Dysfunctional Hemostasis in Patients under Extracorporeal Life Support. A Rapid Diagnostic Approach with Therapeutical Guidance Intentions

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INTRODUCTION

- Short-term cardiopulmonary extracorporeal life supports (ECLS) are invasive devices whose use has increased exponentially during the COVID-19 pandemic.
- Major bleeding is a main cause of morbi-mortality in ECLS-patients and acquired von Willebrand disease (aVWD) could justify this complication.
- There are no specific guidelines for the clinical management of the severe bleeding in ECLS patients.

AIM

- To investigate the primary hemostasis alterations profile in ECLS-patients
- To evaluate a potential substitutive treatment when ECLS-patients present with major bleedings.

PATIENTS AND METHODS

Patients in ECLS at our center since June 2021 were included (n=25).

Primary hemostasis was evaluated by:

- ✓ Measuring Von Willebrand Factor antigen (VWF:Ag) and activity (VWF:GPIbM) (immunoturbidimetry)
- ✓ Evaluating VWF multimers (agarose-gels and immunoblotting),
- ✓ Analyzing platelet performance in the PFA-200 with collagen/epinephrine (Col/EPI) and collagen/ADP (Col/ADP) cartridges.
- ✓ Determining platelet activation antigens' expression (CD62P and CD63, by flow cytometry).
- ✓ Assessing global hemostasis with the Total Thrombus-Formation Analysis System (T-TAS®) in patients with major bleeding, before and after *in vitro* addition of purified VWF.

Studies were performed at different time-points:

- ✓ 24h after implant of the ECLS device
- ✓ Each 7 days
- ✓ In the first week after ECLS extraction
- ✓ If major bleeding, to perform the analysis with T-TAS®

RESULTS

PRIMARY HEMOSTASIS LABORATORY EVALUATION

	VWF:Ag (60-160%)	VWF: GPIbM (60-160%)	Ratio (N>0.7)	PFA Col/EPI (<137s)	PFA col/ADP (<105s)	Ristocetin 1mg/ml (N>44%)	HCT (%)	Platelet count x10⁹/L	Bleeding events
V-V									
1	598	411	0.68	>300	>300	57	40	396	-
2	224	124	0.5	>300	>300	42	30	398	+
3	594	382	0.64	>300	>300	12	31	88	+
4	424	272	0.64	>300	198	57	30	279	-
5	479	232	0.48	>300	>300	41	29	135	-
6	377	300	0.79	>300	145	60	40	144	+
7	649	584	0.89	197	182	40	30	146	-
8	456	114	0.25	>300	>300	51	31	201	+
9	263	289	1.09	>300	157		27	85	+
10	446	300	0.67	>300	>300	16	26	136	+
11	387	268	0.69	>300	>300	35	27	255	+
12	305	149	0.48	>300	266		28	93	-
13	448	250	0.55	>300	>300		27	32	+
14	559	516	0.92	>300	>300	36	37	101	+
15	>600	145	0.24	226	107	40	32	233	-
16	370	225	0.60	>300	>300	41	30	242	+
17	475	276	0.58				28	160	-
18	242	202	0.83	>300	>300	44	29	96	-
19	632	467	0.73	>300	163	51	32	233	-
20	263	289	1.09	>300	157		27	85	+
V-A									
1	>600	550	0.9	>300	>300	26	24	141	+
2	224	124	0.55	>300	>300	42	28	210	-
3	553	416	0.75	>300	>300		29	156	-
4	164	132	0.8	>300	>300		28	113	-
5	481	301	0.62	>300	>100	17	31	103	+

Table 1. Primary hemostasis parameters from first samples (+24h). V-V:venous-venous ECLS; V-A: venous-arterial ECLS. Ratio: VWF:GPIbM/VWF:Ag. Ristocetin refers to the slope observed in the aggregometry studies (turbidimetry). HCT: hematocrit.

- Day 1 after implant: all patients showed increased VWF:Ag. Sixty % had an altered VWF:GPIbM/VWF:Ag ratio (<0.7), with loss of VWF high molecular weight multimers (HMWM) (**Figure 1**).
- Prolonged occlusion times at the PFA-200 were observed in all cases with both cartridges. Although differences were not statistically significant, a trend to an increased expression of platelet-activation antigens was observed in 5 patients (**Figure 2**).
- *In vitro* addition of purified VWF to blood samples from bleeding patients, reduced significantly the occlusion time (reduction of 384s) and increased the AUW (370%) at T-TAS (**Figure 3**).
- All patients who survived to explant (n=16) showed a rapid normalization of VWF:GPIbM/VWF:Ag ratio (>0.7), the VWF multimeric pattern and the PFA-200 values.

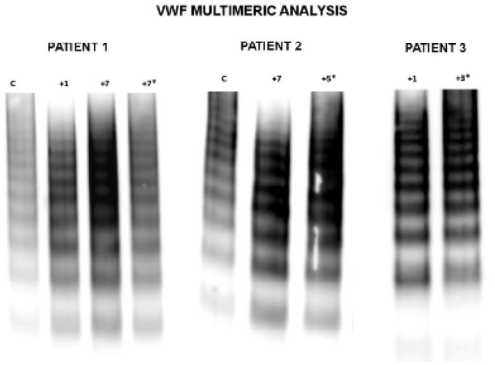

Figure 1. VWF multimeric analysis, by electrophoresis in agarose gels (1.2%) and immunoblot, of plasma samples from 3 ECLS-patients at the time-points indicated. C: control; + *n* days after ECMO cannulation; + *n** days after ECMO decanulation. Dilution of samples 1/90 to normalize VWF concentration.

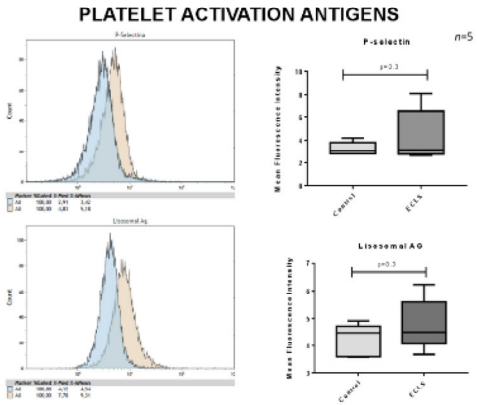

Figure 2. Platelet activation measured by P-Selectin and Lisosomal antigen analysis by flow cytometry (Mean Fluorecescence Intensity). The blue peak corresponds to a healthy donor sample and the orange peak to a patient sample after 7 days in ECLS.

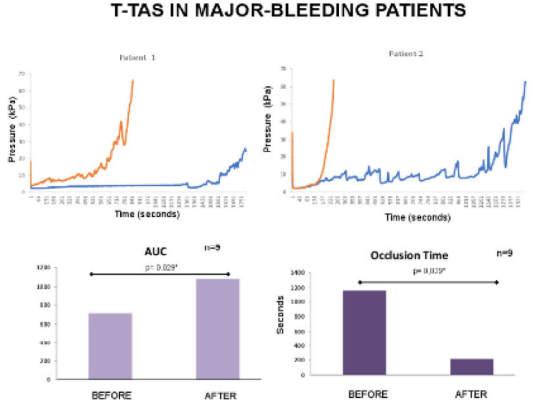

Figure 3. Upper graphs (T-TAS®): kinetics of the thrombi formation for the sample before (blue) and after adding purified VWF (Haemate-P) (red) from 2 ECLS-treated patients with bleeding complications. Graphs below: comparison of the pre- and post- purified VWF factor-administration's values.

CONCLUSIONS

- ✓ ECLS devices caused critical alterations of the primary hemostasis leading to aVWD and platelet activation.
- ✓ These alterations appeared within the 24h after ECLS initiation and were solved early after support removal.
- ✓ The hemostatic deficiency in ECLS-bleeding patients, with lack of HMWM, was corrected by in vitro addition of concentrates containing functional VWF.

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Authors declare no conflicts of interest.

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