Early initiation of Extracorporeal Blood Purification using the AN69ST (oXiris®) hemofilter as a treatment modality for COVID - 19 patients: a single-centre case series

Petar Ugurov
Zan Mitrev Clinic
Dijana Popevski
Zan Mitrev Clinic
Tanja Gramosli
Zan Mitrev Clinic
Dashurie Neziri
Zan Mitrev Clinic
Dragica Vuckova
Zan Mitrev Clinic
Emil Stoicovski
Zan Mitrev Clinic
Lidija Veljanovska-Kiridjievska
Zan Mitrev Clinic
Katerina Ignevska
Zan Mitrev Clinic
Sanja Mehandziska
Zan Mitrev Clinic
Elena Ambarkova
Zan Mitrev Clinic
Rodney Alexander Rosalia (rodney.rosalia@zmc.mk)
Zan Mitrev Clinic https://orcid.org/0000-0002-7763-092X
Zan Mitrev
Zan Mitrev Clinic

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Abstract

Introduction: Our understanding of the COVID-19 disease has been steadily evolving since the original outbreak in December 2019. Advanced disease is characterised by a hyperinflammatory state, systemic coagulopathies and multiorgan involvement, in particular respiratory distress.

We here describe our initial experience with treating of COVID-19 patients based on early initiation of extracorporeal blood purification, systemic heparinisation and respiratory support.

Methods: 15 patients were included; 2 were females. We monitored real-time several biochemical, immunological and coagulation biomarkers associated with disease severity following admission to our dedicated COVID-19 intensive care unit. To guide personalised treatment, we monitored among others levels of IL-6, IL-8, TNF-α, C-Reactive Protein (CRP), Neutrophil-to-Lymphocyte ratios, Thrombocyte counts, D-Dimers, Fibrinogen, and Activation Clotting time (ACT).

Treatment consisted of individualised respiratory support supplemented with 1 - 4 cycles of 24-hour Extracorporeal Organ Support (ECOS) and Blood Purification using the AN69ST (oXiris®) hemofilter. We administered heparin (300 U/kg) to counter suspected hypercoagulability (= elevated Fibrinogen or D-dimers) states to maintain ACT ≥ 180 seconds.

Results: N = 10 presented with severe to critical disease (= dyspnoea, hypoxia, respiratory rate > 30/min, peripheral oxygen saturation < 90%, or > 50% lung involvement on X-ray imaging). A single case was admitted with a critical condition (= respiratory failure). One patient died after 5 days of hospitalisation after developing Acute Respiratory Syndrome. 8 Patients have been discharged - average ICU length-of-stay was 9.9 ± 2.4 days. Clinical improvement was associated with normalisation (increase) of thrombocytes, white blood cells, stable levels of IL-6 (< 50 ng/mL) and a decrease of CRP and Fibrinogen.

Conclusion: Means to monitor COVID-19 disease severity during hospitalisation are crucial to control disease progression and prevent hyperinflammation and irreversible multiorgan failure. We present here a real-time monitoring system accounting for biochemical, immunological, coagulation parameters and radiological imaging.

The combination of systemic heparin anticoagulation regimens and blood purification may prevent hyperinflammation, thromboembolism during hospitalisation and thus support clinical recovery.

Introduction

The current coronavirus disease 2019 (COVID-19) pandemic is manifesting itself as an unprecedented threat to the global population. The outbreak of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) started in Wuhan of Hubei Province, China [1]. Since then, it has spread in a rapid, deadly, pace throughout the world instigating the WHO to classify COVID-19 disease a global epidemic on February 28, 2020 [2]

Severe COVID-19 disease is characterised by (infectious) pneumonia; complications typically include acute respiratory distress syndrome (ARDS) [3]. COVID-19 has also been linked with acute cardiac injury [4, 5], kidney malfunction [6] and secondary infections [7].

COVID-19 disease progression is associated with dysregulated immunity, commonly referred to as cytokine storm [8], in particular aberrant IL-6 levels [9–12] that promote numerous pathological downstream effects. Hyperinflammation is a well-established trigger of multiorgan failure, for example, acute kidney injury. Moreover, recent reports point to a link between hyper inflammation and COVID-19 induced coagulopathy [13, 14] as a result of increased production of clotting factors by the liver [15].

Despite several lines of evidence pointing to a potential clinical benefit of controlling hyper inflammation triggered by COVID-19 [8], management of COVID-19 remains mostly supportive built around continuous respiratory support. [16–19]

To this end, considering the underlying immunological character of COVID-19 disease and the high risk of SARS-CoV-2 hyper inflammation to trigger ARDS, hypercoagulability and AKI, we have established a treatment protocol for COVID-19 disease. We follow selected biochemical, immunological and coagulation risk factors to tailor therapy; our approach centres around the 1) early initiation of blood purification using the oXiris® (AN69ST) filter [20, 21] 2) systemic heparinisation and 3) respiratory support, CPAP and physical therapy [22].

With this initial report, we present a preliminary overview of biochemical, immunological, inflammatory, and coagulation biomarkers assessed, and offer insights into their correlations with clinical status. Finally, we report the early results in regards to treatment outcome.

Materials And Methods

This single-centre case series included 15 consecutive patients with confirmed COVID-19 treated in June 2020. The study designed is presented in the *STrengthening the Reporting of OBservational studies in Epidemiology* (**STROBE**) diagram, **Figure 1**.

Patients were classified according to their clinical presentation in 4 severity degrees:

1. **Mild cases**

The clinical symptoms are mild, with no apparent sign of pneumonia on imaging.

2. **Moderate cases**

Showing fever and respiratory symptoms with radiological findings of pneumonia.

3. **Severe cases**

    A. Respiratory distress (30 breaths/min)

B. Oxygen saturation < 90% at rest

C. Arterial partial pressure of oxygen (PaO_2)/ fraction of inspired oxygen (FiO_2); 300mmHg (1 mmHg = 0.133 kPa).

Cases with chest imaging that show lesion > 50% progression within 24 hours shall be managed as severe cases.

4. **Critical cases**

A. Respiratory failure requiring mechanical ventilation

B. Shock

C. With organ failure that requires ICU care.

Inclusion Criteria:

- Written or (temporary, verbal) informed consent
- Adult > 18 years
- Confirmed COVID-19 Pneumonia using; RT-PCR, X-Ray and/or Computed Tomography

Exclusion Criteria:

- Pregnancy
- Heart failure; severe systolic dysfunction, left ventricular ejection fraction < 25% requiring urgent surgery
- Aortic Aneurysms, dissection or rupture requiring urgent surgery
- Recent Myocardial Infarction; cardiovascular disease patients requiring urgent surgery

Biochemistry Analysis

Blood samples were collected from each patient at the time-points shown for routine blood analysis: White blood cell count (WBC), Lymphocyte count (LYM), Neutrophil count (NEU), Thrombocyte count (PLT), Monocyte count (MONO) and Eosinophil count (EO) were determined as well as the Neutrophil-to-Lymphocyte ratio (NEU/LYM) and the systemic immune-inflammation index PLT*(NEU/LYM). Moreover, blood biochemistry parameters such as Na^+, K^+ Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Bilirubin, Urea, C-Reactive Protein (CRP), as well as Procalcitonin, lactate dehydrogenase (LDH), were assessed using Siemens ADVIA Centaur XP Immunoassay System.

Data on coagulation parameters were obtained from all patients; coagulation tests included D-dimers, Fibrinogen (FIB) and international normalised ratio (INR). Tests were performed using a Sysmex CA-600 automatic coagulation analyser.

Luminex

Analyses of human cytokines IL-6, IL-8/CXCL-8 and TNF-α in serum samples were performed as described elsewhere using the Human Magnetic Luminex® assay (R&D Systems, USA), According to the manufacturer's instructions. The measurements were performed in triplicates using a Luminex® 100/200 System.

Statistical analysis

Categorical parameters were summarised as absolute numbers and percentages. Continuous data are shown as mean ± SD; alternatively, non-parametric data are presented as median + Interquartile range (IQR). Continuous variables were evaluated using the D'Agostino and Pearson normality test. The data were analysed with the statistical programs GraphPad Prism, version 7.03.

Treatment

The treatment protocol is shown in **Figure 2** and follows practice safety recommendations, treatment strategies and up-to-date sepsis management guidelines [23-26].

The multidisciplinary care and therapeutic approach consist of the early initiation of blood purification using the AN69ST (oXiris®) hemofilter initiated within 4 - 12 hours of admission and high dosed heparinisation. We opt for an aggressive non-invasive respiratory therapy, including Continuous Positive Airway Pressure (CPAP) on-mask, physical therapy in an attempt to avoid mechanical ventilation. In the case of secondary infections, we administer targeted antibiotic therapy.

- **Extracorporeal Organ Support (ECOS) and Blood Purification**

The Prismaflex® oXiris® system was mounted in the ICU and connected 4 - 12 hours after admission upon establishing control of the haemostasis, ACT = Activated Coagulation Time of 180 secs. The patient is connected to the Prismaflex® oXiris® system via a double lumen catheter placed in the femoral vein or vena subclavia.

Flow rates were maintained as follow; effluent dose 35 mL/Kg/h, dialysate 14 – 16 mL/Kg/h, blood 150 mL/min, replacement 16 -18 mL/Kg/h; patient fluid removal is tailored to the individual's volume status, ≈ 100 - 250 mL/h. Blood purification is initiated within 4 - 12 hours of admission and the oXiris® ECOS modality was chosen according to the patient's kidney function; continuous venovenous hemofiltration (CVVH), continuous venovenous hemodiafiltration (CVVHDF) or slow continuous ultrafiltration (SCUF).

- **Heparinisation**

An initial 25000 IU bolus injection (≈ 300 IU/kg) followed by continuous infusion of 300 IU/kg dissolved in physiological buffer (0.9% NaCl) administered at 6 - 8 mL/h flow rate; target ACT ≥ 180s during hospitalisation.

- **Respiratory support:**

Oxygen therapy: Patients with severe symptoms should receive nasal cannulas or oxygen masks and timely assessment of respiratory distress and/or hypoxemia should be performed

Non-invasive ventilation (NIV): CPAP on mask for patients with SpO_2 86-90%; prone position.

Invasive mechanical ventilation: Lung protective ventilation strategy, namely low tidal volume (4 - 6ml/kg of ideal body weight) and low level of airway platform pressure (< 30 cm H_2O) should be used to perform mechanical ventilation to reduce ventilator-related lung injury.

While the airway platform pressure maintained 30 cm H_2O, high positive end-expiratory pressure (PEEP) can be used to keep the airway warm and moist. Sedation and muscle relaxants were used according to the clinical condition and preferably in a prone position. Furthermore, anaesthesia regimens are tailored to promote early weaning from mechanical ventilation.

Antibiotic Therapy:

- Empiric administration of Azithromycin in the first 48 hours; antibiotic therapy is discontinued, switched to targeted according to the antimicrobial susceptibility testing [27, 28].

Medical therapy:

Individual medical therapy was continued according to the patient's pre-existing conditions and comorbidities.

Results

We admitted in a total of 15 patients with confirmed SARS-CoV-2 infection manifesting as COVID-19 were treated at our clinic in June 2020. Table 1. shows the basic patient characteristics. Of the 15 cases, 2 were females – the mean age of the cohort was 60.2 years (range 27–83). The patients were referred to us from peripheral hospitals across the country.

Table 1
Basic Patient Characteristics

Age (years)	**60.2 ± 12.8**
Female gender (%)	2 (13%)
BSA (m^2)	1.9 ± 0.14
BMI	26.7 ± 2.4
Diabetes	2
Hypertension	6
Obesity (BMI > 35 Kg/m^2)	2
Glucose (mmol/L)	6.6 (5.7–12.8)
Creatinine (µmol/L)	70.9 ± 14.3
Urea (mmol/L)	4.5 (3.1–6.2)
Aspartate transaminase (U/L)	58.9 ± 23.2
Alanine transaminase (U/L)	75.1 ± 40.4
Bilirubin (µmol/L)	6.68 ± 0.73
Lactate dehydrogenase (U/L)	330.5 (258.8–453.5)
Hemoglobulin (g/dL)	13.2 (12.3–14.0)
Hematocrit (%)	37.90 (36.30–40.40)
Na^+ (mmol/L)	137.3 ± 2.6
K^+ (mmol/L)	3.8 ± 0.54
Procalcitonin (ng/mL)	0.07 ± 0.04
C-Reactive Protein (mg/mL)	74.1 (55.1–127.8)
White Blood Cell counts (*10^3 counts/µL)	6.3 (2.9–10.1)
Platelets (*10^3 counts/µL)	140 (108 to 208)
NEU (%)	83.11 (64.3–89.2)
LYM (%)	9.8 (7.3–21.5)
MONO (%)	3.3 (2.6–6.9)

NLR = Neutrophil (NEU)-to-lymphocyte (LYM) ratio

SII = Systemic immune-inflammation index, PLT*(NEU/LYM)

Age (years)	60.2 ± 12.8
EO (%)	0.1 (0.04–0.44)
NLR (*10^3 counts/µL)	8.3 (3.5–12.2)
Systemic immune-inflammation index	1311 (406.2–2791)
D-dimers (ng/mL)	790.0 (395.0–1980) (1980
Fibrinogen (g/L)	7 (3.6–8)
NLR = Neutrophil (NEU)-to-lymphocyte (LYM) ratio	
SII = Systemic immune-inflammation index, PLT*(NEU/LYM)	

Primary symptoms reported were dyspnea, fever and low peripheral saturation; 10 cases presented with severe disease; all patients had advanced COVID-19 pneumonia Fig. 3.

Patients presented with elevated levels of CRP, 74.1 mg/mL (IQR55.10–127.8), mild thrombocytopenia 140*10^3 counts/µL (IQR108–208) and significantly increased values of D-dimers 790.0 ng/mL (IQR395–1980) and Fibrinogen 5.8 ± 2.4 g/L. Lactate Dehydrogenase and NLR were elevated at admission with values of 330.5 IU (IQR 258.8–453.5) and 8.3 (IQR3.5–12.2), respectively (Table 1). Two patients were intubated within 24 hours of admission; one of these cases did not recover and died on the 5th hospitalisation day. The single mortality case was admitted with signs of acute respiratory distress syndrome (ARDS) and multiorgan failure.

Treatment led to a gradual normalisation of biochemical parameters (Fig. 4); in particular, we observed a linear trend, r = 0.40, [CI95% 0.21 to 0.57], p < 0.0001, between platelet numbers, white blood cells, r = 0.37, [CI95% 0.18 to 0.54], p = 0.0003) and the clinical picture during hospitalisation suggesting that an increase of thrombocytes was associated with recovery. A similar trend was observed for the white blood cells. In contrast, clinical recovery was associated with a decrease in Fibrinogen levels, r = -0.45, [CI95% -0.63 to -0.21], p = 0.0004 and CRP, r = 0.39 [CI95% -0.57 to -0.20] (Fig. 5A).

IL-6 is the primary cytokine leading to hepatic CRP production; we observed that early initiation of oXiris® blood purification was associated with stable, or decreasing levels of IL-6, IL-8 and TNF-α which in turn led to a gradual reduction of systemic CRP levels across the whole cohort (Fig. 4 **and** Fig. 6).

The treatment approach led to an improvement in SpO_2, decrease of inflammatory mediators and an increase in the number of thrombocytes.

In one particular case, a 50-year-old male admitted with a SpO_2 of 92% on 2L of oxygen (Fig. 2I **and J**) with previous episodes of high body temperature received in addition to the two cycles of oXiris blood purification 8 mg/kg Tocilizumab given over 120 minutes via intravenous infusion (Fig. 6B). The latter

was administered on the explicit, consented request of his family. Administration of IL-6r blocking antibody led to a transient spike of IL-6 levels as reported before [29]. He was also treated with Azithromycin which was adapted to Ciprofloxacin after multiplex RT-PCR identified Methylin-Resistant Staphylococcus aureus and Klebsiella pneumoniae; he was discharged after 15 days.

The clinical course was in some cases complicated because of bacterial co-infections; a case of a 56-year-old male with dyspnea, SpO_2 of 90% on room air and a body temperature of 38 °C at admission were challenging due to a Klebsiella pneumoniae infection.

The same pathogen was detected in a 70-year-old female. We also confirmed Streptococcus beta haemolyticus in her respiratory samples and vancomycin-resistant Enterococcus in urine samples taken within 24 after admission. We successfully treated her with two cycles of blood purification and antibiotics consisting of Azithromycin and Ampicillin/Sulbactam.

Another male presenting with high fever (38.8 °C), dry cough, dyspnea and SpO_2 of 85% had a co-infection of Streptococcus pneumoniae in his throat swabs detected using RT-PCR. We treated him with two cycles of oXiris® blood purification and Azithromycin. He was discharged after 8 days with markedly recovered symptoms: C-reactive protein level was 6.4 mg/L, white blood cell (WBC) count of 4.5 × 10^3/µL, and normalised platelet count was $186*10^3$/µL.

The single case of mortality was an 83-year-old male with dyspnoea, tachypnoea and extremely low SpO_2 of 65% despite 6L oxygen suggesting ARDS. Biochemical analysis revealed significant abnormalities; CRP was 279.9 mg/L and LDH of 671 U/L. He was immediately placed on extracorporeal blood purification; we also discovered Streptococcus beta haemolyticus in his throat, and nasal swab detected. Targeted therapy with Ampicillin/Sulbactam was initiated. However, despite intensive treatment including mechanical ventilation, and a total of 3 cycles of oXiris hemofiltration, the patient's condition deteriorated over the next days as he developed multiorgan failure; he passed away 5 days after his admission (Fig. 6H).

The 2nd critical case pertained to a 73-year-old male admitted with dyspnoea, tachypnoea and severely reduced SpO_2 < 70% on room air and elevated LDH was elevated at 527 U/L. Despite 2 cycles of oXiris blood purification SpO_2 levels were not improving. We were able to stabilise his condition with mechanical respiratory support. His condition is sensitive due to the discovery of Klebsiella pneumoniae in his bronchial secretion. For this reason, we switched antibiotherapy to include Ampicillin/Sulbactam. Still, despite systemic heparinisation, the levels of D-dimers (Fig. 5B) were increased to 31400 ng/mL on the 9th hospitalisation day. Towards the end of his $four^{th}$ oXiris® cycle, we observed notable improvements, and we were able to extubate him in the subsequent 48 hours (Fig. 6O).

In summary, our treatment approach based on early initiation of blood purification using the AN69ST (oXiris®) hemofilter, systemic heparinisation, respiratory support, targeted antibiotic therapy and real-time monitoring of biomarkers pointed supported clinical recovery.

Discussion

We present with this work our initial case series of 15 COVID-19 patients treated with early initiation of extracorporeal blood purification using the oXiris® (AN69ST) hemofilter, systemic heparinisation and respiratory support; we monitored several biochemical, immunological, inflammatory, and coagulation biomarkers to tailor therapy to the individual requirements.

In numbers, as of July 2020, there are 11.8 million confirmed cases of COVID-19 worldwide with an estimated mortality rate of 3.7% [3]. About 5% of the infected population will develop advanced disease requiring intensive care, often necessitating extracorporeal organ support therapies. Of this critically ill subgroup, the mortality rate is high 40–50% [6]. The role of lung injury in COVID-19 is well-established; however, recent observations point to a high risk for acute kidney injury (AKI) in COVID-19 patients [6] but also hypercoagulability [13], Several lines of evidence have implicated a role for pro-inflammatory cytokines in the pathology of COVID-19, especially advanced cases.

COVID-19 has shown to elicit a two-phase immune response; in the initial (asymptomatic, pre-incubation) phase the adaptive immune response plays a critical role in its attempt to kill infected epithelial cells and thereby by preventing viral replication [30]. The second phase points to a failure of the adaptive immunity to clear the virus; consequently, SARS-CoV-2 propagates. Interestingly, the cytokine profile seen in COVID-19 resemble observations previously reported for secondary hemophagocytic lymphohistiocytosis. The hyperinflammatory syndrome is characterised by fulminant and fatal cytokine storm leading to multiorgan failure. ARDS is a major complication in severe cases of COVID-19, affecting 20% − 41% of hospitalised patients. [31–33]. Respiratory failure from ARDS is recognised as one of the leading causes of mortality.

Ruan et al. described that the critically-ill patients had higher systemic levels of IL-2, IL-7, IL-10, GSCF, IP-10, MCP1-, MIP-1A, TNF-α and IL-6 [7]. Aberrant IL-6 levels were indicative of an adverse outcome. Another marker associated with disease severity and adverse outcomes is the NLR [34, 35]. In addition, hypercoagulability is now considered as one of the hallmarks of COVID-19 disease progression with both D-dimers [36] and Fibrinogen levels [37] suggested having predictive power in establishing disease severity [14].

The intensive monitoring of the aforementioned parameters (Figs. 4, <link rid="fig6">6</link> and <link rid="fig6">6</link>) guides our clinical practice and allows us to tailor our treatment to the acute needs of the patient. Treatment focuses on limiting lung injury and on promoting physiological breathing using daily intermittent physical therapy regimens combined with CPAP-ventilation and prone position. Secondly, hypercoagulability and possibility of thromboembolism were countered through systemic administration of high dosages of heparin to maintain ACT above 180 seconds. It is noteworthy to mention that even bolus dosages of 25000 IU were not sufficient to reach ACT values of > 200 seconds, pointing to severe dysregulation of the coagulation cascade in COVID-19 patients. Thirdly, hyper inflammation was controlled using oXiris® hemofilter based extracorporeal blood purification.

Control of systemic levels of cytokines (IL-6, IL-8/CXCL8/TNF-α) (Fig. 6) was achieved using the Prismaflex® system (Baxter International Inc. Deerfield, Illinois) mounted with the oXiris® hemofilter. The oXiris® filter is a hollow fibre acrylonitrile and methanesulfonate (AN69ST) membrane [20] that removes larger molecular weight molecules. Approved first in Europe in 2009, its initial CE-marked indication was extended in 2017 for patients who require blood purification, including those requiring continuous renal replacement therapy (CRRT), and in conditions with excessive endotoxin and inflammatory mediator levels. The system also received emergency FDA authorisation for COVID-19 Treatment in April [38].

The oXiris® filter uses a modified AN69ST membrane and has an affinity for both endotoxins and cytokines. The modified oXiris® membrane has 3-fold more polyethyleneimine for optimal endotoxin adsorption. Additional (10-fold) higher amount of immobilised heparin efficiently reduces thrombogenicity [39]. It has shown a superb capacity to adsorb cytokines and endotoxins [40] control abnormal levels of systemic cytokines [41] and improve haemodynamic parameters [42, 43]. To this end, our COVID-19 treatment bundle is based on the use of oXiris® blood purification to counter the multidimensional inflammatory attack on the body triggered by the SARS-CoV-2 virus.

Our treatment approach is built on our previous experience [44] and our ongoing partnerships with European experts on the treatment of sepsis and related infections diseases [45].

Blood purification has been evaluated in mechanically ventilated COVID-19 cases [46]. The authors reported promising results, an effective reduction in pro-inflammatory cytokines and recovery in most of the treated patients.

Our results further provide empiric evidence for the effectiveness of blood purification to prevent control and reduce hyperinflammation in COVID-19. However, our treatment approach differs on three key points, 1) the blood purification device; oXiris® vs CytoSorb®; the latter is a CE-marked device containing polymer beads to adsorb cytokines used in blood pump circuits vs the modified AN69ST membrane, 2) we performed blood purification in moderate to severe cases within 4–12 hours of admission, intending to prevent disease progression and the need of mechanical ventilation and 3) we use repetitive cycles whenever the inflammation markers are increasing.

The first cases of COVID-19 in the Republic of North Macedonia (NMK) were confirmed in early March 2020. The country has seen a sudden rise in the confirmed case since restrictions were lifted in May 2020; the number of cases is slowly outnumbering the national ICU-bed capacity.

We report here our initial case series, compared to global numbers a relatively small cohort. However, we only received permission from the Ministry of Health to hospitalise COVID-19 patients since June 2020. Confirmed COVID-19 patients between March and June were treated at the public clinic of infectious diseases.

Collectively, the work here presents a promising outlook on the treatment possibilities using a standardised procedure based on 1) control of excessive pro-inflammatory cytokines through early

initiation of blood purification (Fig. 6), 2) prevention of hypercoagulability through systemic heparinisation (Fig. 5) and intensive physical therapy combined with CPAP-respiratory support (Fig. 2).

Patients fared well using this approach and were discharged on average around the 10th day of hospitalisation. In summary, we observed that clinical recovery was associated with an increase in thrombocytes and white blood cells, whereas a decrease in CRP and Fibrinogen was observed in patients with improving clinical conditions.

Blood purification to control excessive inflammation has gained acceptance as a treatment modality for COVID-19 [21, 47] and was successfully used in one case [48].

Limitations

Our findings propose a base for further evaluation and should be appraised with caution due to the limitations of single centre observational studies [49] and the small cohort. Although we provide several lines of evidence that our treatment is clinically effective. The long-term assessment is warranted to establish the speed of complete recovery, especially the resolution of COVID-19 pneumonia and recuperation of lung function.

Conclusion

An early initiation of blood purification using the oXiris® hemofilter was effective in preventing aberrant pro-inflammatory levels of COVID-19 patients. Furthermore, we observed no cases of thromboembolism which might be linked to the systemic heparinisation regimen.

Collectively, we show that real-time digital monitoring of vital signs, biochemical, immunological, coagulation markers and X-ray imaging in COVID-19 patients offer the opportunity to track disease severity and tailor therapy based on cytokine-hemofiltration, heparin anticoagulation and respiratory support.

Finally, a multi-centre randomised study is warranted to adequately scrutinise the clinical effectiveness of extracorporeal blood purification in the treatment of COVID-19.

Abbreviations

IL
interleukin
TNF
tumor necrosis factor
CRP
C-reactive protein
ARDS

acute respiratory distress syndrome
AKI
Acute Kidney Injury
CPAP
Continuous Positive Airway Pressure
ECOS
Extracorporeal Organ Support
CVVH
continuous venovenous hemofiltration
CVVHDF
continuous venovenous hemodiafiltration (CVVHDF)
SCUF
slow continuous ultrafiltration
NIV
Non-invasive ventilation

Declarations

Ethics approval and consent to participate

The local ethical committee of the Zan Mitrev Clinic reviewed and approved the clinical practice, treatment procedures described and the results reported in this manuscript and approved the submission, #EBPZ.357. Trial registration: ClinicalTrials.gov, NCT04478539. Registered 14th of July 2020 - Retrospectively registered, https://clinicaltrials.gov/ct2/show/NCT04478539

Consent for publication

Written informed (or temporary verbal) consent was obtained from all patients for publication of this manuscript and any accompanying images; the use of all health and medical information for scientific research and manuscript preparation was approved. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Availability of data and material

All original data described in this case report can be submitted for evaluation upon reasonable request.

Competing interests

Dr Zan Mitrev is the hospital director at the *Zan Mitrev Clinic*.

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Authors' contributions

ZM is the study director; PU, DP, TG, DN, DV, ES, and ZM were responsible for diagnostics and patient care. L.V-K. performed the radiological examinations. SM and RR performed the cytokine analysis. RR provided academic assistance, coordinated data collection, analysed the data and wrote the manuscript with the assistance of PU, DP and DN.

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Figures

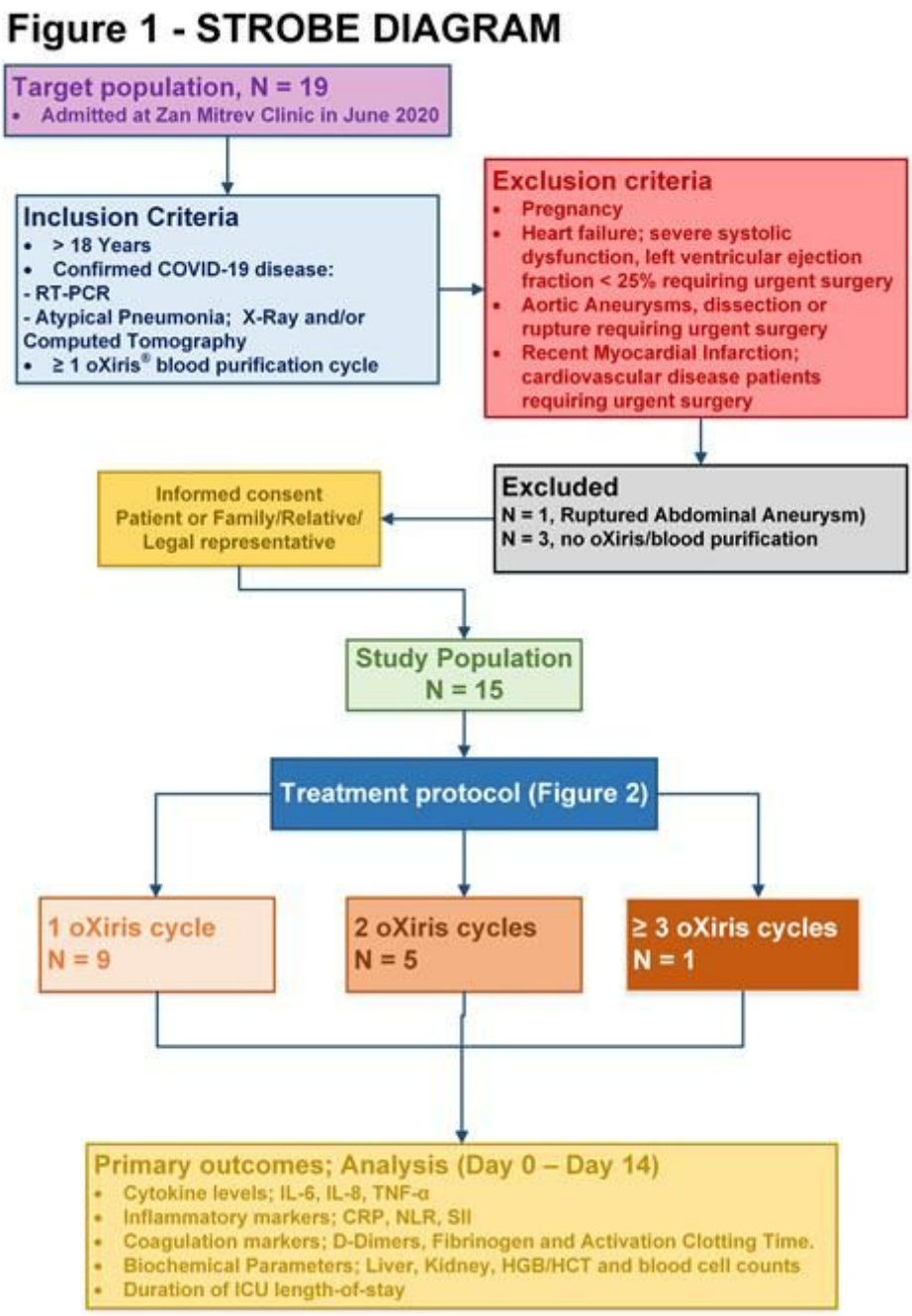

Figure 1

STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) diagram.

Figure 2 – Treatment Protocol

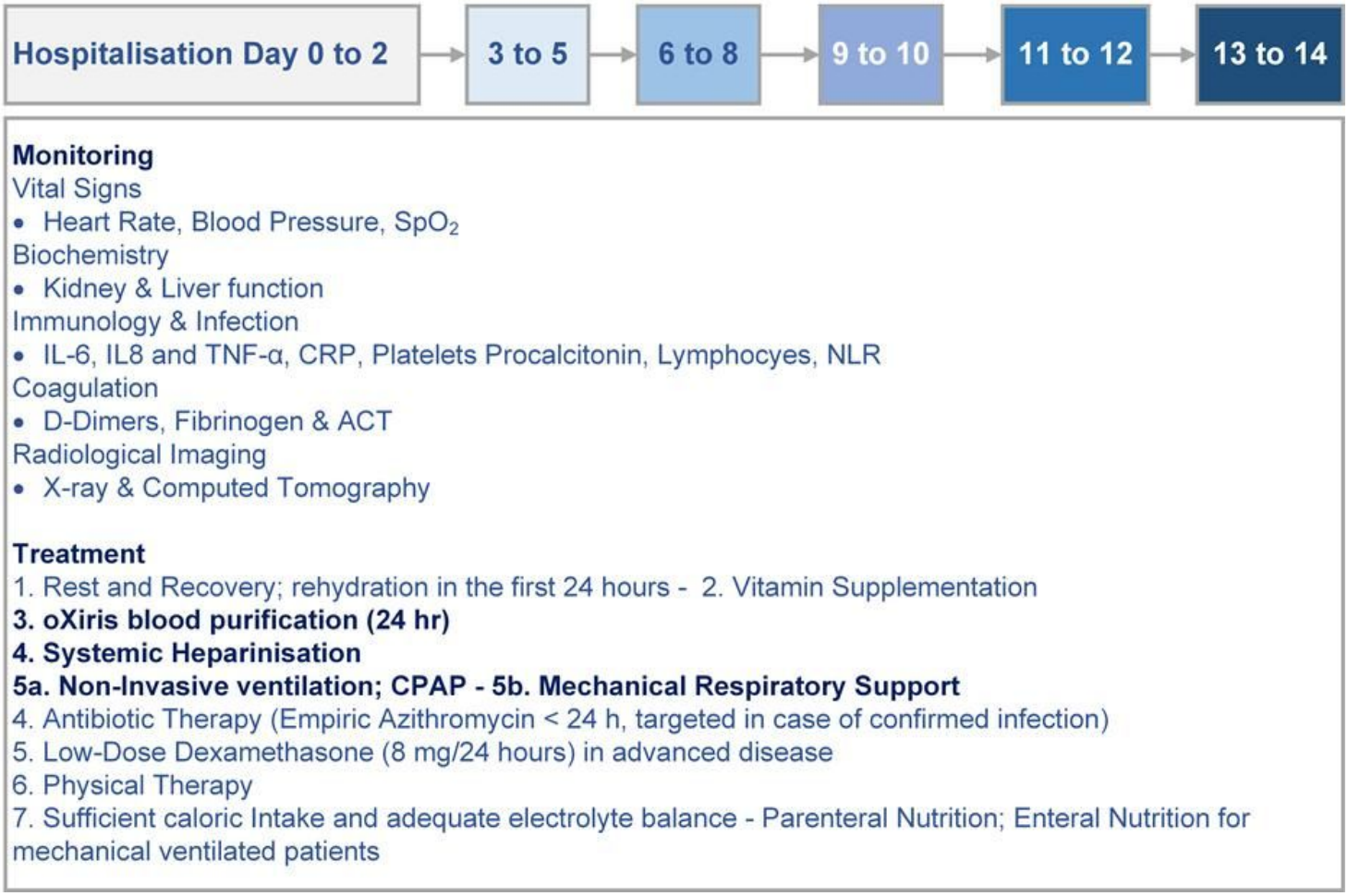

Figure 2

Treatment protocol for COVID-19 patients In addition to blood purification, systemic heparinisation and physical therapy under continuous positive airway pressure (CPAP), the general care protocol consisted of rest and recovery; sufficient caloric intake and adequate electrolyte balance; aggressive rehydration in the first 24 hours; parenteral, enteral nutrition for mechanically ventilated patients; low-dose Dexamethasone therapy (8 mg/24 hours), antibiotic therapy, and biochemical and chest X-ray imaging for monitoring

Figure 3

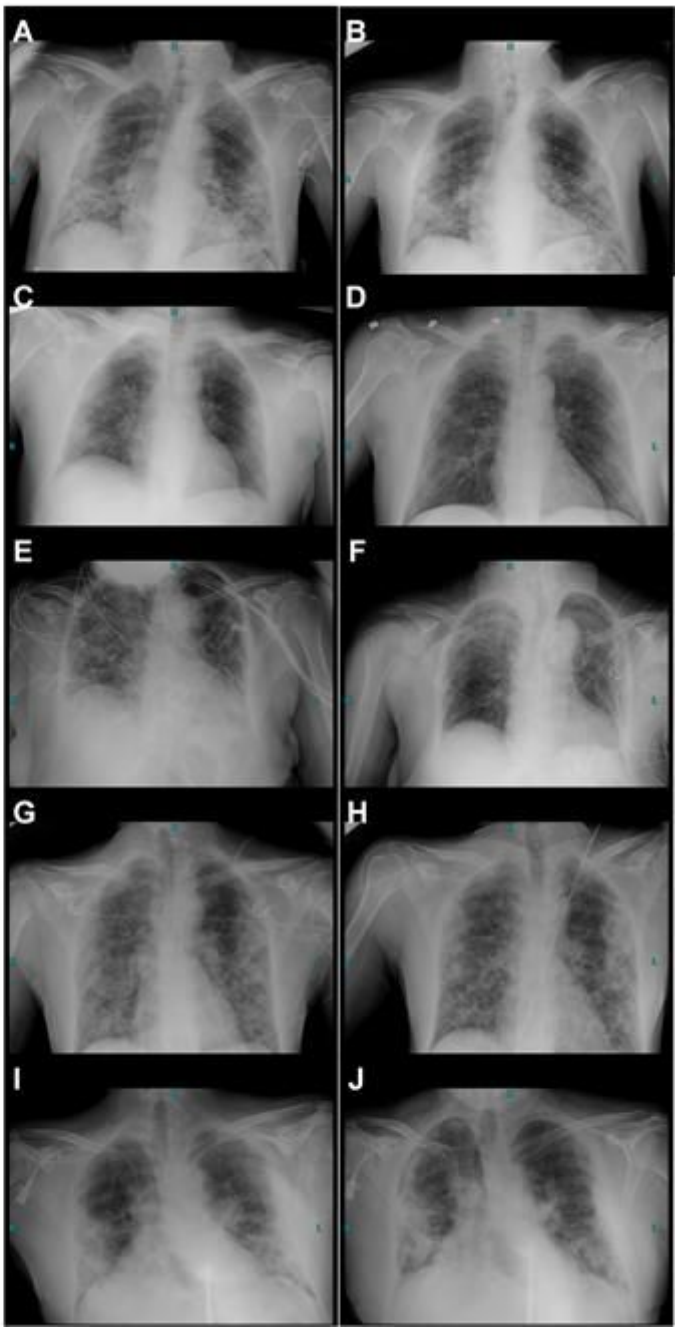

Figure 3

Pre- and Post-treatment X-ray images (A) A 62-year-old male (Fig 6M) was admitted with a SpO2 of 93%, fatigue and breathing difficulties. Before his admission, he had several episodes of high body temperature (39°C). chest radiography on admission showed bilateral patchy reticular areas of opacifications, perihilar and peripheral distribution with lower zone predominance and subsegmental atelectasis in the mid-zone of the left lung (B) Control x-ray showing minor regression of baseline findings. (C) A male of 67 years with severe COVID-19 was admitted with breathing difficulties and SpO2 of 85% and Staphylococcus aureus (cytokine profile shown in Fig 6F), we noted patchy bilateral areas of opacifications with lower zone predominance, right perihilar and left peripheral distribution (D) We discharged him after 10 days with significantly improved SpO2 96% and regression of x-ray findings. (E)

We admitted a 70-year-old hypertensive febrile (38ºC) female (Fig 6I) with SpO2 of 85%, dyspnoea, tachypnoea COVID-19 pneumonia; we observed bilateral reticulonodular areas of opacifications perihilar and peripheral distribution, with consolidation in upper right lung and evidence of right pleural effusion. Her condition was complicated because of Klebsiella pneumoniae, Streptococcus beta haemolyticus co-infection in respiratory samples and Vancomycin-Resistant Enterococcus in urine samples taken within 24 after admission. At discharge, we observed minimal regressions in findings of consolidation and resolution of the right pleural effusion (F). Panel (G) shows the first x-ray image taken of a 51-year-old male (Fig 6J); chest x-ray findings point to bilateral perihilar and peripheral extensive patchy opacifications and a prominent zone of consolidation in the mid- and upper peripheral section, the left lobe was more affected (H) Treatment resulted in the normalisation of peripheral oxygen saturation values. Still, x-ray images suggested a minor progression of initial findings; non-resolving bilateral consolidations with bigger consolidation zone in the left upper peripheral lung. Panel (I) shows patchy bilateral consolidations, perihilar and peripheral distribution of a 50-year-old male (Fig 6B) admitted with a SpO2 of 92% and previous episodes of high body temperature. He received two cycles of oXiris blood purification and on the explicit, consented request his family relatives he was also treated with 8 mg/kg Tocilizumab given over 120 minutes via intravenous infusion. (J) Chest X-rays showed progression; bilateral consolidations, pleural effusion and small apical pneumothorax in the right lobe. He was initially treated with Azithromycin which was adapted to Ciprofloxacin after multiplex RT-PCR identified Staphylococcus aureus and Klebsiella pneumoniae; he was discharged after 15 days.

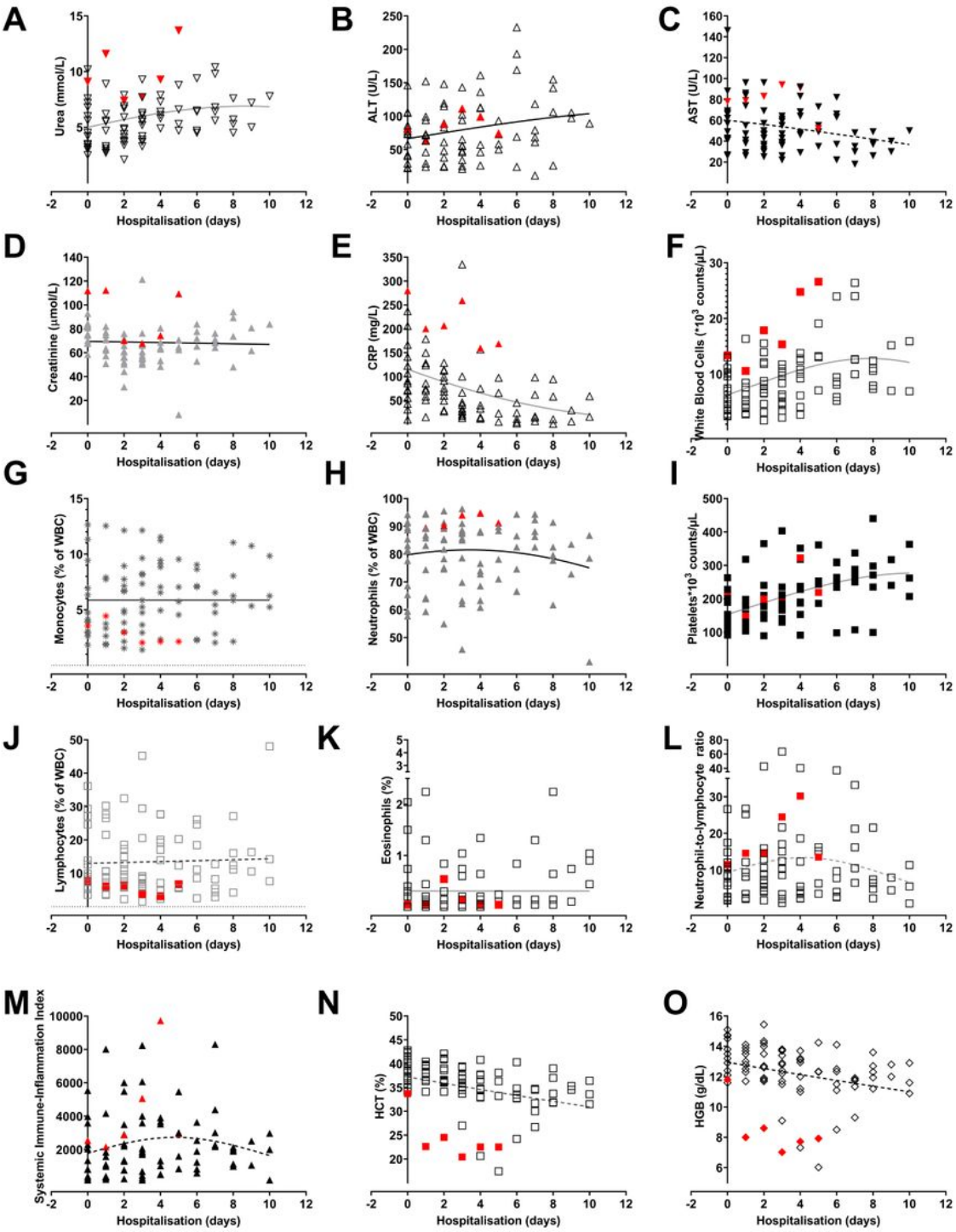

Figure 4

Biochemical parameters during hospitalisation Graphs present an overview of selected biochemical parameters monitored for COVID-19 disease severity. Red lines show a general trendline during hospitalisation. The red coloured symbols pertain a the patient who succumbed as a result of ARDS.

Figure 5 - Coagulation Marker Analysis

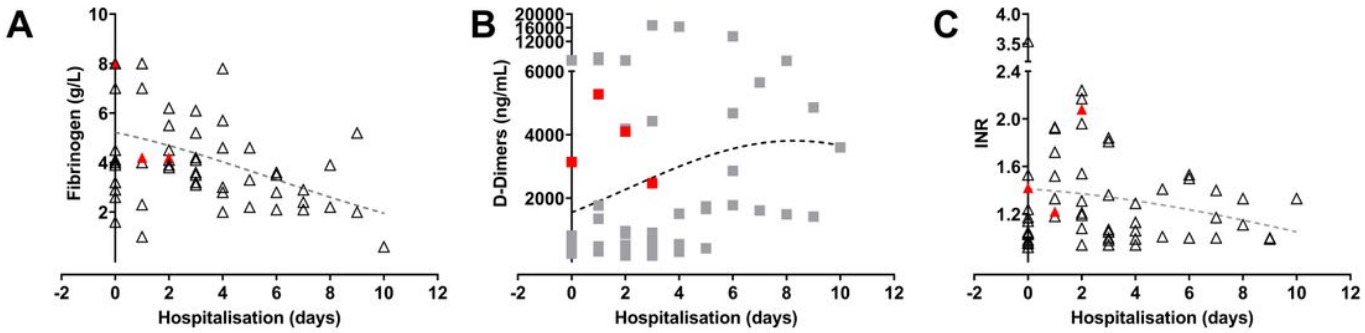

Figure 5

Analysis of coagulation markers Patients receive an initial 25000 IU bolus injection (≈ 300 IU/kg) followed by continuous infusion of 300 IU/kg dissolved in physiological buffer (0.9% NaCl) administered at 6 - 8 mL/h flow rate; target ACT ≥ 200s during hospitalisation. Patient's coagulation status were tracked by evaluating Fibrinogen, D-Dimers and the international normalised ratio (INR).

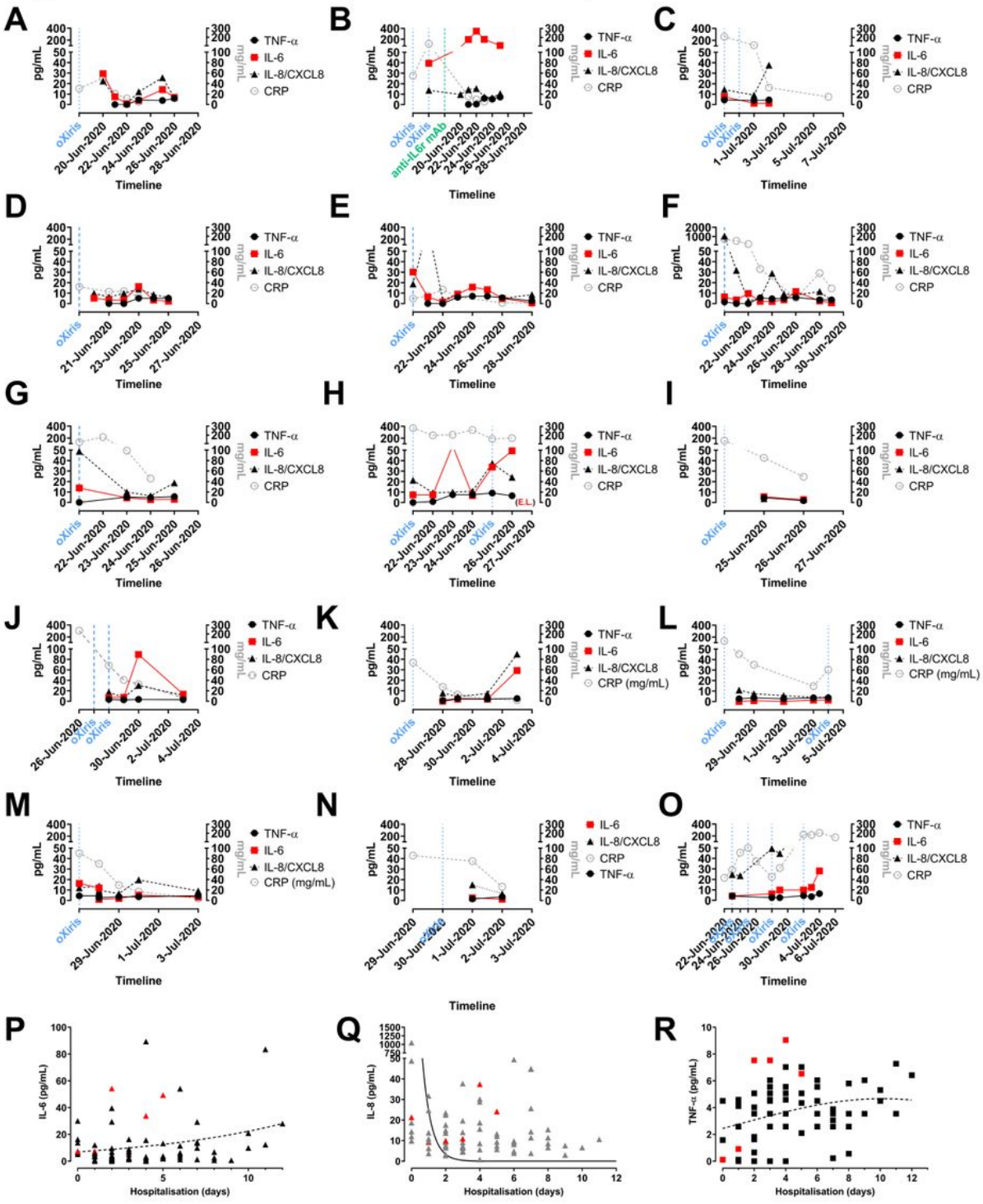

Figure 6

Inflammatory mediator analysis; systemic levels of IL-6, IL/CXCL-8 and TNF-α Individual cytokine profile (A – O) IL-6, IL-8 and TNF-α are plotted on the left y-axis (pg/mL), and CRP is plotted on the right y-axis (mg/mL). The start of oXiris® Hemofiltration 24-cycle is shown on the x-axis. One patient (Panel B) also received Tocilizumab (= anti-IL6 receptor mAb). Cytokine data are plotted on the left y-axis; C-reactive protein (grey checkered line) values are plotted on the right y-axis. Panels (P, Q and R) show combined

data during hospitalisation for IL-6, IL-8 and TNF-α. The red coloured symbols show the values for the single mortality case.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- IRBandECApprovalEBPZ.357.pdf
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