score was calculated at baseline and after 24 h.

Evaluation of Microvascular Perfusion

The sublingual microcirculation was evaluated at each time point with sidestream dark field (SDF) videomicroscopy (Microscan, Microvision Medical, Amsterdam, the Netherlands). Details on the SDF imaging technique have been extensively described elsewhere [11]. Three videos of adequate quality were selected for each time point and analysed offline with the Automated Vascular Analysis software version 3.2 (Microvision Medical, Amsterdam, the Netherlands). The following parameters were obtained for small vessels (diameter ≤20 mm) as described elsewhere [12]: total vessel density, perfused vessel density (PVDs), percentage of perfused vessels and microvascular flow index.

Tissue Oxygenation and Microvascular Reactivity

At each time point near infrared spectroscopy (NIRS; InSpectraTM Model 650, Hutchinson Technology Inc., Hutchinson, MN, USA) was performed with a 15-mm sized probe to measure peripheral muscle tissue oxygen saturation (StO2) and tissue haemoglobin index on the thenar eminence [13] before and during a vascular occlusion test [14]. This was performed by inflating a sphygmomanometer cuff placed on the forearm to 50 mm Hg above the systolic blood pressure. Arterial inflow was thus arrested until the StO2 decreased to 40%. StO2 was recorded during the ischemic and the reperfusion phases until stabilization [14]. The StO2 downslope was calculated from the regression line of the first part of StO2 decay after occlusion, providing an index of tissue oxygen extraction rate [14]. The StO2 upslope during the reperfusion phase and the area under the curve of the hyperaemic response (area of hyperaemia) were calculated as indices of microcirculation reactivity [14].

Statistical Analysis

Statistical analysis was performed using Graphpad Prism 6 (GraphPad Software, La Jolla, CA, USA). The data distribution was explored using a Kolmogrov-Smirnov test. Data were expressed as mean and SD for normally distributed variables or median and interquartile range for non-normally

distributed variables. Oneway analysis of variance for repeated measurements with Bonferroni post hoc test or the Friedman test with Dunn's post hoc test for repeated measurements test were used as appropriate to evaluate changes over time. A paired *t* test or the Wilcoxon test was used as appropriate to evaluate changes between 2 time points. The alpha level of significance was set a priori at 0.05. We calculated a sample size of 10 patients, sufficient to detect a 20% increase in the PVDs after 24 h of treatment with a power > 80% and an alpha error of 0.05.

Results

A total of 446 patients were screened in total. Ten patients (5 males and 5 females) were enrolled. Plotting clinical and laboratory variables, one out of 10 patients was discarded for the final analysis because acting as an outlier, resulting in a sample of 9 patients. The mean age was 63 ± 18 years. The mean Simplified Acute Physiology II score was 62 (24–71). The sources of infection were respiratory (2 cases), abdominal (2 cases), soft tissues (2 cases), urinary tract (2 cases) and mediastinitis (1 case). Microbiological details are shown in online supplemental Material 1 (see www.karger.com/doi/10.1159/000502540). The SOFA score was 12 ± 3 at baseline and decreased significantly to 10 ± 1 after 24 h of treatment with CytoSorb ($p = 0.039$). No significant change was observed over time in any haemodynamic or blood gas variables during the observation (Table 1). All patients required norepinephrine infusion throughout the 24-h study period and no significant reduction in norepinephrine dosage was observed over time. Changes in plasma levels of cytokines are shown in Figure 1. A significant decrease was found only for IL-8 from 6 to 24 h of CytoSorb treatment, while levels of the other cytokines did not vary significantly. Procalcitonin levels tended to decrease after 24 h of treatment (34 [6–157] vs. 6 [3–176] ng/mL, $p = 0.074$). All sublingual microvascular variables tended to improve over time and a significant increase was found for PVDs after 6 and 24 h of treatment (13.9 [13.3–16.4] vs. 15.7 [15–17.3] and vs. 17 [14.8–18.6] respectively, $p = 0.003$) and for total vessel densities after 24 h (14.9 [13.9–16.9] vs. 17.9 [15.3–20], $p = 0.0015$) as shown in Figure 2. NIRS-derived variables did not change significantly over time (Table 2).

Table 1. Haemodynamic and blood gas variables

	Baseline	6 h	24 h	*p* value*
Heart rate, bpm	86 (74 to 94)	98 (74 to 107)	79 (75 to 97)	0.143
MAP, mm Hg	70 (65 to 80)	75 (71 to 85)	73 (65 to 79)	0.569
Cardiac index, L/min/m^2	3.62 (2.48 to 4.05)	3.45 (2.25 to 4.58)	3.13 (2.67 to 3.52)	0.971
Global end diastolic index, mL/m^2	861 (655 to 1,002)	746 (643 to 964)	763 (650 to 977)	0.531
Systemic vascular resistance index, dynes * s/cm^5/m^2	1,690 (1,413 to 2,008)	1,824 (1,255 to 2,450)	1,922 (1,562 to 2,081)	0.813
Extravascular lung water index, mL/kg	14 (8 to 19)	11 (9 to 13)	11 (9 to 16)	0.734
Norepinephrine dose, µg/kg/min	0.50 (0.16 to 0.87)	0.50 (0.18 to 1.25)	0.50 (0.11 to 1.05)	0.078
Dobutamine dose (number of patients), µg/kg/min	3 (1 to 4.53), 5	3.33 (2.5 to 4.53), 5	3.57 (2.66 to 4.5), 5	0.999
pH	7.38 (7.35 to 7.40)	7.41 (7.35 to 7.48)	7.43 (7.30 to 7.51)	0.118
PaO_2, mm Hg	98 (75 to 116)	106 (93 to 157)	116 (83 to 128)	0.741
$PaCO_2$, mm Hg	38 (33 to 44)	40 (34 to 41)	38 (33 to 40)	0.813
PaO_2/FiO_2, mm Hg	218 (163 to 305)	238 (224 to 363)	270 (248–372)	0.187
Base excess, mmol/L	–3.2 (–5.9 to 2.4)	–2.5 (–4 to 4.35)	–1.1 (–4.6 to 6)	0.262
Arterial lactate, mmol/L	2.6 (1.7 to 6.65)	2.7 (1.25 to 4.45)	2 (1 to 3.8)	0.026
$ScvO_2$, %	81 (73 to 86)	86 (75 to 89)	83 (72 to 86)	0.645

All data is reported as median (1st–3rd quartile).
* Friedman test; corrected *p* for repeated measures significance <0.017.
MAP, mean arterial pressure.

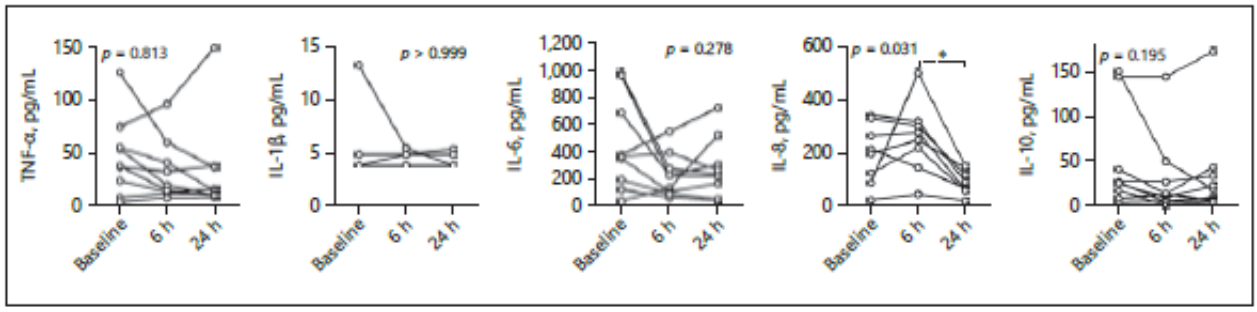

Fig. 1. Changes in plasma levels of cytokines. Data is expressed as median (1st – 3rd quartile). Friedman test with Dunn's test for repeated measures; * corrected *p* for repeated measure significance <0.017. TNF-alpha, tumor necrosis factor-alpha; IL, interleukin.

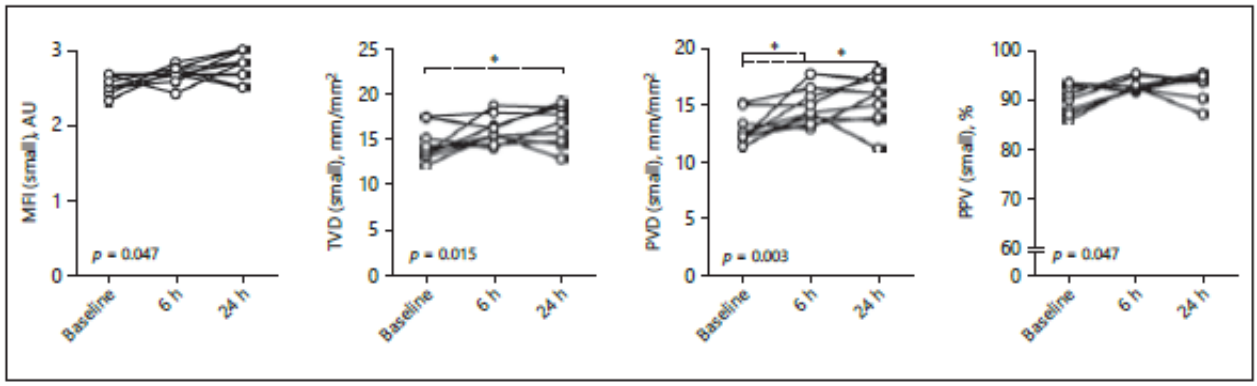

Fig. 2. Individual changes in sublingual microvascular parameters. Data is expressed as median (1st – 3rd quartile). Friedman test with Dunn's test for repeated measures; * corrected *p* for repeated measure significance <0.017. MFI, microvascular flow index; TVD, total vessel density; PVD, perfused vessel density; PPV, percentage of perfused vessels.

Table 2. Sublingual microcirculation and NIRS-derived variables

	Baseline	6 h	24 h	*p* value*
Total small vessel density, mm/mm^2	14.9 (13.9 to 16.9)	17 (16 to 18.7)	17.9 (15.3 to 20)#	0.015
Perfused small vessel density, mm/mm^2	13.9 (13.3 to 16.4)	15.7 (15 to 17.3)#	17 (14.8 to 18.6)##	0.003
MFI, AU	2.50 (2.37 to 2.62)	2.67 (2.62 to 2.75)	2.83 (2.58 to 3.00)	0.046
Percentage of perfused small vessels	89 (86 to 92)	92 (91 to 93)	93 (92 to 94)	0.048
StO_2, %	83 (74 to 91)	87 (82 to 90)	85 (83 to 88)	0.528
StO_2 downslope, %/min	−6 (−10.8 to −5.3)	−7.7 (−10.4 to −3.3)	−9 (−13.9 to −7.5)	0.442
StO_2 upslope, %/min	153 (106 to 186)	164 (118 to 253)	144 (58 to 194)	0.654
Area of hyperemia, % * min	13.8 (5.3 to 15.2)	10.7 (5.1 to 17.8)	10.6 (6.7 to 18.4)	0.764
Tissue haemoglobin index, AU	10.6 (6.5 to 16.7)	12.9 (9.8 to 18.4)	11.1 (9.1 to 13.7)	0.236

All data is reported as median (1st–3rd quartile).
* Friedman test; corrected *p* for repeated measures <0.017.
# $p < 0.05$ versus baseline.
$p < 0.01$ versus baseline.
NIRS, near infrared spectroscopy; MFI, microvascular flow index; AU, arbitrary units; StO_2, oxygen saturation.

Discussion

This small pilot prospective observational study in 9 septic patients undergoing CVVHD and a 24-h treatment with CytoSorb, showed a reduction in IL-8 plasma levels, whereas TNF-alpha, IL-1beta, IL-6 and IL-10 did not decrease significantly. Haemodynamic parameters and vasopressor requirement remained substantially stable during the 24-h treatment; nonetheless an increase in sublingual microcirculatory density was observed and microvascular flow quality tended to improve over time. Given the central role of the overwhelming systemic inflammation in the pathophysiology of sepsis-induced organ failure, several attempts have been recently made to develop therapies aimed to damper the cytokines storm. Nonetheless, specific antagonists for pro-inflammatory cytokines and treatments designed to control the early cytokines activation during sepsis failed to improve outcome [2]. Blood purification therapy is a non-selective and broad-spectrum strategy able to remove both pro- and anti-inflammatory mediators from the bloodstream, thus restoring the immune homeostasis [15]. In addition, by performing a concentration-dependent removal of molecules, blood purification acts as a self-tailored therapy. Haemoadsorption with CytoSorb was able to reduce plasma levels of IL-6 and IL-10, increase blood pressure and improve survival in rat models of sepsis [8, 9]. In recent years, multiple reports described a successful use of CytoSorb treatment in different disease categories, including patients with non-infectious systemic inflammatory response [16], acute

respiratory distress syndrome [17] or those undergoing cardiopulmonary bypass for cardiothoracic surgery [18–21]. A number of case reports and case series suggested a beneficial effect of CytoSorb during sepsis, with attenuation of the inflammatory reaction, haemodynamic stabilization and potential survival benefit [22–24]. In refractory septic shock, the use of CytoSorb as a rescue therapy was associated with improved lactate clearance and shock reversal in two-thirds of patients [25]. In this study, a significant reduction in plasma levels of IL-8 during the 24-h CytoSorb treatment was detected. However, levels of the other measured cytokines remained substantially unaltered; a non-significant trend towards a reduction was observed for TNF-alpha. These results seem to contrast with data from some preclinical studies [8, 9] and clinical reports [21]. In a multicentre randomized controlled trial in patients with severe sepsis/septic shock, haemoadsorption with CytoSorb was able to remove IL-6 with a 5–18% elimination per blood pass through the filter (calculated by dosing IL-6 levels from the arterial [inlet] and the venous [outlet] blood); however, this did not lead to lower plasma IL-6 levels in treated patients as compared to controls [26]. The stability of plasma cytokines levels despite effective extraction from the blood may be explained by a cytokines' shift from the interstitium into the blood compartment (cytokinetic theory [27]), exacerbated by a continuous production during the treatment period. Unfortunately, the elimination rate of cytokines, in order to demonstrate the effective removal of the haemoadsorption device, has not been measured in the present study. Moreover, the inability of this study to reproduce the effectiveness of Cytosorb in reducing cytokines levels could suggest other positive effects of this filter on septic patients leading to a microvascular improvement. Peng et al. [28] already postulated a different potential beneficial effect of sorbents, other than cytokines removal, in experimental model of sepsis. A plausible hypothesis is the capability of Cytosorb to remove free haemoglobin, a well-known nitric oxide scavenger, which can induce vasoconstriction and a reduction in microvascular density [29]. All the enrolled patients had sepsis/septic shock with high Simplified Acute Physiology II and SOFA scores. They received standard resuscitation therapies according to current guidelines [10] including antibiotics, fluids, vasopressors and/or inotropes before enrolment. The indication for CRRT was

based on persisting acute renal failure with oliguria/anuria despite maximal treatment and CytoSorb adsorber was applied as a rescue therapy. Initial haemodynamic stabilization had been achieved in most patients at enrolment, as indicated by a baseline MAP ≥65 mm Hg in 8 patients out of 9 and a central venous StO2 > 70% in 8 patients out of 9. Despite this, microvascular perfusion was significantly impaired at the time of enrolment, with 7 patients out of 9 showing a microvascular flow index < 2.6, a cut off already used in previous study to identify patients with microvascular impairment [12,30]. This may reflect a loss of haemodynamic coherence, a condition that frequently occurs during sepsis and is a predictor of adverse outcome [31]. During the 24-h treatment with CVVHD and CytoSorb, haemodynamic parameters remained stable, as well as vasopressor and/or inotrope requirements. Nonetheless, microcirculatory alterations were attenuated over time, with a significant increase in microvascular density and a trend towards an improvement in blood flow quality. These findings support the idea that extracorporeal cytokines' removal during sepsis may produce beneficial effects by preventing (or ameliorating) microvascular dysfunction. David et al. [32] evaluated the effects of extracorporeal cytokines' removalon vascular barrier integrity in vitro using human umbilical vein endothelial cells: alterations in endothelial morphology and function were prevented when human umbilical vein endothelial cells were challenged with serum from a septic shock patient collected after CytoSorb treatment, suggesting a protective effect. The use of a CytoSorb adsorber in an isolated kidney perfusion system was able to improve mean renal blood flow and reduce the inflammatory response due to ischemia/reperfusion injury, although this did not lead to better renal function [33]. The improvement in microcirculatory perfusion in the present study was not accompanied by a significant decrease in lactate levels during the treatment, although substantial inter-individual variability was seen. We cannot exclude that a longer follow-up period was necessary to detect improvements in lactate clearance as a sign of restored aerobic metabolism, as well as to record a reduction in norepinephrine requirement. No variations in NIRS-derived parameters that could reflect changes in tissue oxygenation or microvascular reactivity during CytoSorb treatment were observed. This discrepancy with the improvement seen sublingually may be explained by

physiological differences between the 2 microvascular beds examined. The sublingual mucosa has been selected for the assessment of microvascular blood flow as an easily accessible element that shares the embryologic origin with the gut, and is thus seen as a possible window to splanchnic microcirculatory perfusion [34], although a dissociation with the gut microcirculation was found in some studies [35, 36]. On the contrary, thenar-NIRS evaluates peripheral oxygenation in the skeletal muscle tissue, which is likely to respond differently to cardiovascular insults or vasoactive stimuli such as norepinephrine infusion [14]. The study has several limitations. First, the observational design and the lack of a control group prevent the possibility to demonstrate any cause-effect relationship between CytoSorb treatment and microvascular changes: we cannot exclude that the improvement in microcirculatory perfusion was a result of other treatments. Second, the small sample size may have been responsible for a type-II error: the study may be underpowered to detect significant changes in some variables. However, this was conceived as an exploratory observational study aimed to detect any improvement in the microcirculation during haemoadsorption therapy in sepsis and these findings warrant further investigations. Third, a longer follow-up period could have been required to observe changes in some variables, such as haemodynamic parameters and arterial lactate. Lastly, the CytoSorb filter elimination rate of cytokines by sampling blood from the arterial and venous branches of the circuit, in order to verify the methodological efficacy of the technique, was not assessed. To conclude, even with some limitations, the present pilot study suggests a potential beneficial effect of treatment with CytoSorb on microcirculatory perfusion of septic patients going beyond the cytokines' removal. These preliminary data warrant further investigations to clarify the mechanisms of this effect in a wider setting.

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Chapter 2

IgM-enriched immunoglobulins (Pentaglobin) may improve the microcirculation in sepsis: a pilot randomized trial

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Abstract

Background: Polyclonal or IgM-enriched immunoglobulins may be beneficial during sepsis as an adjuvant immunomodulatory therapy. We aimed to test whether the infusion of IgM-enriched immunoglobulins improves microvascular perfusion during sepsis.

Methods: Single-centre, randomized, double-blind, placebo-controlled phase II trial including adult patients with a diagnosis of sepsis or septic shock for less than 24 h. Patients received an intravenous infusion of 250 mg/kg (5 mL/kg) per day of IgM-enriched immunoglobulins (Pentaglobin, n = 10) for 72 h or placebo (NaCl 0.9%, n = 9). At baseline and after 24 and 72 h of infusion, the sublingual microcirculation was assessed with Incident Dark Field videomicroscopy. Thenar near-infrared spectroscopy (NIRS) was applied with a vascular occlusion test to assess tissue oxygenation and microvascular reactivity. Levels of interleukin (IL) 1-beta, IL-6, IL-8, IL-10 and tumour necrosis factor alpha were measured in the serum.

Results: The perfused vessel density (PVD) for small vessels (diameter < 20 micron) increased in the Pentaglobin group (from 21.7 ± 4.7 to 25.5 ± 5.1 mm/mm2) and decreased in the placebo group (from 25 ± 5.8 to 20.7 ± 4.1 mm/mm2, p for interaction < 0.001, two-way analysis of variance). The absolute between-group difference at 72 h was 4.77 (standard error 2.34), p = 0.140. The microvascular flow index for small vessels increased at 24 h in the Pentaglobin group (from 2.68 [2.38-2.78] to 2.93 [2.82-3], p < 0.01) and decreased at 72 h in the placebo group (from 2.83 [2.60-2.97] to 2.67 [2.48-2.73], p < 0.05). Changes in general parameters, cytokines and NIRS-derived parameters were similar between the two groups, except for IL-6 and IL-10 that significantly decreased at 72 h only in the Pentaglobin group.

Conclusions: A 72-h infusion of IgM-enriched immunoglobulins (Pentaglobin) in patients with sepsis or septic shock may be associated with an increase in sublingual microvascular perfusion. Further studies are needed to confirm our findings.

Keywords: Immunoglobulins; Immunomodulation; Microcirculation; Pentaglobin; Sepsis.

Background

Sepsis is a major healthcare problem, with high mortality and morbidity: even if some reports showed a decline in crude hospital mortality in the last decade [1], sepsis survivors remain at higher risk of infections, cardiovascular events, acute renal failure or the development of new physical disability or cognitive impairment [2]. At present, sepsis treatment is non-specific and mainly based on antibiotics and hemodynamic support [3].

Sepsis is characterized by a dysregulated host response to an infection, with uncontrolled activation of both pro- and anti-inflammatory pathways [4]. Increasing evidence suggests that a state of immunoparalysis is mainly responsible for adverse outcome. A recent meta-analysis showed a significant reduction in circulating B cells and immunoglobulin M (IgM) levels in sepsis non-survivors as compared to survivors [5]. The administration of polyclonal or IgM-enriched immunoglobulins as an adjuvant immunomodulatory therapy gave encouraging results in both pre-clinical and clinical studies [6], although the evidence supporting a reduction in mortality is still too weak to justify a widespread use in septic patients [7]. The potential benefits of immunoglobulins (especially IgM-enriched preparations) are related not only to their anti-inflammatory activity (pathogen recognition and clearance, toxin scavenging, inhibition of inflammatory mediators production, cytokine neutralization, complement-scavenging properties) but also to their anti-apoptotic effects on immune cells [8]. Pre-clinical studies showed a potential role in the regulation of endothelial cell function, leukocyte adhesion and capillary perfusion [9, 10]. Nonetheless, no clinical studies exist that evaluated the microvascular effects of immunoglobulins in septic patients.

We hypothesized that the intravenous administration of IgM-enriched immunoglobulins in patients with sepsis as an adjunctive therapy could improve microvascular perfusion. This may result in better tissue oxygenation and preserved organ function. The primary goal of this study was to evaluate whether the infusion of IgM-enriched immunoglobulins was able to increase the sublingual perfused vessel density (PVD) after 72 h as compared to a placebo. Secondary endpoints were parameters of microcirculatory flow quality, peripheral (skeletal muscle) tissue oxygenation and microvascular reactivity.

Methods

This single-centre, randomized, double-blind, placebo-controlled phase II trial was conducted in the Intensive Care Unit of Azienda Ospedaliera Universitaria "Ospedali Riuniti" of Ancona in Italy. The study protocol was approved by the local ethics committee (Comitato Etico Regionale Marche) and registered in www.ClinicalTrials.gov (Identifier: NCT02655133, date of registration 7th January 2016, https://www.clinicaltrials.gov/ct2/show/NCT02655133). Written informed consent was obtained before enrolment from all patients or their legal representatives in accordance with current Italian legislation. A deferred consent procedure was applied in case of temporary inability.

This manuscript adheres to the 2010 Consolidated Standards of Reporting Trials statement.

Participants

We included adult (≥ 18 years old) patients with severe sepsis or septic shock according to the 2001 International Sepsis Definition Conference criteria [11], as the original study protocol was approved before the publication of the Sepsis-3 definitions [12]. Severe sepsis was defined by the

presence of at least one sepsis-induced organ dysfunction; septic shock was defined as persistent hypotension despite adequate fluid resuscitation, requiring vasopressor infusion [11]. Nonetheless, the term "sepsis" (instead of severe sepsis) will be used hereafter, as the current concept of sepsis now includes the presence of an organ dysfunction induced by a dysregulated response to infection [12]. In addition, the term "septic shock" will refer to a condition of persistent arterial hypotension despite adequate fluid resuscitation and lactate levels > 2 mmol/L, based on the current definition [12]. All patients were enrolled within the first 24 h of sepsis development. Exclusion criteria were: contraindications to immunoglobulin treatment; sepsis/septic shock for more than 24 h; history of chronic renal failure; life expectancy < 24 h; lack of informed consent; pregnancy; factors impeding the sublingual microcirculation evaluation (recent oral surgery or maxillo-facial trauma); inclusion in other interventional studies. Patients with a history of chronic renal failure were excluded since previous studies showed a higher risk of osmotic-induced renal damage following intravenous immunoglobulins infusion in those with pre-existing renal insufficiency [13].

Interventions

Patients were randomly assigned to one of two study groups. Patients in the Pentaglobin group received 250 mg/kg (5 mL/kg) per day of IgM-enriched immunoglobulins (Pentaglobin, Biotest Pharma GmbH, Dreieich, Germany) as a continuous intravenous infusion for 72 h. Patients in the placebo group received the same volume of normal saline solution (NaCl 0.9%) within a period of 72 h. Saline solution was chosen as the placebo treatment as being an inert substance with no expected biological effect at the volume infused in this study. A simple randomization was performed by a pharmacist through sealed envelopes with a 1:1 allocation ratio. The study treatment or placebo was then provided by the Hospital Pharmacy in identical bottles masked in opaque green plastic bags: neither the attending physicians nor the investigators nor the patient

were aware of the study group. All other therapies (including fluids and vasoactive agents) were provided according to individual needs and based on the Surviving Sepsis Campaign guidelines [3].

Measurements

All measurements were performed at baseline, 24 h after starting Pentaglobin/placebo infusion and at the end of infusion (72 h). Mean arterial pressure (MAP), heart rate (HR) and norepinephrine requirements were recorded. Arterial and central venous blood samples were collected in order to measure blood gases, arterial lactate, haemoglobin (Hb), white blood cell (WBC) count, procalcitonin, interleukin (IL) 1 beta, IL-6, IL-8, IL-10, tumour necrosis factor alpha (TNF-α). The Simplified Acute Physiology Score (SAPS) II, Acute Physiology and Chronic Health Evaluation (APACHE) II score were obtained at admission, and Sequential Organ Failure Assessment (SOFA) score at the study time-points.

The sublingual microcirculation was evaluated by means of incident dark field (IDF) videomicroscopy (Cytocam, Braedius Medical, Amsterdam, The Netherlands). The Cytocam-IDF is a third-generation handheld microscope that enables real-time in vivo visualization of the microcirculation. It consists of an illumination unit based on IDF imaging with a 4× magnification lens. The illumination light is emitted with a short pulse time of 2 ms (synchronized with a computer-controlled image sensor) and a wavelength of 548 nm, ensuring the highest absorption of oxyhaemoglobin and deoxyhaemoglobin, whereby flowing red blood cells are visible within the vessels as dark moving globules against a clear background [14]. After removal of secretions with a gauze, the probe was gently applied on the sublingual mucosa. At least 5 videos from different areas were recorded with adequate contrast and focus, and all efforts were made to avoid

pressure artifacts. The image quality was checked offline [15], and videos of inadequate quality were discarded. Three videos per time-point were analysed offline with dedicated software (Automated Vascular Analysis 3.2, Microvision Medical, Amsterdam, NL) [16]. In brief, the image was divided by three equidistant horizontal and three equidistant vertical lines; the De Backer score was calculated as the number of vessels crossing the lines divided by the total length of the lines. For each vessel crossing the lines, perfusion was categorized as continuous, sluggish, intermittent or absent. The percentage of perfused vessels (PPV) was estimated as follows: $100 * [(\text{total number of grid crossings} - [\text{no flow} + \text{intermittent flow}])/\text{total number of grid crossings}]$ [16]. The total vessel density (TVD) was calculated as the total length of vessels divided by the total area of the image. The perfused vessel density (PVD) was estimated by multiplying TVD by PPV as estimated with the De Backer method. The microvascular flow index (MFI) was calculated semiquantitatively as described elsewhere [16]. The flow heterogeneity index (FHI) was also calculated as the highest MFI minus the lowest MFI, divided by the mean MFI, providing an index of heterogeneous microcirculatory perfusion [16]. The analysis was focused on small vessels (≤ 20 μ in diameter).

Near-infrared spectroscopy (NIRS) (InSpectra™ Model 650; Hutchinson Technology Inc., Hutchinson, MN, USA) with a 15-mm-sized probe was used to measure microvascular oxygen saturation (StO2) and tissue Hb index (THI) on the thenar eminence at baseline and during a vascular occlusion test (VOT) [17]. The StO2 downslope (%/min) was calculated as an index of oxygen consumption, while the StO2 upslope (%/min) and the area under the curve (AUC) of the hyperemic response were obtained to assess microvascular reactivity [17].

Sample size calculation

The sample size was calculated based on the primary outcome of the study, i.e. we hypothesized that the infusion of Pentaglobin would be able to induce an increase in the PVD at 72 h. We calculated that the inclusion of 9 patients per group would be sufficient to detect a difference of at least 4 mm/mm2 (standard deviation: 3 mm/mm2) [18] between the two groups at 72 h with a power of 80% and an alpha error of 0.05. Ten patients per group were enrolled in total to allow for dropouts.

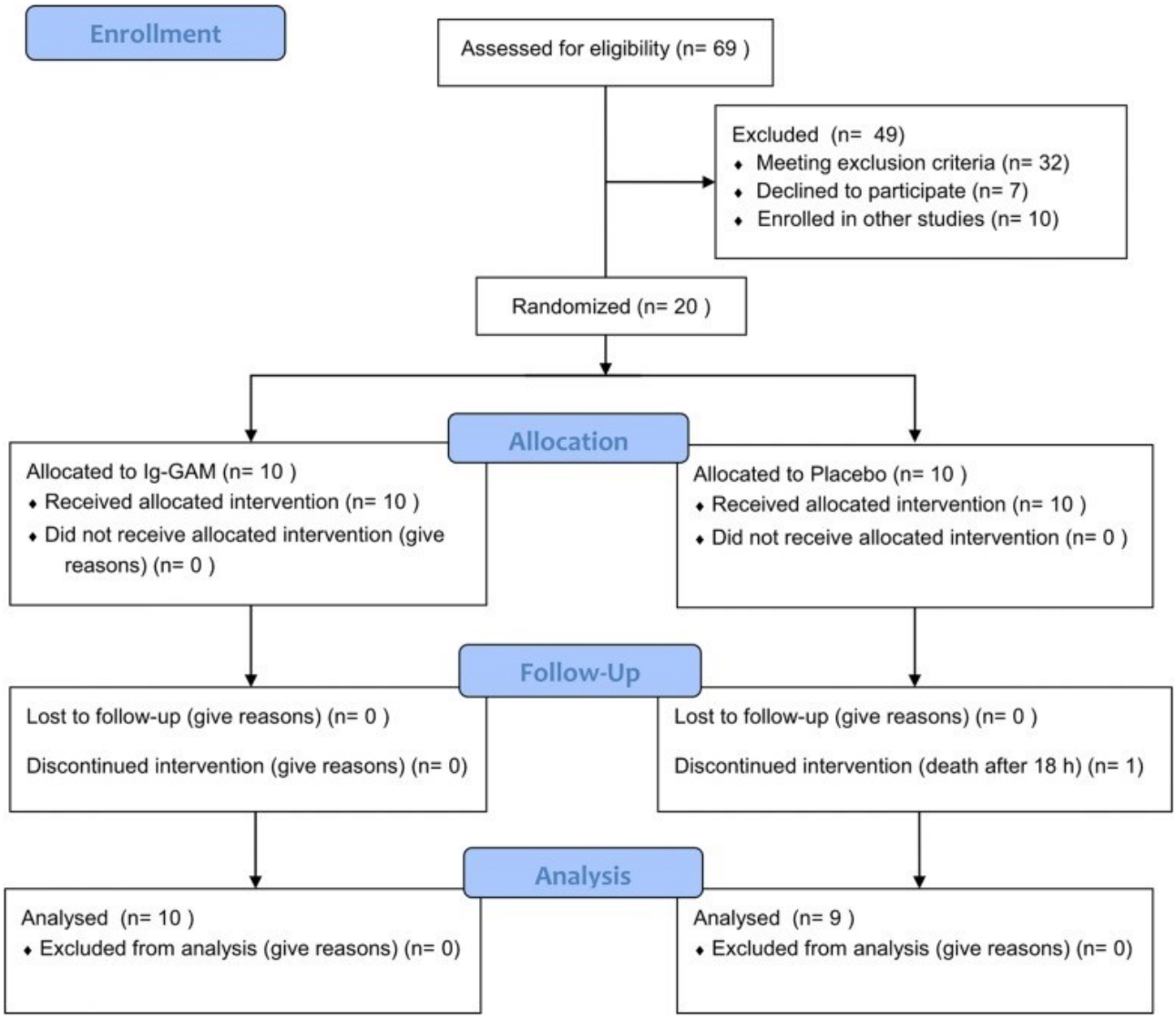
Statistical analysis

This was performed using GraphPad Prism version 6 (GraphPad Software, La Jolla, CA, USA) and IBM Statistical Package for Social Science version 21 (Armonk, NY: IBM Corp.). Normality of distribution was checked using the Kolmogorov–Smirnov test. The data were expressed as mean ± standard deviation (SD) for normally distributed variables or median [1st–3rd quartiles] for non-normally distributed variables. For normally distributed variables, we applied a two-way analysis of variance (ANOVA) for repeated measures to test the effect of treatment (Pentaglobin versus Placebo) and time on the variables of interest. A Sidack's post hoc test was used for multiple comparisons. For non-normally distributed variables, the Mann–Whitney U test was applied to evaluate differences between the two groups at each time-point and the Friedman test with the Dunn's test for multiple comparisons was applied to evaluate differences between time-points in each group. Since we found significant inter-individual variability in microcirculatory parameters at baseline, we performed a secondary analysis by calculating the delta values (changes from baseline at 24 and 72 h) in each group and performed an analysis of covariance (ANCOVA) for repeated measures to evaluate the interaction between the factors "time" and "treatment" by controlling for the baseline value of the outcome of interest (in order to correct for

the “regression to the mean” phenomenon), with the Bonferroni post hoc test to assess differences between the two group at each time-point. The Pearson r (or the Spearman rho) was calculated to evaluate correlation between variables. A two-tailed $p < 0.05$ was used to define statistical significance.

Results

From January 2016 to December 2017, 20 patients were enrolled in the study and randomized to receive Pentaglobin or placebo. One patient in the placebo group died 18 h after randomization, leaving 19 patients in total for the final analysis (Fig. 1).



Baseline characteristics for the two study groups are reported in Table 1.

Table 1 Baseline characteristics

Patient demographics	Pentaglobin ($n=10$)	Placebo ($n=9$)	p
Age (years)	62 ± 20	67 ± 16	0.545
Gender (nr of males, %)	7 (70%)	8 (89%)	0.582
Comorbidities (n, %)			0.659
Arterial hypertension	4 (40%)	3 (33%)	
Cardiopathy	1 (10%)	1 (11%)	
Diabetes	2 (20%)	1 (11%)	
Malignancy	3 (30%)	2 (22%)	
SAPS II (admission)	42 ± 17	45 ± 12	0.690
APACHE II (admission)	17 ± 8	21 ± 7	0.243
SOFA (admission)	9 ± 4	10 ± 3	0.663
Source of sepsis (n, %)			0.532
Abdominal	4 (40%)	2 (22%)	
Pulmonary	3 (30%)	2 (22%)	
Uro-genital	1 (10%)	3 (33%)	
Soft tissues	1 (10%)	2 (22%)	
Other	1 (10%)	0 (0%)	
Multi-drug-resistant pathogen (n, %)	4 (40%)	5 (56%)	0.656
Shock[a] (n, %)	3 (30%)	2 (22%)	0.999

SAPS Simplified Acute Physiology Score, *APACHE* Acute Physiology and Chronic Health Evaluation, *SOFA* Sequential Organ Failure Assessment

[a] Persistent arterial hypotension despite adequate fluid resuscitation and hyperlactatemia (lactate levels > 2 mmol/L)

Changes in sublingual microvascular and NIRS-derived parameters are shown in Fig. 2 and Table 2. A two-way ANOVA showed a significant interaction effect of treatment and time on the PVD with an F ratio of F (degree of freedom = 2, error = 34) = 9.84 ($p < 0.001$). The Sidack's post hoc test showed that the PVD was increased at 72 h in the Pentaglobin group ($p < 0.05$ versus baseline), while it was reduced in the Placebo group ($p < 0.01$ versus baseline). The between-group comparison at 72 h showed an absolute difference of 4.77 (standard error = 2.34), $p = 0.140$ (Student's t test, $p = 0.039$). A comparison of delta values (adjusted for the baseline value) showed

opposite variations of the PVD at 72 h (Pentaglobin: +3.8 ± 3.8, versus Placebo: − 4.2 ± 4.7, p = 0.003, Additional file 1, Fig. 3). The MFI was increased at 24 h in the Pentaglobin group, while it decreased at 72 h in the placebo group (Fig. 2, Table 2) and the comparison of delta values showed divergent changes at 72 h (+ 0.2 ± 0.2 versus − 0.2 ± 0.2, p = 0.035). The PPV was higher in the Pentaglobin group as compared to the placebo group at 72 h (Fig. 2); however, variations from baseline did not differ between the two groups. An example of sublingual microcirculation before and 72 h after Pentaglobin infusion is shown in Fig. 4.

Table 2 Changes in sublingual microcirculation and NIRS-derived parameters

	Baseline	24 h	72 h	*p* (time)[a]	*p* (interaction)[b]
PVDs (mm/mm^2)				0.869	< 0.001
Pentaglobin ($n = 10$)	21.7 ± 4.7	23.4 ± 6.0	25.5 ± 5.1*		
Placebo ($n = 9$)	25.0 ± 5.8	23.8 ± 4.4	20.7 ± 4.1**		
MFIs (AU)					–
Pentaglobin ($n = 10$)	2.68 [2.38–2.78]	2.93 [2.82–3.00]**	2.82 [2.65–2.95]	0.002	
Placebo ($n = 9$)	2.83 [2.60–2.97]	2.93 [2.62–2.93]	2.67 [2.48–2.73]*	0.016	
TVDs (mm/mm^2)					–
Pentaglobin ($n = 10$)	25.5 [17.9–27.1]	24.9 [18.7–27.6]	27.0 [19.3–29.6]	0.436	
Placebo ($n = 9$)	26.6 [21.1–30.3]	25.2 [20.5–28.1]	22.5 [17.8–26.6]	0.154	
De Backer score (n/mm)				0.887	0.144
Pentaglobin ($n = 10$)	13.1 ± 2.2	13.3 ± 3.4	14.2 ± 2.7		
Placebo ($n = 9$)	13.9 ± 2.8	13.2 ± 2.4	12.8 ± 2.7		
PPVs (%)					–
Pentaglobin ($n = 10$)	96 [87–98]	99 [98–100]	98 [97–100]	0.050	
Placebo ($n = 9$)	98 [96–99]	98 [97–99]	96 [94–98][#]	0.154	
FHI (AU)					–
Pentaglobin ($n = 10$)	0.31 [0.16–0.45]	0.03 [0–0.13]	0.07 [0–0.18]	0.032	
Placebo ($n = 9$)	0.18 [0.03–0.30]	0.07 [0.07–0.18]	0.11 [0.03–0.26]	0.495	
StO_2 (%)					–
Pentaglobin ($n = 10$)	80 [79–84]	84 [79–89]	84 [77–88]	0.316	
Placebo ($n = 9$)	85 [72–86]	81 [78–84]	84 [75–87]	0.658	
StO_2 downslope (%/min)					–
Pentaglobin ($n = 10$)	− 8.4 [− 10.8, − 6.1]	− 10.9 [− 11.9, − 8.2]	− 11.5 [− 16.3, − 9.1]	0.367	
Placebo ($n = 9$)	− 8.0 [− 14.2, − 5.7]	− 6.6 [− 13.9, − 6.1]	− 10.2 [− 12.3, − 6.7]	0.813	
StO_2 upslope (%/min)				0.005	0.681
Pentaglobin ($n = 10$)	157 ± 37	224 ± 82**	207 ± 77		
Placebo ($n = 9$)	120 ± 57	162 ± 68	146 ± 73		
Area of hyperemia (%*min)				0.002	0.544
Pentaglobin ($n = 10$)	16.7 ± 10.5	12.7 ± 8.3	10.3 ± 5.9*		
Placebo ($n = 9$)	15.3 ± 10.3	13.9 ± 7.4	7.1 ± 6.8*		
THI (AU)					–
Pentaglobin ($n = 10$)	12 [9–15]	11 [8–12]	10 [8–14]	0.601	
Placebo ($n = 9$)	11 [7–13]	10 [7–13]	8 [7–12]	0.654	

Data are expressed as mean ± standard deviation or median [1st–3rd quartile], as appropriate

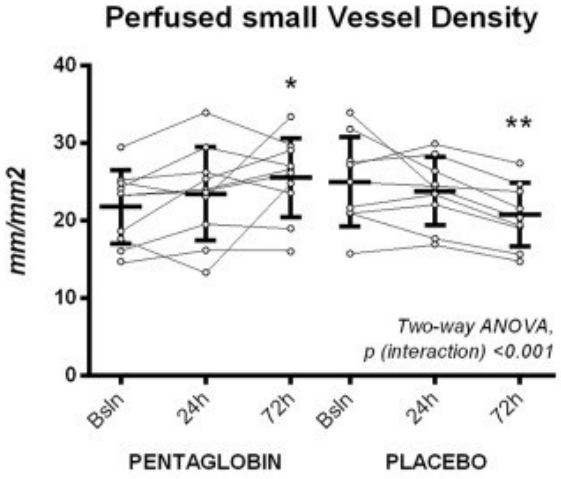
NIRS near-infrared spectroscopy, *PVD* perfused vessel density, *MFI* microvascular flow index, *TVD* total vessel density, *PPV* percentage of perfused vessels, *FHI* flow heterogeneity index, StO_2 tissue oxygen saturation, *THI* tissue haemoglobin index, *AU* arbitrary units

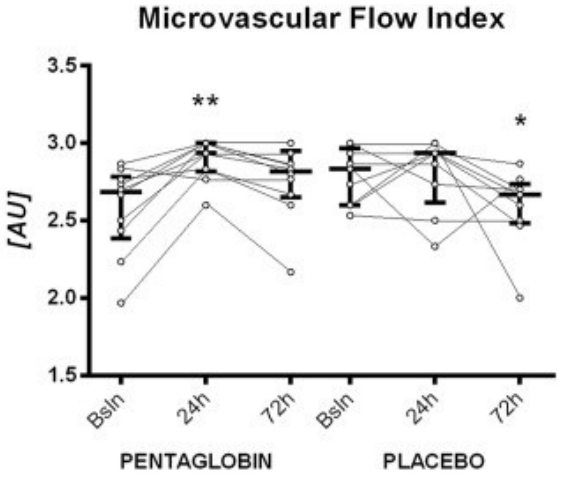
[a] Two-way analysis of variance for repeated measures (testing the effect of time) or Friedman test, as appropriate

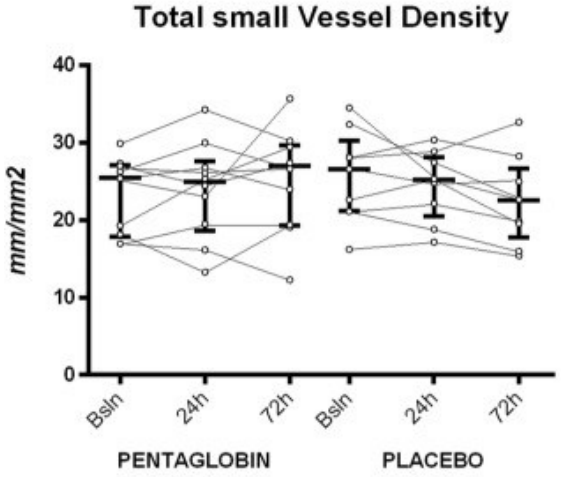
[b] Two-way analysis of variance for repeated measures (testing for the interaction between time and treatment), when applicable

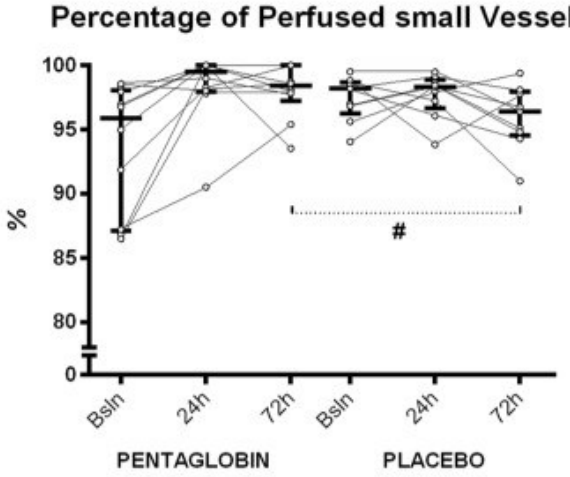
* $p < 0.05$, ** $p < 0.01$ versus baseline, Two-way analysis of variance for repeated measures with Sidack's post hoc test or Friedman test with Dunn's post hoc test for multiple comparisons, as appropriate

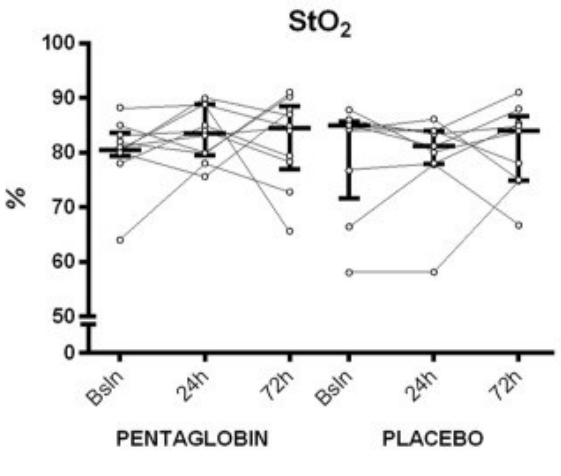
[#] $p < 0.05$ versus Pentaglobin group, Mann–Whitney *U* test

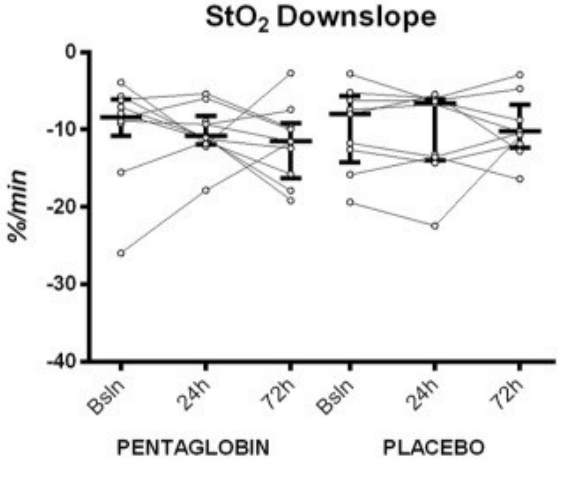

Perfused small Vessel Density
mm/mm2
40
30
20
10
0
*
**
Two-way ANOVA,
p (interaction) <0.001
Bsln
24h
72h
Bsln
24h
72h
PENTAGLOBIN
PLACEBO

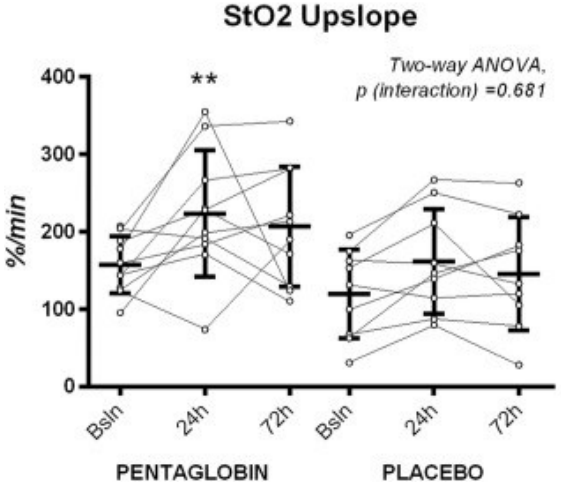

Microvascular Flow Index
[AU]
3.5
3.0
2.5
2.0
1.5
**
*
Bsln
24h
72h
Bsln
24h
72h
PENTAGLOBIN
PLACEBO


Total small Vessel Density
mm/mm2
40
30
20
10
0
Bsln
24h
72h
Bsln
24h
72h
PENTAGLOBIN
PLACEBO


Percentage of Perfused small Vessels
%
100
95
90
85
80
0
#
Bsln
24h
72h
Bsln
24h
72h
PENTAGLOBIN
PLACEBO


StO2
%
100
90
80
70
60
50
0
Bsln
24h
72h
Bsln
24h
72h
PENTAGLOBIN
PLACEBO


StO2 Downslope
%/min
0
-10
-20
-30
-40
Bsln
24h
72h
Bsln
24h
72h
PENTAGLOBIN
PLACEBO


StO2 Upslope
%/min
400
300
200
100
0
**
Two-way ANOVA,
p (interaction) =0.681
Bsln
24h
72h
Bsln
24h
72h
PENTAGLOBIN
PLACEBO

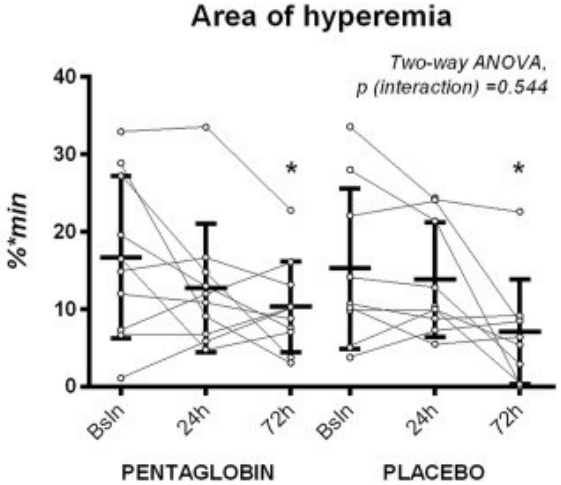

Area of hyperemia
%*min
40
30
20
10
0
*
*
Two-way ANOVA,
p (interaction) =0.544
Bsln
24h
72h
Bsln
24h
72h
PENTAGLOBIN
PLACEBO

*Fig. 2 Comparison of microcirculatory and NIRS-derived parameters. Data are expressed as mean and standard deviation of median [interquartile range]; lines indicate individual changes. *p < 0.05, **p < 0.01 versus baseline, Two-way ANOVA for repeated measures with Sidack's post hoc test or Friedman test with Bonferroni post hoc test, as appropriate. #p < 0.05 versus placebo, Two-way ANOVA for repeated measures with Sidack's post hoc test or Mann–Whitney U test, as appropriate*

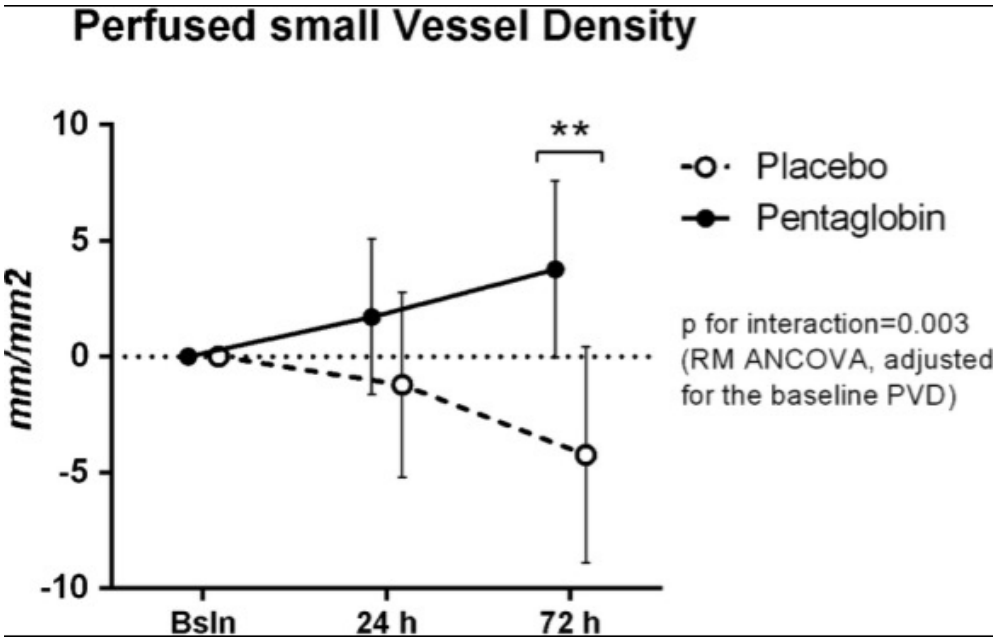


Fig. 3 Comparison of the delta values (variations from baseline) for the perfused vessel density

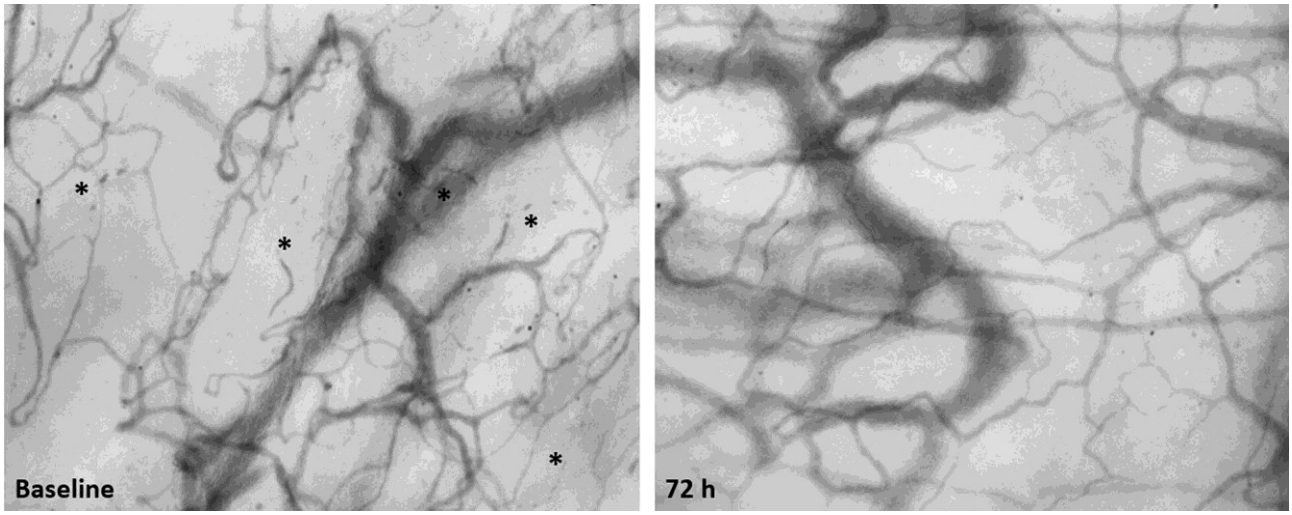


Fig. 4 Images of the sublingual microcirculation of a patients at baseline and after 72 h of Pentaglobin infusion. Non-perfused vessels are indicated with stars

Two-way ANOVA showed significant effects of both time (with an F (2, 34) 6.29, p = 0.005) and treatment (with an F (1, 17) 4.51, p = 0.049) on the StO2 upslope, with no significant interaction (p = 0.681). Changes from baseline were similar between the two groups (Additional file 1). We found a significant effect of time on the area of hyperemia with an F (2, 34) 7.19 (p = 0.002), but variations over time did not differ between the two groups (Fig. 2, Additional file 1). No other differences were observed for microvascular and NIRS-derived parameters.

The administration of Pentaglobin did not induce any significant variation in MAP or HR, while norepinephrine dosage was decreased in the placebo group at 72 h (Table 3). A greater decrease in central venous O2 saturation (ScvO2) was found in the placebo group compared with the Pentaglobin group at 24 h (Table 3). Changes in the other parameters and SOFA score were similar between the two groups. Similarly, changes in WBC count, procalcitonin and cytokine levels in the Pentaglobin group did not differ from those in the placebo group, although a significant decrease in IL-6 and IL-10 at 72 h was only found in the Pentaglobin group (Table 4). ICU-mortality was similar between the two groups (20% in the Pentaglobin group and 22% in the placebo group, p = 0.999), as well as the ICU length of stay (19 ± 13 days in the Pentaglobin group versus 16 ± 12 days in the placebo group, p = 0.649).

Table 3 Changes in hemodynamic, blood gas parameters and organ function

	Baseline	24 h	72 h	*p* (time)[a]	*p* (interaction)[b]
MAP (mmHg)				0.083	0.309
Pentaglobin ($n=10$)	80 ± 14	82 ± 9	86 ± 15		
Placebo ($n=9$)	85 ± 15	75 ± 17	89 ± 13		
HR (bpm)				0.662	0.169
Pentaglobin ($n=10$)	84 ± 23	85 ± 11	90 ± 21		
Placebo ($n=9$)	95 ± 19	89 ± 30	80 ± 25		
Norepinephrine tartrate (*n*, mcg/kg/min)					–
Pentaglobin ($n=10$)	8, 0.13 [0.03–0.39]	8, 0.12 [0.03–0.23]	7, 0.11 [0–0.38]	0.616	
Placebo ($n=9$)	8, 0.38 [0.23–0.70]	8, 0.50 [0.14–0.70]	6, 0.10 [0–0.40]*	0.004	
Arterial pH				0.110	0.377
Pentaglobin ($n=10$)	7.43 ± 0.07	7.42 ± 0.06	7.45 ± 0.07		
Placebo ($n=9$)	7.45 ± 0.03	7.47 ± 0.05	7.49 ± 0.07		
PaO_2/FiO_2 (mmHg)				0.875	0.474
Pentaglobin ($n=10$)	300 ± 89	311 ± 151	320 ± 89		
Placebo ($n=9$)	311 ± 89	280 ± 74	262 ± 85		
$ScvO_2$ (%)				< 0.001	0.053
Pentaglobin ($n=10$)	80 ± 5	78 ± 10	72 ± 9*		
Placebo ($n=9$)	79 ± 9	68 ± 9**#	71 ± 7*		
Base excess (mEq/L)				< 0.001	0.659
Pentaglobin ($n=10$)	0.4 ± 4.9	2.5 ± 3.8	5.6 ± 4.9**		
Placebo ($n=9$)	1.2 ± 4.7	4.0 ± 4.8	5.1 ± 4.4*		
Arterial lactate (mmol/L)					–
Pentaglobin ($n=10$)	1.7 [1.3–3.5]	1.5 [1.0–2.2]	1.9 [0.9–2.7]	0.682	
Placebo ($n=9$)	1.6 [1.0–2.2]	1.4 [1.0–2.2]	1.2 [1.1–1.7]	0.755	
Haemoglobin (g/dL)				0.460	0.356
Pentaglobin ($n=10$)	10.4 ± 1.5	10.5 ± 0.9	9.6 ± 0.9		
Placebo ($n=9$)	9.8 ± 1.8	10.1 ± 1.3	10.0 ± 1.1		
Platelets ($*10^3$/mmc)				0.170	0.036
Pentaglobin ($n=10$)	158 ± 98	141 ± 80	153 ± 83		
Placebo ($n=9$)	163 ± 86	168 ± 84	136 ± 67		
Creatinine (mg/dL)				0.284	0.522
Pentaglobin ($n=10$)	1.1 ± 0.7	1.0 ± 0.5	0.9 ± 0.4		
Placebo ($n=9$)	1.9 ± 1.0	2.1 ± 1.6	1.8 ± 1.2		
Bilirubin (mg/dL)					–
Pentaglobin ($n=10$)	0.7 [0.5–1.7]	0.8 [0.5–1.6]	0.9 [0.5–1.7]	0.356	
Placebo ($n=9$)	1.0 [0.5–1.3]	0.9 [0.5–2.3]	0.7 [0.6–2.4]	0.515	
Glasgow Coma Scale					–
Pentaglobin ($n=10$)	14 [13–15]	15 [13–15]	15 [11–15]	0.999	

Table 3 (continued)

	Baseline	24 h	72 h	*p* (time)[a]	*p* (interaction)[b]
Placebo ($n=9$)	14 [10–14]	13 [9–14]	12 [10–14]	0.999	
SOFA score					–
Pentaglobin ($n=10$)	9 [7–11]	9 [5–10]	7 [6–8]	0.229	
Placebo ($n=9$)	10 [7–13]	12 [6–12]	10 [6–12]	0.544	
Propofol (mg/kg/h, *n*)					–
Pentaglobin ($n=10$)	1.2 [0–2.4], 7	0 [0–2.6], 4	0 [0–2.6], 4	0.790	
Placebo ($n=9$)	0.8 [0–1.6], 5	0 [0–1.1], 4	0 [0–1.6], 3	0.518	
Remifentanil (mcg/kg/min, *n*)					–
Pentaglobin ($n=10$)	0.08 [0.04–0.09], 8	0.05 [0–0.08], 7	0.06 [0–0.10], 7	0.366	
Placebo ($n=9$)	0.06 [0–0.10], 8	0.05 [0–0.08], 7	0.06 [0–0.10], 7	0.991	

Data are expressed as mean ± standard deviation or median [1st–3rd quartile], as appropriate

MAP mean arterial pressure, *HR* heart rate, $ScvO_2$ central venous oxygen saturation, *SOFA* Sequential Organ Failure Assessment

[a] Two-way analysis of variance for repeated measures (testing the effect of time) or Friedman test, as appropriate

[b] Two-way analysis of variance for repeated measures (testing for the interaction between time and treatment), when applicable

* $p<0.05$, ** $p<0.01$ versus baseline, Two-way analysis of variance for repeated measures with Sidack's post hoc test or Friedman test with Dunn's post hoc test for multiple comparisons, as appropriate

# $p<0.05$ versus Pentaglobin group, Two-way analysis of variance for repeated measures with Sidack's post hoc test or Mann–Whitney *U* test, as appropriate

Table 4 White blood cells, procalcitonin and cytokine levels

	Baseline	24 h	72 h	*p* (Friedman test)
White blood cell count (n/mmc)				
Pentaglobin (*n* = 10)	9070 [5560–18,660]	10,710 [6260–13,790]	10,100 [7388–11,720]	0.763
Placebo (*n* = 9)	12,000 [6145–23,300]	12,560 [8185–25,090]	12,990 [7275–26,220]	0.569
Procalcitonin (ng/mL)				
Pentaglobin (*n* = 10)	14.4 [3.4–48.6]	14.6 [4.8, 28.8]	7.1 [3.2–14.9]*	0.026
Placebo (*n* = 9)	20.0 [4.5–95.1]	19.7 [4.6–79.9]	5.2 [2.4–33]**	< 0.001
Interleukin-1 BETA (pg/mL)				
Pentaglobin (*n* = 10)	5.3 [4–12.8]	4.5 [4–6.7]	4 [4–5.6]	0.057
Placebo (*n* = 9)	4 [4–5.6]	4 [4, 5]	4 [4–5.8]	0.376
Tumour necrosis factor alpha (pg/mL)				
Pentaglobin (*n* = 10)	32 [20–84]	18 [14–38]	16 [12–28]	0.078
Placebo (*n* = 9)	30 [24–77]	39 [25–51]#	19 [16–42]	0.010
Interleukin-6 (pg/mL)				
Pentaglobin (*n* = 10)	350 [104–1418]	166 [61–781]	151 [41–296]*	0.030
Placebo (*n* = 9)	212 [52–971]	98 [36–217]	69 [21–141]	0.154
Interleukin-8 (pg/mL)				
Pentaglobin (*n* = 10)	138 [52–1268]	74 [29–177]	75 [49–108]	0.262
Placebo (*n* = 9)	146 [66–302]	62 [32–79]	57 [45–115]	0.278
Interleukin-10 (pg/mL)				
Pentaglobin (*n* = 10)	30 [10–118]	9 [5–15]*	8 [6–13]**	0.003
Placebo (*n* = 9)	20 [8–71]	19 [6–29]	10 [6–13]	0.685

Data are expressed as median [1st–3rd quartile]

* $p < 0.05$, ** $p < 0.01$ versus baseline, Friedman test with Dunn's test for multiple comparisons

No correlation was found between changes (delta 72 h-baseline) in PVDs and changes in MAP (Pearson r = − 0.073, p = 0.765), norepinephrine dose (r = 0.325, p = 0.175), ScvO2 (r = 0.171, p = 0.483) and cytokine levels (Spearman rho for TNF-alpha = − 0.125, p = 0.610; Il-6 = − 0.040, p = 0.870; Il-10 = − 0.146, p = 0.552).

No unintended effects were reported for any of the two study groups.

Discussion

Microcirculatory dysfunction plays a key role in the pathogenesis of sepsis [19,20,21,22]. Persistent microcirculatory alterations during septic shock are associated with organ failure and death [23, 24]. In this single-centre, randomized, double-blind, placebo-controlled phase II trial, we showed that a 72-h infusion of IgM-enriched immunoglobulins (Pentaglobin) as an adjunctive

therapy during sepsis may be associated with an increase in the sublingual microvascular density and blood flow quality. These changes did not correlate with variations in macro-hemodynamic parameters or cytokine levels. Although exploratory, these data would support a potential role of Pentaglobin therapy in favouring microvascular recruitment and tissue perfusion during sepsis.

A number of clinical studies suggested a beneficial effect of IgM-enriched immunoglobulins in sepsis; however, the quality of the available evidence remains low [7]. The use of immunoglobulins was introduced with the rationale of modulating the inflammatory reaction and supporting the immune system in the fight against pathogens [25]. In septic pigs, the infusion of Pentaglobin was able to shift the inflammatory response towards an anti-inflammatory profile [26]. In experimental sepsis models, Pentaglobin normalized capillary perfusion at 24 h by reducing venular leukocyte adhesion [10] and alleviated the histopathological injury in the lungs and small intestine [27, 28]. In a rat model of pneumonia, IgM-enriched immunoglobulins enhanced the anti-inflammatory response by increasing blood IL-10 levels and reducing TNF-alpha in bronchoalveolar lavage fluid [29]. In this study, we failed to detect a clear impact of Pentaglobin on the cytokine profile. The heterogeneity of sepsis syndrome likely influenced the variation in cytokine levels. In addition, we could have missed changes in cytokines occurring earlier than the first 24 h of treatment. Our study was not powered to detect changes in cytokine levels, which are extremely variable during sepsis.

Although it cannot be excluded that macro-hemodynamic changes unrelated to immunoglobulin therapy were responsible for the observed variations in the microcirculation, we could not find any correlation between changes in microvascular perfusion and variations of macro-hemodynamic parameters. While the infusion of Pentaglobin was able to improve the sublingual microcirculation without inducing any significant change in MAP or vasopressor dose, in the placebo group the PVD and MFI were reduced at 72 h despite a decrease in norepinephrine requirements. This loss of coherence between the macro- and the microvascular responses has

been described during sepsis and shock states [30] and emphasizes that targeting systemic hemodynamic parameters may not be sufficient to ensure an optimization of tissue perfusion.

Pentaglobin had no consistent impact on tissue oxygenation and microvascular reactivity as assessed by NIRS. Thenar NIRS with a VOT enables to investigate alterations in tissue oxygen delivery and consumption, and to test the microvascular reserve capacity following a short period of ischemia. Reduced StO2 and slower StO2 downslopes and upslopes are generally found during sepsis and are associated with worse outcome [31]. Nonetheless, NIRS shows alterations in peripheral (skeletal muscle) oxygenation and may not be sensitive enough to detect a hypoperfusion in inner organs, whereas the capillary perfusion of the sublingual mucosa is generally explored as a window to the splanchnic microcirculation [32].

Our study has several limitations. First, the small sample size carries a high risk of type-I error. The study may be underpowered to detect differences in some parameters (including cytokine levels). Moreover, the two groups may be unbalanced for some baseline characteristics (e.g. norepinephrine dose and lactate levels). Therefore, our results should be considered as exploratory and need confirmation by future studies. No differences were observed in the SOFA score between the groups: unfortunately, however, our study was not powered to detect differences in mortality or other major outcomes (organ failures, shock reversal, ICU length of stay). Second, the comparison of absolute PVD values at 72 h (as per pre-planned statistical analysis) did not reach statistical significance. However, we believe that this is (at least partly) due to a between-group variability at baseline. In order to control for this confounder, we included a comparison of delta values (adjusted for baseline) that confirmed the different trend observed in the two groups. Third, most of the enrolled patients were already hemodynamically stable and less than 30% were in shock based on the Sepsis-3 definitions [12]. Consistently, we did not find severe microvascular alterations at baseline: an MFI < 2.6 [16] was observed only in 5 patients out of 19 and the PPV was < 90% only in 4 cases. As the infusion of Pentaglobin could produce a bigger effect in patients

with more severe microcirculatory dysfunction, the presence of an altered microcirculation should be among the inclusion criteria in future studies. Fourth, we did not measure baseline immunoglobulin levels. The infusion of IgM-enriched immunoglobulins could have been more effective in patients with more severe hypo-IgG or hypo-IgM, who may represent the best target for this immunomodulatory therapy [33]. Lastly, data of cardiac output were not evaluated as available only for a small number of patients.

Conclusions

In this single-centre, randomized, double-blind, placebo-controlled, phase II trial, a 72-h infusion of IgM-enriched immunoglobulins (Pentaglobin) in patients with sepsis or septic shock was associated with an increase in sublingual microvascular perfusion. Given the small sample size, these results must be seen as exploratory and need to be confirmed by other studies.

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Chapter 3

Mid-Regional Proadrenomedullin (MR-proADM) and Microcirculation in Monitoring Organ Dysfunction of Critical Care Patients With Infection: A Prospective Observational Pilot Study

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Abstract

Introduction: Microvascular alterations are involved in the development of organ injury in critical care patients. Mid-regional proadrenomedullin (MR-proADM) may predict organ damage and its evolution. The main objective of this study was to assess the correlation between MR-proADM and microvascular flow index (MFI) in a small cohort of 20 adult critical care patients diagnosed with infection, sepsis, or septic shock. Further objectives were to evaluate the correlation between the clearance of MR-proADM and the variables of microcirculation and between MR-proADM and the Sequential Organ Failure Assessment (SOFA) score.

Materials and Methods: This is a prospective observational pilot study. Inclusion criteria: consecutive adult patients admitted to intensive care unit (ICU) for or with infection-related illness. Daily measurement of MR-proADM and calculation of the SOFA score from admission in ICU to day 5. Repeated evaluations of sublingual microcirculation, collection of clinical data, and laboratory tests.

Results: Primary outcome: MR-proADM was not significantly correlated to the MFI at admission in ICU. A clearance of MR-proADM of 20% or more in the first 24 h was related to the improvement of the MFIs and MFIt [percentual variation of the MFIs + 12.35 (6.01-14.59)% vs. +2.23 (-4.45-6.01)%, p = 0.005; MFIt +9.09 (4.53-16.26)% vs. -1.43 (-4.36-3.12)%, p = 0.002].

Conclusion: This study did not support a direct correlation of MR-proADM with the MFI at admission in ICU; however, it showed a good correlation between the clearance of MR-proADM, MFI, and other microvascular variables. This study also supported the prognostic value of the marker. Adequately powered studies should be performed to confirm the findings.

Keywords: infection; microcirculation; mid-regional proadrenomedullin; organ failure; sepsis; septic shock.

Introduction

Sepsis is a life-threatening syndrome characterized by a widespread tissue and microvascular injury (1, 2). Organ failure is one of the main challenges of septic patients and hemodynamic optimization is a cornerstone of adequate organ perfusion for prevention and treatment of organ dysfunction (3). However, organ sufferance may occur even after restoration of systemic hemodynamics. The mechanisms underlying this phenomenon are multifactorial and not completely clear, but there is increasing evidence that alterations of the microvascular blood flow are strongly implicated. Sepsis affects endothelial cell function; it determines endothelial barrier disruption and leakage and it leads to microcirculatory alterations that directly contribute to organ dysfunction (4–7). Adrenomedullin (ADM) is an endogenous peptide hormone of 52 amino acids synthesized widely through tissues (including bone, adrenal cortex, kidney, lung, blood vessels, and heart). ADM is biologically active and its effects include vasodilator, positive inotropic, diuretic, natriuretic, and bronchodilator actions; ADM is also an inhibitor for the secretion of insulin, aldosterone, and adrenocorticotropic hormone (8, 9). Previous studies showed that ADM increases in inflammatory diseases, including sepsis and septic shock, in order to stabilize the microcirculation and to protect against endothelial hyperpermeability (10–14); they also suggested that the variation in plasmatic levels of ADM may act as a marker of severity of the endothelial damage (15, 16). Mid-regional proADM (MR-proADM) is a fragment of ADM with no known function. It is produced in ratio of 1:1 to ADM. Its half-life is numerable in hours and it proportionally reflects the activity of ADM (8). Mid-regional proADM was already described as biomarker in community-acquired pneumonia and it has been proposed as a prognostic marker with potential clinical role in sepsis (17, 18). In the study of Valenzuela-Sánchez et al. (19), MR-proADM showed good correlation with organ dysfunction related to infection and to mortality in

critical care patients. The authors evaluated the clearance of MR-proADM during the first days of intensive care unit (ICU) admission and they demonstrated an enhanced clearance of MR-proADM in survivor patients. In our small-scale preliminary study, we aimed to investigate if MR-proADM, as biomarker of organ failure and of endothelial damage, could be correlated with microvascular alterations in critical care patients admitted in ICU for or with different degrees of infection-related illness.

Materials and Methods

Population, Enrolment, and Data Collection

This is a prospective observational pilot study performed in the 14-bed General and Traumatic ICU of Azienda Ospedaliera Universitaria Ospedali Riuniti of Ancona (Italy).

Inclusion criteria: 20 adult (age equal or superior to 18 years old) critically ill patients, consecutively admitted in ICU for or with different degrees of infection-related illness (infection, sepsis, and septic shock), with a length of stay (LOS) in ICU inferior to 24 h before the enrollment in this study. Infection, sepsis, and septic shock were determined according to the Third International Consensus Definitions for Sepsis and Septic Shock (20).

Exclusion Criteria to enrollment were: age inferior to 18 years old, LOS in hospital longer than 48 h, conditions that prevented adequate monitoring of sublingual microcirculation, end-of-life care, and refusal to consent.

The primary objective of this study was to study a correlation between plasma levels of MR-proADM and the microcircular flow index (MFI) at admission in ICU. Sample size was calculated on the primary endpoint. It was also purpose of the study to examine the relationship between MR-proADM in the 5-day period of observation and other microvascular variables [total vessel

density (TVD), De Backer score, perfused vessel density (PVD), and proportion of perfused vessels (PPV)] and to verify the association of MR-proADM with the Sequential Organ Failure Assessment (SOFA) score (as marker of organ dysfunction) and between the SOFA score and the microvascular indices. We predetermined to calculate the clearance of MR-proADM and to evaluate the relation between the first 24 h clearance and the evolution of microvascular parameters.

The study was articulated in 5 days of monitoring (day 1 to day 5) from admission in ICU.

Plasmatic levels of MR-proADM were dosed for all the timepoints. The Simplified Acute Physiology Score (SAPS) II and the Acute Physiologic Assessment and Chronic Health Evaluation Classification System II (APACHE II) scores were calculated at admission in ICU. The SOFA score was evaluated at admission (day 1) and daily to day 5.

Anthropometric and demographic data were collected at baseline including age, sex, weight, and height of the patients.Microbiological parameters were assessed to categorize patients [infection, sepsis, or septic shock; source of infection; and presence of multidrug resistance (MDR)].

For each of the five timepoints, we recorded clinical, hemodynamic, and laboratory parameters [systolic, diastolic, and mean arterial pressure; heart rate; cardiac output where available; respiratory parameters and mechanical ventilation; venous and arterial blood gas variables; vasoactive therapy; main parameters for renal, hepatic, and hematological function; and procalcitonin (PCT)]. At day 1 (<24 h from ICU admission), day 2 (24 h after the first assessment), and day 5, the sublingual microcirculation was assessed by using incident dark field (IDF) technology.

Microvascular Assessment

Sublingual microcirculation was registered by using a high-resolution video microscopy camera (CytoCam, Braedius Medical BV, Huizen, Netherlands, UK) with IDF technology and the microcirculatory parameters were, then, derived offline with the Automated Vascular Analysis (AVA) software (version 3.2; Microvision Medical, Amsterdam, Netherlands, UK). Microvascular assessment and analysis were performed by experienced operators and in compliance with the "second consensus on the assessment of sublingual microcirculation in critically ill patients" and "the microcirculation image quality score" (21, 22). The MFI, TVD, PVD, and PPV were calculated for both the small-size vessels and total vessels in all the videos analyzed. The De Backer score was analyzed for total vessels. The MFI is a semi-quantitative measure of perfusion quality; it is calculated by dividing the image into four quadrants in which the observer reports the predominant type of flow by using an ordinal scale (0 for absent flow, 1 for intermittent flow, 2 for sluggish flow, and 3 for normal flow).The average of the four quadrants is the final MFI. Total vessel density and De Backer score are indices of vessel density. The first is the total length of vessels divided by the total surface of the analyzed area, while the second one is calculated as the number of vessels crossing horizontal and the vertical arbitrary grid lines divided by the total length of the lines. PPV is the percentual number of perfused vessels divided by the total number of vessels; PVD is derived by multiplying vessel density by the PPV and reflects the functional vessel density.

Measurement of MR-proADM

Arterial blood samples were collected and immediately centrifuged. Plasma samples were, then, stored at −80°C for subsequent measurement of MR-proADM.

Plasmatic levels of MR-proADM were measured by using Time Resolved Amplified Cryptate Emission (TRACE) technology with Thermo Scientific™ B·R·A·H·M·S™ KRYPTOR Compact PLUS (Dasit). The reference limit for this method was 0.55 nmol/l.

Ethics

In compliance with national applicable laws, informed consent was obtained from the subject before inclusion by signing the appropriate informed consent paperwork. Patients temporarily unable to consent were included in the study with deferred subject consent in a later phase and written informative for the next of kin. The study protocol was approved by the Local Ethics Committee [Comitato Etico Regione Marche (CERM); protocol number 212639, NCT03931967] and it conformed to the principles of Helsinki declaration (last revision, Edinburgh 2000).

Sample Size Calculation

Sample size calculation was calculated on the basis of the primary endpoint of the study (correlation between plasma levels of MR-proADM and the MFI at admission in ICU-T1): 19 patients were shown to be sufficient to detect a statistically significant correlation coefficient (higher than 0.6) with a power of 80% and an alpha error of 0.05.

Statistical Analysis

Statistical analysis was performed by using IBM SPSS statistic software (version 17.0) (IBM Corporation, New York, USA). According to the distribution of the main variables (assessed with the Kolmogorov–Smirnov test) and to the limited size of the sample, non-parametric statistics predominated. Data are presented as median and interquartile ranges (IQRs) for continuous variables and number and percentage for discrete variables. The Spearman's rank correlation coefficient was used to summarize the strength and direction (negative or positive) of the relationship between MR-proADM and the MFI as primary outcome measure with further parameters of microcirculation and with the severity scales. The non-parametric Mann–Whitney

U test was used for comparisons between independent samples. The Friedman test with the Dunn's post-hoc pairwise comparison was used for repeated measures of the same variable. In order to take into account the factor “time” in the comparison between groups, the two-way ANOVA for repeated measures was also performed (after normalization of the data through Box-Cox transformation) for the parameter of microcirculation with the Sidack's post-hoc test. The area under the receiver operating characteristic (ROC) curve was calculated to sample the ability of MR-proADM to discriminate the severity of patients. Differences were considered significant at $p < 0.05$.

Results

Descriptive of the Sample

From November 2018 to June 2019, a total of 29 patients were screened for the study and 20 of them were enrolled after obtaining the informed consent. A total of 9 patients were not enrolled for exclusion criteria. Patients were predominantly males (65%) with a median age of 70 (51–74) years. At admission in ICU, the SAPS II score was 52.5 (35.50–75.05), the APACHE II score was 19.5 (12.25–30.00), and the SOFA score was 11 (8–14). The SOFA score at admission corresponded to SOFA score at time of enrollment, as all the patients were enrolled in the first 24 h of ICU stay (Table 1).

TABLE 1 | Descriptive of the study population.

Age, years	70 [51–74]
Males	13 (65)
SAPS II score, AU	52.50 [35.50–75.05]
APACHE II score, AU	19.50 [12.25–30.00]
SOFA score, AU	11 (8–14)
Source of infection	
Respiratory	13 (65)
Abdominal	3 (15)
Genito-urinary	2 (10)
CNS	2 (10)
Septic shock	10 (50)
Sepsis	5 (25)
Infection without sepsis	5 (25)
PCT at admission, ng/ml	6.42 [1.01–24.08]
WBC at admission, cell * 10^3/mm^3	9.71 [7.7–13.06]
LOS in ICU, days	12.50 [9.00–16.75]
Mortality	3 (15)

Median (IQR), n (%), AU.
IQR, interquartile range; SAPS II, Simplified Acute Physiology II; APACHE II, acute physiology and chronic health evaluation II; SOFA, Sequential Organ Failure Assessment; PCT, procalcitonin; WBC, white blood cell; LOS, length of stay.

Half of the 20 patients were in septic shock at recruitment and 5 of 20 patients were septic. The origin of infection was respiratory in the vast majority of them (65%). Of 13 patients with low respiratory tract infections, five patients were diagnosed with type 1 influenza virus. Mortality rate was 15% and all the non-survivors were in septic shock. The LOS in ICU was 12.50 (9.00–16.75) days, but 11 of 20 patients were transferred to other ICUs for further treatments (Table 1). Median LOS for patients who did not survive was 21 (9–21) days. Mid-regional proADM was 3.42 (1.73–4.17) nmol/l at day 1, 3.02 (1.55–3.73) nmol/l at day 2, 2.05 (1.35–3.59) nmol/l at day 3, 1.8 (1.34–3.10) nmol/l at day 4, and 1.62 (1.27–3.20) nmol/l at day 5.

We calculated the clearance of MR-proADM (daily clearance in percentage) during the 5 days of recruitment: the median clearance of MR-proADM was 11.44 (−12.29–23.90)% at day 2, 10.79

(0.16–26.94)% at day 3, 1.80 (−6.82–16.38)% at day 4, and 8.82 (−2.35–20.61)% at day 5. In Table 2 we report the median values of MR-proADM and of PCT at admission in ICU (day 1 of enrollment) according to the diagnosis (infection, sepsis, and septic shock). No significant difference was evidenced for MR-proADM and PCT in the comparison between infected, septic, and septic shock patients.

TABLE 2 | Day 1 plasmatic values of MR-proADM and procalcitonin (PCT).

	Infection	Sepsis	Septic shock	p value
MR-proADM, nmol/l	1.44 [0.94–1.84]	2.16 [1.35–4.00]	3.99 [3.58–7.04]	ns
PCT, ng/ml	1.20 [0.52-6.93]	0.8 [0.59-3.73]	25.95 [1.79-86.62]	ns

Median (IQR).
MR-proADM, mid-regional proadrenomedullin.

The median values of MR-proADM at admission were higher in septic shock confronted to sepsis and of sepsis confronted to infection; these differences are not statistically significant. PCT did not show a linear increase in the three subgroups and resulted in very wide IQRs. MR-proADM and PCT showed a weak linear correlation in the population, when evaluated at T1 (Spearman's rank correlation coefficient + 0.672, p = 0.001). The correlation was more solid in the subgroup of patients with septic shock (Spearman's rank correlation coefficient + 0.758, p = 0.011).

Mid-Regional proADM and Microvascular Flow Index

Table 3 presents median values of the parameters of microcirculation in the general population at the three timepoints.

TABLE 3 | Descriptive of microvascular parameters at day 1, day 2, and day 5.

	Day 1	Day 2	Day 5	*p* value
MFIs, AU	2.83 [2.67–2.83]	2.92 [2.75–2.00]	2.75 [2.58–2.92]	ns
MFIt, AU	2.87 [2.75–2.92]	2.92 [2.86–3.00]	2.83 [2.75–2.96]	ns
TVDs, mm/mm^2	21.20 [17.07–23.85]	18.98 [16.99–23.11]	20.31 [18.29–23.17]	ns
TVDt, mm/mm^2	21.87 [18.21–25.38]	19,60 [18.33–23.68]	20.94 [19.15–24.10]	ns
PVDs, mm/mm^2	20.10 [16.55–23.04]	18.88 [16.88–23.00]	18.70 [17.49–22.72]	ns
PVDt, mm/mm^2	20.85 [17.70–24.86]	19.41 [18.21–23.57]	20.72 [18.83–23.52]	ns
PPVs, %	97.01 [95.47–98.01]	98.57 [96.26–99.39]	96.86 [95.14–98.71]*	0.023
PPVt, %	97.04 [96.53–98.35]	98.65 [96.61–99.41]	97.11 [95.05–98.59]	ns
De Backer score, 1/mm	12.99 [10.29–13.96]	11.28 [10.18–13.27]	11.77 [11.14–13.83]	ns

*Median (IQR) (Friedman test with Dunn's post-hoc test. *$p < 0.05$ in the comparison $t_{(5)}$ on $t_{(2)}$].*
MFIs, microvascular flow index of small vessels; MFIt, microvascular flow index of total vessels; TVDs, total vessel density; TVDt, total vessel density of total vessels; PVDs, perfused vessel density; PVDt, perfused vessel density of total vessels; PPVs, proportion of perfused small vessels; PPVt, proportion of perfused vessels for total vessels.

The MFI of small vessels (MFIs) at admission in ICU was lower in the subgroup of septic shock patients [2.72 (2.5–2.85)], but not statistically different from that of septic [2.83 (2.83–2.96)] and infected [2.83 (2.58–2.87)] patients. Similar results were evident for MFI of total vessels (MFIt). Both the MFIs and MFIt at admission in ICU were not correlated to MR-proADM (the Spearman's rank correlation coefficient not statistically significant) in the general population nor in the three subgroups of patients (infected, septic, and shocked). We determined an arbitrary cutoff of 20% of the clearance of MR-proADM in the first 24 h of recruitment by dividing the patients in two groups: patients who showed a clearance of MR-proADM higher and equal-to-lower than 20%. We measured the percentage of variation of the MFI of small and total vessels in the same time frame. The MFI improved significantly more in patients that showed a clearance of MR-proADM > 20% compared to those patients where the clearance of MR-proADM was ≤ 20% [the Mann–Whitney U test, percentual variation of the MFIs +12.35 (6.01–14.59)% vs. +2.23 (−4.45–6.01)%, p = 0.005; percentual variation of MFIt + 9.09 (4.53–16.26)% vs. −1.43 (−4.36–3.12)% p = 0.002] (Figure 1).

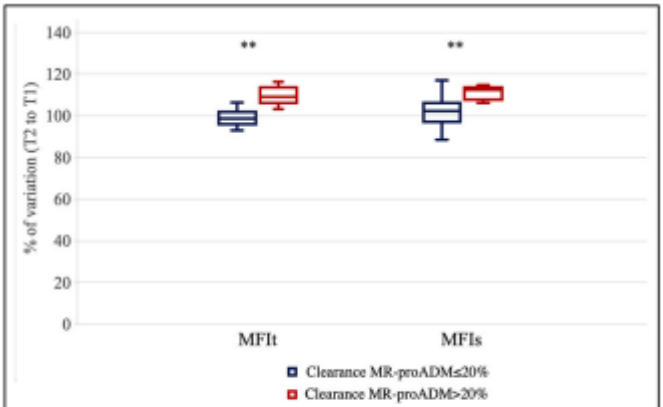

FIGURE 1 | Percentage of variation for microvascular flow index of total vessels (MFIt) and MFI of small vessels (MFIs) in the first 24 h of evaluation in the two groups of patients [clearance of mid-regional proadrenomedullin (MR-proADM) inferior-to-equal or higher than 20%]. $^{**}p < 0.01$.

Patients with reduced clearance of MR-proADM showed lower clearance also at T5 (clearance T5 to T2 19.2 vs. 24.3%) and deterioration in the MFIs [negative variation of −6.1% (−10.5–0) vs. 0% (−6.33–(+3.1); p = 0.017]. The trend was similar for MFIt, but not statistically significant (p = 0.06). The Friedman test for repeated measures was statistically significant for the MFIs in the group of patients with clearance of MR-proADM ≤ 20% (p = 0.035, Dunn's post-hoc test not significant; p = 0.057 for patients with clearance of MR-proADM > 20%) (Figure 2).

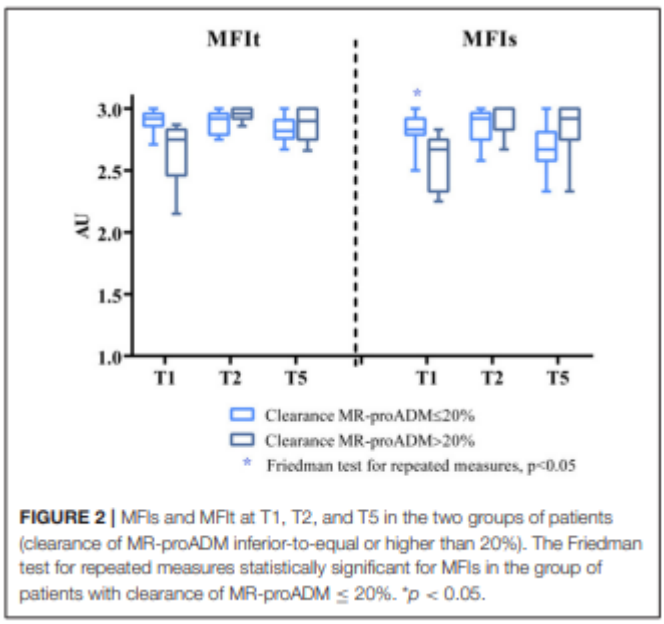

FIGURE 2 | MFIs and MFIt at T1, T2, and T5 in the two groups of patients (clearance of MR-proADM inferior-to-equal or higher than 20%). The Friedman test for repeated measures statistically significant for MFIs in the group of patients with clearance of MR-proADM ≤ 20%. $^{*}p < 0.05$.

The two-way ANOVA for repeated measures, performed after normalization of the data through Box-Cox transformation, showed for the MFIs a significant interaction between time and group ($p = 0.015$), but no significant effect of time ($p = 0.611$) or group ($p = 0.836$) per se. The Sidack's multiple comparisons test revealed a significant difference at baseline between the two groups ($p = 0.043$) and no other differences at the other time points [t(2): $p = 0.848$; t(5): $p = 0.419$]. Moreover, there was a significant increase in the MFIs at day 5 only in the group of patients with clearance of MR-proADM > 20% ($p = 0.045$ vs. baseline). The two-way ANOVA was also performed on the percentage of variation of the MFIs from baseline and it showed a significant interaction between time and group ($p = 0.005$) and a significant effect of both the time ($p = 0.008$) and group ($p = 0.004$). The Sidack's multiple comparisons test revealed a significant difference between the two groups at 24 h ($p = 0.009$) and at day 5 ($p < 0.001$). A significant increase in the MFIs was found only in the group of patients with a clearance of MR-proADM > 20% either at 24 h ($p = 0.002$ vs. baseline) and 5 days ($p = 0.017$ vs. baseline), while the MFIs did not significantly change over time in patients with a clearance of MR-proADM ≤ 20% (Figure 3).

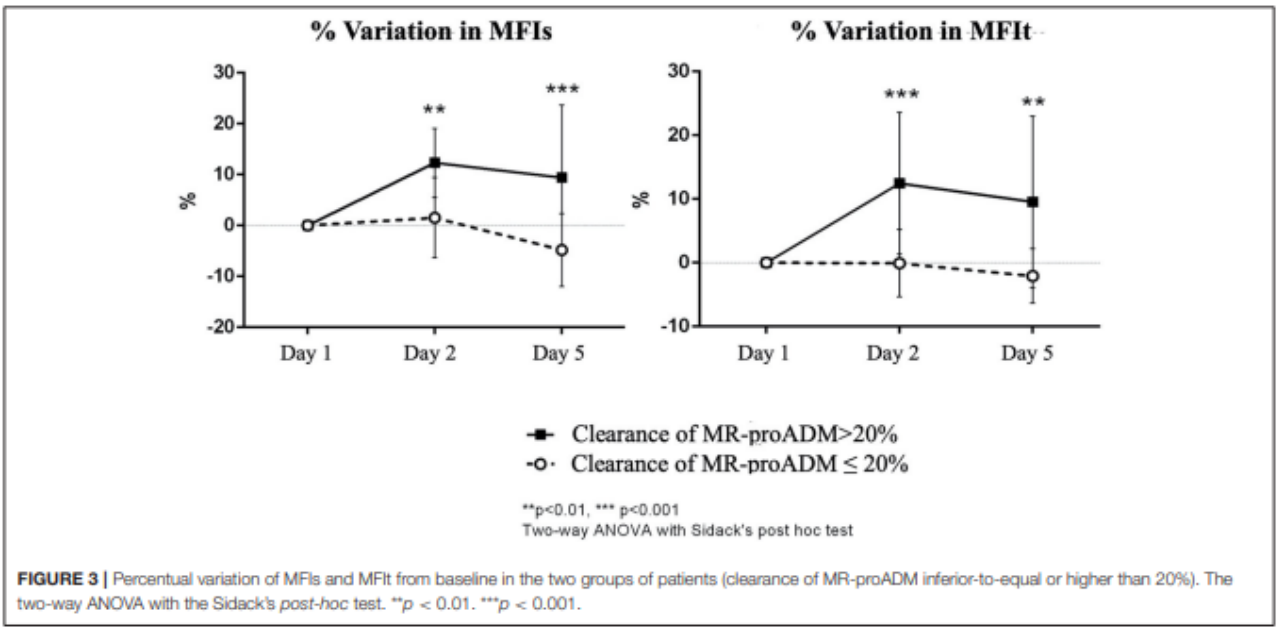

FIGURE 3 | Percentual variation of MFIs and MFIt from baseline in the two groups of patients (clearance of MR-proADM inferior-to-equal or higher than 20%). The two-way ANOVA with the Sidack's *post-hoc* test. $^{**}p < 0.01$. $^{***}p < 0.001$.

For MFIt, the two-way ANOVA test showed a significant interaction between time and group ($p = 0.004$). No significant effect of time ($p = 0.561$) or group ($p = 0.937$) was noticed. There was a significant difference at baseline between the two groups ($p = 0.029$; Sidack's multiple comparisons test) and no other differences at the other time points [t(2): $p = 0.273$; t(5): $p = 0.780$]. Moreover, there was a significant increase in MFIt at day 2 only in the group of patients with clearance of MR-proADM > 20% ($p = 0.018$ vs. baseline). The analysis performed on percentage of variation of MFIt from baseline showed a significant interaction between time and group ($p = 0.001$) and a significant effect of both the time ($p = 0.003$) and group ($p = 0.006$). The difference between the two groups was significant at 24 h ($p < 0.001$) and at day 5 ($p = 0.002$) and a significant increase in MFIt was found only in the group of patients with clearance of MR-proADM > 20% either at 24 h ($p < 0.001$ vs. baseline) and 5 days ($p = 0.002$ vs. baseline) (Figure 3).

Mid-Regional proADM and Other Parameters of Sublingual Microcirculation

Mid-regional proADM was not correlated to the parameters of sublingual microcirculation at admission in ICU. The proportion of perfused small and total vessels were weakly correlated to MR-proADM at day 2 (Spearman's rho correlation coefficient for PPVs−0.648, $p = 0.002$; for PPVt−0.578, $p = 0.008$). The Mann–Whitney U test showed a difference in the percentual variation of PVDs and PVDt day 2 to day 1 between patients with a clearance of MR-proADM > 20% and ≤ 20%, respectively; the percentual variation of PVDs was +10.51 [−9.28–(+)15.09]% vs. −9.93 [−19.32–(–)3.94]%; $p = 0.024$] and the percentage of variation of PVDt was 6.94 [−8.98–(+)23.12]% vs. −9.65 [−16.15–(–)4.35]%; $p = 0.024$]. The difference between the two groups was shown also in the interval day 5 to day 1 [percentage of variation of PVDs +20.19 (2.26–33.57)% vs. −14.24 [−27.05–(+)0.17]%; $p = 0.005$] [PVDt + 15.43 (0.68–32.67)% vs. −11.27 [−23.23–(+)3.42]%; $p = 0.01$].

The Friedman test for repeated measures was statistically significant for PVDs and PPVs in the group that showed a reduced clearance of MR-proADM ($p = 0.039$ for PVDs, the Friedman test for repeated measures, the Dunn's post-hoc test significant in the comparison T5 to T1, $p = 0.043$; $p = 0.027$ for PPVs, the Dunn's post-hoc test significant in the comparison T5 to T2, $p = 0.024$). The two-way ANOVA for repeated measures (Box-Cox transformation) showed for PVDs a significant interaction between time and group ($p = 0.004$). The Sidack's test was not significant at any time points; however, there was a significant increase in PVDs at day 5 in the group of patients with clearance of MR-proADM > 20% ($p = 0.024$ vs. baseline) and a significant decrease in PVDs at day 5 in the group of patients with clearance of MR-proADM ≤ 20% ($p = 0.035$ vs. baseline). The two-way ANOVA performed on percentage of variation of PVDs from baseline found a significant interaction between time and group ($p = 0.002$) and a significant effect of group ($p < 0.001$). A difference between the two groups was evident at 24 h ($p = 0.012$) and at day 5 ($p < 0.001$). A significant increase in PVDs was found in the group of patients with clearance of MR-proADM > 20% at 5 days ($p = 0.022$ vs. baseline), while a significant decrease was found in patients with clearance of MR-proADM ≤ 20% at day 5 ($p = 0.021$ vs. baseline) (Figure 4).

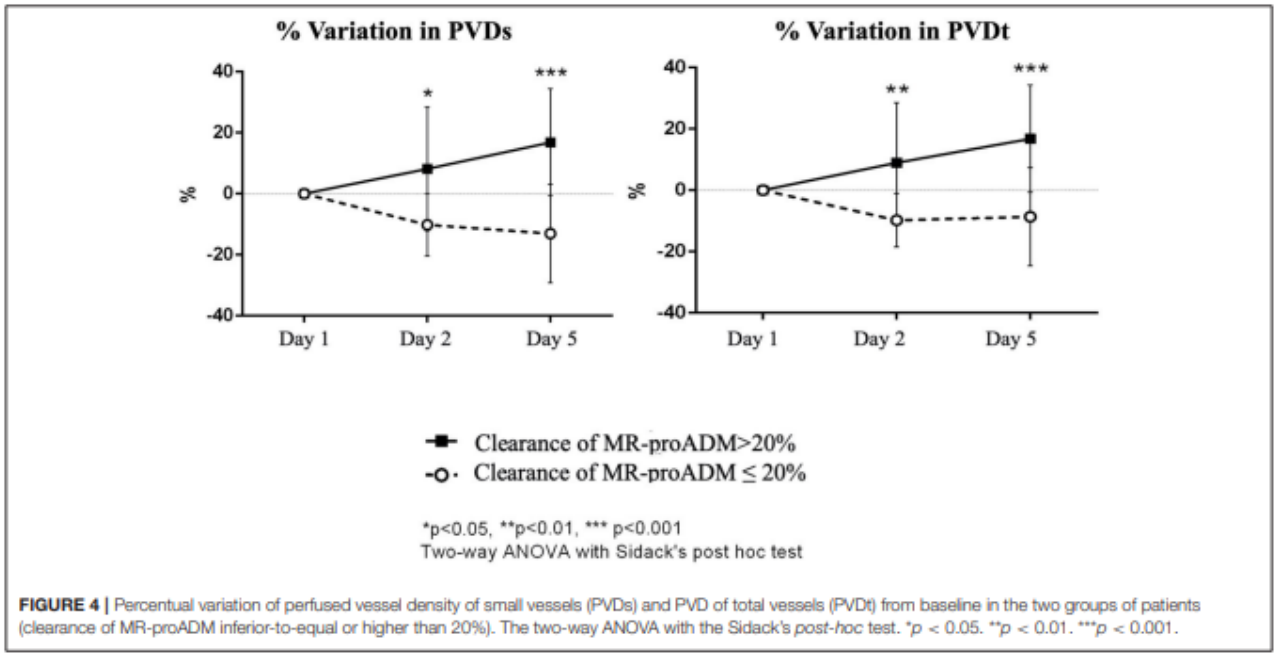

FIGURE 4 | Percentual variation of perfused vessel density of small vessels (PVDs) and PVD of total vessels (PVDt) from baseline in the two groups of patients (clearance of MR-proADM inferior-to-equal or higher than 20%). The two-way ANOVA with the Sidack's *post-hoc* test. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

For PVDt, a significant interaction was evidenced between time and group ($p = 0.015$); the Sidack's multiple comparisons test was not significant. PVDt tended to increase at day 5 in the group of patients with clearance of MR-proADM > 20% ($p = 0.066$ vs. baseline) and decrease at day 5 in the group of patients with clearance of MR-proADM ≤ 20% ($p = 0.088$ vs. baseline); however, the changes were not statistically significant. There was a significant interaction between time and group ($p = 0.006$) and a significant effect of group ($p = 0.001$) in the two-way ANOVA performed on percentage of variation of PVDt from baseline with a significant difference between the two groups at 24 h ($p = 0.007$) and at day 5 ($p < 0.001$). A significant increase in PVDt was only found in the group of patients with clearance of MR-proADM > 20% at 5 days ($p = 0.019$ vs. baseline) (Figure 4). For PPVs and PPVt, no significant interaction between time and group and no significant effect of time were found. The Sidack's multiple comparisons test revealed no significant difference between the two groups at any time points. Moreover, there was no significant change over time in PPVs or PPVt in either group. The test also showed a significant interaction between time and group for De Backer score ($p = 0.022$) and for TVDs ($p = 0.018$); the Sidack's test was not statistically significant. No other correlation was evidenced between MR-proADM and the De Backer score or TVD of total and small vessels.

Mid-Regional proADM and the SOFA Score

A clearance of MR-proADM ≤ 20% in the first 24 h of ICU stay discriminated a worsening of the SOFA score from T2 to T5 (AUC 0.938, CI 0.776–1, $p = 0.025$) with the median SOFA score at day 5 of 13 (9–15) in the group where MR-proADM clearance was lower than 20 vs. 8% (6–11) in the group with higher clearance.

The SOFA Score and Microvascular Variables

The SOFA score was correlated to microvascular variables at admission in ICU only in septic shock patients with the Spearman's rho correlation coefficient for MFIs of −0.698 ($p = 0.025$) and for PPVs of −0.720 ($p = 0.017$). At day 2, the SOFA score was weakly correlated to PPVs (the Spearman's rho correlation coefficient −0.459; $p = 0.042$) also in the general population. The correlation was stronger in the group with clearance of MR-proADM > 20% (the Spearman's rho correlation coefficient −0.893; $p = 0.007$) than in the group with clearance of MR-proADM ≤ 20% (the Spearman's rho correlation coefficient −0.663; $p = 0.014$). Similar correlation was evident at day 2 between the SOFA score and PPVt (the Spearman's rho correlation coefficient −0.461; $p = 0.041$ (−0.883, $p = 0.08$ in the group with clearance of MR-proADM > 20% and −0.682, $p = 0.01$ for the group with clearance of MR-proADM ≤ 20%, respectively). The SOFA score was not correlated to microvascular variables at day 5.

Discussion

In this small-scale preliminary study, we consecutively recruited 20 adult patients admitted in ICU with or for infection, sepsis, or septic shock. We monitored them for 5 consecutive days, analyzing the plasmatic levels of MR-proADM, the main clinical parameters, and the scores of severity. We evaluated the sublingual microcirculation to understand if MR-proADM, as biomarker of organ failure and of ADM-activity on the endothelium, could be associated with alterations of the variables of microcirculation and in particular to the MFI at admission in ICU. We calculated the clearance of MR-proADM over the first 24 h of recruitment and we compared it to the evolution over time of microvascular variables and of the SOFA score.

Mid-regional proADM at recruitment (and admission in ICU) tented to be higher in septic shock patients, than in septic and infected ones, but the difference was not statistically significant. The first 24 h clearance showed relation with the SOFA score. Although MR-proADM was not statistically related to the MFI at admission in ICU, the reduction of plasmatic levels of MR-proADM in the first 24 h of intensive care treatment was associated with an improvement of the MFI that was more evident than in patients with reduced or no clearance of the marker. Patients in which MR-proADM cleared showed a substantial stability of the MFI toward the first 5 days and an improvement in the SOFA score, while the opposite group suffered a deterioration of sublingual microcirculation in terms of the MFI and showed the statistically higher SOFA score at day 5. Similar evolution was evident for other parameters of microcirculation, in particular PVD and PPV of small vessels that relate to the quality of the flow and to microvascular perfusion. The proportion of perfused vessels was inversely correlated to the SOFA score at day 2, strongly in patients with higher clearance of MR-proADM. The SOFA score was correlated to microvascular variables at admission in ICU, but just in the small group of patients with septic shock. Sepsis and septic shock are characterized by increased endothelial permeability, endothelial barrier dysfunction, proinflammatory activation of endothelial cell, reduced deformability of red blood cells, alterations of the glycocalyx, and leukocyte adhesion and rolling; further mechanisms lead to microcirculatory flow disturbance in septic pattern (23–25). Damaged microcirculation is involved in the pathophysiology of organ dysfunction: it compromises tissue perfusion through the impairment of both the diffusion (reduced and heterogeneous capillary density) and convection (altered capillary flow) of oxygen and nutrients; the altered blood flow furtherly acts as trigger to tissue inflammation (23–25). In this situation, as in further inflammatory diseases, ADM increases and MR-proADM parallelly to ADM (10, 23). The increase of ADM participates to stabilize the endothelial barrier and to optimize the junctional integrity; it protects endothelial cells and the microcirculation (9, 10, 26–29). This effect was demonstrated on endothelial cells from different vascular beds (lung pulmonary artery, umbilical

vein, and brain) in ex-vivo and in-vivo models and on the ileal microcirculation of experimental rat model of Staphylococcus aureus alpha-toxin-induced sepsis (13, 26, 29). The relevance of ADM on the integrity of the microvasculature in sepsis is the trigger for this study. In this study, the single value of MR-proADM was not correlated to microvascular variables at admission in ICU, but the 24 h clearance of MR-proADM was correlated with the quality of the microvascular perfusion, the density of microcirculation, and with the severity of organ dysfunction. From the results of this study, we could hypothesize that patients who showed enhanced clearance of MR-proADM and where organ dysfunction and microcirculation both improved could be considered as patients who controlled the inflammatory source that triggered production of ADM (ratio 1:1 with MR-proADM) and in which the endothelial damage of microcirculation resolved (or was more controlled). The opposite result in the group where MR-proADM cleared more slowly may indicate persistence of synthesis and release of ADM due to an active organ dysfunction and also of microvascular dysfunction. Full-scale prospective studies will be needed to confirm the hypothesis, but it would be consistent with previous studies and in particular with the study of Valenzuela-Sánchez et al. (19), where they reported that ongoing MR-proADM levels at 48 h following admission in ICU and clearance of the marker on day 5 following admission helped to determine unfavorable evolution in 104 patients with severe sepsis. As a pilot study on a small number of patients, our investigation cannot provide any conclusive answer on the correlation between MR-proADM and the microvascular and organ perfusion for several and important limitations. As the estimation of the sample size was aimed to the primary objective of this study (correlation of the single value of MR-proADM and the MFI at admission in ICU), the population could be statistically underpowered for the secondary analysis performed on the clearance of MR-proADM and the MFI, the SOFA score, and other microvascular parameters. The decision to choose an arbitrary cutoff of the 24 h clearance of MR-proADM could influence the results of this study and the use of a different cutoff could lead to different results. There is limited literature about the clearance of MR-proADM; none about the cutoff of clearance at 24 h and this threshold

value requires further validation (8, 30, 31). These factors should be carefully considered when extrapolating the data reported. The results could be also affected by the difference in the rate of disappearance of MR-proADM and the rate of changes in the microcirculation. Moreover, this study was intentionally designed to include a heterogeneous population of patients with wide different degrees of infection-related illness and, therefore, different expression of MR-proADM and degrees of microvascular alterations. The two groups analyzed showed baseline differences and this determinant influences the reliability of the results. Some of the patients enrolled presented sepsis related to viral origin in which MR-proADM could have performed within given limits, if considered just as single value (admission value) without examining a trend, although few studies suggest it as effective prognostic tool (8, 32) and the advanced average age of our population may also have affected the results and should be cautiously considered in the limitations of our data. The microvascular damage in our population was, indeed, less severe of what we can expect in a population of septic and septic shock patients (in particular for the MFI and PPV), as we also included patients with infection, but without sepsis where the microvascular impairment is less studied and less homogeneous; most of the patients presented with an MFI <3 at admission in ICU and this result was consistent with our previous study, the MicroDAIMON study (33), where we reported an abnormal MFI on day 1 of admission in 20.6% of the patients and in 55.7% of cases during ICU stay; on the other hand, the median value of the MFI was still acceptable even in septic shock patients and the damage of the microcirculation was expressed heterogeneously among patients. The density of vessels (that is more strongly related to outcome in septic patients) was less impaired than expected, while the dominant alteration was the quality of the perfusion (6, 34). For all the limits of this study explained, future full-scale studies should be performed in a more homogeneous population.

Although we suggest caution in reading our findings for the limitations that we already explained, we believe that our results may justify further research projects to evaluate if the trend of MR-proADM toward days could be able to relate to the evolution of microvascular dysfunction and of organ injury. If MR-proADM confirms the correlation found, it may play a clinical role as a biomarker in predicting the microvascular response to infections, sepsis, or septic shock. There are limited publications on the usefulness of MR-proADM in the field of critical care; we suggest this will be a field of interest (15, 32, 35–38).

Conclusion

In our small cohort of adult patients admitted in ICU with infections, sepsis, and septic shock, MR-proADM and the MFI were not correlated at admission in ICU; however, a correlation existed between the first 24 h clearance of MR-proADM and the MFI and between the clearance of the molecule and other indices primarily connected with the quality of perfusion of the sublingual microcirculation. We believe that the relation between the expression of MR-proADM and the infection-related microvascular impairment merits further investigation: the clearance of MR-proADM could be a variable of interest in a comprehensive evaluation of this type of patients, but full-scale studies are needed to confirm our findings.

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Chapter 4

Variation in the Outcome of Norepinephrine-Dependent Septic Patients After the Institution of a Patient-Tailored Therapy Protocol in an Italian Intensive Care Unit: Retrospective Observational Study

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Abstract

Objective: To evaluate the outcome of patients with septic shock after the institution of a patient tailored therapy protocol in our Intensive Care Unit (ICU).

Methods: Single-center retrospective observational study including 100 consecutive septic patients (≥ 16 years) requiring norepinephrine infusion, admitted to our ICU between 2018 and 2019 after the institution of a patient-tailored therapy protocol, compared with a historical control group of 100 patients admitted between 2010 and 2013 (historical controls). The patient-tailored therapy protocol included the use of IgM-enriched immunoglobulins for patients with low plasma IgM levels, blood purification strategies for patients with high plasma levels of cytokines or endotoxin, albumin correction and modulation of vasoactive agents. Clinical and therapeutic parameters were noted at the time of initiation of norepinephrine infusion and for the 1st 24 h. The primary outcome was ICU mortality.

Results: ICU-mortality was lower in the patient-tailored therapy cohort as compared to historical controls (32 vs. 57%, $p < 0.001$). Patient-tailored therapy was associated with a lower risk of ICU-mortality even after adjusting for the main clinical severity indices (adjusted odds ratio 0.331 [95% confidence interval 0.166-0.658], $p = 0.002$). After propensity score matching, 48 patients in historical control group and 48 patients in the patient-tailored therapy cohort with similar general characteristics were selected. ICU-mortality was lower in the patient-tailored therapy matched subgroup as compared to historical controls (40 vs. 60%, $p = 0.037$).

Conclusions: An individualized therapeutic approach in septic patients may be associated with a survival benefit. However, the use of an historical control group of patients admitted between 2010 and 2013 may introduce substantial bias. Further adequately designed studies are needed to demonstrate the impact of patient-tailored therapy on outcome.

Keywords: mortality; outcome; patient-tailored therapy; sepsis; septic shock.

Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (1). Septic patients are a heterogeneous population (2), not only because of the pathophysiological complexity underlying this syndrome, but also because of the different basal characteristics of individual patient: for this reason, it is difficult to find a single therapy that may be effective for everyone (3, 4). For each patient, the therapeutic strategy must be based on the underlying physiological reserve, pre-existing comorbidities and organ dysfunction severity (2), with a patient tailored therapy approach. For example, recent evidence suggests that hemodynamic management must be optimized in each patient based on dynamic evaluation of clinical and laboratory parameters, indicative of organ perfusion (5), and on the previous clinical history. A multicentre trial showed that a higher blood pressure target may be required in patients with a history of arterial hypertension (6), suggesting that an individualized approach may be preferable. Similarly, different immunomodulation treatments, including intravenous immunoglobulins or extracorporeal blood purification techniques, taken individually, did not show a clear positive correlation with outcome (5), but a more careful patient selection (based on immunoglobulin and cytokine levels) may be necessary to better show an impact on survival. Starting from 2018, a patient tailored therapy protocol has been applied in our Intensive Care Unit (ICU) for the management of sepsis and septic shock. This protocol involves the use of adjunctive therapies, modulated according to the patient's characteristics, including intravenous immunoglobulins, extracorporeal removal strategies and hemodynamic support.

The primary goal of this study was to evaluate the outcome of patients with sepsis/septic shock admitted to our ICU after the institution of this patient tailored therapy protocol, in comparison to an historical control group of patients who were treated in an earlier period. Secondary endpoints were hospital mortality, ICU length of stay, maximum dose of norepinephrine and total fluid intake in the 1st 24 h after sepsis diagnosis, fluid balance at 24 h.

Materials and Methods

This single-center retrospective observational study was conducted in the Intensive Care Unit of Azienda Ospedaliero-Universitaria "Ospedali Riuniti" of Ancona, in Italy. The study protocol was approved by the local ethic committee (Comitato Etico Regionale delle Marche) before the data were accessed. The patient records were de-identified before the data were accessed, and the data were analyzed anonymously. Written informed consent was not necessary due to the retrospective nature of the study and because the data were analyzed anonymously. We included 100 consecutive patients (≥ 16 years old) with sepsis (1) requiring norepinephrine infusion, admitted to our ICU between July 2018 and September 2019 (patient-tailored therapy group). Clinical records were reviewed in order to select those patients who required an infusion of norepinephrine for persistent sepsis-induced hypotension. Persistent hypotension was defined by a systolic arterial pressure below 90 mmHg, or mean arterial pressure lower than 60 mmHg, or a reduction in systolic blood pressure of more than 40 mmHg from baseline, despite adequate volume resuscitation, in the absence of other causes of hypotension, requiring the infusion of vasopressors (7). According to current and previous guidelines of the Surviving Sepsis Campaign (8, 9), adequate volume resuscitation was defined by the absence of hemodynamic improvement after a fluid challenge either based on dynamic or static hemodynamic variables. Septic shock was defined as an acute circulatory failure characterized by persistent hypotension despite adequate fluid resuscitation, requiring vasopressor infusion, with arterial lactate levels > 2 mmol/l

(1). As historical controls, we used a group of 100 consecutive patients with norepinephrine-dependent sepsis admitted to our ICU between December 2010 and January 2013, who had been already enrolled for a previous retrospective study (10). That multicentre study was aimed to test the association between tachycardia and mortality and involved adult patients with a diagnosis of septic shock requiring norepinephrine as the first-line vasopressor despite adequate volume resuscitation (10), according the 2001 definitions (7). Exclusion criteria were in both studies: age < 16 years; duration of norepinephrine infusion < 6 h or survival time <6 h after the introduction of norepinephrine (10).

Patient-Tailored Therapy Protocol

Starting from 2018, a patient-tailored therapy protocol for the management of sepsis has been systematically applied in our ICU. This protocol is shown in details in Figure 1. After diagnosis of sepsis, dosage of plasma levels of immunoglobulins, cytokines (interleukin (IL) 1-beta, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF) alpha), endotoxin and albumin is performed in all patients. When plasma levels of IgM are low, septic patients receive IgM-enriched immunoglobulins solutions; when plasma levels of cytokines or endotoxin are high, extracorporeal removal strategies are applied; albumin replacement is indicated for patients with low plasma levels. To achieve a value of mean arterial pressure ≥ 65 mmHg, in patients who would require high norepinephrine dosage, terlipressin is added. All other therapies were based on the indications of the Surviving Sepsis Campaign (8). Before 2018, all septic patients were treated according to current guidelines and based on the indications of the attending physician, without a protocolized approach.

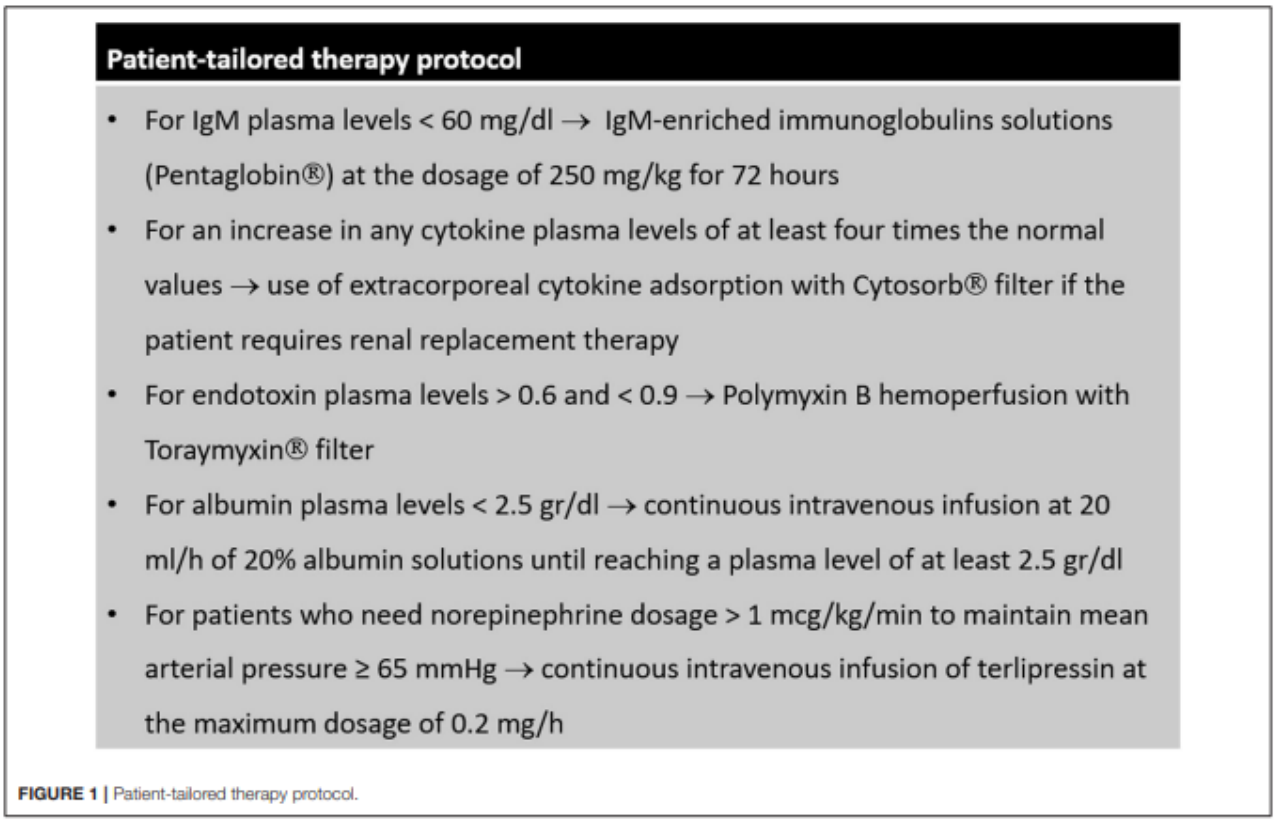

FIGURE 1 | Patient-tailored therapy protocol.

Data Collection

Data collection was performed by different researchers for the historical and the interventional cohorts, using an identical data extraction form. Data extracted for the 2018–2019 cohort were double-checked by the investigator who had collected data for the 2010–2013 cohort, in order to make sure that the same criteria had been applied for selecting patients and that those who had received norepinephrine for hypotension due to other causes were excluded. For each patient, source of infection, pathogen involved, multidrug resistance [defined as: Gram positive – methicillin-resistant; Gram negative – resistant to at least 3 antibiotic classes; Candida – resistant to fluconazole (10)] and state of immunosuppression [defined as: neutrophils <500 cells/mm3 or chemotherapy administration in the previous 2 weeks or prednisone 20 mg/day or equivalent (10)] were noted. The Sequential Organ Failure Assessment (SOFA) score, Simplified Acute Physiology Score (SAPS II), and the Acute Physiology and Chronic Health Evaluation

(APACHE) score of the 1st 24 h after ICU admission were calculated. At the time of initiation of norepinephrine infusion and for the 1st 24 h the following parameters were registered: body temperature, heart rate, heart rhythm, systolic arterial pressure, diastolic arterial pressure, mean arterial pressure, norepinephrine and other vasoactive drugs dosage, cardiac output (if available), sedative drugs dosage, main mechanical ventilation parameters, need for renal replacement therapy. We calculated SOFA score at the time of initiation of norepinephrine and for the 1st 24 h and the fluid balance for the 1st 24 h. The primary outcome was ICU mortality. We also noted in-hospital mortality, ICU length of stay, hospital length of stay, cause of death.

Sample Size

In the previous retrospective study 100 patients diagnosed with septic shock (7) were enrolled. These patients were used as historical controls. We arbitrarily decided to collect data of an equal number of patients admitted to our ICU after the institution of this patient tailored therapy protocol.

Statistical Analysis

This was performed using GraphPad Prism Version 6 (GraphPad Software, La Jolla, CA, USA) and IBM Statistical Package for Social Science version 21 (Armonk, NY: IBM Corp.). Normality of distribution was checked using the Shapiro-Wilk test. The data were expressed as mean ± standard deviation (SD) for normally distributed variables or median [1st−3rd quartiles] for non-normally distributed variables. The Chi-squared test was applied to evaluate nominal variables. To compare quantitative variables between groups the Student's T-test and the Mann-Whitney U-test were used, as appropriate. A multivariate logistic regression analysis with a forward conditional method was performed to evaluate the association between patient tailored therapy

and ICU-mortality, adjusted for arbitrarily selected variables that were deemed to be relevant for the outcome. These included variables related to the type of infection (source of infection, multidrug-resistance), patient's severity on ICU-admission (APACHE II score), available data on comorbidities (chronic heart failure, diabetes mellitus), immunosuppression, indices of organ function (SOFA score, heart rate, mean arterial pressure, lactate levels). In order to obtain more robust evidence of the outcome, we also decided to perform a propensity score-matched analysis. A Propensity Score for the likelihood of being part of the group treated with the patient tailored therapy was obtained by means of multiple logistic regression. The following clinically relevant variables were included in the score: source of infection, multidrug-resistant pathogens infection, immunosuppression, SOFA score, heart rate, mean arterial pressure, lactate in the 1st h after initiation of norepinephrine infusion, APACHE II at ICU admission, comorbidities (chronic heart failure, diabetes mellitus). Matching was then performed in a 1:1 fashion with a caliper of 0.1 (11) in order to account for the different characteristics between the patient tailored therapy group and historical controls. Comparisons between propensity matched groups were made by means of paired t-test for normally distributed variables or Wilcoxon test for non-normally distributed variables and McNemar's test for proportions. In order to show the magnitude of differences between the two groups, we reported the correlation coefficient r as effect size for all comparisons: a value of ± 0.1 indicates a small effect, ± 0.3 a medium effect, ± 0.5 a large effect (12). A two-tailed $p < 0.05$ was used to define statistical significance.

Results

The study flow chart is reported in Figure 2. The full dataset of this study is available at https://doi.org/10.17026/dans-zyf-qvax.

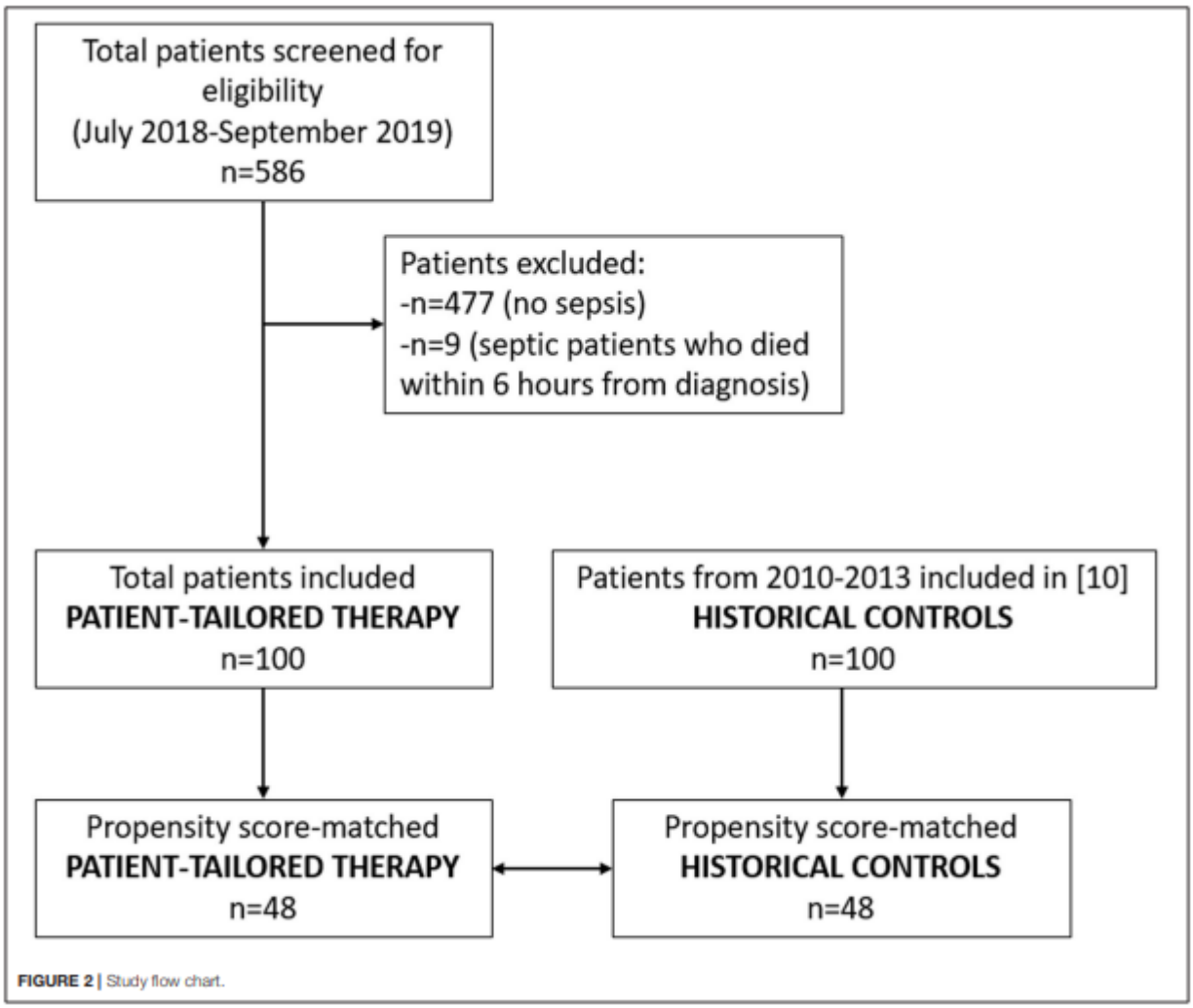

FIGURE 2 | Study flow chart.

General characteristics of the historical controls and patient-tailored therapy cohort are reported in Table 1. Patients in the patient-tailored therapy group had higher SAPS II on ICU-admission but lower SOFA score at the time of initiation of norepinephrine and the number of those with pre-existing immunosuppression tended to be lower in this group as compared to historical controls. Although the main source of infection was respiratory in both groups, among historical controls we observed a higher prevalence of abdominal sepsis while the patient-tailored therapy group showed more cases of genito-urinary sepsis and bactaeremia. The distribution of pathogens was similar between the 2010–2013 and the 2018–2019 cohorts ($p = 0.227$): gram-positive (21 vs. 28%), gram-negative (40 vs. 41%), virus (8 vs. 5%), fungi (6 vs. 4%), atypical (0 vs. 2%), unknown (31 vs. 20%). The cytokine profile in the patient-tailored therapy cohort was: IL-1beta

4 [4-9] pg/ml; IL-6 354 [507–1,980] pg/ml; IL-8 317 [119–2,439] pg/ml; IL-10 35 [11-365] pg/ml; TNF-alpha 34 [14-82] pg/ml.

TABLE 1 | General characteristics of the two cohorts.

	Unmatched entire cohort				Propensity-score matched cohort			
	Historical controls (n = 100)	Patient-tailored (n = 100)	Effect size (r)	p	Historical controls (n = 48)	Patient-tailored (n = 48)	Effect size (r)	p
Age (years)	69 [55–75]	67 [49–76]	−0.046	0.517	72 [58–78]	60 [50–72]	0.229	0.027
Males	59	56	−0.030	0.668	31 (65%)	25 (52%)	−0.127	0.301
Comorbidities (n, %)								
Chronic heart failure	10	8	−0.035	0.806	3 (6%)	3 (6%)	0	0.999
Diabetes mellitus	24	21	−0.036	0.735	12 (25%)	12 (25%)	0	0.999
SAPS II (ICU admission)	57 ± 17	61 ± 16	0.141	0.047	59 ± 17	57 ± 15	0.051	0.731
APACHE II (ICU admission)	23 [19–30]	23 [19–28]	−0.033	0.641	24 ± 6	23 ± 6	0.179	0.228
SOFA score (1st h)	11 ± 3	10 ± 2	0.193	0.007	11 ± 3	11 ± 3	0.080	0.609
MAP (mmHg) 1st h	76 ± 13	75 ± 15	0.026	0.719	75 ± 15	78 ± 16	0.140	0.338
Norepinephrine (mcg/kg/min) 1st h	0.40 [0.16–0.77]	0.30 [0.16–0.50]	−0.116	0.101	0.39 [0.14–0.77]	0.28 [0.13–0.49]	0.118	0.252
Lactate (mmol/L) 1st h	2.4 [1.1–4.2]	2.3 [1.5–3.4]	−0.007	0.919	2.4 [1.1–5.2]	2.3 [1.5–4.7]	0.086	0.406
HR (bpm) 1st h	100 ± 22	98 ± 19	0.056	0.430	99 ± 24	99 ± 20	0.014	0.926
Immunosuppression	18	9	−0.132	0.063	6 (12%)	7 (15%)	0.030	0.999
Source of infection (n, %)			0.199	<0.001			0.039	0.906
Respiratory	51	49			24 (50%)	25 (52%)		
Abdominal	35	11			13 (27%)	11 (23%)		
Genito-urinary	2	12			2 (4%)	3 (6%)		
Bacteraemia	2	9			2 (4%)	1 (2%)		
Skin and soft tissue	7	7			5 (10%)	6 (12%)		
Other	1	2			0 (0%)	1 (2%)		
Unknown	2	10			2 (4%)	1 (2%)		
MDR infection	33	24	−0.100	0.110	16 (33%)	14 (29%)	0.045	0.826
ICU LOS (days)	11 [3-22]	12 [7-22]	−0.070	0.324	11 (4-34)	11 (7-24)	0.046	0.660
ICU Non-survivors (n, %)	57	32	−0.251	<0.001	29 (60%)	19 (40%)	−0.208	0.037
Hospital Non-survivors (n, %)	63	36	−0.270	<0.001	33 (69%)	19 (40%)	−0.293	0.007

Propensity score analysis: patients were matched for source of infection, multi-drug resistant pathogen, pre-existing immunosuppression, pre-existing chronic heart failure, diabetes mellitus, APACHE II on ICU-admission, SOFA, HR, MAP, Lactate at the 1st hour of sepsis diagnosis.
For the effect size r: a value of ± 0.1 indicates a small effect, ± 0.3 a medium effect, ± 0.5 a large effect.
SAPS II, Simplified Acute Physiology Score II; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; MAP, mean arterial pressure; HR, heart rate; MDR, multi-drug resistant; ICU LOS, length of stay in the Intensive Care Unit.

Patients in the patient-tailored therapy group received a lower amount of fluids in the 1st 24 h of norepinephrine infusion and a lower dose of norepinephrine (Table 2). A higher number of patients underwent renal replacement therapy in the 1st 24 h as compared to historical controls. The use of hemodynamic monitoring in the 1st 24 h of norepinephrine administration was greatly increased in our ICU in 2018–2019 in comparison to 2010–2013. A substantial number of patients in the patient-tailored therapy group received albumin, pentaglobin and blood purification therapies, while these treatments were not used in the period 2010–2013 (Table 2). The use of steroids as adjunctive therapy for sepsis was similar in the two time periods.

TABLE 2 | Comparison of treatments between the two study cohorts.

	Unmatched entire cohort				Propensity-score matched cohort			
	Historical controls ($n = 100$)	**Patient-tailored ($n = 100$)**	**Effect size (r)**	**p**	**Historical controls ($n = 48$)**	**Patient-tailored ($n = 48$)**	**Effect size (r)**	**p**
RRT (1st 24 h)	10	25	0.197	0.005	6 (12%)	15 (31%)	0.227	0.047
Mechanical Ventilation (1st 24 h)	99	98	−0.041	0.561	48 (100%)	48 (100%)	0	0.999
Fluid balance 1st 24 h*	513 [−96, 2,351] ($n = 88$)	347 [−854, 1,720] ($n = 93$)	−0.107	0.150	430 [−90, 2,500] ($n = 41$)	182 [−634, 1,725] ($n = 41$)	0.098	0.395
Total fluid 1st 24 h (ml/kg)*	59 [47–79] ($n = 88$)	50 [38–71] ($n = 93$)	−0.164	0.027	54 [45–72] ($n = 41$)	47 [35–69] ($n = 41$)	0.128	0.264
Norepinephrine MAX 1st 24 h (mcg/kg/min)	0.61 [0.27–1.02]	0.40 [0.21–0.71]	−0.211	0.003	0.66 [0.19–1.04]	0.42 [0.20–0.67]	0.248	0.014
Other vasoactive/inotropic agents (1st 24 h)								
Dobutamine	20	24	0.048	0.495	9 (19%)	9 (19%)	0	0.999
Dopamine	9	3	−0.126	0.074	2 (4%)	1 (2%)	−0.060	0.999
Levosimendan	4	10	0.118	0.096	2 (4%)	3 (6%)	0.047	0.999
Terlipressin	0	9	0.217	0.002	0 (0%)	3 (6%)	0.160	0.242
Pentaglobin, n	0	18	0.314	<0.001	0 (0%)	11 (23%)	0.360	0.001
Cytosorb, n	0	15	0.285	<0.001	0 (0%)	8 (17%)	0.301	0.006
Toraymyxin, n	0	5	0.160	0.059	0 (0%)	3 (6%)	0.160	0.242
Albumin, n	0	71	0.742	<0.001	0 (0%)	35 (73%)	0.757	<0.001
Steroids, n	14	9	−0.078	0.376	7 (15%)	4 (8%)	−0.098	0.523
Hemodynamic monitoring** (1st 24 h), n	10	63	0.550	<0.001	5 (10%)	31 (65%)	0.559	<0.001

Patients were matched for source of infection, multi-drug resistant pathogen, pre-existing immunosuppression, SAPS II on ICU-admission, SOFA, HR, MAP, Lactate at the 1st hour of sepsis diagnosis.
In order to show the magnitude of differences between the two groups, we reported the effect size r: a value of ± 0.1 indicates a small effect, ± 0.3 a medium effect, ± 0.5 a large effect.
RRT, renal replacement therapy.
**Fluid balance and total fluid intake in the 1st 24 h are calculated only for patients surviving for more than 24 h (number of patients is shown in parenthesis).*
***This includes invasive or mini-invasive measurement of cardiac output by means of either trans-pulmonary thermodilution or pulse contour analysis.*

ICU-mortality was lower in the patient-tailored therapy cohort as compared to historical controls (32 vs. 57%, $p < 0.001$). The main causes of death were multi-organ failure (79% in historical controls, 75% in the patient-tailored therapy cohort), respiratory failure (14 and 12%, respectively), and intractable hypotension (7 and 6%, respectively). Patient-tailored therapy was associated with a lower risk of ICU-mortality even after adjusting for source of infection, presence of multi-drug resistant pathogen, pre-existing immunosuppression, SOFA, heart rate, mean arterial pressure, lactate levels at the 1st h of norepinephrine infusion, APACHE II score on ICU-admission, pre-existing chronic heart failure and diabetes mellitus (Table 3).

TABLE 3 | Multivariate binary logistic regression for ICU-mortality.

Variable	Odds ratio [95% CI]	*p*
Patient-tailored therapy	0.331 [0.166–0.658]	0.002
SOFA (1st h)	1.238 [1.077–1.423]	0.003
Immunosuppression	3.297 [1.106–9.825]	0.032
MAP (1st h)	0.968 [0.943–0.993]	0.014
Lactate levels (1st h)	1.239 [1.095–1.401]	0.001

Variables included in the model were source of infection, multidrug-resistant pathogens infection, immunosuppression, SOFA score (1st h of norepinephrine infusion), heart rate, mean arterial pressure, lactate levels, APACHE II on admission, pre-existing chronic heart failure and diabetes mellitus. Forward conditional method.
MAP, mean arterial pressure; APACHE II, Acute Physiology and Chronic Health Evaluation Score II.

After propensity score matching, we selected 48 patients in historical control group and 48 patients in the patient-tailored therapy cohort with similar general characteristics (Table 1). ICU-mortality was lower in the patient-tailored therapy matched subgroup as compared to the historical control subgroup (40 vs. 60%, p = 0.037). A comparison of treatments in the two propensity matched groups is shown in Table 2.

Patients With Septic Shock

A total of 56 patients among historical controls and 58 patients in the patient-tailored therapy cohort had high lactate levels, meeting the criteria for septic shock according to the Sepsis-3 definitions. Among these patients, ICU-mortality was 43.1% in the patient-tailored therapy cohort and 73.2% in the standard therapy cohort (p = 0.001). Patient-tailored therapy was associated with lower ICU-mortality independent of the source of infection, presence of multi-drug resistant pathogen, pre-existing immunosuppression, SOFA at time of initiation of norepinephrine, heart rate, mean arterial pressure, lactate levels, pre-existing chronic heart failure, diabetes mellitus and APACHE on ICU-admission (adjusted odds ratio 0.269 [95% CI 0.112–0.644], p = 0.003, Supplementary File 2). After propensity score matching (for source of infection, multi-drug resistant pathogen, pre-existing immunosuppression, APACHE on ICU-admission, chronic heart failure, diabetes mellitus, SOFA, HR, MAP, lactate levels and initial dose of norepinephrine),

ICU-mortality was 47% in the patient-tailored therapy group (n = 30) and 77% in the historical control group (n = 30) (p = 0.039).

Adherence to the Patient-Tailored Therapy Protocol

In the patient-tailored therapy cohort, five patients did not receive Pentaglobin infusion despite IgM plasma levels <60 mg/dl. Hemoadsorption was not applied in 5 patients who met the criteria for blood purification therapy. In 17 cases, albumin was not administered although plasma levels were <2.5 g/dl. Five patients requiring a norepinephrine dosage > 1 mcg/kg/min did not receive terlipressin infusion.

Discussion

In this single-center retrospective observational study, patients with norepinephrine-dependent sepsis admitted to our ICU after the institution of a patient-tailored protocol showed significantly lower mortality in comparison to a group of patients admitted between 2010 and 2013. The association between patient-tailored therapy and reduced mortality remained after adjusting for the main clinical severity parameters or restricting the analysis to patients who met the Sepsis-3 definitions for septic shock (1). Nonetheless, we must recognize that such association does not imply a cause-effect relationship. The outcome of septic patients may have changed over the last years for many other factors unrelated to this personalized approach, which need to be discussed. The therapeutic approach to septic patients has changed a lot in our ICU in the last years. The approach to hemodynamic stabilization, as regards both fluid therapy and vasoactive agents, has changed. The reduction in norepinephrine dosage in the 1st 24 h and the increase in the use of terlipressin, together with the lower fluid intake in the 1st 24 h, suggest that we now tend to avoid an excessive fluid administration and limit the use of excessive doses of norepinephrine, in favor of an earlier association with other vasoactive agents. Moreover, the widespread use of

hemodynamic monitoring in 2018–2019 as compared to 2010–2013 likely reflects a different approach to the hemodynamic stabilization of septic patients, with the implementation of a fluid resuscitation guided by a more thorough hemodynamic assessment, which may have contributed to limit the amount of fluids administered and the vasopressor dosage. In addition, a higher number of patients currently receive RRT in the 1st 24 h. A restrictive fluid administration strategy, allowed by an earlier initiation of RRT and more rational use of vasoactive and inotropic agents, could have a beneficial effect on the outcome since many recent studies demonstrated that fluid overload can lead to several complications such as pulmonary oedema, congestive heart failure, delayed wound healing and impaired bowel function (13). At present, all septic patients in our ICU with low serum albumin levels receive human serum albumin, while this was not used in 2010–2013. This practice was implemented in our unit after the publication of the results of the ALBIOS trial (14) that showed significant hemodynamic advantages with albumin administration and a possible reduction in mortality in the subgroup of patients with septic shock. The use of albumin in our patient-tailored therapy cohort may have contributed to the reduction in norepinephrine dosage. Different therapies, such as immunomodulation and extracorporeal removal strategies, has been introduced. Despite absence of clear recommendations for the use of immunoglobulin preparations and blood purification techniques in Surviving Sepsis Campaign (8), a number of clinical studies has shown a beneficial effect of IgM-enriched immunoglobulin solutions and blood purification with Cytosorb adsorber during sepsis. In a meta-analysis of RCTs, we previously showed that the infusion of immunoglobulins may reduce mortality in septic patients, although the overall quality of the available evidence remains low (15). In a recent randomized controlled study by our group, IgM-enriched immunoglobulins were able to improve sublingual microvascular perfusion during sepsis (16). In patients with septic shock requiring RRT, we showed that extracorporeal blood purification with Cytosorb adsorber was associated with an improvement in sublingual microcirculation (17). It is known that during sepsis a macro-hemodynamic improvement may not be accompanied by a parallel restoration of microvascular

perfusion (18). Massive cytokine release in sepsis leads to leukocyte activation, oxidative stress, endothelial glycocalyx dysfunction and impaired nitric oxide pathway, resulting in impaired hemorheology, loss of microvascular tone, microcirculatory shunting, tissue oedema and oxygen extraction deficit (17). The implementation of cytokine removal strategies, by acting on the main pathophysiological mechanism of microcirculatory dysfunction, may have a beneficial impact on tissue perfusion and organ function. It is reasonable to think that these treatments could show the best impact on outcome if administered to selected patients (e.g., those with lower plasma IgM levels or more severe inflammatory response) following an individualized, patient-tailored approach. Of note, we used the Cytosorb cartridge in combination with RRT as a dedicated extracorporeal circuit is not available in our ICU. However, RRT is generally not a pre-requisite for hemoadsorption/hemoperfusion therapies: their use as stand-alone therapies with dedicated extracorporeal circuits could allow an earlier implementation of blood purification, with a potentially better impact on outcome. Our data are in line with the current evidence. Due to the extreme heterogeneity of septic patients (2), most trials failed to show a mortality benefit from treatments including immunoglobulins or blood purification techniques (19–22). For this reason, an individualized approach is most frequently required, that will take into account the basal patient characteristics, underlying infection, organ dysfunction severity and the individual immune response (5). By performing a Latent class analysis of the PROWESS Shock study, Gardlung et al. highlighted that septic shock is a complex entity characterized by different phenotypes, and each of these phenotypes would benefit from targeted therapies based on patient characteristics (23). Consistently, Zhang et al. identified four subclasses of septic patients that showed different responses to fluid resuscitation with different outcomes (24). Of note, in the present study, the adjunctive treatments, if individually analyzed, do not show this positive correlation with survival. This is in line with the concept of “bundles of care” that was endorsed by the Surviving Sepsis Campaign based on the assumption that the association of different treatments will be able to provide the best impact on outcome (25).

Our study has several limitations. First of all, due to its retrospective design and the use of an historical control group, this study does not allow to discriminate the potential benefit of patient-tailored therapy from the impact of other practice changes that occurred from 2010 to 2019. We cannot exclude that the observed difference in mortality was determined by factors not exclusively related to the individualized therapeutic approach or was influenced by therapeutic elements that we may have missed to consider. Among these, the type of fluids infused: it is likely that a lower amount of synthetic colloids was used in the 2018–2019 cohort, according to the most recent recommendations of the SSC guidelines (8) after the publication of RCTs showing a higher risk of mortality and acute kidney injury with hydroxyethyl starches (26). Unfortunately, we do not have access to data on the specific type of fluids (crystalloids or colloids) for the 2010–2013 cohort. Similarly, data on antimicrobials were not available. Even if the distribution of pathogens was similar between the two cohorts, changes in antimicrobial strategies over years could have played a relevant role in determining patient outcome. This study cannot demonstrate any cause-effect relationship between the individualized approach and patients' outcome, but can only suggest associations. Even if we made all efforts to adjust for the main clinical severity indices, unmeasured confounders may still bias our results. We used a group of historical controls, consisting of patients admitted to our ICU in the period between 2010 and 2013, for which some information of therapeutic elements were no longer available. Differences in the base populations from which the patients were selected may limit the comparability between the two cohorts. While at present (as well as in the period 2018–2019) our unit is an exclusively medical and trauma ICU, in the period 2010–2013 also post-operative patients were admitted. This may at least partly explain the higher prevalence of abdominal sepsis cases in the standard therapy group. Moreover, the SOFA score was slightly lower in the 2018–2019 cohort. A general improvement in the care of septic patients including an earlier recognition of this condition, also related to the diffusion of the use of quick SOFA (1) outside the ICU, may justify such difference and could have contributed to mortality reduction. These potential confounding factors were included in

multivariate logistic regression and propensity score analyses. The prevalence of comorbidities in the two cohorts could not be analyzed in details, as only incomplete information of the patients' medical history was available for the 2010–2013 dataset. Unfortunately, we could not collect data from a more recent control group of patients, as our electronic medical reports are fully available and easy accessible only for 3 years before being archived. Finally, the sample size was arbitrarily chosen based on the size of the old sample available. Even if the study is retrospective and observational, the lack of protocol registration to any public register does not allow verification of design and statistical analysis.

Conclusion

In a group of 100 patients with norepinephrine-dependent sepsis admitted to our ICU after the institution of a protocol of patient-tailored therapy, we found a significantly lower mortality in comparison to a group of historical controls. This study cannot demonstrate a clear effect of the patient tailored therapy on the outcome. Nevertheless, our results encourage the design of future studies specifically aimed to test the impact of an individualized therapeutic approach on the outcome of septic patients.

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Chapter 5

Microvascular alterations in patients with SARS-COV-2 severe pneumonia

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Multiple evidences suggest that pulmonary microcirculatory dysfunction may play a key role in the pathogenesis of SARS-COV-2 severe pneumonia. SARS-COV-2 uses the angiotensin converting enzyme 2 (ACE2) as its receptor [1]. ACE2 normally functions as a negative regulator of the renin–angiotensin system (RAS) [1]. RAS dysregulation leads to increased vascular permeability, inflammation and pneumocyte apoptosis [1]. Pulmonary microvascular leakage may result in lung oedema and impaired lung function. Severe Coronavirus disease 2019 (COVID-19) is frequently complicated by coagulopathy and markedly elevated D-dimer is associated with poor prognosis [2]. The formation of micro-thrombi in the lung vessels likely contributes to ventilation/perfusion mismatch and impairs gas exchange. In this study, we reviewed data from mechanically ventilated patients with SARS-COV-2 severe pneumonia admitted to an intensive care unit (ICU) of Ancona (Italy) in March 2020, who underwent an evaluation of the sublingual microcirculation by means of incident dark field videomicroscopy (Cytocam, Braedius Medical, Amsterdam, NL). The protocol of this retrospective observational study was approved by the local Ethics Committee (Comitato Etico Regionale delle Marche).

The Cytocam is a third generation handheld video-microscope that enables the non-invasive, real-time, in vivo visualization of the microcirculation [3]. This technique is routinely applied in our ICU to monitor microvascular perfusion. Three videos from different sublingual areas were recorded with adequate contrast and focus and without pressure artefacts. The videos were analysed offline with dedicated software (Automated Vascular Analysis 3.2, Microvision Medical, Amsterdam, NL) to obtain parameters of vessel density (total vessel density [TVD], perfused vessel density [PVD]) and blood flow quality (microvascular flow index [MFI], percentage of perfused vessels [PPV] and flow heterogeneity index [HI]), as described elsewhere [3].

Data are presented as mean (± standard deviation) or median [1st–3rd quartile], based on the distribution of the variable of interest. The Spearman's rho was calculated to evaluate correlations between variables with a significance level of 0.05 (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Among 29 patients with SARS-COV-2 severe pneumonia who were admitted to our ICU during the study period, 12 patients underwent microcirculatory evaluation. Patients' characteristics are reported in Table 1. Microvascular parameters for vessels smaller than 20 μm were: TVD 15.3 [14.5–17.1] mm/mm2; PVD 14.9 [14.1–16.9] mm/mm2; PPV 97.3 [95.1–98.8] %; MFI 2.9 [2.6–3]; HI 0.3 [0–0.4]. D-Dimer levels were inversely correlated with PVD (Spearman rho = − 0.70, p = 0.016) and TVD (rho = − 0.645, p = 0.032) (Fig. 1). D-Dimer levels were also inversely correlated with PaO2/FiO2 (rho = − 0.609, p = 0.047). PVD tended to decrease with increasing driving pressure values (rho = − 0.691, p = 0.086).

Table 1 Patients' characteristics

Male (*n*, %)	10 (83.3%)
Age (years)	56 (10)
BMI (kg/m^2)	31.6 (5.4)
Comorbidities (*n*, %)	
Dyslipidemia	4 (33.3%)
Hypertension	3 (25.0%)
Diabetes type 2	2 (16.7%)
Ischemic cardiomyopathy	2 (16.7%)
Tidal volume (ml)	421 (190)
RR (breath/min)	13 (3)
Pplat (cmH_2O)	27 (5)
PEEP (cmH_2O)	10 (8.6; 13.8)
ΔP (cmH_2O)	13 (4)
Cstat (ml/cmH_2O)	52 (37)
FiO_2	0.40 (0.35; 0.48)
PaO_2/FiO_2 (mmHg)	207 (88)
VV-ECMO (*n*, %)	6 (50.0%)
CRRT (*n*, %)	2 (16.7%)
MAP (mmHg)	88 (13)
HR (beat/min)	86 (23)
Lactate (mmol/l)	1.16 (0.41)
WBC ($\times 10^9$/l)	14.12 (5.13)
IL-6 (pg/ml)	138 (18.5; 338)
D-Dimer (ng/ml)	788 (717; 5536)
Noradrenaline	
n (%)	9 (75%)
mcg/kg/min	0.24 (0.14)
Propofol	
n (%)	9 (75%)
mg/kg/h	2.5 (0.46)
Midazolam	
n (%)	9 (75%)
mg/kg/h	0.26 (0.12)
Remifentanil	
n (%)	12 (100%)
mcg/kg/min	0.1 (0.85; 0.1)

Data reported as *n*. (%); mean (standard deviation); median (interquartile range)

BMI body mass index, *CRRT* continuous renal replacement therapy, *Cstat* static compliance of respiratory system, FIO_2 inspiratory fraction of oxygen, *HR* heart rate, *IL-6* interleukin 6, *MAP* mean arterial pressure, PaO_2 arterial partial pressure of oxygen, ΔP driving pressure, *PEEP* positive end expiratory pressure, *Pplat* plateau pressure, *RR* respiratory rate, *VV-ECMO* veno-venous extracorporeal membrane oxygenation, *WBC* white blood cells

Sublingual microcirculation of two patients with SARS-COV2 severe pneumonia. Patient (a) showed a significantly lower perfused vessel density as compared to patient (b). D-dimer levels were 6367 ng/ml in patient (a) and 741 ng/ml in patient (b). PaO2/FiO2 ratio was 74 mmHg in patient (a) and 247 mmHg in patient (b)

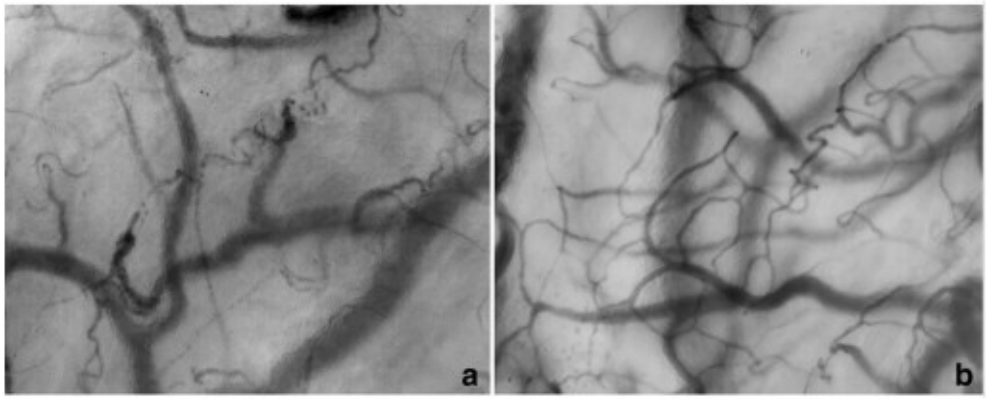

Fig. 1 Sublingual microcirculation of two patients with SARS-COV2 severe pneumonia. Patient (**a**) showed a significantly lower perfused vessel density as compared to patient (**b**). D-dimer levels were 6367 ng/ml in patient (**a**) and 741 ng/ml in patient (**b**). PaO_2/FiO_2 ratio was 74 mmHg in patient (**a**) and 247 mmHg in patient (**b**)

This is the first study that evaluated microcirculatory blood flow in COVID-19 patients. Microvascular alterations are associated with mortality in critically ill patients [3]. In a general population of 97 critically ill patients, we previously reported a PVD of 19.3 ± 4.4 mm/mm2 [3], which seems significantly higher in comparison with the value observed in this sample of COVID-19 patients. Varga et al. recently reported signs of endotheliitis in several organs in patients with SARS-COV-2 infection, suggesting systemic microvascular dysfunction that may account for tissue hypoperfusion, inflammation and a procoagulant state [4]. Sublingual microcirculatory blood flow was significantly compromised in patients with severe influenza A (H1N1) infection [5]. In acute respiratory distress syndrome, increased heterogeneity of sublingual microvascular perfusion was related to an increase in dead-space ventilation, suggesting a role of microcirculatory dysfunction in ventilation/perfusion mismatching [6]. Our report supports a link between coagulopathy and microvascular perfusion disturbances in patients with SARS-COV-2 severe pneumonia. Further studies are needed to demonstrate a cause–effect

relationship, clarify the role of microcirculatory disturbances on lung function and indicate potential implications for therapy.

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Chapter 6

Microvascular dysfunction in pediatric patients with SARS-COV-2 pneumonia: report of three severe cases

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Abstract

The coronavirus 19 (COVID-19) pandemic has affected hundreds of millions of people worldwide: in most of cases children and young people developed asymptomatic or pauci-symptomatic clinical pictures. However authors have showed that there are some categories of childhood more vulnerable to COVID-19 infection such as newborns or children with comorbidities. We report for the first time to the best of our knowledge about microvascular dysfunction in three pediatric clinical cases who developed COVID-19 infections with need of pediatric critical care. We found that sublingual microcirculation is altered in children with severe COVID-19 infection. Our findings confirmed most of data already observed by other authors in adult population affected by severe COVID-19 infection, but with distinct characteristics than microcirculation alterations previous observed in a clinical case of MIS-C. However we cannot establish direct correlation between microcirculation analysis and clinical or laboratory parameters in our series, by our experience we have found that sublingual microcirculation analysis allow clinicians to report directly about microcirculation dysfunction in COVID-19 patients and it could be a valuable bedside technique to monitor thrombosis complication in this population.

Keywords: COVID-19; Children; Coagulation; Newborns; Pediatric critical care; Sublingual microcirculation analysis.

1. Introduction

The coronavirus 19 (COVID-19) pandemic has affected hundreds of millions of people worldwide so far and caused over 3 million deaths (Ludvigsson, 2020). The existing evidence suggests that children generally have a milder disease course and better prognosis than adults, and deaths are extremely rare among the pediatric population (Ludvigsson, 2020). Even if elevated inflammatory markers are less common in children with mild COVID-19 disease, those pediatric patients who develop severe COVID-19 show not only a great increase in pro-inflammatory cytokines but also markers of endothelial dysfunction (Diorio et al., 2020a). COVID-19-related endothelitiis has been recently described in several organs by some authors (Varga et al., 2020), suggesting an impaired microvascular function in different vascular beds as a consequence of viral involvement and host inflammatory response (Varga et al., 2020). Furthermore sublingual microcirculation alterations have been described in a young girl affected by multisystem inflammatory syndrome COVID-19 correlated (MIS-C): the sublingual microcirculation analysis (SMA) evidenced low values of Mean Flow Index (MFI), high heterogeneity index (HI) (Bottari et al., 2021). SMA has allowed the researchers to report directly about microcirculation dysfunction in COVID-19 patients (Favaron et al., 2021; Kanoore Edul et al., 2021; Damiani et al., 2020; Carsetti et al., 2020) showing some correlations between microvascular variables and clinical or laboratory parameters in this population.

We present the first clinical report about sublingual microcirculation analysis in three children with severe COVID-19 infection who required admission to the pediatric Intensive Care Unit (PICU). Five videos per patients were recorded within a week from PICU admission with a single time-point assessment using handheld vital microscope based on incident dark field microscopy imaging (Braedius Medical, Huizen, The Netherlands). Three videos of the best quality were selected (Massey et al., 2013). Videos were analysed offline with dedicated software (Analysis Manager V2) (Braedius Medical, Huizen, The Netherlands) (Carsetti et al., 2017) by two

independent operators (GB and VC) (Ince et al., 2018; De Backer et al., 2007). The following parameters were calculated: De Backer score (De Backer et al., 2007), total small vessel density (TVD); proportion of perfused vessels (PPV); perfused microvascular density (PVD). Semi-quantitative analysis of the microcirculatory flow was performed as previously described by Boerma et al. (2005). Each image was divided into four equal quadrants and for each one a quantification of flow was scored (no flow: 0; intermittent flow: 1; sluggish flow: 2; continuous flow: 3). MFI was determined by eye on the basis of the predominant type of flow in each quadrant and averaged over the values obtained in each one. We also calculated the heterogeneity index (HI), following the method of Trzeciak et al. (Trzeciak et al., 2007 based on MFI and PPV values.

Patient number 1 (Pt1), a 14 year-old boy, with a relapsed bone lymphoblastic B lymphoma. He showed already in the previous weeks a blood cytopenia because of chemiotherapy treatments. He was admitted to the PICU due to a COVID-19 infection inducing hypoxic respiratory failure with a clinical picture of acute respiratory distress syndrome (ARDS) and need for oro-tracheal intubation and invasive mechanical ventilation. SARS-CoV2 was confirmed before and after PICU-admission by means of real-time polymerase chain reaction (RT-PCR) on nasopharyngeal swab and bronco-alveolar lavage. At day 1 blood chemistry evidenced White Blood Cells (WBC) 0.22 103/uL, C-Reactive Protein (CRP) 10.52 mg/dL, Ferritin 4879 ng/mL, Procalcitonin (PCT) 1.05 ng/mL, D-dimers 2.65 μg/mL. He was treated with a protective ventilation strategy, prono-supine position, dexamethasone (0.15 mg/kg/ day) and low molecular weight heparin (100 U/kg bpd). In Table 1 we show the microvascular parameters, as well as ventilatory and hemodynamic variables and the main biomarkers of inflammation measured at the same time of sublingual microcirculation analysis (day 7). As showed in Table 1 he developed a severe ARDS (PEEP > 5 PaO2/FiO2 < 100, Oxygen Index 23.9) with bilateral opacities at the chest X Ray. Fig. 1 reports sublingual microcirculation and chest X-ray performed at the same time point. Microbiological

work-up did not evidence other positive microbiological agents responsible for concomitant infections except for polymerase chain reaction test (blood sample) positive for Candida krusei (day 3), which was not confirmed by standard blood culture and become negative by day 7. During the PICU-stay the patient was generally hemodynamically stable and required only low dose of Noradrenaline (0.03–0.06 μg/kg/min). The patient developed a massive bilateral pneumothorax at day 7 with progressive refractory hypoxemia in the following days and despite the medical and pharmacological therapies he did not improve and died at day 14.

Table 1
Patients' characteristics, respiratory, hemodynamic, laboratory and microcirculatory parameters. MAP = Mean arterial pressure; WBC=White Blood Cells; RBC = Red Blood Cells; CRP=C-Reactive Protein; PT = prothrombin time ratio, LDH = lactose dehydrogenase; TVD = total small vessel density; PVD = Perfused Vessels Density; PPV = Proportion of Perfused Vessels; HI = heterogeneity index.

Patient's characteristics	Case 1	Case 2	Case 3
Age	14 ys	1 years 6 month	15 days
Weight	47	9,5	3,4
Comorbidity	Lymphoma	Cystic fibrosis	
Ventilation setting			
PIP	32	20	18
Peep	15	7	6
PaO2/FiO2	96	220	250
FiO2 (%)	80	40	35
ECMO VA setting			
LPM (l/min)		0.58	
RPM		3250	
FiO2 (%)		75%	
Hemodynamic variables			
MAP (mmHg)	70	85	65
Noradrenaline (μg/kg/min)	0.03	0.04	–
Lactate (mmol/L)	1.5	0.6	1.3
Laboratory parameters			
WBC (10^3/μL)	0.26	25.24	5.56
RBC (10^6/μL)	3.54	3.61	4.08
Platelets (10^3/μL)	26.000	139.000	183.000
Hemoglobin (gr/dL)			
Ferritin (ng/mL)	4879	501	531
CRP (mg/dL)	10.5	1.55	0.36
D-dimer (μg/mL)	2.65	1.46	2.45
PT-ratio (%)	1.34	1.89	1.09
LDH (U/L)	239	339	285
Microcirculation parameters			
TVD (mm/mm^2)	18.5	16.36	12.7
PVD (mm/mm^2)	9.31	14.9	12.4
PPV (%)	79.3	89.7	98.7
MFI (AU)	1.3	2.6	2.6
HI	2.25	0.28	0.18
Outcome			
Survival PICU discharge	No	Yes	Yes
Survival at 28 days	No	Yes	Yes

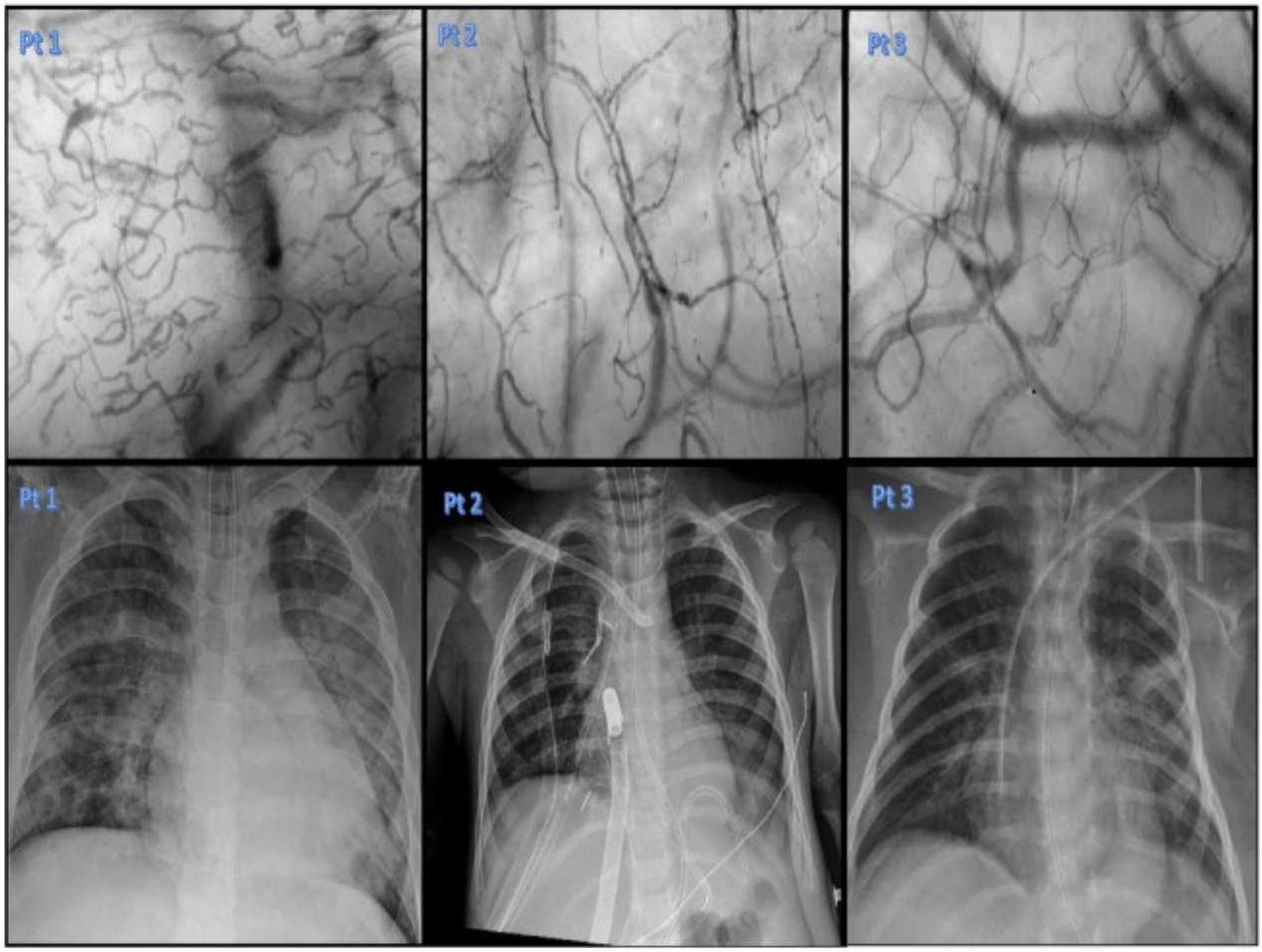
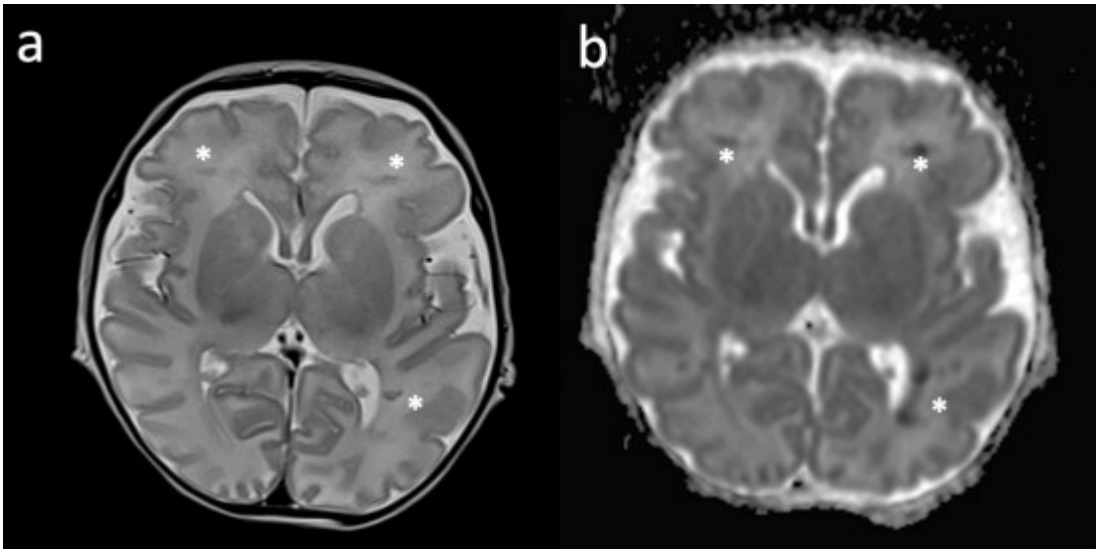


Fig. 1. Upper section: Chest x-rays performed during the COVID-19 respiratory infection in our series. **Lower sections:** sublingual microcirculation pictures of our series. The video captions of sublingual microcirculation analysis has been performed the same day of the chest x-rays reported in the upper section.

Patient 2 (pt 2): a 20-month old boy with cystic fibrosis was admitted to the PICU in November 2020 for respiratory distress. At admission, RT-PCR on nasopharyngeal swabs showed positivity to Sars-COV-2 and Rhinovirus. After 24 h the patient developed respiratory failure and refractory hypercapnia (Oxygen Index 28; PaO2/FiO2 68; pCO2 > 70 mmHg) requiring Venous-Arterial (VA) Extra Corporeal Membrane Oxygenation (ECMO). At day 1 blood chemistry evidenced WBC 8.46 103/uL CRP 0.07 mg/dL, Ferritin 97 ng/mL, PCT 0.07 μg/mL, D-dimers 0.59 μg/mL. Thorax chest-X ray evidenced a basal pneumonia associated to a pleural effusion and diffuse thickening of the peri-broncovascular interstitium. He received dexamethasone (0.15 mg/kg/ day) and low molecular weight heparin 100 U/kg/bpd. Table 1 shows microvascular parameters together with ventilatory and hemodynamic variables and inflammatory biomarkers (measured at day 7). Fig. 1 reports the sublingual microcirculation and chest X-ray performed at the same time

point. Microbiological workup did not evidence other positive microbiological agents responsible for infection. During the PICU-stay the patient was hemodynamically stable and received only low dose of Noradrenaline 0.03–0.06 μg/kg/min. He was weaned from VA-ECMO at day 10 and was discharged from the PICU at day 16. He survived to hospital discharge.

Patient 3 (pt3): a 15 day-old newborn was admitted to the PICU for gastro-intestinal bleeding. He had been admitted to the hospital for fever in the previous week and resulted positive to Sars-Cov2. Need for surgery had been excluded, but 48 h after he showed a progressive respiratory failure with progressive hypercapnia not responsive to non-invasive mechanical ventilation, therefore he required oro-tracheal intubation ($PaCO_2$ > 65 mmHg). At day 1 blood chemistry evidenced WBC 7.84 103/uL, CRP 0.74 mg/dL, D-dimers 1.77 μg/mL. Thorax chest-X ray diffuse thickening of the peri-broncovascular interstitium. He received hyper-immune plasma 15 mL/kg for three days, dexamethasone 0.15 mg/kg/ day, remdesivir 2.5–5 mg /kg/die and low molecular weight heparin 100 U/kg bpd. Table 1 shows microvascular, ventilatory and hemodynamic parameters and the most important biomarkers of inflammation (measured at day 6). Fig. 1 reports the sublingual microcirculation and chest X-ray performed at the same time point. A first attempt of weaning from mechanical ventilation was made on day 7, but it failed for progressive respiratory distress and the baby required re-intubation. We performed MRI for an hypo-reactive baby during the mechanical ventilation weaning trial: MRI evidenced thrombosis of deep medullary veins with ischemic lesions in the related distribution areas. In Fig. 2 we report the MRI images reporting thrombosis of deep medullary veins in association to a second report of the sublingual microcirculation where we have observed obstructed or sluggish flow. On day 18 he was successfully extubated and he was discharged from the PICU on day 22. He survived to hospital discharge.



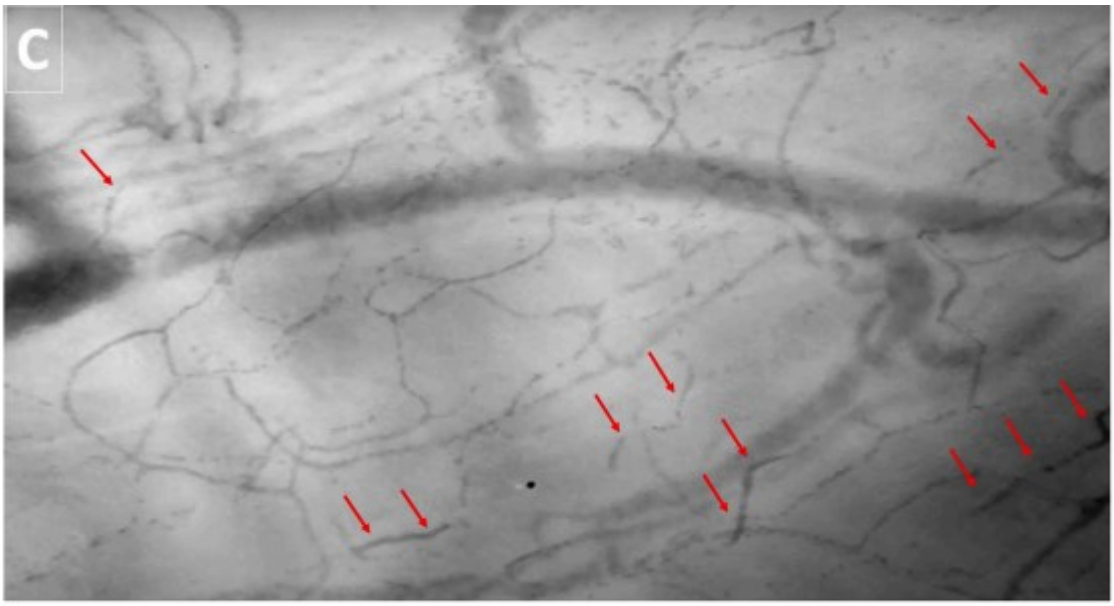


Fig. 2. Upper section: MRI shows focal linear hypointensity on T2 weighted transverse image (a), mainly located into the deep white matter on both hemispheres (asterisk). Lesions appears hypointense on ADC map of DWI (b) and correlate with focal deep venous thrombosis with restricted diffusion. Typically cortex is spared. **Lower section:** sublingual microcirculation picture: red arrows show obstructed blood flow in small vessels. Sublingual microcirculation analysis and MRI has been performed the same day. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Discussion

Recently other authors (Favaron et al., 2021; Kanoore Edul et al., 2021; Damiani et al., 2020; Carsetti et al., 2020) described microvascular alterations in adult patients affected by COVID-19. Despite the evidence that children with COVID-19 are often asymptomatic or pauci-symptomatic,

endothelial damage associated with hyper-cytokinemia due to COVID-19 was recently described also in the pediatric population (Varga et al., 2020; Diorio et al., 2020b). We report for the first time on sublingual microvascular analysis on three children with severe COVID-19 disease. Microvascular alterations consisted in an increased number of small vessels with stopped, intermittent or sluggish flow. However normal values in pediatric populations are still not validated, we have observed TVD values very similar to what reported by other authors (Favaron et al., 2021): increase in TVD in these patients may be correlated with the hypoxia or with a neoangiogenesis that was described in COVID-19 patients (Kanoore Edul et al., 2021). PVD, PPV and MFI values were clearly disturbed than normal in all patients: these findings are consistent with data reported in adult patients with severe COVID-19 infection (Kanoore Edul et al., 2021; Damiani et al., 2020; Carsetti et al., 2020). Some common findings on microcirculation has been observed between our series and the recent case report on microcirculation alteration in MIS-C (Bottari et al., 2021) in particular regarding the alterations in MFI and in HI, on the other hand the most important differences observed are related to the TDV values that were clearly disturbed in the report of the patient affected by MIS-C.

The most severe alteration in blood flow quality, with increased flow heterogeneity (Trzeciak et al., 2007), was observed in patient 1: this could suggest a correlation between microvascular alterations and outcome, similarly to what happens during sepsis (Trzeciak et al., 2007), which merits further investigations in future studies. Furthermore MFI < 2.5 correlated with an adverse outcome. This patient had a pre-existing relevant comorbidity of a methastatic lymphoma with a chemotherapy-induced cytopenia. The observed microvascular alterations were not related to sepsis since the patient did never show signs of septic shock during the clinical course. A previous report (Karvunidis et al., 2012) reported no statistical difference in microvascular parameters between cytopenic patients and healthy control. Therefore we can assume that COVID-19 infection played a potential role in determining microcirculatory dysfunction in this case. The

MFI < 2.5 correlated with an adverse outcome. Microcirculatory alterations in COVID-19 patients may be associated with clotting abnormalities and formation of microthrombi. In this case however, despite severe alterations in microcirculatory perfusion, D-dimer levels were never severely increased throughout the PICU stay. Patient number 2 had an MFI of 2.6 suggesting some degree of impairment in microvascular flow even in absence of significant hemodynamic instability. Previous reports did not show a correlation between microvascular alterations in patients undergoing ECMO and the extracorporeal treatment per se (Carsetti et al., 2020; Erdem et al., 2019). Furthermore this type of alteration did not correlate with other biomarkers of inflammation. Previous evidences suggest that COVID-19 in newborn induces more severe symptoms in comparison to older children (De Rose et al., 2020). In patient 3 we observed respiratory failure with need of mechanical ventilation associated with thrombotic complications. It is relevant to underline that in this patient we found images of obstructed blood flow in small and we have observed also thrombotic complications involving central nervous system (Fig. 2). We can suppose that there is a relationship between these findings and COVID-19 infection and this is also a plausible hypothesis if we consider previously reported data in adults of an inverse relationship between PVD and D-dimers levels (Damiani et al., 2020). Due to the small number of patients studied, we cannot establish correlations among microvascular parameters and laboratory values or mechanical ventilation variables. Our patients had severe and moderate ARDS, an increase of D-dimers and a mild increase in ferritin levels. Even if they were hemodynamically stable and two of them received only low doses of vasopressors, they showed sublingual microvascular alterations. Other markers of endothelial damage such as Il-8 ore C3a and C5a (Diorio et al., 2020a; Diorio et al., 2020b) could better correlate with our sublingual microcirculation analysis, but unfortunately these were not measured in our patients.

The potential harmful role of viral agents against vascular endothelium has been already reported in scientific literature: Senchenkov and others have showed that persistent CMV infection induces adhesion molecules upregulation finally responsible of leukocyte and platelets recruitment and microvascular dysfunction (Khoretonenko et al., 2014; Senchenkov et al., 2011). However our patients had not evidence of CMV infections, a similiar model could be hypothesized to explain the potential mechanisms of microvascular dysfunction COVID-19 related. Although we are aware that we cannot draw definitive conclusions, some important observations could be done based on these clinical cases. The three patients had very different severity of clinical conditions and also a different prognosis. Our experience confirms that children with comorbidity, in particular with congenital and acquired immunodeficiencies could show a higher risk of severe COVID-19 infections (Brisca et al., 2021). At present, limited evidences exist on the impact of COVID-19 in newborns, although a higher vulnerability has been described by some authors (De Rose et al., 2020). We confirmed that, in children affected by COVID-19, the sublingual TVD is normal or increased, whereas flow parameters (convective) MFI and PPV are decreased and may be correlated with the outcome. A limitation of this report is that we performed a single time point evaluation: evaluating the evolution of microvascular alterations in these patients would have provided additional important information on the possible relationship between the course of microcirculatory dysfunction and the patient prognosis. Furthermore with multiple time points of sublingual microcirculation evaluation we could better evaluate the impact of the main pathology of each patient than the impact of the COVID-19 infection itself.

3. Conclusions

Children affected by COVID 19 with need of PICU show a wide spectrum of clinical pictures. Pre-existing comorbidities can impact of the clinical course of this infection which is mild in the

most healthy children (Brisca et al., 2021). In children with severe COVID-19 microvascular parameters are altered.

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Chapter 7

Sublingual microcirculation in patients with SARS-CoV-2 undergoing veno-venous extracorporeal membrane oxygenation

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Abstract

Veno-Venous Extracorporeal Membrane Oxygenation (VV-ECMO) is a rescue treatment for severe acute respiratory failure refractory to conventional ventilation. We examined the alterations of sublingual microcirculation in patients with SARS-CoV-2 during VV-ECMO treatment and assessed the relationship between microvascular parameters and ventilation, hemodynamics, and laboratory tests. Nine patients were included in the study and the following microcirculatory parameters were estimated: TVD 16.81 (14.46-18.6) mm/mm2; PVD 15.3 (14.09-17.96) mm/mm2; PPV 94.85% (93.82%-97.79%); MFI 2.5 (2.5-2.92); HI 0.4 (0.18-0.4). TVD and PVD were inversely related to D-dimer levels (rho = -0.667, p = 0.05 and rho = -0.733, p = 0.025 respectively), aspartate aminotransferase (AST) (rho = -0.886, p = 0.019 and rho = -0.886, p = 0.019 respectively) and alanine aminotransferase (ALT) (rho = -0.829, p = 0.042 and rho = -0.829, p = 0.042 respectively). Our results showed an altered sublingual microcirculation in patients receiving VV-ECMO for severe SARS-CoV-2 and suggest a potential contribution of endothelia dysfunction to determine microvascular alteration.

Keywords: Extracorporeal membrane oxygenation; Microcirculation; SARS-CoV-2.

1. Introduction

Veno-Venous Extracorporeal Membrane Oxygenation (VV-ECMO) is a rescue treatment for severe cases of acute respiratory failure refractory to conventional mechanical ventilation, neuromuscular blockade, and prone positioning (Sen et al., 2016). During the current pandemic outspread, many patients with severe acute respiratory syndrome due to novel Coronavirus (SARS-CoV-2), unresponsive to first line therapies, underwent VV-ECMO (Pravda et al., 2020). Multiple evidences suggest that the assessment of sublingual microcirculation may have a role to guide clinical decisions during ECMO treatment (Yeh et al., 2018). Monitoring of sublingual microcirculation provides clinically relevant information representative of microcirculatory alterations of other organ beds (Guven et al., 2020). We recently reported the presence of microcirculatory alterations in patients with SARS-CoV-2 (Damiani et al., 2020). Thus, the aim of this study was to describe the alterations of sublingual microcirculation in patients with SARS-CoV-2 treated with VV-ECMO and to assess relationship between microvascular parameters and ventilation, hemodynamics, and laboratory tests.

2. Materials and methods

A retrospective data collection has been performed including patients with SARS-CoV-2 admitted to Intensive Care Unit at Ospedali Riuniti of Ancona (Italy) between February 2020 and April 2020 receiving VV-ECMO support for severe respiratory failure unresponsive to conventional mechanical ventilation and pronation. The study has been approved by Ethical Committee of Regione Marche (record ID: 2020 121/6152).

We retrieved information of patients that received assessment of sublingual microcirculation as routine clinical practice. Sublingual microvascular videos were recorded using the Cytocam camera. The Cytocam is a third generation handheld videomicroscope that enables the non-invasive, real-time, *in vivo* visualization of the microcirculation. Three videos from different sublingual areas were

recorded with adequate contrast and focus and without pressure artefacts. The videos were analyzed offline with dedicated software (Automated Vascular Analysis 3.2, Microvision Medical, Amsterdam, NL) to obtain parameters of vessel density (total vessel density [TVD], perfused vessel density [PVD]) and blood flow quality (microvascular flow index [MFI], percentage of perfused vessels [PPV], and flow heterogeneity index [HI]), as described elsewhere (Scorcella et al., 2018). Only vessels with a diameter less than 20 μm were considered.

Data distribution was assessed using the Kolmogorov-Smirnov test. Data were reported as mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate. The Pearson or Spearman correlation has been used to assessed relationship between microvascular and other clinical parameters.

3. Results

Nine patients with severe SARS-CoV-2 received VV-ECMO support within the study period. The main clinical characteristics are presented in Table 1 and the parameters regarding sublingual microcirculation are summarized in Table 2 . TVD and PVD were inversely related to D-dimer levels (rho = −0.667, $p = 0.05$ and rho = −0.733, $p = 0.025$ respectively), aspartate aminotransferase (AST) (rho = −0.886, $p = 0.019$ and rho = −0.886, p = 0.019 respectively) and alanine aminotransferase (ALT) (rho = −0.829, $p = 0.042$ and rho = −0.829, p = 0.042 respectively). No other correlation was found between microcirculation parameters and hemodynamics or other laboratory tests.

Table 1
Clinical characteristics.

Male (n; %)	8 (89%)
Age (years)	51 (47–60)
BMI (kg/m^2)	31.14 (27.1–36.9)
Murray Score	3.5 (3.5–3.8)
Tidal volume (ml)	350 (200–400)
Pplat (cmH_2O)	26 (22–29)
ΔP (cmH_2O)	16 (12–19)
PEEP (cmH_2O)	10 (8–10)
RR (breath/min)	10 (10 – 13)
Cstat (ml/cmH_2O)	18 (13 – 30)
FIO_2	0.4 (0.4–0.55)
pH	7.45 (7.36–7.48)
PaO_2 (mmHg)	75 (59–85)
$PaCO_2$ (mmHg)	45 (41–53)
Lactate (mmol/l)	0.8 (0.7–0.95)
MAP (mmHg)	86 (68–98)
HR (beat/min)	93 (70–96)
AST (U/l)	54 (33.5–97.25)
ALT (U/l)	85.5 (38.75–172.75)
D-dimer (ng/ml)	1428 (628.25–5743.75)
aPTT (sec)	41.5 (35–45)

Data reported as median (interquartile range).
ALT: alanine aminotransferase; AST: aspartate aminotransferase; aPTT: activated partial thromboplastin time; BMI: body mass index; Cstat: static compliance ore respiratory system; HR: heart rate; FIO_2: fraction of inspired oxygen; MAP: mean arterial pressure; $PaCO_2$: arterial partial pressure of carbon dioxide; PaO_2: arterial partial pressure of oxygen; ΔP: driving pressure; PEEP: positive end expiratory pressure; Pplat: plateau pressure; RR: respiratory rate.

Table 2
Parameters of sublingual microcirculation for small vessels.

TVD (mm/mm^2)	16.81 (14.46–18.6)
PVD (mm/mm^2)	15.3 (14.09–17.96)
PPV (%)	94.85 (93.82–97.79)
MFI	2.5 (2.5–2.92)
HI	0.4 (0.18–0.4)

Data reported as median (interquartile range).
HI: heterogeneity index; MFI: microvascular flow index; PPV: proportion of perfused vessels; PVD: perfused vessel density; TVD: total vessel density.

4. Discussion

VV-ECMO is an established technique to support lung function during severe respiratory failure refractory to conventional mechanical ventilation and prone position (Peek et al., 2009). However, its role in SARS-CoV-2 needs to be clarified.

Microvascular parameters of patients with SARS-CoV-2 supported with VV-ECMO reported in our report were worse than those recorded in a mixed population of critically ill patients (Scorcella et al., 2018) but comparable to our previous report including SARS-CoV-2 patients not supported by ECMO (Damiani et al., 2020). This is an indirect evidence that ECMO *per se* is not able to improve microvascular alterations. SARS-CoV-2 causes endothelial dysfunction (Jung et al., 2020) and the blood contact with ECMO circuit components may increase inflammatory state. Both previous conditions are likely to affect organ microcirculation and our findings showing impairment of both density and flow parameters suggest that monitoring sublingual microcirculation may provide clinically relevant information regarding the severity of the SARS-CoV-2. Moreover, we confirmed results from our previously report showing an inverse correlation between density parameters of microcirculation and D-dimer levels (Damiani et al., 2020). Interesting, this relation was confirmed in patients undergoing extracorporeal support with prolonged activated partial thromboplastin time (aPTT) due to heparin anticoagulation. Even if sepsis and SARS-CoV-2 may share common pathophysiological pathways like cytokine storm and endothelial dysfunction, the mechanisms of coagulopathy may be different (Iba et al., 2020). Anyhow, both diseases manifest themselves in a similar way at microvascular level, with reduced capillary density and altered flow. Although D-dimer was not previously related with capillary density in septic patients, it was associated with severity of illness (Angstwurm et al., 2004). Finally, the relationship between capillary density and liver enzymes may suggest that microvascular alteration may have a role in organ dysfunction. Unfortunately, the small number of patients considered in our analysis is the major limitation of the study and did not allow to demonstrate further relationship between microcirculation and other clinical parameters.

5. Conclusion

In our report considering patients with SARS-CoV-2 undergoing VV-ECMO, we found altered density and flow microvascular parameters and a relationship between capillary density and D-dimer

level, suggesting a potential role of endothelial disfunction to determine microvascular alteration. Further studies are needed to clarify the relationship between microcirculation and other clinical parameters.

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Chapter 8

Too much tolerance for hyperoxemia in mechanically ventilated patients with SARS-CoV-2 pneumonia? Report from an Italian intensive care unit

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Abstract

Background: In COVID-19 patients requiring mechanical ventilation, the administration of high oxygen (O2) doses for prolonged time periods may be necessary. Although life-saving in most cases, O2 may exert deleterious effects if administered in excessive concentrations. We aimed to describe the prevalence of hyperoxemia and excessive O2 administration in mechanically ventilated patients with SARS-CoV-2 pneumonia and determine whether hyperoxemia is associated with mortality in the Intensive Care Unit (ICU) or the onset of ventilator-associated pneumonia (VAP).

Materials and methods: Retrospective single-center study on adult patients with SARS-CoV-2 pneumonia requiring invasive mechanical ventilation for ≥48 h. Patients undergoing extracorporeal respiratory support were excluded. We calculated the excess O2 administered based on the ideal arterial O2 tension (PaO2) target of 55-80 mmHg. We defined hyperoxemia as PaO2 > 100 mmHg and hyperoxia + hyperoxemia as an inspired O2 fraction (FiO2) > 60% + PaO2 > 100 mmHg. Risk factors for ICU-mortality and VAP were assessed through multivariate analyses.

Results: One hundred thirty-four patients were included. For each day of mechanical ventilation, each patient received a median excess O2 of 1,121 [829-1,449] L. Hyperoxemia was found in 38 [27-55]% of arterial blood gases, hyperoxia + hyperoxemia in 11 [5-18]% of cases. The FiO2 was not reduced in 69 [62-76]% of cases of hyperoxemia. Adjustments were made more frequently with higher PaO2 or initial FiO2 levels. ICU-mortality was 32%. VAP was diagnosed in 48.5% of patients. Hyperoxemia (OR 1.300 95% CI [1.097-1.542]), time of exposure to hyperoxemia (OR 2.758 [1.406-5.411]), hyperoxia + hyperoxemia (OR 1.144 [1.008-1.298]), and daily excess O2 (OR 1.003 [1.001-1.005]) were associated with higher risk for ICU-mortality, independently of age, Sequential Organ failure Assessment score at ICU-admission and mean PaO2/FiO2. Hyperoxemia (OR 1.033 [1.006-1.061]), time of exposure to hyperoxemia (OR 1.108 [1.018-

1.206]), hyperoxia + hyperoxemia (OR 1.038 [1.003-1.075]), and daily excess O2 (OR 1.001 [1.000-1.001]) were identified as risk factors for VAP, independently of body mass index, blood transfusions, days of neuromuscular blocking agents (before VAP), prolonged prone positioning and mean PaO2/FiO2 before VAP.

Conclusion: Excess O2 administration and hyperoxemia were common in mechanically ventilated patients with SARS-CoV-2 pneumonia. The exposure to hyperoxemia may be associated with ICU-mortality and greater risk for VAP.

Keywords: ARDS; COVID-19; SARS-CoV-2; hyperoxia; oxygen; ventilator-associated pneumonia.

Introduction

Supplemental oxygen (O2) is a life-saving therapy in hypoxemic patients in order to guarantee adequate tissue O2 delivery. Nonetheless, excessive O2 administration may also exert deleterious effects (1). In recent years, several studies supported the use of more conservative oxygenation strategies in Intensive Care Units (ICUs), whereas liberal O2 therapy and the exposure to arterial hyperoxia in critically ill patients were associated with adverse outcomes (2–4). The lung is the first organ affected by O2 toxicity. Hyperoxia induces oxidative stress and inflammation in the lung (1) and may impair the surfactant system, thus causing alveolar collapse and the reduction in pulmonary compliance (5). Excess O2 administration may also compromise muco-ciliary clearance and the anti-microbial capacity of the immune cells, thus contributing to the development of ventilator-associated pneumonia (VAP) (6). In moderate or severe acute respiratory distress syndrome (ARDS), the administration of high inspired O2 fractions (FiO2) is frequently required to maintain normoxemia (arterial O2 tension [PaO2] 80–100 mmHg) and this may predispose to additional hyperoxia-induced lung injury. In order to limit the exposure to hyperoxia, the ARDS Network recommends using a PaO2 target of 55–80 mmHg in mechanically ventilated patients (7). Nonetheless, ARDS patients are frequently exposed to excessive FiO2 levels (8) and even undergo a condition of hyperoxemia (PaO2 > 100 mmHg) in a substantial number of cases (9). In a meta-analysis of randomized controlled trials (RCTs), excessive O2 administration (FiO2 > 50%) resulting in PaO2 levels above the protocol goal (>80 mmHg) was associated with mortality and lower ventilator- and hospital-free days (10). A recent multicentre RCT was aimed to compare a conservative (target PaO2 55–70 mmHg, SpO2 88–92%) with a liberal oxygenation strategy (target PaO2 90–105 mmHg, peripheral O2 saturation [SpO2] ≥ 96%) in ARDS patients, however, this study was prematurely stopped due to safety concerns (higher mortality and five mesenteric ischemic events in the conservative O2 group) (11). Therefore,

which is the safest oxygenation target for mechanically ventilated patients with ARDS remains an open question. This is a problem of major importance for patients with severe acute respiratory syndrome due to novel Coronavirus (SARS-CoV-2). A large Italian cohort study showed that 12% of these patients received FiO2 up to 100% and an FiO2 ≥ 50% was necessary in 89% of total (12). Hyperoxia-induced lung injury may add to the inflammatory process caused by the viral infection. Moreover, these patients often require long periods of mechanical ventilation and are at risk of exposure to high O2 concentrations for several days (13). Despite the key role of O2 in the treatment of SARS-CoV-2, the potential adverse effects of a prolonged exposure to hyperoxia in this patient category remain unexplored. The primary aim of this study was to explore whether mechanically ventilated patients with SARS-CoV-2 pneumonia in our ICU received an excessive amount of O2 and were exposed to hyperoxemia. In addition, we evaluated if hyperoxemia was associated with mortality or the onset of VAP.

Materials and methods

This retrospective observational study was conducted in the "General, respiratory and major trauma Intensive care Unit" of the Azienda Ospedaliera Universitaria "Ospedali Riuniti Umberto I-Lancisi-Salesi" of Ancona, Italy. During the pandemic phase, this ICU provided 18 beds for COVID-19 patients. The study protocol was approved by the local Ethics Committee (Comitato Etico Regionale Marche). Written informed consent was not requested due to the retrospective study design. This study included all consecutive adult (>18 year old) patients with SARS-CoV-2 pneumonia admitted to the ICU between February 2020 and May 2021, who required endotracheal intubation and invasive mechanical ventilation for at least 48 consecutive hours. COVID-19 infection was confirmed by means of real time polymerase chain reaction on nasopharyngeal swab or bronchoalveolar lavage. Exclusion criteria were: duration of mechanical ventilation < 48 h; ICU-discharge or death within 48 h; use of extracorporeal membrane

oxygenation (ECMO) or extracorporeal carbon dioxide removal (ECCO2R); admission from another ICU with a length of stay > 48 h; re-admissions after a previous ICU admission for SARS-CoV-2 pneumonia; COVID-19+ patients without pneumonia admitted to the ICU for different reasons.

Patient management

According to the most recent guidelines for the management of ARDS and COVID-19 (7, 14, 15), all patients received a lung protective ventilation strategy with a tidal volume (TV) of 4–6 ml/kg of ideal body weight, while maintaining a plateau pressure (Pplat) ≤ 30 cmH2O and a driving pressure ≤ 15 cmH2O. A positive end-expiratory pressure (PEEP) ≥ 5 cmH2O was applied in all patients, using an open-lung strategy. Neuromuscular blocking agents (NMBA) (continuous infusion of cisatracurium or rocuronium) were used in the early phase of mechanical ventilation in cases of refractory hypoxemia despite deep sedation to facilitate lung protective ventilation, during prone positioning, in cases of patient-ventilator dyssynchrony and/or in presence of high respiratory drive despite optimal sedation (15). Prone positioning was used in patients with a PaO2/FiO2 < 150 mmHg for a duration of at least 16 h per session (15). During the COVID-19 pandemic, we implemented and applied a protocol of prolonged prone positioning with a duration of 36 consecutive hours per session, which showed to be feasible and potentially more effective in improving oxygenation (16). Inhaled nitric oxide was administered as a rescue therapy in cases of refractory hypoxemia despite the use of NMBA and prone ventilation. Veno-venous ECMO was used in patients with refractory hypoxemia despite all other therapies (17). A VAP prevention strategy was routinely applied, including: oral care with chlorhexidine three times a day; head of bed elevation (30° whenever possible); check (3 times a day) and maintenance of the cuff pressure of the endotracheal tube (25 cmH2O); routine aspiration of tracheal secretions by means of a closed suction system; frequent check of the correct positioning and function of the nasogastric tube; weaning from mechanical ventilation as soon as possible; tracheostomy in cases of

anticipated difficult weaning (18). During prone ventilation, the administration of enteral nutrition was discontinued or, alternatively, continued at low dosage (10–20 ml/h) with frequent check of the gastric residual volume and possible episodes of regurgitation. Stress ulcer prophylaxis (pantoprazole 40 mg/day) was administered in patients with risk factors for gastro-intestinal bleeding (including coagulopathy, mechanical ventilation >48 h, hypotension), who represented the vast majority of the patients enrolled. Deep venous thrombosis prophylaxis was applied in all patients with low molecular weight heparin (at least 4,000 UI/day). In patients with VAP, antibiotic therapy was based on local written protocols in accordance with the most recent guidelines (19).

Data collection

For each included patient we collected: age, gender, body mass index (BMI), comorbidities (obesity, arterial hypertension, diabetes mellitus, chronic obstructive pulmonary disease, ischemic cardiomyopathy), the Sequential Organ Failure Assessment (SOFA) score at ICU admission, arterial lactate levels at ICU admission, PaO2/FiO2 at ICU admission, ICU length-of-stay, duration of invasive mechanical ventilation, ICU mortality, antibiotics prior to ICU admission, steroids prior to ICU admission, total steroid dose administered in the ICU (adjusted for body weight, mg/kg/day), episodes of VAP (early/late onset, causal pathogen, antibiotic resistance), use of NMBA, prone positioning (number of sessions, prolonged prone ventilation), use of proton pump inhibitors, blood transfusions (number of units). The onset of VAP was defined by the detection of a causative agent in a respiratory sample (tracheal aspirate or bronchoalveolar lavage) associated with a new or progressive lung infiltrate, plus at least two clinical criteria (fever or hypothermia, leukocytosis or leukopenia, purulent secretions), after at least 48 h of invasive mechanical ventilation (18). In cases of multiple VAP episodes, only the first episode was considered. An episode of VAP occurring within 4 days after endotracheal intubation was defined

as “early onset VAP” (18). Starting from endotracheal intubation and for the whole duration of invasive mechanical ventilation, we collected data from all arterial blood gas (ABG) analyses, including PaO2, SaO2, pH, PaCO2 with date and time. In our ICU, ABG are generally performed at least every 8 h in all patients. For each ABG we recorded data on mechanical ventilation: TV (ml), respiratory rate (RR), minute ventilation (l/min), PEEP, FiO2, and PaO2/FiO2. In addition, for each ABG we noted if the patient was on NMBA, prone ventilation, and measures of respiratory mechanics if available (Pplat, driving pressure, static compliance [Cstat]).

Based on the ARDS Network protocol, we considered a PaO2 of 80 mmHg as the upper limit of the oxygenation target. For each ABG showing a PaO2 > 80 mmHg, we estimated the “ideal FiO2” that would be sufficient to obtain a PaO2 of 80 mmHg based on the PaO2/FiO2 ratio, according to the following formula: $FiO2ideal = (80 * FiO2actual)/PaO2actual$. We then estimated the excess O2 being administered as follows: Excess O2 (L/min) = Minute ventilation (L/min)$*$ (FiO2 actual − FiO2 ideal). We then multiplied the value obtained for the time (minutes) elapsed since the previous ABG and calculated:

- Total excess O2 (L): total cumulative amount of excess O2 administered during the whole duration of invasive mechanical ventilation;

- Daily excess O2 (L/day): average amount of excess O2 administered in each day of invasive mechanical ventilation;

- Three-days excess O2 (L): cumulative amount of excess O2 administered during the first 3 days of invasive mechanical ventilation;

- Daily excess O2 before VAP (L/day): average amount of excess O2 administered in each day of invasive mechanical ventilation before the first diagnosis of VAP.

Episodes of "hyperoxemia" were defined by a PaO2 > 100 mmHg. We defined as "uncorrected hyperoxemia" any episode of hyperoxemia that was not followed by a reduction in the FiO2. In addition, we defined as "hyperoxia + hyperoxemia" any episode of FiO2 > 60% in presence of a PaO2 > 100 mmHg. Herein, the term "hyperoxia" (generally defined as the administration of any O2 dose > 21%) was used to indicate a high FiO2 (>60%). We then calculated the prevalence of hyperoxemia and hyperoxia + hyperoxemia for the whole duration of invasive mechanical ventilation, in the first 3 days of mechanical ventilation and for the days prior to VAP diagnosis. The duration of exposure to hyperoxemia was estimated as follows:

1. Daily time of exposure to hyperoxemia (hours per day of MV): whenever an ABG showed a PaO2 > 100 mmHg, we considered as time of exposure to hyperoxemia the time elapsed since the previous ABG; the total sum of hours was then divided by the number of days of MV.

2. Time of exposure to hyperoxemia in the first 3 days of MV: total sum of hours of hyperoxemia in the first 3 days of MV.

3. Daily time of exposure to hyperoxemia before VAP: average amount of hours of hyperoxemia in each day of invasive mechanical ventilation before the first diagnosis of VAP.

In addition, we calculated: mean FiO2, mean PaO2, mean PaO2/FiO2, highest FiO2, highest PaO2.

Statistical analysis

Statistics was performed with GraphPad Prism version 6 (GraphPad Software, La Jolla, CA, United States) and Statistical Package for Social Science software, version 17.0 (SPSS Inc., Chicago, IL, United States). Normality of distribution was checked with the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation or median [1st–3rd quartile],

as appropriate. Unpaired t-test or Mann Whitney U-test were used for comparisons of two groups. The chi-square test was used for nominal variables. We constructed multivariate binary logistic regression models in order to evaluate the independent association between the exposure to hyperoxemia (prevalence of hyperoxemia, prevalence of hyperoxia + hyperoxemia, daily excess O2) and the outcomes of interest (ICU mortality, diagnosis of VAP). Separate models were constructed for each index of exposure to hyperoxemia in order to avoid multi-collinearity. The basic assumptions for conducting logistic regression analyses were verified, including the absence of multi-collinearity and the linearity of the logit for each continuous independent variable (20). Covariates included in the models were selected based on their well-established association with the outcome of interest (6, 20). A p-value < 0.05 was used to indicate statistical significance.

Results

Between February 27, 2020 and May 12, 2021, a total of 207 COVID-19 patients was admitted to our ICU. Of these, exclusions were: 43 patients who underwent ECMO or ECCO2R; 7 patients who had an ICU length of stay < 48 h; 1 patient who was extubated before 48 h; 1 non-intubated patient; 13 patients transferred from a different ICU in which they stayed for more than 48 h; 6 re-admissions; 2 COVID-19+ patients without pneumonia. Therefore, we included 134 patients in total. The vast majority of patients was male, and the most frequent comorbities were obesity and arterial hypertension (Table 1). ICU-mortality was 32%. ICU Non-survivors were older, had a higher prevalence of ischemic cardiomyopathy and worse PaO2/FiO2 and SOFA score at ICU-admission (Table 1).

TABLE 1 General characteristics of the included patients.

	All patients ($n = 134$)	ICU survivors ($n = 91$, 68%)	ICU non-survivors ($n = 43$, 32%)	p
Age (years)	66 [57–74]	62 [55–72]	72 [66–75]	0.0004
Gender (n, % of males)	106 (79%)	72 (68%)	34 (32%)	0.995
Body mass index	29 [26–33]	29 [26–34]	28 [24–33]	0.138
Comorbidities (n, %)				
Arterial hypertension	71 (53%)	44 (62%)	27 (38%)	0.118
Obesity	52 (39%)	36 (69%)	16 (31%)	0.794
Diabetes mellitus	22 (16%)	17 (77%)	5 (23%)	0.303
Ischemic cardiomyopathy	15 (11%)	6 (40%)	9 (60%)	0.014
Chronic obstructive pulmonary disease	6 (4.5%)	4 (67%)	2 (33%)	0.947
Immunosuppression	2 (1.5%)	1 (1%)	1 (2.3%)	0.540
ICU length of stay (days)	17 [11–27]	17 [11–29]	16 [9–24]	0.129
Duration of mechanical ventilation (days)	14 [8–26]	14 [8–28]	16 [9–24]	0.859
SOFA score (admission)	7 [6–8]	7 [6–8]	7 [7–9]	0.0029
Lactate levels (admission, mmol/L)	1.3 [1–1.6]	1.3 [1.1–1.6]	1.3 [1.1–1.7]	0.578
PaO_2/FiO_2 (admission, mmHg)	111 [81–173]	122 [90–188]	101 [73–123]	0.0095

ICU, Intensive Care Unit; SOFA, Sequential Organ Failure Assessment.

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Excess O2 and hyperoxemia

We analyzed 9,583 ABGs in total. A PaO2 > 80 mmHg was found in 68.8% of cases. All the analyzed patients received an excessive amount of O2 in relation to the oxygenation target indicated by the guidelines (55–80 mmHg). We estimated that, for the whole duration of invasive mechanical ventilation, each patient received an average excess O2 of 17,741 [8,950–27,248] L, corresponding to a daily excess O2 of 1,121 [829–1,449] L (minimum: 319 L; maximum: 3,818 L). In each patient, hyperoxemia (PaO2 > 100 mmHg) was present in 38 [27–55]% of ABGs, and 11 [5–18]% of ABGs showed a condition of hyperoxia + hyperoxemia (FiO2 > 60% + PaO2 > 100 mmHg). Hyperoxemia was more frequently associated with higher PaO2/FiO2 and possible

indicators of less severe pulmonary dysfunction (lower Pplat and driving pressure) (Table 2). Figure 1 show the prevalence of hyperoxemia stratified by the FiO2 and PaO2/FiO2.

TABLE 2 Comparison of all arterial blood gases with or without hyperoxemia (PaO_2 > 100 mmHg).

	Hyperoxemia (PaO_2 > 100 mmHg, n = 3,517)	**No hyperoxemia ($PaO_2 \leq$ 100 mmHg, n = 6,066)**	***p***
PaO_2 (mmHg)	120 [109–140]	81 [71–90]	<0.0001
SaO_2 (%)	99.4 [99.1–99.9]	98.5 [97.3–99.4]	<0.0001
FiO_2 (%)	50 [40–60]	50 [40–60]	<0.0001
PaO_2/FiO_2 (mmHg)	257 [211–302]	170 [128–220]	<0.0001
Ventilation mode (n, %)			<0.0001
Volume controlled	890 (25%)	803 (13%)	
Pressure-controlled ventilation-volume guaranteed	1,091 (31%)	1,460 (24%)	
Pressure controlled	586 (17%)	1,207 (20%)	
Pressure support	767 (22%)	2,329 (38%)	
Continuous positive airway pressure	91 (2%)	93 (2%)	
Spontaneous breathing	92 (3%)	174 (3%)	
Minute ventilation (L/min)	9 [8–10.5]	9.1 [8–11]	0.0002
Peep (cmH_2O)	10 [10–12]	10 [8–12]	<0.0001
pH	7.44 [7.39–7.48]	7.45 [7.39–7.48]	<0.0001
$PaCO_2$ (mmHg)	45 [40–51]	45 [40–52]	<0.0001
Cstat (mL/cmH_2O)	47 [38–56]	42 [33–53]	<0.0001
Plateau pressure (cmH_2O)	23 [21–25]	24 [22–26]	<0.0001
Driving pressure (cmH_2O)	11 [9–13]	12 [10–14]	<0.0001

PEEP, Positive End-Expiratory Pressure; Cstat, Static compliance.

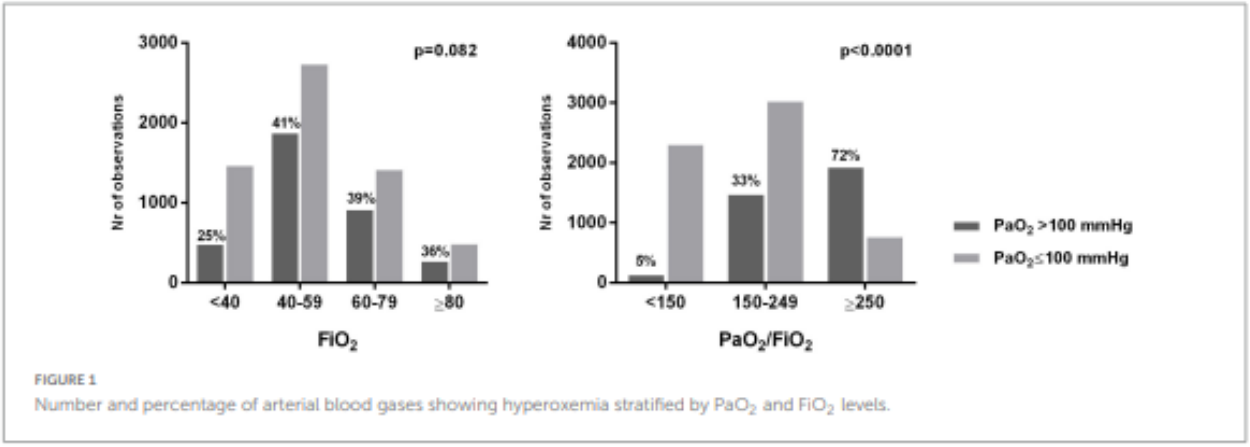

FIGURE 1

Number and percentage of arterial blood gases showing hyperoxemia stratified by PaO_2 and FiO_2 levels.

In 69 [62–76]% of cases, an ABG showing hyperoxemia was not followed by a reduction in the FiO2 (uncorrected hyperoxemia). The FiO2 was more likely to be reduced in presence of higher PaO2 and initial FiO2 levels, although even in presence of a PaO2 ≥ 150 mmHg or an initial FiO2 ≥ 80% no change was made in about 40% of cases (Figure 2). The choice of reducing the FiO2 was not influenced by PaO2/FiO2 or PEEP levels (Figure 2).

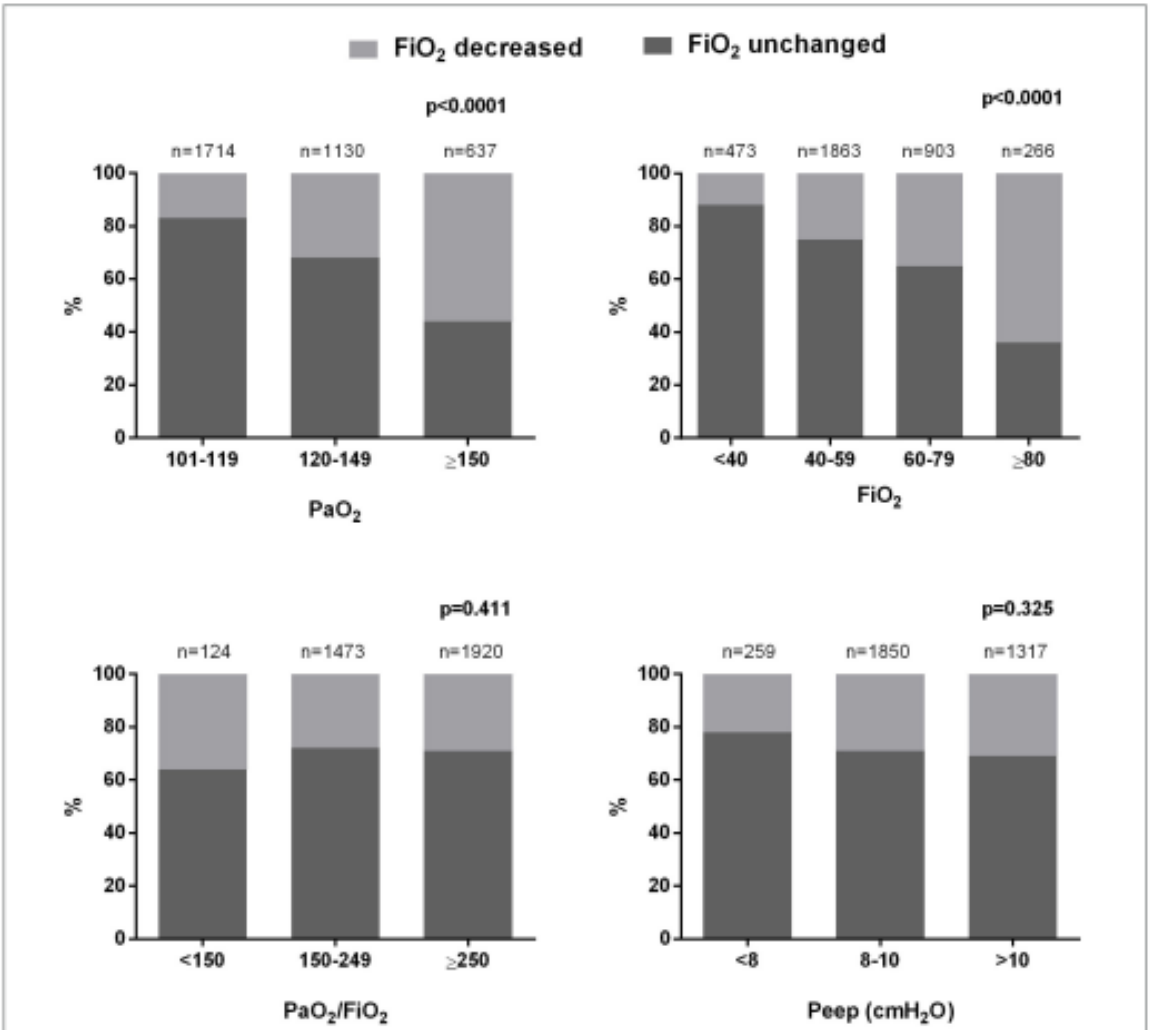

FIGURE 2
Episodes of uncorrected hyperoxemia stratified by PaO_2, FiO_2, PaO_2/FiO_2, and PEEP levels.

Hyperoxemia and intensive care unit-mortality

Intensive care unit-survivors showed higher mean PaO2/FiO2, mean PaO2 and maximum PaO2 as compared to Non-survivors, however mean and maximum FiO2 were significantly lower (Table 3). Hyperoxemia was more frequent among ICU-survivors, and the daily time of exposure was higher, even if Non-survivors showed a higher prevalence of hyperoxia + hyperoxemia (both for the whole duration of mechanical ventilation and in the first 3 days) (Table 3). After adjusting for age, SOFA score at ICU-admission and mean PaO2/FiO2, the prevalence of hyperoxemia, the duration of exposure, the prevalence of hyperoxia + hyperoxemia and daily excess O2 were

independently associated with ICU-mortality (Table 4). The total excess O2 and the excess O2 in the first 3 days were not significantly associated with mortality in the logistic regression analysis.

TABLE 3 Comparison of oxygenation variables between ICU-survivors and Non-survivors.

	ICU-survivors ($n = 91, 68\%$)	ICU non-survivors ($n = 43, 32\%$)	p
Mean PaO_2 (mmHg)	105 [97–114]	94 [88–100]	< 0.0001
Mean FiO_2 (%)	45 [42–47]	63 [56–69]	< 0.0001
Mean PaO_2/FiO_2 (mmHg)	242 [222–275]	163 [137–177]	< 0.0001
Max PaO_2 (mmHg)	219 [179–260]	197 [166–227]	0.011
Max FiO_2 (%)	100 [80–100]	100 [100–100]	0.0007
Hyperoxemia, % of ABGs	44 [30–63]	34 [24–41]	0.0002
Time of exposure to hyperoxemia, hours/day of MV	11 [7–14]	8 [5–10]	0.0006
Uncorrected hyperoxemia, % of ABGs	70 [61–77]	69 [63–75]	0.937
Hyperoxia + Hyperoxemia, % of ABGs	8 [4–14]	17 [11–23]	< 0.0001
Hyperoxemia (first 3 days), % of ABGs	64 [50–75]	58 [35–69]	0.066
Time of exposure to hyperoxemia, total hours in the first 3 days	47 [37–59]	44 [26–61]	0.204
Hyperoxia + Hyperoxemia (first 3 days), % of ABGs	20 [13–36]	31 [20–47]	0.0063
Total excess O_2 (L)	17,449 [8,912–27,118]	19,575 [11,038–29,055]	0.662
Daily excess O_2 (L)	1,145 [809–1,480]	1,049 [890–1,393]	0.860
Three-days excess O_2 (L)	6,098 [4,875–7,728]	6,270 [4,531–7,863]	0.689

All variables were calculated for the whole duration of invasive mechanical ventilation unless otherwise specified. ABGs, arterial blood gases.

TABLE 4 Binomial logistic regression models for the association between hyperoxemia and ICU-mortality.

	Odds ratio (95% confidence interval)	*p*
Model 1		
Hyperoxemia, % of ABGs	1.300 [1.097–1.542]	0.003
Mean PaO_2/FiO_2 (mmHg)	0.805 [0.712–0.911]	0.001
SOFA score (ICU admission)	2.043 [0.941–4.435]	0.071
Age (years)	1.034 [0.948–1.127]	0.452
Model 2		
Time of exposure to Hyperoxemia, hours/day of MV	2.758 [1.406–5.411]	0.003
Mean PaO_2/FiO_2 (mmHg)	0.773 [0.658–0.907]	0.002
SOFA score (ICU admission)	1.892 [0.898–3.985]	0.094
Age (years)	1.041 [0.949–1.141]	0.396
Model 3		
Hyperoxemia (first 3 days), % of ABGs	1.077 [1.022–1.135]	0.005
Mean PaO_2/FiO_2 (mmHg)	0.877 [0.822–0.935]	< 0.001
SOFA score (ICU admission)	1.775 [0.906–3.477]	0.095
Age (years)	1.014 [0.931–1.105]	0.744
Model 4		
Time of exposure to Hyperoxemia, total hours in the first 3 days	1.074 [1.017–1.135]	0.010
Mean PaO_2/FiO_2 (mmHg)	0.879 [0.825–0.936]	< 0.001
SOFA score (ICU admission)	1.779 [0.990–3.194]	0.054
Age (years)	1.007 [0.928–1.093]	0.863
Model 5		
Hyperoxia + Hyperoxemia, % of ABGs	1.144 [1.008–1.298]	0.037
Mean PaO_2/FiO_2 (mmHg)	0.916 [0.878–0.956]	< 0.001
SOFA score (ICU admission)	1.393 [0.800–2.424]	0.241
Age (years)	0.997 [0.931–1.068]	0.933
Model 6		
Daily excess O_2 (L)	1.003 [1.001–1.005]	0.008
Mean PaO_2/FiO_2 (mmHg)	0.889 [0.840–0.940]	< 0.001
SOFA score (ICU admission)	1.384 [0.790–2.422]	0.256
Age (years)	1.002 [0.932–1.076]	0.964

All variables were calculated for the whole duration of invasive mechanical ventilation, unless indicated otherwise.

Model 1: This model was statistically significant with a χ^2 (df 4) = 141.754 and $p < 0.0001$. This model explains 91.3% (Nagelkerke R^2) of variance for the outcome ICU-mortality and correctly classifies 96.3% of cases.

Model 2: This model was statistically significant with a χ^2 (df 4) = 142.335 and $p < 0.0001$. This model explains 91.5% (Nagelkerke R^2) of variance for the outcome ICU-mortality and correctly classifies 95.5% of cases.

Model 3: This model was statistically significant with a χ^2 (df 4) = 131.543 and $p < 0.0001$. This model explains 87.5% (Nagelkerke R^2) of variance for the outcome ICU-mortality and correctly classifies 93.3% of cases.

Model 4: This model was statistically significant with a χ^2 (df 4) = 128.237 and $p < 0.0001$. This model explains 87.5% (Nagelkerke R^2) of variance for the outcome ICU-mortality and correctly classifies 93.3% of cases.

Model 5: This model was statistically significant with a χ^2 (df 4) = 124.466 and $p < 0.0001$. This model explains 86.2% (Nagelkerke R^2) of variance for the outcome ICU-mortality and correctly classifies 92.5% of cases.

Model 6: This model was statistically significant with a χ^2 (df 4) = 127.466 and $p < 0.0001$. This model explains 85.8% (Nagelkerke R^2) of variance for the outcome ICU-mortality and correctly classifies 93.3% of cases.

ABGs, arterial blood gases; SOFA, Sequential Organ Failure Assessment.

Hyperoxemia and ventilator-associated pneumonia

Sixty-five patients (48.5% of total) had at least one episode of VAP during the ICU-stay. The vast majority (91%) of VAP episodes was late-onset. The most frequently isolated pathogens were: Staphylococcus Aureus (29 cases); Acinetobacter Baumanii (20 cases); Enterobacteriaceae (Klebsiella spp., Escherichia Coli, Serratia spp., Enterobacter spp., 34 cases); Pseudomonas Aeruginosa (14 cases); Corynebacterium Striatum (10 cases); Proteus Mirabilis (10 cases); Streptococcus Pneumoniae (3 cases); Aspergillus spp. (7 cases). Multi-drug resistant pathogens were isolated in 33% of cases.

Patients with VAP had longer ICU-stay, more frequently underwent prolonged prone positioning, and showed lower PaO2/FiO2 than those with no episodes of VAP (Table 5). Patients with at least one episode of VAP showed higher prevalence of hyperoxia + hyperoxemia (before VAP and in the first 3 days of mechanical ventilation) and received a higher excess O2 (daily and in the first 3 days of mechanical ventilation) (Table 5).

TABLE 5 Comparison between patients whit at least one episode of VAP and those without any episode of VAP.

	No VAP (n = 69, 51.5%)	VAP (n = 65, 48.5%)	p
Age (years)	66 [55–73]	67 [58–74]	0.236
Gender (n, % of males)	56 (81%)	50 (77%)	0.547
BMI (kg/m^2)	28 [25–32]	29 [27–35]	0.056
ICU length of stay (days)	12 [8–17]	25 [19–36]	<0.0001
Days of mechanical ventilation (before VAP)	9 [6–14]	8 [6–12]	0.415
SOFA score (ICU admission)	7 [6–8]	7 [6–8]	0.245
PaO_2/FiO_2 (ICU admission, mmHg)	130 [91–193]	101 [72–133]	0.003
Antibiotics before ICU admission (n, %)	29 (42%)	20 (31%)	0.176
Steroids before ICU admission	40 (58%)	40 (61%)	0.674
Steroids in ICU, mg/kg/die	0.06 [0.03–0.09]	0.06 [0.05–0.08]	0.282
NMBA (days before VAP)	3 [1–6]	4 [3–6]	0.143
Prone positioning (number of sessions before VAP)	2 [1–3]	2 [1–3]	0.056
Prolonged prone positioning, number of patients (%)	43 (62%)	55 (85%)	0.004
RBC before VAP (number of units)	0 [0–2]	0 [0–2]	0.753
Mean PaO_2 (mmHg)	105 [94–116]	107 [99–116]	0.348
Mean FiO_2 (%)	46 [41–55]	53 [48–59]	<0.001
Mean PaO_2/FiO_2 (mmHg)	244 [182–281]	211 [185–238]	0.004
Max PaO_2 (mmHg)	206 [162–249]	196 [173–249]	0.872
Max FiO_2 (%)	100 [75–100]	80 [60–100]	0.099
Hyperoxemia (% of ABGs)	47 [27–65]	45 [38–60]	0.492
Time of exposure to hyperoxemia, hours/day before VAP	11 [8–16]	12 [9–15]	0.285
Uncorrected hyperoxemia (% of episodes)	68 [59–78]	69 [55–75]	0.587
Hypoxia + hyperoxemia (% of ABGs)	11 [8–19]	18 [9–29]	0.002
Hyperoxemia (first 3 days, % of ABGs)	64 [46–75]	60 [48–72]	0.425
Time of exposure to hyperoxemia, total hours in the first 3 days	47 [31–60]	47 [40–59]	0.664
Hypoxia + Hyperoxemia (first 3 days, % of ABGs)	23 [13–34]	30 [17–42]	0.016
Daily excess O_2 (L/day)	1,209 [781–1,535]	1,443 [1,154–1,952]	0.001
Three-days excess O_2 (L)	5,642 [3,915–7,763]	6,343 [5,161–7,763]	0.048

VAP, ventilator-associated pneumonia; BMI, body mass index; ICU, Intensive Care Unit; SOFA, Sequential Organ Failure Assessment; NMBA, neuromuscular blocking agents; RBC, red blood cells; ABGs, arterial blood gases.

After adjusting for BMI, blood transfusions, days of NMBA (before VAP), prolonged prone positioning and mean PaO2/FiO2 before VAP, a higher prevalence of hyperoxemia was associated with a higher risk of VAP (Table 6): the adjusted risk for VAP increased by 3.3% for each unitary increase in the percentage of ABGs with hyperoxemia. Similarly, the time of exposure to hyperoxemia before the diagnosis of VAP, the prevalence of hyperoxia + hyperoxemia, as well as the daily excess O2, were independently associated with the risk of VAP (Table 6). Moreover, patients in the highest tertile of daily excess O2 showed a 4.3 times greater adjusted risk of developing VAP as compared to those in the lowest tertile. The total amount of excess O2 received before the diagnosis of VAP was not significantly associated with the onset of VAP, nor were the prevalence of hyperoxemia or hyperoxia + hyperoxemia in the first 3 days of mechanical ventilation and the duration of hyperoxemia in the first 3 days.

TABLE 6 Binomial logistic regression models for the association between hyperoxemia and VAP.

	Odds ratio (95% confidence interval)	*p*
Model 1		
Hyperoxemia, % of ABGs (before VAP)	1.033 [1.006–1.061]	0.015
Mean PaO_2/FiO_2 (mmHg)	0.983 [0.973–0.993]	0.001
Prolonged prone positioning (yes/no)	3.089 [1.228–7.767]	0.017
NMBA (days before VAP)	0.919 [0.814–1.037]	0.170
BMI (kg/m^2)	1.031 [0.977–1.088]	0.266
RBCs before VAP (number of units)	1.014 [0.880–1.169]	0.843
Model 2		
Time of exposure to hyperoxemia, hours/day before VAP	1.108 [1.018–1.206]	0.018
Mean PaO_2/FiO_2 (mmHg)	0.982 [0.973–0.992]	<0.001
Prolonged prone positioning (yes/no)	3.040 [1.178–7.846]	0.022
NMBA (days before VAP)	0.908 [0.804–1.025]	0.118
BMI (kg/m^2)	1.039 [0.985–1.097]	0.160
RBCs before VAP (number of units)	0.994 [0.861–1.147]	0.931
Model 3		
Hypercoia + Hyperoxemia, % of ABGs (before VAP)	1.038 [1.003–1.075]	0.035
Mean PaO_2/FiO_2 (mmHg)	0.993 [0.985–1.002]	0.122
Prolonged prone positioning (yes/no)	3.138 [1.244–7.920]	0.015
NMBA (days before VAP)	0.925 [0.822–1.042]	0.199
BMI (kg/m^2)	1.030 [0.976–1.087]	0.287
RBCs before VAP (number of units)	1.017 [0.885–1.169]	0.809
Model 4		
Daily excess O2 (L/day)	1.001 [1.000–1.001]	0.007
Mean PaO_2/FiO_2 (mmHg)	0.988 [0.980–0.997]	0.006
Prolonged prone positioning (yes/no)	3.102 [1.193–8.064]	0.020
NMBA (days before VAP)	0.900 [0.796–1.018]	0.095
BMI (kg/m^2)	1.016 [0.962–1.073]	0.562
RBCs before VAP (number of units)	1.045 [0.906–1.206]	0.543
Model 5		
Daily excess O2 (L/day)		
First tertile (≤1,136)	Reference	*p* for trend 0.014
Second tertile (≤1,561)	1.712 [0.672–4.363]	0.260
Third tertile (>1,561)	4.332 [1.595–11.767]	0.004
Mean PaO_2/FiO_2 (mmHg)	0.988 [0.980–0.997]	0.006
Prolonged prone positioning (yes/no)	2.666 [1.035–6.865]	0.042
NMBA (days before VAP)	0.913 [0.811–1.028]	0.134
BMI (kg/m^2)	1.024 [0.970–1.081]	0.389

(Continued)

TABLE 6 (Continued)

	Odds ratio (95% confidence interval)	p
RBCs before VAP (number of units)	1.044 [0.905–1.204]	0.554

All variables were calculated before the onset of VAP.
Model 1: This model was statistically significant with a χ^2 (df 6) = 23.035 and $p = 0.001$. This model explains 21.1% (Nagelkerke R^2) of variance for the outcome VAP and correctly classifies 66.4% of cases.
Model 2: This model was statistically significant with a χ^2 (df 6) = 27.746 and $p = 0.001$. This model explains 24.9% (Nagelkerke R^2) of variance for the outcome VAP and correctly classifies 65.7% of cases.
Model 3: This model was statistically significant with a χ^2 (df 6) = 21.622 and $p = 0.001$. This model explains 19.9% (Nagelkerke R^2) of variance for the outcome VAP and correctly classifies 67.2% of cases.
Model 4: This model was statistically significant with a χ^2 (df 6) = 25.094 and $p < 0.001$. This model explains 22.8% (Nagelkerke R^2) of variance for the outcome VAP and correctly classifies 69.4% of cases.
Model 5: This model was statistically significant with a χ^2 (df 6) = 25.784 and $p = 0.001$. This model explains 23.3% (Nagelkerke R^2) of variance for the outcome VAP and correctly classifies 67.9% of cases.

Discussion

This retrospective single-centre study on 134 mechanically ventilated patients with SARS-CoV-2 pneumonia showed that: first, the dose of supplemental O2 administered was often excessive in comparison to the oxygenation target indicated by current guidelines and the exposure to hyperoxemia was frequent; second, in most cases of hyperoxemia the FiO2 was not varied, adjustments in O2 dose were made more frequently in presence of higher PaO2 or higher initial FiO2; third, the exposure to hyperoxemia was independently associated with higher risks of ICU-mortality and VAP. Our data are consistent with those of previous studies showing that clinicians generally tolerate higher PaO2 values than those commonly recommended. In a retrospective study in critically ill mechanically ventilated patients, de Graaf et al. showed a prevalence of hyperoxemia (PaO2 > 120 mmHg) of 22% and adjustments in the FiO2 were rarely made, especially if this was ≤40% (21). Suzuki et al. calculated that an excess O2 dose of 3,472 L per patient was administered on average during mechanical ventilation and no change in the FiO2 was made in most cases of hyperoxemia if the initial level was 30–40% (22). In a recent study in COVID-19 mechanically ventilated patients, the prevalence of hyperoxemia in the ICU using a standard oxygenation protocol was 75.9% in the first day (23). Similarly in our study, the FiO2

was more likely to be reduced in presence of more severe hyperoxemia, whereas PaO2 values of 100–119 mmHg were accepted in more than 80% of cases. The initial FiO2 also determined the clinicians' behavior against hyperoxemia: if this was <40% no change was made in almost 90% of cases, while an initial FiO2 ≥ 80% was corrected in more than 60% of cases. A possible explanation of this too liberal attitude toward O2 therapy is a lack of perception of the risks associated with hyperoxemia. A survey by Helmerhorst et al. showed that most clinicians recognize the potential deleterious effects of a prolonged exposure to excessive O2 concentrations, including hyperoxia-induced lung injury, and show little tolerance toward even mild hyperoxemia (24). Nonetheless, a large proportion of their ICU patients was exposed to higher arterial O2 levels than self-reported target ranges (24). These data suggest that, in actual clinical practice, clinicians tend to tolerate a certain degree of hyperoxia, as perceived as a safety buffer against hypoxemia. In the context of the COVID-19 pandemic, such a liberal attitude may be partly justified by a greater fear against episodes of hypoxemia in a clinical scenario in which a ready accessibility to the patient is not always guaranteed due to the isolation precautions, and an SpO2 of 98–100% on the monitor could appear more reassuring than an SpO2 of 90–92%. As a matter of fact, O2 toxicity and hyperoxia-induced lung injury are relatively slow processes in comparison to more acute conditions that can rapidly induce pulmonary oedema, respiratory failure and shock (such as aspiration pneumonia or sepsis-induced ARDS). A continuous exposure to hyperoxia for days/weeks can lead to diffuse alveolar damage, respiratory failure and high risk of death, while prolonged exposures to sub-lethal O2 doses generally induce pulmonary fibrosis (1). In healthy volunteers, breathing 95% O2 for 17 h caused a significant alveolar-capillary leak, due to a progressive destruction of the alveolar-capillary membrane, alveolar hemorrhage, formation of microthrombi, and intrapulmonary shunt (25). Moreover, oxidative stress compromises the surfactant system, thus causing atelectasis and reduction in lung compliance (26). Patients with ARDS may be particularly susceptible to O2 toxicity, since the ongoing inflammatory process may already compromise their adaptive and anti-oxidant

capacities. Moreover, hyperoxia may aggravate and predispose the lung to the deleterious effects of positive pressure ventilation (5). A recent meta-analysis of RCTs showed that the use of higher oxygenation targets in the critically ill may increase mortality, even if with a very-low level of evidence (27). The LOCO2 trial failed to prove the safety and efficacy of a more restrictive oxygen therapy in patients with ARDS (11), however, these results cannot be conclusive. Similarly, the multi-center HOT-ICU trial did not show any difference in 90-day mortality with a lower oxygenation target as compared to higher PaO2 target in patients with acute hypoxemic respiratory failure (28). Our study showed that the exposure to hyperoxemia in mechanically ventilated patients with SARS-CoV-2 pneumonia may be associated with higher ICU mortality, independently of other risk factors such as the mean PaO2/FiO2, SOFA score at admission and age. Moreover, a higher prevalence of hyperoxemia, as well as an excess O2 administration, was independently associated with a greater risk of VAP. Ventilator-associated pneumonia is the most common ICU-acquired infection among mechanically ventilated patients, leading to higher mortality, longer need for mechanical ventilation, and an increase in healthcare cost (18). For the implementation of effective preventive strategies, it is crucial to gain a deep understanding of the pathophysiology and risk factors for VAP. There is ample evidence that a long-term exposure to hyeroxia can impair pulmonary innate immunity and bacterial phagocytosis capacity (29). In a retrospective study on mechanically ventilated critically ill patients, the presence of hyperoxemia (defined as PaO2 > 120 mmHg) at ICU-admission and the number of days of exposure to hyperoxemia were independently associated with the onset of VAP (6). Preclinical studies in animal models also supported a role of hyperoxia in the pathogenesis of VAP. Entezari et al. showed that a prolonged exposure to hyperoxia can compromise the ability of alveolar macrophages to phagocytose Pseudomonas Aeruginosa (30) and increased mortality in infected mice (31). Hyperoxia increased mortality in mice with Acinetobacter pneumonia, in which the administration of procysteine was able to improve survival by increasing the phagocytic activity of alveolar macrophages under hyperoxic conditions (32). By applying a too liberal O2 therapy,

we may be losing the opportunity to control for a significant risk factor for VAP, a complications that occurred in almost 50% of patients in our cohort. In our study, the use of prolonged prone positioning (sessions of up to 36 consecutive hours) was also associated with an increased risk of VAP. From a theoretical point of view, prone positioning could prevent the onset of VAP by facilitating the drain of respiratory secretions and limiting ventilator-induced lung injury, although most clinical studies showed no significant impact on the actual incidence of VAP (33). Several factors may explain the association observed, including the need for prolonged administration of NMBA and deep sedation, the increase in abdominal pressure with a higher risk of aspiration of gastric content, the need to limit the dose of enteral nutrition. ICU-acquired weakness is a frequent complication in COVID-19 patients, especially in those who received higher doses of NMBA and sedatives and lower caloric/protein intake, and may be responsible for a longer duration of mechanical ventilation and prolonged exposure to the risk of VAP (34).

Our study has several limitations. First, the retrospective design that does not allow to define a cause-effect relationship between the exposure to hyperoxia and mortality or VAP, but only enables to describe associations. Second, the relatively low sample size and the involvement of a single center, which limits the generalizability of our results. The sample size limited the number of confounders that could be included in multivariate regression models, since the inclusion of too many independent variables would lead to a mathematically unstable outcome (20). Third, we based our analysis on ABG data that can only provide a partial picture of the exposure to hyperoxemia, limited to the moment in which the ABG was made. Unfortunately, we could not collect SpO2 data, which could have been useful for a more continuative evaluation of the oxygenation status and the responses of clinicians to SpO2 values above the target. Fourth, the calculation of the “ideal FiO2” and “excess O2” can only provide an imprecise estimate of the amount of O2 administered in excess. In fact, factors such as PEEP, prone positioning or use of iNO will determine the most appropriate FiO2 of the patient by influencing gas exchange:

therefore, calculating the ideal FiO2 merely on the basis of the PaO2/FiO2 ratio may be reductive. Despite its limitations, to the best of our knowledge, this is the first study that describes the prevalence of hyperoxemia in mechanically ventilated COVID-19 patients and explores the potential effects of excess O2 doses on outcome. Future larger studies are needed to confirm our findings.

Conclusion

In mechanically ventilated patients with SARS-CoV-2 pneumonia admitted to our ICU, the administration of O2 was often excessive in comparison to the PaO2 target indicated by the guidelines, and the exposure to hyperoxemia was frequent. In addition, most episodes of hyperoxemia were not followed by a reduction in the FiO2; changes were made more frequently in the presence of higher PaO2 or higher initial FiO2. The prevalence of hyperoxemia was independently associated with a greater risk of ICU-mortality, as well as with a greater risk of developing VAP. The retrospective nature of our study does not allow to draw conclusions on a possible cause-effect relationship between the exposure to excessive amounts of O2 and outcome. However, these data add to the ample literature that warns against the possible deleterious effects of a too liberal O2 therapy. In the absence of strong evidence of the safety of hyperoxemia in critically ill patients (in particular in those with ARDS and SARS-CoV-2 pneumonia), more efforts should be made to avoid the exposure to excessive amounts of supplemental O2. Further studies are needed to define the best oxygenation target for this patient category.

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Chapter 9

Antibiotic Treatment of Acinetobacter baumannii Superinfection in Patients With SARS-CoV-2 Infection Admitted to Intensive Care Unit: An Observational Retrospective Study

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Abstract

Introduction: In COVID-19 patients on mechanical ventilation, VAP from Acinetobacter baumannii remains a crucial risk factor for death. Antibiotic resistance represents an important problem in treating this infection. This study aims to describe the evolution of the superinfection from PDR Acinetobacter baumannii in patients with acute respiratory failure from SARS-CoV-2 infection admitted to ICU and compare the impact of two different antibiotic strategies on microbiological negativization.

Methods: Single-center observational retrospective study, including patients admitted to our ICU from March 2020 to May 2021 for acute respiratory failure from SARS-CoV-2 infection who developed PDR Acinetobacter baumannii superinfection. Clinical data at ICU admission were collected, as well as the timing of isolation of Acinetobacter baumannii, its resistance profile, the site of infection, and the antibiotic therapy.

Results: Of the 32 patients enrolled, 10 patients (31.2%) were treated with the combination of high-dose ampicillin/sulbactam, high-dose tigecycline, intravenous and inhaled colistin (Protocol), the other 22 (68.8%) were treated with the combination of two antibiotics (Control). Of the 10 patients in the Protocol group, 8 patients (80%) received also fosfomycin. All patients (100%) in the Protocol group had microbiological negativization, while in the Control group microbiological negativization was observed in 8 (36.4%) patients, $p < 0.01$.

Conclusion: Our report shows microbiological negativization in all patients treated with the combination therapy of nebulized and intravenous colistin, high-dose tigecycline, and high-dose ampicillin/sulbactam. This combination of antibiotics seems to be a useful alternative when other treatments are not available or fail.

Keywords: Acinetobacter baumannii; SARS-CoV-2; acute respiratory failure; antibiotics; superinfection.

Introduction

In December 2019 new cases of pneumonia of unknown origin came to light in China (1). The new virus was recognized as a coronavirus able to cause the severe acute respiratory syndrome (2). Therefore, it was named SARS-CoV-2, and the pathology derived from it was called Coronavirus Disease 2019 (COVID-19) (2). It is widely known that the clinical presentation of the illness may vary considerably. In some cases, the disease may be asymptomatic, while 5–15% of patients may experience dyspnea and respiratory effort and require endotracheal intubation and mechanical ventilation (3, 4). In the case of intubated patients in intensive care units (ICUs), Ventilator-Associated Pneumonia (VAP) remains a crucial risk factor for death (5). A VAP is diagnosed when new pneumonia is detected after 2 days from the patient being intubated and mechanically ventilated. As for causative agents, the most common pathogens include Staphylococcus spp., Enterococcus spp., Klebsiella pneumonia, Enterobacter spp., Escherichia coli, Acinetobacter spp., and Pseudomonas spp. (6). Furthermore, a significant percentage of patients, admitted to ICU, is treated with broad-spectrum antibiotics, which increase the risk of developing hospital-acquired infections, particularly from multi-drug resistant (MDR) pathogens. Among them, Multi-Drug Resistant Acinetobacter baumannii (MDR-AB) represents a causative agent for almost half of Ventilator-Associated Pneumonia (VAP) (7) and is a severe problem in patients with COVID-19 in ICU (8, 9). The most important risk factors for VAP from Acinetobacter baumannii are high blood pressure, chronic obstructive pulmonary disease (COPD), length of stay in ICU, at least one organ failure, chronic renal impairment, and reduced blood oxygenation level. Interestingly, these features are usually common to COVID-19 patients in ICU, who, therefore, become highly susceptible to the infection (10–12). Acinetobacter

baumannii is a Gram-negative bacterium, opportunistic, pleomorphic, and non-motile. It can colonize dry surfaces and devices surviving up to 33 days (13–15). Moreover, the pathogen can develop resistance to numerous classes of antibiotics more rapidly than other bacteria. Therefore, it has been considered a major health problem in the international medical community (16). In regards to antimicrobial therapy, in the case of a Multi Drug-Resistant Acinetobacter baumannii, carbapenems still represent the treatment of choice. Unfortunately, the resistance to carbapenems has increased making the pathogen eXtensively Drug-Resistant (XDR), while other strains have been named Pan Drug-Resistant (PDR) when they showed resistance to polymyxins, especially colistin, and tigecycline (17). As for XDR AB, one of the last options is colistin which is highly nephropathic and neurotoxic (18). However, by changing the way of administration, the risk of nervous and renal damage can be decreased. When colistin is given by inhalation, the systemic distribution of the drug is reduced (19). Therefore, nebulized colistin seems to be a reasonable choice in the case of Carbapenem-Resistant Acinetobacter baumannii in patients with COVID-19 in ICU (5). The higher incidence of Pan Drug-Resistant Acinetobacter baumannii causing VAP is observed particularly in Greece, Spain, and Italy, implying the need for new therapeutic strategies (20). Thus, some authors proposed to use of a combination of antibiotics to exploit the synergistic effect of different classes (21). In 2019, Assimakopoulos et al. reported positive results in treating 10 ICU patients with VAP from Pan Drug-Resistant Acinetobacter baumannii with a combination of antibiotics, which consisted of a high dose of tigecycline and ampicillin/sulbactam, and colistin, given both by inhalation and intravenously (22). As for sulbactam, its use is justified by its intrinsic activity against several strains of AB (23, 24).

The present brief report aimed to describe retrospectively the evolution of the superinfection from PDR Acinetobacter baumannii in patients with SARS-CoV-2 infection admitted to ICU. In addition, it assessed the incidence of negativization between patients treated with the combination

of at least three antibiotics, according to a treatment protocol applied in our ICU, and those who received a combination of two antibiotics.

Materials and Methods

We retrospectively collected the data from adult patients admitted to a single COVID-ICU (Anesthesia and Intensive Care Unit, University Hospital "Ospedali Riuniti" of Ancona, Italy) for acute respiratory failure from SARS-CoV-2 infection and Acinetobacter baumannii superinfection. We collected demographic data, including age, sex, body mass index (BMI) and comorbidities, and clinical data at ICU admission among which respiratory parameters and blood tests including lymphocytes, leukocytes, and procalcitonin. We calculated the SOFA (Sequential Organ Failure Assessment) score and the Charlson Comorbidity Index at ICU admission. The immunomodulatory and immunosuppressive therapies, if administered before admission, were noted. Any microbiological tests performed at the beginning and during the stay in ICU were reviewed. We noted the date of positivity to SARS-CoV-2, detected with the reverse transcriptase-polymerase chain reaction (RT-PCR) on the nasopharyngeal swab, performed before ICU admission. We also noted the precise timing of isolation of Acinetobacter baumannii, its resistance profile (MDR, XDR, PDR), and the site of infection. Acinetobacter baumannii strains from all kinds of cultures were identified in our microbiology laboratory with the new-generation mass spectrometry microbial identification system, VITEK® MS PRIME (bioMérieux, Marcy-l'Étoile, France). To test the antimicrobial susceptibility was used the VITEK®2 System (bioMérieux, Marcy-l'Étoile, France) for all antibiotics. The resistance to colistin detected with the VITEK®2 System was confirmed with the broth microdilution method. The results were interpreted following the latest EUCAST breakpoints for Acinetobacter baumannii spp. available.

Following the definition of the resistance profile (17), we considered as Multi Drug-Resistant (MDR) Acinetobacter baumannii resistant to at least three classes of antimicrobial agents (all penicillins and cephalosporins, including inhibitor combinations, fluoroquinolones, and aminoglycosides), eXtensively Drug-Resistant (XDR) the MDR Acinetobacter baumannii resistant also to carbapenems and Pan Drug-Resistant (PDR) the XDR Acinetobacter baumannii resistant to polymyxins and tigecycline. In addition, data regarding antimicrobial treatment were collected. Starting from the second wave of the pandemic, when the problem of PDR Acinetobacter baumannii superinfection became very consistent in COVID-19 patients admitted to our ICU, impacting the length of stay and the outcome, we started to apply a protocol of antibiotic therapy based on the case series study of Assimakopoulos et al. (22). Patients with PDR Acinetobacter baumannii superinfection received combination therapy with intravenous colistin at the loading dose of 9 million IU followed by a maintenance dose of 4.5 million IU every 12 h, intravenous tigecycline at the dose of 100 mg every 12 h, intravenous ampicillin/sulbactam, administered in a continuous infusion, at the dose of 12 gr per day and inhaled colistin at the dose of 3 million IU every 6 h, added in case of respiratory tract infection. Sometimes, in patients with particularly severe clinical conditions, we added also fosfomycin at the dose of 12 gr per day. The maintenance dose of intravenous colistin in patients with the impaired renal function was adjusted with the use of the colistin calculator, based on the pharmacokinetic modeling data published by Garonzik et al. (25). We also reduced the dose of ampicillin/sulbactam in patients with a creatinine clearance less than 30 ml/min, according to the Cockcroft-Gault equation. Considering that rapid molecular systems to detect the pathogen were not routinely used in the period of study, we used to start this combination of antibiotics about 48–72 h after the cultural tests, as soon as the microbiological examinations reports were made available. Before the application of this protocol of the three antibiotics in combination, patients with PDR Acinetobacter baumannii were treated with nebulized and intravenous colistin alone or combined with an antibiotic of another class. To define the resolution of the infection, we considered both the clinical improvement in the signs of

infection and the laboratory or instrumental parameters and the negativization from Acinetobacter baumannii in control culture tests. We also reported the complications of antibiotic therapy. According to the KDIGO guidelines (26), we defined Acute Kidney Injury (AKI) as the presence of any of the following criteria: an increase in serum creatinine by ≥ 0.3 mg/dl (≥ 26.5 μmol/l) within 48 h or an increase in serum creatinine to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days, or urine volume < 0.5 ml/kg/h for 6 h.

Statistical Analysis

The statistical analysis was performed using STATA 17.0 BE – Basic Edition (StataCorp, Texas, United States). Categorical data were expressed as absolute and relative frequencies, numerical data as mean ± standard deviation, if normally distributed, or median [interquartile range], if not normally distributed. Normality of distribution was assessed using the Shapiro-Wilk test. Dichotomous data were compared using the chi-square test or Fisher's exact test, as appropriate. Continuous variables were compared using the Student's t-test for unpaired data or the Wilcoxon rank-sum test, as appropriate. A $p < 0.05$ was used to indicate the statistical significance.

Given the descriptive nature of the primary objective, a sample size calculation was not necessary.

Ethical Aspects

The study protocol was approved by the local Ethics Committee (Comitato Etico Regionale delle Marche). All the data were anonymously analyzed. Written informed consent was not applicable due to the retrospective nature of the study.

Results

We considered 32 patients, admitted to our ICU from March 2020 to May 2021 for acute respiratory failure consequent to SARS-CoV-2 infection who developed PDR Acinetobacter baumannii superinfection. The MIC (Minimum Inhibitory Concentration) values of the PDR Acinetobacter baumannii in the study population are presented in Table 1. In 30 patients (93.7%) the site of PDR Acinetobacter baumannii superinfection was the respiratory tract, in 2 patients (6.3%) the microorganism was isolated firstly in the rectal swab and then also in the respiratory tract cultures. The median age of patients was 59.5 [54–66] years and 28 (87.5%) were males. Of the 32 patients, 10 patients (31.2%) were treated with the combination of high-dose ampicillin/sulbactam, high-dose tigecycline, intravenous and inhaled colistin (Protocol), the other 22 (68.8%) were treated with the combination of two antibiotics (Control). Of the 10 patients in the Protocol group, 8 patients (80%) received also fosfomycin. In all the 10 patients of the Protocol group, the PDR Acinetobacter baumannii was isolated only in the respiratory swab. The demographic and clinical characteristics of the two groups of patients are presented in Table 2.

TABLE 1 | Minimum Inhibitory Concentration (MIC) values of the *PDR Acinetobacter baumannii* in the study population.

Antimicrobial agent	MIC values (mg/L)	MIC breakpoints (mg/L)* R >
Amikacin	≥ 64	8
Ciprofloxacin	≥ 4	1
Colistin	> 2	2
Gentamicin	≥ 16	4
Meropenem	≥ 16	8
Trimethoprim/Sulfamethoxazole	≥ 320	4
Tigecycline	2-4	–

**EUCAST Clinical breakpoints tables, v. 10.0 and 11.0.*
R = resistant; – = no EUCAST breakpoint available.

TABLE 2 | Demographic and clinical characteristics at Intensive Care Unit (ICU) admission of the two groups of patients.

Characteristics	Protocol (n = 10)	Control (n = 22)	p-value*
Male sex, n (%)	8 (80)	20 (90.9)	0.57
Age, years	58.5 [55–66]	60 [53–66]	0.97
BMI, kg/m^2	33.5 [26–35.9]	27.8 [25.5–31.2]	0.12
Charlson Comorbidity Index, points	2.2 ± 2.2	2.1 ± 1.5	0.84
PaO_2/FiO_2	74.5 [63–82]	78.5 [61–87]	0.67
WBC, ×10'3/mm	12.1 [7.8–14]	11.8 [7.8–19.2]	0.81
Lymphocytes, ×10'3/mm	0.98 [0.63–1.24]	0.56 [0.4–0.69]	0.07
Procalcitonin, ng/ml	0.33 [0.17–0.93]	0.39 [0.11–1.03]	0.96
SOFA score	6.5 ± 1.6	7.7 ± 2.3	0.13

Data are presented as absolute and relative frequencies, mean ± standard deviation, median [interquartile range].
**Chi-squared test or Fisher's exact test for categorical variables and Student's t-test or Wilcoxon rank-sum test for numerical variables, as appropriate.*
BMI = body mass index; PaO_2/FiO_2 = ratio of partial pressure of arterial oxygen to fraction of inspired oxygen; WBC = white blood cells; SOFA = Sequential Organ Failure Assessment.

Between the two groups of patients, no significant differences were observed in demographic and clinical characteristics at admission to the ICU. Considering the therapy received before the ICU admission, 12 patients (54.5%) in the Control group and 7 (70%) in the Protocol group had already been treated with antibiotics, p = 0.28. There was no significant difference in the duration of the steroid therapy received before the ICU admission [8 (2–11) days in the Control group vs. 4 (1–10) days in the Protocol group, p = 0.52]. The mean length of stay in the ICU of patients in the Control group was 25.2 ± 17.3 days, instead, for patients in the Protocol group was 36.1 ± 32.6 days, p = 0.36. All patients, 100% (95% CI: [69–100]%), in the Protocol group had microbiological negativization, while in the Control group microbiological negativization was observed in 36,4% (95% CI: [17–59]%) of patients, p < 0.01. Considering the side effects of the antibiotic therapy, 40% (95% CI: [12–73]%) of patients in the Protocol group developed AKI, while in the Control group only 4,5% (95% CI: [0,1–22]%) of patients, p = 0.01. All patients with AKI, in both groups, received renal replacement therapy and, in all patients, the renal function recovered before ICU discharge. No other relevant side effects related to antibiotic therapy were observed in both groups. All patients, 100% (95% CI: [69–100]%) in the Protocol group were

discharged alive from ICU, while, in the Control group, 36.4% (95% CI: [17–59]%) of patients survived, $p < 0.01$, Table 3.

TABLE 3 | Outcomes in the study population.

Outcomes	Protocol* (*n* = 10)	Control° (*n* = 22)	p-value**
Negativization, n (%)	10 (100)	8 (36.4)	<0.01
Complication – AKI, n (%)	4 (40)	1 (4,5)	0.01
ICU Survivors, n (%)	10 (100)	8 (36.4)	<0.01

**Protocol = colistin 9 million IU + 4.5 million IU every 12 h, intravenous tigecycline 100 mg every 12 h, intravenous ampicillin/sulbactam 12 gr per day and inhaled colistin 3 million IU every 6 h.*
°Control = nebulized and intravenous colistin alone or combined with an antibiotic of another class.
***Chi-squared test or Fisher's exact test, as appropriate. AKI = acute kidney injury.*

Discussion

The present brief report aimed to retrospectively describe the evolution of the superinfection from PDR Acinetobacter baumannii in patients with SARS-CoV-2 infection admitted to ICU and assess the incidence of negativization between patients treated with the combination of at least three antibiotics, according to the protocol applied in our ICU, and those who received a combination of two antibiotics. Our study shows that all patients in the Protocol group had microbiological negativization together with the clinical resolution of the infection and all of them were discharged alive from ICU. Considering the side effects of the antibiotic therapy, the patients in the Protocol group had a significantly higher incidence of AKI, which was managed in all cases with renal replacement therapy. However, the renal function recovered without sequelae in all patients before ICU discharge. Regarding the outcome, it is important to mention that the causes of death of patients in the Control group were not exclusively related to the complications of the PDR Acinetobacter baumannii superinfection. In fact, this study was focused on this single specific infection and the impact of this treatment protocol on microbiological negativization. No other co-infections, as well as other possible complications, were considered and the study itself was not designed to assess a cause-effect relationship with the outcome. However, considering

the impact of this superinfection, the fact that all patients in the Protocol group survived was important to point out.

Carbapenem-Resistant Acinetobacter baumannii, as well as Enterobacterales and Pseudomonas aeruginosa resistant to carbapenems, were first on the WHO's list of resistant bacteria for 2016-2017 as they threaten public health globally (27). In particular, among the 12000 annual infections in the United States, more than 60% of them were caused by MDR Acinetobacter baumannii, as remarked by the American CDC report in 2013 (28). The management of MDR Acinetobacter baumannii is currently based on carbapenems if the isolated microorganisms show susceptibility to this antibiotics class (17). With regards to XDR Acinetobacter baumannii, it is associated with a mortality rate higher than 50% (29, 30). Its recommended treatment consists of polymyxins and tigecycline. Whether colistin alone or in a combined therapy gives advantages or not, is still debatable (17, 30). As regards tigecycline, although standard doses did not seem to have an effect, high-dose tigecycline, defined as a loading dose of 200 mg followed by 100 mg every 12 h, lead to better results in terms of outcome (31).

A recent metanalysis by Jung et al., regarding MDR/XDR Acinetobacter baumannii, showed that sulbactam, both at a normal and at a high dose, had the best survival benefit. Fosfomycin and colistin came second, followed by a combination of colistin given both by inhalation and intravenously, while monotherapy with high-dose of tigecycline and colistin came last (32). Only sulbactam showed activity against Acinetobacter baumannii but in most European countries, such as Greece and Italy, the only available combination is ampicillin/sulbactam (33). As Acinetobacter baumannii becomes Pan-Drug Resistant, treatment options significantly decrease in number. The problem of Pan-Drug Resistant Gram-negative bacteria is increasing worldwide, but the management of the PDR Acinetobacter baumannii infections is particularly hard (21). Karakonstantis et al., in their cohort study, showed that in-hospital mortality is significantly higher in patients with PDR Acinetobacter baumannii infections compared to those with PDR

Acinetobacter baumannii colonization (34). Moreover, typically affecting patients with critical illness, multimorbidity, and exposure to invasive procedures, the PDR Acinetobacter baumannii infections considerably prolong the length of hospitalization (34). To cope with the lack of effective treatments available, several studies have been performed to establish the effectiveness of current options available such as ceftazidime/avibactam and ceftolozane/tazobactam, but they did not show any significant advantages (35). Following this, if some data regarding the effectiveness of antibiotics against XDR Acinetobacter baumannii exist, no clinical data are available for PDR Acinetobacter baumannii (36–38). For this reason and given the difficulty in treating PDR Acinetobacter baumannii, Assimakopoulos et al. used a combination therapy, which seemed to have promising results in vitro. By administering colistin both nebulized and intravenous with high-dose tigecycline and high-dose ampicillin/sulbactam, they demonstrated a high rate of clinical response and the hitherto highest percentage of survival at 28 days (90%) (22).

Nonetheless, we must mention one of the newest cephalosporins, cefiderocol, which was inserted into the list of antimicrobials suitable for MDR Gram-negative infections, mainly for MDR Acinetobacter baumannii (39). As Bassetti et al. remarked in their review, in Europe, it has been used since 2020, while in the United States it was already approved in 2019 (39). Given its high costs and its initial no-refunds policy in Italy, it was not used routinely. The Italian Drug Agency (AIFA) did not approve its refundability until June 2021 and only for patients with limited or no further options of treatment. Nevertheless, cefiderocol showed advantageous clinical cure rates compared to the best available therapy in Gram-negative pneumonia caused by carbapenem-resistant Enterobacteriaceae, complicated urinary tract infections, bloodstream infections, and sepsis. Despite this, the all-cause mortality was found higher in patients treated with cefiderocol (39). Therefore, its use is restricted to those aged equal to or more than 18 years old with no other options (39).

Our study has some limitations. First of all, the retrospective design of the study does not allow to control for all confounding factors. Moreover, our attention, as mentioned above, was focused on a single infection and the impact of the treatment protocol, applied in our ICU, on microbiological negativization: we did not collect data about other possible co-infections. Regarding the side effects of antibiotic therapy, it was difficult to assess and report the exact incidence of the neurotoxicity of colistin. It is established that colistin, interacting with neurons, can cause a wide spectrum of neurological manifestations, such as peripheral and orofacial paresthesias, visual disturbances, vertigo, mental confusion, ataxia, and seizures (40). All these manifestations are difficult to assess in patients sedated and intubated in ICU. Furthermore, it is now known that the SARS-CoV-2 infection itself can lead to neurological effects (41) as well as hospitalization in ICU, which is related to the "critical illness polyneuropathy" (40). Furthermore, to date, we have not yet collected the data on COVID-19 patients, admitted to our ICU in a period following the study population, who developed PDR Acinetobacter baumannii superinfection, treated with cefiderocol. It may be useful to compare the two treatment strategies in terms of effectiveness and side effects.

Conclusion

Our brief report shows microbiological negativization as well as the clinical resolution of the PDR Acinetobacter baumannii superinfection in all patients treated with the combination therapy of nebulized and intravenous colistin, high-dose tigecycline, and high-dose ampicillin/sulbactam. This combination of antibiotics seems to be a useful alternative to eradicate PDR Acinetobacter baumannii when cefiderocol is not easily accessible or may fail therapeutically.

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Chapter 10

Discussion and conclusions

As mentioned in the introduction, this thesis collects a series of articles in which it is possible to identify a before and an after. The event that establishes this before and this after is the pandemic. The pandemic has suddenly stopped the routine of most intensive care units and all the research activity conducted up to that moment. Intensivists faced an enormous challenge. On the one hand, they were busy managing the huge number of critically ill patients resulting from SARS-CoV-2 infection, applying knowledge learned from other pandemics or other diseases; on the other hand, they tried to conduct valid research to understand the pathophysiology and the best treatment strategy of a new disease which immediately showed peculiar characteristics, not comparable to any other pathology. Conducting valid research was not easy, especially at first. The intensivists came from years in which research had made enormous progress, allowing the passage from "poorly characterized" to "personalized" medicine.[1]

1. From "poorly characterized" to "personalized" medicine in ICU.

Personalized medicine represents the integration of molecular data with clinical data from individual patients to enhance diagnosis and treatment and tailor the management to the individual characteristics of each patient.[2,3] The concept of personalized medicine focuses on the individual and the disease, taking into account the inter-individual differences, making possible more effective, targeted treatments, aiming to "treat the individual and not the disease".[2] Over the last decades, thanks to a profound revolution of research, often more integrated with technology, critically ill patients have become beneficiary of personalized medicine.[4] Patients admitted to the ICU present different physiological statuses, determined by a certain genetic profile and characterized by biomarkers, specific to the individual and not exclusive to the disease.[5] Moreover, they have a series of multiple and intricate dysfunctions as well as complex and rapidly changing pathophysiological mechanisms underlying their disease[4], which sometimes overlap

with pre-existing different pathological conditions. *Can Ince* propose that personalized medicine is composed of four pillars relevant to the critically ill patient.[5] Pillar 1 is defined by the frailty and fitness of patients and their physiological reserve to cope with the stress of critical illness and therapy.[5] Pillar 2 involves monitoring the key physiological variables of the different organ systems and their response to disease and therapy.[5] Pillar 3 concerns the evaluation of the success of resuscitation by assessment of the hemodynamic coherence between the systemic and microcirculation and parenchyma of the organ systems.[5] Pillar 4 is defined by the integration of the physiological and clinical data into a time-learning adaptive model of the patient to provide feedback about the function of organ systems and to guide and assess the response to disease and therapy.[5] Concerning Pillar 3, it is important to mention that many critical conditions can cause the loss of hemodynamic coherence, among which is sepsis. During sepsis, the inflammatory mediators and the oxidative and nitrosative stress factors cause endothelial and erythrocyte injury resulting in obstruction of the capillaries.[6] This causes a heterogeneous microcirculatory flow and functional shunting in parts of the microcirculation, resulting in reduced oxygen extraction capability.[6] This loss of hemodynamic coherence can occur when all the factors affecting the microcirculation are not corrected by resuscitation procedures.[5] The assessment of the presence or absence of hemodynamic coherence requires the simultaneous measurement of the response of the macro- and the microcirculation.[5] The first two studies, presented in Chapter 1 and Chapter 2, aimed to assess the effects of additional therapies for sepsis on microcirculation. We demonstrated that both the treatment with hemoadsorption with Cytosorb and the therapy with IgM-enriched immunoglobulins can improve microvascular perfusion. The latest *Surviving Sepsis Campaign guidelines* made no recommendations on blood purification techniques and suggested against using intravenous immunoglobulins.[7] These indications were based either on the results of poor-quality studies at high risk of bias or the negative results of trials or meta-analyses. In this way, *Cohen et al.* suggested changing or reconsidering several aspects, among which the choice of the primary outcome and the patient selection, of the trial design of studies on sepsis to improve the

quality and maximize the possibility to identify effective treatments.[8] Indeed, as demonstrated in our studies, these additional treatments could be beneficial in improving the microcirculation in selected patients. The need to divide septic patients according to their characteristics and identify subgroups has been remarked on in several studies.[1,9] We underlined also in our study, presented in Chapter 4, the importance of patient selection who may benefit from the different therapies proposed for sepsis. We demonstrated that patients with norepinephrine-dependent sepsis admitted to our ICU after the institution of a patient-tailored protocol showed significantly lower mortality in comparison to a group of patients admitted before the application of this protocol. In this study, we hypothesized that all these adjunctive treatments could show the best impact on outcome if administered to selected patients following an individualized, patient-tailored approach. Over the years, has become clear how the characterization of patients and, consequently, the creation of subgroups request more than physical signs and physiological variables.[1] Multiple biomarkers have been proposed and studied but none was adeguate in terms of specificity. For this reason, the identification of subgroups should consider a combination of clinical and laboratory parameters and different biomarkers, among which some are possibly new. In our study, presented in Chapter 3, although the limitations, we identify the possible role of MR-proADM as potential biomarker in predicting the microvascular response to infections, sepsis, or septic shock. We observed that patients, in which MR-proADM cleared, showed a substantial stability of the microcirculation toward the first 5 days and an improvement in the SOFA score, while the opposite group suffered a deterioration of sublingual microcirculation in terms of the MFI and showed the statistically higher SOFA score at day 5. These results need to be confirmed by further studies and the challenges for the future will be the definition of panels of biomarkers, combined with integrated clinical and instrumental data, able to characterize specific subgroups of patients and the transfer of these informations into the clinical practice.

2. What has happened with the pandemic?

From the beginning of the pandemic, SARS-CoV-2 affected every aspect of medical practice in ICU and research. The intensivists, at first, attempted to apply knowledge from other pandemics or other diseases to optimize the management of COVID-19 critically ill patients. Initially, they tried to treat COVID-19 pneumonia as a "classic" ARDS, but soon, thanks also to the first reports, they realized that it had some peculiar characteristics. *Gattinoni et al.* proposed, very early from the beginning, the presence of two types of patients ("non-ARDS," type 1, and "ARDS", type 2) with different pathophysiology and distinguishable by CT scans.[10] They also described the (micro)thrombosis and the associated ischemic events, suggesting a daily check of coagulation parameters, including D-dimer levels.[10] It became clear how important it was to change the approach to research in the intensive care setting, returning to pathophysiology. For this reason, we tried to apply the tools we had available to obtain some more information on this pathology. We conducted two studies, presented in Chapter 5 and Chapter 7, and we cooperated with a pediatric intensive care unit to integrate the information and obtain the results of a small case series, presented in Chapter 6. With our reports, we supported the link between coagulopathy and microvascular perfusion disturbances in patients with SARS-COV-2 severe pneumonia and we found a relationship between capillary density and D-dimer level, suggesting a potential role of endothelial dysfunction to determine microvascular alterations. Our first results were then confirmed by subsequent studies, which highlighted the different mechanisms of endothelial dysfunction, including reduced nitric oxide (NO) bioavailability, oxidative stress, endothelial injury, glycocalyx/barrier disruption, hyperpermeability, inflammation/leukocyte adhesion, senescence, hypercoagulability, thrombosis.[11] Thus, COVID-19 has been deemed a (micro)vascular and endothelial disease[11], that in a variable percentage of patients required endotracheal intubation and mechanical ventilation[12]. Having clarified some pathophysiological aspects, the research then concentrated on identifying the risk factors for mortality, with more

structured studies and larger populations. Possible effective treatments already available were tested, as well as new drugs. Acute and chronic complications were described to implement all the strategies aimed at preventing or managing them. In this way, we conducted two studies, presented in Chapter 8 and Chapter 9, in which we investigated the risk factors for ventilator-associated pneumonia (VAP) and described an effective antibiotic treatment strategy, adopted in our ICU. We found that in mechanically ventilated patients with SARS-CoV-2 pneumonia the administration of O_2 was often excessive in comparison to the PaO_2 target indicated by the guidelines, and the exposure to hyperoxemia was frequent. In addition, most episodes of hyperoxemia were not followed by a reduction in the FiO_2, and the prevalence of hyperoxemia was independently associated with a greater risk of ICU mortality, as well as with a greater risk of developing VAP. From the literature, it is known that for causative agents the most common pathogens include *Staphylococcus spp., Enterococcus spp., Klebsiella pneumonia, Enterobacter spp., Escherichia coli, Acinetobacter spp.*, and *Pseudomonas spp.*[13] and *Multi-Drug Resistant Acinetobacter baumannii* (MDR-AB) represents a causative agent for almost half of Ventilator-Associated Pneumonia (VAP).[14] Considering the high mortality rate in patients with *Acinetobacter Baumannii* infections [15], we applied an antibiotic treatment protocol in our ICU and we observed a microbiological negativization as well as the clinical resolution of this superinfection in all COVID-19 patients treated with the combination therapy of nebulized and intravenous colistin, high-dose tigecycline, and high-dose ampicillin/sulbactam. With these two studies, we tried to share useful information about the prevention and the management of this possible complication of COVID-19 critical illness. The future perspective and the evolution of the research into the field of COVID-19 could be the application of the concept of personalized medicine.

3. *Conclusions*

This thesis has presented the progress of the approach to critical illness and the research from sepsis to COVID-19, highlighting the challenges for the intensivists of the past and present.

The key message is that research should always be conducted to provide clinically applicable and useful information.

These three years have taught me that the center of the intensivist's practice, both clinical and research, must always be the patient, with unique history and characteristics.

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