Challenges in extracorporeal membrane oxygenation

CHALLENGES IN EXTRACORPOREAL MEMBRANE OXYGENATION: PRECISION MANAGEMENT TO IMPROVE SURVIVAL

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Chapter 1

General Introduction

Introduction

The technical improvement of easy-to-use technology, the increase of survival under extracorporeal membrane oxygenation (ECMO) support in many centers, the constant requests by governments, and public opinion of escalating the support to reduce the mortality in the ICU has tremendously increased the number of ECMO centers, and application of ECMO worldwide (Figure 1 and 2).[1-3] This increase has been prompted by positive results of ECMO both in cases of respiratory cases and in cases of cardiac and cardio-respiratory support.[4,5] In fact, ECMO for post-cardiotomy shock as well as in case of cardiogenic shock due to myocardial infarction is currently an established and valuable tool to reduce mortality in these cases, though morbidity and mortality are still high. [6-8] Moreover, the veno-venous ECMO design has been shown to be associated with better outcomes in adults with severe stage of acute respiratory distress syndrome (ARDS) to rescue acute lung injury (EOLIA trial), and this has been confirmed by a post-hoc Bayesian analysis of the same trial, by a global meta-analysis (based on a limited number of trials) and by a recent systematic review and individual patient data meta-analysis. [9-12] And, last but not least, ECMO is currently adopted for a number of pre- or post-operative conditions needing cardio/respiratory support as a bridge to decision, to the operating room or to further escalation of the long-term support, including the bridge to lung and heart transplantation. [13-20] It is also applied for cardiac arrest as extra-corporeal cardiopulmonary resuscitation (ECPR). [21-23]

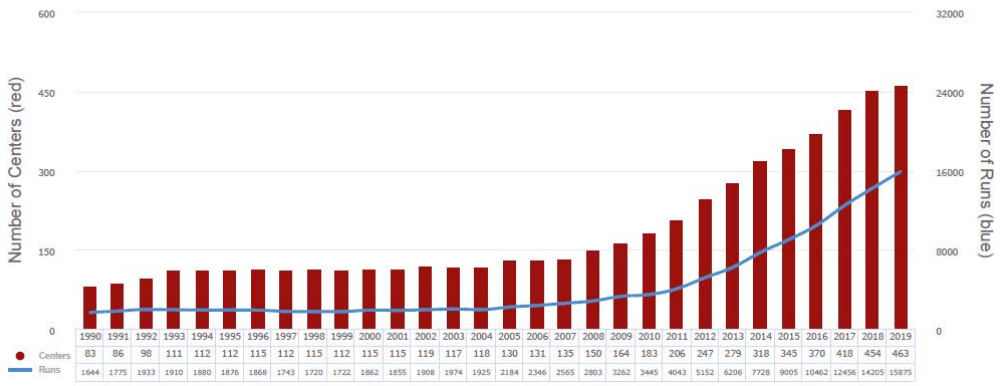

Figure 1

Evolution of the number of centers practicing ECMO worldwide according to the ELSO Registry (International summary – July, 2020).

However, despite such an increase in use, the basic scientific evidence of ECMO's superiority to other conventional or experimental treatments does not lie in classical evidence-based medicine and, moreover, a number of issues are unknown or neglected. [23,24,25] In this regard, little is really understood about the impact of ECMO use on organ tissues and systems, and daily clinical management is still based on personal beliefs, center history and attitude and, finally, complications (despite constant reduction) are not yet understood completely in terms of pathophysiology. [26,27] Additionally, the type of management in several respects, starting from indications and continuing to anticoagulation and prevention of bleeding and neurological injury, is not standardized by concrete guidelines.[28-30]

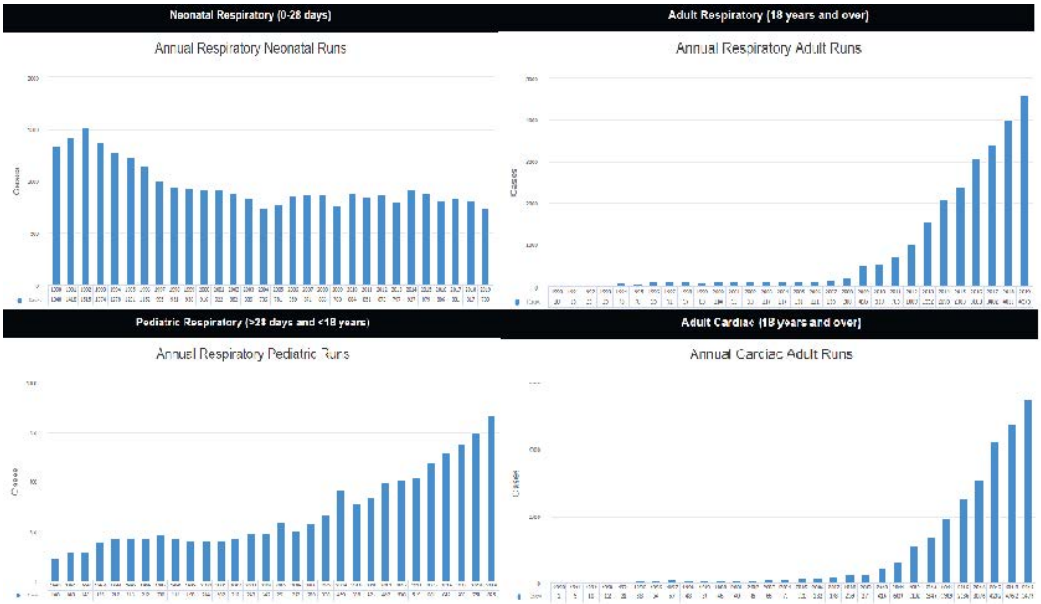

Figure 2

Evolution of ECMO use in neonatal, pediatric patients, and adults over time according to the ELSO Registry (International summary – July, 2020)

The COVID-19 pandemic has highlighted these unexplored aspects in a paradigmatic way. [31-33] Therefore, since ECMO is a costly and complex therapy, at the beginning of the pandemic, when the disease was still generally unknown (many things have recently been discovered about COVID-19, and the community was still far from a complete understanding of the disease, treatment, and patient stratification), many experts, stakeholders, and scientific societies were initially very reluctant to apply ECMO in such patients.[33-36] The risk of applying a therapy with still many unresolved challenges to an unknown disease spreading as a pandemic, with its consequent impact on bed occupancy, respiratory

and, resource availability, was overwhelming the potential (hypothesized or realized) benefits. [31,37-39] But, since the beginning ECMO was had the clear potential to be a means of support in COVID-19 patients presenting with an ARDS-like picture of the severe COVID-19 disease, the frequent cause of death represented by fatal arrhythmias, myocarditis and pulmonary embolism. [40] At the same time, the ECMO practitioners were a reservoir of knowledge for coagulation derangements and anticoagulation therapy and management. [41-43] Therefore, the lessons learned by ECMO specialists in terms of ultra-protective ventilation and prone position, fluid administration and hemoglobin management, coagulation monitoring and anticoagulation methods, cardiac support and monitoring, was fundamental during the care of COVID-19 patients in the general ICUs more than in the ECMO environment, and even outside the ICU. [44,45] Similarly, in recent years, the ability to work and manage extracorporeal circuits and properly define oxygenation, decarboxylation and adequate metabolic sustainment have been successfully lent to other fields, such as organ preservation and organ reconditioning. [17,46-48]

However, critical care medicine is perhaps now evolving faster than the attainment of medical evidence. After the sporadic use documented by case reports and case series, ECMO has been applied massively worldwide, with a reasonable difference among countries due to the immediate burden of the pandemic and the economic situation.[32] But as demonstrated by the EuroELSO Survey, as well as by relevant case series, ECMO was largely applied with a reliable ability to reduce mortality in the most severe cases.[32,40,49,50]

Historical perspective

Despite all these premises, from the first use of a heart/lung machine by John Gibbon in 1953 at the Mayo Clinic and the subsequent development of artificial membrane lungs in the 1960s, ECMO has travelled a long and successful way, largely in the last two decades.[51-54]

Respiratory support was first adopted outside the operating room in 1971, and cardiac support in 1972, while ECMO for newborn and neonatal respiratory failure began in 1975. Initially, the outcomes were highly favorable for neonates and children; consequently, the number of pediatric ECMO patients was more than in adults. From then to the 2000s, a number of technical and theoretical principles have been established regarding the biomaterials utilized, the vascular accesses, physiology, anticoagulation, gas exchanges, and patient management. [55-57] In the last 20 years, the progressive improvement in outcomes has changed the pattern of ECMO use, as seen in the ELSO registry, and currently the use in adults is the preponderant application.[12]

Moreover, year after year, new applications and techniques have been proposed and widely accepted, such as extracorporeal carbon dioxide removal, extracorporeal cardiopulmonary resuscitation, and bridging to heart and lung transplantation, as well as bridging from heart and lung transplantation in cases of short-term incomplete graft recovery.[58,59]

Finally, the H1N1 influenza pandemic in 2008 and 2009 prompted a diffuse application of ECMO for respiratory failure and, accompanied with successful randomized conventional ventilator support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR), legitimized the use for respiratory failure.[60,61] Definitively, the recent EOLIA trial, despite some methodological controversies and the failing to prove traditional statistical significance, demonstrated that ECMO can be applied safely, does not cause additional harm and, likely, assures better survival than conventional accurate ventilation, particularly if applied early.[9,62]

At the same time, ECMO, in its veno-arterial (V-A) configuration and mainly with peripheral cannulation, has currently become the first-line support in case of cardiogenic shock unresponsive to standard therapy, as well as for post-cardiotomy shock in select cases, as suggested by recent guidelines.[63] In this case, V-A ECMO serves as a bridge to myocardial recovery or to other therapies, such as transplantation or the implantation of long-term ventricular assist devices.[64]

ECMOlogy, a summary of challenges

During this recent but complex itinerary, the evolution in biomaterials, extracorporeal surface coating, cannulas, and circuit coating has seen a steep improvement, and the availability of advanced critical care has arrived in countries and populations devoid of that in the past, with a new public interest in the topic of advanced critical care systems and performance. [65] These two general considerations have made current times a new era for ECMO and its specific management. All concepts of physiology applied to critical care are taken together to reach desired outcomes. ECMOlogy has to consider the circuit and its implications, the fluid circulating inside (blood with its cellular and liquid components), and the patients with the complex clinical picture and its complications due to the underlying disease and potentially also due to the interaction with the extracorporeal surface or the therapy specifically applied, such as anticoagulation. These characteristics associated with the new notions learned in critical care make ECMO a continually evolving state of the art that needs constant updates and essential multidisciplinary contribution. Some of the open topics in this field are considered in this dissertation, prevalently in case of the V-V configuration, but also with considerations on the V-A ECMO design.

V-V ECMO: end organ oxygenation is in the intersection between gas exchanges and the carrier

The first aim of the V-V ECMO is oxygenation of the blood to increase the oxygen content. The V-V ECMO mimics an "*in series"* circuit, on the venous side of the circulation, able to provide highly oxygenated blood to the right atrium before the blood passes through the lungs. The essential equation of the oxygen transport of the extracorporeal circuit already contains the main topics that turn out to be fundamental in daily clinical practice, and they will be discussed later in more than one chapter in the course of the thesis: $VO_{2ECMO} = ECBF \cdot (CaO_{2outlet} - CaO_{2inlet})$.[51] In this equation VO_{2ECMO} is the oxygen transport by the extracorporeal circuit expressed as ml/min, ECBF is the extracorporeal blood flow in ml/min and the CaO_2 is the content of oxygen in ml/dl of whole blood. The well known equation of the oxygen content is, $CaO_2 = 1.39 \cdot [Hb] \cdot Sat_{Hb} + (0.003 \cdot PO_2)$, and demonstrates that, when the hemoglobin is completely saturated (a constant situation in case of a properly functioning membrane lung), the increase in CaO_2 can be relevantly reached by a variation in the hemoglobin content.[52] The essential application of the VO_{2ECMO} equation guides the two main ways to increase the oxygen provided to the whole body (consequently to the patient) by ECMO: to increase the blood flow and to increase the oxygen content (CaO_2). These two actions taken together and contemporarily with the general and specific care of the patient make the difference. In fact, as a classic definition also reported in the ELSO red book or other ECMO manuals, the blood flow is able to restore the oxygenation function while the sweep gas flow is responsible for ventilation. [66] But the blood flow is strongly determined by the patient's volume status. ECMO, as a modified cardiopulmonary bypass circuit without a reservoir for real-time adjustment of blood volume, dictates active management in order to optimize the patient's intravascular volume status, which involves administration of both fluids and blood products. [67] In this light it is reasonable to avoid excessive fluid administration and prefer a small amount of packed red blood cells that are able to keep the blood flow more constant, do not have the risk of rapid leak to the interstitial fluids, and it is able to assure a better organ perfusion. Finally the advantage of an adequate and constant ECMO blood flow is the main result: an adequate support to the rest of the treatment. [68] Consequently, a patient stable from the blood flow side (and consequently oxygenation) may be prone more easily and for more hours without reaction to desaturation to stop the prone therapy; a better fluid mass may reduce the need for neuromuscular paralysis, letting the patient breathe spontaneously reducing long term ventilation by using the diaphragm and reducing the risk of critical illness neuropathy. [69] Finally, the judicious use of blood products may reduce the use of fluids and consequently render the fluid balance more negative (or less positive) a factor clearly associated with a higher rate of ECMO weaning. [70] Surely, in ECMO patients, hemorrhage, hemolysis and reduced red cells lifespan are frequent and these, associated with the risk of thrombocytopenia and coagulopathies (not considering the anticoagulation by heparin or other drugs), coexist and are common findings determining a substantial transfusion requirement.

Returning to the fundamental equations, the initial approach in ECMO is considered to be to restore a more physiologic level of hemoglobin to determine a significant increase in the oxygen content. In fact, guidelines in ECMO still suggest keeping the hemoglobin between 12 and 14 g/dL. Is this still valid? In recent years, several trials and observational studies have dealt with anemia in critically ill patients. [71] The general notion is that a higher level of transfusion (and consequently a higher trigger of hemoglobin) may have harmful effects, such as transfusion-related acute lung injury, transfusion-associated fluid overload, and immunomodulating effects finally resulting in higher incidence of severe invasive infections. [72] The major price that has to be payed for transfusions during ECMO is the triggering of inflammation that is already in place and caused by the extracorporeal circuit and may be worsened by multiple and diverse transfusions. The landmark study on the topic is the transfusion requirements in critical care (TRICC), but the approach, called restrictive, has been questioned for the more severe and complex patients in whom comorbidities or the severe status make the state of anemia very risky for hypoperfusion, and critical for distribution of nutrients.[73-75] Actually, transfusions were the first case of clinical application of precision medicine after the discovery of blood groups.[76] But opposite to the precision of genotyping and blood matching, today the decision of administering a transfusion still relies on a relatively small amount of defined data. [77] The first one is the so-called "hemoglobin trigger."[78] And the precision medicine still has difficulties in becoming precise medicine or integrating medicine. [79] Since in ECMO the potential amount of transfusion is indefinite, data should be gathered to build protocols able to avoid reckless transfusions (ending also in uncontrolled expenses and lack of blood products for real emergencies) and avoid a too restrictive administration that put patients at risk of experiencing the negative effects of anemia.[80] These considerations have an impact also on the future interventional trials, since as long as no defined data on what we define as parameters for transfusions are available, no prospective interventional trial is destined to be informative on this topic.[81] Notably, as said earlier, the clinical practice gives an idea of the trend in use, but is far from fair evidence. In fact in the EOLIA trial, which enrolled patients from 2011 to 2017, the Hb level was set per protocol between 8 and 10 g/dl (with a planned possible increase in the case of persistent hypoxemia), far from the level recommended in the CESAR trial (protocolized between 12 and 14 g/dl), which included patients ten years before. In addition, the recent international survey on the topic sheds light on a diffuse practice of reduced hemoglobin trigger in ECMO, but it does not arrive at the number of 7 g/dl suggested in the recent trials in critically ill patients.[80]

These last considerations involve the strategy of managing the ARF patient on ECMO support, the rationale of which is to provide variable, but always partial, oxygenation of the entire cardiac output. Before administering a transfusion to increase the DO2 it is considered important to check for blood flow, optimize ventilation when possible, and reduce oxygen expenditure, all of which constitute a plausible pathway toward the application of the principles of precision medicine for all ECMO patients.

[82] A protocolized approach has recently been proposed and, though every ECMO patient is so unique that he or she needs a personalized approach, a protocol on that issue may help in always having in mind the pathophysiological principles needed to adequately manage the patient with the help of the machine and to not enter the vicious thinking loop to make the ECMO pump work properly "using" the patient as for fluid optimization. [68] All this should also be evaluated in the framework of indications. ECMO has an indisputable lifesaving role in case of severe or extreme hypoxia due to ARDS when the other conventional therapies like prone position and nitric oxide fail to stabilize the clinical picture, and in case of severe respiratory acidosis with high plateau pressures. This explain how ECMO is the way to allow other therapies to work on the initial conditions. In this light it is really important to reduce transfusions to avoid armful practices and define the best practices to apply the mainstay of ARDS treatment: negative fluid balance, prone position and protective or ultra-protective ventilation (often associated with paralysis).

In this dissertation a single-center experience on transfusion practice is presented with the protocol in use at IRCCS-ISMETT. Actually, it underlines how a transfusion protocol is far from a computer-based decision and still needs a specialist to decide on every single transfusion act, since transfusions are a benefit for the whole community, a finite resource, and may also be harmful for the patient.

ECMO and coagulation: a never ending battle

Another topic relevant during ECMO is related to coagulation. In brief, when ECMO is applied to a patient, there is the activation of the inflammatory system accompanied by the consumption of soluble factors. This activation occurs when the blood touches the external surface; consequently, the use of new and more biocompatible materials or coating products is one of the recent advances in ECMO-related technology. [83] More research is needed to reduce ECMO-induced coagulopathy, and the use of biocompatible materials (such as bioline-coated cannulas and circuits as well as shorter circuits) should be a priority in the coming years. Thinking that even a slight improvement is currently worth pursuing, mortality is probably not the best outcome variable in the ECMO cohort, where the survival of these very severe patients has already exceeded 65%. Likely more are surrogate outcomes, such as the number of transfusions, changes of circuits and, even better but more expensive from the research point of view, changes in hematological parameters (cells and soluble factors).[24,84] In this thesis, the topic of coating is approached for the new cannulas available on the market, and starting from this topic we have integrated the considerations for circuit use with larger drainage cannulas and shorter cannulas in a femoro-jugular configuration. Actually the femoro-jugular configuration is considered the type of configuration able to guarantee less recirculation, but is deemed to be more sensible for

respiratory swinging and, consequently, the blood flow produced may be more reduced by several factors reducing the flow in the inferior vena cava. That is one of the reasons adopted by the supporters of the jugulo-femoral cannulation, able to be less influenced by spontaneous breathing. In our single-center protocol we propose the use of coated cannulas, with higher diameter and a shorter length (38 cm), with promising results on intermediate outcomes such as the number of transfusions and the length of ECMO stay.

But coagulation is relevant also in the need for anticoagulation. Unfractionated heparin (UFH) is the current mainstay for anticoagulation during ECMO, but this drug, and this concept is often neglected, works indirectly to anticoagulate the blood but acts via a co-worker represented by antithrombin. Moreover, the coagulation profile of the ECMO patient largely varies during the run since a deep consumption of coagulation factors arrives during the stay. The acquired von Willebrand syndrome has been widely described and effectively is experienced when the ECMO run lasts longer, but also other coagulation factors are consumed, and antithrombin is the principal consumed factor during the heparin-based anticoagulation, and is its effector. The consumption of coagulation factors is even more relevant when we consider that long-staying critically ill patients often experience a malnutrition state, and their ability to synthetize protein and factors is also critically impaired. But, first, coagulation is impaired by the inflammatory state that has several checkpoints that operate in common between the inflammatory and coagulation patterns. This is relevant also from the therapeutic point of view in special circumstances like in case of acute myocardial infarction where the systemic inflammation plays a role in the worsening of the clinical picture. The activation of the bradikinine-kallikreine system from the activate factor XIII (from the extracorporeal circuit) may enhance the intrinsic coagulation pathway conducting to an uncontrolled thrombin generation. At the same time an increase in the spontaneous hydrolysis of the complement molecule C3 boost the inflammation and favor cell lysis. On this topic extracorporeal removal of inflammatory mediators has a strong pathophysiologic rationale and could be applied in case of ECMO associated with shock. The limitation is the poor availability of evidence on the field, but the basic knowledge of metabolic and biochemical processes, as well as, intracellular signaling with microRNA may contribute to define better the picture in ECMO and will revela of paramount importance in evaluating further advancements. Moreover, during ECMO the liver function may be impaired also by the underlying disease (without considering the possible presence of a liver comorbidity). An example is fairly given by the alteration of the liver function tests that is sees rarely in case of respiratory support; in this case is mainly due to pulmonary hypertension exerting a right ventricular failure with congestion of the splancnic organs, including the liver, often associated with the toxic effect of antibiotics. More often it is seen in case of cardiogenic shock due to poor perfusion, venous congestion, metabolic dysregulation, pharmacological effects, lack of pulsatility and occasionally direct injury. Liver dysfunction is a known predictor of worse outcomes in the general ICU population

and specifically in patients on ECMO liver dysfunction appears to be associated with a high 30 day mortality. While a majority of the patients can have some form of elevated liver enzymes after initiation of VA ECMO, some markers have been shown to have poor outcomes. Among liver enzymes, elevated alkaline phosphatase, total bilirubin (related to the excretory role of the liver) and low albumin level (related to synthetic role) are the known markers that predict 30 day mortality. In addition, anti-thrombin III activity has been studied as an indicator for reduced functional reserve of the liver and can predict acute liver failure. The principles of management of liver dysfunction on ELS are similar to that of other splanchnic organ system. The primary focus being on improving forward flow, perfusion and reducing venous congestion. Any relative sudden spike in enzymes should arouse the suspicion of ongoing liver injury and the ECMO flows should be re-evaluated, or, in patients who progress to fulminant hepatic failure their indication for ECLS needs to be reassessed.

Heparin-based anticoagulation is widely adopted, but many criticisms make its use more empirical than scientifically-based. In fact, even the clinical monitoring of heparin anticoagulation during ECMO has several important limitations. Two contact-activated tests, whole-blood activated clotting time (ACT), and plasma-based activated partial thromboplastin time (aPTT) are the most commonly used, but their results are rather poorly correlated and known to be variable among different reagents and devices. [85] Moreover, the test considered the most precise and advanced to monitor unfractionated heparin (UFH), the anti–factor Xa assay, has some biases in cases of antithrombin (AT) deficiency. In fact, this test provides a measure of heparin efficacy on factor Xa (not as sometimes misconsidered UFH concentration) and is also dependent on the concentration of AT that many assays supplement. Consequently, when the value of anti-Xa is low in the presence of a low AT level, UFH should not be increased, but through AT supplementation should increase heparin sensitivity. This is just to highlight how heparin based anticoagulation should be accompanied by a higher level of knowledge of the function, production and level of antithrombin. We therefore tried to gather information from the sporadic literature on the field of antithrombin in a narrative review that summarizes the pathophysiology, the current evidence on the coagulation profile, and on the outcomes.

Actually, antithrombin supplementation represents one of the key components of our protocol for anticoagulation since it is based on an integrative reasoning also in the coagulation evaluation. If ECMO is not the therapy able to achieve the clinical cure of the underlying disease, the practice should be aimed at keeping the all the homeostasis processes more balanced. Coagulation is part of it, and the aim, in an unbalanced coagulation profile may be to restore the antithrombin activity level with its innate anticoagulation/anti-inflammatory activity, and adding the amount of heparin to the system to decrease the coagulability. All this is reasonable, but should be tested adequately in future independent and randomized trials. In the meantime, large observational collaborative and prospective data sets will

have to provide actual data on worldwide practice of anticoagulation monitoring and administration in terms of survival, and also bleeding and number of circuits use.

ECMO and complications: will we get rid of them with "precise" medicine?

Complications during the ECMO stay are a relevant cause of morbidity, more rarely a direct cause of mortality.[86,87] Bleeding is a leading challenge, and cause of morbidity lately, leading to mortality.[29,88]According to the rule of thumb repeated in every ECMO curse, "don't touch the patient," every site can be a potential bleeding source. [89] That is the reason for a reasoned management of venous and arterial accesses, drainages and tubes in contact with the patient. [90] But, considering that the topic of bleeding is largely explored by the literature we focused on a specific setting, which probably, considering the large importance of cardiocirculatory and respiratory systems, is largely neglected: the gastrointestinal tract. [91] Two specific topics are explored by the data of a single center: the gastrointestinal bleeding through an advanced endoscopic treatment, and a practical way and flow chart to afford the occurrence of intra-abdominal hypertension during ECMO, a critical complication able to impair the ability of abdominal cannula drainage capability. [92]

Looking at gastrointestinal bleeding during ECMO, it has been reported in 4.2% of patients with cardiac support, and in 5.2% with respiratory support.[93] There is little data on GI bleeding during ECMO, but these patients, in addition to the typical causes for bleeding related to the ECMO application (such as large bore cannulation, coagulopathy, or administration of heparin) have a specific risk for stress ulcer due to the high severity and inflammatory state and, in the case of veno-arterial (V-A) ECMO, likely as a result of non-pulsatile blood flow, and reduced gastric perfusion and PH. [94,95] Therapeutic management of GI bleeding can be a challenge even for an experienced endoscopist, though several effective hemostatic techniques have been developed in recent years. Data on GI bleeding under ECMO are highly fragmentary and mostly published as case reports and very limited case series. Moreover, the treatment of such a significant complication is not codified by guidelines or expert opinions, and varies from a wait-and-see approach, invasive surgical methods with no or limited experiences to less invasive techniques particularly appropriate for ECMO patients. A pro-active approach with advanced hemostatic ability taking advantage of multidisciplinary approach (like the endoscopy service at our center used to manage frequent and severe gastrointestinal patients in liver transplant candidates and recipients) can contribute to reducing overall mortality, and even maintain mortality that is similar to those patients without bleeding. The same concept of multidisciplinary treatment should be applied in every field, but the gastrointestinal tract is particularly likely to take some steps forward in the coming years. But it is still there, and data from the ELSO registry

demonstrate that it is a major determinant of mortality when complications occur. Interestingly, in our series the mortality among patients experiencing gastro-intestinal bleeding was similar to that of the patients without gastro-intestinal bleeding. With all the limitations of a single-center retrospective study this is noteworthy data that should be taken into account. In fact, this is an example of how a non-invasive treatment of complications during ECMO may have a game-changer role for future impact on mortality due strictly to complications.

Another topic considered is the occurrence of intra-abdominal hypertension (IAH). [96] IAH in the more severe and continued forms may lead to an acute abdominal compartment syndrome (ACS), one of the most terrifying syndromes in the ICU, with a very high risk of mortality. [97] In the general ICU patients there are available guidelines with a step-up escalation of the treatment according to well established flow charts, including medical management with paralysis, adequate sedation, increase of hemoglobin, and reduction of fluid overload with continuous renal replacement therapy accompanied by the curative treatment of the underlying disease causing the IAH. [96] Unfortunately, as often realized, ECMO poses new and complex challenges. In fact, during ECMO, patients are at risk of IAH due to the severity of their critical illness and the need for multiple transfusions and fluids. Moreover, IAH can impair the venous drainage, reducing its ability to provide an adequate oxygenated blood flow. [98,99] We hypothesized that ECMO malfunction can be considered an aspect of ACS; consequently, the appearance of IAH during ECMO would likely necessitate a personalized approach. This reflects on the treatment, since while some series have described positive outcomes for decompressive laparotomy, such an invasive treatment is not plausible to be the treatment of choice during ECMO with all the severity and risk for infections and bleeding of the ECMO patients. [100] [101] New strategies should be explored but, as often in these highly-severe critically ill patients, when the syndrome has established itself it may be too late to interrupt the vicious circle of inflammation, organ dysfunction, and organ cross-talk; consequently, preventive or early-active strategies should be applied. Again, in this field, the multidisciplinary approach can be applied, and we present our precocious approach to the IAH with total water-assisted colonoscopy, a less-invasive technique able to avoid the over-distention of the colon and reduce the pressure gradient. This approach is feasible and has highlighted a relevant lack of knowledge in the guidelines for ACS. ECMO should receive the attention of a dedicated chapter, and a different flow chart should be elaborated since even minimal IAH can prompt the use of more fluids, sedation, and blood transfusions, all together a critical avoidable harmful treatment during ECMO.

On the V-A ECMO configuration side we explore a never-ending much discussed complication: the occurrence of limb ischemia during V-A ECMO with peripheral cannulation. No guidelines or concrete suggestions are available on the topic, and consequently a wide consideration of the literature was taken into account. For sure, in recent years, the peripheral approach to cannulation in V-A ECMO has been

progressively preferred because it does not need the chest opening, can be quickly established, can be applied percutaneously, and is less likely to cause bleeding and infections than central cannulation. Consequently the vascular complications have risen too. The mechanisms of such adverse events are often multifactorial, including suboptimal arterial perfusion and hemodynamic instability due to the underlying disease, peripheral vascular disease, and placement of cannulas that nearly occlude the vessel. The effect of femoral artery damage and/or significant reduced limb perfusion can be devastating because limb ischemia can lead to compartment syndrome, requiring fasciotomy and, occasionally, even limb amputation, thereby negatively impacting hospital stay, long term functional outcomes, and survival. Once again this complication needs to be prevented, or treated early with non-invasive approaches. Giving the lack of RCTs and inadequate sample of data for a meta-analysis, the collection of series and experts opinions was able to define some flow charts. In a general overview the concept that probably should guide the treatment is the repeated monitoring via multiple signs and an early or preventive use of cannula for distal leg perfusion. Regarding the monitoring, this should be based on the responsible use of all the available resources as suggested by the crew resource management practice, based on clinical monitoring by nurses and ECMO specialists at every shift or multiple times in the same shift using also new methodology like the Doppler ultrasonography and the near infrared spectroscopy. The treatment and prevention should integrate some tricks, such as the decision of using bilateral legs for cannulation to avoid the venous congestion due to the compression exerted by the concomitant presence of the arterial cannula, the use of new devices like the bidirectional cannula (a promising device that still has to be tested in large series for efficacy and complications), and the protocolized use of a distal perfusion cannula. As already stated, all the presented features are a part of a precise management that should rely on locally-based education, team training, and specialists' engagements. To treat a patient with ECMO is not simple, and the reason for the need of centralization of patients shown by higher survival in high volume centers is probably due to the specialized human resources available in these centers rather than the availability of more and updated devices released on the market.

ECMO and indications: the intersection of enthusiasm and patient characteristics

Lastly, a major point of discussion in ECMO is patient selection, since this high level of support is used either as a rescue, in desperate cases, or as a concrete support in patients with high chances of survival.[102,103] Patient selection currently takes place following the ELSO guidelines and local practice. Even considering very innovative case report and series. [104] Severity scores with probability of survival are available, but fail when applied to all patients because of the widening of indications and concomitant diseases, and complications. [105,106] Often scores are even applied to vast populations, but

mixing V-A configuration and V-V configuration with results that do not apply to the real patient, not because the score has no value but principally because it is applied to a population including completely different physiology status and severity. Moreover, as already underlined earlier, by definition, the severity score cannot consider the exceeding mortality due to the specific inherent complications in ECMO: fatal bleeding, neurologic complications, cardiac mortality.

A paradigm of that situation is represented by the acute respiratory distress syndrome (ARDS). In this field, as highlighted in the landmark study, Large Observational Study to Understand the Global Impact of Severe Acute Respiratoty Failure (LUNG SAFE), by Bellani and coll., worldwide, 67.2% of patients fulfill the ARDS diagnosis, according to the Berlin definition, at least once during their ICU stay. [107,108] Among this large cohort recruited in 50 countries, the overall hospital mortality was 40%, while it was 46.1% in the severe ARDS cohort (patients fulfilling the ARDS criteria, with PEEP at least 5 cmH2O, and a PaO2/FiO2 ratio ≤ 100 mmHg). This last segment is the application of ECMO principally, and this level of mortality is usually what we want reduce by ECMO support. [109] But despite the efforts and promising results, this reduction of mortality is not consistently reached, and the application of ECMO in COVID-19 has demonstrated that the main goal is to apply the support to patients that have a real chance of survival and to apply the support at the right time. [32,49,50]

One possible approach is personalized medicine to underline the biological processes common among different subpopulations in the same wide-ranging clinical category or definition. [110] This has been highlighted in recent years in ARDS and recently also tested in COVID-19, but may also be considered in cardiogenic shock. [111,112] It is plausible that the immediate inflammatory response caused by the extracorporeal surface works in pair with the different cascades of inflammation, coagulation, and innate immunity. All this processes are different according to personal tendency, and this personal contribution or predisposition can be explored only by personalized signals. [113] All these processes are likely involved in the disruption of homeostasis, as when we see a septic shock or a cytokine storm, but a different regulation of their proportion is also involved in the recovery processes. It is reasonable to suppose that their modulation, may contribute to fostering recovery. In this thesis we provide very preliminary, but sound, data on microRNAs in patients affected with ARDS and supported by ECMO, showing that at least looking at the severity of patients, groups are different also at the biological level (microRNA), while the clinical outcomes in terms of survival are not related to the baseline conditions. MiRNAs are a promising tool to personalize the approach, not on a genomic level, but at a signaling level between organs, systems, and biological pathways. [114,115] They are a class of small non-coding RNA (19–25 nucleotides) responsible for silencing specific genes able to modulate specific pathways. [116] Circulating miRNAs likely play important roles in cellular communication, regulating gene expression and the phenotype of the recipient cells. Therefore, in the presence of harmful stimuli, the composition of blood

miRNAs can be altered, making themselves excellent candidates as peculiar biomarkers. They have been described as biomarkers for physiological responses (like the response to physical training, but also for cardiovascular disease and cancer), thus indicating that they might also play a role as signals of ongoing pathological processes. Inflammation, platelet function, and immunity (innate and adaptive) probably act together, with still unknown connections. [117,118] The missing piece of the puzzle is management of the patient, and complications, but at least this is one plausible way to verify the patient's tendency toward different recovery patterns, and may, with future validation, define new targets to see the effects of newly introduced interventions. [119]

The future in management of ECMO and patients on ECMO will be refined year after year, but is a incessant process, based on the sharing of knowledge, practices, and also evidence. [120] With all the contributions, from unusual case reports to randomized controlled trials, we are constructing a specific body of knowledge. [121-123] All these considerations are difficult and under doubt in our global society, where short communication, flavors of the day, and lack of evidence move the attitudes and thoughts of vast populations of humans. ECMO, with its inherent hope for survival of patients otherwise destined to die, is the avant-garde of critical care medicine, with indisputably improving results. The community of providers and stakeholders working together is at the core of this success. [124] Precision medicine is commonly equated with genomic medicine; however, other technical advances, such as new technologies and monitoring tools, knowledge of deep pathophysiological states, and diffuse multidisciplinary practice, can facilitate a more personalized management as well.

Aim and outline of the thesis

The purpose of this thesis was to elucidate clinical data and basic knowledge regarding several challenges in ECMO practice to build more solid data on current practice, and guide future management strategies and studies. To reach this goal, several investigations were undertaken by means of original studies and reviews.

A single-center experience at ISMETT (Palermo, Italy) presenting a restrictive approach in anticoagulation and transfusion management was conducted to assess the impact on amount of transfusions and survival in patients supported by V-V ECMO for acute respiratory failure. Following, the impact of bioline cannulas and a circuit configuration with shorter drainage cannula on the topic of transfusions was explored. Worthy of note, the topic of transfusions in ECMO for respiratory support was also investigated at an international level in the TRAIN-ECMO survey on hemoglobin trigger and other parameters in transfusion practice among 447 participants from 42 countries.

A relevant challenge in V-A ECMO, the need for distal reperfusion arterial cannula, was also reviewed by gathering information from a large number of well-conducted studies, highlighting the current practice in high volume centers, the risk factors for limb ischemia, and creating a flow chart for adequate monitoring and management.

Moreover, new insight into a frequent and likely neglected theme is given on gastrointestinal hemorrhage during V-V ECMO, given the actual prevalence and outcomes in a high-volume ECMO center, and suggesting a new advanced endoscopy management that was actually able to reduce mortality compared with other series with the same complications and standard management. The topic of intra-abdominal hypertension has been approached as well, underlying the utility of the total water-assisted colonoscopy.

Taking into account the promising data from our previous studies, which showed a positive association between the level of antithrombin during ECMO and survival, we reviewed information from the currently available literature on the supplementation of antithrombin during ECMO, given its plausible heparin-sparing effect, endothelial preservation, and anti-inflammatory effect.

Finally, a new approach to personalized medicine is described for ARDS patients. In an explorative pilot study on a cohort of patients under V-V ECMO for respiratory failure, the variation of microRNA was explored, and a short list of them was definitely associated with patients' severity of condition, opening the pathway to new potential biomarkers to monitor the evolution of the disease and to be used as a target to test eventual interventions.

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Chapter 2

Anticoagulation and Transfusions Management in Veno-Venous Extracorporeal Membrane Oxygenation for Acute Respiratory Distress Syndrome: Assessment of Factors Associated with Transfusion Requirements and Mortality

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Abstract

Purpose: We describe an approach for anticoagulation and transfusions in VV-ECMO, evaluating factors associated with higher transfusion requirements, and their impact on mortality.

Methods: Observational study on consecutive adults supported with VV-ECMO for acute respiratory distress syndrome (ARDS). We targeted an activated partial thromboplastin time of 40-50 seconds, and a hematocrit of 24-30%. Univariate and multiple analyses were done to evaluate factors associated with transfusion requirements, and the influence of increasing transfusions on mortality during ECMO.

Results: In a cohort of 82 VV-ECMO patients (PRESERVE score 4, IQR 3-5, RESP Score 2, IQR -2-4), 76 patients (92.7%) received at least one unit of packed red-blood cells (PRBC) during the intensive care unit stay related to ECMO (median PRBC/day 156 ml, IQR 93-218; median ECMO duration 14 days, IQR 8-22). A higher requirement of PRBC transfusions was associated with pre-ECMO hematocrit, and with the following conditions during ECMO: platelet nadir, antithrombin III (ATIII), and stage 3 of acute kidney injury (all $p<0.05$). Sixty-two patients survived ECMO (75.6%). Pre-ECMO hospital stay, PRBC transfusion, and septic shock were associated with mortality (all $p<0.05$).

The adjusted odds ratio for each 100ml/day increase in PRBC transfusion was 1.9 (95%CI:1.1-3.2, $p=0.01$); for the development of septic shock it was 15.4 (95%CI:1.7-136.8, $p=0.01$), and for each day of pre-ECMO stay it was 1.1 (95%CI:1-1.2, $p=0.04$).

Conclusions: Implementation of a comprehensive protocol for anticoagulation and transfusions in VV-ECMO for ARDS resulted in a low PRBC requirement, and an ECMO survival comparable to data in the literature. Lower ATIII emerged as a factor associated with increased need for transfusions. Higher PRBC transfusions were associated with ECMO mortality. Further investigations are needed to better understand the right level of anticoagulation in ECMO, and the factors to take into account in order to manage personalized transfusion practice in this select setting.

Introduction

Mortality associated with acute respiratory distress syndrome (ARDS) remains high despite continual improvement in mechanical ventilation and extracorporeal membrane oxygenation (ECMO). [1] Technical simplifications and its use during the 2009 H1N1 influenza pandemic prompted a sharp increase in the use of ECMO worldwide. [2-4] The survival rate in large-volume centers has improved over recent years thanks to increased expertise of ECMO centers. [5, 6]

The optimal management of anticoagulation target and transfusion practice in veno-venous-ECMO (VV-ECMO) patients is still under debate. [7, 8] Traditionally, the threshold for transfusions of packed red blood cells (PRBC) in ECMO aimed to keep hemoglobin (Hb) values in the normal range (12-14 g/dL). [9] Even if strict triggers have been questioned by several experts because they appear to be too simplistic, a liberal transfusion strategy in critically ill patients is associated with increased morbidity and mortality, as many randomized controlled studies have shown.[10-13]

There is also some evidence that a higher number of transfusions in ECMO patients is associated with worse outcomes. However, such a relationship is difficult to assess given the large number of factors influencing transfusion practice in this setting: target of anticoagulation, frequent bleeding episodes, and the peculiar characteristics of the circuits that can cause mechanical damage, activation of inflammation, and consumption of platelet and coagulation factors, to name but a few.[14]

Considering the conventionally accepted restrictive strategy for different populations of critically ill patients, as well as the increasing experience with ECMO management and improvement in the equipment, the aim of reaching a normal physiologic range of Hb should be reconsidered also in ECMO. Moreover, available data on transfusions during ECMO are fragmentary, usually including VV-ECMO and veno-arterial-ECMO (VA-ECMO) configuration, and mixing adults with neonatal and pediatric cohorts, which are historically more represented. [15, 16]

After a short learning curve, and in light of a more "physiologic" way of managing ECMO patients, since the beginning of our ECMO program (2006), we target activated partial thromboplastin time (APTT) and hematocrit (Htc) at a lower level than described in the literature. Therefore, we have defined and put into place a comprehensive protocol, described below, on anticoagulation and transfusions during ECMO.

In this study we present data from our prospectively collected registry of VV-ECMO patients affected with ARDS with a threefold aim: 1) to describe the effects of our transfusion protocol, 2) to identify factors associated with increased transfusion requirements and, 3) to define their effects on short-term mortality in ECMO with a follow-up of at least six months after ECMO weaning.

Materials and Methods

The study was approved by ISMETT's Institutional Research Review Board and by the local Ethics Committee (Comitato Etico Palermo 2. Reference Code: 88-ISMETT-2016). All the patients in their full mental capacities, or their relatives, signed an institutional consent for publication in manuscripts as anonymous aggregate data.

This is a single center study of consecutive patients supported on ECMO from December, 2006 to December, 2015. The patients were affected with ARDS according to the 1994 ARDS consensus or to the Berlin definition. [17, 18] Before the decision to start VV-ECMO, patient management was optimized with ventilator support according to the ARDSnet protocol plus rescue therapy (proning, nitric oxide or prostaglandin). Exclusion criteria for the study were < 18 years of age, need for ECMO as bridge, or intraoperative during lung transplant or other surgical procedures, and veno-arterial configuration (combined respiratory/cardiac disease). [19]

Medical history, demographics, biometrics, and labs were collected prospectively through our electronic medical record system (Sunrise Clinical Manager, Allscripts Healthcare Solutions, Inc). ECMO predictive scores PRESERVE (PRedicting dEath for SEvere ARDS on VV-ECMO), RESP (Respiratory Extracorporeal Membrane Oxygenation Survival Prediction), and ECMONet (the latter only for H1N1 patients) scores were recorded, as well. [20] Acute kidney injury (AKI) was assessed according to the Acute Kidney Injury Network (AKIN) stage classification. [21]

Anticoagulation was achieved with heparin, and assessed by measuring activated partial thromboplastin time (APTT) and antithrombin III (ATIII) activity. Transfusion requirement for PRBC was described as the total number of units and median of ml transfused per day of ECMO support. Fresh frozen plasma (FFP) and platelets (PLT) were counted as the number of products transfused.

Bleeding episodes were classified as follows: general (any kind of bleeding), minor (episode without any intervention beyond stopping anticoagulation), major (episodes needing more than 2 PRBC transfusions or needing an interventional/surgical approach, including gastrointestinal tract endoscopic procedure, nasal/pharyngeal tamponade or cauterization, surgical intervention), and fatal (intracerebral fatal hemorrhage). [22, 23]

ECMO management was defined as type of device (RotaFlow or CardioHelp System – Maquet, Getinge Group, Rastatt, Germany), cannulation configuration, and blood and sweep gas flow. ECMO outcomes are described as weaning from support, effective intensive care unit (ICU) discharge, and six-month mortality follow up.

Institutional protocol for anticoagulation and transfusions during ECMO

Our protocol is based on integration of the Hb values with other parameters: SvO2, urine output, lactate values, need for vasopressors. Htc is maintained at least between 24% and 30% unless severe hypoxia is present despite other maneuvers. Every PRBC transfusion is given using anti-leucocyte filters at bedside. [24] Anticoagulation is maintained with heparin at a target range of APTT between 40 and 50 seconds, checked every four hours to assure frequent adjustment, and stopped in every case of bleeding. ATIII is checked daily and replaced by ATIII concentrates, as needed, to keep the activity as near as possible at 100%. All circuits are heparin-bounded tip-to-tip with polymethylpentene oxygenators.

Platelets are transfused when the level falls below 40,000-50,000 /ml, according to the identification of possible bleeding sources. Blood waste is avoided: need for blood sampling is re-evaluated daily, and at ECMO weaning the blood in the circuit is auto-transfused to the patient.

Statistical analyses

Categorical variables are described as frequencies and percentages, continuous variables as mean and standard deviation or as median (25^{th}-75^{th} percentile interquartile range – IQR), when appropriate, according to data variability.

The comparison of the PRBC transfusion among categorical variables was made with the two-sample t-test or Wilcoxon rank sum and analysis of variance where appropriate. In order to estimate the odds ratio, univariate logistic regressions were also applied. The associations with continuous variables were assessed with Pearson's correlation coefficient and univariate linear regressions.

The associations between the binary outcome ECMO survival and categorical variables were assessed with Fisher's exact test or the chi-square test when appropriate. Odds ratios are also reported. A stepwise multiple regression analysis was applied (multiple linear regression for PRBC, and multiple logistic regression for ECMO survival). The stepwise selection procedures were computed considering significance levels of 0.1 and 0.2 for entering and removing, respectively, for both models.

Odds ratios (ORs) are reported with a 95% interval confidence (CI 95%). All tests were two sided, and a p value of <0.05 was indicative of statistical significance. Data handling and analyses were done with SAS 9.4 software (SAS Institute Inc, Cary, NC, USA).

Results

During the study period, 138 patients required ECMO support for respiratory causes and were screened. However, 56 patients were excluded for the following reasons: 8 (2006-2007) because of incomplete data, 3 were < 18 years-old, 1 died during transport to our institute, 1 VV-ECMO run intra-operative for tracheal resection, 2 veno-arterial configuration to perform cesarean section in severe pulmonary hypertension, 38 related to the preoperative or intraoperative time of lung transplantation, 2 affected with hematologic malignances (inappropriate to explore the topic), 1 because at too high a risk of death (Simplified Acute Physiology Score II at ICU admission score 66, and Sequential Organ Failure Assessment score 18), (this was the only patient with an ICU stay of < 48 hours).

Therefore, we included 82 patients in our study cohort. Patient characteristics are illustrated in Table 1. The most common underlying diagnoses for ARDS were H1N1 Influenza A (42, 51.2%), bacterial pneumonia (23, 28%), primary lung graft failure (5, 6.1%), pneumocystis jirovecii pneumonia in HIV-AIDS (3, 3.7%), polytrauma (2, 2.4%) and post-pneumonectomy (2, 2.4%). The following diagnoses were made in only one patient (1.2%): chemical pneumonia, bacterial pneumonia associated with primary graft failure, bacterial pneumonia associated with H1N1, mediastinitis, and pleural empyema.

The main associated conditions/comorbidities were diabetes (16%), polytrauma (11%), hypertension (10%), immunosuppression (10%), coronary artery disease (8%), previous lung transplant (8%), puerperium (5%), human immunodeficiency virus (4%), chronic obstructive pulmonary disease (3%), and pulmonary fibrosis (3%).

Patients' characteristics *n* = 82	
Pre-ECMO Data	
Age, year	42 ± 11
Male gender, *n* (%)	62 (75.6
BMI, kg/m2	29 ± 6
SAPS 2 ICU admission	38 [32-46]
SOFA score ICU admission	9 ± 3
Hospital LOS pre-ECMO, days	7 [3-12]
ICU LOS pre-ECMO, days	5 [2-9]
Mechanical Ventilation, days	4 [2-8]
PaO2/FiO2	59 [51-67]
Creatinine, mg/dl	1.3 [0.8-1.9]
Htc pre-ECMO, %	33 ± 6
Bilirubin, mg/dl	0.9 [0.6-1.3]
Charlson Comorbidity Index	1 [0-2]
1 Comorbidity	36 (43.9 %)
2 Comorbidities	33 (40.2 %)
PRESERVE score	4 [3-5]
ECMOnet score	5.5 [5-7.5]
RESP Score	2 [-1-4]
ECMO and Transfusion Management	
Blood Flow, L/min	3.9 ± 0.7
Air Flow, L/min	3.5 ± 1.5

Plateau Pressure, cmH2O	23 [21-24]
PEEP, cmH2O	12 ± 2
Femoro-Jugular, *n* (%)	76 (93)
Femoro-Femoral, *n* (%)	6 (7)
Pronation, *n* (%)	36 (43.9 %)
Device RotaFlow, *n* (%)	37 (45.1 %)
Device CardioHelp, *n* (%)	45 (54.9 %)
N. Circuits 1, 2, 3	66(80.5%), 11(13.4%), 5(6.1%)
Htc during ECMO, %	31 ± 3
Platelet count nadir, mcL	73 [42-130]
Mean APTT during ECMO, *sec*	45.6 ± 4.9
Antithrombin III, %	92 ± 15
Heparin dose, iu/kg/h	11.2 [7.8-14.8]
Total pRBC unit, *n*	8 [4-18]
PRBC ml/day ECMO	156 [93-218]
NO PRBC, *n* (%)	6 (7.3 %)
NO FFP, *n* (%)	72 (87.8 %)
Mean total FFP, ml	894 [500-1750]
NO PLT, *n* (%)	57 (69.5 %)
Mean total PLT, ml	496 [250-954]
Total bleeding episodes, *n* (%)	34 (41.4 %)
Major bleeding, *n* (%)	12 (14.6 %)
Fatal bleeding, *n* (%)	3 (3.7 %)
Surgical procedure, *n* (%)	6 (7.3 %)
Mechanical Ventilation post ECMO, days	10 [5-18]
Acute kidney injury stage 3, *n* (%)	45 (54.9 %)
Septic shock, *n* (%)	52 (63.4 %)
>24 h Vasopressor use, *n* (%)	53 (64.6 %)
Clinical Outcomes	
ECMO support duration, days	14 [8-22]
ECMO survived, *n* (%)	62 (75.6 %)
ICU Discharge, *n* (%)	56 (68.3 %)
6 month survival, *n* (%)	50 (60.9 %)

Table 1: Patients' characteristics, ECMO anticoagulation and transfusion management, clinical outcomes.

ECMO and anticoagulation management, bleeding episodes, and transfusion requirements

Mean ECMO blood flow after stabilization was 3.9 ± 0.7 L/min, with a prevalent configuration as femoro-jugular cannulation (76, 93%), and the use of two devices: RotaFlow (37, 45.1%) and CardioHelp (45, 54.9%), with the latter becoming prevalent in recent years. Anticoagulation was maintained by heparin at a mean dose of 11.2 IU/kg/h (7.8-14.8 IQR), with a mean APTT of 45.6 ± 4.9, while the mean daily AT activity was 92 ± 15, and we recorded the following bleeding episodes according to our definitions: 34 (41.4%) total episodes, 12 (14.6%) major bleeding (3 hemothorax, 4 intracerebral, 5 gastric/duodenal) and, among these, 3 (3.7%) fatal intracranial hemorrhage. Six patients needed surgical or interventional procedure while on ECMO (7.3%).

All the patients who survived ECMO underwent CT scan in the subsequent hospital stay. We recorded just one patient with an inferior vena cava partial thrombosis, resolved after 6 months of oral anticoagulant therapy. The majority of patients (76, 92.7%) received at least one PRBC transfusion during a median duration of ECMO support of 14 (8-22 IQR) days. The amount of PRBC transfused was 8 (4-18 IQR) units and 156 (93-218 IQR) ml/day. Mean Htc during ECMO support was 31±3%. FFP was administered to 10 patients (12.2%), and platelets to 25 (30.5%).

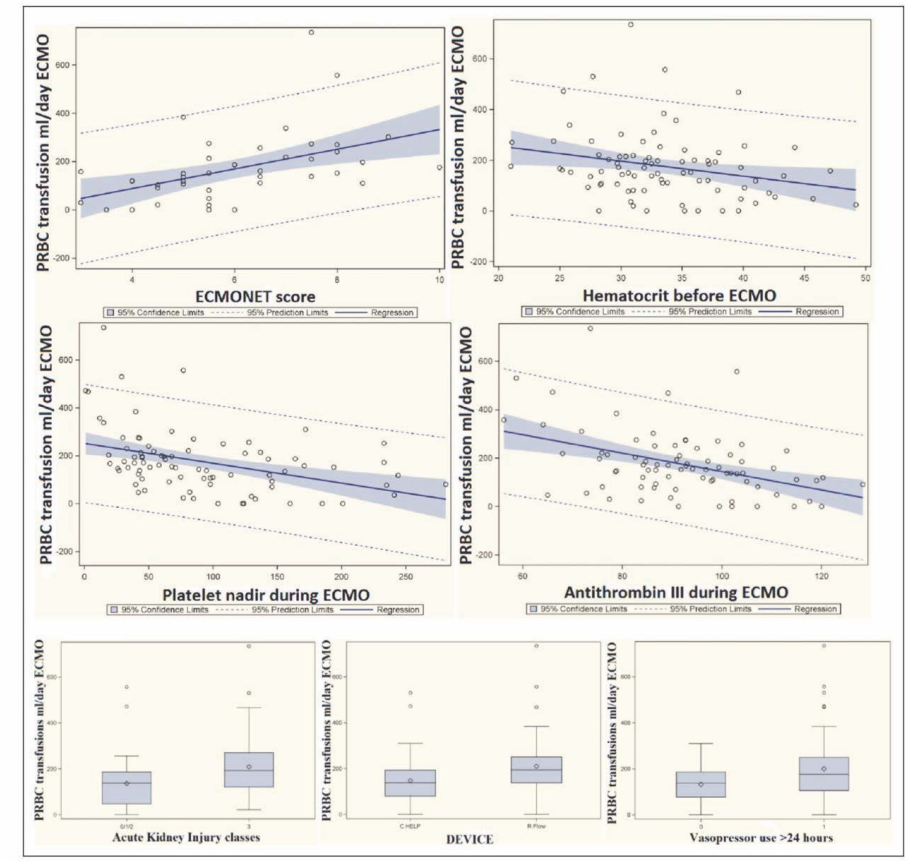

Figure 1: Parameters associated with increased transfusion requirements.

Factors associated with PRBC transfusion

Analyzing the continuous variables, we found that higher needs for PRBC transfusions during the VV-ECMO support were significantly associated with a lower pre-ECMO Htc (p=0.02), a higher

ECMONet score in H1N1 patients ($p<0.01$), a lower platelet nadir ($p<0.01$), and a lower mean daily ATIII activity ($p<0.01$). For the categorical variables, we found a significant association between increased amount of PRBC transfusions and the presence of at least one comorbidity ($p=0.04$), the use of an older device, namely the Rota-Flow device ($p=0.03$), the development of AKI stage 3 ($p=0.01$), and the use of vasopressors for at least 24 hours ($p<0.01$) (Figure 1). The complete results of univariate analyses, Pearson correlation coefficients, and t test are presented in Table 2.

Continuous Variables	**Pearson Correlation Coefficients**	**P Value**
Age	-0.17	0.13
BMI	-0.12	0.29
SAPS 2	0.07	0.51
SOFA	0.16	0.15
P/F	-0.05	0.60
Creatinine	0.21	0.05
Htc pre-ECMO	-0.25	0.02
Bilirubin	0.01	0.95
Charlson C.I.	0.01	0.93
PRESERVE	0.03	0.77
ECMONet	0.47	<0.01
RESP Score	-0.13	0.25
Blood flow	-0.07	0.52
Sweep gas flow	-0.09	0.40
Htc in ECMO	-0.12	0.28
PLT nadir	-0.41	<0.01
APTT	-0.08	0.49
ATIII	-0.42	<0.01
Heparin	-0.18	0.11
ECMO days	0.05	0.63
Categorical variables	**T test**	**P value**
Gender (F/M)	212.0 ± 140.9 vs. 164.4 ± 128.8	0.16
Comorbidity (Y/N)	209.5 ± 116.2 vs. 149.7 ± 139.7	0.04
Device R.Flow/C.Help	210.4 ± 148.1 vs. 147.6 ± 112.1	0.03
Prone early (Y/N)	159.8 ± 147.2 vs. 188.6 ± 119.9	0.33
Prone late (Y/N)	171.0 ± 113.8 vs. 178.0 ± 140.4	0.82
AKI class (0-1-2/3)	136.1 ± 119.0 vs. 208.7 ± 135.3	0.01
Septic shock (Y/N)	193.1 ± 140.4 vs. 146.2 ± 113.8	0.12
Vasopressor (Y/N)	200.0 ± 147.7 vs. 132.1 ± 84.7	<0.01
Surg. proc. (Y/N)	176.8 ± 65.2 vs. 175.9 ± 136.7	0.98
Bleeding (Y/N)	196.1 ± 125.5 vs. 161.7 ± 136.7	0.24
Major bleeding (Y/N)	206.8 ± 98.5 vs. 170.7 ± 137.4	0.38

Table 2: Parameters associated with ECMO survival, continuous and categorical variables.

After adjustment for confounders in the multiple regression model, following a stepwise procedure, four covariates remained correlated with transfusions: pre-ECMO Htc, PLT nadir, ATIII, and AKI stage 3 in ECMO. The estimated increase in PRBC transfusion were as follows: 5 ml/day for every

point reduction in Htc, 5 ml/day for every 10,000 PLT count reduction, 3 ml/day for every reduction point of ATIII, and 50 ml/day for the occurrence of AKI stage 3.

Factors associated with ECMO survival

The overall survival rate of the ECMO run was 75.6% (62 patients), and 68.3% (56 patients) were discharged from the ICU. At sixth months after ICU discharge the survival rate was 60.9% (50 pts).

Among the baseline pre-ECMO variables, a shorter hospital length of stay ($p<0.01$), a shorter duration of mechanical ventilation ($p=0.04$), and a higher RESP Score ($p=0.04$) were associated with ECMO weaning.

Nadir of PLT count (survived: 87 $*10^3$, 44-146 IQR versus deceased: 48 $*10^3$, 20-72 IQR; $p<0.01$), mean ATIII activity (survived: 94% ± 13 versus deceased: 84% ± 16; $p=0.03$), and the amount of PRBC transfused (survived: 140 ml/day, 80-200 IQR versus deceased: 209 ml/day, 161-347 IQR; $p<0.01$) were correlated with ECMO survival. Moreover, positively associated with ECMO survival were the use of the CardioHelp device ($p<0.01$), the absence of AKI stage 3 ($p<0.01$), and the absence of septic shock ($p<0.01$) (Table 3).

In the subsequent multiple model, the variables that remained associated with mortality in VV-ECMO were the amount of transfusion, with an OR of 1.9 per each 100 ml/day (95% CI: 1.1-3.2, $p=0.01$), the occurrence of septic shock (OR 15.4, 95% CI: 1.7-136.8, $p=0.01$), and the length of hospital stay before ECMO (OR 1.1, 95% CI: 1-1.2, $p=0.04$) (Table 4).

Parameters associated with ECMO survival						
Variable	Survived ECMO *n* 62 ()	Deceased on ECMO *n* 20 ()	P Value	Odds Ratio	95% CI	P Value
Age	42.3 ± 10.9	41.8 ± 12.4	0.85	0.99	0.95-1.04	0.86
BMI	29.5 ± 6.1	27.9 ± 6.0	0.29	0.95	0.87-1.04	0.29
SAPS 2	39.3 ± 10.5	41.5 ± 12.4	0.42	1.02	0.97-1.07	0.42
SOFA	8.6 ± 3.1	8.5 ± 2.3	0.86	0.99	0.83-1.17	0.86
Hosp. LOS	8.5 ± 9.0	15.6 ± 11.4	<0.01	1.07	1.01-1.12	0.01
ICU LOS	6.0 ± 7.7	10.2 ± 9.5	0.05	1.05	0.99-1.12	0.07
Mech. vent.	5.8 ± 7.6	9.9 ± 9.7	0.04	1.06	0.99-1.12	0.07
P/F preECMO	59.7 ± 14.2	58.3 ± 9.5	0.67	0.99	0.95-1.03	0.67
Creatinine	1.9 ± 1.7	1.7 ± 1.6	0.71	0.94	0.68-1.30	0.70
Htc preECMO	33.2 ± 5.6	34.3 ± 6.1	0.45	1.03	0.95-1.13	0.45
Bilirubin	1.2 ± 1.2	1.3 ± 0.9	0.67	1.09	0.72-1.66	0.67
Charlson C.I.	1.2 ± 1.6	1.5 ± 0.7	0.51	1.10	0.82-148	0.51
PRESERVE	3.8 ± 1.9	4.3 ± 1.7	0.32	1.15	0.87-1.52	0.32
ECMOnet	5.8 ± 1.6	7.1 ± 1.6	0.05	1.67	0.96-2.88	0.06
RESP Score	1.8 ± 3.1	0.2 ± 2.9	0.04	0.85	0.72-0.99	0.04
Blood flow	3.9 ± 0.7	3.8 ± 0.9	0.67	0.86	0.42-1.76	0.67
Air flow	3.5 ± 1.4	3.6 ± 1.6	0.99	1.01	0.71-1.41	0.99
Plateau press.	22.9 ± 2.6	22.4 ± 1.9	0.47	0.93	0.76-1.13	0.46
PEEP	12.2 ± 2.3	12.3 ± 2.4	0.84	1.02	0.82-1.27	0.83
Htc	31.0 ± 2.8	30.9 ± 3.0	0.87	0.98	0.82-1.18	0.86
Antithrombin III	94 ± 13	84 ± 16	0.02	0.96	0.92-0.99	0.03
PLT nadir	87 (44-146)	48 (20-72)	<0.01	0.98	0.96-0.99	<0.01
PRBC unit	9.6 ± 13.5	19.2 ± 13.0	<0.01	1.05	1.01-1.11	0.03
PRBC ml/day	140 (80-200)	209 (161-347)	<0.01	1.01	1.00-1.01	<0.01
ECMO days	15.7 ± 12.9	21.1 ± 13.9	0.11	1.03	0.99-1.07	0.14
Association with ECMO survival– Categorical Variable						
Variable	N. (% *)	N. (%)	P Value			
Gender Female	16 (80)	4 (20)	0.77	0.71	0.20-2.47	0.76
Male	46 (74)	16 (26)				
Device C.Help	43 (96)	2 (4)	<0.01	0.05	0.01-0.23	<0.01
R.Flow	19 (51)	18 (49)				
N. Circuit 1	53 (80)	13 (20)	0.05			
2	7 (64)	4 (36)				
3	2 (40)	3 (60)				
FFP Yes	8 (80)	2 (20)	1.0	1.33	0.26-6.86	1.0
No	54 (75)	18 (25)				
PLT Yes	17(68)	8 (32)	0.40	0.57	0.19-1.62	0.40
No	45 (79)	12 (21)				
AKI stage 1,2,3	33 (89)	4 (11)	<0.01	0.22	0.07-0.73	<0.01
stage 3	29 (64)	16 (36)				
Septic shock Yes	33 (63)	19 (37)	<0.01	0.06	0.01-0.48	<0.01
No	29 (97)	1 (3)				
Procedure Yes	4 (67)	2 (33)	0.63	0.62	0.10-3.67	0.63
No	58 (76)	18 (24)				
Bleeding Yes	25 (74)	9 (26)	0.79	0.83	0.30-2.28	0.79
No	37 (77)	11 (23)				
Major bleed Yes	7 (58)	5 (42)	0.15	0.38	0.11-1.38	0.15
No	55 (79)	15 (21)				

Table 3: Univariate analyses of factors associated with PRBC transfusion.

Multiple linear regression analysis			
	PRBC estimate (ml/day)	**Std. Error**	**p Value**
Htc pre-ECMO	-5	2.3	0.04
Platelet nadir *	-5	2.2	0.02
Antithrombin III	-3	0.9	<0.01
Acute kidney injury stage 3	50	29.0	0.09
Multiple logistic regression analysis			
	Odds Ratio	**95% CI**	**p Value**
Hospital LOS pre-ECMO	1.1	1-1.2	0.04
Septic shock	15.4	1.7-136.8	0.01
PRBC 100 ml/day ECMO	1.9	1.1-3.2	0.01

Table 4: Multiple linear regression analysis for PRBC transfused and multivariate logistic regression for ECMO survival.

To investigate the effect of PRBC on mortality, we did a Kaplan-Meier survival analysis, dividing the patients into two groups according to the median PRBC transfusion (156 ml/day). We found lower 90-day survival among patients with increased transfusion requirements (survival for the group with lower PRBC transfusion of 89.9% versus survival for increased transfusion of 62.7%, log-rank p<0.01) (Figure 2).

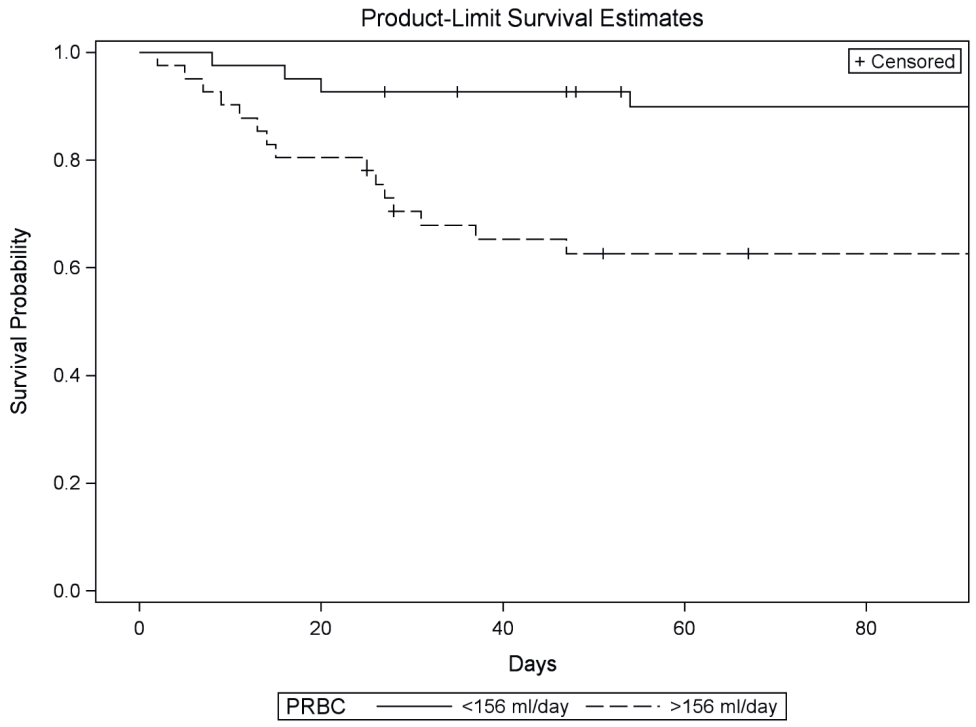

Figure 2: Kaplan-Meier survival analysis. Cut off was set at the daily median PRBC transfusion amount 156 ml/day. $p < 0.01$

Discussion

In this study we evaluated transfusion management in VV-ECMO in light of the anticoagulation protocol, considered together given the relevant activation of the interdependent cascades of inflammation and coagulation caused by the ECMO circuit *per se* and the need for anticoagulation. [25-29]

There is still no consensus on the preferred dose of heparin (recently, some authors have explored the possibility of a low dose), the range of APTT, and the role of ATIII in ECMO (clear recommendations have been released only for pediatric ECMO). [30-33] Our anticoagulation protocol is based on a low median heparin dose, restricted range of APTT, and a high median ATIII, which may cause less derangement of the physiological coagulation/anticoagulation balance combined with the circuits' higher biocompatibility. In a relatively large cohort we found that using a relatively low target of APTT did not cause an increase in thrombotic complications, and observed an acceptable rate of bleeding episodes.

To the best of our knowledge, few ECMO centers have reported the maintenance of a range of ATIII among their anticoagulation criteria. ATIII showed an inverse association with amount of transfusion, and also with mortality. Though lower levels of ATIII are found in more severe patients, the high level of AT activity may contribute to reducing the heparin dose, causing less of an impact on the coagulation system. Its relevant anti-inflammatory role has recently been explored. [34] [35]. Keeping a higher AT activity may reduce endothelial activation, coagulopathy, and inflammation, thus limiting the need for transfusion. [36]

AKI stage 3 was a factor associated with increased need for transfusion. [37] This finding could be explained by the higher severity of such patients, and the unavoidable use of another extracorporeal circuit (continuous renal replacement therapy, CRRT) to manage the fluid balance, since all these patients were in CRRT for at least 24 hours. [38] This result is in line with data in the literature, considering the high prevalence of AKI during ECMO due to organ crosstalk, systemic inflammation, and hemodynamic variation. [39]

The amount of daily transfusion was considerably less than one unit per day, and the mean Htc shows how the target was respected. This is an important result when compared with data in the literature, and suggests that the Hb and APTT ranges are feasible, and, taking into account the survival rate, may also be safe. [14]

The pathophysiological rationale for VV-ECMO treatment is to provide a partial oxygenation of the whole cardiac output. [40] When the patients are severely ill (as in our cohort, with a median PaO2/FiO2 ratio of 59 despite maximal support), even after any reassessment for possible recirculation, it is often difficult to assure a complete oxygenation; so one possible strategy is to increase DO2 to increase the

Hb level. [41, 42] In addition, given the risk of hidden bleeding, a strict trigger of Hb at 7 g/dl may reveal itself to be too risky. [42, 43]

Finally the circulating volume has a role in assuring an adequate blood flow in ECMO. Because first-line management in ECMO is aimed at reducing the extravascular lung water, this strategy could have an impact on the circulating volume. In some cases, perhaps, it would be more efficient to transfuse a limited amount of PRBC more than fluids, thus increasing DO2 by blood flow and Hb, and avoiding hemodilution.[44-46]

The survival was encouraging, particularly in light of the ECMO weaning, the discharge from the ICU, and the return to almost normal life for patients with a long life expectancy, providing further evidence of the safety of our approach. These results, even if influenced by several management decisions and the difficult generalizability of the study design, may reflect our anticoagulation and transfusion protocol, considering the high negative impact of transfusion requirements on mortality. Moreover, the impact of illness severity at admission was not significantly associated with the end points taken into account.

Our study has several limitations. First, it is a single center study and was carried out over a long period of time. Consequently, our data were influenced by the learning curve. We have progressively reduced the number of transfusions over the years, following an increase in the number of treated patients. In addition, a confounding factor may be the later use of new devices (CardioHelp), which have better and integrated monitoring of Hb and SvO2. Second, we could not identify a definitive trigger able to guide transfusion management. But as mentioned before, this will probably be an arduous task given the strong interdependence of the several factors influencing survival.

Despite such limitations we believe that our study has a number of strengths. To the best of our knowledge, this is the largest available homogeneous cohort of adult VV-ECMO patients affected with ARDS, and studied in terms of transfusion within a protocol of restrictive management of anticoagulation. Moreover, we were able to investigate the effects of PRBC transfusions on survival, with a longer follow up. In addition, our data collection methods were consistent and computer-based. Finally, the study was carried out in accordance with the highest ethical standards, in light of emerging concerns over privacy and consent in critically ill patients.

Conclusions

The implementation of a comprehensive protocol for anticoagulation in VV-ECMO for ARDS was feasible and resulted in low PRBC transfusion requirements. The ATIII activity level was associated with transfusion requirements. Consequently, ATIII should be taken into account in an anticoagulation protocol. The factors independently associated with mortality in ECMO, apart from the pre-ECMO

hospital stay, were the occurrence of septic shock, and the amount of transfusion, prompting the adoption of restrictive policies.

Future studies will be needed to better delineate anticoagulation management and transfusion thresholds for such patients.

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Chapter 3

Impact of Cannulae Design on Packed Red Blood Cell Transfusions: Technical Advancement to Improve Outcomes in Extracorporeal Membrane Oxygenation

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Abstract

Background: Technological improvement has contributed to making veno-venous extracorporeal membrane oxygenation (VV-ECMO) safer and easier, spreading its use in acute respiratory failure (ARF).

Methods: This is a retrospective observational study carried out in the ECMO center at IRCCS-ISMETT, a medical center focused on end-stage organ failure treatment in Italy. We investigated the effect of different cannulae designs on the amount of blood product transfused. Eighty-nine consecutive patients affected with ARF on VV-ECMO from 2008 to 2016 were compared according to type of cannulation: older percutaneous cannula (Standard group, 52 patients) and HLS© BIOLINE-coated, but with shorter drainage cannulae (BIOLINE group, 37 patients).

Results: The two study groups were comparable in terms of baseline characteristics (age, BMI, SAPS-II, SOFA, PRESERVE score) and ECMO management (median hematocrit, platelet nadir, antithrombin III, heparin, activated partial thromboplastin time). In the BIOLINE group, a lower amount of packed red blood cells (PRBC) was transfused considering both total number (4 units, IQR 1-9 vs. 12 units, IQR 5.5-21; $p<0.01$) and ml of PRBC/day of ECMO support (91, IQR 21-158 vs. 193.5, IQR 140.5-254; $p<0.01$). In the BIOLINE group, a trend in reduction of ECMO days ($p=0.05$) and length of ICU stay was found ($p=0.06$), but no differences in rates of ECMO weaning and ICU discharge were evidenced. The BIOLINE group constituted a saving of €1,295.20 per patient/treatment, counting the costs for cannulation and PRBC administration.

Conclusions: More biocompatible and shorter drainage cannulae may represent one of the contributing factors to a reduction in transfusions and costs of VV-ECMO in the current ongoing technological improvement in ECMO.

Background

Over the last decade, extracorporeal membrane oxygenation (ECMO) has emerged as a promising intervention to provide supportive care to patients affected with acute respiratory failure (ARF) unresponsive to conventional and rescue therapies (1).

The survival rate of patients affected with ARF and supported with ECMO, in this case mainly in its veno-venous (VV) configuration, is progressively improving, but mortality is still high, even when bearing in mind that such support is usually initiated primarily in younger patients (2).

The improvement in clinical management of ECMO and its eventual complications on the one hand, and technological progress (3) on the other, have increased survival in specialized high-volume centers where a centralization process is established (4).

The rationale for ECMO use is to provide an adequate delivery of oxygen (DO2), which is assured by hemoglobin (Hb) content and blood flow in the circuit. For years now, Hb has been set at values close to the normal range (12-14 g/dl), but recent observational series (5,6) have suggested the feasibility of a lower transfusion threshold following the general reduction of transfusions in critically ill patients (7-9).

Apart from clinical considerations, the success of ECMO depends largely on the ability of the circuit to assure adequate blood flow, with oxygenation and decarboxylation over time (10). In the last few years, oxygenators and circuits have undergone an important improvement in efficacy and biocompatibility (11). The cannulae (12) and the interface between patient and circuit have also changed, and cannula design has to be focused on maximizing the blood flow while causing minimal damage to the blood (13), the least possible coagulation activation (14), and easy placement. It is in this light that the HLS© BIOLINE-coated cannulae have recently been introduced (15).

The aim of this study was to evaluate the impact of the introduction of new cannulae specifically designed for ECMO on the number of packed red blood cells (pRBC) transfused in adult patients undergoing VV-ECMO, as well as its potential impact on reducing transfusion-related costs.

Methods

This study was approved by our Institutional Research Review Board and by the local Ethics Committee. All the patients in their full mental capacities, following recovery, signed an institutional consent for publication of manuscripts as anonymous aggregate data. The deceased patients were included, in accordance with Authorization N° 9 of the Italian Privacy Law.

This is a single center retrospective study of consecutive adult patients supported on ECMO from February, 2008 to March, 2016. The patients were affected with ARDS as defined by the 1994 ARDS consensus or, later, the Berlin definition. Exclusion criteria for the study were < 18 years of age, pre- or intra-operative support during lung transplantation or other surgical procedures (16), and veno-arterial configuration (combined respiratory/cardiac disease).

Medical history, demographics, biometrics, and lab tests were collected prospectively in our ECMO database through an electronic medical record system (Sunrise Clinical Manager, Allscripts Healthcare Solutions, Inc). ECMO predictive scores PRESERVE (PRedicting dEath for SEvere ARDS on VV-ECMO), RESPscore (Respiratory Extracorporeal Membrane Oxygenation Survival Prediction), and severity of illness scores SAPS-II (Simplified Acute Physiology Score) and SOFA (Sepsis-related Organ Failure Assessment) score were recorded at admission, as well. Acute kidney injury (AKI) was assessed according to the Acute Kidney Injury Network (AKIN) stage classification during the ECMO stay.

Transfusion requirement for pRBC was defined as the total number of units, total amount as ml, and median of ml transfused per day of ECMO support. Fresh frozen plasma (FFP) and platelets (PLTs) were counted as the number of patients who received transfusions and the volume of products transfused.

Bleeding episodes were classified as follows: overall (any kind of bleeding), minor (no intervention beyond stopping anticoagulation), major (> 2 pRBC transfusions triggered by the bleeding event or interventional/surgical approach, including endoscopy, nasal/pharyngeal tamponade or cauterization), and fatal (intracerebral fatal hemorrhage) (17).

ECMO configuration and type of cannulae

ECMO management was defined as type of device (ROTAFLOW or CardioHelp System – Maquet, Getinge Group, Rastatt, Germany), cannulation configuration, and blood and sweep gas flow at the beginning and the last day of support. ECMO outcomes were defined as weaning from support, and effective intensive care unit (ICU) discharge.

All cannulae were placed peripherally and percutaneously, and preferential ECMO configuration at our institute is with femoral drainage and right internal jugular reinfusion, while the femoro-femoral circuit is adopted when there are specific contraindications to jugular cannulation. Since the beginning of our ECMO program, we have placed our standard peripheral cannulae for extracorporeal circulation (Edwards Lifesciences Corporation – Irvine, USA). The drainage cannulae were thin-walled and wire-reinforced, with extended drainage holes: 20 Fr and 22 Fr, 55 cm length, or 24 Fr and 68 cm for drainage. For reinfusion, we employed 16 Fr or, mainly, 18 Fr, 15 cm length for the jugular vein, and 20 Fr, 22 Fr, 55 cm length, or 24 Fr and 68 cm length for femoral reinfusion. In November, 2014 we

introduced into our practice the HLS© cannulae (Maquet - Getinge Group – Rastatt, Germany) with thin and biocompatible polyurethane bodies reinforced with a flat wire, alternating pairs of side holes, and coated with a BIOLINE matrix composed of albumin and heparin for an increased tip-to-tip biocompatible circuit certified for an extended use of up to 30 days: 23 Fr and 25 Fr, 38 cm, and 17 Fr or 19 Fr, 15 cm length for jugular vein reinfusion (in one case 23 Fr, 55 cm as femoral reinfusion).

Institutional protocol for anticoagulation and transfusions during ECMO

Our protocol for transfusions and anticoagulation (5) is based on integration of the Hb values with other parameters: SvO2, urine output, lactates, and hemodynamics. Hematocrit (Htc) is maintained between 24% and 30% unless severe hypoxia is present despite rescue maneuvers. Anticoagulation is maintained with heparin at a target range of APTT between 40 and 50 seconds, checked every four hours, and stopped in any case of bleeding or in case of spontaneous coagulopathy. ATIII activity is checked daily and replaced by ATIII concentrates, as needed, to keep the activity as near as possible at 100%. Blood waste is avoided: need for blood sampling is re-evaluated daily, and at ECMO weaning the blood in the circuit is reinfused in the patient.

PLTs are transfused when the level falls below 40,000-50,000 /ml, according to the identification of possible bleeding sources. FFP is transfused when there is evidence of relevant bleeding in the presence of coagulopathy (18).

Statistical analyses

Categorical variables are given as frequencies and percentages, continuous variables as mean and standard deviation or as median and IQR (25^{th}-75^{th} percentile interquartile range), when appropriate, according to data distribution.

The comparison between the BIOLINE group and the Standard cannulae group was made with the two-sample t-test for continuous variables or Wilcoxon rank-sum test, and by Fisher's exact test or chi-square test for categorical variables, when appropriate.

All tests were two-sided, and a p value of <0.05 was considered indicative of statistical significance. Data handling and analyses were done with SAS 9.4 software (SAS Institute Inc, Cary, NC, U.S.A.).

Results

During the study period, 92 patients required ECMO support for ARF at our institute. Eighty-nine (89) patients comprised our study cohort since 3 were excluded for the following reasons: 2 affected with hematologic malignancies (inappropriate to explore the topic of transfusion), 1 because at too high a

risk of death (SAPS-II at ICU admission 66 and SOFA score 18) and with less than 48 hours of ICU stay.

The patients were divided into two groups according to the type of cannulation: group 1, named Standard, was composed of 52 patients with Edwards cannulae, and group 2, named BIOLINE, and composed of 37 patients with HLS© BIOLINE cannulae.

The underlying diagnoses were H1N1 influenza A (27 patients in the Standard group and 17 patients in the BIOLINE group), bacterial pneumonia (12 Standard and 16 BIOLINE), lung graft failure (6 cases in the Standard group), polytrauma (2 Standard, 1 BIOLINE), pneumocystis jirovecii pneumonia in HIV-AIDS (1 Standard, 2 BIOLINE), ARF post-pneumonectomy (2 cases in the Standard group), mediastinitis (1 case in the Standard group), chemical pneumonia (1 case in the Standard group), and 1 case of transfusion-related lung injury in the BIOLINE group.

Patient characteristics of the combined cohort and separate groups are summarized in Table 1. The two groups were comparable in terms of the majority of baseline and pre-ECMO characteristics.

Variable	Overall *n. 89*	BIOLINE *n. 37*	Standard *n. 52*	*P value*
Pre ECMO Data				
Age, years	43 (33-52)	45 (31-55)	43 (34-48)	0.56
Gender male, N (%)	66 (74)	30 (81)	36 (69)	0.23
Body mass index, Kg/m	27.8 (24-31.1)	29 (24-33)	27.7 (24-31)	0.88
SAPS 2 at admission	38 (32-45)	39 (33-47)	38 (30-44)	0.27
SOFA score at admission	8 (6-10)	8 (6-11)	8 (6.5-10)	0.44
Hospital length of stay, days	7 (3-12)	5 (2-12)	8 (5-13)	0.14
ICU LOS, days	5 (2-9)	3 (1-8)	5 (2-11.5)	0.08
Mechanical ventilation, days	3 (2-8)	3 (1-7)	5 (2-10)	0.06
PaO2/FiO2 ratio	59 (52-67)	58 (52-68)	59 (51.5-64.5)	0.86
Creatinine, mg/dl	1.3 (0.80-1.9)	1.4 (0.8-2.1)	1.2 (0.8-1.8)	0.84
Bilirubin, mg/dl	0.86 (0.60-1.30)	0.8 (0.58-1.59	0.9 (0.6-1.3)	0.60
Hematocrit, %	32.5 (29.8-37.10)	32.1 (29.7-39.6)	32.5 (29.8-35.5)	0.09
Charlson Comorbidity Index	1 (0-2)	1 (0-3)	1 (0-1.5)	0.13
PRESERVE score	4 (3-5)	4 (3-5)	4 (3-5)	0.91
RESPscore	2 (-1-4)	2 (1-4)	1 (-1-4)	0.04
ECMO circuit and management				
ROTAFLOW, n(%) **CardioHelp, n(%)**	38 (42.7) 51 (57.3)	7 (18.4) 30 (58.8)	31 (81.6) 21 (41.2)	< 0.01
Drainage, Fr	23 (22-24)	25 (23-25)	22 (22-24)	0.02
Return, Fr	18 (18-19)	19	18	0.34
Blood flow 1st day, l/min	3.8 (3.3-4.2)	3.8 (3.4-4.3)	3.85 (3.25-4.05)	0.33
Air flow 1st day, l/min	3 (2.5-4)	3 (2-4)	3 (3-4)	0.43
Plateau pressure 1st day, cmH2O	22 (21-24)	23 (22-24)	22 (20-24)	0.47
PEEP, 1st day	12 (10-14)	12 (10-14)	12 (10-14)	0.80

Prone position, N (%)	15 (17)	5 (14)	10 (19)	0.57
CRRT, N (%)	50 (56)	20 (54)	30 (58)	0.82
Septic shock, N (%)	56 (63)	24 (65)	32 (62)	0.82
Hematocrit	30.5 (29-32.3)	31 (28.7-32.9)	30.15 (29-31.75)	0.78
Platelets nadir	71 (42-130)	81 (40-145)	65.5 (42.5-129)	0.36
Activated Partial Thromboplastin Time	45.2 (41.7-49.4)	45 (41.8-47)	45.5 (41.5-51.1)	0.28
Antithrombin III	91.8 (83.8-102)	94.1 (84.1-107.1)	90.8 (82.7-100.2)	0.42
Heparin	10.9 (7.5-14.8)	10.8 (8.6-15.2)	11.5 (7.5-14.6)	0.53
Overall bleeding, N (%)	37 (41)	15 (40)	22 (42)	1.00
Major bleeding, N (%)	14 (16)	3 (8)	11 (21)	0.14
Fatal bleeding, N (%)	3 (3)	0	3 (6)	0.26

Table 1: Baseline characteristics and data on ECMO management.

ECMO management

No relevant between-group differences were found in ECMO management apart from the type of console, and diameter and length of drainage cannula. All parameters for the targets for transfusions and anticoagulation (hematocrit, platelet nadir, APTT, antithrombin III, heparin) were comparable between the two groups, and in range for our protocol (all p values were > 0.05) (Table 1). In the BIOLINE group there was a higher prevalence of CardioHelp adoption, while in the Standard group there was more frequent use of the ROTAFLOW console and pump ($p<0.001$). In the BIOLINE group there was a higher median diameter of the inflow cannula: 25 Fr, IQR 23-25 vs. 22 Fr, IQR 22-24, $p=0.027$.

Transfusion management and bleeding

The overall cohort received a reduced number and amount of pRBC transfusions related to our adaptable and restrictive protocol: the median number of pRBC units transfused was 8 (IQR 3-18), and the amount in ml adjusted for the duration of ECMO was 158 ml pRBC/day of ECMO support (IQR 91-214), with 9 patients who did not require any pRBC units (Table 2).

Investigating different management in the two groups we found significantly fewer pRBC transfusions in the group with BIOLINE cannulation (Table 2). This is evident considering the total number of transfused units during the ECMO stay: 4 (IQR 1-9) in the BIOLINE group, 12 (IQR 5.5-21) in the Standard group ($p=0.004$). The evidence is even more relevant considering the daily amount: 91 ml/day ECMO (IQR 21-158) in the BIOLINE and 193.5 ml/day ECMO (IQR 140.5-254) in the Standard group ($p<0.001$). Moreover, in the BIOLINE group, 9 patients received no pRBC transfusion during the ECMO duration (mean ECMO length 9 ± 6 days).

Variable	**Overall** ***n. 89***	**BIOLINE** ***n. 37***	**Standard** ***n. 52***	***P value***
Total PRBC during ECMO support, unit	8 (3-18)	4 (1-9)	12 (5.5-21)	< 0.01

Total PRBC during ECMO support, ml	1926 (858-4530)	1050 (250-2244)	3000 (1415-5285)	< 0.01
PRBC / day of ECMO support, ml/day	158 (91-214)	91 (21-158)	193.5 (140.5-254)	< 0.01
Patients without fresh frozen plasma transfusions, N (%)	78 (87)	32 (86)	46 (88)	1.00
Total Fresh frozen plasma*, ml	789 (400-1750)	1000 (789-1000)	450 (250-1750)	-
Patients without platelets transfusions, N (%)	61 (69)	28 (76)	33 (63)	0.25
Total Platelets*, ml	498 (250-1500)	954 (314-1966)	496 (250-900)	-

Table 2: Transfusion management during ECMO support.
PRBC: Packed red blood cells.

*: calculated in patients who received at least one unit of fresh frozen plasma or platelets during the ECMO stay.

FFP was transfused in 11 of the 89 patients, and in a comparable way between the two groups: 5 patients in the BIOLINE group, and 6 patients in the Standard group. PLTs were transfused in 28 of the 89 patients: 9 in the BIOLINE group, and 19 in the Standard group.

There was no statistically significant difference between the groups in terms of bleeding episodes (Table 1). Apart from 3 fatal bleedings, i.e., intracranial hemorrhage, we registered overall bleeding in 15 cases in the BIOLINE group, and 22 in the Standard group. Episodes of major bleeding were less frequent with the adoption of the new cannulae, though not statistically significant (p=0.140). In the BIOLINE group we registered 3 major bleedings: 1 due to duodenal gastric ulcer, 1 due to rhinopharyngeal and abdominal bleeding in a polytrauma patient, 1 due to cannulation-site-relevant bleeding. In the standard group we encountered 11 major bleedings: 4 were intracerebral hemorrhage (in 3 cases they were fatal, while in 1 case a surgical evacuation of hematoma allowed recovery); 3 were hemothorax (2 patients on ECMO for lung graft failure, and 1 polytrauma patient who underwent a thoracotomy during ECMO support, all 3 successfully weaned); 2 were severe gastric bleeding (one recognized as an incidental diagnosis of a gastric bleeding cancer); 1 case was a rhinopharyngeal and gastric bleeding with several interventional procedures to stop the bleeding in a patient with a long ECMO stay and an acquired von Willebrand disease; and 1 case of cannulation bleeding in a patient with heparin-induced thrombocytopenia.

Clinical outcomes

The number of days on ECMO evidenced a borderline statistical significance, with a reduction using the new and more biocompatible devices (p=0.050) (Table 3) This reduction in ECMO duration is also reflected in the trend of reduction in total ICU length of stay, while there was no difference in the median number of days of mechanical ventilation post-ECMO. ECMO survival (namely the successful weaning of the extracorporeal device) and effective discharge from the ICU were comparable in the two groups.

Clinical Outcomes	Overall *n. 89*	BIOLINE *n. 37*	Standard *n. 52*	*P value*
Mechanical ventilation post ECMO, days	10 (5-18)	9 (3.5-17.5)	11 (6-18)	0.89
ECMO support, days	14 (8-24)	13 (7-18)	15 (9.5-27-5)	0.05
ICU length of stay, days	27 (16-41)	22 (14-33)	31.5 (16.5-47)	0.06
ECMO weaning, N (%)	66 (74)	29 (78)	37 (71)	0.47
ICU discharge, N (%)	60 (67)	27 (73)	33 (63)	0.36

Table 3: Clinical Outcomes.
ECMO weaning indicates the number of patients successfully weaned from ECMO, and consequently represents the survival on ECMO.

ICU Discharge indicates the number of patients discharged alive from the ICU, and consequently represents the survival in the ICU.

Costs of transfusions and cannulae

Considering that at our institute every unit of pRBC has a total cost of €224.40, the median cost for pRBC during ECMO in the BIOLINE group was €897.60 per treatment per patient, while in the Standard group the median cost was €2,692.80. This gain in cost effectiveness was partially offset by the higher cost of the cannulae: we calculated an increased cost for cannulation of €500.00 in the BIOLINE group. Consequently, the implementation of the new BIOLINE cannulation resulted in a median saving of about €1,295.20 considering just pRBC and cannulae, and not including the potential reduction in ECMO duration and ICU length of stay.

Discussion

In this study, we evaluated different transfusion results in VV-ECMO patients treated with two different types of cannulae.

Currently, several approaches are adopted to improve the biocompatibility of extracorporeal circuits (19-21) , based on the use of antithrombotic biomolecules such as heparin (22), polymeric molecules, and glycoprotein because the ECMO circuit is one of the largest and longest surface areas and volume for blood contact (23) in any medical device for prolonged use (10,24).

The BIOLINE coating combines albumin and heparin polypeptide absorbed into the components of the circuits' surfaces, forming a steric hindrance. With heparin, a negatively charged hydrophilic complex polysaccharide acid, molecules are attached to the polypeptides via covalent bonds and ionic interaction. It has been hypothesized that the circuits' higher biocompatibility, combined with a low median heparin dose, restricted range of APTT, and a high median ATIII(25), may cause less derangement of the physiological coagulation/anticoagulation balance(26).

In terms of ECMO management, we found a difference in pump and console use because the more recent cases were treated mainly with the CardioHelp system (27). Therefore, we progressively switched

from ROTAFLOW use to CardioHelp (both polymethylpentene membrane oxygenators) because almost all our patients are referred by other hospitals (on-site cannulated) and, consequently, we used the properly miniaturized new console (28) for transport (29). This difference may have influenced the results since the CardioHelp provides a slightly lower priming volume, and the difference in the circuit may reduce the formation of vortices, thus reducing the potential for hemolysis. The dynamic differences between the two systems have been not completely elucidated by the literature, but there are some data suggesting a reduced pressure drop with the CardioHelp system, and the ability to achieve desired flow rates at lower rotational speeds (30). Moreover, the CardioHelp offers the opportunity of close monitoring of the circuit's pressures and Hb values, which can help for a more tailored fluid and blood administration. This is the main bias of our data collection since these two systems are quite different in terms of biodynamics and monitoring; but at the same time it is an evidence of updated clinical practice, which is also the basis of research in biomedical technology.

The second difference between groups concerns the drainage cannula. In the current literature there is consensus concerning the best configuration (2) - femoro-jugular, based on expert advice, though there is no ultimate definition of the best cannula type, size, and length (31) (32). In the evolution of our cannulation approach a relevant role has been played by the different designs (33), accompanied by a shorter length of the drainage cannula, an active way to reduce circuit length. In fact, adequate management of the patient's intravascular volume status is critical during ECMO; therefore, we can speculate that a lower speed of the pump with a stable blood flow may translate into a lower number of pRBC transfusions, and better oxygenation may reduce the peripheral tissue hypoxia and organ failure, favoring the patient's recovery (34).

These last considerations involve the strategy of managing the ARF patient on ECMO support, the rationale of which is to provide variable but partial oxygenation of the entire cardiac output. Before administering a transfusion (35) to increase the DO2 it is considered important to check for blood flow, optimize ventilation when possible, and reduce oxygen expenditure, all of which constitute a plausible pathway toward the application of the principles of precision medicine for all ECMO patients.

Considering the bleeding complications, we found a reduced number of overall episodes in the BIOLINE group, but without statistical significance. Theoretically, a less relevant derangement of physiologic coagulation may reduce the episodes of bleeding due to coagulopathy, but several considerations may put into question definitive conclusions from our series since the differences among the episodes of bleeding in the two groups were more related to the case mix and the reduced number of ECMO runs, though we cannot exclude a potential role in the reduced number of days of ECMO support.

Finally, we looked at costs of transfusions. This is a non-negligible "side-effect" of transfusions considering the costs incurred for the health care system, and the shortage of pRBC in blood banks. The use of BIOLINE cannulae is much more expensive than the older cannulae, but considering the potential for reduction by one-third of pRBC administration, expenses can be considered cost-effective (36). This hypothesis is even more relevant in light of potentially reduced length of ECMO support, and should receive increasing interest in the ECMO community since the applicability of ECMO (37, 38) is constantly spreading, even toward pathologies not traditionally associated with ECMO support (39-42). Obviously this is not a cost-analysis since even the change in the ECMO system is expensive and increases the costs, though it is an example of the indirect clinical costs and savings that can be derived from a careful consideration of the available techniques.

Our study has several limitations. First, it is a single center study and with a retrospective design. Second, apart from a few cases, the two groups were not treated contemporaneously, since the standard cannulae were adopted mainly in a previous period; consequently, our data are probably influenced by the learning curve and the general improvement in ICU knowledge. In addition, a confounding factor may be the later use of new devices, which have an integrated monitoring of Hb and SvO2.

Despite such limitations, we believe that our study has some strengths. To the best of our knowledge, this is the first study to compare different types of cannulae in transfusions. This is a large and homogeneous cohort of adult VV-ECMO patients affected with ARF, and despite the almost before-after design of the study the principles of management did not change over the years, as evidenced by the similar levels of hematocrit, APTT, and heparin. In addition, our data collection methods were consistent and computer-based, also adding the costs of transfusions and ECMO technology, thus offering a practical and "real life" picture of the field. Lastly, our conclusions are hypothesis-generating more than evidence statements and highlight the importance of careful consideration of circuit configurations and type of cannulae use.

Conclusions

Our results suggest that the use of shorter and more-biocompatible cannulae, associated with a restrictive policy of anticoagulation and transfusion, may contribute to a reduction in transfusions during VV-ECMO and to a potentially indirect more cost-effective practice. Our results should be considered hypothesis-generating and highlight the importance of further investigation in prospective studies of the specific effects of the clinical advancements determined by new and evolving technologies during ECMO.

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Chapter 4

Hemoglobin Trigger and Approach to RBC Transfusions during veno-venous Extracorporeal Membrane Oxygenation: The International TRAIN-ECMO Survey

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Abstract

Introduction: Optimal red blood cell (RBC) transfusion practice during veno-venous extracorporeal membrane oxygenation (VV-ECMO) is still under debate. This survey aimed to assess the Hb trigger (also comparing with other critically ill patients) and major physiologic determinants considered for transfusions during VV-ECMO.

Methods: Voluntary Web-based survey, endorsed by the European Society of Intensive Care Medicine, of ECMO practitioners worldwide.

Results: Four hundred forty-seven (447) respondents worldwide answered the questionnaire: 277 (61.9%) from Europe, 99 (22.1%) from North America, 36 (8.2%) from Asia and Oceania, and 35 (7.8%) from Central and South America. Among the respondents, 59.2% managed less than 12 ECMO runs/year, 19.4% between 12 and 24 runs/year, and 21.4% more than 24 runs/year. Of the respondents, 54.4% do not use a pre-defined Hb trigger in VV-ECMO, and, while the rate of adoption of a defined trigger varied worldwide, the effective value of Hb did not differ significantly among macro-regions. In patients on VV-ECMO, the Hb trigger to initiate RBC transfusion, was higher than in other critically ill patients: 9.1 ± 1.8 g/dl versus 8.3 ± 1.7 g/dl, $p < 0.01$. The Hb trigger was lower in centers with more than 24 ECMO/year (8.4 mg/dl [95% CI 7.7-8.9]); (8.9 mg/dl [95% CI 8.2-9.7]) in centers with between 12 and 24 ECMO/year; and (9.6 mg/dl [95% CI 9.1-10.0]) in centers with fewer than 12 ECMO/year ($p<0.01$). Several and variable adjunctive parameters are considered in cases of uncertainty for transfusion: the principal are hemodynamic status, SvO2, lactates, and fluid balance.

Conclusions: Though the use of a pre-defined Hb trigger is still under-adopted among centers with low or median ECMO case volume, the majority of respondents use a higher Hb trigger for VV-ECMO patients compared with other critically ill patients. Higher volume centers tolerate lower Hb levels.

Introduction

Mortality associated with acute respiratory distress syndrome (ARDS) remains high despite continual improvement in mechanical ventilation and extracorporeal membrane oxygenation (ECMO). [1-4] Across the last decade, survival rates of ECMO patients have certainly improved, particularly in large-volume centers, though, despite publication of recent randomized controlled trials, the benefit of ECMO and its timing are still questioned. [5-7] In addition, several important aspects of daily patient management during ECMO support remain a matter of debate. [8] One of them is the red blood cell (RBC) transfusion policy, and the hemoglobin (Hb) level, which is considered a trigger. Past guidelines from the Extracorporeal Life Support Organization (ELSO) commonly suggested maintaining the Hb level within normal limits (12-14 g/dL), with the rationale that this will maintain the arterial content of oxygen and, therefore, the delivery of oxygen in patients with severe impaired oxygenation. [9] This notion has been challenged by several case series and the recent EOLIA trial, which reported on lower transfusion thresholds and good outcomes in critically ill patients. [6, 10-12] Moreover, some evidence suggests that a greater number of transfusions in ECMO patients is associated with worse outcomes, [13, 14] and a liberal transfusion strategy seems to be associated with increased morbidity and mortality. [15, 16] Such a relationship can be confounded by several factors influencing transfusion practice, such as target of anticoagulation, bleeding episodes, hemolysis, activation of inflammation, and consumption of platelet and coagulation factors. [17, 18] Given these premises, targeting at lower Hb in ECMO still lacks evidence and, consequently, ELSO guidelines, even reporting on the possibility of a restrictive approach, still recommend a hematocrit of about 40%. [9]

Since defined guidelines in this specific setting are not available, it is important to assess current transfusion practices of ECMO physicians, and to understand how the reports on lower Hb targets have been interpreted among different centers around the globe. [19]

Two previous surveys in the field of transfusions in ECMO, one in 2010 and one in 2016, had some limitations: the first was conducted before the evidence of potential harm of transfusions was put forth, [20] and the second had a limited number of responders. [21]

The present international survey was thus planned and conducted to investigate, at a global level, the management of anemia and RBC transfusion practice during VV-ECMO, including the Hb threshold for transfusion and the physiological integrative parameters adopted to assess RBC transfusion, and eventually compare the adopted Hb threshold between VV-ECMO patients and "non-ECMO" critically ill patients.

Methods

This study was approved and endorsed by the European Society of Intensive Care Medicine (ESICM) Research Committee. Given the nature of the study, no ethics approval was needed for the participants.

Design and Administration

This is a descriptive, semi-structured, and voluntary, anonymous, open and self-administered cross-sectional survey.

The survey was in English and submitted online using a SurveyMonkey platform (SurveyMonkey, San Mateo, CA, USA) from August 4th to November 30th, 2017. It was active on a dedicated Web page on the ESICM Web site, sent by e-mail to ESICM members (potentially 16,173 recipients) on September 7th, and was made public in social media of the Society (Facebook 4th August, Twitter 11th August, LinkedIn 4th October). Moreover, it was sent to several national societies and associations (a list of them is provided in the acknowledgement section) and by personal e-mail to international experts.

After completion, no survey re-entry was allowed. All responses were anonymous, and no reimbursement or benefit was offered for completion of the questionnaire.

Survey Development

The survey and its cover letter were developed after a review of clinical practice, the literature, and guidelines on survey design and conduction. [22-24] Once a consensus was achieved, the survey was submitted to the ESICM Research Committee, which released its endorsement after a peer review process (two reviewers and committee evaluation) and request for some minor changes.

It was conducted using 22 multiple-choice questions and 2 questions presenting uncertain cases of possible transfusion in VV-ECMO to evaluate the parameters taken into account to administer RBC (Additional File 1). The survey was organized into four domains. The first group of questions aimed at defining the general characteristics of respondents according to country, personal experience, type of ECMO circuit adopted, and volume of activity of their ECMO center. The second group of questions investigated the presence of institution-specific guidelines indicating transfusional triggers for blood product administration in critically ill patients. The third part of the survey focused on the transfusion triggers in ECMO: whether a pre-specified trigger (Hb and hematocrit) was adopted and, possibly, what the value was. Moreover, in this section, to understand the practice for prevention of anemia, the use of iron measurement and supplementation, and the use of erythropoietin were investigated. Finally, clinicians were questioned about the physiological parameters adopted in cases of uncertainty (i.e., when Hb has a borderline value but the transfusion indication is not clearly present). In two clinical representative cases (Model Patient 1: young patient affected with ARDS; Model Patient 2: young patient with history of stroke as main comorbidity) in which the RBC transfusion may have been

uncertain, the participants were asked if they would administer a transfusion, and what threshold of hemoglobin they would use in such a patient.

Statistical Analysis

After completion of the survey, participants were grouped into their geographical origin (i.e., Europe, North America, Central and South America, Asia and Oceania), and by their ICU experience since they completed their residency.

ECMO centers were categorized in three groups according to the number of VV-ECMO runs managed per year: fewer than 12 per year, between 12 and 24 per year, and more than 24 per year, as suggested by the position paper published by the ECMONet. [5]

Categorical variables are expressed as count (percent), and continuous variables as mean (standard deviation) or median (IQR), as appropriate.

Categorical variables and continuous variables (stratified according to macro-regions and to the centers' volume of ECMO management) were compared using chi-square or Fisher's exact tests, t-test or Wilcoxon Rank Sum test, and ANOVA or Welch's ANOVA test, when appropriate.

All tests were two-sided, and a p value of <0.05 was considered indicative of statistical significance. Data handling and analyses were done with SAS 9.4 software (SAS Institute Inc, Cary, NC, U.S.A.)

Results

Survey Response and Characteristics of Participants

The characteristics of respondents are summarized in Table 1. In total, 447 respondents from 42 countries (listed in Additional File 2) answered the questionnaire.

The majority of participants (n=355, 79.4%) work in teaching hospitals, and looking at the type of ICU, the majority of respondents work in a mixed ICU (292 participants, 65.3%) or in a cardiac surgery ICU (109 respondents, 24.4%). The main working environment was intensive care for n=365 (81.7%), operating room for n=78 (17.5%), and emergency medicine for n=4 people (0.8%). The median years of working experience after completion of the residency program was 10 years (IQR 5-18 years).

Table 1 **Characteristics of Participants**

Geographical Differences, N (%)					
Europe	North-America	Asia and Oceania	Central/South America		
277 (61.9%)	99 (22.1%)	36 (8.2%)	35 (7.8%)		
Type of Hospital, N (%)					
Teaching Hospital	Non-teaching Hospital		Private Hospital		
355 (79.4%)	71 (15.9%)		21 (4.7%)		
Type of Intensive Care Unit, N (%)					
Mixed	Cardiac Surgery	Medical	Surgical	Cardiology	Neurology
292 (65.3%)	109 (24.4%)	23 (5.2%)	20 (4.5%)	2 (0.4%)	1 (0.2%)
Working Environment, N (%)					
Intensive Care	Operating Room	Emergency Medicine			
365 (81.7%)	78 (17.5%)	4 (0.8%)			
VV-ECMO Center Volume (N of VV-ECMO run per year), N (%)					
< 12 runs/year	12-24 runs/year		> 24 runs/year		
216 (59.2%)	71 (19.4%)		78 (21.4%)		
VA-ECMO Center Volume (N of VA-ECMO run per year), N (%)					
< 12 runs/year	12-24 runs/year		> 24 runs/year		
174 (47.7%)	89 (24.4%)		102 (27.9%)		

With regard to the centers' volume, for VV-ECMO, n=216 (59.2%) manage fewer than 12 runs per year, n=71 (19.4%) participants manage between 12 and 24 runs per year, and n=78 (21.4%) more than 25 runs per year; 75.6% of the respondents currently use tip-to-tip heparin-bonded circuits.

Transfusion thresholds

A slight majority of participants responded that they do not adopt a pre-specified threshold Hb value as a trigger for RBC transfusions, in either the general population of critically ill patients (56.4%) or in VV-ECMO patients (54.4%).

Similarly, no threshold hematocrit value is adopted as a transfusional trigger by a high number of participants, either for the general critically ill patients (80.6%) or VV-ECMO patients (79.9%).

Looking at geographical differences, there is a wide heterogeneity for the adoption of Hb trigger at hospitals for critically ill patients: 54.3% of respondents from Asia/Africa/Oceania, 55.9% from Central and South America, 47.24% from Europe, and 27.8% in North America, (p value < 0.01) (Figure 1A). The lowest Hb threshold was reported in Europe.

Instead, for VV-ECMO, the adoption of an Hb trigger is significantly lower in North America (33.3%) than in Asia/Africa/Oceania (66.7%), in Central and South America (67.7%), and in Europe (44.3%) (p value < 0.01). But in this specific setting, the threshold Hb value was not significantly different among the different geographical regions (Figure 1B).

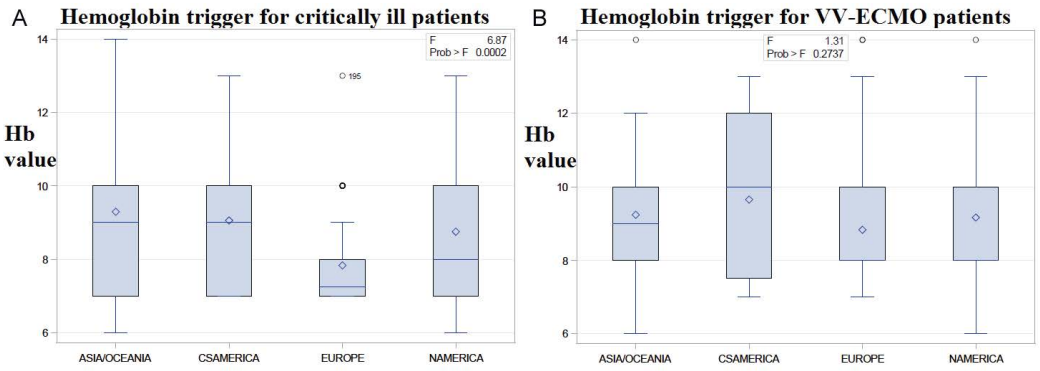

Figure 1. Hemoglobin trigger adopted by respondents according to predefined macro-regions.

1A: critically ill patients

Respondents – Total number: 155; Asia/Oceania: 17; Central/South America: 18; North America: 28; Europe: 92.

1B: VV-ECMO patients.

Respondents – Total number: 151; Asia/Oceania: 19; Central/South America: 20; North America: 31; Europe: 81.

CSAMERICA: Central and South America; NAMERICA: North America.

In each region there was a difference between the thresholds in critically ill patients and in ECMO, with higher values for ECMO patients. This finding is confirmed by the cumulative data.

When a predefined Hb trigger is adopted, the mean value was significantly higher in patients on VV-ECMO than in other critically ill patients (9.1 ± 1.8 g/dl versus 8.3 ± 1.7 g/dl, p < 0.01).

In VV-ECMO, such a rate of adoption of a protocol for RBC transfusion based on a fixed Hb level was influenced by the centers' volume of activity, with a higher adoption of protocolized transfusion strategies based on a pre-defined Hb value in the higher volume centers (Table 2).

Table 2 **Adopted Triggers according to ECMO Center Volume**

	Number of VV-ECMO cases managed per year			P value
Hemoglobin trigger	**<12 per year**	**12-24 per year**	**>24 per year**	
Yes (%)	37.6	48.5	63.6	<0.01
No (%)	62.4	51.5	36.6	
Hematocrit trigger				
Yes (%)	20.0	25.0	15.8	0.39
No (%)	80.0	75.0	84.2	

Similarly, Figure 2 illustrates how the level of Hb trigger is significantly lower in higher volume centers (8.4 g/dl, 95% Confidence Interval 7.7-8.9, in centers with more than 24 ECMO/year), while it was not affected by the quartiles of years of working experience.

Use of Iron and Erythropoietin

The iron level is regularly checked by just 9.8% of respondents, and the type of iron supplementation utilized is heterogeneous, with ferric carboxymaltose the main type, followed by ferric gluconate, ferric sulphate, and iron sucrose.

Erythropoietin is administered to prevent anemia or avoid RBC transfusions by only 7.9% of respondents.

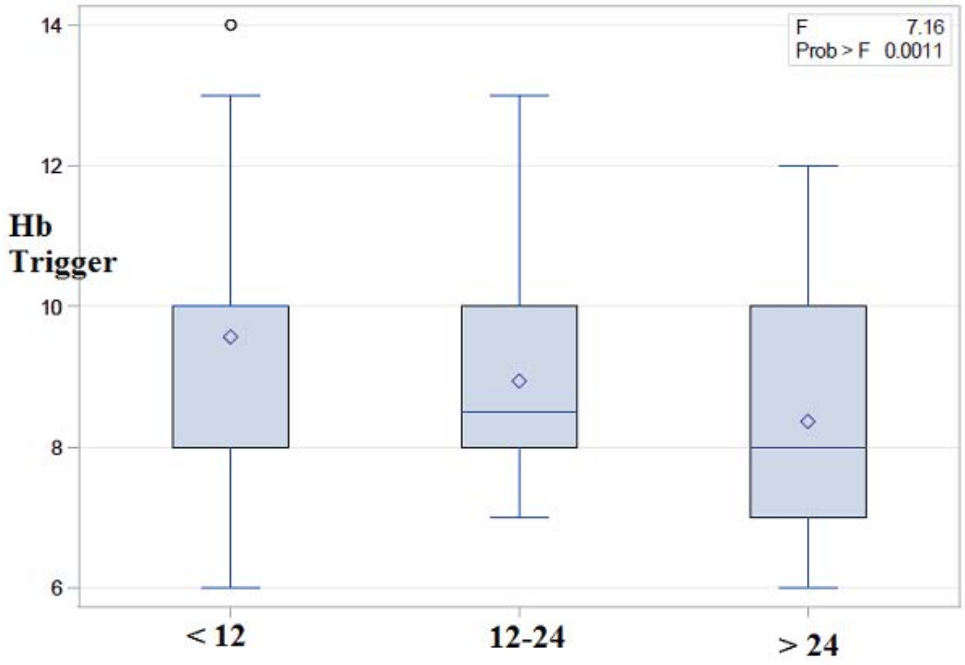

Figure 2. Different hemoglobin triggers adopted according to VV-ECMO centers' volume per year.

Hb: Hemoglobin.

< 12: fewer than 12 ECMO run per year. 12-24: between 12 and 24 ECMO run per year. >24: more than 24 ECMO run per year.

Integrated Parameters to Administer Transfusions

A number of physiological parameters are utilized as transfusion triggers in cases of uncertainty. As expected, the first parameter indicated remains Hb (28.9% of respondents), followed by several other variables related to oxygenation: SvO2 (18.2% of respondents), SaO2 (6.4%), DO2 (6.1%). Bleeding is considered a relevant and specific trigger by 7.5% of participants. The third group of variables is related to hemodynamics and global perfusion: lactates (7.9% of participants), hemodynamic instability (7.1%), clinical signs of hypoperfusion (3.9%), cardiac function (2.5%), and volume status (1.1%).

Figure 3 shows the responses to a question about the variables that should guide RBC transfusions in ARDS patients on VV-ECMO when the need for transfusion is uncertain. Those more frequently taken into account by respondents ("highly likely" taken into account) were hematocrit (41.2%), SvO2 (52.9%), hemodynamics (52.5%), and lactates (47.6%).

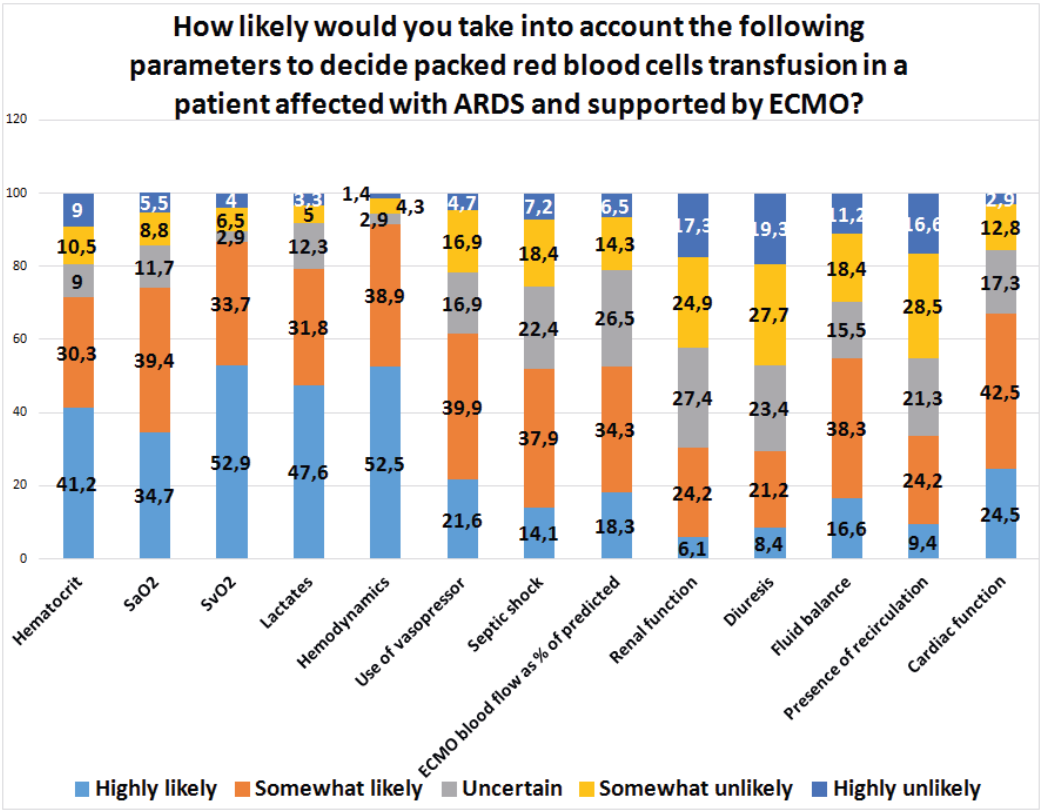

Figure 3. Variables that should guide RBC transfusions in ARDS patients on ECMO

For every variable, the rate of agreement by the respondents is reported in the axis of ordinates.

Figure 4 illustrates which variables, in the participant's opinion, must be optimized before administering RBC: the main determinants are the control of bleeding sources (60.9%), followed by the evaluation of global organ perfusion (53.4%).

Model Patients for Decision on Transfusion

Two model patients were included as ECMO cases with a questionable decision for transfusion. In both cases the clinical picture was stable, and oxygenation was adequate on full ECMO support.

For Model Patient 1, a young woman without relevant comorbidities affected with ARDS due to H1N1 influenza with an Hb level of 6.8 g/dl, 66% of respondents declared they would have transfused the patient. For those who answered they would not have transfused the patient the median Hb considered as trigger is 7 g/dl (IQR 6-10).

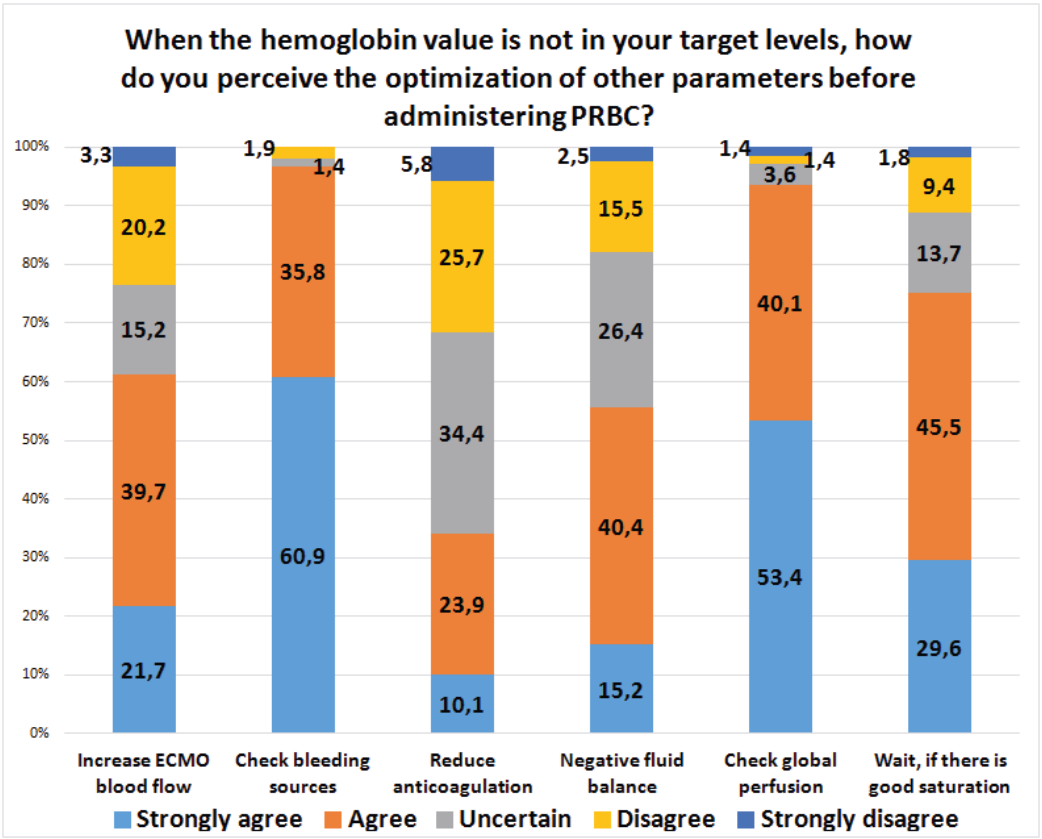

Figure 4. Variables, in the participants' opinion, must be optimized before administering RBC

For every variable, the rate of agreement by the respondents is reported in the axis of ordinates.

For Model Patient 2, a man with a recent ischemic stroke and an Hb level of 7.6 g/dl, precisely 50% of respondents would have transfused the patient. For those who answered they would not have transfused the patient the median Hb considered as trigger is 7 g/dl (IQR 7-9).

Discussion

This survey is the largest one to provide data on the worldwide approach of ECMO practitioners to Hb thresholds and physiological triggers for RBC transfusion in adult patients supported by VV-ECMO.

Less than half of respondents reported using an Hb trigger in ECMO patients. A higher threshold Hb value is adopted during VV-ECMO than in the general population of critically ill patients. As a principal finding, the level of Hb selected as transfusional trigger was not related to geographical

differences, while it is strongly influenced by the center's volume of procedures. Indeed, consistently in each region the threshold for transfusion has a higher value for the ECMO patients, and a lower Hb value is tolerated in centers performing a higher number of procedures. [5, 25, 26]

Two other surveys have evaluated transfusion practices in patients in ECMO support. In the first study, in 2010, Bembea et al. collected 121 responses from ECMO medical directors and coordinators at 187 ELSO centers (located mainly in the U.S.). [21] In this survey, 72% of respondents reported having a written institutional protocol for anticoagulation and blood product management during ECMO. The main transfusion trigger in adults programs was hematocrit, with a median cutoff value for transfusion of 30% (ranging from 20 to 40). In the second survey, in 2017, Esper et al. administered the questionnaire to 166 institutions, obtaining 54 responses (36 were from North America). [20] In that study, the Hb trigger threshold in VV-ECMO was extremely heterogeneous: <7 mg/dl for 15.6% of respondents, between 7.1 and 8 mg/dl for 26.7%, between 8.1 and 10 g/dl for 33.3%, between 10.1 and 11 g/dl for 6.7%, and more than 11 g/dl for 6.7%. In addition, 11.1% of respondents reported considering the clinical signs of bleeding as an isolated trigger. Both surveys found wide variations in transfusion practices among the different institutions, probably due to the lack of evidence-based guidelines and randomized controlled trials in this specific population. [27, 28] This heterogeneity is still present, and confirmed by our data, but the progressive influence of guidelines on transfusions in critically ill patients and recent studies exploring this topic, also in ECMO patients, likely played a role in determining the level of triggers reported in our responses. [29-31] This evolving scenario is proven by published data; indeed recent ELSO guidelines cautiously recommend adopting a hematocrit trigger of about 40%, highlighting how several centers are lowering the Hb and hematocrit thresholds for transfusion during VV-ECMO. [9] Notably, in the EOLIA trial, which enrolled patients from 2011 to 2017, the Hb level was set per protocol between 8 and 10 g/dl (with a planned possible increase in the case of persistent hypoxemia), far from the level recommended in the CESAR trial (protocolized between 12 and 14 g/dl), which included patients ten years before. [6, 32]

In this survey, some interesting, and somewhat surprising data were collected, observations that have not been reported previously. First, the adoption of a transfusion protocol based on Hb trigger is not widely adopted by intensivists. This was relatively unexpected, considering that several recent guidelines and recommendations suggest a restrictive approach to RBC transfusions. [31, 33, 34] The data grouped per macro-regions highlighted how this tendency is diffuse worldwide, and it can be hypothesized that it is probably more difficult to introduce and follow new guidelines for sicker patients. However, during ECMO, the decision to transfuse RBC should take into account a number of physiological parameters, and the need for patient oxygenation should be matched, together with the general conditions, also with the specific need for the ECMO circuit. [35]. In fact, ECMO, as a modified cardiopulmonary bypass

circuit without a reservoir for real-time adjustment of blood volume, dictates active management in order to optimize the patient's intravascular volume status, which involves administration of both fluids and blood products. [36, 37] It might explain the higher Hb threshold reported in VV-ECMO patients compared with other "non-ECMO" ICU patients.

The second main result of our survey is the effect of the centers' volume of activity on the transfusion threshold. This is quite relevant, since there is wide debate over centralization of ECMO patients to optimize the management of several aspects of daily care (first of all, fluids and RBC), and of possible complications with the most advanced approaches, but the spread of the technique has led to a number of centers with a limited case load as the majority of respondents suggests. [5] In recent years, several authors have highlighted the association of increased mortality with higher volume of transfusions. [38, 39] Consequently, the adoption of more restrictive transfusion protocols, inferred from the lower accepted Hb, may be one of the contributing factors in higher survival and better clinical outcomes. [40]

The impact of geographical differences was present, but limited in its effect. Surprisingly, there was a higher prevalence of fixed Hb trigger in Central/South America and in the Asia/Oceania group than in Europe and North America. However, the Hb trigger level was not statistically different among the different macro-regions, though there is a trend toward the adoption of lower cutoff values in European centers. However, it is difficult to know whether these observations reflect a different familiarity with the published literature or just personal practice, or if it is influenced by the type of hospital and type of reimbursement. [41-44]

The final result of the survey involved the use of integrated physiologic parameters to decide on a transfusion, since the treatment of anemia is too complex to be referred to just a single number (Hb): comorbidities, volume status, and vital organ perfusion should be better integrated in a transfusion algorithm decision process in the future. But, at the same time, the strong variability of preferred values indicates how there is no consensus on what should guide the administration of RBC, and further prospective studies are warranted. As an example, the use of SvO2 was considered a valuable parameter by a large number of participants, though it is possible that this parameter is frequently altered during VV-ECMO due to recirculation, which is more likely in longer support. But considering the parameters to be set before transfusion, the participants stated how it is important to manage the bleeding (it can be argued that interventions to stop bleeding are decided early in ECMO), and another important topic is postponing transfusion if the oxygenation is adequate. This is likely a modern approach to transfusion through a global evaluation of the patient and not only fixed triggers.

The high number of participants underscores the interest in the ECMO community regarding the critical topic of Hb values and transfusion, but, despite being the largest survey, and with the greatest

geographical distribution of worldwide practices in the field to date, this survey has several limitations that have to be acknowledged. First, given its anonymous nature, as a general survey bias the "self-reported data" rely on the memory and honesty of respondents. Second, it was not possible to provide the response rate among the target respondents, since the survey was sent to a very high number of ESICM subscribers and colleagues. Third, the number of respondents per center could not be controlled. Therefore, it cannot be ruled out that high volume ECMO centers may more likely provide more responders, which could have over-expressed high volume ECMO centers' management in final results. Lastly, this survey did not assess clinical outcomes associated with different Hb triggers employed by respondents. The reported data should be considered a baseline to design future prospective studies on blood product management and ECMO outcomes.

Conclusions

This survey shows that, in the absence of clear guidelines, the approach to RBC transfusions during ECMO varies widely among international ECMO centers, and that there is no consensus on the Hb trigger value among ECMO practitioners.

Higher volume centers more frequently adopt a protocolized transfusion practice, and tolerate lower Hb levels. This may reinforce the benefit of centralizing ECMO patients in high volume centers.

The current approach to anticoagulation and transfusion during ECMO needs further prospective investigation to define the effective current triggers adopted, and to better understand the relation between transfusions and outcomes.

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Chapter 5

Limb Ischemia in Peripheral Veno-Arterial Extracorporeal Membrane Oxygenation: a Narrative review of Incidence, Prevention, Monitoring, and Treatment

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Abstract

Veno- arterial extracorporeal membrane oxygenation (V-A ECMO) is an increasingly adopted life-saving mechanical circulatory support for a number of potentially reversible or treatable cardiac diseases. It is also started as a bridge-to-transplantation/ventricular assist device in the case of unrecoverable cardiac or cardio-respiratory illness. In recent years, principally for non-post-cardiotomy shock, peripheral cannulation using the femoral vessels has been the approach of choice because it does not need the chest opening, can be quickly established, can be applied percutaneously, and is less likely to cause bleeding and infections than central cannulation. Peripheral ECMO, however, is characterized by a higher rate of vascular complications. The mechanisms of such adverse events are often multifactorial, including suboptimal arterial perfusion and hemodynamic instability due to the underlying disease, peripheral vascular disease, and placement of cannulas that nearly occlude the vessel. The effect of femoral artery damage and/or significant reduced limb perfusion can be devastating because limb ischemia can lead to compartment syndrome, requiring fasciotomy and, occasionally, even limb amputation, thereby negatively impacting hospital stay, long term functional outcomes, and survival. Data on this topic are highly fragmentary, and there are no clear-cut recommendations. Accordingly, the strategies adopted to cope with this complication vary a great deal, ranging from preventive placement of antegrade distal perfusion cannulas to rescue interventions and vascular surgery after the complication has manifested.

This review aims to provide a comprehensive overview of limb ischemia during femoral cannulation for VA-ECMO in adults, focusing on incidence, tools for early diagnosis, risk factors, and preventive and treating strategies.

Background

Veno-arterial extracorporeal membrane oxygenation (V-A ECMO) is an increasingly adopted temporary strategy of circulatory support in cases of refractory cardiac or cardiopulmonary failure, with a constant widening of indications (1–6). In adults, there are two possible VA-ECMO configurations: central (cV-A ECMO), in which direct cannulation of the right atrium and ascending aorta are obtained, or, more frequently, peripheral (pV-A ECMO), in cases of femoral or axillary vessel cannulation (7). Central cannulation is more frequently performed in cases of post-cardiotomy shock (PCS), and its reliability in supplying better cerebral and upper body perfusion has to be weighed against an increased number of complications, such as bleeding, infections, and need for transfusions (8–10). The emergent nature of the shock, as in cardiac arrest scenarios, and the faster and easier accessibility at the bedside, make the peripheral cannulation, and particularly the femoral vessels, the preferred site for percutaneous or surgical cutdown cannula insertion (9,11). However, arterial femoral cannulation can cause ipsilateral limb ischemia related to reduced blood flow and oxygen delivery to the distal leg below the insertion point of the cannula, with multiple mechanisms (9,12–18).

Recent studies have demonstrated that limb ischemia negatively affects patient mortality and survivor's quality of life (19,20). Therefore, early diagnosis and prevention of leg ischemia appear to be of paramount importance (19,21,22). However, clear evidence-based recommendations are still lacking, and the literature on this peculiar V-A ECMO-based aspect is composed primarily of case reports, case series, retrospective cohort studies, and a low number of prospective studies (4,11,23). Depending on the type of cannulation and local protocols, several strategies have been adopted as a preventive approach or rescue treatment of emergent leg ischemia in pV-A ECMO. Moreover, new solutions and devices have become available specifically addressing this ECMO-related shortcoming.

This narrative review of the literature focuses on the incidence, identified risk factors, pathophysiology, monitoring techniques, prevention strategies, and treatment options for distal limb ischemia during pV-A ECMO in order to provide a comprehensive overview of this complicated issue in the era of increasing ECMO support.

Methods

A literature review was carried through PubMed to identify any study on adults (18 years or older) published from January, 2008 to November, 2018 to evaluate this condition in the most recent ECMO setting. The terms searched for were "(ECMO OR ECLS) AND (((limb OR leg) AND (ischemia OR hypoperfusion)) OR ((peripheral OR arterial) AND cannulation))." Only papers published in English were analyzed.

The flow chart of the literature review and screening is shown in Figure 1. We obtained 184 articles, but only manuscripts including more than 10 patients and reporting cannulation details and leg-related complications for arterial femoral pV-A ECMO were considered for this review. Using a customized form, data were extracted from the 28 remaining articles and stored in an electronic database.

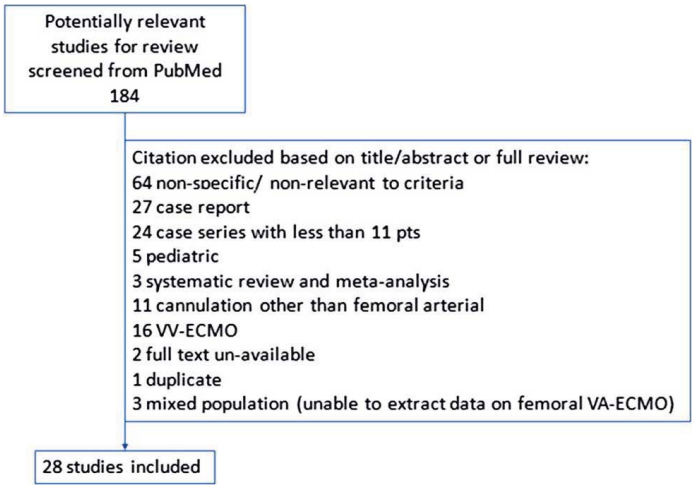


Table 1 summarizes the principal findings of the selected articles. Where applicable, the following data were abstracted: study design, number of patients included, age, main comorbidities, percentage of patients with limb ischemia, duration of ECMO run, hospital mortality, cannulation and decannulation strategy, modality and timing for distal perfusion cannula (DPC) placement, and other strategies to prevent or treat limb ischemia.

After a careful evaluation of the literature by two authors (E.B. and G.M.), double checked by two others (V.L.C. and C.S.), considering the fragmentary data, the different populations mixed in the same studies and the variability of outcomes and interventions, data were considered inadequate to be pooled in a meta-analysis without arriving at potentially erroneous conclusions.

Table 1: Manuscript included for Review

Author, Year	Type of study	Patient population	Study endpoint	Main comorbidity	Mean ECMO duration	H survival	Arterial cannula size	Cannulation technique	Decannulation technique	Limb ischemia	DPC timing	DPC size	Ischemia therapy /limb outcome
Sabashnikov, 2018(24)	R	28 pts (15 under CPR): 3 (11%) ARDS 1 (3%) DCM 17 (61%) ICM 5 (18%) PAE 1 (3%) MIO 1 (3%) PCS	Primary: early and mid-term overall cumulative survival (2 ys follow-up) Secondary: -incidences of ECMO-related complications, -impact of CPR on outcome and changes in hemo- dynamics -tissue perfusion factors 24 h after cannulation	NA	96±100 h	11 (40%)	21-23 Fr	PC 27 (90%) SCD 1 (10%)	NA	3 (10%)	Pre-emptive 19 (68%)	6.5 (6,5-8)	Surgical exploration of the femoral artery and embolectomy using a Fogarty catheter.
Park, 2018 (25)	R	255 pts with HF and/or ARF	Identify risk factors for lower limb ischemia	CAD 83 (32.5%) PVD 5 (2%)	89.8 h	NA (30 days survival 69.8%)	16.5±1.8	PC	NA	24 (9.4%)	Pre-emptive 23 (9%) Rescue 14 (5.5%)	5-7 Fr	2 surgical catheter removal (functional deficit). 14 rescue DPC (Of those, 2 needed surgical intervention and survived with functional deficit.)
Yen, 2018 (14)	R	139 pts: LI group n=46 No LI group n= 93	Identify pre-cannulation variables that are associated with limb ischemia and selection criteria for using DPC for prevention of limb ischemia	No LI group: DM 16 (17%) HT 28 (30%) Uremia 10 (11%) PVD 8 (9%) LI group: DM 10 (22%) HT 17 (37%) Uremia 8 (17%) PVD 11 (24%)	NA	No LI goup: 69 (74%) LI group: 25 (54%)	16.5 ±0.8	PC	NA	46 (33%)	Rescue	6 Fr	NA
Burrell, 2018 (26)	R	144 pts	complications and outcomes of patients who were commenced on ECMO at a referring hospital	S 35 (26%) CAD 35 (26%) DM 16 (12%) HF 69 (53%) CT 18 (13%)	7 (4-11) days	105 (72.9%)	17-19 Fr	PC	NA	1 (0.7%)	Pre-emptive	9 Fr	Resolved after DPC insertion at the referral center

			compared with patients who had ECMO in a referral center for ECMO.										
Voicu, 2018 (27)	R	46 pts with refractory CA	Analyze the feasibility and the time interval required for percutaneous cannulation versus anatomic landmark cannulation for va ECMO.	S 21 (46%) DM 5 (11%) HT 17 (37%) HL 15 (33%)	NA	4 (9%)	15-17-19 Fr	PC	NA	0	Pre-emptive	4 Fr	NA
Salna, 2018 (28)	R	192 pts with CS: 35% AMI 23% PCS 18% ADHF 15% PGD 8.9% other	Incidence of in-hospital lymphocele formation in VA-ECMO patients and identify predictors for its development	DM 65 (33.9%) CKD 52 (27.1% PVD 19 (9.4%)	4 (2-6) days	120 (62.5%)	15-17 Fr	SCD 88 (45.8%)	Surgical	16 (8.3%)	Preventive based on Doppler signal at cannulation	6-10 Fr	NA
Lamb, 2017(29)	R	91 patients: CS 73 (80%); ARF 14 (15%) PE 3 (4%) VAD failure 1 (1%)	Evaluation of an ischemia prevention protocol	HT 53 (58%) DM 26 (29%) HL 34 (37%) OB 30 (33%) CLD 15 (17%) PVD 6 (7%) CKD 27 (30%)	9 days	38 (42%)	16-24Fr on pressure-flow curve and pts size	PC	Surgical	12 (13%) all in patients without preventive DPC	Preventive 55 (60%) Rescue 7 (8%)	5 Fr	DPC 2 (2.2%) DPC+ Fasciotomy 5 (5.5%) Fasciotomy 4 (4.3%)
Pasrija, 2017 (30)	R	20 pts with PE	<u>Primary outcome</u>: in-hospital and 90-day survival. <u>Secondary outcomes:</u> -acute kidney injury that required renal replacement therapy, -new hemodialysis at discharge, - sepsis, -tracheostomy, -RV dysfunction at discharge -ECMO-related complications (bleeding that required blood product, stroke after cannulation and vascular complications)	NA	5.1 (3.7-6.7) days	19 (95%)	17-19 Fr	PC	NA	0	Pre-emptive	6 Fr	1 vascular injury due to retrograde type B dissection after ECMO cannulation. Required central cannulation.

Vallabhajosyula, 2016 (31)	R	105 pts on femoral VA-ECMO: G1=no DPC G2=PC DPC G3=Sugical DPC	assessed if the type of limb perfusion strategy influenced the rate and severity of ipsilateral limb ischemia in peripheral ECLS patients	DM 24 (33%) HT 39 (37%) S 22 (21%)	G1 87.7±119 h G2 88.5±121 h G3 89.2±120 h	G1 21 (60%) G2 14 (61%) G3 32 (68%)	16-20 Fr	NA	NA	G1 7 (20%) G2 6 (26%) G3 1 (2.1%)	Pre-emptive 70 (67%)	7 Fr	4 tromboembolectomy+ artery repair 4 fasciotomy 3 cannulation revision 1 amputation
Yeo, 2016 (32)	R	151 pts: G1=pre-emptive DPC (44pts) G2=rescue DPC (107 pts)	Evalute the efficacy of pre-emptive DPC during ECMO support in term of limb ischemia prevention	DM 25 (16.4%) HT 39 (25.7%) CKD 6 (3.9%) S 27 (17.8%) PVD 11 (7.2%) CVD 5 (3.4%)	G1 4.9±4.9 days G2 6.0±5.4 days	(overall mortality G1 66 (61.7%) G2 17 (38.6%))	G1: 17.2±2.1 Fr G2 17.9±1.8 Fr	PC	NA	10 (6.7%) all in G2	Pre-emptive G1 Rescue G2	5-8 Fr	2 DPC 2 fasciotomy 1 amputation 5 died before therapeutic intervention
Avalli, 2016 (33)	R	100 pts: G1 with vascular complications 35 (35%) G2 without vascular complications 65 (65%)	Primary endpoint was early vascular complication rate. Secondary endpoint was 1-month and 6-months survival	PVD 8 (8%) CAS 4 (4%) HT 59 (59%) DM 19 (19%) S 25 (25%) HL 20 (20% OB 13 (13%)	G1 5 (3-6) days G2 4.5 (2-9) days	G1 15 (43%) G2 13 (20%)	15-17 Fr	PC	Manual compression 30'+ SafeGuard	34 (34%)	Rescue	7-9 Fr	30 DPC 6 fasciotomy 1 amputation
Tanaka, 2016 (19)	R	84 pts on pVA-ECMO. 17/84 with vascular complication (G1) 67/84 without vascular complication (G2)	impact of vascular complications on survival in patients receiving VA ECMO by means of femoral percutaneous cannulation.	S 28 CAD 34 PVD 3 DM24 COPD 10	G1: 14.6 ±6.7 G2: 10.6 ±7.5	G1: 3 (18%) G2 32 (48%)	G1: 19.8±2.3 G2: 19.7±1.7	PC	Surgical	10 (12%)	Pre-emptive exept 7 (41%) G1 10 (15%) G2	NA	Prophylactic fasciotomy
Ma, 2016 (34)	R	70 pts PCS 44 (63%) ECPR 21 (30%) ARF 5 (7%)	To identify predictive factors for vascular complications, and provide insight into how to reduce these complications	NA	NA	NA	15-24 Fr	44 (63%) SCT 25 (36%) PC 1 not recorded	Surgical	14 (20%)	33 Pre-emptive 6 Rescue	6-8.5 Fr	6 DPC rescue 1 embolectomy 1 fasciotomy 1 embolectomy+ femoral artery repair 1 amputation
Esper, 2015 (35)	R	18 pts with ACS complicated by CS	Single center experience	DB 5 (27.8%) HT 9 (50%) HL 2 (11.1%)	3.2 ± 2.5 days	67%	15-17 FR	PC	NA	4 (22%)	Rescue	NA	DPC

				S 3 (16.7%) PVD 3 (16.7%)									
Takayama,2015 (36)	R	101 Group L : (n 51) Group S (n 50)	To compare the clinical outcomes of 2 strategies: conventional approach (using a 15F-24F cannula- Grop L) or smaller cannula of15 Fr (Group S)	Group L CAD 22 (43) Ht 26 (51) HL 15 (29) DM 17 (33) COPD 17 (14) Group S CAD 31 (62) HT 33 (66) HL 23 (46) DM 16 (32) COPD 5 (10)	Group L 3.4 (1.0-6.1) days Group S 3.1 (1.9-5.1) days	Group L 31 (61%) Group S 27 (54%)	Group L 17 to 24Fr Group S 15 Fr	Group L PC 22 (43) SCD 29 (57) Group S PC 44 (88) SCD 6 (12)	NA	Group L 2 (4) Group S 2 (4)	Group L 19% Group S 18% Inserted if distal doppler signal is lost	NA	NA
Truby, 2015(37)	R	179 pts with CS	Trends in device usage, and analysis of clinical outcomes	CAD 82 (45.8%) HL 72 (40.2%) HT 103 (57.5%) CLD 16 (8.8%) DB 52 (29.1%)	3.58 days	69 (38.6%)	15-23 Fr	NA	NA	25 (13.9%)	9 Rescue	NA	2 Fasciotomy
Saeed, 2014 (38)	R	37 pts: 25 p VA ECMO	Compare outcome of cECMO versus pECMO patients in the immediate postoperative period.	DM 3 (12%) HT 13 (52%) HL 8 (32%) CAS 3 (12%) CKD 9 (36%) Re-do surgery 5 (20%)	5.8±4.3 days	(30-day mortality 60%)	18-22 Fr	NA	NA	4 (16%)	Pre-emptive	NA	All required surgical intervention
Aziz, 2014 (39)	R	101 pts	Incidence of peripheral vascular complication	HT 33 (32.7%) DM 22 (21.8%) HL 22 (21.8%) S 20 (19.8%)	7.3 days	59 (58.4%)	15-17 Fr	PC	S	8 (8%)	77 (77%) Pre-emptive	NA	8 Arterial cannula removal; 4 Femoral endoarterectomy with patch angioplasty 1 amputation
Papadopoulos, 2014 (40)	R	Total: 360 PCS. 120 (37%) femoral pVA-ECMO	Identification of risk factors for adverse outcome (failed ECLS weaning or in-hospital mortality)	COPD 32 (9%) HT 227 (63%) PH 31 (17%) DM 151 (42%) CVD 22 (6%) PVD 65 (18%) S 122 (34%) CKD 40 (11%)	7±1 days	108 (30%)	NA	Seldinger or 8-mm Dacron Graft	NA	20 (17% of femoral pVA-ECMO)	NA	NA	Fasciotomy 18 (5% of total pts) NA data on femoral pVA-ECMO pts.
Stub, 2014 (41)	SC-POT	26 pts ECPR (24 cannulated)	Primary outcome: Survival with good neurologic recovery	HT 11 (42%) HL 11 (42%) DM 2 (8%) HF 5 (19%) CAD 4 (15%)	2 (1-5) days	14 (54%)	15 Fr	PC	S	10 (42%)	As soon as possible after ICU admission	8.5 Fr	9 Femoral artery repair and surgical

			Secondary outcomes: rates of ROSC, successful weaning from ECMO support and ICU and hospital length of stay.										placement of DPC 1 fasciotomy
Mohite, 2014 (42)	R	45 pts: 14 ADHF 8 PCS 6 CS 15 Post CT 2 Bridge to LungT	compared pts outcomes focusing on the distal limb perfusion methods (perfusion cannula VS introducer sheat)	NA	Perfusion cannula group: 11.9±9.1 days Introducer sheat group 7.7±4.3	19 (42.2%)	19-21 Fr	20 (44.5%) PC 14 (31%) SCT 11 (24%) Hybrid	NA	9 (20%)	Pre-emptive	Perfusion cannula 10-12 Fr Introducer sheat 6-8 Fr	5 (11.2%) conservative 4 (8.8%) surgery 1 amputation
Spurlock, 2012(43)	R	On 154 patients (data on 36 patients in PTA-DPC)	Posterior tibial artery for DPC placement	NA	5.8 days	63 (41%)	15-24 Fr on surgeon decision	PC	Direct pressure 30 mins	Available only for PTA-DPC group) 3 (8.3%)	DPC in 68 (44%) PTA-DPC in 36 (24%): 20 (58%) within 6h of ECMO; 16 (42%) after 6h of ECMO	6-8 Fr	(available only for PTA-DPC group) 2 amputation 1 neuropathy
Wong, 2012 (44)	R	20 pts: 17 (85%) on VA-ECMO	Report single center experience on cerebral and lower limb NIRS	NA	7 (2-26) days	NA	NA	PC	NA	6 (35%) diagnosed with drop in unilateral lower limb NIRS tracings	Pre-emptive	NA	4 two-compartment prophylactic fasciotomy
Wernly, 2011 (45)	R	51 pts with Hantavirus cardiopulmonary syndrome	Evaluate the outcome of ECMO support in Hantavirus cardiopulmonary syndrome (HCPS) patients	NA	121.7 h	34 (66.6%)	15-21 Fr	PC 18 (35.3%) SCD 33 (64.7%)	SCD	4 (8%)	Pre-emptive	8-10 Fr	2 thrombectomy, embolectomy, and insertion of an additional cannula in the superficial femoral artery. 2 Amputations
Ganslmeier, 2011 (46)	NA	158 pts	Reviews cannulation strategies and	NA	3.6±5.2 days	32 (20%)	13-15-17-19 Fr	PC	Safeguard system	13 (8.2%)	NA	NA	50% Surgical revision and

			associated vascular complications					SCT if femoral vessels were small during sonography					vascular reconstruction 100% prophylactic fasciotomy
Bisdas,2011 (15)	R	143 pts with ECMO VA	To evaluate such complications to outline basic technical principles for their prevention.	HT 77 (44%) CKD 53 (30%) CAD 47 (27%) COPD 25 (14%) DM 29 (17%) PAD 15 (9%)	6 days (range, 1 to 11 days).	26%	15F or 17F	percutaneous cannulation in 136 (95%) and by open vessel exposure in 7 (5%).	manual compression, and femoral compression system	8 pts	Pre-emptive	6F	2 amputation
Foley, 2010 (47)	R	43 pts on femoral pVAECMO	examined the outcomes of patients placed on ECMO, including the rate of limb ischemia	NA	NA	NA	Li group 16.9 ±1.1 No li group 18.0±1.7 Pre-emptive DPC group 17.7±1.8	PC	Surgical	7 (21%)	10 pre-emptive 3 Rescue	NA	4 Decannulation and fasciotomy 3 rescue DPC 1 amputation
Arlt, 2009 (48)	R	13 pts: 10 (77%) CS 3 (27%) Septic shock	Report 9 years emergency ECMO application	NA	3.5±2.9 days	8 (62%)	15-17 Fr	PC	NA	6 (46%)	Not used	NA	Resolved limb ischemia after cannula switch from the femoral artery to the right subclavian artery.

List of abbreviations:
ADHF: acute decompensated heart failure; AF: atrial fibrillation; AMI: acute myocardial infarction ; ARDS: acute respiratory distress syndrome; ARF: acute respiratory failure; CAD: Coronary Artery Disease; CAS: carotid artery stenosis; cECMO: centrally inserted ECMO; CKD: chronic kidney disease; CO: cardiac output; COPD: chronic obstructive pulmonary disease; CPF: cardiopulmonary failure; CRA: cardiorespiratory arrest; CS: cardiogenic shock; CT: cardiac transplantation; CVD: cerebrovascular disease; DCM: dilatated cardiomyopathy ; DM: diabetes; DPC: distal perfusion cannula; ECMO: extracorporeal membrane oxygenation; ECPR: extracorporeal membrane oxygenation assisted cardiopulmonary resuscitation ; ESPF: end stage pulmonary fibrosis; HF: heart failure; HL: hyperlipidemia; HT: arterial hypertension; IABP: intra-aortic balloon pump; ICM: ischemic cardiomyopathy; ICU: Intensive care unit; IQR: interquartile range; LI: limb ischemia; LungT: Lung transplantation; MIO: myocarditis ; MR: multicenter retrospective; NA: not available; NIRS: near infrared spectroscopy; OB: obesity; PC: percutaneous ; PC-DC:

Limb ischemia associated with femoral peripheral pV-A ECMO has a reported incidence ranging from 10% to 70% (49,50). That highly variable incidence is due to studies performed in populations that are different in baseline characteristics, ECMO indications, cannulation techniques, limb ischemia definition, detection tools, and DPC modalities and timing of insertion (51,52).

Yang et al., in their large study of major vascular complications in PCS adults receiving femoral–femoral pV-A ECMO support by surgical cut-down, reported a lower incidence of limb ischemia (8.6%), which may be explained in part by the potential advantages of surgically inserted cannulas, with a preventive DPC placement in the majority of the cohort (49). Nonetheless, in a retrospective series of 84 adult patients on V-A ECMO for cardiac or respiratory failure, Tanaka found a 12% incidence of distal limb ischemia requiring fasciotomy, even in the presence of a prophylactically inserted DPC (19), in line with the findings of Yen et al., who reported that limb ischemia occurred in 33% of patients, even with the use of DPC (14).

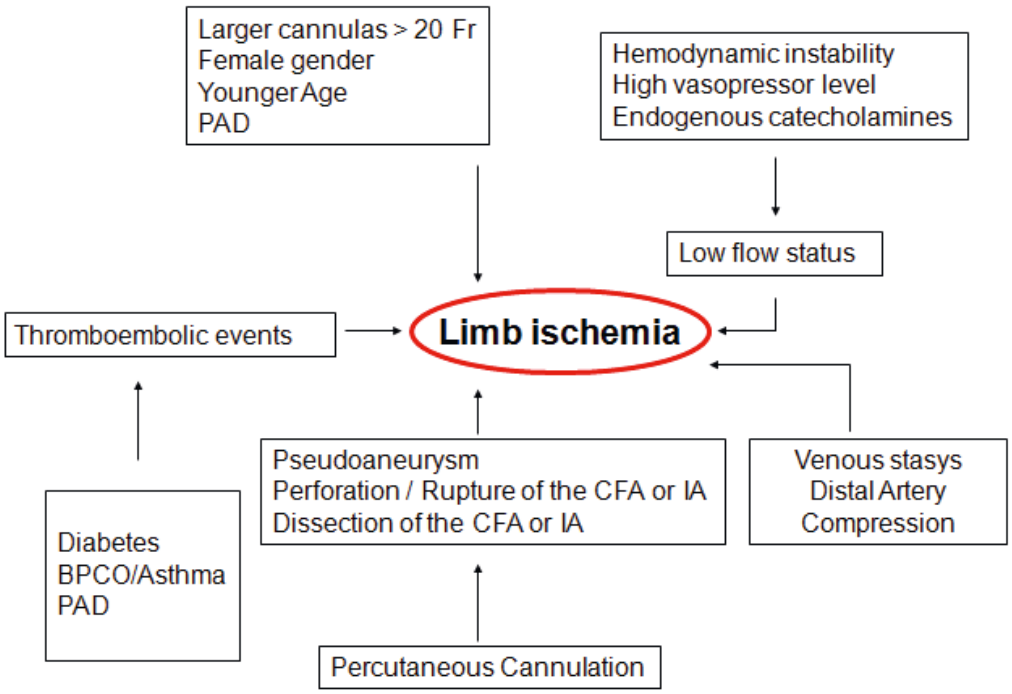
With the aim of differentiating the incidence of complications among groups, only two manuscripts can be considered together for cardiogenic shock: one, on 109 patients, reported 16 episodes of limb ischemia (14.7%), 9 fasciotomies (8.3%), and just one case of distal amputation (0.9%) (29,35). Three studies dealt with limb ischemia in the ECPR setting: pooling data from these studies on 253 patients, 27 episodes of limb ischemia (10.6%) were detected, though it should be highlighted that in the study by Voicu et al. the mortality was high, and the absence of peripheral complications may be likely related to the marked early mortality (25,27,41). Two studies distinctly considered the concomitant use of V-A ECMO and intra-aortic balloon pump (IABP), describing limb complications. Pooling the data, on 55 patients, we found 4 episodes of limb ischemia (7.2%), with an even protective role for the IABP placement in this setting (25,31).

Though the comparison of risk of limb complications among the different short-term ventricular assist devices by means of ECMO, Impella, IABP, Tandem heart, is beyond the purpose of this study, this adverse event might be significant when these devices are used in combination as left ventricular (LV) unloading strategy. Recently, Russo et al. reviewed 17 observational studies including 3,997 patients: among them, 1,696 (42%) patients received a concomitant LV unloading strategy while on V-A ECMO, IABP was combined in 91.7% of cases, the Impella percutaneous ventricular assist device in 5.5%, and pulmonary vein or transseptal left atrial cannulation in 2.8%). In this meta-analysis, limb ischemia (RR: 1.07; 95% CI: 0.90 to 1.27; p=0.47) was not significantly different in patients treated with V-A ECMO associated with another cannulation for left ventricular unloading strategy compared with patients with V-A ECMO support alone (53).

Pathophysiology and risk factors

Limb ischemia in pV-A ECMO patients has a multifactorial genesis that can act at any stage of the ECMO run like at time of cannulation, during support, and at or after decannulation (Figure 2).

Figure 2: Summary of mechanisms determining leg ischemia during peripheral V-A ECMO run.



PAD :peripheral vascular disease, CFA: common femoral artery, IA: iliac artery

The principal mechanism is a reduced blood flow and related oxygen supply, which arises from an absolute or relative deficit of arterial blood flow to distal tissues. It may be due to a nearly occluding arterial cannula, selective perfusion of the *arteria femoralis profunda*, femoral or iliac vessel damage during cannulation, inadequate peripheral perfusion to match tissue demand, high level of vasopressors, an extrinsic compression of the distal arterial vessel by the same arterial or venous cannula, or atherosclerotic arterial disease, especially in the absence of collateral circulation (14,15,19,25,54). Indeed, larger cannulas (> 20 Fr), female gender, younger age, and the presence of peripheral vascular disease are the main risk factors. The use of large cannulas is intuitively associated with limb ischemia due to flow obstruction (54). However, several studies have not demonstrated such an association, perhaps because the cannula diameter per se is not the cause, but, rather, is the relationship between the cannula and arterial diameter. The catheter/vein ratio frequently adopted in venous cannulation is not widely used in arterial cannulation (55). A lower incidence of limb ischemia was found when the relationship between body surface area (BSA) and cannula size is greater than 11 (54). In addition, the

cannula may also exert a so-called "downstream compression" effect, which limits the blood flow below its insertion point (18,56).

Younger patients, who lack collateral circulation, seem to have smaller femoral arteries, which increase in diameter with age (57). For the same reason, women have a higher incidence of ischemic complications (19,29). Pre-existing atherosclerotic disease can increase the risk of plaque dislodgment and embolism during both cannulation and decannulation, as well as increase the technical complexity of the procedure, with higher risk of dissection, or significantly reduce the antegrade flow (15,19,58). Moreover, an increased venous pressure, with consequent reduced perfusion pressure, may contribute to tissue hypoxia (56). Furthermore arterial compression, distally to the cannulation site, may be induced also by an incorrect lateral course of venous cannula.

Considering the comorbidities, diabetes and respiratory diseases are independent risk-factors for limb ischemia during pV-A ECMO. Diabetes is characterized by a proinflammatory state, with macro- and micro-vascular alterations that can exacerbate limb hypoperfusion during a low flow state (12,59). Pulmonary diseases, such as asthma and chronic obstructive pulmonary disease (COPD), are characterized by a state of chronic hypoxia, which induces endothelial damage, inflammatory state, and development of atherosclerotic disease (60). Danial and colleagues found limb ischemia independently associated with the SOFA score at ECMO cannulation, suggesting that the patient's condition (and a proinflammatory state), namely the compensatory capacity for peripheral hypoperfusion, may be more relevant than the single mechanical procedure (61).

Limb ischemia does not account for only local vascular damages. The persistence of prolonged ischemia can lead to an irreversible damage of the leg, with the most severe cases complicated by compartment syndrome, eventually requiring fasciotomy or even limb amputation (62). Furthermore, reperfusion of the ischemic limb by re-establishing or enhancing distal flow may represent an additional threat because of proinflammatory and wasting mediators released into the systemic circulation, causing rhabdomyolysis, systemic inflammatory state, and multi-organ dysfunction (63,64)

Diagnosis

The Intersociety Consensus for the Management of Peripheral Arterial Disease (TASC II) defines acute limb ischemia as a sudden decrease in limb perfusion that causes a potential threat to limb viability (65). The latest AHA/ACC guidelines include a specific section on limb ischemia during hemodynamic support and called "Asymptomatic Artery Disease," the obstructive disease in patients who require large-diameter catheter access for life-saving procedures (66). Diagnostic tools for early diagnosis are summarized in Table 2.

Monitoring distal perfusion in pV-A ECMO is of paramount importance in order to timely detect and treat ischemia, with favorable limb and patient outcomes. As in other acute conditions, "time is tissue," but, nevertheless, there is no standard of care regarding monitoring. Several tools have been adopted, and they can be grouped into clinical examination, the extensive use of ultrasound and Doppler ultrasonography and, recently, the use of near infrared spectroscopy (NIRS) as a surrogate for distal perfusion. As a general rule, during pV-A ECMO, any suspicion of limb ischemia should conduct to an increase in monitoring to reach a complete diagnosis: clinical examination should be followed by Doppler sonography and eventually leading to angiography and complete involvement of a multidisciplinary team.

	Every Hour	Every Shift	Altered Perfusion
Bedside Nurse	Bilateral Clinical Evaluation Temperature Appearance Refilling Time	Doppler Pulse Check	Doppler Pulse Check
ECMO Specialist		Bilateral Clinical Evaluation	Bilateral Clinical Evaluation
		ECMO Flow Check	ECMO flow Check
		Vasopressor Balance	Vasopressor Balance
		DPC Flow Check	DPC Flow Check
NIRS	NIRS	NIRS	NIRS
Radiologist			ECHO Doppler
			Angiography

Table 2. Summary of diagnostic tools for early detection of limb ischemia during V-A ECMO

Clinical Signs and Diagnostic Tools

The clinical pattern of acute limb ischemia was described by Pratt, in 1954, as the 6 Ps signs: paleness, pulselessness, paraesthesia, paralysis, pain, and poikilothermia (67). Clinical evaluation should be routinely performed several times per shift (68). High level of suspicion for ischemia can arise from skin temperature (cold), appearance (pale, mottling), compared with the contralateral limb, and refilling time (42). Guidelines recommend ultrasound (US)-guided vascular access in order to reduce immediate and late complications (55,69). US can be useful in pV-A ECMO at the time of cannulation in order to select the optimal cannulation site, avoiding atherosclerotic arteries, sparing the deep femoral artery origin with its collateral flow to the limb and, finally, providing information regarding vessel size and measurement to guide cannula selection and implantation. First-pass success and reduced groin hematoma rates have been described when US-guided vascular access is compared with landmark techniques (70,71). No studies have investigated the relationship between common femoral artery and cannula diameter in determining leg ischemia (54). During pV-A ECMO support, if Doppler flow is audible, distal limb perfusion pressure can be evaluated by placing a sphygmomanometric cuff at the ankle just proximal to the Doppler probe. A perfusion pressure of less than 50 mmHg indicates limb ischemia (72). Moreover, Doppler ultrasonography (D-US) can be used to monitor peak systolic

velocity (PSV) of distal arteries, such as the posterior tibial or dorsalis pedis. Feasibility of Doppler-derived flow velocity in pV-A ECMO patients as a monitoring tool for leg ischemia has been reported by Breeding et al. (73). However, PSV was positively correlated with pulse pressure and negatively with ECMO pump flow, making its usefulness unclear in fully-supported ECMO patients.

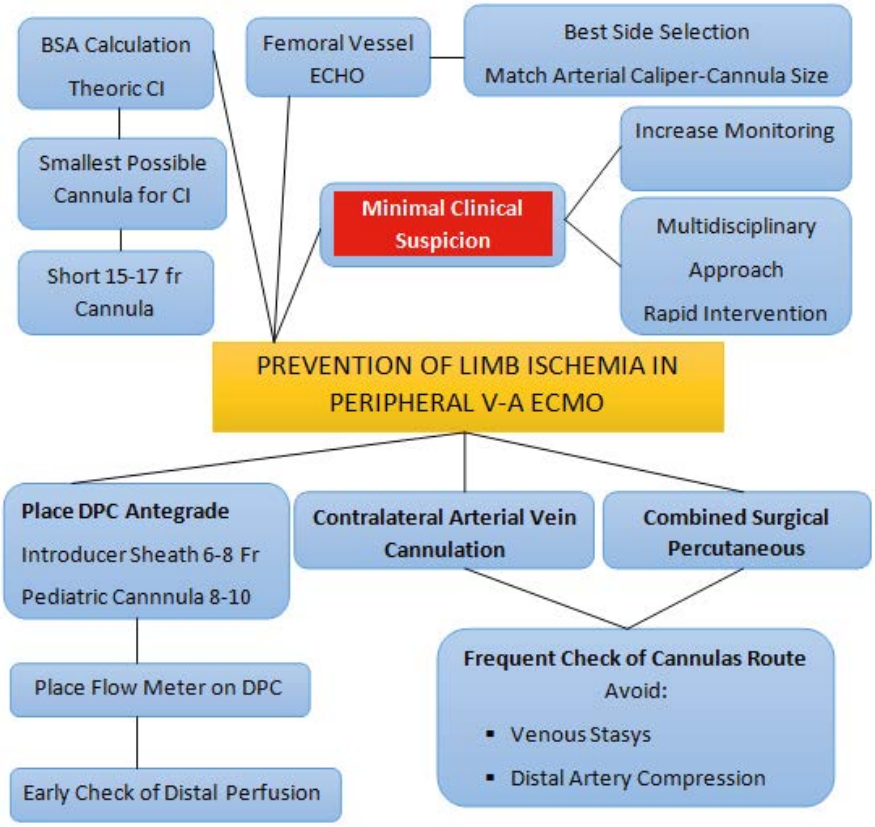
NIRS use is increasing in adult anesthesia and critical care (74). It employs light of near infrared wavelengths (700-1000 nm) emitted and detected by a probe applied to a body region. Differently from a pulse oximeter, NIRS monitors the difference between oxy- and deoxygenated hemoglobin (HBO-HBD), and a pulsatile blood flow is not a prerequisite for its functioning. HBO-HBD reflects oxygen uptake in the tissue bed, and is defined as regional oxygen saturation (rSO_2) (75). Because of the independence of pulsatile blood flow, rSO_2 comprises arterial and venous contribution, the latter being the most important (76).

Wong et al. first described NIRS in ECMO patients to concomitantly monitor both cerebral and limb perfusion (77). They included NIRS monitoring into the treatment protocol, and identified clinically significant events that warranted intervention when rSO2 dropped below 40 or more than 25% from baseline (44,78). More recently, NIRS monitoring in both cannulated and non-cannulated leg in pVA-ECMO patients has been used to differentiate between cannula-related obstruction (delta-rSO_2 between cannulated and non-cannulated leg <15%) and other causes of hypoperfusion (77) . All patients with clinical evidence of limb ischemia had rSO_2 below 50% for longer than 4 minutes, and a positive predictive value of 86% was calculated (77).

Limb ischemia prevention

Many prevention strategies have been proposed to avoid limb ischemia in pV-A ECMO patients: cannula size and cannulation side selection, cannulation technique, and placement of a smaller cannula for anterograde or retrograde (ankle) distal perfusion (79). A summary of proposed preventive strategy is illustrated in Figure 3.

Figure 3: Proposed flow-chart illustrating strategies for limb ischemia prevention.



General Considerations for Arterial Cannula Selection

The selection of type and size of the arterial cannula should be based on a balance between the targeted flow rate and anatomical considerations. Generally, the first consideration starts from the evaluation of the patient's BSA and, conventionally, cannulas are chosen to obtain a flow equivalent to a cardiac index (CI) of 2.2-2.5 L/m^2/min (80).

This accepted rule should be considered as the starting of the decision making process since it is challenged by the fact that the main determinant of the ECMO flow is the capacity of the drainage cannula (determined by the size, the number of side holes and the position – preferred in the right atrium), and that generating a full flow is not always necessary during V-A ECMO. In some cases it is even detrimental when the peripheral inflow determines an excessive increase in the ventricular afterload, with consequent left ventricular distension (9).

In this light, combining the targeted flow, the US-doppler of the femoral arteries, and - in case of surgical cut - also the inspection and palpation, the smallest possible cannula should be preferred. Moreover, the arterial cannulas are also shorter to provide less resistance to the flow. According to the

center's strategy for cannulation and flow support, the arterial cannulas usually range from 15 Fr to 23 Fr. Takayama et al. have documented a protocol of using a small size cannula, 15 Fr diameter, with promising results of comparable clinical support, but a lower rate of complications (36).

Cannulation Technique

Arterial and venous cannulation can be achieved with a surgical cut-down, or a percutaneous approach. In the open technique, a surgical exposure of the femoral vessels can be obtained by a longitudinal or transverse skin incision of the groin and dissection of subcutaneous tissue and fascia. Identification of ligament, common femoral artery, and the bifurcation are important in detecting the proper cannulation site. Inspection and palpation of vessels contribute to an adequate cannula-size selection and avoid dangerous calcifications. A 4/0 or 5/0 polypropylene purse-string is then performed on the vessel. The purse-string should be in the longitudinal direction, and as small as possible, in order to avoid stenosis of the artery after cannula removal and purse-string knotting. The venous and arterial cannulas are placed using a modified Seldinger technique. The venous cannulation should be performed first, followed by the arterial cannulation because of the anatomic relationship and course of the vein compared to the artery. Alternatively, after distal and proximal vascular clamp placement, a transversal incision is made on the artery and the cannula is gently introduced. In these circumstances, longer vessel isolation is advisable, with a vessel loop placement around the vessel to achieve better control during the cannula implantation. Purse-strings are tightened around the vessel entry and secured to the cannula by snuggers long enough to allow sufficient prolene length for final knotting at cannula removal. The plastic snuggers are looped and hidden in the groin pouch. In the so-called "pseudo-percutaneous approach," the femoral vessels are exposed with an open approach, but the cannulas are tunneled through two separated small incisions at 3-4 cm distally from the groin vessel exposure, allowing complete closure of the femoral incision (18). Further justification for such an approach is the reduced risk of bleeding and infections post-ECMO implantation, easier nursing care, and easier device removal, though it still requires an open surgical closure, allowing better control of the vascular entry site and embolectomy in case of distal or proximal clots. Femoral artery perfusion can be also achieved through a Dacron or Hemashield prosthetic graft (6–8 mm) anastomosed end-to-side onto the femoral artery, thus maintaining antegrade as well as retrograde arterial flow to the ipsilateral lower limb (81–84). This approach is aimed at establishing the flow through a small femoral artery, and simplifying the decannulation procedure. However, excessive arterial flow to the limb and reduced flow to the rest of the body can occur. A distal venous draining catheter connected to the ECMO venous cannula may be needed in order to limit limb edema (17,83).

The percutaneous cannulation technique is performed using the Seldinger technique under ultrasound guidance and, whenever available, transesophageal echocardiography can guide the entire procedure,

detecting the location of the guidewire and any new or increasing pericardial collection (85,86). After ultrasound identification, femoral artery is cannulated using a percutaneous kit, avoiding a lateral or backwall puncture rather than to achieve a front-wall puncture. The wire is advanced to the abdominal aorta under fluoroscopic or transesophageal guidance, whenever available, and, after dilation, the cannula is introduced.

Compared with percutaneous cannulation, surgical cannulation is adopted mainly in PCS, and associated with fewer vascular complications (87). A propensity-score-matched study explored the differences in the rate of limb ischemia at the same center between the percutaneous and surgical approaches, and found no significant difference, though the trend was in favor of the percutaneous approach (61). Recently, Deschaka et al. described a hybrid V-A ECMO configuration in which the ascending aorta was cannulated via an 8 mm prosthesis directed subxyphoidally, and the femoral vein was percutaneously cannulated in order to limit limb ischemia due to the femoral artery cannulation, at the same time avoiding the risks of an open thorax (88). Saeed et al. adopted a similar approach in 9 cases of PCS, demonstrating its feasibility (89).

Cannulation Site Selection

The puncture of the femoral artery can be performed ipsilaterally or contralaterally according to the center and the surgeon's preference (18). The most appropriate site for pV-A ECMO cannulation has not been well identified, but bilateral groin cannulation (one cannula in one groin, the other in the contralateral one) might be preferable due to the reduction of vessel compression and the avoidance of the association of reduced perfusion flow and venous congestion (90,91).

Distal Perfusion Cannula (DPC)

The Extracorporeal Life Support Organization (ELSO) guidelines state that "if distal arterial flow to the leg is inadequate a separate perfusion line is placed in the distal superficial femoral artery by direct cutdown, or in the posterior tibial artery for retrograde perfusion." The most adopted preventive strategy is the placement of a DPC in the proximal superficial femoral artery. The insertion of the DPC can be performed percutaneously with ultrasound or fluoroscopy guidance and, in this case the wire for antegrade distal perfusion cannula should be placed at the time of main femoral cannula placement, based on a better exposure and puncture without the proximal cannula in place. In the case of surgical cut-down, it can be performed either by surgical arteriotomy or by direct vision with a modified Seldinger technique, and in a recent meta-analysis the limb ischemia was lower with DPC placement by open access (92).

There is a significant variability of the DPC type and caliper among centers. This catheter is usually connected to the side port of the arterial cannula using a 6-inch extension tubing with an intervening

three-way stopcock for regular check of the flow and eventual line to administer arterial vasodilator. DPCs are reported in sizes from 5 to 14 Fr; the most adopted are central venous catheter and vascular introducer sheaths (usually 6-8 Fr) (93).

The use of pediatric armed arterial cannulas (8 or 10 Fr) are also reported (illustrated in Figure 4), and there are likely some advantages, such as direct connection between the shunt line and the DPC, avoiding a stopcock, and allowing a better configuration in terms of flow patterns and preventing dangerous kinking. This was investigated by Mohite et al. showing lower limb ischemia comparing to the use of the introducer sheath (42). Rao et al. reported a case of DPC insertion from the contralateral femoral artery and angiographically guided to restore perfusion of both the superficial and profound femoral artery of the cannulated leg (94). A relevant trick with this technique is to place a flow meter also on the DPC applied along the DPC circuit to recognize the effective distal flow and counteract if the flow is reduced but also partially reducing the flow by a clamp in case of excessive distal flow.

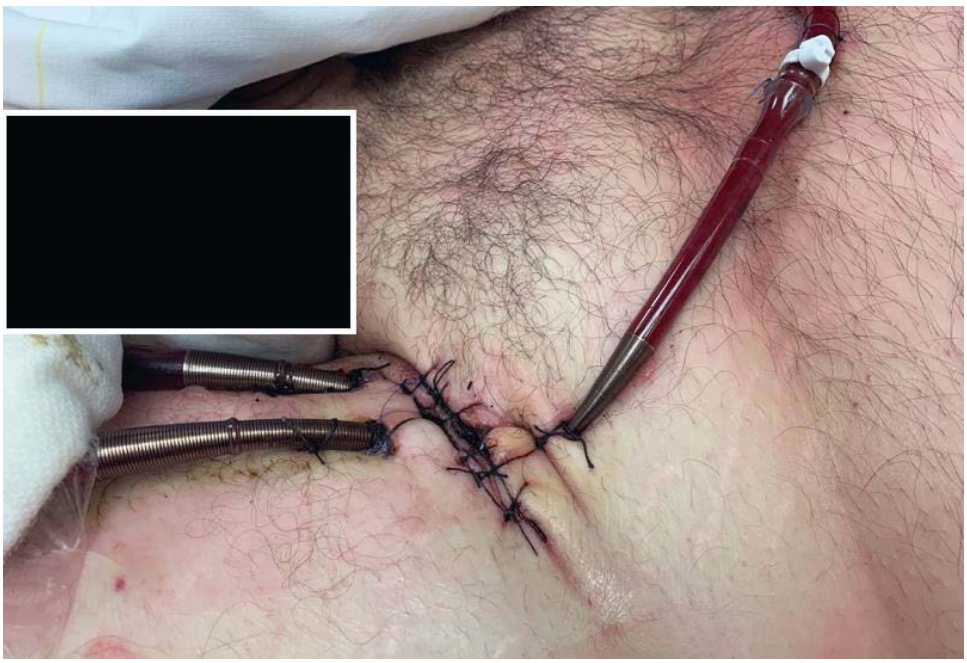


Figure 4: Possible contralateral cannulation during V-A ECMO: bi-groin Cannulation with combined surgical/Percutaneous approach. The distal perfusion cannula is a pediatric 10 Fr cannula connected without a stopcock to the side port of the femoral cannula. (Original photo provided by R.L.)

Bidirectional Cannula

Recently, to overcome the distal limb ischemia, a new bidirectional femoral arterial cannula (LivaNova PLC, Arvada, CO, USA) has been proposed and tested during cardiopulmonary bypass (CPB) in 15 patients (95). This cannula, similar to a standard femoral arterial cannula, has a 120-degree angled elbow with a side hole for antegrade perfusion to alleviate the compression of the femoral artery below the

insertion point. The external diameter of the distal section of the 19 Fr bidirectional cannula is 7 mm, and the external diameter, obliquely at the cannula elbow, is 8.4 mm. This cannula showed appropriate bypass flows in the extracorporeal circuit, satisfactory line pressures, mean arterial pressures adequate to provide organ perfusion, and allowed an adequate distal flow in 14 of 15 patients checked with NIRS, with no ischemic complications (95). A study using a percutaneous insertion technique of the femoral bidirectional cannula in patients requiring V-A ECMO is currently in progress, and the promising results in the short-term support of CPB, if confirmed in longer support for V-A ECMO, may offer the community a relevant technological improvement for clinical use in a number of different perfusion settings.

ECMO weaning and decannulation strategy

Danial et al. found higher rates of vascular complications after decannulation in a percutaneous group compared with a surgical cut-down group (14.7% vs. 3.4%, $p<0.01$) (61). This result is rarely highlighted, and not confirmed by the available literature. Surgical closure at decannulation may enhance safer decannulation, with reduced bleeding, pseudoaneurysm formation, compression time likely associated with local thrombosis, check for distal flow, and allow repair in case of vessel damage or structural impairment (61). It is indeed advisable to perform an immediate control after cannula withdrawal of the distal artery pulsatility and of the presence of flow at distal leg portion since embolization, when it does occur, is usually observed just after decannulation.

Weaning trial of V-A ECMO will also decrease flow through the distal perfusion cannula such that ischemia may result from a prolonged duration of low ECMO flow despite the presence of a distal perfusion catheter; consequently, in patients with critical limb perfusion the length of weaning trial should be reduced (58).

A new method of percutaneous arterial closure proposed recently is the use of specific closure devices, usually imported into the V-A ECMO practice from the interventional cardiology environment. These devices have been used for closure in case either of percutaneous or surgical approach, and seems able to reduce bleeding and surgical site infections, but are challenged by the need of expert users who are not always involved in V-A ECMO management. Their use is still restricted to some centers, and documented in short reports. Majunke et al. proposed the combined use of the Perclose ProGlide system (Abbott Vascular) and the AngioSeal device (St. Jude Medical), while Montero-Cabezas et al. reported the use of the MANTA vascular closure device (Essential Medical Inc, Malvern, Pennsylvania)(96,97). Further prospective-focused studies should explore this field in order to understand the feasibility of such an approach.

Treatment

The key to deciding the treatment of limb ischemia during V-A ECMO is to distinguish a threatened from a nonviable extremity, bearing in mind that the determination of whether ischemia is reversible is rather subjective (largely based on appearance of soft tissue and amount of necrotic tissue). Often, it can be determined only after conservative management has failed, but the longer the symptoms are present, the less likely the possibility of limb salvage.
According to the Society of Vascular Surgery standard, the loss of the Doppler arterial signal indicates that the limb is threatened (stage II). The absence of both arterial and venous Doppler signal indicates that the limb may be irreversibly damaged and non-salvageable (stage III) (72).
Limb ischemia in femoral cannulation ECMO is largely transient and completely reversible with the removal of the cannula or the insertion of DPC. In a small percentage of patients, it is irreversible, with refractory muscle damage eventually leading to leg amputation (up to 14% of cases) or even contributing to patient death. When the ischemia is considered irreversible the potential amputation should not be delayed since tissues necrosis may extend with higher risk of sepsis, bleeding, intractable acidosis and systemic release of toxic mediators.

Acute compartment syndrome (ACS) is a severe clinical condition caused by increased tissue pressure, inducing a reduction of the perfusion, with consequent further ischemia. It can lead to severe functional impairment due to muscular necrosis and neurological damage, or to ischemic muscle shrinking, with consequent limb deformity.

When limb ischemia is ongoing, a thorough evaluation should be constantly performed to balance between the need for adequate systemic flow, vasopressors use, and the risk associated with further surgical procedures that are in any case at risk of bleeding and further vascular damage in VA-ECMO patients (98). Consequently, first, the amount of vasopressor should be considered and eventually reduced or discontinued, also optimizing volemia and oxygen transport by hemoglobin. In case of mild reduced perfusion, optimizing peripheral temperature is a general adopted care, while the administration of peripheral vasodilator through the DPC may help in diagnosing the reversibility of reduced perfusion due to excessive vasoconstriction. Moreover, during limb ischemia, in the absence of bleeding, anticoagulation should be kept at the highest level according to the center's range.

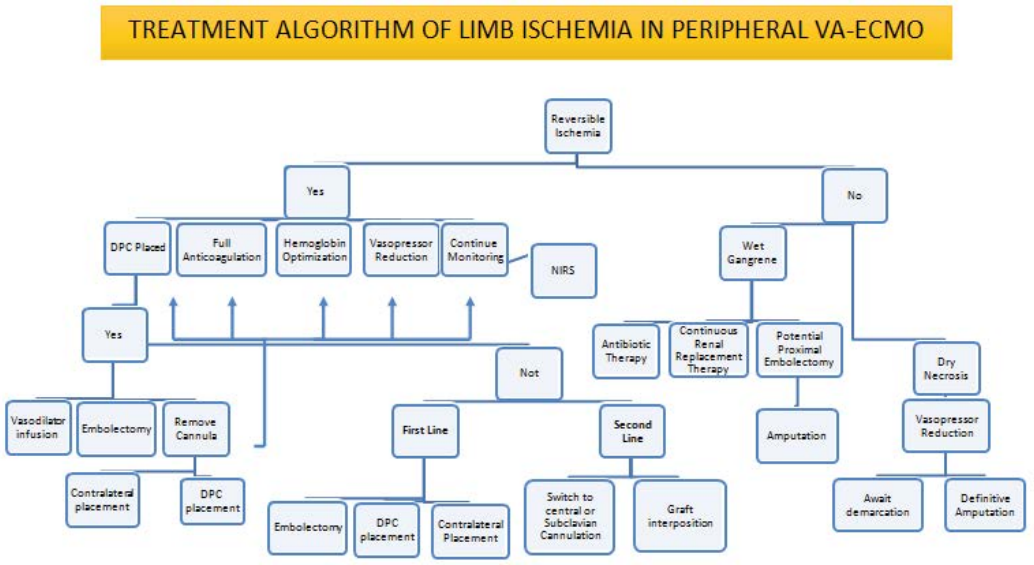
The invasive therapies include removal and repositioning of the cannula (contralateral limb, subclavian, or aortic cannulation) and repair of the artery with suture and/or bovine pericardial patch angioplasty, Fogarty catheter-based embolectomy, limb fasciotomy or amputation (19,29,47).

Yau et al. found that in their cohort of 34 patients with limb ischemia after V-A ECMO, 3 required lower extremity amputation, and 7 needed fasciotomy for a compartment syndrome (12,15,19). Moreover, Tanaka reported an independent association between major vascular complications and mortality in 84 patients on V-A ECMO, with 20% experiencing major ischemic injury, and 12% requiring fasciotomies (19).

Endovascular methods, including balloon angioplasty or stenting, can be additional options. In these circumstances, open reconstruction of the femoral vessels with endarterectomy and patch angioplasty or femoral-femoral bypass grafting can help to improve the arterial flow.

A proposed flow chart for limb ischemia treatment considering general clinical and surgical approach is shown in Figure 5.

Figure 5: Proposed flow-chart for the treatment of limb ischemia in V-A ECMO.



Conclusion

V-A ECMO is a life-saving procedure that provides mechanical circulatory support for advanced heart failure. Advances in technology, portability, and easy-to-use devices have led to its use worldwide, even outside the cardiac surgery setting, with a progressive improvement in survival.

In cases of peripheral cannulation, limb ischemia is still frequent, particularly if preventive strategies are not adopted, and the consequences of this complication can impact negatively on the survival or the long-term functional outcomes.

A strict monitoring protocol for early detection, and timely interventional strategy to guarantee an adequate peripheral flow restauration are mandatory to reduce the incidence and improve the prognosis and outcome of the V-A ECMO patient.

V-A ECMO is a complex, resource-intense, and high-risk type of mechanical support. Future research should focus on complications, providing more clues as to the effectiveness of different preventive and therapeutic strategies to guide a further increase in survival.

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Chapter 6

The Role of Endoscopy as Non-Invasive Procedure to Manage Gastrointestinal Complications during Extracorporeal Membrane Oxygenation

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Abstract

Introduction

Gastrointestinal (GI) bleeding is a life-threatening complication in patients undergoing extracorporeal membrane oxygenation (ECMO) support. Despite data on increased mortality due to GI bleeding, there is little data on the treatment of such conditions under ECMO, and on the possibilities of advanced endoscopic therapy (ET) to non-invasively solve these bleeding complications. No clear treatment in the case of ECMO support is recommended in the guidelines.

Methods

Retrospective observational cohort study including 134 veno-venous (V-V) ECMO patients for acute respiratory failure from 2009 to 2018 at IRCCS-ISMETT (Italy). Patients were divided into two groups according to GI bleeding episodes, and reviewed for type of ET. GI bleeding group was characterized for pre-ECMO characteristics, management variables - including amount of transfusions, and clinical outcomes.

Results

Fourteen (14) patients (10.4%) experienced upper (n=13) or lower (n=1) GI bleeding. GI bleeding and no-GI bleeding group had similar characteristics apart from higher creatinine in the GI bleeding group (1.9 mg/dL (1.3-4.9) vs. 1.2 mg/dL (0.7-1.8), p=0.03). In 3\14 patients (21%) endoscopy showed no signs of active bleeding (nasogastric or feeding tube decubitus), and no specific intervention was performed. Active bleeding was recognized in 11\14 patients (79 %). No patients died of fatal bleeding in the GI bleeding group. ET was feasible, with a complete bleeding control in all the cases: 5 Hemospray, 2 fibrin glue, 2 metallic clips, 1 combined approach metallic clips with epinephrine, and 1 cyanoacrylate. The ECMO course was significantly longer in the GI bleeding group: 19.5 days (15-36) vs. 13.5 days (8-25), p= 0.01. No significant differences in mortality were found between the two groups (all p values > 0.05).

Conclusions

Advanced ET during V-V ECMO may contribute to reducing the negative effects on mortality for GI bleeding episodes.

Introduction

Bleeding is one of the most significant complications of extracorporeal membrane oxygenation (ECMO) and, even today, with more biocompatible and easier-to-use devices, hemorrhage is still one of the main causes of mortality during ECMO, above all for intracranial hemorrhage [1-5]. Causes of bleeding in ECMO are multifactorial, and include heparin administration, coagulopathy, thrombocytopenia, platelet dysfunction, acquired von Willebrand syndrome, and hyperfibrinolysis [6,7].

The main sites of bleeding during ECMO according to the Extracorporeal Life Support Organization (ELSO) Registry are cannulation site, chest, nose, central nervous system, and gastrointestinal (GI) system [8,9]. GI bleeding has been reported in 4.2% of patients with cardiac support, and in 5.2% with respiratory support. There is little data on GI bleeding during ECMO, but these patients, in addition to the typical causes for bleeding related to the ECMO application (such as large bore cannulation, coagulopathy, or administration of heparin), have a specific risk for stress ulcer due to the high severity and inflammatory state and, in the case of veno-arterial (V-A) ECMO, likely as a result of non-pulsatile blood flow, and reduced gastric perfusion and PH [10]. Moreover, ECMO-related GI bleeding is associated with high mortality as seen in data from the ELSO Registry, showing a percentage of survival after GI bleeding of 44% in adult respiratory ECMO support, and 23% in adult cardiac ECMO support [8].

In the veno-venous (V-V) ECMO setting, the controversial question is the balance between the benefits of an advanced and well-established support, and the potential (reduced but still present) complications [11-16]. Among these complications, GI bleeding is a concrete challenge for ECMO teams, and it is likely that is a this is a neglected topic [17].

Therapeutic management of GI bleeding can be a challenge even for an experienced endoscopist, though several effective hemostatic techniques have been developed in recent years. In particular, management of profound venous or arterial bleeding and malignant lesions with large surface area are not frequently amenable to traditional endoscopic hemostatic techniques, especially in patients with impaired coagulation. Endoscopy has a high clinical success rate in GI bleeding control with a mini-invasive approach, especially compared with interventional radiology or surgery [18].

Data on GI bleeding under ECMO are highly fragmentary and mostly reported as case reports and very limited case series. Moreover, the treatment of such a significant complication is not codified by guidelines or expert opinions, and varies from a wait-and-see approach, invasive surgical methods with no or limited experiences to less invasive techniques, particularly appropriate for ECMO patients [19,20].

This study is focused on GI bleeding in V-V ECMO patients in order to provide a homogeneous cohort according to clinical conditions, blood flow, and circulatory state. We present a series of 14 cases of GI bleeding and its related approach through advanced endoscopy management, focusing on the different

characteristics of the patients with GI bleeding, and comparing the survival rate to the no-GI bleeding cohort.

Methods

This study was approved by the Ethics Committee of IRCCS-ISMETT (88/ISMETT/16). All patients at the ISMETT ECMO center undergoing endoscopy for evidence or suspected GI bleeding while supported on V-V ECMO from 2009 to 2018 were retrospectively reviewed. The population of this study was restricted to V-V ECMO configuration in order to consider a homogeneous population, thereby avoiding biases caused by higher anticoagulation strategies, different indications for support, and lower splanchnic organ perfusion usually observed in V-A ECMO. All the patients received daily stress ulcer prophylaxis with omeprazole 20 mg.

Definitions of GI bleeding and endoscopic treatment

Upper or lower GI bleeding was defined as hematemesis or coffee-ground emesis, hematochezia, or melena with significant decrease of hemoglobin (Hb) levels compromising the hemodynamic status of the patient. Initial medical therapy included administration of high-dose omeprazole (80 mg) intravenous bolus followed by continuous infusion (8 mg/h) of proton pump inhibitors, antibiotics, and intravascular volume replacement with hemodynamic support according to the ESGE guidelines [18]. A prompt endoscopy in the GI bleeding group was performed within 12 hours in a properly equipped setting by qualified teams and operators. Anticoagulation protocol, as previously reported, is based on low heparin dose and antithrombin supplementation, with an activated partial thromboplastin time (aPTT) target of 40–50, stopped in any case of bleeding [21,22].

All endoscopies were performed directly at the bed of the patients in the ICU unit. Pre-endoscopic risk stratification, such as the Glasgow-Blatchford Bleeding Score [18], was not applicable due to the ICU characteristics, and all the patients were considered at high-risk. All patients were closely monitored and placed in a supine position, allowing greater mobility of the operator to secure airways and manage the ECMO cannulas. All endoscopy procedures were carried out with a therapeutic endoscope (Olympus, Tokyo, Japan) with a 3.7-mm operative channel, allowing complete aspiration of blood and clots in order to locate the target bleeding site. A water pump was always available to irrigate the lumen, also for water-assisted colonoscopy. The bleeding activity was classified using the Forrest classification, which includes [18,23]: Forrest Ia (spurting bleeding) and Ib (oozing bleeding) represent active hemorrhage stigmata; Forrest IIa (non-bleeding visible vessel), IIb (adherent clot), and IIc (flat pigmented spots) are signs of recent hemorrhage; and Forrest III (clean-based ulcer) represents lesions without active bleeding. Endoscopic evaluation was also focused on detecting possible multiple bleeding sites.

Our endoscopic approach was based on injection therapy using diluted adrenaline (1:10.000), mechanical therapy using endoclips, apposition of fibrin glue or tissue adhesive, such as cyanoacrylate that forms as a hard plug at the bleeding point when the injection is complete [24]. Following the guidelines, the combination therapy, including epinephrine injection with a second hemostasis modality, was preferred. After the first introduction for endoscopic purposes in 2011 [18,25] the Hemospray (Cook Medical, Winston-Salem, North Carolina, USA) was preferred for a complete hemostasis because of its technical characteristics. Hemospray is a *non-contact* hemostatic agent that has an effective and safe profile for endoscopic hemostasis, with easy application and diffuse apposition, especially in the presence of multiple sites of active bleeding. The non-contact nature of Hemospray makes it desirable in situations involving larger areas of mucosa that would not otherwise be amenable to standard, targeted therapies, particularly on antithrombotic therapy [26].

Bleeding recurrence was defined as a decrease in Hb level of 2 g/dL or more after achieving a stable Hb level, new onset of fresh hematemesis, fresh hematochezia, or melena. However, no routine second endoscopic look was performed. In cases of new onset of GI bleeding, another prompt endoscopic therapy (ET) was provided, with particular preference for Hemospray. Transfusion of packed red blood cells (PRBCs) was aimed at keeping the hemoglobin value between 7 g/dL and 10 g/dL, but every decision regarding transfusion was taken considering several components, including blood flow, circuit pressure, hemodynamic state, peripheral perfusion and lactates.

The primary end point of the study was survival in V-V ECMO. The secondary end point was length of ECMO stay, estimate of the ratio of transfused PRBCs and compared with the non-bleeding population, and evaluation of GI bleeding-related mortality, such as acute hemorrhagic shock or progressive general decompensation within 28 days of the last ET.

Statistical analysis

Categorical variables are given as frequencies and percentages, continuous variables as mean and standard deviation or as median and interquartile range (25th–75th percentile IQR), when appropriate, according to data distribution. The comparison between the group without GI bleeding and the GI bleeding group was made with the two-sample t-test for continuous variables or Wilcoxon rank-sum test, and by Fisher's exact test or chi-square test for categorical variables, when appropriate. All tests were two-sided, and a p value of <0.05 was considered statistically significant. Data handling and analyses were done with SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Statistical power for survival findings between the proportions of the two groups was calculated using the Likelihood Ratio Test (PASS 16 Ample Size – NCSS Statistical Software).

Results

During the 10-year study period, 134 patients were supported with V-V ECMO for acute respiratory failure at our institute. No patients were excluded since the aim of the study was to describe the approach in case of GI bleeding. Among them, 14 patients (10.4%) experienced upper (n=13) or lower (n=1) GI bleeding. Comparative pre-ECMO patient characteristics are reported in Table 1. The two groups were homogeneous for severity and pre-ECMO course, with significantly higher values only for creatinine in the group who experienced GI bleeding [1.9 mg/dL (1.3-4.9) vs. 1.2 mg/dL (0.7-1.8), p value = 0.03]. This difference remained statistically significant, also categorizing patients for creatinine > 1.8 mg/dL (as 2 per standard deviation) p value = 0.039.

Variable	Overall N = 134	No GI Bleeding N = 120	GI Bleeding N = 14	P value
Age, years	43.1 ± 12.5	42.8 ± 12.6	45.4 ± 11.1	0.46
Male gender N(%)	101 (75)	88 (73)	13 (93)	0.11
BMI, Kg/m^2	28.7 ± 6.3	28.5 ± 6.1	30.4 ± 7.6	0.29
Saps II score	40.6 ± 11.4	40.4 ± 11.5	41.6 ± 10.9	0.71
SOFA score	8.5 ± 3.1	8.4 ± 3	9.8 ± 3.7	0.08
Hospital LOS, days	7 (3-13)	7 (3-13)	7 (3-21)	0.57
ICU LOS, days	5 (2-9)	5 (2-9)	4 (3-9)	0.50
Mech. Vent, days	4 (2-8)	4 (2-8)	4 (2-9)	0.43
PaO_2/FiO_2 ratio	59 ± 13.7	58.9 ± 13.9	59.8 ± 11.1	0.82
Murray score	3.5 ± 0.2	3.5 ± 0.2	3.5 ± 0.3	0.40
Creatinine, mg/dl	1.3 (0.8-1.9)	1.2 (0.7 – 1.8)	1.9 (1.3 – 4.9)	0.02
Hematocrit, %	32.8 ± 6	33 ± 5.9	31.1 ± 6.6	0.27
Bilirubin, mg/dl	0.9 (0.6-1.3)	0.9 (0.6 – 1.3)	0.8 (0.5 – 2.1)	0.76
Charlson Comorbidity Index	1 (0 – 2)	1 (0 – 2)	2 (1 – 3)	0.07
Preserve score	3.9 ± 1.9	3.8 ± 1.8	4.1 ± 2.8	0.72
RESP Score	0.9 ± 3.2	0.9 ± 3.2	0.9 ± 3	0.98
Prone position before ECMO, N (%)	20 (15%)	20 (17)	0 (0)	0.10
Nitric oxide before ECMO, N (%)	18 (14)	16 (14)	2 (15)	0.87

Table 1. Pre-ECMO population characteristics

Variables are presented as number and percentage, mean value ± standard deviation, median value and interquartile range according to the type of variable and distribution. GI: gastrointestinal; BMI: body mass index; SOFA: Sequential Organ Failure Assessment; LOS: length of stay; ICU: intensive care unit.

Characteristics, type of endoscopic evaluation and treatment, as well as outcomes of patients with GI bleeding are presented in Table 2. The endoscopy evaluation was performed in all patients with suspected GI bleeding as soon as possible after diagnosis of bleeding (very early timing <12 hours). In 21% (3\14) of the patients, endoscopy showed no signs of active bleeding, only nasogastric or feeding tube decubitus, and no specific interventions were performed. Active bleeding due to a peptic ulcer disease and stress-induced hemorrhagic gastritis was recognized in 79 % (11\14) of the patients. In detail, gastric IIa ulcer

(n=4), cecum IIa ulcer (n=1), gastric Ib ulcer (n=2), duodenal IIa ulcer (n=1), multiple gastric Ib ulcers (n=1), diffuse hemorrhagic gastritis with oozing bleeding (n=2) were found. No patient died of fatal bleeding in the GI bleeding group, while 3 out 120 of the no-GI bleeding patients died of fatal bleeding for other causes (intracranial hemorrhage).

The ET was feasible, with a complete and safe bleeding control in all the cases: 5 patients required Hemospray, 2 cases needed fibrin glue, 2 cases were treated with metallic clips, 1 case was treated with combined approach of metallic clips with epinephrine, and cyanoacrylate was adopted in 1 case. Clinical success after the first endoscopic treatment was achieved in 4/11 patients, and recurrent bleeding was observed in 7\11. A new ET session was effective in all the remaining patients, without need for radiological intervention (such as transcatheter angiographic embolization) or surgery. In particular, the combination of clips and endoloop was adopted only in one case because the mineral powder was not then available on the market for clinical application. The remaining 6 cases were responsive to the Hemospray, likely due to its mini-invasiveness and higher efficacy for multiple sites or major bleeding area. Definitive clinical success was 100% (mean number of endoscopy 1.6±0.85), with no episodes of hemorrhagic shock or GI bleeding-related mortality.

Looking at the data concerning ECMO management (Table 3), as expected, significant differences were found in transfusion requirements in the two groups. In the GI bleeding group, the amount of PRBC transfused was higher (p value = 0.04), but this association did not reach statistical significance when the amount of PRBC was adjusted for the number of ECMO days, considering the transfusions as amount in ml of PRBC transfused per day: 160.5 ml (138 – 240) in the GI bleeding group vs. 147 ml (63 – 214); p value = 0.45. No differences were found between the two groups in the administration of other blood products (fresh frozen plasma and platelets), or the number of hours without heparin.

Table 2. Demographics, endoscopy (type and treatment), outcomes. BMI: body mass index; PRBC: packed red blood cells; ET: endoscopy treatment; **: clinical era before Hemospray availability on the market.

Patient	Gender	Age	BMI	Preserve score	RESP score	Indication for ECMO	Comorbidities	ECMO days	Total PRBC	Endoscopy	Endoscopy treatment	Recurrent bleeding	Timing successive ET	Successive ET	Total number endoscopy	Outcome (days after last ET)
1	F	38	30.9	3	4	H1N1	Pregnancy	31	5,100	20 mm gastric IIa[a] ulcer	Clips + adrenaline[b]	Yes	14 days	Clips and Endoloop[®b]	2	Discharge (48 days)
2	M	51	25.5	7	−1	H1N1	\	12	2,884	40 mm cecum IIa[a] ulcer	Fibrin glue	Yes	1 day	2 Hemospray sessions	3	Discharge (30 days)
3	M	63	29.4	10	−3	Pneumonia	Coronary artery disease	27	2,715	Multiple gastric Ib[a] ulcers	Hemospray	Yes	19 days	1 Hemospray sessions	2	Death (28 days)
4	M	41	26	4	4	H1N1	Hereditary acondroplasia	90	24,717	Diffuse hemorrhagic gastritis with oozing bleeding	Hemospray	Yes	2 days	2 Hemospray sessions	3	Discharge (115 days)
5	M	52	36	3	1	H1N1	Chronic obstructive pulmonary disease	18	2,500	30 mm gastric Ib[a] ulcer	Hemospray	Yes	6 days	2 Hemospray sessions	3	Discharge (20 days)
6	M	16	21.8	4	−3	Poly-trauma	Bilateral pneumothorax	102	17,192	30 mm gastric IIa[a] ulcer	Clips	No	\	\	1	Death (90 days)
7	M	43	26.4	−2	1	H1N1	\	20	3,904	25 mm duodenal IIa[a] ulcer	Clips	Yes	4 days	1 Hemospray session	2	Discharge (35 days)
8	M	54	25	6	0	H1N1	Pneumonia	36	8,022	20 mm gastric Ib[a] ulcer	Hemospray	No	\	\	1	Death (30 days)
9	M	48	25.6	7	−4	Aspiration pneumonia	Tuberculosis	11	3,070	Diffuse hemorrhagic gastritis with oozing bleeding	Hemospray	Yes	4 days	1 Hemospray session	2	Discharge (30 days)
10	M	45	43	3	3	H1N1	Pneumonia	21	0	Nasogastric tube decubitus	No active bleeding	No	\	\	1	Discharge (28 days)
11	M	48	41.2	3	4	H1N1	Diabetes and hypertension	17	1,642	Feeding tube decubitus	No active bleeding	No	\	\	1	Discharge (23 days)
12	M	37	44.3	1	7	H1N1	\	14	0	No sign of active bleeding	No active bleeding	No	\	\	1	Discharge (21 days)
13	M	44	27.4	5	3	H1N1	Polytrauma	54	8,200	25 mm gastric IIa[a] ulcer	Cyanoacrylate[b]	No	\	\	1	Death (30 days)
14	M	56	23	4	−1	H1N1	Coronary artery disease, hypertension, and atrial fibrillation	15	5,070	20 mm gastric IIa[a] ulcer	Fibrin glue[b]	No	\	\	1	Death (7 days)

Variable	Overall N = 134	No GI Bleeding N = 120	GI bleeding N = 14	P value
Femoro-jugular cannulation, N (%)	120 (89)	108 (90)	12 (86)	0.62
Bioline circuit, N (%)	79 (59)	71 (60)	8 (57)	0.85
Cardiohelp Device, N (%)	87 (65)	77 (64)	10 (71)	0.83
Drainage cannula, Fr	24 (22-25)	24 (23 – 25)	24 (22 – 25)	0.92
Return cannula, Fr	19 (18-19)	19 (18 – 19)	19 (18 – 21)	0.20
Number of circuit changed	1 (1-1)	1 (1 – 1)	1 (1 – 2)	0.15
Blood flow first day, L/min	3.9 ± 0.8	3.8 ± 0.8	3.9 ± 0.8	0.75
Sweep gas flow first day, L/min	3.4 ± 1.5	3.3 ± 1.5	3.5 ± 1.4	0.63
Tidal volume first day, ml	216.3 ± 123.1	213.8 ± 123.2	236.2 ± 125	0.54
Peak pressure first day, cmH2O	22.7 ± 2.7	22.8 ± 2.6	21.8 ± 3.2	0.37
PEEP first day, cmH2O	11.9 ± 2.7	11.8 ± 2.8	12.3 ± 1.6	0.35
Mean hematocrit in ECMO, %	29.7 ± 3.5	29.8 ± 3.5	28.9 ± 3.2	0.40
Platelet nadir in ECMO,	68 (40-124)	68 (42 – 125)	51 (30 – 102)	0.40
APTT, sec	45.2 ± 5.9	45.1 ± 5.9	45.4 ± 5.2	0.88
Antithrombin, %	90.8 (78.8-102.4)	89.7 (78.8 – 102.4)	95.5 (92.7 – 101.8)	0.45
Heparin dose,U/kg/h	11.4 ± 5.1	11.59 ± 5.18	9.45 ± 4.03	0.15
Heparin, hours	273 (141-485)	241.5 (139 – 448)	501 (335 – 706)	< 0.01
No heparin, hours	27 (8-110)	25.5 (9 – 73)	38 (0 – 212)	0.67
Total PRBC, ml	1926 (780-4643)	1795 (750 – 4530)	3487 (2500 – 8022)	0.04
Total PRBC, unit	8 (3-19)	7 (3 – 18)	13.5 (10 – 32)	0.04
Fresh frozen plasma*[1], N (%)	18 (13)	15 (12)	3 (21)	0.36
Platelet transfusion*[2], N (%)	45 (34)	39 (33)	6 (43)	0.45
Acute kidney injury, N (%)	95 (71)	83 (73)	12 (86)	0.21
CRRT use N (%)	78 (58)	66 (55)	12 (85)	0.02
Septic shock, N (%)	81 (66)	73 (66)	8 (72)	0.64
Fatal bleeding N (%)	3 (2)	3 (2)	0 (0)	0.55
Major bleeding N (%)	21 (16)	14 (12)	7 (50)	< 0.01

Table 3. Variables during ECMO management

Variables are presented as number and percentage, mean value ± standard deviation, median value and interquartile range according to the type of variable and distribution.

APTT: activated partial thromboplastin time; PRBC: packed red blood cells; CRRT: continuous renal replacement therapy.

*[1]: Number of patients who received at least one unit of fresh frozen plasma

*[2]: Number of patients who received at least one unit of platelets.

Clinical outcomes are shown in Table 4. Despite the absence of mortality due to GI bleeding, the ECMO course was significantly longer in the GI bleeding group:19.5 days (15-36) vs. 13.5 days (8-25) in the no-

GI bleeding group, p value = 0.01. Nonetheless, no significant differences were found for the length of post-ECMO ICU stay, total ICU length of stay, and hospital length of stay.

Variable	Overall N = 134	No GI Bleeding N = 120	GI bleeding N = 14	P value
Mech. Vent. after ECMO, days	11 (6-19)	11 (6 – 19)	11 (6 – 17)	0.97
ECMO LOS, days	14.5 (8-26)	13.5 (8 – 25)	19.5 (15 – 36)	0.01
ICU after ECMO LOS, days	10 (2-19)	10 (3 – 19.5)	6.5 (0 – 17)	0.54
Total ICU LOS, days	28 (17-42)	27.5 (16 – 41)	36.5 (23 – 54)	0.09
Hospital LOS, days	33 (20-53)	32.5 (19.5 – 51.5)	39 (23 – 54)	0.24
ECMO successful weaning N (%)	99 (74)	89 (74)	10 (71)	0.82
ICU survival, N (%)	93 (69)	84 (70)	9 (64)	0.66
Hospital survival, N (%)	92 (69)	83 (69)	9 (64)	0.71

Table 4. Clinical outcomes

Mech. Vent.: mechanical ventilation; LOS: length of stay; ICU: intensive care unit

Regarding in-hospital survival, GI bleeding did not have a significant impact on survival or weaning of V-V ECMO, or on ICU stay, and hospital discharge (all p values not significant). In order to assess the power of these explorative results, we compared the percentage of survival within our cohort with the percentage of survival in the international registry (44% on 795 reported patients). The estimated power for the absence of differences between groups was 52.4%. The survival over the first 180 days was also explored with the Kaplan-Meier estimator. The difference was not statistically significant (p = 0.65).

Discussion

In this study, we describe the incidence of severe and persistent GI bleeding during V-V ECMO and management through an advanced endoscopic assessment and treatment. Moreover, we recognized the characteristics of GI bleeding population on ECMO, and evaluated the effect of GI bleeding on mortality in light of the endoscopic hemostatic treatment.

GI bleeding is associated with high mortality in critically ill patients, and even though stress ulcer prophylaxis is currently a mainstay in supportive therapy of the ICU patients, it is still frequent [27]. Patients on ECMO support are a subset at increased risk of abdominal complications and GI bleeding for multifactorial reasons, like systemic anticoagulation and consumption coagulopathy, systemic inflammation, low platelet count, hypoxia, and low GI tract perfusion [17,28-30]. In the case of acquired coagulopathy, such as consumption of vonWillebrand factor, GI bleeding is often associated with diffuse bleeding without specific lesions. Furthermore, GI bleeding in ECMO patients, given the previous considerations, may be more severe, and the cause of refractory anemia and hypovolemia. These circumstances may also induce, though rarely, prolonged need for support, and increased need for transfusions, with obvious worsening impact on overall clinical outcome and mortality.

In fact, data from the ELSO registry has already shown that in the presence of GI bleeding, the survival rate is significantly lower than in the entire cohort [8]. Interestingly, in our cohort we did not find a statistically significant difference in survival in the GI bleeding vs. the no-GI bleeding groups.

Thanks to advancements in endoscopic equipment and management, effective minimally invasive techniques are feasible and applicable, even in the presence of severe GI bleeding [24,31]. Previously, endoscopists may have achieved effective hemostasis by combining different types of hemostatic therapy or using various devices with other specific applications. In fact, our first and only case of recurrent bleeding prior to 2011 was effectively treated by the combination of clips and Endoloop (Olympus Europe) thanks to the mechanical traction and tangential force of the clips and the endoolop itself, though this latter device is commonly used as a prophylactic maneuver for post-endoscopic resection bleeding [32]. More recently, Hemospray, which is a small mineral hemostatic powder composed of topical granules, sprayed onto the bleeding site without direct mechanical contact, has shown immediate results in terms of effective hemostasis [33]. The administration of Hemospray is simple, and its action lasts about 12-24 hours, with optimal outcomes [34]. Though published data on the incidence and efficacy of ET in ECMO patients are lacking in the literature, our findings indicate that a proper ET and its application, also for recurrent bleeding, has improved the overall survival in ECMO-related GI bleeding patients. We may hypothesize that in an a tertiary referral center with a highly specialized endoscopy team, GI bleeding episodes can be resolved earlier and may have less impact on the overall survival. In current times of very easy-to-use devices, circuits and cannulas, expertise in ECMO management relies strongly on the early, effective and less-invasive procedures to approach the decreasing (but still present and eventually catastrophic) complications, which may be better achieved in higher volume centers.

Looking at the significant clinical outcomes, we must highlight the differences among transfusion quantity, length of ECMO stay, and patient survival. PRBC transfusions were significantly higher in the GI bleeding group, but no significant difference was found normalizing the data for the ECMO length of stay. This data may contribute to support the effectiveness of the ET in stopping bleeding. The length of ECMO stay and patient survival are likely correlated. In fact, there was no statistical difference between the two groups in regards to survival (weaning from ECMO, discharge from the ICU, or discharge from the hospital) and this data strongly differed from the data reported in the ELSO registry. Usually, increased survival results in longer ICU stay, as early deaths are avoided. This is another supporting issue to the hypothesis of the effectiveness of ET in contributing to a mortality reduction.

This study has some limitations. First, this is a retrospective, single-center study and, though this is the largest specific study on the topic, the patient number is still limited. Second, the endoscopy team at our institute is a highly trained group in cases of bleeding in liver transplantation recipients on the waiting list or after transplant; consequently, this practice should not be considered immediately generalizable to other centers. Finally, we did not include a control group with patients who developed GI bleeding and

were treated by conservative therapy or surgery. We chose to focus on and include cases of clinically evident or suspected GI bleeding (we cannot rule out diffuse oozing in patients that did not undergo endoscopy).

This study, however, also has some strengths: there are no specific guidelines for ECMO patients with GI bleeding (despite the fact that this relevant in terms of mortality), and its appropriate management is largely not addressed in the ECMO setting. In almost all patients, endoscopy treatment was able to avoid an early worse outcome, and we can confidently speculate that the cause of death was never related to the GI bleeding in this series. Moreover, the cohort was extensively explored and no other incidental endoscopy exams or treatment was performed within the cohort.

Conclusions

GI bleeding in V-V ECMO is not uncommon, and represents a threatening complication. No guidelines are available on this topic. Advanced endoscopy treatments are a valuable tool to treat GI bleeding in complex situations, and should be available in large ECMO centers to treat such complications non-invasively and to reduce mortality.

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Chapter 7

A Narrative Review of Antithrombin Use during Veno-venous Extracorporeal Membrane Oxygenation in Adults: Rationale, Current Use, Effects on Anticoagulation, and Outcomes

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Abstract

Background: During extracorporeal membrane oxygenation (ECMO), the large contact surface between the blood and the extracorporeal circuit causes a continuous activation of coagulation and inflammation. Unfractionated heparin, a glycosaminoglycan that must bind to antithrombin (AT) as a cofactor, is currently the standard anticoagulant adopted during ECMO. AT, beyond being a potent natural anticoagulant, acts in the cross-talk between coagulation and inflammatory system through anticoagulation and coagulation-independent effects.

Objectives: In this review we describe, in the adult setting of veno-venous (V-V) ECMO, the pathophysiological rationale for AT use, the current practice of administration, and the effects of AT on anticoagulation, bleeding, and outcomes.

Data Sources: Studies on adults (18 years or older) on V-V ECMO published from 1995 to 2018 in order to evaluate the use of AT.

Results: In adults on V-V ECMO, AT supplementation has a highly pathophysiological rationale since coagulation factor consumption, systemic inflammatory response syndrome and endothelial activation are triggered by ECMO. Eleven manuscripts are focused on the topic but among the authors there is no consensus on the threshold for supplementation (ranging from 70% to 80%) as well as on the dose (rarely standardized) and time of administration (bolus versus continuous infusion). Consistently, AT is considered able to achieve better anticoagulation targets in or not in the presence of heparin resistance. The impact of AT administration on bleeding still shows contrasting results.

Conclusions: AT use in V-V ECMO should be investigated on the threshold for supplementation, dose, and time of administration.

Key Words: Anticoagulation, microRNA, disseminated intravascular coagulation, heparin, endothelial activation

Introduction

During extracorporeal membrane oxygenation (ECMO), the large and continuous contact surface between the blood and the circuit causes a systemic activation of coagulation that, in extreme conditions, may lead to thrombosis and disseminated intravascular coagulation. Unfractionated heparin (UFH) is the current mainstay for anticoagulation during ECMO [1, 2].

Antithrombin (AT) is a single-chain glycoprotein (58.000-Da molecular weight) synthesized by the liver, member of the serine protease inhibitor (SERPIN) super family, considered the most important physiological inhibitor of the coagulation cascade. UFH is a glycosaminoglycan (GAG) that binds to AT and facilitates anticoagulant actions [3]. The heparin–AT complex, accelerating the formation of the thrombin–antithrombin (TAT) complex, deactivates numerous factors of coagulation [4]. In addition, AT exerts anti-inflammatory function via inhibition of thrombin and via coagulation-independent mechanisms [5]. However, proinflammatory conditions may arise in some patients who receive heparin anticoagulation due to the formation of heparin and platelet factor 4 (PF4) complex, an antigen which triggers heparin-induced thrombocytopenia (HIT) [6, 7].

Clinical monitoring of heparin anticoagulation during ECMO has several important limitations. Two contact-activated tests, whole-blood activated clotting time (ACT), and plasma-based activated partial thromboplastin time (aPTT) are the most commonly used, but their results are rather poor correlated and are known to be variable among different reagents and devices [8, 9]. Moreover, the test considered the most precise and advanced to monitor UFH, the anti–factor Xa assay, has some biases in cases of AT deficiency. In fact, this test provides a measure of heparin efficacy on factor Xa (not as sometimes misconsidered UFH concentration) and is also dependent on the concentration of AT that many assays supplements [10]. Consequently, when the value of anti-Xa is low in the presence of a low AT level, UFH should not be increased, but through AT supplementation should increase heparin sensitivity [11]. In cardiopulmonary bypass (CPB), heparin "resistance" or "insensitivity" (cases requiring a higher dose of heparin, 300-400U/kg) is usually considered when ACT failed to increase above 400 seconds, and administration of AT concentrate is a preferred method to restore heparin response [12, 13]. In ECMO, there is no consensus on the definition of heparin resistance or anticoagulation management [1, 14-16]. Furthermore, pathophysiological mechanisms of ECMO-induced coagulopathy is multifactorial and involves both humoral and cellular coagulation elements as well as inflammatory system [17-19]. Acquired AT deficiency is not uncommon due to prolonged heparin usage on ECMO, and AT may reduce the risk of in vivo or intracircuit thrombus formation [18, 20, 21]. Accordingly, since children have AT deficiency at least until they are 6months/1 year old, AT has been administered during ECMO in critically ill children in case of disseminated intravascular coagulation due to sepsis and liver impairment [22-26].

Over the last decade, several reports and case series have suggested a potential usefulness of AT replacement in adults during veno-venous (V-V) ECMO, demonstrating lower heparin requirement, better control of anticoagulation, or lower consumptions of coagulation factors and platelets [27-30]. These findings are yet to be confirmed by prospective randomized controlled trials (RCTs), but it is thus reasonable to consider AT supplementation as a means to mitigate endothelial activation and systemic inflammatory response syndrome (SIRS) triggered during ECMO runs [31, 32].

To elucidate the actual available information and role of AT, this narrative review was undertaken to describe, in the adult setting of V-V ECMO, the pathophysiological rationale for AT supplementation and to review as well as discuss current evidence and clinical impacts of AT usage during V-V ECMO.

Methods

A literature review was performed through PubMed to identify studies on adults (18 years or older) published from 1995 to 2018 to evaluate the use of AT during V-V ECMO. The terms used for the literature search were "(ECMO OR ECLS) AND (ANTITHROMBIN)." In this review, we considered papers on adult patients on V-V ECMO or combined cases V-V/veno-arterial (V-A) ECMO, published in English language. We obtained 110 articles.

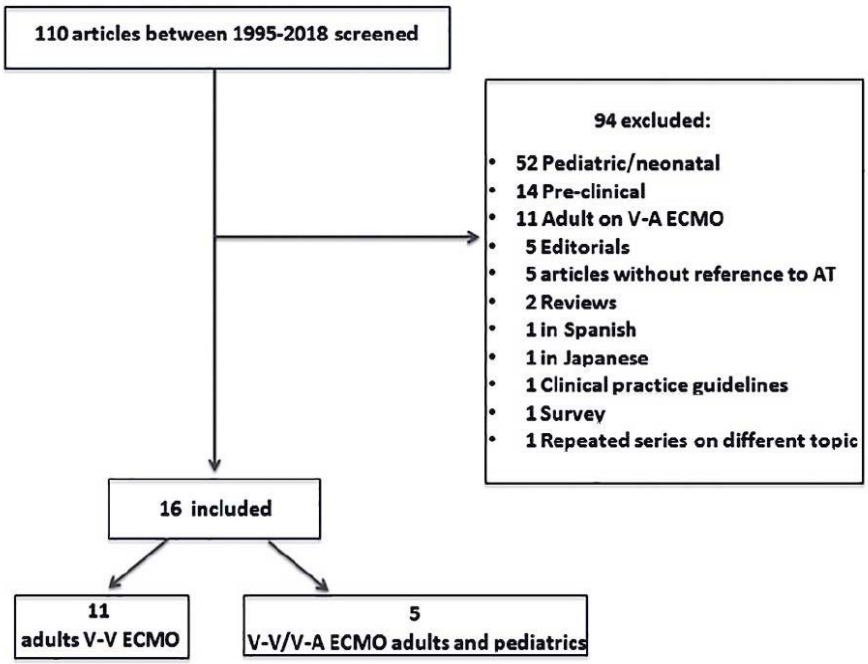


Figure 1. Study selection process

Therefore, we excluded studies on patients younger than 18 years (n=52); pre-clinical studies (n=14); V-A ECMO on adult patients (n=11); and reviews, editorials, repeated case series, and surveys (n=17). The decision to exclude V-A ECMO manuscripts was dictated by the aim of having a homogeneous cohort in terms of anticoagulation targets, complications, and outcomes. Therefore, the field of anticoagulation has significant differences among V-A and V-V patients due to the presence, in cases of V-A ECMO, of a natural reservoir (the heart and the circulatory system) where there is blood stagnation, determining the need for higher anticoagulation targets and, consequently, with a different rate of complications in thrombosis and bleeding. Even though strict anticoagulation are not definitely accepted, guidelines and experts opinion suggest the higher anticoagulation level to be used [33-37].

A flowchart of the literature review and screening is shown in Figure 1. The data were extracted from 16 articles. The following data were collected: study design, number of patients included, details of ECMO circuit/ components, dosage of UFH, modality of coagulation monitoring and time, AT activity level at baseline, AT activity level trigger and target, intensive care unit (ICU) discharge, and ECMO support days (Tables 1 and 2). Complications (bleeding and thrombotic events) and packed red blood cell transfusion are shown in Table 3.

After a careful evaluation of literature by two authors, double checked by two others, the sample of articles was considered adequate for a narrative review, while the data were considered not suitable for pooling in a larger scale.

Table 1. Summary of articles applying AT concentrate treatment during V-V ECMO.

References	Patients (N)	ECMO circuit	UFH mean dose (U/kg/h)	Anticoagulation target	AT target (%)	AT before supplementation or mean AT (%)	AT trigger (%)	AT supplementation (yes/no)	AT dose	ECMO support (days)	ECMO weaning (%)	ICU discharge
Martucci et al.[29] Retrospective	89	Rotaflow Cardiohelp	10.9 (7.5-14.8)	aPTT: 40-50 (every 4h)	100	91.8 (83.8-102)	<80%	Yes	Not reported	14 (8-24)	66	60%
Iapichino et al.[28] Retrospective	66	Rotaflow Cardiohelp	21 ± 6 (PTT < 1.5) 17 ± 6 (PTT > 1.5)	aPTT ratio: 1.5-2	80-120	Low (53 ± 10) in 47 (71%) subjects Normal (87 ± 19) in 19 (29%) subject	<70%	Yes	15-30 UI/kg/day	Not reported	Not reported	73%
Hoshino et al.[44] Retrospective	10	Not reported	Not reported	aPTT ratio: 1.5-2.5 ACT: 180-220	Not reported	Circuit clot: 57 (49-78) Circuit no clot: 70 (59-85)	Not reported	No	No	10 ± 13	50	40%
Sin and Lopez[67] Case report	1	Rotaflow	Not reported	aPTT: 50-60 ACT: 160-180	Not reported	Not reported	Not reported	No	Not reported	Not reported	Not reported	Not reported
Williams et al.[71] Case report	1	Not reported	Not reported	aPTT: 40-60	75-135	39 (pre-TPE)	Not reported	Yes	500 UI + 2 FFP units	4	Yes	Yes
Malfertheiner et al.[32] Prospective	54	Cardiohelp Dideco ECC. O5. Deltastream	1 UI/h	aPTT: 50-60 (every 4h)	Not reported	Before ECMO: (IQR: 57-85) Day 5: 90 (IQR: 75-106)	No	No	No	13.5 (4-70)	81	Not reported
Lavandosky[74] Case report	1	Rotaflow Cardiohelp	15	ACT: 160-180	80-120	47%	<70%	Yes	2,100 IU followed by 480 IU/h	Not reported	Yes	Yes
Jyoti et al.[68] Case report	1	Medos hilite	>31 U/kg/h frequent boluses (4,000-8,000 IU)	aPTT: 60-80 ACT: 200-220	No	31%	No	No	No	23	Yes	Yes
Phillips et al.[89] Case report	1	Not reported	Not reported	ACT: 170-200	No	No	No	No	No	7	Yes	Yes
Dolch et al.[72] Case report	1	Jostra Quadrox D oxygenator	Not reported	aPTT: 45-60	No	No	No	No	No	114	No	No
Dager et al.[30] Case report	1	Medtronic Affinity hollow-fiber	Not reported	ACT: 150-200	No	No	No	No	No	11	Yes	No

AT: antithrombin; V-V: veno-venous; ECMO: extracorporeal membrane oxygenation; UFH: unfractionated heparin; ICU: intensive care unit; aPTT: activated partial thromboplastin time; ACT: activated clotting time; TPE: therapeutic plasma exchange; FFP: fresh frozen plasma; IQR: interquartile range.

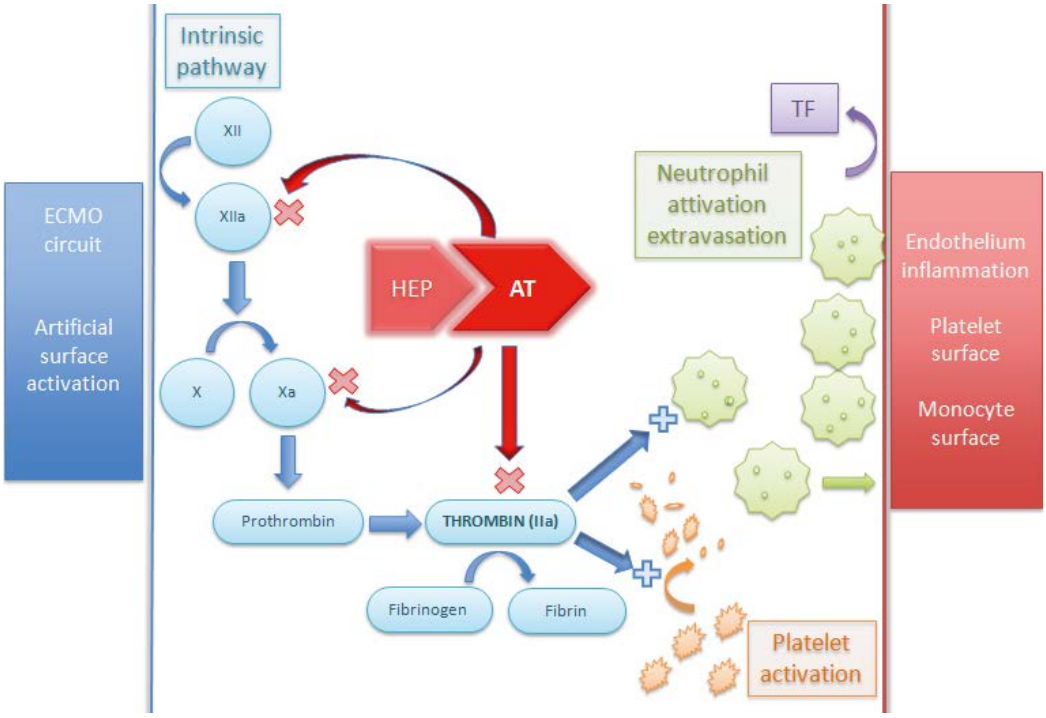
Table 2. Summary of articles applying AT concentrate treatment during V-V/V-A ECMO.

References	Patients (N)	ECMO circuit	UFH mean dose (U/kg/h)	Anticoagulation target	AT target (%)	AT before supplementation or mean AT (%)	AT trigger (%)	AT supplementation (yes/no)	AT dose	ECMO support (days)	ECMO weaning (%)	ICU discharge
Morrisette et al.[20] Retrospective	14 (VV-VA)	Medtronic circuit with a Carmeda bioactive heparin coating and Maquet Bioline Quadrox D Oxygenator	7.35 (pre-AT supplementation) 6.81 (post-AT supplementation)	Anti X-a (U/mL): 0.3-0.5 (bleeding protocol) 0.5-0.7 (no bleeding protocol)	100	Not reported	<50	Yes	Not reported	4.1	9	Not reported
Kim et al.[73] Retrospective	10 TOT 5 (VV) 5 (VA)	Not reported	Not reported	aPTT (s): 55-75 (every 4 h) ACT (s): 150-180	Not reported	Not reported	Not reported	No	Not reported	36 (25-58)	Not reported	Not reported
Northrop et al.[70] Retrospective	349 TOT 121 (VA) 89 (ECLS) 139 (VV)	Stöckert Centrifugal Pump Console Trolley System with Revolution centrifugal cone (Sorin Biomedical)	Not reported	Anti X-a (U/mL): 0.3-0.7	100	Not reported	<80 Dose of heparin: >60 U/kg/h	Yes	Not reported	4.3 median (pre-protocol) 4.4 median (post-protocol)	Not reported	Pre-protocol: 43% Post-protocol: 51%
Pieri et al.[90] Retrospective	20 TOT 10 (VV) 10 (VA)	Not reported	Starting dose: 3 U/kg/h	aPTT (s): 45-60 (every 8 h)	Not reported	Not reported	Not reported	Yes	Not reported	4.5 (UFH) 8 (bivalirudin)	Not reported	Not reported
Fletcher-Sandersjöö et al.[82] Retrospective	253 TOT 161 (VV) 92 (VA)	Biomedicus 550 consoles/Rotaflow pump/ Stockert CAPS Roller pumps/CentriMag pump. Medos Hilite 7000LT/Quadrox	Not reported	aPTT (s): 60-80 (every 8 h)	Not reported	ICH: 90 (78-106) ICH-: 93 (82-109)	Not reported	Yes	Not reported	ICH: 12 (5-19) ICH-: 7 (4-15)	Not reported	Not reported

AT: antithrombin; V-V: veno-venous; V-A: veno-arterial; ECMO: extracorporeal membrane oxygenation; UFH: unfractionated heparin; ICU: intensive care unit; TOT: total number of cases; aPTT: activated partial thromboplastin time; ECLS: extracorporeal life support; ICH: intracranial hemorrhage.

An extensive interaction exists between humoral and cellular components of coagulation and inflammation pathways, both over-expressed and activated during ECMO [38]. The two principal mechanisms of activation are supraphysiological shear stress and interactions between the foreign material and blood components (Figure 2) [39-42]. As soon as ECMO blood flow commences, plasmatic proteins (in particular, coagulation factors), fibrinogen, and von Willebrand factor (vWF) are consumed by the cleavage by the ADAMTS-13 protein [37, 43]. Fibrinogen may contribute to thrombus formation, while loss of high-molecular vWF may contribute to a bleeding tendency [44]. Platelets can be activated by multiple mechanisms: contact with biomaterial interface causes exposure of surface integrins and adhesion to the circuit; high shear stress, due to blood flow through the pump and oxygenator, induces activation of surface proteins, spreading the process, and exposing the phospholipids [45]. Moreover, thrombin (activated factor II (FIIa)) is a crucial procoagulant protease which supports physiological hemostasis but also causes pathological thromboembolic and proinflammatory responses. [39]

Figure 2. Patophisiology of coagulopathy during ECMO and anticoagulant mechanism of the Antithrombin. AT = antithrombin; HEP = heparin; TF = tissue factor.



In this context of cross-talk, AT is not only a physiological anticoagulant, but is strongly involved in the modulation of inflammation. The interaction of AT with heparin-like GAGs on the endothelial cell

surface involves the release of prostacyclin, which inhibits leukocyte activation by decreased release of interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF) [4]. Antiinflammatory functions of AT are partly mediated through anticoagulation activity, since inhibition of thrombin reduces platelet activation, neutrophil– endothelial cell interactions, and endothelial up-regulations [44].

In patients on ECMO, acquired AT deficiency is a result of hemodilution, blood coagulation activation, and consumption due to the use of UFH [20]. The binding of AT to UFH competes with the binding of AT to endothelial GAGs, thus increasing the likelihood of inflammation-related complications. In this light, low levels of AT can increase the risk of either thrombotic or hemorrhagic complications, the first because of reduced effect of heparin, and the second due to relevant concomitant inflammatory response, organ damage, and concomitant coagulation factor consumption [4, 45-47].

In these complex interactions, recent studies demonstrated that microRNAs (miRNAs) can function as a regulator of the immune and coagulation systems [48-51]. In the following section, potential implications of miRNAs in the pathophysiology of the inflammation/coagulation process during ECMO will be briefly discussed.

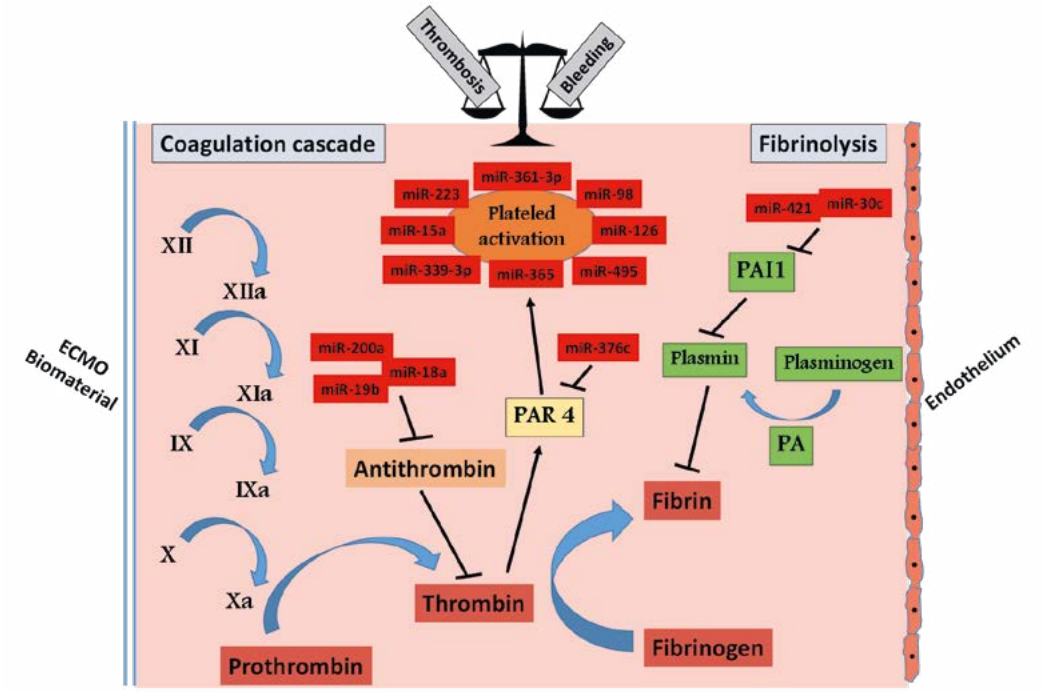
Predictive value of circulating miRNAs: potential epigenetic regulation of coagulopathy during ECMO

MiRNAs belong to a class of small, noncoding RNAs that regulate the expression of target genes at the posttranscriptional level, acting as fine-tuners of gene expression. They are able to regulate both platelet activation and protein involved in the regulation of hemostatic process including AT (Figure 3) [52, 53]. Interestingly, miRNAs play an important role also in the regulation of gene expression during the pathogenesis of acute respiratory distress syndrome (ARDS) [54]. Indeed, previous studies showed that plasma miRNAs levels are different between pulmonary and extrapulmonary ARDS patients [55]. In this framework, the potential correlation between ARDS, ECMO, coagulation process, AT, and miRNAs is paramount.

Two proteins play a critical role in the homeostatic function during the coagulation process: AT and the plasminogen activator inhibitor 1 (PAI-1) [56]. It has been shown that miR-18a and miR-19b are inversely correlated with AT mRNA levels, while miR-200a is inversely correlated with the expression of the two sialyltransferases involved in AT synthesis [51, 57]. Specific cooperative miRNAs have been recognized able to act synergistically to regulate the hemostasis [58]. In addition, miR-421 and miR-30c have a regulatory effect on PAI-1 expression, and also fibrinogen, a key factor of hemostatic process, is targeted by miR-29 family through HNF4α [59, 60]. Thus, the role of miRNAs in hemostasis does not seem to be restricted to the direct control of proteins but could be extended to include indirect effects on elements involved

in transcriptional processes of protein implicated in hemostasis. This regulation may have potential importance for anticoagulant therapy because the interindividual variations of miRNAs may contribute to a different individual response to anticoagulation. According to polymerase chain reaction (PCR) array human miRNA analysis, our preliminary data showed that coagulation in V-V ECMO appears to be regulated by miRNAs timedependently [58]. A deregulation of specific blood-circulating miRNAs that regulate crucial protein involved in hemostasis was observed (Table 4) [61-63], but further studies are needed to investigate miRNAs as diagnostic tools associated with sensitivity to anticoagulant treatments.

Figure 3. Schematic representation of coagulation cascade and fibrinolysis pathways modified from KEGG PATHWAY database (https://www.genome.jp/kegg/pathway.html).
MicroRNAs and corresponding genes target involved in the regulation of hemostasis are shown.



Results and discussion

Current trend in use worldwide

The most important data derived from the analysis of the studies is summarized in Table 1 (for V-V ECMO cases) and Table 2 (for V-V/V-A cases). The Extracorporeal Life Support Organization (ELSO) anticoagulation guidelines do not give specific recommendations concerning AT administration because of evidence of its use derives only from case reports or single-center studies [64]. Only 43% of the articles

reviewed administered AT without a consensus of target (normal range as indicated by ELSO) and trigger value (AT <70%). Different dosing strategies have been reported, including a weight-based dose of 15-30U/kg, or a dosing strategy based on the desired level to achieve [28].

UFH is the gold standard for the anticoagulation for patients on ECMO, though there is the risk of developing HIT [65, 66]. When HIT is suspected, direct thrombin inhibitors (DTIs) can be used instead of UFH. In 57% of the articles reviewed, AT was monitored but not replaced. The likely explanation is the use of an anticoagulant other than UFH, like bivalirudin and argatroban can be used safely as an alternative anticoagulant in patients with thrombocytopenia on extracorporeal life support (ECLS) [66-69]. Actually, DTIs have several advantages, such as AT-independent anticoagulant effect, less wide-spread protein binding with a more dose predictable effect (this has to be demonstrated in larger populations with multiorgan failures), action of clot-bound and circulation thrombin (UFH acts only on circulating thrombin), and the lack of known immune mediated reactions. But if we consider the wide overlapping actions of AT in the coagulation and inflammation systems, its measurement and potential administration remains a possibility that should be investigated.

A prospective study on 57 patients with three different ECMO systems did not show a different activation of hemostasis, and the value of AT was in the expected range [41]. AT values increased from 70% (interquartile range (IQR), 57-85%) before ECMO to 90% (IQR, 75-106%) on day 5 ($p< 0.001$), suggesting that the increase in the value could be interpreted as a good control of underling sepsis.

Effects on anticoagulation

AT, essential for UFH, is considered a potential tool to reach more quickly the desired anticoagulation targets. However, in a retrospective analysis of 66 adults, low AT activity was not always related to a low aPTT value ($p=0.983$), and AT replacement does not facilitate the achievement of the aPTT target if not accompanied by elevated circulating levels of C-reactive protein [28]. Mean AT activity level was 70% in 47 of 66 patients (71%), and the aPTT ratio was < 1.5 in 20 of 66 patients. The aPTT ratio target was between 1.5 and 2, and the UFH dosage remained constant. In contrast, our group has found that AT supplementation may contribute to a lower daily dose of heparin infusion as well as transfusions [21, 29]. Interestingly, when procoagulant and anticoagulant factors are directly measured in the first days of ECMO start, despite a low AT level, heparin seems to be able to reduce thrombin generation anyway. This scenario might probably be completely different if we consider longer ECMO periods and should be balanced by deep knowledge of the inflammatory pattern.

AT activity level monitoring is very often part of anticoagulation protocols to reduce bleeding and thrombotic complications and to increase the average life of the circuit. Northrop et al. [70], in a

retrospective study of 349 patients (neonatal, pediatric, and adult V-A and V-V ECMO), showed an association between anticoagulation laboratory protocol using Xa assay, thromboelastography, and AT measurements, with a decrease in blood product transfusions ($p < 0.001$) and an increase in circuit life (circuit change 70/19 pre-protocol/post-protocol ($p=0.08$), with no statistically significant difference on survival to discharge, $p=0.06$). AT is usually checked once a day or if the heparin dosage increases. It is replaced if AT activity is < 80% and if dose of heparin is >60U/kg/h. The target level of AT is 100%. When the AT bolus is detected, the ACT is monitored, and if it increases the target level (200-220 seconds), the heparin dosage is reduced. However, this reported study should be considered with caution because of the impact of a large number of pediatric patients in whom the UFH dose is usually very high compared with adults and due to the well-known age related differences in hemostatic proteins and response to anticoagulant therapies.

Recently, Morrisette demonstrated how AT administration was associated with longer periods to maintain therapeutic anti-Xa levels, and this is reasonable considering the premises on the test discussed earlier. A case of acquired AT deficiency, in a patient with V-V ECMO as bridge-to-transplant for pulmonary fibrosis with intraoperative therapeutic plasma exchange, has been reported [71].38 The target level was 75-135% and the AT activity pre-TPE was 39%. The dose of heparin was not reported, but the level of AT activity was very low (~10%) causing resistance to heparin and subsequent circuit clotting. Caution should be applied in case of thrombocytopenia, and not only when it is heparin induced [72, 73]. On the other side, in an adult patient with V-V ECMO support for respiratory failure, the AT activity level was 47%, and the heparin dosage to reach an ACT 160-180 was 15U/kg/h [74]. Moreover, in patients on ECMO, the definition of heparin resistance lacks consensus, but Northrop et al. set it at 60U/kg/h, so while 15U/kg/h is a value that we can consider normal, the target ACT value in this study was lower.

Role of AT in bleeding

During ECMO, bleeding is still one of the major complications, often fatal in case of intracranial hemorrhage [75-79], even though bleeding complications have declined over the past years permitting the use of ECMO also in unconventional settings [80, 81]. According to ELSO, bleeding events are defined as a drop in hemoglobin greater than 2g/dL over 24hours, or if there is a hemothorax, central nervous system, retroperitoneal bleeding, or if bleeding requires an intervention or an administration of two or more RBC units in 24hours. Higher aPTT, as well as higher heparin dosage, is associated with bleeding.

AT is a potent endogenous anticoagulant, and its administration, in the presence of UFH, may theorically be associated with increased bleeding risk, though this correlation remains to be determined. Recently, our group has highlighted how a higher level of AT and its supplementation do not increase the risk of

bleeding, probably through a lower dose of heparin and reduced imbalance of the natural anticoagulation and coagulation activity [21].

From the analysis of selected articles, there is no constant correlation between the administration of AT and the increased risk of bleeding (summary in Table 3). In a study on predictors of intracranial hemorrhage in adult patients on ECMO, AT was significantly higher in patients who developed an ICH, but many ICH patients have the value of AT within normal ranges, so it is difficult to draw definitive conclusions [82]. There are various case reports with minor bleeding (cannula site bleeding, nasal–oral bleeding, blood-tinged endotracheal secretions). In these cases, the anticoagulant of choice was not UFH, and AT was not administered. Despite in all the reviewed articles, there was mention that AT was measured daily, the value was not reported. Multiple monitoring systems integrated with closer control after administration of AT or in case of instability probably decreases bleeding and the need for blood transfusions [70]. Moreover on this topic, some pharmacokinetic considerations should be highlighted since AT may exert a strong anticoagulant function when administered as a bolus; therefore, a continuous infusion (or extended infusion over 4-6hours) may be suggested to avoid the peaks of bolus administration.

Finally, on AT administration and coagulation, a debated topic is the presence of latent forms of AT that may have thrombogenic actions. Latent AT is an AT conformation, present in a certain level also in healthy people, that seems to have a prothrombotic and antiangiogenetic role [83, 84]. The concern with exogenous AT administration is that AT human concentrate contains variable concentration of latent AT, but there are not consistent data on differences between several products [85]. More data on the topic from basic sciences are warranted, but the current knowledge does not support the hypothesis that latent AT might have clinical significance.

Relation between AT and mortality during V-V ECMO

The possible role of AT on mortality in critically ill patients has long been proposed. The first trials with AT supplementation began in the early 1990s but did not find benefits in terms of survival, though some degree of improvement in single organ function was observed [86, 87]. An extensive review of AT use in critical illness was published in 2016 by Cochrane database, including 30 trials that, however, were considered at high risk of bias. There was no clear effect of AT on mortality (relative risk (RR) 0.95, 95% confidence interval (CI) 0.88-1.03), instead, AT increased significantly the risk of bleeding events in this large cohort (RR 1.58, 95% CI 1.35-1.84)[88].

The administration of AT during V-V ECMO cannot be currently considered a common practice, since the effect on patient outcome has not been well defined, despite the fact that some studies have shown

a strong association between lower AT level and higher mortality. However, this last concept is far from being clarified if a higher level due to endogenous increase (potentially due to a less intense inflammatory state or factor consumption) can be comparable with an exogenous replacement effect. Iapichino et al. [28] stressed the utility of administration of AT in patients with evidence of heparin resistance with signs of hypercoagulability and inflammation (represented by higher level of C-reactive protein), but the difference in ICU survival was not statistically significant (p value=0.3). This was a single center retrospective study on 66 adults, and the cutoff of AT considered was 70%, though the target value was not identified. Northrop et al. [70] used an anticoagulation laboratory protocol based on anti–factor Xa assays, thromboelastography, and AT measurements. The value target of AT in this study was 100% and was associated with decreased blood product administration, decreased hemorrhagic complications, and increased ECMO circuit life, though survival was not considered. In literature, there are few studies in adult patients with V-V ECMO that analyze the direct correlation between mortality and AT administration in contrast to patients with sepsis in whom the hypothesis that low levels of AT may be related to an increase in mortality has been taken into consideration since 1980, but at the moment, the opinions are conflicting. Considering that new and alternative anticoagulation strategies are currently explored, the role of AT may be worth to be further investigated in the ECMO setting [89, 90].

Conclusion

AT during ECMO support has been used since many years, but despite several reports highlighting its ability to reduce thrombosis, heparin resistance, and heparin infusion dose, it is still recognized just as a second-line intervention in case of heparin resistance. AT may have a role to reduce the imbalance between procoagulant and anticoagulant factors and may be an effective tool to reduce bleeding and thrombosis to achieve a further increase in survival, but thorough research in this setting is still warranted to define the appropriate dose, and type and modality of administration.

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Chapter 8

Management of Intra-Abdominal Hypertension during ECMO: Total Water-Assisted Colonoscopy as a Step-Up Mini-Invasive Treatment, and a Literature Review

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Abstract

BACKGROUND AND STUDY AIMS

During extracorporeal membrane oxygenation (ECMO), intra-abdominal hypertension (IAH), can impair ECMO venous drainage, reducing its ability to provide an adequate oxygenated blood flow. When medical therapy is ineffective in managing IAH, guidelines recommend a decompressive laparotomy (DL), though the procedure is associated with several complications and poor outcomes.

PATIENTS AND METHODS

Case series of IAH in patients affected with acute respiratory distress syndrome (ARDS) on veno-venous (V-V) ECMO, in whom we performed total water-assisted colonoscopy (t-WAC) to treat IAH.

RESULTS

In three patients who underwent t-WAC, we report a real-time intra-procedural reduction of IAH, normalization of ECMO blood flow, and a reduction of vasopressors and lactates. t-WAC was performed in a context of evident abdominal compartment syndrome with multi-organ failure, and in one case was performed because of IAH and ECMO impairment.One patient was discharged alive, while the other two died of multi-organ failure, though the cause of death was apparently not secondary to IAH.

CONCLUSIONS

During ECMO, in select cases,T-WAC may represent a first-line non-invasive approach.

Introduction

Intra-abdominal hypertension (IAH) is frequent in critically ill patients, and can cause abdominal compartment syndrome (ACS), which is a severe complication resulting from an acute and sustained increase in intra-abdominal pressure (IAP)[1].

During extracorporeal membrane oxygenation (ECMO), patients are at risk of IAH due to the severity of their critical illness and the need for multiple transfusions and fluids[2]. Moreover, in the specific setting of ECMO, IAH can impair the venous drainage, reducing its ability to provide an adequate oxygenated blood flow[3]. The ECMO malfunction can be considered an aspect of ACS; consequently, the appearance of IAH during ECMO would likely necessitate a personalized approach[4].

Decompressive laparotomy (DL) is the recommended next step when medical treatment fails in IAH[5]. However, as a surgical procedure, it can be burdened by severe complications, such as bleeding and intra-abdominal infections, and may not always be feasible, especially in unstable patients[6]. Data on DL during ECMO show a still high mortality; consequently, as in other complications during ECMO, any effort to treat them non-invasively should be carried out, and gastrointestinal system with advanced endoscopy has proved to be a fertile field for this purpose.

Considering the absence of a clear standardization in the management of ECMO patients with IAH, total water-assisted colonoscopy (t-WAC) has been applied as a less-invasive approach in order to avoid the over-distention of the colon, and reduce the pressure gradient. [7]

Patients/Materials and Methods

We present a case series of three veno-venous (V-V) ECMO patients affected with IAH treated with t-WAC, which effectively provided a successful reduction of IAP.

The IAP was measured at the end of expiration, with the patient in a supine position, using a catheter through the bladder with an instillation of 25 ml of sterile saline, and the transducer zeroed at the mid-axillary line[8]. Patient characteristics and peri-procedural values are detailed in Tables 1 and 2.

Case	Cause of ARDS	Cannulation	IAH-pre (mmHg)	IAH-post (mmHg)	IAH Delta (mmHg)	Lactates pre tWAC (mmol\L)	Lactates post tWAC (mmol\L)	Norepinephrine pre tWAC (mcg\kg\min)	Norepinephrine post tWAC (mcg\kg\min)	Outcome (day after last endoscopy)
1	Polytrauma	Femoro-Jugular	20	9	11	5.71	2.85	0.15	0.08	Death (65 day:
2	H1N1	Femoro-Jugular	13	3	10	1.12	0.93	\	\	Discharge (37 days)
3	Pnneumocystis Jirovecii	Femoro-Femoral	16	12	4	1.69	1.18	0.1	0.02	Death (15 day:

Table 1. Values of Intra-Abdominal Pressure, Vasopressor Needs and Lactates.

Results

Case 1

A 16-year-old male on V-V ECMO for polytrauma with bilateral pneumothorax and pulmonary contusions, cerebral intra-parenchymal hemorrhage, multiple fractures (maxilla and mandibula with dental avulsion, thoracic vertebraefrom T4 to T7 and pelvis). The clinical course was complicated by acute renal failure requiring continuous renal replacement therapy (CRRT), hospital-acquired pneumonia due to multi-drug resistant germs (*Klebsiellapneumoniae*, *Acinetobacter baumanii*, *E. Coli*ESBL and *Pseudomonas aeruginosa*), and two episodes of massive hemothorax treated with thoracoscopic surgical evacuation, and subsequently evolving into pleural empyema.

On the 37th day after admission, duringa new episode of septic shock,the patient developed IAH up to 20 mmHg, associated with instability of the ECMO flows, hemodynamics, and lactates up to 5.71 mmol/L. Vasopressors (norepinephrine and vasopressin), and an intense volume filling with repeated packed red blood cell (PRBC) transfusions were needed. ACT scan showed over-distension of the colic frame and hydroplane levels. Advanced medical treatment was implemented focusing of reduction of IAP: neuromuscular blockade with rocuronium in continuous infusion was started, gastric content was evacuated by naso-gastric tube, fluids were reduced favoring PRBC, and CRRT was continued. Considering the unstable clinical picture, we proceeded with t-WAC as a step prior to any surgical decompression. The procedure was effective, providing reduction of the IAP (9 mmHg) and lactates (2.85 mmol/L), restoring ECMO blood flow, and reducing the vasopressor dosage. In the following days, we observed the reappearance of canalization. Due to the septic complications and lung failure, the patient required continuous ECMO and CRRT support and, unfortunately, died on the 111th ECMO day.

Case 2

A 43-year-old man presenting with acute ARDS due to human influenza A (H1N1) virus, requiring CRRT for hemodynamic instability and oliguria, and three endoscopic sessions for bleeding from a gastric stress ulcer.

On the 11th day, abdominal distension was observed, with impaired ECMO flow. IAP was 13 mmHg, lactate level was 1.12 mmol/L, and no hemodynamic instability was observed, though it was necessary to reduce the blood flow from 5.5 to 4.3 liters/minute while the patient was entirely ECMO-dependent. Strategies to stabilize and eventually increase the ECMO blood flow was started: the patient was fully sedated and paralyzed to reduce diaphragm swinging and consequent negative

pressures in the drainage cannula, fluids were administered judiciously since echocardiography showed a fully replete vena cava without any change in respiratory activity.

	Case 1	Case 2	Case 3
Gender	Male	Male	Male
Age, years	16	43	43
Weight (kg)	63	81	58
Height (cm)	170	175	165
BMI	22	26	21
Saps II at admission	31	43	64
Sofa score at admission	7	8	7
PRESERVE score	4	-2	4
RESP Score	-3	1	-2
Pre-ECMO Length of hospital stay, days	30	3	9
Pre-ECMO Mechanical ventilation, days	30	2	7
Pre-ECMO P/F ratio	46	\	56
Murray Score	3.75	\	3.75
Pre-ECMO Creatinine (mg/dL)	0.58	1.62	0.64
Hematocrit (%)	27	19	28.5
Drainage (F)	25	25	25
Return	19	21	23
Mean hematocrit value (%)	27.57	23.04	30.25
Total PRBC transfused (unit)	69	16	27
Prone position during ECMO (Yes/No)	No	Yes	Yes
CRRT	Yes	Yes	Yes

Table 2. Summary of Patient Characteristics.

In this case, t-WAC was performed to prevent the development of ACS since the team was not able to deliver proper ECMO support. IAP dropped to 3 mmHg, restoring the blood flow to 5.5. The clinical course in the ICU was complicated by the development of hospital-acquired pneumonia and severe sepsis due to carbapenemase-producing multidrug-resistant *Klebsiella pneumoniae*. After adequate antibiotic therapy, it was possible to proceed with weaning from ECMO support, on the 19th day, weaning from CRRT on the 31st day, and discharge from the ICU on the 37th day.

Case 3

A 43-year-old male patient with a recent diagnosis of human immunodeficiency virus (HIV) placed on V-V ECMO for *Pneumocystis jirovecii* pneumonia. Septic shock secondary to a blood-stream infection due to *Enterococcus faecium* occurred. We subsequently observed a new onset of anasarca, ascites, and bilateral pleural effusion requiring thoracic drainage and, subsequently, complicated by hemothorax.

On the 4th ECMO day, IAH (IAP = 16 mmHg) appeared, requiring an increase of norepinephrine, full sedation and neuromuscular blockade. The septic shock picture prompted the use of CRRT to manage fluids and reduce overload. The blood ECMO flow become constantly inadequate, so after all the explored medical strategies revealed to be ineffective, a new drainage cannula was placed at the jugular level (becoming a femoral-jugular-jugular design). With the abdominal picture, this was not yet sufficient to restore an adequate drainage capacity, and continuous fluid administration as well as transfusions were needed to keep the patient saturation (through the ECMO blood flow) at a level compatible with life. As rescue, we performed t-WAC, and the IAP decreased to 12 mmHg. On the 7th day, abdominal infection of *Clostridium difficile* developed, with the appearance of paralytic ileus and a new ACS framework: lactates up to 5.92 mmol/L, further increase of norepinephrine, and continuing acute renal failure requiring CRRT. An abdominal CT scan revealed the presence of focal ischemia at the right colon and rectum and also in the liver, kidney, and spleen. Despite the high surgical risk, the patient underwent exploratory/decompressive laparotomy and resection of the ischemic colon. The procedure was effective, with ACS resolution, reduction of lactates and norepinephrine, but the septic framework never resolved, and the patient died on the 20th day of ECMO support.

Discussion

This series shows the potential feasibility and effectiveness of the procedure in this setting, even though the mortality in cases of ACS remains high. [9]

The survival rate in cases of IAH during ECMO is still extremely variable among centers worldwide, as we demonstrated in a systematic review of the scant literature on the subject (Table 3). A standardized approach is lacking in this setting, also considering that the values adopted to evaluate the ACS grade may be inadequate when the ECMO drainage cannula does not reach proper flow due to compressed abdomen. [10]

Author, year	Journal	Study Design	Patients N	ECMO Configuration	Cannulation	ECMO Indication	Age	Treatment	Reason for IAH Treatment	Resolution of IAH	ECMO LOS, days
Feddy,	Anesthesiol Intensive Ther	Case Series	3	V-V	2 Avalon, 1 femoro-jugular	ARDS due to Severe Pancreatitis	30-46 *	Conservative	MOF, Impairment of ECMO drainage	Yes	26.5, 9.1, 15
Glowka, 2018	J Crit Care	Retrospective	11	4 V-V 7 V-A	NA	4 ARDS, 1 Trauma, 3 eCPR, 2 MI, 1 PCS	36-80 *	DL	Clinical Decision High volume reuscitation in the previous 24 h	Unspecified. SOFA score reduced in 1/11	NA
Boulos, 2020	ASAIO J	Retrospective	9	V-V	6 Femoro-Jugular, 3 Femoro-femoral	6 ARDS, 2 DLTx, 1 Hypothermia	44 (31-55)	DL	Hypervolemia (44%) Bleeding (33%) Bowel ischemia (22%)	Yes	14

Table 3: Summary of Studies Included in the Systematic Review.Given the Scantity of the Available Studies on PubMed, Only Two Retrospective Studies Reported the Efficacy of DL, with Different Outcomes in Terms of Definitive Results. The Other Report Showed an Effective Resolution of IAH and Higher Survival Rates with Conservative Treatment.

A less-invasive solution, such as t-WAC, could be a therapeutic alternative. T-WAC was first introduced in diagnostic endoscopy because its principal potential benefits included higher cecal intubation rates, lower sedation requirements, and lower patient pain scores, especially in unsedated procedures [11], as well as an increase in the adenoma detection rates.

Differently from an "open" abdomen extra-visceral decompression, such as laparotomy, t-WAC is a mini-invasive technique that provides a visceral intraluminal decompression, with easy reproducibility and without serious side effects. Nonetheless, its initial diagnostic function should not be underestimated since it may also contribute to diagnosing intraluminal colonic lesions that may be neglected even with laparotomy. Technically, using a standard or pediatric diagnostic video colonoscope (CF-H 190L and PCF-H 190L, Olympus Europe, Hamburg, Germany) and generally with the addition of a standard irrigation pump, t-WAC provides the intraluminal instillation of NaCl 0.9%, followed by the complete aspiration of the residual intra-luminal air and opaque saline water. With water instillation, the proximal colon expands regularly without distention of the upstream intestinal loops that worsen the IAP, as can occur with air. Then, the residual gas and water are completely removed by direct suction during the withdrawal, making this strategy very suitable for critically ill patients, and guarantees the reduction of the wall stress and the normalization of intra-luminal pressure.

Moreover, the water method can ease the passage of the colonoscope by straightening angulated sections, such as the sigmoid colon, weighing the colon down, and lubricating the interface between the scope and

the mucosa. All these characteristics make the t-WAC a perfect solution for patients in mandatory supine position, as in the case of ECMO patients due to the presence of the cannulas.

In addition, in our opinion, the small bowel preparation is not mandatory and can be avoided because the purpose of decompressive t-WAC in this setting is solely therapeutic and not diagnostic. Even with the lack of bowel preparation, in expert hands and using a sufficient quantity of saline solution the goal of the colonoscopy can be technically satisfied by the direct and indirect mechanical decompression of major numbers of focal areas of intra-luminal air. As a result, we do not believe in the need to always reach and intubate the cecum but, in our opinion, a sufficient therapeutic effect can be effectively achieved if the intra-procedural IAH measurement detects a stable reduction of values during the entire withdrawal time. In fact, in our case series, we found a real-time and intra-procedural reduction of IAH during the phase of fluid aspiration through the endoscope, and a gradual normalization of ECMO blood flow in all the cases. These conditions were deemed parameters of "adequacy" for the procedure.

In summary, t-WAC, in cases of mild or severe IAH during ECMO can contribute early and non-invasively to the diagnosis and treatment of colonic distension. This condition is not infrequent during ECMO, either in its veno-venous or veno-arterial configuration, in particular when septic shock, low cardiac output, and fluid overload are coexisting. It may act as a first step before the eventual DL, may be applied as a point-of-care treatment since it does not require the transfer of unstable patients, and does not require adjusting the patient position.

The technical algorithm focalized on the DL, as suggested by guidelines, could likely be implemented with the t-WAC as an intermediate point in a more complete "step-up" approach (Figure 1).

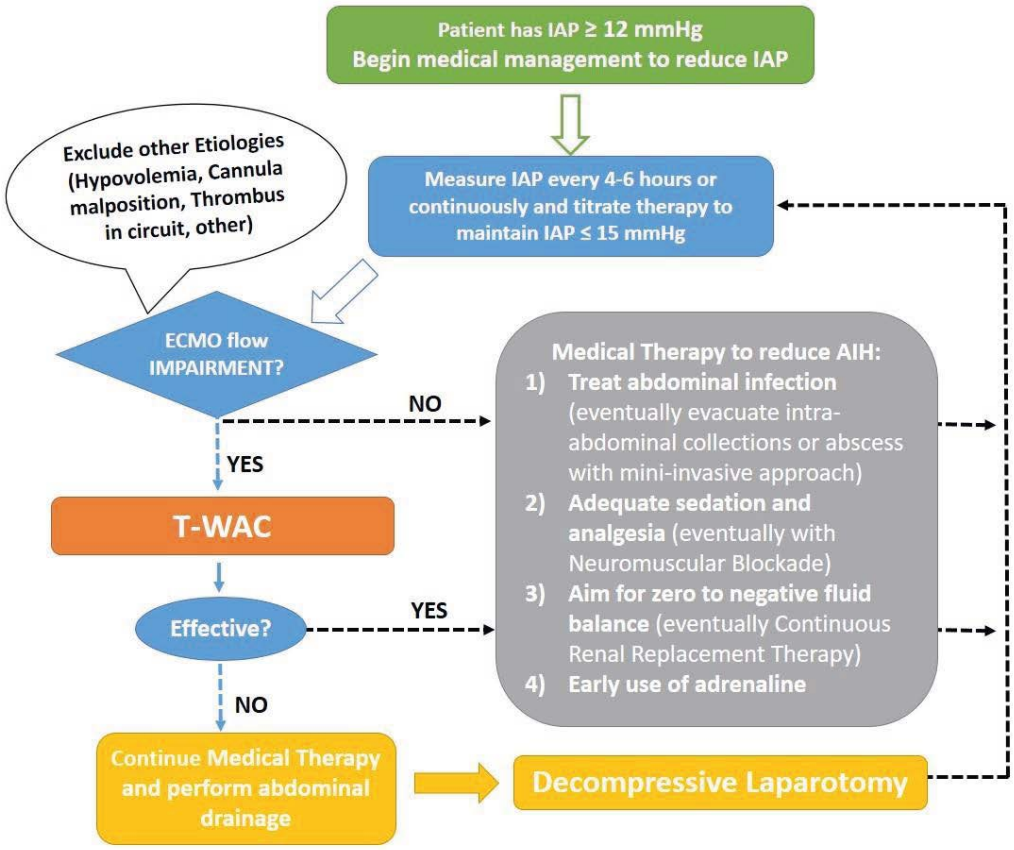

Figure 1: Proposed Flow Chart for the Management of Intra-Abdominal Hypertension during ECMO. IAP: intra-abdominal pressure. T-WAC: total water-assisted colonoscopy

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Chapter 9

Identification of a Circulating miRNA Signature to Stratify Acute Respiratory Distress Syndrome Patients

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Abstract

There is a need to improve acute respiratory distress syndrome (ARDS) diagnosis and management, particularly with Extracorporeal Membrane Oxygenation (ECMO), and different biomarkers have been tested to implement a precision-focused approach. We included ARDS patients on veno-venous (V-V) ECMO in a prospective observational pilot study. Blood samples were obtained before cannulation, and screened for the expression of 754 circulating microRNA (miRNAs) using high-throughput qPCR and hierarchical cluster analysis. The miRNet database was used to predict target genes of deregulated miRNAs, and the DIANA tool was used to identify significant enrichment pathways. A hierarchical cluster of 229 miRNAs (identified after quality control screening) produced a clear separation of 11 patients into two groups: considering the baseline SAPS II, SOFA and RESP score, cluster A (n=6) showed higher severity compared to cluster B (n=5); p values<0.05. After analysis of differentially expressed miRNAs between the two clusters, 95 deregulated miRNAs were identified and reduced to 13 by in silico analysis. These miRNAs target genes implicated in tissue remodeling, immune system and blood coagulation pathways. The blood levels of 13 miRNAs are altered in severe ARDS. Further investigations will have to match miRNA results with inflammatory biomarkers and clinical data.

Keywords: ARDS subphenotypes, miRNAs, biomarkers, lung injury, inflammation

Introduction

Acute respiratory distress syndrome (ARDS) is an acute inflammatory lung disease characterized by the loss of lung endothelial barrier integrity and invasion of alveoli by fluids, proteins, and inflammatory cells [1]. It is an evolutive picture that is schematically divided into an exudative phase followed by or overlapping with a proliferative phase [2]. Thus, ARDS is characterized by persistent injury stimuli and the failure of lung tissue repair that evolves into chronic lung repair, resulting in marked changes in lung structure and function [3]. In this clinical picture, the Berlin definition [4] has helped to define the syndrome, and the subsequent LUNGSAFE study described its application worldwide [5]. Nevertheless, ARDS still comprises a number of different etiologies and likely also different biological patterns differently integrating in the same syndrome, with potentially different response to therapeutic actions and outcomes. The appearance of COVID-19 has cast into question the presence of a "classical" ARDS and a "new" form, with the same clinical definition but different pathophysiology [6-8].

To overcome this controversy, in the last years several biomarkers have been explored to differentiate these patients [9-11]. These markers are not defined tools for clinical use but interestingly, Calfee at al. have identified two ARDS subphenotypes (hyperinflammatory and non-hyperinflammatory), with different management needs and outcomes [12]. But, still, in such cohorts, there is a high variability of severity that usually is measured by specific severity scored designed for critically ill patients like the sequential organ failure assessment (SOFA) score and the simplified acute physiology score II (SAPS II), or effectively designed for extracorporeal membrane oxygenation (ECMO) patients like the respiratory ecmo survival prediction score (RESP score) and the predicting death for severe ARDS on VV ECMO (PRESERVE) score.

In the most severe cases of ARDS, ECMO, more frequently in its veno-venous (V-V) configuration, is the only rescue therapy able to gain time and hasten the evolution of the lung healing processes [13, 14]. However, ECMO presents a new, confounding problem: contact of the blood with an artificial surface (despite advancements in biocompatible materials) can prompt the activation of coagulation factors with further stimulation inflammatory and tissue repair pathways [15, 16].

Considering that in ECMO (a resource-consuming support from both an economic and human workload perspective), mortality is still high, new biomarkers, posed in the direction of precision medicine, may help in characterizing the heterogeneous ARDS population to discriminate groups with different prognoses, and that could be adopted to follow the disease progression, and look at the effects of the treatment given.

From a biopathological point of view, ARDS is characterized by cellular injury pathways, such as endothelial and epithelial injury, pro-inflammatory injury, coagulation, fibrosis, and apoptosis [17]. Pathophysiological differences in these processes in different ARDS severity statuses can likely be used to better understand the role of various biomarkers in ARDS. In this light, microRNAs (miRNAs) are a

class of small non-coding RNA (19–25 nucleotides) responsible for silencing specific genes able to modulate specific pathways. Circulating miRNAs likely play important roles in cellular communication regulating gene expression and the phenotype of the recipient cells [18]. Moreover, in the presence of harmful stimuli, the composition of blood miRNAs can be altered, making themselves excellent candidates as biomarkers [19, 20]. Indeed, miRNAs have been proposed as sensor-biomarkers in physiological processes such as muscular hypertrophy [21]. Otherwise, several miRNAs have been described as biomarkers for cardiovascular disease [22] and cancer [23], thus indicating that they might also play a role as signals of ongoing pathological processes.

Therefore, we aimed to explore whether the identification of a circulating miRNA signature can lead to discover new biomolecules useful for further characterizing ARDS patients in terms of severity status. Thus, we screened the expression of 754 circulating miRNAs in the blood of ARDS patients using high-throughput quantitative PCR to identify differentially expressed miRNAs.

2. Materials and Methods

2.1 Patient recruitment, characteristics, and sample acquisition

This study was approved by the IRCCS-ISMETT Ethics Committee (EC Code: IRRB/34/19) and was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained initially from the next of kin and later confirmed directly by the patients if recovered. Patients were enrolled if they were affected with ARDS and supported by V-V ECMO. In July 2018 we implemented miRNAs measurement for ARDS patients, and subsequently the patients were enrolled in the present proof-of-concept study. Patients were categorized for anthropometric data (age, weight, height) and severity of disease (SAPS II, SOFA score, creatinine and bilirubin). Moreover, they were also categorized for PaO2/FiO2 ratio, specific ECMO related scores like the PRESERVE score and RESP score (a part from RESP score that has inverse values to the survival probability – lower or negative values indicate higher severity and lower probability of survival – all the scores are proportional to the severity – higher values correspond to higher severity), and pre-ECMO length of stay (LOS) in the hospital and in the intensive care unit (ICU) as well as the duration of mechanical ventilation (MV). During the ECMO run the need for continuous renal replacement therapy (CRRT) and the length of ECMO course were assessed, together with ICU- and hospital-related survival. Venous whole blood was collected in PAXgenetubes (Qiagen, Hilden, Germany), and unprocessed samples were immediately stored at −80 °C until further analysis. In addition, blood samples were collected to evaluate the following markers: creatinine, bilirubin, hematocrit, and platelets.

2.2 Real- time PCR analysis of miRNAs by TaqMan low density arrays

MiRNAs were profiled using the TaqMan Array Human MicroRNA panels A and B v3.0 accordingly manufacturer's instructions (Thermo Fisher Scientific, USA). Total RNA was extracted with RiboPure™ RNA Purification Kit, blood (Thermo Fisher Scientific, USA). The purity and quantity of isolated RNA were determined by OD260/280 using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA). Then, 300 ng of RNA were reverse-transcribed with the high capacity RNA-to-cDNA kit protocol (Thermo Fisher Scientific, MA, USA) in order to produce single-stranded cDNA. QRT-PCR of 754 human miRNAs was done with the Applied Biosystems 7900 HT Real-Time PCR system, and for each miRNA, the initial data output was in SDS software v2.4. The expression level was determined with the equation 2-ΔΔCT using the U6 as housekeeping gene. Furthermore, hierarchical cluster analysis of miRNA expression was used to group patients with a similar expression pattern. MiRNA expression data were grouped using Euclidean distance algorithms in the Cluster 3.0 program, and a heat map was generated using the Java TreeView program.

2.3 Target gene prediction

The miRNet [24] online database (https://www.mirnet.ca/faces/home.xhtml) was used to examine the pathways in which differentially expressed miRNAs were implicated. The database is a comprehensive atlas of miRNA-target interactions that can integrate the information resulting from 11 existing miRNA-target prediction programs (TarBase, miRTarBase, miRecords, miRanda, miR2Disease, HMDD, PhenomiR, SM2miR, PharmacomiR, EpimiR, and starBase). The software uses standard enrichment analysis based on the hypergeometric tests after adjustment for false discovery rate (FDR). In this study, to investigate the functional implications of miRNA deregulation in ARDS, we generated with miRNet pathway annotations a protein-protein interaction network of molecules targeted by at least three of our deregulated miRNAs which are directed toward at least 3 genes.

2.4 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

Enrichment of the deregulated miRNAs in the biological processes GO terms was performed using the online annotation tool mirPath v.3 on the DIANA Web site (http://snf-515788.vm.okeanos.grnet.gr/). In addition, the same miRNAs were analyzed using the KEGG database on the DIANA Web site. The statistical significance threshold level for both GO enrichment and KEGG pathway analyses was $P < 0.05$.

2.5 Statistical Analysis

Baseline demographic and clinical characteristics of patients, as well as the blood markers during ECMO are reported as medians and 25th and 75th percentiles for continuous data and frequencies, and

percentages for categorical data. All miRNA data are expressed as mean ± SD. Data from different clusters were compared using computerized statistical software with the ANOVA test. The data were further analyzed with Dunnett's t-test. Differences were considered statistically significant at $p < 0.05$. To detect differences in miRNA expression with respect to severity of ARDS, miRNA expression was compared between two groups (cluster A and B, which differed significantly for SAPS II, SOFA, and RESP score) using the two-tailed Mann–Whitney U test. Both sensitivity and specificity of miRNAs to discriminate between the two clusters were assessed with receiver operating characteristic (ROC) curve analysis. All confidence intervals are reported at 95%. All tests were two-sided, and a p value of <0.05 was considered indicative of statistical significance. Data handling and analyses were done with SAS 9.4 software (SAS Institute Inc, Cary, NC, U.S.A.).

3. Results

3.1 Patient characteristics, and stratification of ARDS severity by miRNA expression

From July 2018 to March 2019, 13 patients were supported by V-V ECMO for ARDS at our institution. In two cases the blood samples deteriorated during the laboratory phase, thereby leaving 11 patients available for adequate blood samples miRNAs analyses. Characteristics of the overall population are presented in Table 1. Causes of ARDS were bacterial pneumonia (n=6), viral pneumonia due to H1N1 Influenza A (n=4), and severe polytrauma with pulmonary contusion and super-infection with bacteria in one case.

To identify deregulated miRNAs associated with ARDS severity status, starting with a panel of 754 analyzed miRNAs, we identified 229 miRNAs after quality control screening (Figure 1). Among them, hierarchical clustering analysis showed systematic variations in the miRNA expression between two different groups, and produced a clear separation of patients into two clusters (Figure 2a).

Table 1. Patient demographics and clinical characteristics

Variable	Overall	Cluster A (n=6)	Cluster B (n=5)	p value (Cluster A *vs.* B)
Gender, N (%)	Male 9 (82%)	Male, 5 (83%)	Male, 4 (80%)	-
Age (years)	54 (44, 59)	51 (44, 59.5)	54 (53.5, 57)	0.84
Weight (Kg)	84 (68.5, 86.5)	80.5 (64.25, 84.75)	86 (83, 99.75)	0.48
Height (cm)	170 (163, 178)	168 (161.5, 173)	178 (175, 178)	0.53
BMI (Kg/m^2)	26.6 (24.6, 28.4)	26.4 (23.8, 27.42)	28.4 (27.47, 32.4)	0.58

SAPS II	36 (33, 51.5)	51.5 (40.5, 59.5)	35 (33.5, 36)	0.03
SOFA score	5 (3.5, 9)	9 (5.75, 10)	3.5 (3, 4.5)	0.03
RESP score	0.5 (-2.5, 4.75)	-2 (-3.75, -1)	4.5 (3.5, 5)	0.02
HOSP. LOS PRE-ECMO (days)	6 (2.5, 13)	10 (6.5, 13.5)	2.5 (1.75, 3.5)	0.63
ICU LOS PRE-ECMO (days)	3 (2, 6.5)	6.5 (3.75, 7)	2.5 (1.75, 3.5)	0.04
MV PRE-ECMO (days)	3 (1.5, 6.5)	6.5 (3.75, 7)	2 (1, 3.5)	0.03
PaO2/FiO2 PRE-ECMO (mmHg)	61 (57.5, 70)	60 (56.25, 60.75)	67 (60, 70)	0.26
Creatinin (mg/dl)	1.36 (0.7, 3.05)	2.08 (0.94, 3.75)	1.3 (0.9, 2.02)	0.34
Hematocrit (%)	30.5 (30, 38.8)	30.1 (28.5, 30.42)	36.6 (32.4, 40.67)	0.38
Bilirubin (mg/dl)	0.92 (0.62, 1.26)	0.89 (0.79, 1.15)	1.15 (0.86, 1.30)	0.94
Acute Kidney Injury, N (%)	6 (54%)	4 (66%)	YES 2 (40%)	-
CRRT, N (%)	6 (54%)	3 (50%)	3 (60%)	-
Septic Shock, N (%)	10 (90%)	5 (83%)	5 (100%)	-
ECMO Duration (days)	22 (14, 39.5)	29.5 (11.75, 39.75)	25 (16.5, 39.5)	0.74
ECMO Survival, N (%)	10 (91%)	5 (83%)	5 (100%)	-
LOS ICU POST ECMO (days)	30.5 (19, 46.75)	38.5 (22, 46.75)	19.5 (8.75, 51.25)	0.81
Total Hospital LOS	60 (31.5, 104)	75.5 (38.25, 108.25)	35.5 (31.5, 59)	0.54

Continuous variables are presented as median value (25th to 75th percentile range) and nominal variables are presented as absolute quantity (percentage).

BMI: body mass index; **SAPS II Score**: Simplified Acute Physiology 2 score; **SOFA score**; Sequential Organ Failure Assessment score; **RESP score**: Respiratory Extracorporeal Membrane Oxygenation Survival Prediction score; **LOS HOSP**: length of stay in hospital; **ICU**: intensive care unit; **MV**: mechanical ventilation; **PaO2:FiO2**: ratio of fraction of partial pressure of O2 to inspired O2; **AKI**: acute kidney injury; **CRRT**: continuous renal replacement therapy; **ECMO**: extracorporeal membrane oxygenation.

Cluster A, n=6, comprises patients with higher critical illness severity at baseline, while cluster B, n=5, with lower baseline severity, considering the baseline SAPS 2, SOFA, and RESP score, as well as the length of ICU LOS and duration of mechanical ventilation prior ECMO run (Table 1, $p < 0.05$). Furthermore, as expected by the disease severity, outcomes related to LOS, such as ECMO duration or hospital LOS, show a trend over a longer stay in cluster, A without reaching statistical significance (Table 1).

Then, after the screening, using volcano plot analysis (Figure 2b) for the deregulated genes comparing the two clusters, 95 deregulated miRNAs were identified, and notably, among them, 94 were upregulated, and 1 was downregulated in cluster A compared to cluster B (Figures 2c-d).

3.2 MiRNAs targets network construction

Starting from the 95 deregulated miRNAs, through an in silico analysis, we analyzed the association between ARDS-related pathways (regulation of tissue remodeling, regulation of immune system, regulation of blood coagulation) and deregulated miRNAs from the miRNet database, using miRNA-target interactions and functional associations through network-based analysis.

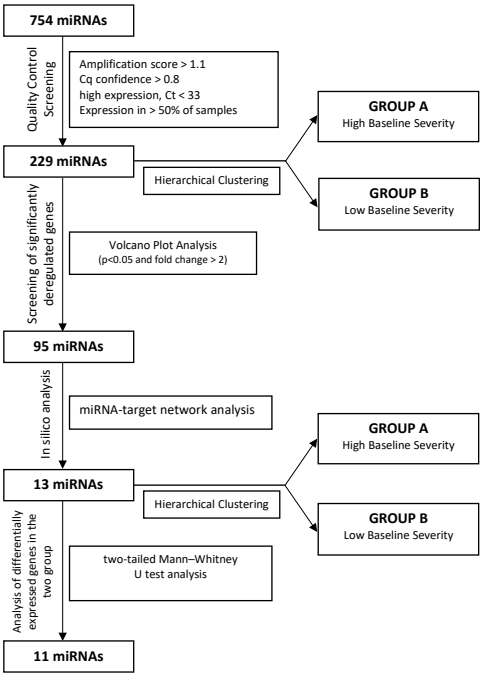

Figure 1. Flow chart illustrating the steps for quality control screening of miRNA expression.

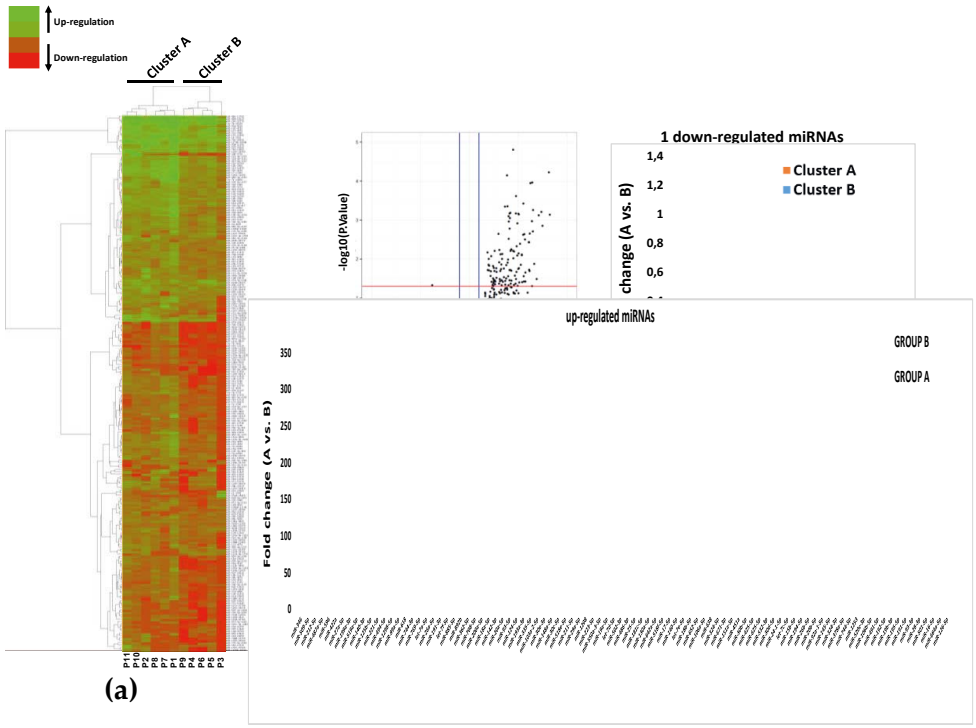

Figure 2. QRT-PCR analysis of miRNAs in ARDS patients. (a) Hierarchical clustering based on miRNA expression levels of 229 miRNAs in ARDS patients. Heatmap colors represent relative miRNA expression normalized to housekeeping. (b) Volcano plot analysis of deregulated miRNAs analyzed between patients in cluster A and patients in cluster B ($p \leq 0.05$ and fold change ≥ 2). (c) Down-regulated miRNAs in cluster A patients vs. cluster B patients. (d) Up-regulated miRNAs in cluster A patients vs. cluster B patients. MiRNA levels were normalized to those of U6. Data are means ± SD.

The 95 deregulated miRNAs were reduced to 13 and, among them, the same 11 miRNAs (miR-93, -99a, -92a, -212, -20a, -19b, -18a, -17, -126, -106b and-769) target genes implicated in all the pathways analyzed, miR-19a target genes implicated in both tissue remodeling and immune system pathways, whereas let-7a was exclusively implicated in blood coagulation pathways (Figure 3 and Table 2).

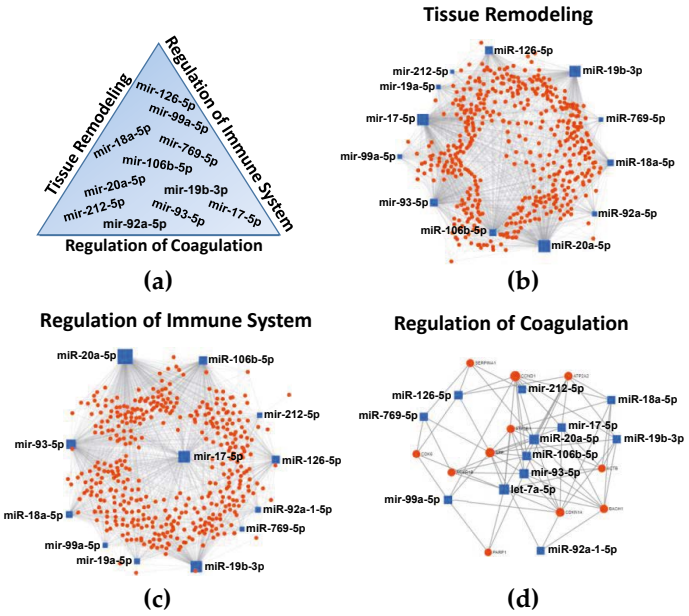

Figure 3. Protein-protein interaction network generated for shared miRNA target genes after miRNET analysis. Only genes targeted by at least three differentially expressed miRNAs are shown. (a) The top ranked deregulated miRNAs belonging to the three analyzed pathways. Images show networks with all interactions between deregulated miRNAs and genes involved in (b) tissue remodeling, (c) regulation of immune system, and (d) regulation of coagulation.

Table 2. Top ranked miRNAs and genes found in the network analysis.

Tissue Remodeling		Regulation of Immune System		Regulation of Coagulation	
TOP miRNAs	**Genes**	**TOP miRNAs**	**Genes**	**TOP miRNAs**	**Genes**
hsa-mir-20a-5p	223	hsa-mir-20a-5p	220	hsa-mir-20a-5p	9
hsa-mir-17-5p	218	hsa-mir-17-5p	194	hsa-mir-93-5p	9
hsa-mir-93-5p	177	hsa-mir-93-5p	169	hsa-let-7a-5p	8
hsa-mir-19b-3p	164	hsa-mir-106b-5p	159	hsa-mir-17-5p	7
hsa-mir-106b-5p	161	hsa-mir-19b-3p	131	hsa-mir-106b-5p	7
hsa-mir-126-5p	104	hsa-mir-126-5p	86	hsa-mir-19b-3p	7
hsa-mir-18a-5p	83	hsa-mir-18a-5p	74	hsa-mir-18a-5p	7
hsa-mir-92a-5p	49	hsa-mir-19a-5p	49	hsa-mir-126-5p	7
hsa-mir-19a-5p	45	hsa-mir-92a-5p	36	hsa-mir-99a-5p	7
hsa-mir-99a-5p	40	hsa-mir-99a-5p	33	hsa-mir-769-5p	7
hsa-mir-769-5p	33	hsa-mir-769-5p	29	hsa-mir-92a-5p	7
hsa-mir-212-5p	22	hsa-mir-212-5p	26	hsa-mir-212-5p	7

Furthermore, many genes had a potential relationship with both each other and with other miRNAs, as shown in Figure 3 and Table 3.

Table 3. Top ranked genes and miRNAs found in the network analysis.

Tissue Remodelling		Regulation of Immune System		Regulation of Coagulation	
TOP Genes	**miRNAs**	**TOP Genes**	**miRNAs**	**TOP Genes**	**miRNAs**
CCND1	9	CCND1	9	CCND1	10
CDKN1A	9	CDKN1A	9	CDKN1A	9
BTG2	9	BTG2	9	APP	9
APP	8	TNRC6A	9	BACH1	8
BMPR2	8	APP	8	ATP2A2	8
MDM2	8	BMPR2	8	ATP2B1	7
PMAIP1	8	MDM2	8	ACTB	6
ARHGAP5	7	PMAIP1	8	ACVR1B	5
ATP2B1	7	TNRC6B	8	SERPINA1	4
E2F1	7	PELI1	8	CDK6	4
EIF4G2	7	AGO3	8	PARP1	3
ELK4	7	CLTC	8		
GABBR1	7	ARHGAP5	7		
MAPK1	7	ATP2B1	7		
VEGFA	7	E2F1	7		
TNFRSF10B	7	EIF4G2	7		
MAP3K2	7	GABBR1	7		
SSX2IP	7	MAPK1	7		
SESN3	7	TXNIP	7		
CALM1	7	FRS2	7		

For better visualization, we defined the proteins that are likely to interact with at least n=3 miRNAs. These thirteen miRNAs (miR-93, -92a, -769, -99a, -212, -20a, -19b, -18a, -17, -126, -106b, -19a and let-7a) were considered as candidate biomarkers, and subjected to further analysis. QRT-PCR showed that these miRNAs were significantly up-regulated from 5- (miR-126, $p=0.04$) to 163- (miR-212, $p=0.0006$) fold in cluster A compared to cluster B (Figure 4a). Interestingly, hierarchical clustering analysis of these miRNAs revealed the same grouping (cluster A and B) (Figure 4b) already observed in the first cluster analysis of initial 229 miRNAs (Figure 2a).

3.3 Enrichment of the deregulated miRNAs in biological processes

We performed both GO enrichment and KEGG pathways analysis with the DIANA database. In the biological process category, among the top 30 GO terms (Figure 4c), all thirteen miRNAs were related to crucial processes potentially involved in the pathogenesis of ARDS. In particular, different terms (e.g., "cellular nitrogen compound metabolic process," biosynthetic process," "response to stress," "EGFR signaling pathway," and "cell death" terms) are involved in the regulation of tissue remodeling. Furthermore, "blood coagulation" and "platelet activation" terms were involved in the regulation of coagulation, while "immune system process" and "innate immune response" terms were involved in the regulation of the immune system. The most enriched KEGG pathways comprising terms also involved in the aforementioned biological processes are summarized in Figure 4d.

3.4 Deregulated miRNAs are associated with the severity of ARDS patients

To explore the clinical relevance of the deregulated miRNAs in ARDS, the thirteen miRNAs were subjected to clinical characteristics based on publicly available online applications. Consistent with the qRT-PCR results, significantly higher expression levels of thirteen miRNAs were observed. In particular, miR-93, -99a, -92a,-212, -20a, -19b, -18a, -17, -126, -19a, and let-7a were significantly up-regulated ($p < 0.05$) in ARDS patients with high severity (cluster A) compared to patients with low severity (cluster B). Instead, for miR-106b and miR-769, though the up-regulation trend was consistent with the qRT-PCR data, the difference in expression level did not reach statistical significance (Figure 5).

In order to test whether clusters A and B were categorized by common indicators to evaluate ARDS patients' clinical illness condition, we analyzed SAPS II, SOFA, and RESP score by ROC analysis, showing that the AUC value was 0.85 ($p=0.02$), 0.83 ($p=0.01$) and 0.86 ($p=0.006$), respectively (Table 4).

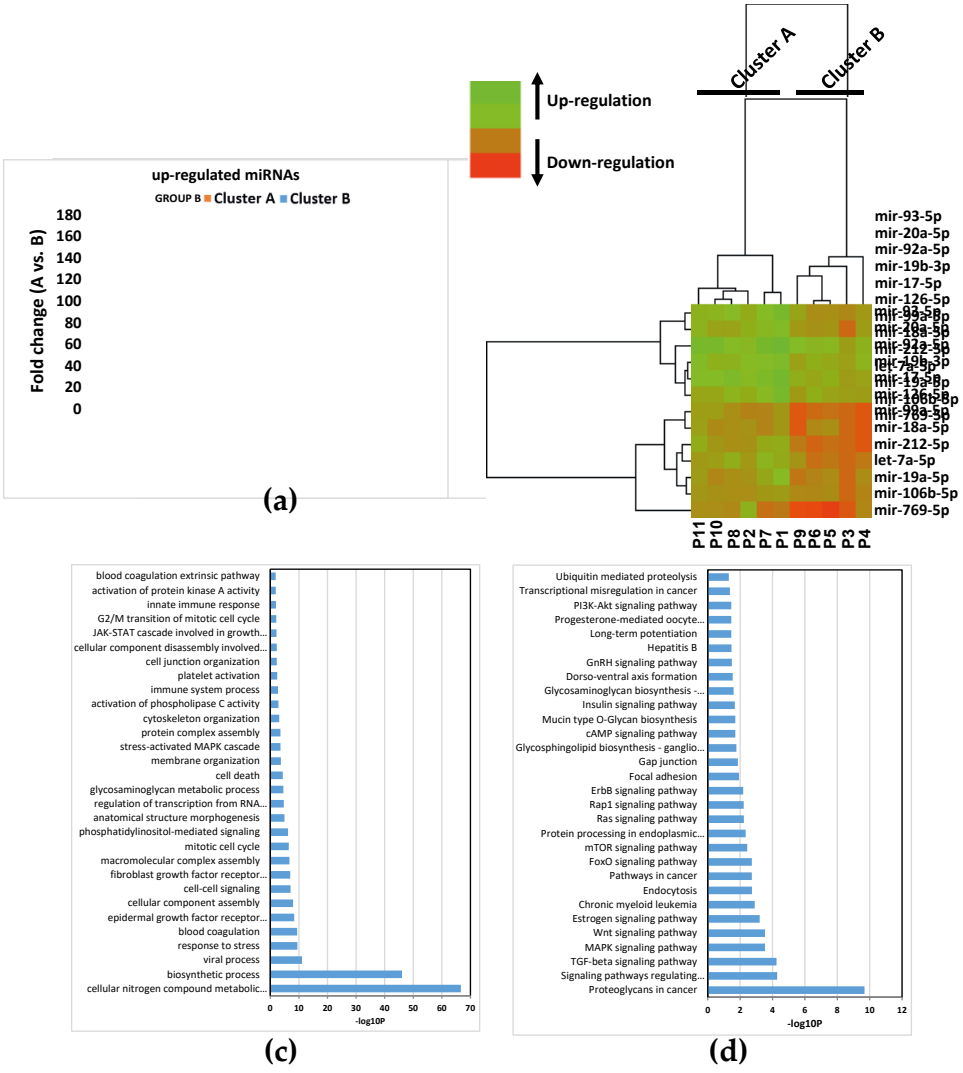

Figure 4. QRT-PCR, GO and KEGG analysis of thirteen miRNAs up-regulated in cluster A vs. cluster B. (a) Up-regulated miRNAs. (b) Hierarchical clustering based on miRNA expression levels of 13 miRNAs in ARDS patients. Heatmap colors represent relative miRNA expression normalized to housekeeping. (c) GO (partial list of biological process) enrichment analysis of thirteen up-regulated miRNA. (d) KEGG pathways (partial list) enrichment analysis of thirteen up-regulated miRNA.

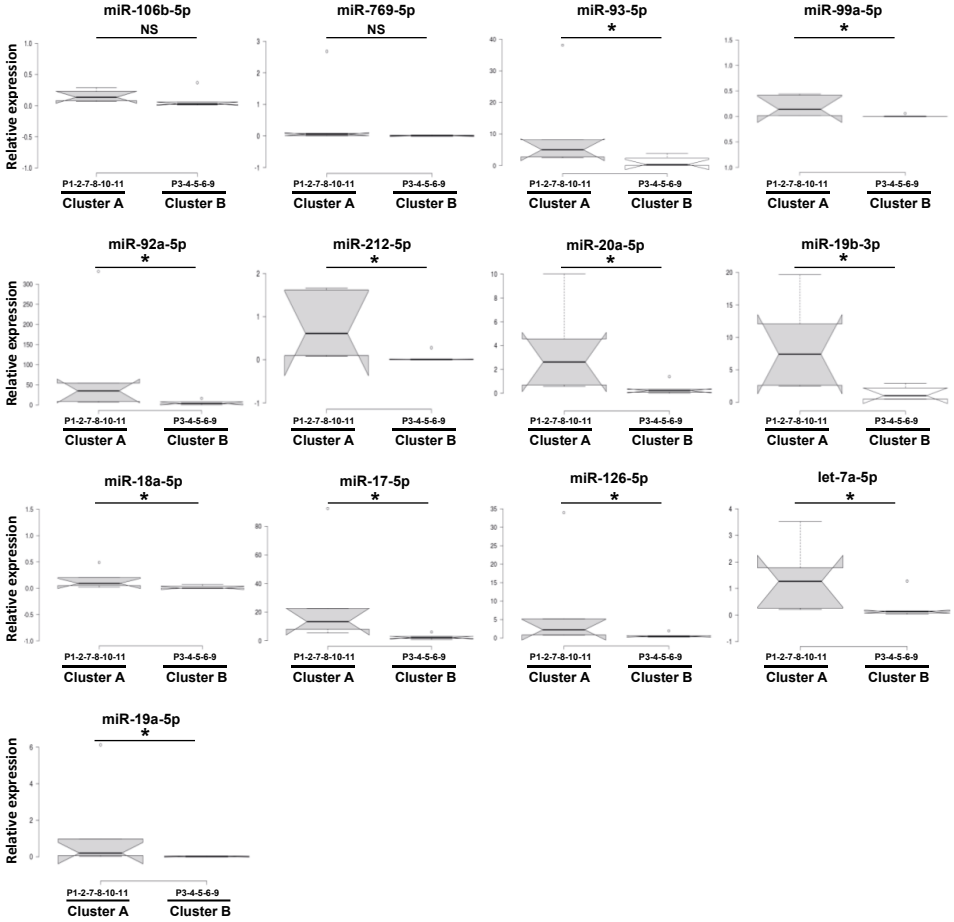

Figure 5. Blood expression levels of thirteen miRNAs in cluster A and cluster B. Data presented as expression relative to U6 (internal control). Box plots are displayed where the horizontal bar represents the median, the box represents the IQR, and the whiskers represent the maximum and minimum values. Comparisons made by Mann–Whitney U test. *p<0.05.

Table 4. Area under the curve (AUC) (95 % confidence interval) for individual severity score of ARDS and for outcome indicators.

Marker	AUC	95% CI for AUC	p-value
SAPS II	0.85	0.55-1	<0.05
SOFA	0.83	0.56-1	<0.05
RESP score	0.86	0.60-1	<0.001
ECMO DURATION	0.51	0.13-0.90	0.93
LOS ICU	0.76	0.42-1	0.12
LOS HOSP	0.53	0.15-0.91	0.86

Also the ability of the thirteen miRNAs (miR-93, -99a,-92a, -212, -20a, -19b, -18a, -17, -126, -106b, -769,-19a and let-7a) to categorize patients with low and high severity was studied. The ROC analysis revealed that the AUC values for individual miRNAs were predictive for distinguishing between low and high severity, with an AUC ranging between 0.80 and 0.96 (Table 5).

Table 5. Area under the curve (AUC) (95 % confidence interval) for individual miRNAs.

Marker	AUC	95% CI for AUC	p-value
miR-93-5p	0.86	0.63-1	<0.01
miR-99a-5p	0.93	0.78-1	<0.01
miR-92a-5p	0.90	0.71-1	<0.01
miR-212-5p	0.90	0.68-1	<0.01
miR-20a-5p	0.93	0.78-1	<0.01
miR-19b-3p	0.93	0.78-1	<0.01
miR-18a-5p	0.88	0.68-1	<0.01
miR-17-5p	0.96	0.87-1	<0.01
miR-126-5p	0.90	0.68-1	<0.01
miR-106b-5p	0.80	0.40-1	0.13
miR-769-5p	0.86	0.63-1	<0.01
let-7a-5p	0.86	0.59-1	<0.01
miR-19a-5p	0.93	0.78-1	<0.01

4. Discussion

In this pilot study we screened 754 circulating miRNAs extracted from the blood of ARDS patients before ECMO cannulation. We found a select pattern of miRNA expression capable of separating patients into two cluster that differ in baseline ARDS severity and, between these clusters, we performed an analysis of the differentially expressed miRNAs.

The current literature is shedding light on different biomarkers to characterize ARDS patients based on the hypothesis that there are distinct sub-classes within a broader group of subjects included in the same clinical definition. In fact, ARDS, as a syndrome, has not found a clearly effective treatment, if we do not consider specific treatments the low volume ventilation, the use of neuromuscular blockade, and prone position that have been demonstrated to reduce mortality [25-27]. Furthermore, the findings of different randomized controlled trials or non-randomized interventional trials that administer the hypothesized effective treatment to a cohort of clinically defined patients, did not reach statistical significance [28-30]. One possible explanation is the different response to treatment found in patients

with different activated biological pathways. In this framework, to increase knowledge of basic biological patterns in ARDS patients will be fundamental to test future treatments, even the more advanced are.

The main achievement in ARDS studies in recent years was the definition of the "inflammatory" and "non-inflammatory" subphenotypes [31]. Recently, this concept has also been tested efficaciously on COVID-19 patients, but the approach, also with combined markers (biological and clinical, like vasopressors) still lacks complete biological explanation [31]. One element that can be explored relatively easily is miRNAs, small non-coding RNA able to modulate specific pathways. Differently from other studies, we attempted to associate miRNA expression with the severity of disease, potentially contributing to define the prognosis, as well as evaluate treatments put in place. Our approach started from the miRNA clusterization, and from this point on defined the two cluster of patients different for the baseline severity characteristics. This approach, was able to define the cluster of miRNA without biases due to the status of the patient, and in our opinion, has strengthened the results.

Currently, knowledge of miRNAs in ARDS is still very preliminary, but miRNAs have been demonstrated to play a relevant role in both physiological and pathophysiological human processes. Different studies have shown that the levels of certain circulating miRNAs involved in inflammation, angiogenesis, and cardiac muscle contractility are modified by the intensity and length of exercising, thus indicating that they might play a role in specific physiological processes [32, 33]. Furthermore, Zhou et al. described miRNAs as potential biomarkers for cardiovascular disease [22], while Lawrie et al. first utilized miRNAs as biomarkers for cancer, in 2008 [23]. MiRNAs also play an important role in the pathogenesis of lung diseases, including ARDS [34-38].

To analyze the potential relationship between miRNAs and the pathogenesis of ARDS, we focused on three main processes potentially involved in ARDS pathogenesis: regulation of tissue remodeling, regulation of the immune system, and regulation of blood coagulation [9]. Accordingly, we analyzed the association between these pathways and miRNAs, showing that 13 deregulated miRNAs (miR-93, -99a, -92a, -212, -20a, -19a, -19b, -18a, -17, -126, -106b, -769 and let-7a) target genes implicated in aforementioned pathways (Figure 3 and Table 2). Interestingly, these 13 miRNAs were previously implicated in ARDS [34-36, 39, 40], and different report showed that these miRNAs clearly infuences cell cycle progression/proliferation (which can potentially regulate tissue remodelling) [41-44], inflammation [45, 46], and coagulation processes [47, 48]. These data were confirmed by GO enrichment and KEGG pathway analysis (Figure 4C-D). Figure 5 show that these miRNAs were up-regulated in cluster A compared to cluster B, though there are no statistical differences for both miR-106b-5p and miR-769-5p when analyzed with Mann–Whitney U test ($p>0.05$). Furthermore, using ROC analysis, our investigation of specificity and sensitivity revealed that these miRNAs (although miR-106b-5p is not significant, $p>0.05$) are predictive for distinguishing between low and high ARDS severity, with the AUC values for each individual miRNA ranging between 0.80 and 0.96 (Table 5). Furthermore, hierarchical

clustering analysis of these miRNAs revealed the same grouping (cluster A and B) observed after the first screening, confirming the same data with a different analysis. These results just confirmed that high expression levels of miR-93, -99a, -92a, -212, -20a, 19a,-19b, -18a, -17, -126, -106b,-769, and let-7a were potentially associated with higher severity of ARDS. Our results indicate that miRNAs are promising biomolecules potentially useful for improving diagnosis, and better stratifying ARDS patients. This hypothesis is supported by fact that different and independent studies provide evidence for the involvement of these miRNAs in dysregulated ARDS signaling pathways [35, 38, 49-51], being implicated in endothelial and/or epithelial cell function, as well as in the regulation of inflammatory responses [52-60] and in the regulation of coagulation [61-65].

Our focus on the baseline values allowed us to categorize patients independent of the outcomes, which is important in recognizing the effective existence of different subgroups. Interestingly, the strength of our results resides in the fact that, with all the caveats of a limited number of cases, our patients were effectively divided into clusters.

However, we also acknowledge the limitations of our study: first, the study population was small despite being part of a peculiar setting of severity (ECMO patients) and the female gender is poorly represented consequently the miRNA may be biased by the high prevalence of males; second, in the period when miRNA assessment was available, ECMO was particularly effective; consequently, in terms of survival, this group of patients might not be completely representative of the general V-V ECMO population. Moreover, our results were only based on 229 (out of 754) miRNAs that passed stringent QC criteria. It is possible that some miRNAs that did not pass QC are functionally related to ARDS. Finally, ARDS is considered a complicated syndrome with multiple etiologies, so a single or a few miRNAs might not evidence strong signals for all ARDS patients.

5. Conclusions

This pilot study shows that the blood levels of 13 miRNAs, strongly related to biological pathways possibly associated with ARDS, are altered in patients with severe ARDS, and may offer diagnostic value as well as contribute to ARDS stratification. Further investigations will have to match miRNA results with inflammatory biomarkers and clinical data to confirm these preliminary observations.

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Chapter 10

General Discussion

ECMO is a resource-demanding procedure because it involves the use of highly expensive technology, with continual improvement and product refining, but also because it relies on the application by specialized providers, the so-called ECMO specialists, in dedicated centers with high volume of activity either for V-V or V-A configuration.[1,2] This feature derives from the complexity of patients, disease, and device and its management. [3,4] In this light, ECMO has peculiar potential complications, para-physiological values, and potential challenges. [5] Some of these are the focus of this dissertation, which aims to illustrate data in a single center, from propensities worldwide, and the vast literature. The topics taken into account are critical ones, and still debated; but all of them should be assets available at any ECMO center.[6] In fact, the number of patients is increasing, as are the number of centers, but every center should try to define its management practice, and aim for innovation and life-long learning experience since the ECMO devices and available applications are constantly increasing. [7]

A relevant field that has to be explored is the characteristics of the patient and their complex interaction with ECMO, and the consequences on the hematological side. Looking at the severe respiratory failure, the LUNG SAFE study on patients affected with the severe form of the acute respiratory distress syndrome (ARDS) in 50 countries, found a mortality of 46.1%.[8] These data require a global intervention, and ECMO may be one of the contributing tools. [9] In fact, the ventilator-induced lung injury (VILI), has been reduced over years by the application of protective ventilation, but in the most severe patients it is still present and contribute to the severity of the patients' picture, mainly when the patient has a very low compliance and high pressures are reached continuously in the airways. VILI is the paramount lung injury due to invasive mechanical ventilation, and determined by multiple factors like alveolar over-distension, excessive positive pressure ventilation and continuous opening and closing of alveoli and local (in the lungs) and systemic inflammation. For the treatment and prevention of VILI in case of ARDS, ECMO is still an appealing approach since it allows to apply protective or even ultra-protective ventilation. The common syllogism would infer that in patients with the same severity (ARDS with relevant gas exchanges derangements) and with the same pathophysiological process (VILI) should have the same outcomes. This reasoning does not take into account personal patient's characteristics and the underlying disease. Therefore, as we showed in our pilot series on microRNA, the same patients on ECMO have different underlying physiological patterns of severity, and also underlying signal strategies like the miRNAs. [10] This is one of the most interesting and promising topics raised in recent decades: the definition of subphenotypes among ARDS patients to answer to the question *why there are different clinical trajectories and different outcomes within the same syndrome.* [11] Therefore the answer is already in the Berlin definition of ARDS, a very useful definition from the clinical standpoint since it unifies and integrates the clinical picture and the management via the

presence of the application of a PEEP of at least of 5 cmH2O, but convenient also for the research side since it allows clinicians to unify patients in more uniform studies. [12] But the definition describes a syndrome, which is likely triggered by a number of different causes that act via the immune response, the host inflammatory and counter-inflammatory responses, and several cross-talking pathways that have the lungs as the effector and cause interstitial edema, that associated with the iatrogenic effect of the ventilator induced lung injury finally results in the catastrophic ARDS picture. [13-15] In this sense, the proposed differentiation between pulmonary and extra-pulmonary ARDS, or the ones based on clinical risk factors may not reach the goal. [16] As has been seen in sepsis, as we recently verified with COVID-19, probably, it is likely also in ARDS more than the *"cytopathic"* effect of the cause of the disease is the host response to act in an essential role in the development of the syndrome. [17] Subphenotypes, biomarkers, microRNA might shed light on this field and contribute in the near future to follow up the disease, test interventions and, unfortunately, also recognize earlier the patients with no chances of survival, even with ECMO. [18-21] This last consideration is not just trivial or speculative, in fact, in a pandemic scenario, like the one we are living since 2020, due to a steep increase in ARDS incidence, ECMO requirements may overcome the availability of ECMO-capable facilities. In this light, and with the concrete risk of further upcoming pandemic respiratory diseases, to have tools to evaluate more precisely the severity and the chances of survival will be paramount to allocate in a proper way such a useful resource.

As stated by the common thread of this dissertation, the precise approach is important to take into account also for complications and the possibility to treat them in a non-invasive fashion may contribute to enlarging the applications of the ECMO with broader indications (and fewer contra-indications). [22-25] The innovation in terms of materials, techniques, and procedures is part of the precise management, and with the current fast spreading of knowledge worldwide, is able to reach even peripheral centers very far from the proposing center of a technique. [26,27] All this also has concrete implications for the current pandemic scenario. [28] In fact, after a review of available early data, in COVID-19 the disruption of the coagulation system and its implications for inflammation in a multi-organ involvement, determines an altered coagulation profile that is too simplistic to define as a simple pro-coagulant pattern; therefore, the simple use of heparin in continuous infusion was not able to reduce mortality and even respiratory failure, but it represents anyway a mainstay of the treatment in the most severe cases. [29-31] The parallelism between COVID-19 coagulation/inflammation patterns and ECMO has been highlighted, and demonstrates how the large amount of data available in ECMO patients may be indirectly critical for a large number of critically ill patients.

After considering the patient and the disease the patient is affected with, the critical issue is the device. It should be considered in itself and in the circulating fluid, the blood. [32] In the circulating blood, the

critical issues are hemoglobin as the carrier of oxygen to peripheral tissues, but also as the circulating mass that allows the pump to develop a flow, the anticoagulation, which is critical in managing the interfacing of cells with an extracorporeal surface, and inflammation, which is strictly linked to the above. [33,34] All this should be accompanied by the prevention and timely and adequate treatment of the complications related to the procedure. If in critically ill patients, with several studies describing the worse effects of liberal transfusion practice, and with patients, at least currently in Europe according to the recent data, being less transfused than in the past, we have to consider that is still unclear whether a restrictive strategy is recommended in patients with comorbidities and in the most severe cases.[35-37] Hemoglobin is a major determinant of the arterial oxygen content and consequently also of the oxygen delivery (DO_2). Saying that, red blood cells (RBC) transfusion is the widespread strategy to increase the DO_2 during ECMO support, either in the V-V or in the V-A configuration. Furthermore, since ECMO patients experience frequently episodes of bleeding (even during the most simple and common procedures) the transfusion of RBC represents one of the most performed and repeated actions during an ECMO run. Just because of that and because PRBC transfusion is not free from adverse events, it should be performed with the most possible integration of several parameters, and with a further step of self-reflection in any case a transfusion is apparently needed. Since that, the research of a restrictive strategy in transfusions during ECMO is still ongoing. In a single center experience we demonstrated the feasibility and safety of a restrictive approach balanced by the use of several clinical variables.[38,39] The tolerance of lower hemoglobin values (up to 8 mg/dL) was linked to a very reduced transfusion amount and an increasing mortality associated with increase of transfusions. [40 41] At the same time, other methods should be adopted to reduce the blood waste (generally and daily), and the current undisputable role of red blood cell transfusion is related to cases with hypoxia unresponsive to maximal ECMO flow, when the increase in hemoglobin is absolutely critical to increase the venous and, consequently, also the arterial oxygen content.

This is a debated question, and many authors and stakeholders are waiting for the holy grail of the next randomized controlled trial on transfusions in ECMO. But the amount of baseline data is so scant that trials with this aim are destined to fail. On the topic of transfusions, if we do not imagine a health care process just managed by blood bank related to the medical charts identifying the patients with a low hemoglobin level, the community needs large and thorough observational data to understand and to share the *"how to do"* and *"how others do,"* what is possible, and what the effects of local protocols are. This is reflected also in the research field on the topic. Recently, a retrospective study illustrating the hemoglobin trigger for red blood cells transfusion in ARDS patients, compared the 28-day mortality between patients with two arbitrary hemoglobin concentrations utilized as trigger (8 g/dl versus 10 mg/dl). [42] The conclusion is that a lower hemoglobin trigger was not associated with an increase in 28-day mortality, but these results were achieved via an exclusion of a number of patients from a large

cohort: patients without transfusions (they are often the classic example of deviation from the protocol), and patients with an effective trigger intermediate between the two. The conclusions are similar to our hypothesis, but rely on a cohort derived from the exclusion of a number of patients, and considering in the same study ECMO and non-ECMO patients, opening the discussion to the many biases, and low generalizability of the results. Unfortunately, that's the picture of cohort studies in ECMO, often mixed patients with respiratory and cardiac indication with a mixture of different cases and often the impossibility to define clear outcomes. For that reason our group has conducted a large multicenter international study (PROTECMO study) to understand the real practice on transfusions, anticoagulation management and bleeding rate in different centers worldwide and hypothesize, this time with more reliability, strategies to be tested in interventional studies.

On this topic, as well for the others, the term precision has a distinct meaning. Precision for ECMO means innovations in terms of technology and knowledge. [43] [7] Precision is also precision medicine, to direct our efforts through the recognizing of patients at higher probability of benefitting from this type of support, accompanying the ones destined to die in a more dignified way. [18,44] In this fashion, precision means also ethics and the ability and responsibility of avoiding futile treatment, with higher social costs and false expectations.[45] But precision is also teamwork, a process of continual learning and the use of all the resources available in the hospital, with a multidisciplinary approach among practitioners aware of the specific ECMO features. [46,47] It is also important to collect biological data and features from different fields, e.g., the question of coagulation, which may be well known by other specialists with advanced monitoring systems that are starting to be used, like the viscoelastic tests (rotational thromboelastometry and thromboelastography) that, despite the fact that they are probably inapplicable in routine management, may help in cases of challenging hemorrhagic or thrombotic conditions. [48,49] This would help in decipher a crucial topic in ECMO since the same mechanisms are at the base of alterations of the hemostasis in the sense of thrombosis and bleeding: direct contact between blood and non-biological surfaces, shear stress and patient factors. In the aim of this thesis anticoagulation couldn't be neglected. We approached the topic through antithrombin, the real effector of heparin activity, highlighting how this derived blood product (it exist also in a recombinant form), may have several potential positive features adapt to the ECMO practice. In fact its anti-inflammatory properties and its action in protecting the glycocalyx and endothelium may reduce the systemic inflammatory response syndrome typical of the patient on ECMO. Current studies focused more on the anticoagulant effect of antithrombin and on potential reduction of heparin dose by its supplementation. Starting from the evidence in patients with heparin resistance, our narrative review highlight the role of antithrombin in contribute to equilibrate the balance between anticoagulation and pro-coagulation status, with effects also independent from the dose or presence of heparin.

In conclusion, ECMO is definitely complex and challenging for the health care system and for the care of any single case. Its use is seeing a steep ascending trajectory worldwide, and its effect has been demonstrated to be able to reduce mortality in the most severe cases of respiratory failure and cardiogenic shock. [50] The occurrence of COVID-19 has contributed to further increasing its application, but this new pandemic as well as adding applications of extracorporeal technology like the bridge to heart and lung transplantation and the trauma, burn, and post-operative settings have highlighted how the knowledge of inflammatory and general changes during ECMO is paramount in adequately using the machine. [51,52]

Pragmatist philosophy is based on the concept of continuity between experience and knowledge in a profound unity, as a fundamental base. Medical research and advancement fits very well into this approach, and the application of a new and demanding technology like ECMO may benefit by the use of a complete synthetic pattern to progressively substitute preceding "gestures" with unprecedented and better performing "gestures." In an evolutionary view of the ECMO science, from the seventies, the first 30 years were dedicated to the "invention" of the procedure, from the 2000s the subsequent twenty years were dedicated to a proper function of the gas exchanges and adequate flow of the blood in the circuit. The new era will gather all the knowledge into one to increase the rate of survival by precise application and management, taking advantage of transparent and ethically sound use of new technology provided by the industry.

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Chapter 11

Impact

This thesis deals with several unresolved challenges in the field of ECMO.

In the case of V-V configuration, the principal challenges are the decisions on anticoagulation and transfusion thresholds, which are discussed from different sides. In the field of anticoagulation, a protocol based on heparin administration at a low-dose (and stopped in case of any bleeding), accompanied by daily supplementation of antithrombin has been proposed, as well as a tolerance of thrombocytopenia and case-based decisions regarding platelet transfusion according to the risk of bleeding more than rather the mere number of platelets. In this large series, one of the lower heparin mean doses was reported, despite the fact that the total number of bleedings was not lower than the one reported in the literature, the number of severe and fatal bleedings reported was low. Moreover, strictly linked to this approach the transfusion practice for PRBC was also proposed. This was proposed with a lower trigger, and mainly was considered always in the light of several clinical considerations related to the patient, concomitant comorbidities or clinical conditions (e.g., septic shock), and also taking into account the function of the ECMO circuit, which, it should always be remembered, is a modified cardiopulmonary bypass system that has no reservoir other than the patient. This protocol and these concepts were also a source of inspiration for other centers to define protocolized optimization of blood flow and, as reported in a recent meta-analysis, was able to reach very successful outcomes in terms of survival.

As said, one of the key component of the anticoagulation protocol was antithrombin. This blood-derived component was of interest in sepsis management in the 1990s, but lately has been removed from daily practice and interventional trials because it is an expensive drug and (with methodological biased studies) it was impossible to gather solid evidence in favor of its use. But as we highlighted in our review, ECMO may be the field of concrete application of this blood product. In fact, currently the standard of care in terms of circuit anticoagulation is heparin, which needs antithrombin. In cases of heparin resistance in the cardiopulmonary bypass, the restoration of antithrombin levels is the treatment of choice. In pediatric ECMO there is vast experience in the use of that and, finally, as we largely elucidated, antithrombin is a link between inflammation and anticoagulation also at a genomic level, with many microRNA involved in its synthesis and in the coagulation process. This may contribute to the preservation of the endothelium, which is heavily damaged when the blood flows through an extracorporeal circuit.

Further investigations regarding the ECMO circuit highlighted the impact of the use of shorter and bioline-coated cannulas on the transfusion of PRBC. This observation stresses the concept of patient-machine fluid interaction in a wide vision. The amount of fluids administered should be linked to the hemoglobin available and to the inflammation and damage of the cellular part of the blood.

The hemoglobin level accepted in ECMO has been explored also by an international survey endorsed by the European Society of Intensive Care Medicine. With all the caution that the use of a survey should be taken with, this survey aimed at covering the knowledge gap about the current use of hemoglobin triggers. In fact, in critically ill patients the comparison between the ABC study and the ICON study at a distance of about ten years has demonstrated that the hemoglobin trigger is lower today compared with the past, and currently ICU patients are transfused (at least in Europe) less frequently, but are also older and sicker. Among the sicker patients there is certainly also the increased number of ECMO patients. Our survey highlighted how, in ECMO, the tolerated hemoglobin is lower than in the past, but currently higher than the last recommendations for general critically ill patients. This was also confirmed in 2020 by the meta-analysis by Abbasciano and collaborators, with a select number of studies.

Strictly related to hemoglobin management there are the episodes of bleeding during ECMO, and among them gastro-intestinal bleeding is one of the major ones. The occurrence of stress ulcer is frequent in critically ill patients due to altered gastrointestinal perfusion, drug administration, and imbalance of the gastric acid production, but when it occurs in ECMO it can become an uncontrolled bleeding, prompting an increase of blood product transfusion, impairing the ability to use adequate anticoagulation and, finally, increasing mortality. The use of advanced endoscopy in our series was able to arrive at a mortality comparable to that of the ECMO population without gastro-intestinal bleeding. The management of complications in proper way is one of the key components for fostering increased survival in higher volume centers.

Finally, in peripheral V-A ECMO, one of the unsolved questions is the occurrence of limb ischemia in the leg, where the arterial cannula is placed. In our thorough review of the literature, the risk factors as well as the need for adequate planning and prevention are highlighted, and an operative algorithm is proposed.

All these considerations taken together may contribute to a more precise ECMO management, and contribute to better outcomes in this resource-demanding procedure. All the explored topics will need further data gathered worldwide to build the current practice of ECMOlogy. All the studies collected in this thesis were fundamental pieces in creating a prospective multicenter observational study, the PROTECMO study, which is focused on transfusions, anticoagulation management, and type and severity of bleeding, and that will arrive at its first results in the near future.


PROTECMO
Study

Chapter 12

Summary

Chapter 1. This chapter is a general introduction to the entire thesis. Here the topic of the changing discipline of ECMO is introduced, and the several unresolved questions in this field are reported, as well as an outline of the relevant studies included.

Chapter 2. In this chapter we present a single-center experience in the management of anticoagulation and transfusions in V-V ECMO. Here is presented a protocol for anticoagulation based on low-dose heparin, low anticoagulation range based on frequent measurement of activated partial thromboplastin time and a tolerance for lower hemoglobin levels, considering the transfusion a medical act that should be considered only in the wider context of the patient's condition and not just related to a trigger. The results showed one of the lowest available mean dosages of heparin infusion during ECMO, and a significant overall survival, which was significantly better in patients with lower amount of PRBC transfused.

Chapter 3. This study is a single center retrospective analysis of different effects of changing some part of the ECMO circuit. In this case we compare a group with a standard cannulation and circuit and the following group where shorter bioline-coated cannulas were applied. In this case we saw a significantly lower amount of transfusions, and a trend toward shorter ECMO stays in the bioline group, with an associated reduction in costs despite the more expensive cannulas.

Chapter 4. In this chapter we illustrate the results of a voluntary Web-based survey, endorsed by the European Society of Intensive Care Medicine, on the hemoglobin trigger for transfusions worldwide. Among 447 respondents, the majority did not use a definitive hemoglobin trigger, but when they used one it was higher than for other critically ill patients. Interestingly, there was a significant difference in the hemoglobin trigger adopted according to the volume of the centers, with higher volume centers tolerating lower hemoglobin values.

Chapter 5. Limb ischemia is a possible complication of peripheral V-A ECMO. In this review we illustrate the incidence and the risk factors for this complication, analyzing the wide range of the recent relevant literature. The main conclusion is that in V-A ECMO, limb ischemia should be prevented and eventually rapidly suspected and treated with a protocolized approach. The mainstay to prevent limb ischemia seems to be a distal perfusion cannula and a dual flow cannula. We also present operative flow-charts to monitor and treat limb ischemia gathered from data in the literature.

Chapter 6. Gastrointestinal bleeding is one of the more frequent complications in critically ill patients under ECMO, and is burdened with higher mortality. In this single center retrospective study we demonstrate that advanced endoscopy management, also including the Hemospray for hemostasis, can contribute to reach a mortality rate similar to the other cohorts of patients on V-V ECMO, despite the increased use of blood products.

Chapter 7. In this narrative review, the use of antithrombin in ECMO is explored. Antithrombin had its day in the sun in the previous decade in cases of sepsis, since it is a relevant link between coagulation and the inflammatory cascade. In fact, beyond being the effector needed for heparin anticoagulation activity, it has anti-inflammatory properties, and is able to protect the endothelium, both activities eventually positive during the ECMO course. High costs, the risk associated with human blood products, and the absence of standard evidence in RCT have restrained its diffusion.

Chapter 8. This is a case series of intra-abdominal hypertension developed in three patients under V-V ECMO support. The case series highlight the issue of intra-abdominal hypertension during ECMO and its effect on the ability of dreinage cannula, describe a novel non-invasive approach through advanced endoscopy (total water-assisted colonoscopy) and illustrate a systematic review of the literature on the topic. A practical flow-chart to approach early and non-invasively the issue of intra-abdominal hypertension with its peculiar effects in ECMO has been also proposed.

Chapter 9. In a pilot study the potential circulating miRNA signature in patients affected with ARDS and lately supported on ECMO is illustrated with the aim of better characterize ARDS patients in their severity and, possibly, in their prognosis. As promising results, the blood levels of 13 miRNAs, strongly related to biological pathways possibly associated with ARDS, are altered in patients with severe ARDS, and may offer diagnostic value as well as contribute to ARDS stratification.

Chapter 10. This chapter is a general discussion of the entire work, were the main topics are summarized and considerations of precision medicine and precision management are illustrated.

Chapter 13

Samenvatting

Hoofdstuk 1. Dit hoofdstuk is een algemene inleiding op het gehele proefschrift. Hier wordt het onderwerp van de veranderende discipline van ECMO geïntroduceerd en worden de verschillende onopgeloste vragen op dit gebied gerapporteerd, evenals een overzicht van de relevante opgenomen onderzoeken.

Hoofdstuk 2. In dit hoofdstuk presenteren we een single-center ervaring in het beheer van antistolling en transfusies in V-V ECMO. Hier wordt een protocol gepresenteerd voor antistolling op basis van een lage dosis heparine, een laag antistollingsbereik gebaseerd op frequente meting van de geactiveerde partiële tromboplastinetijd en een tolerantie voor lagere hemoglobinespiegels, aangezien de transfusie een medische handeling is die alleen in de bredere context van de toestand van de patiënt en niet alleen gerelateerd aan een trigger. De resultaten lieten een van de laagste beschikbare gemiddelde doseringen van heparine-infusie tijdens ECMO zien, en een significante algehele overleving, die significant beter was bij patiënten met een lagere hoeveelheid PRBC-transfusies.

Hoofdstuk 3. Deze studie is een retrospectieve analyse vanuit één centrum van verschillende effecten van het veranderen van een deel van het ECMO-circuit. In dit geval vergelijken we een groep met een standaard canulatie en circuit en de volgende groep waar kortere bioline-gecoate canules werden aangebracht. In dit geval zagen we een significant lager aantal transfusies, en een trend naar kortere ECMO-verblijven in de biolinegroep, met een bijbehorende kostenverlaging ondanks de duurdere canules.

Hoofdstuk 4. In dit hoofdstuk illustreren we de resultaten van een vrijwillige webgebaseerde enquête, goedgekeurd door de European Society of Intensive Care Medicine, naar de hemoglobine-trigger voor transfusies wereldwijd. Van de 447 respondenten gebruikte de meerderheid geen definitieve hemoglobine-trigger, maar wanneer ze er een gebruikten, was deze hoger dan bij andere ernstig zieke patiënten. Interessant genoeg was er een significant verschil in de hemoglobine-trigger die werd aangenomen in overeenstemming met het volume van de centra, waarbij centra met een hoger volume lagere hemoglobinewaarden tolereerden.

Hoofdstuk 5. Ledemaatischemie is een mogelijke complicatie van perifere V-A ECMO. In deze review illustreren we de incidentie en de risicofactoren voor deze complicatie, waarbij we het brede scala van de recente relevante literatuur analyseren. De belangrijkste conclusie is dat bij V-A ECMO ischemie van de ledematen moet worden voorkomen en uiteindelijk snel moet worden vermoed en behandeld met een geprotocolleerde benadering. De steunpilaar om ischemie van de ledematen te voorkomen, lijkt een distale perfusiecanule en een canule met dubbele stroom te zijn. We presenteren ook operatieve stroomdiagrammen om ischemie van ledematen te volgen en te behandelen die zijn verzameld uit gegevens in de literatuur.

Hoofdstuk 6. Gastro-intestinale bloeding is een van de meest voorkomende complicaties bij ernstig zieke patiënten onder ECMO, en gaat gepaard met een hogere mortaliteit. In deze retrospectieve studie in één centrum laten we zien dat geavanceerd endoscopiebeheer, waaronder ook de Hemospray voor hemostase, kan bijdragen aan het bereiken van een sterftecijfer dat vergelijkbaar is met dat van de andere cohorten van patiënten op V-V ECMO, ondanks het toegenomen gebruik van bloedproducten.

Hoofdstuk 7. In deze narratieve review wordt het gebruik van antitrombine bij ECMO onderzocht. Antitrombine had zijn dag in de zon in het vorige decennium in gevallen van sepsis, omdat het een relevante link is tussen coagulatie en de inflammatoire cascade. In feite is het niet alleen de effector die nodig is voor de antistollingsactiviteit van heparine, het heeft ook ontstekingsremmende eigenschappen en kan het endotheel beschermen, beide activiteiten zijn uiteindelijk positief tijdens de ECMO-kuur. Hoge kosten, het risico verbonden aan menselijke bloedproducten en het ontbreken van standaard bewijs in RCT hebben de verspreiding ervan beperkt.

Hoofdstuk 8. Dit is een casus van intra-abdominale hypertensie ontwikkeld bij drie patiënten onder V-V ECMO ondersteuning. De casusreeks belicht de kwestie van intra-abdominale hypertensie tijdens ECMO en het effect ervan op het vermogen van een canule voor ontreiniging, beschrijft een nieuwe niet-invasieve benadering door middel van geavanceerde endoscopie (totale waterondersteunde colonoscopie) en illustreert een systematische review van de literatuur over de onderwerp. Er is ook een praktisch stroomschema voorgesteld om de kwestie van intra-abdominale hypertensie met zijn eigenaardige effecten bij ECMO vroegtijdig en niet-invasief te benaderen.

Hoofdstuk 9. In een pilotstudie wordt de potentiële circulerende miRNA-signatuur bij patiënten met ARDS en recent ondersteund door ECMO geïllustreerd met het doel ARDS-patiënten beter te karakteriseren in hun ernst en mogelijk in hun prognose. Als veelbelovende resultaten zijn de bloedspiegels van 13 miRNA's, sterk gerelateerd aan biologische routes die mogelijk geassocieerd zijn met ARDS, veranderd bij patiënten met ernstige ARDS, en kunnen ze zowel diagnostische waarde bieden als bijdragen aan ARDS-stratificatie.

Hoofdstuk 10. Dit hoofdstuk is een algemene bespreking van het hele werk, waarin de belangrijkste onderwerpen worden samengevat en overwegingen van precisiegeneeskunde en precisiemanagement worden geïllustreerd.


VIA
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Chapter 14

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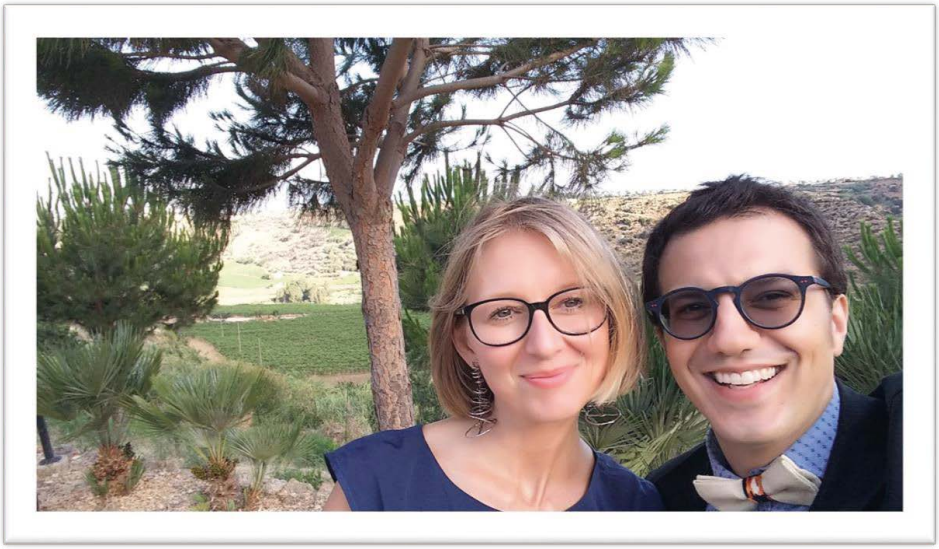

Chapter 15

About the Author

Gennaro Martucci was born in Napoli (Italy) on the 21st of January 1982, under the Aquarius sign. After a childhood spent in friendship, music, natural life and soccer he attended classical studies in high school, becoming passionate about the different and various sides of humanism. He studied Medicine at the Second University of Naples (now Vanvitelli University) and graduated with Honors in 2006 with a thesis on the multimodal treatment of post-operative pain. Later he attended the residency program in Anesthesia and Intensive Care at the Second University of Naples under the supervision of Prof. M. Chiefari and had the opportunity to increase his experience at the University of Turin and at the Catholic University in Rome, with two different fellowships. After working in the intensive care and emergency environment in Napoli, and after one month spent working in the emergency department of the most crowded prison in Europe (Poggioreale Prison in Naples) in December 2011 he moved to Palermo to join ISMETT and University of Pittsburgh Medical Center in Italy to increase the complexity of clinical activity and approach to research programs, earlier as a fellow and now as attending under the guidance of Dott. A. Arcadipane, with whom he has shared clinical practice, research projects, discussions, and clinical and personal growth. His clinical activity is mainly distributed in adult and pediatric liver transplantation care and extracorporeal membrane oxygenation.

During his practice at ISMETT he also had the opportunity to participate in some short visiting fellowships at Vall d'Hebron Hospital (Barcelona) to focus on infections in critical care, at Kings' College (London) to focus on liver transplantation and ECMO, and at XXIIIrd John Pope Hospital (Bergamo) to focus on pediatric liver transplantation and ECMO.

In 2015 he was selected for a Mentorship program in the European Society of Intensive Care Medicine, under the supervision of Prof. K. Amrein, and started a friendship and collaboration on metabolism in critical care that has never ended.

In 2018 he joined the Maastricht PhD program under the supervision of Prof. R. Lorusso, who gave him the opportunity and also the incentive to keep the research activity on one continuous pathway, which resulted ultimately in this thesis, and was able to step up the quality of his work, receiving the interest of International ECMO network, European Society of Intensive Care Medicine and Extracorporeal Life Support Organization.

Since 2017, Gennaro Martucci is a Clinical Assistant Professor at the University of Pittsburgh School of Medicine in the Department of Anesthesia and Perioperative Medicine.

He has had the fortune of sharing his life with Agnieszka Kwiatek, and has two children, Francesco Jan and Elena, who remind him every day just how brilliant life is.

Chapter 16

List of Publications

- Hildreth B, Panarello G, **Martucci G,** et al. ECMO retrieval over the Mediterranean Sea: extending hospital arms. **Membranes 2021**
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COLLABORATION IN MULTICENTER TRIAL

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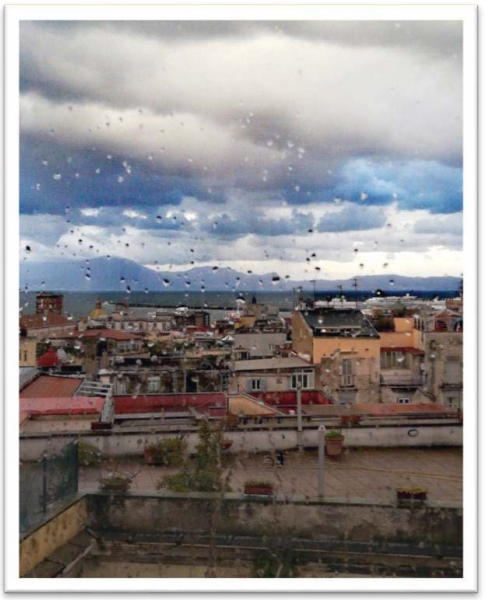

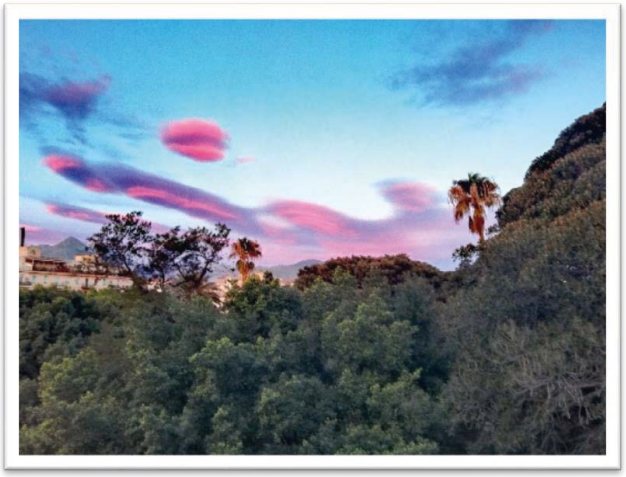

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