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Predictive factors for membrane oxygenator dysfunction in patients with severe COVID-19 under V-V ECMO

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Abstract

Introduction: COVID-19 immune dysregulation and pro-inflammatory state is associated with higher rates of thrombotic events. In patients under venovenous ECMO (V-V ECMO), COVID-19 acts synergically leading to even higher pro-thrombotic activity, with increased membrane oxygenator (MO) dysfunction and need for replacement. This study aimed to assess possible predictors of MO dysfunction.

Methods: This is a retrospective, observational study with a cohort of adult COVID-19 patients requiring V-V ECMO, in an ECMO referral center at a tertiary university hospital, between March 2020 and February 2022. Demographic characteristics, pre-ECMO organ support data (total days under corticotherapy, high-flux nasal cannula, non-invasive and invasive ventilation), total duration of ECMO, carboxy-hemoglobin and coagulation parameters variation during five days prior to each membrane exchange, were collected from electronic files.

Results: MO exchange rate was 51.61% for at least one exchange. The median duration of ECMO run was significantly longer in the case group (35,0 vs 14,0 days, $p<0.001$). Parameters that showed statistical significance, comparing cases and control groups, were total days of corticotherapy pre-ECMO (42 vs 25 days, $p<0.005$), total days under invasive mechanical ventilation pre-ECMO (40 vs 19 days, $p<0.005$), need for neuromuscular blocking agents (13 vs 6 days, $p<0.005$); variation of platelets (W=-21, p 0.0312), D-dimer (W=-21, p 0.0312), aPTT (W=21, p 0.0312), CRP (W=21, p 0.0312), monocytes (W=21, p 0.0312), and carboxyhemoglobin (W=21, p 0.0312), 5 days prior to MO exchange was also significantly different.

Conclusions: The coagulopathy and inflammation induced by COVID-19 could contribute to MO dysfunction, requiring exchange. There is a shortage of studies identifying predictors of MO thrombosis and replacement, however, decrease in platelet count seems to be transversal to studies. These results may raise intriguing questions regarding the increased need for corticotherapy and altered leucogram counts in the case group.

Key words: COVID-19, V-V ECMO, membrane oxygenator dysfunction.

Resumo

Introdução: A desregulação imunológica e estado pró-inflamatório da COVID-19 associa-se a maiores taxas de trombose. Em doentes sob ECMO venovenoso (ECMO V-V), a COVID-19 atua sinergicamente levando a maior atividade pró-trombótica, com aumento da disfunção do oxigenador de membrana (OM) e necessidade de substituição. Este estudo pretendeu avaliar eventuais preditores de disfunção do OM.

Métodos: Estudo retrospetivo, observacional, coorte de doentes adultos com COVID-19 sob ECMO V-V, num centro de referência num hospital universitário terciário, entre março 2020 e fevereiro 2022. Características demográficas, dados sobre suporte de órgão pré-ECMO (dias sob corticoterapia, cânula nasal de alto fluxo, ventilação não invasiva e invasiva), duração total de ECMO, variação da carboxihemoglobina e parâmetros de coagulação nos cinco dias precedentes às trocas de membrana, foram recolhidos de processos eletrónicos.

Resultados: A taxa de troca de OM foi 51,61% para pelo menos uma troca. A duração mediana de ECMO foi significativamente superior no grupo de casos (35,0 vs 14,0 dias, p<0,001). Os parâmetros que apresentaram significância estatística, comparando os casos e controlos, foram o total de dias sob corticoterapia pré-ECMO (42 vs 25 dias, p<0,005), sob ventilação mecânica invasiva pré-ECMO (40 vs 19 dias, p<0. 005), necessidade de bloqueadores neuromusculares (13 vs 6 dias, p<0,005); a variação de plaquetas (W=-21, p 0.0312), D-dímeros (W=-21, p 0.0312), aPTT (W=21, p 0.0312), PCR (W=21, p 0.0312), monócitos (W=21, p 0.0312), e carboxihemoglobina (W=21, p 0.0312) 5 dias antes da troca de membrana mostraram significância estatística.

Conclusões: A coagulopatia e inflamação induzidas pela COVID-19 podem contribuir para disfunção do OM, levando à sua substituição. Há escassez de estudos que identifiquem preditores de trombose do OM, mas a diminuição absoluta de plaquetas parece ser transversal entre estudos. Estes resultados levantam questões interessantes relativamente à maior necessidade de corticoterapia e alteração nas contagens do leucograma no grupo de casos.

Palavras-chave: COVID-19, ECMO V-V, disfunção de oxigenador de membrana

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Glossary

ARDS – Acute respiratory distress syndrome

BMI - Body mass index

CD - Cluster differentiation

COHb - Carboxyhemoglobin

COVID-19 - Coronavirus disease of 2019

CRP - C-reactive protein

DD - D-dimer

$ECCO_2R$ – Extracorporeal carbon dioxide removal

ECLS – Extracorporeal life support

ECMO – Extracorporeal membrane oxygenation

ELSO - Extracorporeal Life Support Organization

H1N1 - Hemagglutinin 1 neuraminidase 1 influenza A virus

HFNC - High-flux nasal cannula

HO-1 - Heme oxygenase 1

ICU – Intensive care unit

IL - Interleukin

IMV - Invasive mechanical ventilation

IQR - Interquartile range

LoS - Length of stay

MO - Membrane oxygenator

NETs - Neutrophil extracellular traps

NF-κB - Nuclear factor kappa-light-chain-enhancer of activated B cells

NIV - Non-invasive ventilation

NMBA - Neuromuscular blockage agents

NO - Nitric oxide

NO MOex - No membrane oxygenator exchange

PAI - 1 - Plasminogen activator inhibitor-1

PFHb - Plasma free hemoglobin

PGI2 - Prostacyclin

RCT - Randomized controlled trials

ROS - Reactive oxygen species

SAPS II - Simplified Acute Physiology Score II

SARS-CoV2 - Severe acute respiratory syndrome coronavirus 2

SOFA - Sequential Organ Failure Assessment

TF - Tissue factor

TFPI - Tissue factor pathway inhibitor

TNF-α - Tumor necrosis factor alpha

UFH - Unfractioned heparin

USA - United States of America

V-A ECMO – Venoarterial extracorporeal membrane oxygenation

V-V ECMO – Venovenous extracorporeal membrane oxygenation

VILI – Ventilator induced lung injury

WITH MOex - With membrane oxygenator exchange

aPTT - Activated partial thromboplastin time

mRNA - Messenger ribonucleic acid

vWF - Von Willebrand factor

INTRODUCTION:

1. Definitions:

Extracorporeal life support (ECLS) includes extracorporeal membrane oxygenation (ECMO) and extracorporeal carbon dioxide removal ($ECCO_2R$). ECMO, on which this paper will mainly focus, has gained increasing relevance due to its ability to partially, or fully, bypass injured lungs, hence allowing for adequate gas exchange and lower intensity protective mechanical ventilation in cases of life-threatening lung injury. (Munshi et al., 2022).

Extracorporeal membrane oxygenators are designed to work as out-of-body, artificial lungs and/or heart, that function by removing carbon dioxide (CO_2) and providing oxygen (O_2) to the blood that flows through the membrane (Combes et al., 2020). There are two main categories of ECMO: venovenous ECMO (V-V ECMO), that can be primarily implemented for respiratory support, and venoarterial ECMO (V-A ECMO), used for cardiac pump support (Munshi et al., 2022). Simplistically, in ECMO the blood can be drawn from the inferior vena cava through one of its tributaries, such as the femoral vein, among various other ECMO circuit designs. Then it flows in an afferent cannula and reaches the membrane of oxygenation where the gas exchange occurs. Afterwards, the blood is pumped to the efferent cannula and is re-injected in the systemic circulation through major veins, such as the jugular vein (V-V ECMO), bypassing only the lungs, therefore being used for lung support. Alternatively, the blood can return to the systemic circulation by major arteries, such as the femoral artery (V-A ECMO), thus bypassing the function of both lungs and heart. (Combes et al., 2020).

Acute Respiratory Distress Syndrome (ARDS) is a severe form of acute respiratory failure. It is induced by a pulmonary or systemic insult, and it is characterized by diffuse alveolar injury with exudates, surfactant depletion and impairment of gas exchanges, alongside with pulmonary capillary leaks, resulting in noncardiogenic pulmonary edema. (Force et al., 2012). The management of ARDS is based on addressing the underlying condition and providing supportive care; the patients are usually admitted in the intensive care unit (ICU) and may require invasive mechanical ventilation. However, positive pressure ventilation can also aggravate the primary lung injury through various mechanisms - so called ventilator induced lung injury (VILI). Accordingly, in ARDS there

is a need to minimize VILI and other iatrogenic interventions by reducing the positive pressure and volumes used during mechanical ventilation, meaning that a lung-protective ventilation should be favored (Munshi et al., 2022).

2. Evidence of V-V ECMO in randomized controlled trials (RCT):

The CESAR trial (2009), randomly assigned 180 patients between being transported to an ECMO center and then considered for ECMO, or to being treated without ECMO, upon severe but possibly reversible acute respiratory failure, refractory to conventional management. The results showed a significant difference regarding survival without severe disability at 6 months, favoring the patients randomly assigned to ECMO management in detriment of those treated without ECMO (63% vs. 47%). However, this study had some limitations, such has significant differences in management within the ECMO and the control group; all in all, ECMO looked promising, but needed more robust evidence (Peek et al., 2009). Afterwards, an international and multicentered study emerged - the Extracorporeal Membrane Oxygenation for Severe Acute Respiratory Distress Syndrome (EOLIA) trial (2018). In this trial, 249 patients were randomized between a control group, who received conventional and protocolized mechanical ventilation with protective settings and prone positioning (90%), and a group who received ECMO within 7 days of mechanical ventilation and prone positioning (66%). The data showed significantly greater treatment failure in the control group (58% vs. 35%; P,0.001); furthermore, the patients in the control group that crossed over to the ECMO, also had higher mortality than those who had ECMO intervention early on. Overall, the results from the EOLIA trial suggested that early V-V ECMO may have a role in reducing mortality in patients with severe forms of ARDS, mainly through its benefit of ameliorating ventilator-induced lung injury (VILI) (Combes et al., 2018).

3. Context:

In recent history, there were two pandemics - H1N1 pandemic and SARS-CoV2 pandemic - in which ARDS was a severe and relatively common complication. In the Influenza A (H1N1) pandemic, the development of ARDS led to a tremendous increase

in the mortality rate, ranging from 17.3% to 41.4% among critically ill patients with H1N1 infection (Guillermo Domínguez-Cherit et al., 2009). Regarding SARS-CoV2, by September 2020, in Europe, mortality estimates among COVID-19 patients with severe ARDS was reported between 13%-73% (Hasan et al., 2020). Therefore, ARDS and V-V ECMO cross paths, since the key benefit in V-V ECMO is the ability to facilitate lung-protective ventilation and further reduce ventilation intensity, allowing for ultra-protective lung ventilation and better healing of the lungs.

In conclusion, there was a paradigmatic shift in ECMO care, since historically ECMO was restricted to patients with refractory, and nearly fatal, severe hypoxemia and recently, in experienced centers, ECMO has become more frequently used beyond its classical indications, for ICU patients with severe ARDS (Abrams et al., 2019). This way, being clear that there is a growing need for advanced medical management of acute respiratory failure in the ICU, it became more relevant to obtain robust scientific evidence on the benefits of ECMO, but also on its complications and their adequate prediction, identification and management, which will be developed throughout this paper.

3.1 Thrombotic complications associated with COVID-19

Since the very beginning of the SARS-CoV2 pandemic, COVID-19 associated coagulopathy has been a landmark of the disease. There is a complex immune dysregulation and enhanced pro-inflammatory state that can lead to coagulopathy that manifests as microthrombi and/or macrothrombi, resulting in damage to multiple organs, including the lungs, heart, brain and kidneys. However, it can also lead to microthrombi in life support devices and their subsequent failure, such as ECMO membrane clotting and failure (Conway et al., 2022a).

The primary coagulopathy milestone is damage to the endothelial cells. Endothelial cells normally express anticoagulant molecules including prostacyclin (PGI2), nitric oxide (NO), tissue factor pathway inhibitor (TFPI), and thrombomodulin. Under conditions of inflammation or stress, endothelial cells become altered, and they express procoagulant molecules, notably tissue factor (TF), but also thromboxane A2,

plasminogen activator inhibitor-1 (PAI-1), and Factor VIII. Simultaneously, there is functional loss of the referred anticoagulant molecules, from the endothelial surface (Mazzeffi et al., 2021). This endothelial damage is thought to be due to direct infection and injury of the endothelium by SARS-CoV2, and/or due to indirect injury to the cells from the "cytokine storm" (Ackermann et al., 2020). Subsequent to endothelial damage, there is an increase in thrombopoietin levels leading to hypercoagulability (Yin et al., 2021). Other factor that contributes to COVID-19 infection associated hypercoagulability is the hyper-inflammatory state that involves neutrophilic recruitment with subsequent release of proteolytic enzymes, reactive oxygen species (ROS) and neutrophil extracellular traps (NETs), which propagates tissue damage and platelet aggregation (Conway et al., 2022; Magro et al., 2020).

Lastly, increased platelet activation resulting from hyper-inflammation, is often seen in COVID-19 patients; this activation occurs in response to soluble coagulation and complement factors, inflammatory cytokines, neutrophils, etc. Through the release of their intracellular granules, platelets help trigger and sustain a thrombotic amplification loop, which contributes to the coagulopathy (Caillon et al., 2022a).

The prevalence of thrombosis in COVID-19 has been described extensively in the literature and has been reported to be between 20% and 30% in hospitalized patients, most of which is diagnosed as pulmonary embolism (Middeldorp et al., 2020; Yusuff et al., 2022). This way, compared with other severe respiratory diseases, COVID-19 is more frequently associated with major clotting events, namely when associated with elevated levels of D-dimers, C-reactive protein and fibrinogen levels (Tang et al., 2020).

3.2 Thrombotic complications associated with ECMO support

The ECMO systems contain several materials which are foreign to our body (Peek et al., 2002). More recently, biocompatible materials have replaced the previous generation of uncoated circuits. Nonetheless, some parts of the ECMO circuits continue to have coagulation issues, namely, foreign surface-triggered inflammation and narrowing of the circuit's internal diameter that leads to turbulent flow and stasis zones.

Furthermore, the membrane oxygenator is a large, porous surface area which also causes turbulent flow and contact activation of coagulation cascade (Thomas et al., 2018).

Overall, during ECMO support, there are notable nonphysiological alterations in the coagulation and hemostasis systems in vivo. The direct exposure of blood components to artificial materials and narrowings of the circuit induces high shear stress and turbulence, and the dilution, activation, and consumption of blood components, significantly impacting the coagulation system (Doyle & Hunt, 2018). This activation of coagulative and inflammatory cascades may lead to extensive endothelial injury and thrombosis (Millar et al., 2016). Furthermore, other conditions correlated with ECMO support also confer increased thrombotic risk: critical illness, need for more frequent blood transfusions, patient immobilization and non-pulsatile blood flow are also important thrombogenic factors (Olson et al., 2021).

The incidence of clotting events in the ECMO patients in general is around 15-22% (Olson et al., 2021) and thrombosis specific to the ECMO oxygenator is described between 10-16% (Doyle & Hunt, 2018).

3.3 Thrombotic complications: interplay between COVID-19 and concomitant V-V ECMO support

It seems the inflammatory state above described regarding COVID-19 disease, amplifies this proinflammatory predisposition of ECMO, leading to more bleeding and coagulation disorders when combined (Kowalewski et al., 2020). Overall, the influence of COVID-19 associated coagulopathy is likely to act synergistically with the hyperinflammatory state associated with COVID-19 and ECMO to produce clinically significant bleeding and thrombosis (Yusuff et al., 2022).

Various studies have reported a higher prevalence of thrombosis in patients with COVID-19 under V-V ECMO support, than with either V-V ECMO or COVID-19 alone. Studies conducted during the first pandemic wave, reported occurrences of thrombotic

events in up to 63-69% of patients, most patients presenting with pulmonary thromboembolism (Arachchillage et al., 2022; Shaefi et al., 2021). This contrasts with the previous COVID-19 related thrombotic events data, described has 20-30% (Middeldorp et al., 2020; Yusuff et al., 2022), or the ECMO related thrombotic events incidence described has 15-22% (Olson et al., 2021). Hence, COVID-19 patients who require ECMO have been noted to be hypercoagulable, apparently requiring more frequent circuit and oxygenator changes (Beyls et al., 2020).

LITERATURE REVIEW REGARDING ECMO CIRCUIT AND MEMBRANE OXYGENATOR EXCHANGE IN OTHER SERIES:

Table 1 consists of a summary of the most relevant literature reporting data regarding ECMO circuit exchange causes, circuit exchange incidence, membrane oxygenator (MO) exchange rate, ECMO total duration, MO lifespan and factors possibly predicting MO exchange.

1. Causes of ECMO circuit exchange

In the literature, there are various described causes for ECMO circuit exchange. The main reason seems to be circuit thrombosis, mainly membrane oxygenator thrombosis (Allen et al., 2011); with rates ranging from 14.7-47%. The Extracorporeal Life Support Organization (ELSO) Registry (2014) described an incidence of oxygenator thrombosis of 12.9%, of 5278 adult ECMO runs, for respiratory support. Other relevant causes for ECMO circuit exchange are intravascular hemolysis representing 18-30% of ECMO circuit replacements, bleeding due to ECMO coagulopathy with 7-12% of cases, and circuit failure accounting for a minority of cases, only 3-4.5%. Circuit failure includes plasma leaks, cannula dislodgement, pump failure and air entrapment in the ECMO circuit (Hoffman et al., 2023; Nagler et al., 2021; Pan et al., 2016).

Regarding COVID-19 patients under V-V ECMO, there seems to be a higher rate of bleeding and coagulation disorders. A multicentered observational study with 152 COVID-19 patients under V-V ECMO support reported a 30.9% rate of bleeding

complications and a 63.1% rate of thrombotic complications, of which 44.7% where venous thromboembolism (TE), 18.6% arterial TE and 10% ECMO circuit related thrombosis (Arachchillage et al., 2022).

2. Incidence of MO exchange and MO lifespan

V-V ECMO in non-COVID-19 patients

There is a shortage of studies reporting incidence of circuit exchanges in V-V ECMO. A large retrospective study with 461 patients under the support of V-V ECMO, described an incidence of at least one circuit exchange of 30.1% (Philipp et al., 2018). A retrospective study with 142 patients under V-V ECMO, V-A ECMO and $ECCO_2R$ reported that 44.4% of patients underwent circuit exchange at least once (Nagler et al., 2021). Another retrospective study with 265 patients under V-V ECMO described an exchange rate of 31%, for at least one circuit exchange (Lubnow, Philipp, Foltan, et al., 2014). A different retrospective study with 100 patients under V-V ECMO, reported the same 31% exchange rate of ECMO circuit (Hoffman et al., 2023). Lastly, a retrospective study with 151 patients under both V-V and V-A ECMO support, reported an incidence of 23.1% of ECMO circuit exchange, at least once (Basken et al., 2019a).

The median duration of ECMO membrane oxygenator (MO) is highly variable in the literature. ECMO circuit age upon exchange is described between 8-20 days (Hoffman et al., 2023; Nagler et al., 2021). Accordingly, the largest retrospective study here analyzed, containing 461 patients under V-V ECMO, reported a median duration of total ECMO support of 19 days, and a median membrane oxygenator lifespan of 9 days (IQR 6-12 days) (Philipp et al., 2018).

V-V ECMO in COVID-19 patients

An international, multicentered large retrospective study, states the complication rates leading to ECMO exchange, and the total duration of V-V ECMO support do not differ with statistically significance between non-COVID-19 and COVID-19 patients (Raasveld et al., 2021). A review study from the international ELSO Registry

provides data on 1035 ECMO-supported patients with COVID-19 and reports a median duration of total ECMO support of 14 days (IQR 8-23 days). This supports the previous statement that complications, ECMO circuit exchange rate and total duration of ECMO support, do not vary significantly between COVID-19 patients and other patients (Barbaro et al., 2020). However, a multicenter observational study with 152 COVID-19 patients under V-V ECMO supported, reports a median duration from initiation of ECMO to thrombosis of membrane oxygenator of 6 days (IQR 2–17 days), which may be slightly shorter than the above-described lifespan of the membrane of oxygenation for non-COVID-19 patients. Another retrospective study with 45 patients under V-V ECMO support, 51% of which had COVID-19 associated ARDS as a cause for ECMO support, reported different results: incidence of ECMO circuit exchange was 42.2%, and 76% of exchanges occurred in COVID-19 patients; median ECMO support duration was reported as 16 days, but for the group with membrane oxygenator exchanges, median duration of ECMO run was 30 days; the median lifespan of the oxygenator was 9 days (IQR 7-14 days) – the same as described for non-COVID-19 patients (van Minnen et al., 2023).

In summary, thrombosis of membrane oxygenator (MO), might affect systemic oxygenation, limiting the expected duration of ECMO circuit and requiring MO exchange, exposing the patients to higher risks. Several parameters have been associated with need for oxygenator exchange, but no guidelines or predictive scores are published; therefore, the delicate balance between oxygenator dysfunction and its exchange needs to be more accurately predicted. Furthermore, it appears COVID-19 patients under V-V-ECMO have higher rates of thrombosis leading to MO dysfunction and exchange, hence, this study aims to identify possible causes and analytical predictors of MO dysfunction in this group of patients.

METHODS:

This is a single-center, retrospective, observational study of a cohort consisting of adult COVID-19 patients requiring V-V ECMO support for acute respiratory failure. The study was conducted on patients that were admitted between March 2020 and February 2022, to an ECMO referral center in a tertiary university hospital. The Santa Maria University Hospital serves the urban area of Lisbon, Portugal, accommodating a population of about 3 million inhabitants. In Portugal there are only three ECMO referral centers, this being one of them and hence, indirectly serving a much bigger number of patients than the referred Lisbon inhabitants.

Inclusion criteria were patients with COVID-19 infection confirmed by polymerase chain reaction laboratory test, being under the support of V-V ECMO, and temporal criteria of being admitted between March 2020 and February 2022. Regarding exclusion criteria, age under 18 years old and need for V-A ECMO lead to exclusion. No other criteria regarding disease severity or timing of ECMO circuit exchanges were applied for exclusion.

We collected demographic characteristics, pre-ECMO organ support such as: total days under corticotherapy, high-flux nasal cannula (HFNC), non-invasive ventilation (NIV) and invasive mechanical ventilation (IMV), all regarding the pre-ECMO period. Data of total duration of ECMO support and total days with need for neuromuscular blockage agents (NMBA) were also collected. Furthermore, number of membrane of oxygenation exchanges was collected and used to divide the patients in case group (patients with MO exchange) and control group (patients without MO exchange). We also collected data of platelet count, D-dimer levels, fibrinogen levels, activated partial thromboplastin time (aPTT), C-reactive protein levels (CRP), leucocyte, neutrophil and monocyte counts and carboxyhemoglobin (COHb) levels, data was collected daily on the five days prior to each MO exchange (in the case group) and on the five days prior to the end of ECMO run (in the control group). In patients with multiple MO exchanges, only the first ones were included in the analysis. Lastly, we collected data of SAPS II and SOFA scores and ICU and in-hospital mortality. Data was collected from electronic files.

All statistical analysis and graphics were done using GraphPad Prism® (GraphPad Software, La Jolla, California, USA). Continuous and qualitative variables were

considered, and overall population characterization was established, including comprehensive demographical data. Descriptive statistics were performed on continuous variables, including quartile, skewness, and kurtosis assessment. Normality tests (Kolmogorov-Smirnov and Shapiro-Wilk tests) were applied to establish whether a normal distribution was present. For non-normally distributed variables, median and inter-quartile range (IQR) were used, while mean and standard deviation were preferred for gaussian distributions. Two different study groups were considered: those who had not required MO exchange (NO MOEx) – control group - and those who had at least one membrane exchange during ECMO run (WITH MOex) – case group. When comparing different variables, parametric tests such as Unpaired T-test (for comparing two variables) or One Way ANOVA (for comparing more than two variables) were used for normally distributed variables. Non-parametric tests Mann-Whitney test (for comparing two variables) or Kruskall-Wallis test (for comparing more than two variables) were selected for non-normally distributed variables. When comparing both groups for continuous variables with repeated measurements over time, Wilcoxon test was performed, for the values were paired; data was used in the form of medians and results presented with a 95% confidence interval (95%).

Primary outcome was to characterize the membrane oxygenator exchange events regarding MO exchange frequency, MO lifespan and total ECMO run duration. Secondary outcome was to identify medical care related factors and hematological factors that may indicate imminent MO thrombosis and need for replacement. The default V-V ECMO circuit used in this center is the Cardiohelp® (Maquet-Getinge Group®) device and the corresponding disposable HLS set advanced 7.0 with BIOLINE coating (Maquet-Getinge Group®) and dedicated BIOLINE coated HLS cannulas (Maquet-Getinge Group®).

RESULTS:

Summary of the demographic results can be consulted in Table 2. Between March 2020 and February 2022, a total of 93 patients were admitted to the referred ICU with COVID-19 related acute respiratory distress in need of V-V ECMO support. Most patients were males (71.0%). Mean age at admission was 50 (±12) years old. Mean body mass index (BMI) was 31.55 (±7.41) kg/m^2. Mean critical care scores at admission were SOFA of 5.7 (±2.9) and SAPS II of 35.59 (±13.68). SOFA at cannulation and at ICU discharge were mean 6.34 (±2.8) and 3.62 (±4.3), respectively. ICU mean length of stay (LoS) was 45.5 (±38.4) days. ICU mortality was 24.73% and hospital mortality was not calculated due to insufficient follow-up data.

Regarding primary outcomes, using the number of ECMO membrane oxygenator exchanges, we divided the cohort in a case group, with one or more MO exchanges (n=48), and a control group without MO exchanges (n=45). There was a total of 72 MO exchanges and the frequency of MO exchange was 51.61% for at least one event. Median duration of total ECMO run was 26 days (IQR 14-42 days) and median MO lifespan was 15 days (IQR 11-27 days). The median number of ECMO membrane exchanges was 1 (IQR 0-1).

We compared the control and the case groups using Mann-Whitney (non-parametric) test, these results can be consulted in Table 3. The results comparing controls and cases showed no statistically significant difference regarding age (median 51 vs 55 years old, p 0.172). Regarding secondary outcomes, there was no statistically significant difference in number of days under high-flux nasal cannulation (HFNC) pre-ECMO (median 1 vs 0 days, p 0.697), number of days under non-invasive ventilation (NIV) pre-ECMO (median 0 vs 0 days, p 0.835), number of days under invasive mechanical ventilation (IMV) pre-ECMO (median 4 vs 5 days, p 0.516) and number of sick days pre-ECMO (median 17 vs 16 days, p 0.429). There was, however, a significant difference regarding number of days of corticotherapy pre-ECMO (median 25 vs 42 days, p 0.0018). About results of variants that refer to the ECMO run period, the difference in number of days under IMV and number of days under neuromuscular blockage agents (NMBA) had statistically significant differences between control and cases (median 19 vs 40 days, p <0.0001 and median 6 vs 13 days, p 0.004 respectively); likewise, regarding

primary outcomes, total days under ECMO showed to be statistically significant between control and cases (median 14 vs 35 days, p <0.0001). On the other hand, median MO lifespan did not show statistically significant differences between the two groups (14 vs 15 days, p 0.65). Lastly, critical care scores namely SAPS II (median 33 vs 33, p 0.386), SOFA at admission (median 5 vs 4, p 0.369), SOFA at canulation (median 6 vs 6, p 0.437) and SOFA at ICU discharge (median 2 vs 2, p 0.552), did not have statistically significant differences.

Furthermore, regarding secondary outcomes, we performed XY tables for the analysis of the hematological and coagulation-related factors and their median values during the five days prior to each MO exchange (cases group), and their values during the five days prior to the end of ECMO run (control group); these results can be found in Tables 4-12 and Figures 1-9.

For comparison of continuous variables paired through time, the Wilcoxon Signed-Rank tests (two-tailed) were performed; these results can be consulted in Table 13. The values that did not show statistical significance were fibrinogen levels, leucocytes and neutrophils count. The median fibrinogen levels in the 5 days prior to MO exchange were superior for patients in the case group than for patients in the control group (W=19, p 0.0625, CI 95%), with a large strength of association (rs=1), hence non statistically significant. The median leucocyte count in the 5 days prior to MO exchange was inferior for patients in the case group than for patients in the control group (W=11, p 0.312, CI 95%) with a small strength of association and opposite tendencies of variation between variables (rs= -0.2), therefore without statistical significance. The median neutrophil count in the 5 days prior to MO exchange was inferior for patients in the case group than for patients in the control group (W=-15, p 0.156, CI 95%), with a large strength of association and opposite tendencies of variation between variables (rs= -0.7), hence non statistically significant. On the other hand, platelet count, D-dimer levels, aPTT, C-reactive protein levels, monocyte count and carboxyhemoglobin levels, all showed to be statistically significant. The median platelet count in the 5 days prior to MO exchange was inferior for patients in the case group than for patients in the control group (W=-21, p 0.0312, CI 95%), with a large strength of association and similar tendencies of variation between variables (rs=1), hence

statistically significant. The median D-dimer levels in the 5 days prior to MO exchange were superior for patients in the case group than for patients in the control group (W=-21, p 0.0312, CI 95%), with a large strength of association and similar tendencies of variation between variables (rs=1), therefore statistically significant. The median aPTT values in the 5 days prior to MO exchange were inferior for patients in the case group than for patients in the control group (W=21, p 0.0312, CI 95%), with a small strength of association and similar tendencies of variation between variables (rs=0.14), therefore statistically significant. The median CRP levels in the 5 days prior to MO exchange were superior for patients in the case group than for patients in the control group (W=21, p 0.0312, CI 95%), with a small strength of association and opposite tendencies of variation between variables (rs= -0.26), hence statistically significant. The median monocyte count in the 5 days prior to MO exchange was inferior for patients in the case group than for patients in the control group (W=21, p 0.0312, CI 95%), with a large strength of association and opposite tendencies of variation of variables (rs= -0.66), therefore statistically significant. Lastly, the median carboxyhemoglobin levels in the 5 days prior to MO exchange were superior for patients in the case group than for patients in the control group (W=21, p 0.0312, CI 95%), with a large strength of association and similar tendencies of variation between variables (rs=0.66), hence statistically significant.

DISCUSSION:

Despite improvement in V-V ECMO technology and management, the most common complication leading to MO exchange remains clot formation within the ECMO circuit (Hoffman et al., 2023). There is a shortage of studies reporting rates, causes and predictors of MO exchange. Our results support the existing data, showing that MO exchange is a relatively common event during a V-V ECMO run, although the 51.61% exchange rate obtained here, is slightly superior to the reported rate in other studies. This finding could be explained by the fact that there aren't any guidelines or scores based on analytical variables implemented for MO exchange, therefore, different ECMO-intensivists and perfusionists could decide to exchange the oxygenator at different timings based on these variables. Also, reported total duration of ECMO run is highly variable among studies (Basken et al., 2019; Hoffman et al., 2023; Lubnow, Philipp, Dornia, et al., 2014; van Minnen et al., 2023).

Regarding MO lifespan, to the extent of our knowledge, this parameter is rarely ever evaluated or reported. Only Philip et al. (2018) and van Minnen et al. (2023), among the studies here analyzed, made reference to MO lifespan (9 days in both). Besides these studies, there is also information obtained from the producing brand, in this case, the disposable HLS set advanced 7.0 with BIOLINE coating (Maquet-Getinge Group®) is a membrane with expected duration of 30 days. Hence the median lifespan of 15 days obtained here is slightly longer than data reported elsewhere; however, it is shorter than expected according to the producing brand. Since both our reported MO exchange rate (51.61%) and MO lifespan (9 days) are superior to that reported by other authors, it could be hypothesized that this increase in MO exchange is also due to longer ECMO runs. Regarding the referred high variability in the criteria for MO exchange amongst different ECMO centers, our center could have longer ECMO runs due to internal protocols or clinical management preferences of the physicians; additionally, the possibility that ECMO runs are longer due to the increased severity of COVID-19 associated ARDS should also be considered, since most studies here analyzed did not incorporate COVID-19 patients.

In terms of ECMO-related medical care and ECMO run duration, as would be expected, the total duration of ECMO support for patients that required more than one

MO system was significantly prolonged [median (IQR), 35 (26-57) days] compared to patients with only one ECMO circuit [median (IQR), 14 (12-26) days; p<0.0001]; this was also described by Philipp et al. (2018). Predictably, total days under mechanical invasive ventilation [median (IQR), 40 (28-48) days; p<0.0001] and number of days under neuromuscular blockage agents (NMBA) [median (IQR), 13 (8-20) days; p <0.005], were also significantly increased in the case group, comparing with the control group [median (IQR), 19 (12-33) days; p<0.0001; and 6 (4-12) days; p<0.005, respectively]. This would be predictable since ultra-protective lung ventilation and neuromuscular blockage of patients are standard-of-care medical procedures, essential for management of V-V ECMO support. As stated, these factors do not seem to be predictors of MO exchange, for logically thinking they ought to be a consequence of MO exchanges and hence prolonged ECMO runs, rather than the cause.

We also found a statistically significant difference in the number of days of corticosteroid therapy before VV-ECMO cannulation between the case and control groups [median (IQR), 42 (25-69) days versus 25 (17-40) days; p <0.005]. There are some robust randomized controlled trials showing benefit of corticosteroids in moderate to severe ARDS, including in COVID-19 disease associated ARDS (Annane et al., 2018; Villar et al., 2020; Ye et al., 2020). Furthermore, a recent systematic review with meta-analysis, showed that corticosteroid use may reduce ICU mortality in critically ill patients with ARDS as well as lead to fewer days of mechanical ventilation (Chaudhuri et al., 2021); however, timing of initiation of therapy, dosage and duration of treatment aren't yet established for maximum benefit. In light of this evidence, our findings seem surprising, for they suggest that prolonged corticosteroid therapy pre-ECMO is correlated with increased MO dysfunction and need for replacement. A plausible explanation for this result could be related to the classical immunosuppressive side effects of systemic corticosteroids, mainly by repressing the activity of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and other key immunomodulatory transcription factors (Coutinho & Chapman, 2011). This could lead to increased risk for secondary infection and, by pathophysiological mechanisms already explored, ultimately lead to increased hypercoagulability and risk for MO thrombosis and need for exchange. However, literature shows that systemic corticosteroid use in

ARDS and sepsis probably does not increase rates of secondary infections (Chaudhuri et al., 2024). Another mechanism that could explain the correlation between total days of corticotherapy before ECMO and need for MO exchanges, could be related to the thrombo-modulatory effects of corticosteroids. *In vitro* studies revealed that dexamethasone, significantly enhances PAI-1 antigen and mRNA expression, indicating that corticosteroids may directly inhibit fibrinolysis (Morange et al., 1999). Additionally, a clear rise in the activity levels of coagulation factors VII, VIII and XI was observed in healthy volunteers under high-dose short-term corticotherapy. Hence, glucocorticoids seem to increase the activity of clotting factors also *in vivo*, which may contribute to the reported increased risk of thrombosis in patients with sustained exposure to glucocorticoids (Brotman et al., 2006). Lastly, a systematic review supports that this hypercoagulable state can also be verified in patients with increased inflammatory activity, and not just healthy individuals, reporting that glucocorticoids lowered fibrinogen levels and increased PAI-1 levels, impairing fibrinolytic activity in sick patients (Van Zaane et al., 2010). All this priming of the hemostatic mechanisms towards a pro-coagulant tendency, could possibly explain why patients with prolonged courses of corticotherapy prior to ECMO cannulation may have increased MO thrombosis and need for exchange. It could also by hypothesized that patients with a more severe ARDS at admission and, therefore, enhanced hyperinflammatory and hypercoagulability states, that would justify, by themselves, an increased risk of MO thrombosis, would beforehand need longer corticotherapy courses to manage their increased disease severity. In this case, MO exchange would be correlated with basal severity of ARDS, rather than directly related to total duration of corticotherapy before ECMO; meaning that corticosteroid administration and course would be a consequence of disease severity, rather than the cause for MO dysfunction.

Further follows the discussion regarding the comparison between hematological factors measured continuously in the 5 days prior to MO exchange; several factors were found to have statistically significant variations. Leucocyte count and neutrophil count variations and their association with need for MO exchange, were not statistically significant, whereas monocyte count variation was statistically significantly between the two groups (Tables 9-11; Table 13; Figures 6-8). Interestingly, all the referred cell counts

have approximately the same starting point, but then diverge in a way that patients without MO exchange (controls) only have a slight fall in total leucocytes, a fluctuant maintenance in neutrophils and a rise and maintenance in monocyte counts. For patients with MO exchanges (cases), leucocytes and neutrophils have a similar tendency of reaching *nadir* values two days before MO exchange (-2) and then slowly recovering until the day of MO exchange (0); monocytes have a decreasing tendency and a reach *nadir* one day before MO exchange (-1), although the values are the lowest since the two days prior to MO exchange and until the day of exchange, not recovering like other cell lines. To understand leucocyte modulation during ECMO, Ki et al. (2021) proposed a "two hit ECMO model" which hypothesizes that leucocytes' *first hit* is related to the immune modulation from the patient's primary disease burden, and that the *second hit* is related with the inflammatory response to ECMO. Such as described previously, upon contact with non-endothelialized surface and non-physiological conditions of the ECMO circuit, the inflammatory cascade is activated; hence, ECMO-mediated release of pro-inflammatory cytokines, chemokines and complement products can lead to further activation of leucocyte subpopulations and endothelial cells (Ki et al., 2021; Rungatscher et al., 2015). This can subsequently elevate leukocyte adhesion onto either the artificial surface of the ECMO circuit or the endothelium itself (Bredthauer A, 2017; Wilm et al., 2018). Furthermore, ECMO environment also activates platelets which release granules that contain a variety of soluble pro-inflammatory and pro-adhesion mediators that can form leukocyte conjugates, predominantly with monocytes and neutrophils. This way, the first cells to become activated in early ECMO are precisely neutrophils and monocytes; these activated leucocytes have shown to have increased expression of cluster differentiation integrins (CD-11 and CD-18) that potentiate their adhesion to the membrane oxygenator and activated endothelium (Ki et al., 2021). Studies report that monocytes and neutrophils rolling and adhering to surfaces may be the initiating stimulus for thrombi development, as they provide a base on to which platelets can adhere and contribute to the progression of the thrombi (Meyer et al., 2020; von Brühl et al., 2012). This platelet-leukocyte interaction induces monocytes to express tissue factor and neutrophils to degranulate and form NETs, leading to a hypercoagulability state (Ki et al., 2021; Laurance et al., 2017; Maugeri et al., 2006). Neutrophil mediated propagation of thrombi is mainly due to NETs for they enhance platelet adherence by

trapping them; additionally, NETs are negatively charged and so they can trigger activation of factor XII, which leads to the activation of intrinsic coagulation cascade (von Brühl et al., 2012). Concluding, the crosstalk between platelets, monocytes and neutrophils seems to be central in thrombi formation and propagation in ECMO's membrane oxygenator. We believe that the observed overall decrease in leucocyte count observed in patients with MO exchange can be explained by this central role neutrophils and monocytes appear to play in thrombogenesis. The hypothesis would be that thrombi formation starts few days before MO exchange; since the first cells to be recruited and adhered to the MO seem to be leucocytes, namely neutrophils and monocytes, this would justify an increased consumption of these cells and, therefore, an accentuated drop in the cell counts, as observed during the two days before the MO exchange. Afterwards, leucocytes become less relevant for maintenance of the thrombi, and platelets gain a more preponderant role, allowing for a slight recuperation in cell counts. Two studies that analyzed oxygenators retrieved from ECMO therapy in adults may strengthen this hypothesis; they performed immunofluorescence techniques on the oxygenators targeted to identify leucocyte deposits and vonWillebrand factor fibers. They found that 33% of oxygenators (Cardiohelp HLS set advanced 7.0, Maquet-Getting Group®) were colonized with single cells or small aggregates of leucocytes; this may reinforce the hypothesis that leucocyte consumption and capture on the MO, may lead to decrease cell counts preceding thrombosis of MO and need for replacement (Bredthauer A, 2017). In conclusion, there is a pathophysiological background that supports the behavior observed in leucocytes, neutrophils and monocytes counts in the days prior to MO exchange. However, to the extent of our knowledge, this is the first study identifying this trend and to find statistically significant differences in monocytes related to MO exchange, which could be related with the fact that monocytes release significant amounts of tissue factor. All these hypotheses should be considered with caution, and further studies should be performed to investigate the behavior of leucocytes with MO exchange.

Another hematological factor here analyzed was platelet count (Tables 4 and 13; Figure 1). Our results show that both groups, with or without MO exchange, have a decreasing tendency when it comes to platelet counts; however, despite the same

kinetic tendency, the differences still showed to be statistically significant. This suggests that, perhaps, there is a cut-off value bellow which MO thrombosis and dysfunction is more likely to happen. Regarding platelet modulation in the context of COVID-19 disease, platelet number fluctuations appear to be correlated with progression and prognosis of SARS-CoV 2 infection: in the beginning there is an increase in platelet count, due to the pro-inflammatory induction of thrombopoietin, and increase in platelet function, with release of their intracellular granules, that contribute to thrombogenesis (Caillon et al., 2022); as the disease evolves and the severity increases, the count drops. Hence, thrombocytopenia (platelet count < 150 $x10^9$/L) is more frequent in patients with severe forms of COVID-19 (Qu et al., 2020; Yin et al., 2021). Regarding ECMO's platelet modulation, it is estimated that approximately 21% of patients under ECMO may experience thrombocytopenia, which means that decreased platelet count is a rather frequent complication of ECMO support (Jiritano et al., 2020). According to the literature, the mechanisms of thrombocytopenia in ECMO can be divided into 2 major groups: 1) circuit related thrombocytopenia and 2) heparin induced thrombocytopenia. Focusing only on circuit related mechanisms, they are caused by the contact between platelets and foreign circuit surfaces, plus high shear stress, during ECMO treatment. These factors have been shown to cause enhanced platelet activation and consumption. Additionally, they have been associated with loss of platelet surface receptors, namely platelet glycoprotein Ibα, important for platelet adhesion, as well as loss of high-molecular-weight von Willebrand factor (vWF) multimers, resulting in decreased binding of vWF to platelets (Abrams et al., 2016; Jiritano et al., 2020; Rauch et al., 2023). These COVID-19 and ECMO related factors could justify why, in this paper, decreasing kinetics in platelet count is seen in both groups, with or without MO exchange. This indicates that platelet drop is a condition intrinsic to both ECMO and severe SARS-CoV2, and not necessarily an indicator of MO dysfunction, just by the decreasing kinetics itself. Accordingly, Basken et al. (2019) hypothesized that during ECMO, a consumptive coagulopathy process occurs with advanced age of the membrane oxygenator, accounting for the increased D-dimer values and decreased fibrinogen and platelet count, reinforcing the idea that platelet count drop is associated with ECMO circuit aging, and not independently associated with MO dysfunction. However, decreasing platelet count has been identified as a common tendency in the days prior to MO

exchange in various studies, and it is said that this could be an indicator of imminent ECMO circuit failure and need for replacement (Basken et al., 2019; Hoffman et al., 2023; Lubnow, Philipp, Dornia, et al., 2014). Our results, appear to concur with this, because even though platelet count drop occurs similarly in both groups, only in the group with MO exchange, patients began with platelet counts <200x10^9/L and reached thrombocytopenic values (platelet count < 150 x10^9/L) 48 hours before MO exchange, which suggests that a severe platelet count drop below a certain cut-off, could predict MO imminent dysfunction and need for exchange.

Fibrinogen levels variation in the days prior to MO exchange did not show statistically significant differences between the two groups (Tables 6 and 13; Figure 3). According with the literature, fibrinogen levels could be important predictors of MO dysfunction, which is, surprisingly, not concordant with our results, although the results were very close to being statistically significant (p 0.0625) and showed a strong correlation between variables (rs=1). D-dimer values showed statistically significant differences between cases and control groups (Tables 5 and 13; Figure 2). Just as explained, direct exposure of blood to artificial, non-endothelized ECMO components, together with narrowings of the circuit, induces high shear stress with turbulence, dilution, activation, and consumption of blood components, significantly impacting the coagulation system (Doyle & Hunt, 2018). Additionally, ECMO patients are universally critically ill, under frequent blood transfusions, prolonged immobilization and non-pulsatile blood flow (Olson et al., 2021). This leads to an imbalance of the plasma coagulation system resulting in a generalized activation of coagulation and fibrinolysis (Webb et al., 1995) As such, it would be expected that coagulation markers that are consumed during thrombogenesis, like platelets and fibrinogen, would be reduced. Similarly, an increase in D-dimers (DD) would be expected, for they are cross-linked fibrin degradation products generated by reactive fibrinolysis (Lubnow, Philipp, Dornia, et al., 2014). Several studies (Table 1) report fibrinogen's decreasing and DD's increasing tendency on the days prior to MO exchange, hypothesizing they could be adequate markers for prediction of MO dysfunction (Basken et al., 2019; Hoffman et al., 2023; Lubnow, Philipp, Dornia, et al., 2014; van Minnen et al., 2023). However, only van Minnen et al. (2023) conducted a study with patients that had, both, COVID-19 (51% of

patients) and concomitant V-V ECMO support; the other studies here analyzed were conducted in patients with ARDS from other causes (not COVID-19) and under V-V ECMO (Basken et al., 2019b; Hoffman et al., 2023; Lubnow, Philipp, Dornia, et al., 2014). Supporting our results regarding DD, Lubnow et al. (2014) demonstrated that a sudden increase in DD levels within 3 days of an MO exchange was a sensitive indicator that could be used to predict an upcoming MO exchange; although D-dimers can be elevated for many reasons in patients receiving ECMO support, a sudden rise could signify incipient failure of the oxygenator. They also found that DD could not be used as a marker of local fibrinolysis in the MO, or as a predictor of MO exchange, in patients with persistently high DD from de beginning of the ECMO run. Other authors also found that increased and persistent high D-dimer levels for more than two days were correlated with increased clot volume in patients with MO dysfunction (Dornia et al., 2015). Concluding, decreased fibrinogen tendency and increase in DD levels are pathophysiologically related with both ECMO, COVID-19 and thrombosis of MO. The fact that fibrinogen has a similar kinetic tendency whether MO exchange is required or not suggests that other factors should be considered to determine its usefulness as a marker of MO dysfunction. Steepness of decrease, or number of days below a certain value, seem to correlate better with the need for MO exchange than the kinetic tendency itself. For example, the decrement of fibrinogen has an increased steepness in the two days before MO exchange, which could be hypothesized to predict an impending MO exchange in the upcoming days.

The results here obtained also showed statistically significant difference in aPTT values between case and control groups in correlation with MO dysfunction and need for replacement (Tables 7 and 13; Figure 4). The behavior of aPTT in severe COVID-19 disease has not yet been clearly established. Some studies reported shortened aPTT in severe disease, however without statistical significance (Gao et al., 2020). Other studies reported prolonged aPTT, but also identified diverse confounding factors, such as anticoagulation with heparin, positive lupus anticoagulant, and elevated C-reactive protein (CRP), which are all underlying conditions often present in severe COVID-19 patients (Christensen et al., 2020; Devreese, 2021). One metanalysis found no statistically significant difference between aPTT values in severe and nonsevere COVID-

19 at admission (Lin et al., 2021). Other study supporting this evidence reported no statistically significant difference in aPTT or thrombin time between severe and nonsevere COVID-19, but also between thrombotic COVID-19 disease and non-thrombotic disease (Jin et al., 2020). Regarding aPTT in ECMO patients, it is a well-established predictor of hemorrhagic risk, and it is frequently used for monitoring therapeutic effect of anticoagulation with heparin or heparin-derivates in ECMO patients. Studies reported that higher aPTT values were correlated with major bleeding events and that a cut-off of aPTT of 72 seconds best discriminated between patients with and without major bleeding, with a sensitivity of 67%, a specificity of 90.6%. Hence, in ECMO, aPTT values >72 seconds have been defined as off-target values associated with a higher risk of major bleeding (odds ratio = 16.4) (Nguyen et al., 2022). When it comes to thrombotic risk in ECMO, Hoffman et al. (2021), Lubnow, Philipp, Dornia, et al. (2014) and van Minnen et al. (2023), evaluated aPTT values and their variation in patients with thrombotic events under V-V ECMO, namely oxygenator thrombosis and exchange; none of them found statistical significant difference in aPTT levels between patients with or without MO exchange. However, another study with patients under V-V ECMO, but without COVID-19, observed a significantly lower aPTT (47.0 ± 12.3 seconds vs. 53.6 ± 12.5 seconds; p = 0.037) in patients diagnosed with venous thrombosis, suggesting decreased aPTT values may be useful for the prediction of thrombotic events. Overall, the primary role of aPTT measurement in the setting of V-V ECMO is to monitor the effectiveness of anticoagulation with heparin derivates or direct thrombin inhibitors, such as bivalirudin. Our institutional protocol recommends measuring aPTT values every 4 hours in these patients, adjusting anticoagulation to target an aPTT ratio between 1.5 to 2, which translates to an aPTT around 50-60 seconds. During ECMO run, patients who underwent MO exchange had different anticoagulant strategies: 28 patients were under anticoagulation with unfractioned heparin (UFH), 19 patients were under anticoagulation with bivalirudin, and one patient was without anticoagulation, due to clinically significant hemorrhage. The statistically significance found between aPTT values in patients with and without exchange suggest that patients in the case group, that had overall lower values of aPTT, where sub-therapeutically anticoagulated; this attests the relevance of anticoagulation protocols and the importance of maintaining aPTT levels out of the desired range, for as little time

as possible. Additionally, although it showed statistically significant difference, the correlation of aPTT values with MO dysfunction in the two groups had small strength of association (rs=0.14), which weakens its statistical power, suggesting this may not be the most adequate hematological factor to predict MO dysfunction and need for exchange.

The correlation between CRP levels and MO thrombosis also showed to have statistically significant difference between patients with and without MO exchange (Tables 8 and 13; Figure 5). CRP is a commonly used biomarker of systemic inflammation and has been studied as a predictor of clinical outcomes in critically ill patients. Various studies described the utility of CRP to triage COVID-19 patients; these papers reported a significant difference in CRP levels between severe and non-severe COVID-19 patients and showed a correlation with mortality risk, suggesting it could be used to differentiate patients according to disease severity (Abdullah et al., 2023; Luan et al., 2021). Another study found CRP to be the strongest univariable predictor for deterioration in COVID-19 patients (deterioration was defined as initiation of ventilatory support in the form of non-invasive ventilation, invasive mechanical ventilation, extracorporeal membrane oxygenation, admission to intensive care unit or death) (Gupta et al., 2021). Regarding the variability of CRP in V-V ECMO supported patients, Lichter et al. (2024), showed statistically significant decrease in CRP levels during the first 5 days of V-V ECMO run in patients without COVID-19, but the results did not reach statistical significance for patients with COVID-19 requiring V-V ECMO support, despite a trend of decline in CRP levels. The authors hypothesized that the marked decrease in CRP levels observed in V-V ECMO patients may be related to the abrupt transition to ultra-protective ventilation immediately after ECMO initiation, therefore mitigating the degree of alveolar stress and strain, and ultimately decreasing the systemic inflammatory process (Lichter et al., 2024). Contrarily, the exposure of blood to the artificial ECMO circuit can induce an inflammatory response and consequent increase in CRP; just as many other variables that may lead to increase in CRP during ECMO, for example, infections. Studies have described that this pro-inflammatory activation, if severe, persistent or unchecked by a compensatory anti-inflammatory response, may lead to endothelial injury, disruption of microcirculation, and end-organ failure, justifying pathophysiologically why thrombus

formation in the MO may occur in states of higher CRP levels (McILwain et al., 2010). Another research suggests that CPR, by itself, can induce endothelial dysfunction and promote oxidative stress, leading to higher thrombotic risk (Schwedler et al., 2007). Hence, we can hypothesize that in patients with infection, systemic inflammatory response syndrome, sepsis or severe ARDS, pro-thrombogenic state is enhanced and signalized by increased levels of CRP. This is supported by another study which identified that MO exchanges were more frequent in a subgroup of V-V supported, COVID-19 patients with sepsis (Rui F. Gomes et al., 2023). On the other hand, a study conducted in patients with COVID-19 requiring V-V ECMO support, found no statistically significant difference in CRP measurements between patients who required MO exchange and those who did not, which is not concordant with our results (van Minnen et al., 2023). However, although our results showed to be statistically significant, they also state that the correlation of CRP levels between case and control group had small strength of association (rs=-0.26), therefore diminishing its statistical power and suggesting CRP levels may not be the best indicator to independently predict MO dysfunction and need for exchange.

Lastly, the levels of carboxyhemoglobin (COHb) on the days prior to MO exchange, showed statistically significant differences between case and control groups (Tables 12 and 13; Figure 9). The production of endogenous carbon-monoxide (CO) depends mostly on increased heme metabolism, as it is seen in hemolysis. Upon destruction of erythrocytes there is an increase in free hemoglobin, inducing heme oxygenase (HO-1), which catalyzes a reaction that converts hemoglobin into biliverdin, releasing CO in the process (Rose et al., 2017). CO has a very high affinity for hemoglobin, thereby forming carboxyhemoglobin (COHb), and reducing oxygen-binding to hemoglobin molecules, hence diminishing oxygen transport and delivery to tissues. Higher concentrations of CO may ultimately lead to tissue hypoxemia and serious organ damage, affecting mitochondrial respiration, and leading to an inflammatory response and formation of free radicals (Rose et al., 2017). In the context of COVID-19, several studies showed elevated carboxyhemoglobin concentrations in severely ill patients' blood; it was hypothesized that increased COHb levels in SARS-CoV2 infection could be due to oxidative stress and inflammation which induced HO-1 activity (Scholkmann et

al., 2020; Yasuda, 2002). Other hypothesis for causes of increased COHb in COVID-19, were auto-immune hemolytic anemia, direct viral infection of erythrocytes, and respiratory insufficiency with decreased clearance of CO (Lazarian et al., 2020; Lechuga et al., 2021). Regarding ECMO, hemolysis is a relatively common complication; literature reviews report up to 29% of hemolysis incidence in V-V ECMO (Appelt et al., 2020). This is thought to occur because centrifugal pumps, as well as tubing, induce very high shear stress that, as the ECMO circuit ages, wears down erythrocytes and eventually leads to hemolysis (Lehle et al., 2015). A retrospective study described that during the course of ECMO therapy, median arterial COHb increased from 1.5% in samples taken during the first day of ECMO support to 1.9% in samples taken from patients with ECMO support >72h, which shows a steady increase in COHb with aging of the ECMO circuit (Bemtgen et al., 2022). This steady increase in COHb levels was also observed in our results in both cases (variation from median 2.3% on day -5 to median 2.6% on day 0) and control groups (variation from median 1.5% on day -5 to median 1.9% on day 0). Thrombosis of the oxygenator and, more importantly, pump head thrombosis, are also factors associated with hemolysis during ECMO; thrombi in the oxygenator create local turbulent flow conditions and higher shear stress, which enhances hemolysis at the cloth site (Lehle et al., 2015; Lou et al., 2014). On the other hand, upon hemolysis, there is a rapid increase in free Hb which scavenges nitric oxide (NO), decreasing available NO, and leading to inappropriate vasoconstriction, endothelial dysfunction and platelet aggregation, hence promoting thrombogenesis (L'Acqua & Hod, 2015; Schaer et al., 2013). This means that hemolysis in ECMO can either occur as a cause or a consequence of MO thrombosis and dysfunction; this way, if COHb is increased as a marker of hemolysis, it would appear to be an adequate marker for MO dysfunction. It would be expected to encounter higher levels of COHb in patients with MO dysfunction than in control groups. Various studies state that COHb has shown progressive increase and subsequent decrease around the time of oxygenator replacement, suggesting a role for COHb values as an early marker of oxygenator-induced hemolysis, which is supported by our results (Hariri et al., 2021; Hoffman et al., 2021, 2023; van Minnen et al., 2023).

To conclude, one interesting other possible marker for MO dysfunction is plasma free hemoglobin (PFHb). This is the direct product of hemolysis, for which COHb is only

a surrogate marker. PFHb is the gold standard for diagnosis and monitoring of intravascular hemolysis in ECMO patients, and its use is recommended by Extracorporeal Life Support Organization (ELSO). On the other hand, the routine use of PFHb has some limitations. Namely its low availability in ECMO centers' laboratories, the possibility of delayed results due to the considerable time it may take to measure, false positive results in cases of traumatic sampling, and the interference of bilirubin levels and lipaemia with spectrophotometric methods of quantification (Hoffman et al., 2021). In our study this marker was not used due to inexistence of laboratory capacity in our ECMO center, but it would be a useful marker to explore in future studies.

Strengths and limitations

One of the main limitations of our study is the fact that this is a retrospective-observational study, subjected to selection bias, incomplete datasets, and lack of strict protocols regarding ECMO management. Although including all V-V ECMO patients during the study period may have reduced the selection bias, the study population was relatively small (N=93); on the other hand, this allowed for a heterogeneous population which has more real-life applicability of the analyzed variables as independent predictors of MO exchange. During the study period we exclusively used the Cardiohelp® (Maquet-Getinge Group®) oxygenators, which makes the results possibly not translatable to other oxygenators. The absence of a consensual definition for oxygenator dysfunction leads to heterogeneity in criteria used when making the decision to perform an oxygenator exchange; as a result, different ECMO-intensivists and perfusionists could decide to exchange the oxygenator at different timings based on the variables here analyzed complemented with other clinical characteristics, such as vital signs. Lastly, this study only analyzed variation in hematological factor in the days preceding MO exchange; this may mask the predicting capacity for MO failure of some factors that have the same kinetic tendency in patients with or without MO dysfunction. Meaning that factors that vary in similar ways in both groups, that may depend on the identification of a cut-off value to be statistically significant different, may not be identified when only the data preceding MO exchange is considered. If the values of the

predicting factors are analyzed in the days before and after MO exchange, we could expect a normalization of their values upon a new MO, which could facilitate the identification of predicting factors for MO dysfunction. Hence, the analysis of data only referring to the period before MO exchange could present as a limitation.

On the one hand, the selected time period for analyzes is a strength for it was a period of high incidence and prevalence of COVID-19 which allowed for a more robust amount of subjects; on the other hand, the fact that only COVID-19 patients were selected limits the applicability of these results to V-V ECMO supported patients, without COVID-19. Furthermore, this study is one of the first to conjugate the analyzes of hematological factors in a dynamic way, during five days, leading up to MO exchange, but also important pre-ECMO medical care characteristics that could impact the same hematological factors even before ECMO was initiated. To conclude, to the extent of our knowledge this is one of the first studies to identify monocytes has predictors of MO exchange, which can open new ideas for future research of surrogate markers of leucocyte consumption as predictors of oxygenator imminent dysfunction.

CONCLUSIONS:

In summary, the divergency of results among various studies suggests that there is no single marker that can consistently be used to fully predict MO dysfunction in these patients. By collecting the combination of demographics, medical interventions, physiological markers and severity of disease and outcomes data, we were able to provide a whole picture of different parameters that could possibly be analyzed to predict MO exchange. Our research aligns with current literature by highlighting the importance of using various indicators to forecast MO exchange; effective management of circuit replacements during ECMO involves taking into account multiple factors including platelet count, leucocyte count, fibrinogen levels, D-dimers, blood gases, plasma-free hemoglobin, flows, pressures, and anticoagulation strategy. Observing these parameters enables timely detection of problems, prompt actions, and optimal ECMO treatment. Uniformed procedures, tailored anticoagulation methods, and non-invasive monitoring can enhance the safety and efficacy of MO exchange. However, further research is needed on factors that predict oxygenator failure with the aim of defining clear cut-off values for the timing of the exchange.

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APPENDIX

Article Title	Design and Methods	Sample size	ECMO type	COVID-19	Circuit exchange causes and respective incidence (%)
Hoffman et al., 2023: The Hematological Effects of Extracorporeal Membrane Oxygenator Exchange	Single-centre retrospective study	N=100	V-V ECMO	No	Circuit thrombosis 57% Bleeding due to ECMO coagulopathy 7% Intravascular hemolysis 27% Circuit failure 4.5% Unknown 4.5%
Basken et al., 2019: Predictors of Oxygenator Exchange in Patients Receiving Extracorporeal Membrane Oxygenation	Single-centre retrospective study	N=151	V-V ECMO V-A ECMO	No	—
Lubnow, Philipp, Dornia, et al., 2014: D-dimers as an Early Marker for Oxygenator Exchange in Extracorporeal Membrane Oxygenation	Single-centre retrospective study	N=24	V-V ECMO	No	Membrane oxygenator thrombosis 66% Bleeding due to ECMO coagulopathy 25% Severe drop in total platelet count
van Minnen et al., 2023: Risk factors for elective and emergency oxygenator exchanges during veno-venous extracorporeal membrane oxygenation	Single-centre retrospective study	N=45	V-V ECMO	Yes (51%)	Oxygenator thrombosis Pump head thrombosis Functional decline of the oxygenator Acquired coagulation disorders (haemorrhagic and thrombotic complications)
Philip et al., 2018: Life span of different extracorporeal membrane systems for severe respiratory failure in the clinical practice	Single-centre prospective study	N=461	V-V ECMO	_	Blood or water leakage of the oxygenator Acute oxygenator thrombosis Pump head thrombosis

Article Title	MO Exchange Rate (%)	Median ECMO run duration (days)	Membrane duration recommendation by the producing brand (hours, days)	Factors associated with ECMO circuit exchange*
Hoffman et al., 2023: The Hematological Effects of Extracorporeal Membrane Oxygenator Exchange	31%	8 days	Quadrox oxygenator (Maquet/ Getting) - 6 hours	** Increase in Plasma free hemoglobin Increase in D-dimer:fibrinogen ratio Increase in D-dimers Increase in carboxyhemoglobin Decrease in fibrinogen Decrease in platelets
Basken et al., 2019: Predictors of Oxygenator Exchange in Patients Receiving Extracorporeal Membrane Oxygenation	23.1%	9 days	Quadrox oxygenator (Maquet/ Getting) - 6 hours	*** Increase in D-dimer Decrease in fibrinogen Decrease in platelet count Decrease in heparin dose
Lubnow, Philipp, Dornia, et al., 2014: D-dimers as an Early Marker for Oxygenator Exchange in Extracorporeal Membrane Oxygenation	28.4%	20 days	Quadrox PLS oxygenator (Maquet/Getting) - 14 days	** Increase in D-dimer Decrease in platelet count
van Minnen et al., 2023: Risk factors for elective and emergency oxygenator exchanges during veno-venous extracorporeal membrane oxygenation	42.2%	ECMO run - 16 days MO lifespan - 9 days	Cardiohelp HLS set advanced 7.0 (Maquet/Getting) - 30 days	Increase in D-dimer Increase in carboxyhemoglobin Decrease in fibrinogen
Philip et al., 2018: Life span of different extracorporeal membrane systems for severe respiratory failure in the clinical practice	30%	ECMO run - 19 days MO lifespan - 9 days	PLS, n = 117 Cardiohelp HLS-set (Maquet/ Getting), n = 107 - 30 days Deltastream-system/Hilite7000LT, n = 135 iLA-activve, n = 15 ECC.O5-system, n = 87	—

*only represented the factors that showed statistical significance ($p<0.05$).

**parameters evaluated during the 72 hours before and after membrane oxygenator exchange.

***parameters evaluated only during the 72 hours prior to membrane oxygenator exchange.

Table 1: Summary of most relevant literature reporting data regarding ECMO circuit exchange causes and their incidence, MO exchange rate, ECMO total duration, MO lifespan and factors possibly predicting MO exchange.

Legend: ECMO – Extracorporeal membrane oxygenation; MO – membrane oxygenator.

Parametrer	Summary of data
Sex[a]	66 males (71%)
Age (years old)[b]	54 (41-58) years old
Body mass index (BMI) (kg/m^2)[b]	30.4 (26.2-36.2) kg/m^2
Ideal body weight (kg)[b]	67 (59-75) kg
Comorbidities[a]:	
- Obesity	53 (56.9%)
- Chronic hypertension	32 (34.4%
- Dislipedaemia	29 (31.2%)
- Diabetes mellitus	10 (10,8%)
- Former smoker	9 (9.7%)
- Pregnancy	6 (6.5%)
- Active smoker	4 (4.3%)
- Ethilic habits	4 (4.3%)
- Hypotiroidism	4 (4.3%)
- COPD/asthma	4 (4.3%)
- Peripheral arterial disease	3 (3.2%))
- Ischemic cardiopathy	2 (2.2%)
- Liver steatosis	2 (2.2%)
- Obstructive sleep apnea	2 (2.2%)
- Others (atrial fibrillation, mitral valve prolapse, aortic stenosis with prosthetic aortic valve, subvalvular congenital aortic stenosis, aortic insufficiency and aortic aneurism with prosthetic aortic valve, diastolic heart failure, status post ischemic cerebral stroke, epilepsy, HIV infection, T cell leukemia/lymphoma of the adult, chronic kidney disease and Alport syndrome with status post kidney transplant)	12 (12.9%)

Parametrer	Summary of data
ICU LoS (days)[b]	35 (21-54) days
SAPS II[b]	33 (26-42)
SOFA at admission[b]	5 (4-7)
SOFA at cannulation[b]	6 (4-8)
SOFA at discahrge from ICU[b]	2 (1-4)
ICU mortality[a]	24.73%
MO lifespan (days)[b]	15 (11-27) days
ECMO total run (days)[b]	26 (14-42) days
MO exchange rate[a]	48 (51.61%)
Number of MO exchanges[b]	1 (0-1)

a –number (percentage); b – median (IQR).

Table 2: Summary of the demographic description of the population.

Legend: BMI – Body mass index; COPD – Chronic obstructive pulmonary disease; HIV – Human immunodeficiency virus; ICU – Intensive care unit; LoS – Length of stay; SAPS II – Simplified acute physiology score II; SOFA – Sequential organ failure assessment; MO – membrane oxygenator; ECMO – Extracorporeal membrane oxygenation.

Parametrer	Summary of data		P value
	With MO exchange, n=48 (51.61%)[b]	Without MO exchange, n=45 (48.38%)[b]	
Age (years old)[a]	55 (43-59) years old	51 (38-59) years old	0.172
Days under HFNC[a]	0 (0-4) days	1 (0-4) days	0.697
Total days under NIV pre-ECMO[a]	0 (0-2) days	0 (0-1) days	0.835
Total days under IMV pre-ECMO[a]	5 (3-6) days	4 (3-8) days	0.516
Total sick days pre-ECMO[a]	16 (11-19) days	17 (12-22) days	0.429
Total days under corticotherapy pre-ECMO[a]	42 (25-69) days	25 (17-40) days	**0.0018**
Total days under ECMO[a]	35 (26-57) days	14 (12-26) days	**<0.0001**
Total days under IMV[a]	40 (28-48) days	19 (12-33) days	**<0.0001**
Total days under NMBA[a]	13 (8-20) days	6 (4-12) days	**0.0004**
SAPS II[a]	33 (26-38)	33 (27-47)	0.386
SOFA at admission[a]	4 (4-7)	5 (4-7)	0.369
SOFA at cannulation[a]	6 (4-8)	6 (4-10)	0.437
SOFA at ICU discharge[a]	2 (1-4)	2 (1-4)	0.552
MO lifespan (days)[a]	15 (9-31) days	14 (12-26) days	0.65

a–median (IQR); b – number (percentage).

Table 3: Summary of the statistical analysis between cases (patients with MO exchange) and controls (patients without MO exchange), using Mann Whitney test.

Legend: HFNC – High-flux nasal cannula; NIV – Non-invasive ventilation; ECMO – Extracorporeal membrane oxygenation; IMV – Invasive mechanical ventilation; NMBA – Neuromuscular blockage agents; SAPS II – Simplified acute physiology score II; SOFA – Sequential organ failure assessment; ICU – Intensive care unit; LoS – Length of stay; MO – membrane oxygenator.

Days prior to MO exchange	Platelet median count (x10^9/L)	
	NO MOex	WITH MOex
-5	206x10^9/L	173.5x10^9/L
-4	194x10^9/L	167.5x10^9/L
-3	198x10^9/L	159.5x10^9/L
-2	171x10^9/L	152.5x10^9/L
-1	171x10^9/L	135x10^9/L
0	152x10^9/L	133x10^9/L

Table 4: Variation in median platelet count during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; -5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Days prior to MO exchange	D-dimer median levels (mg/L)	
	NO MOex	WITH MOex
-5	5,15 mg/L	6,97 mg/L
-4	7,1 mg/L	7,01 mg/L
-3	7,26 mg/L	12,77 mg/L
-2	11,01 mg/L	12,925 mg/L
-1	14,75 mg/L	15,055 mg/L
0	14,085 mg/L	18,57 mg/L

Table 5: Variation in median D-dimer levels during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange (NO MOex).

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; -5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Days prior to MO exchange	Fibrinogen median levels (mg/dL)	
	NO MOex	WITH MOex
-5	414 mg/dL	447 mg/dL
-4	387,5 mg/dL	446 mg/dL
-3	370 mg/dL	372 mg/dL
-2	335 mg/dL	384 mg/dL
-1	326 mg/dL	359 mg/dL
0	281,5 mg/dL	311,5 mg/dL

Table 6: Variation in median fibrinogen levels during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; -5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Days prior to MO exchange	aPTT median values (seconds)	
	NO MOex	WITH MOex
-5	43,3	40,45
-4	45,3	42,35
-3	44,5	41,6
-2	44,35	43,7
-1	46,9	43,85
0	43,55	43,4

Table 7: Variation in median activated partial thromboplastin time during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; aPTT -- activated partial thromboplastin time; -5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Days prior to MO exchange	CRP median values (mg/L)	
	NO MOex	WITH MOex
-5	4,8 mg/L	8,155 mg/L
-4	4,2 mg/L	6,48 mg/L
-3	2,8 mg/L	7,74 mg/L
-2	3,3 mg/L	6,73 mg/L
-1	3,7 mg/L	7,36 mg/L
0	3,9 mg/L	8,38 mg/L

Table 8: Variation in median C-reactive protein values during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; CRP – C-reactive protein; -5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Days prior to MO exchange	Leucocyte median count (x10^9/L)	
	NO MOex	WITH MOex
-5	13.15 x10^9/L	12.25 x10^9/L
-4	13.76 x10^9/L	11.7 x10^9/L
-3	12.77 x10^9/L	11.05 x10^9/L
-2	13.4 x10^9/L	10.8 x10^9/L
-1	12.4 x10^9/L	11.4 x10^9/L
0	12.4x10^9/L	11.7 x10^9/L

Table 9: Variation in median leucocyte counts during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; WBC – white blood cells; -5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Days prior to MO exchange	Neutrophils median count (x10^9/L)	
	NO MOex	WITH MOex
-5	10.57 x10^9/L	9.92 x10^9/L
-4	10.72 x10^9/L	9.72 x10^9/L
-3	10.83 x10^9/L	8.65 x10^9/L
-2	10.44 x10^9/L	7.99 x10^9/L
-1	10.55 x10^9/L	8.17 x10^9/L
0	10.82 x10^9/L	9.65 x10^9/L

Table 10: Variation in median neutrophil counts during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; 5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Days prior to MO exchange	Monocytes median count (x10^9/L)	
	NO MOex	WITH MOex
-5	7.1 x10^9/L	6.42 x10^9/L
-4	7.32 x10^9/L	5.91 x10^9/L
-3	8.54 x10^9/L	6.47 x10^9/L
-2	8.28 x10^9/L	5.56 x10^9/L
-1	8.2 x10^9/L	5.51 x10^9/L
0	7.8 x10^9/L	5.65 x10^9/L

Table 11: Variation in median monocytes counts during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; 5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Days prior to MO exchange	Carboxyhemoglobin median levels (%)	
	NO MOex	WITH MOex
-5	1,5%	2,3%
-4	1,6%	2,3%
-3	1,8%	2,3%
-2	1,7%	2,4%
-1	1,8%	2,7%
0	1,9%	2,7%

Table 12: Variation in median carboxyhemoglobin percentage (%) during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; COHb – carboxyhemoglobin; 5 – five days prior to membrane oxygenator exchange; -4 - four days prior to membrane oxygenator exchange; -3 - three days prior to membrane oxygenator exchange; -2 - two days prior to membrane oxygenator exchange; -1 - one day prior to membrane oxygenator exchange; 0 – day of membrane oxygenator exchange.

Parametrer	W value	P value	rs (strenght of correlation)	Conclusion
Platelet count[a]	-21	0.0312	1.0 (large)	Statistically significant, similar tendency in variation, strong correlation between variables.
D-dimer levels[a]	-21	0.0312	1.0 (large)	Statistically significant, similar tendency in variation, strong correlation between variables.
Fibrinogen levels[a]	19	0.0625	1.0 (large)	Not statistically significant, similar tendency in variation, strong correlation between variables.
APTT[a]	21	0.0312	0.14 (small)	Statistically significant, similar tendency in variation, small correlation between variables.
C-reactive protein[a]	21	0.0312	-0.26 (small)	Statistically significant, opposite tendency in variation, small correlation between variables.
Leucocyte count[a]	11	0.312	-0.2 (small)	Not statistically significant, opposite tendency in variation, small correlation between variables.
Neutrophil count[a]	-15	0.156	-0.7 (large)	Not statistically significant, opposite tendency in variation, large correlation between variables.
Monocyte count[a]	21	0.0312	-0.66 (large)	Statistically significant, opposite tendency in variation, strong correlation between variables.
Carboxyhemoglobin levels[a]	21	0.0312	0.66 (large)	Statistically significant, similar tendency in variation, strong correlation between variables.

a – Absolute values were considered for the statistical analysis.

Table 13: Summary of results from the Wilcoxon Signed-Rank Test (two-tailed), confidence interval 95%.

Legend: aPTT – Activated partial thromboplastin time; W – sum of signed ranks, result of Wilcoxon test; rs – Spearman's correlation coefficient.

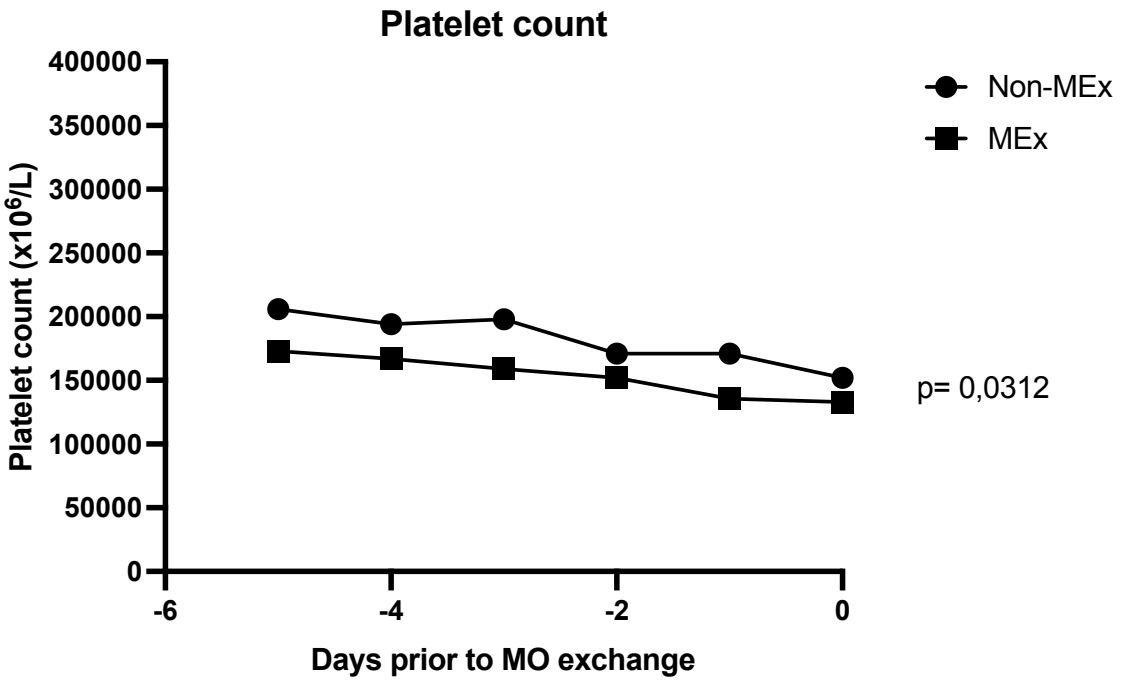

Figure 1: Absolute variation in platelet count during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation.

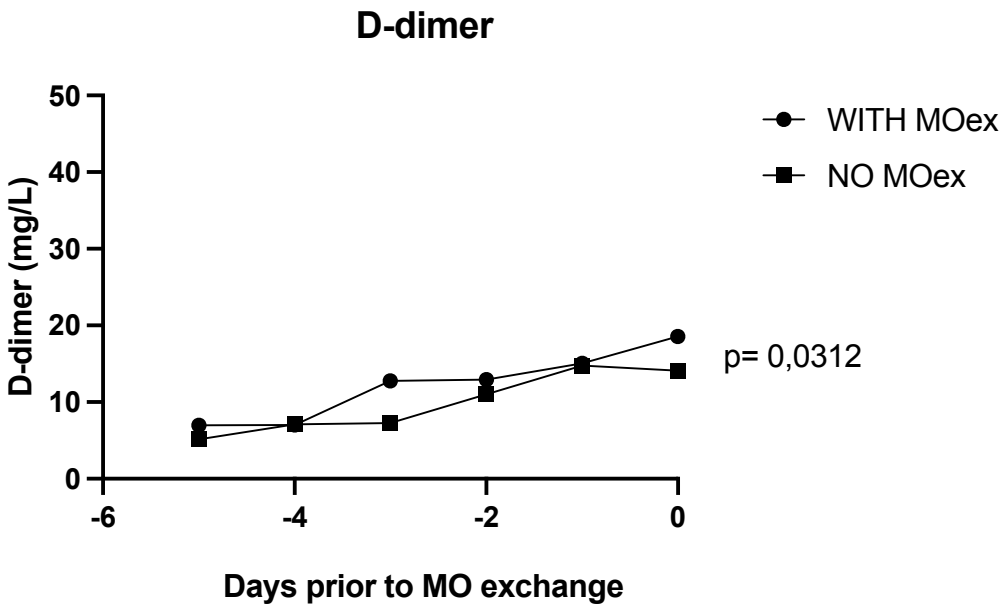

Figure 2: Absolute variation in D-dimer levels during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange (NO MOex).

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation.

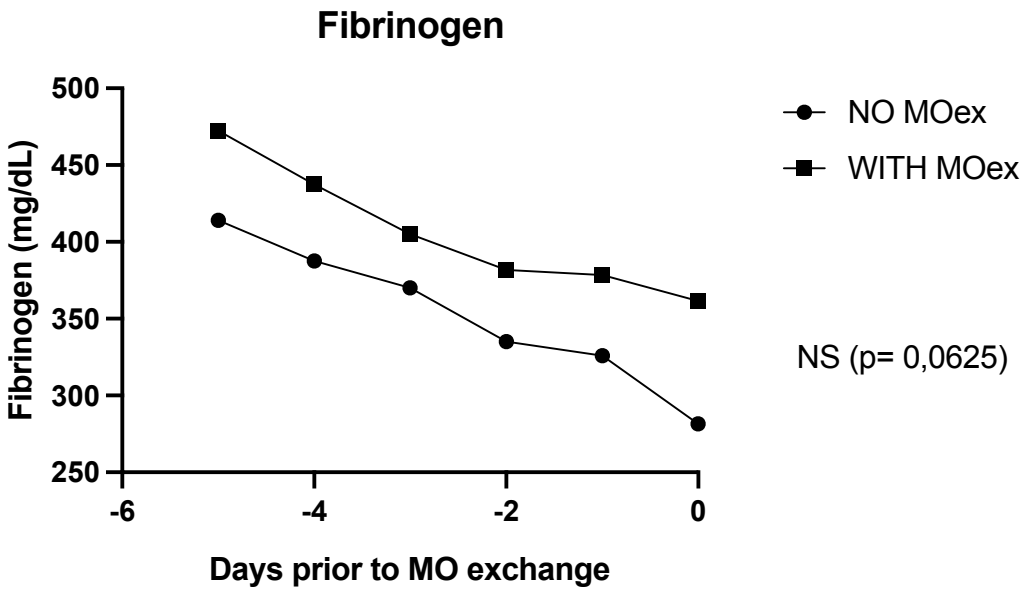

Figure 3: Absolute variation in fibrinogen levels during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation.

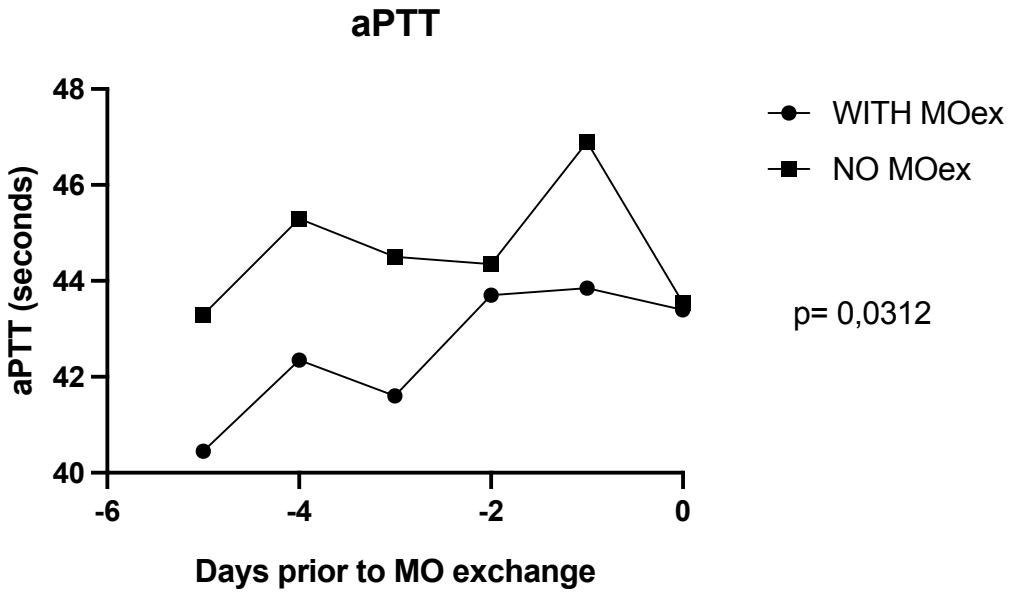

Figure 4: Absolute variation in activated partial thromboplastin time during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; aPTT -- activated partial thromboplastin time.

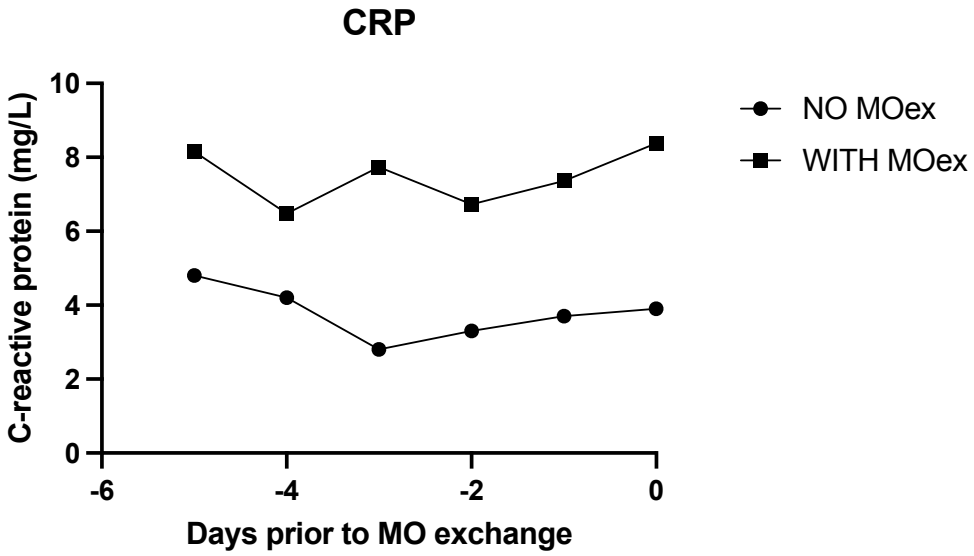

Figure 5: Absolute variation in C-reactive protein values during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; CRP – C-reactive protein.

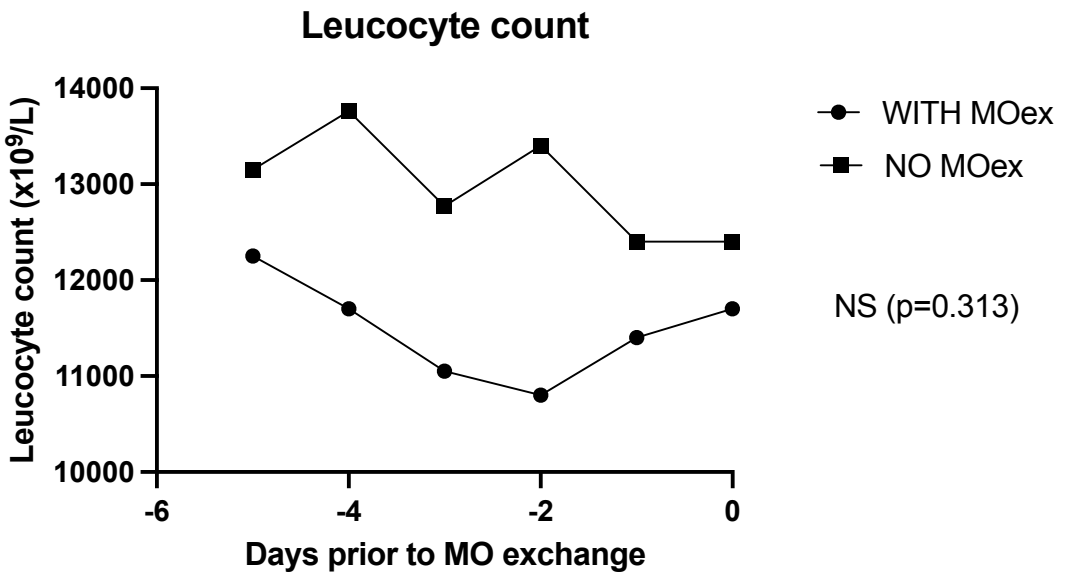

Figure 6: Absolute variation in leucocyte counts during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; WBC – white blood cells.

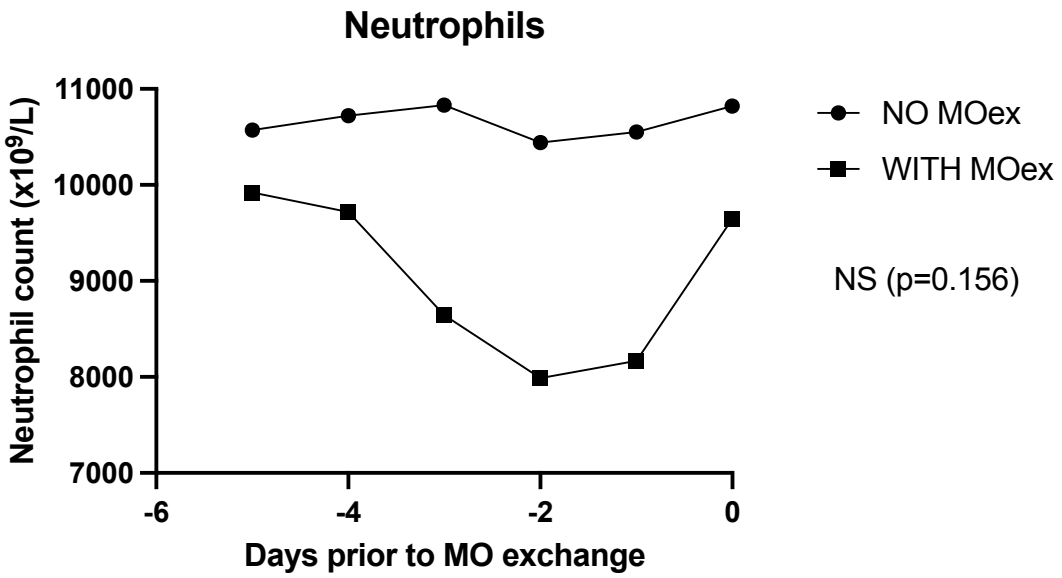

Figure 7: Absolute variation in neutrophil counts during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation.

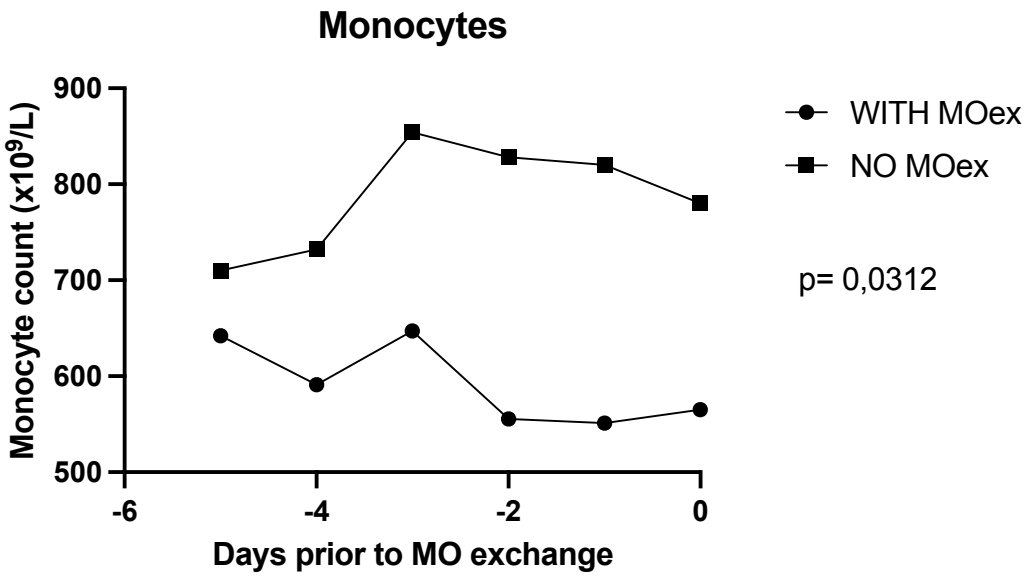

Figure 8: Absolute variation in monocytes counts during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation.

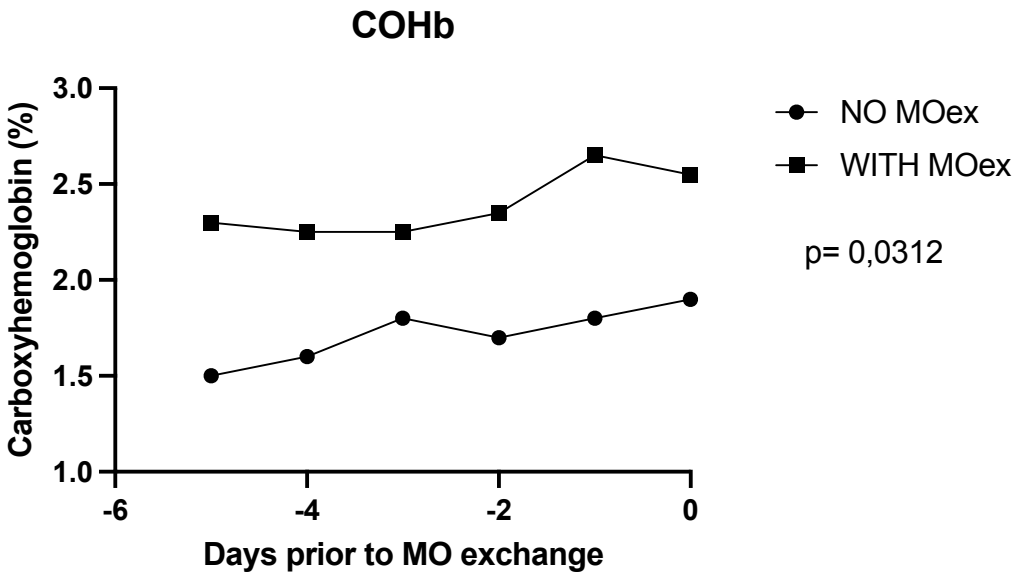

Figure 9: Absolute variation in carboxyhemoglobin percentage (%) during the 5 days prior to first membrane oxygenator exchange in patients with MO exchange, or before end of ECMO run in patients without MO exchange.

Legend: MO – membrane oxygenator; NO MOex – control group, without MO exchanges; WITH MOex – case group, with at least one MO exchange; ECMO – extracorporeal membrane oxygenation; COHb – carboxyhemoglobin.

Contributo individual da aluna para o projeto:

- Pesquisa bibliográfica para introdução e contextualização do tema e para discussão dos resultados;
- Contribuição para a elaboração da base de dados, nomeadamente para a colheita de dados relativos às intervenções médicas e variação de fatores hematológicos ao longo do tempo, em relação com troca de membrana;
- Escrita na íntegra do trabalho final de mestrado, realização autónoma de parte do trabalho estatístico, e apoio na restante análise estatística e interpretação de resultados;
- Escrita autónoma de abstract para posterior apresentação em Congresso adequado ao tema do trabalho de investigação aqui representado.