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1 **Shear forces induced platelet clearance is a new mechanism of thrombocytopenia**

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1 **Abstract**

2 **Background**

3 Thrombocytopenia has been consistently described in patients with Extracorporeal Membrane
4 Oxygenation (ECMO) and associated with poor outcome. However, the prevalence and underlying
5 mechanisms remain largely unknown and a device-related role of ECMO in thrombocytopenia has
6 been hypothesized.

7 **Aims**

8 To investigate the mechanisms underlying thrombocytopenia in ECMO-patients.

9 **Methods**

10 In a prospective cohort of 107 ECMO-patients, we investigated platelet count, functions and
11 glycoprotein shedding. In an *ex vivo* mock circulatory ECMO loop, we assessed platelet responses
12 and von Willebrand factor (VWF)-GPIb α interactions at low- and high-flow rates, in presence or
13 absence of red blood cells. The clearance of human platelets subjected or not to *ex vivo* perfusion was
14 studied using an *in vivo* transfusion model in NOD/SCID mice.

15 **Results**

16 In ECMO-patients, we observed a time-dependent decrease in platelet count starting 1h after device-
17 onset, with a mean drop of 7-, 35- and 41% at 1-, 24- and 48h post-ECMO initiation ($p=0.00013$,
18 $p<0.0001$ and $p<0.0001$, respectively), regardless of the type of ECMO.

19 This drop in platelet count was associated with a decrease in platelet-GPIb α expression (before:
20 47.8 ± 9.1 vs 24h-post-ECMO: 42.3 ± 8.9 mean fluorescence intensity (MFI), $p=0.002$) and an increase
21 in soluble-GPIb α plasma levels (before: 5.6 ± 3.3 vs 24h-post-ECMO: 10.8 ± 4.1 $\mu\text{g.mL}^{-1}$, $p<0.0001$).
22 GPIb α -shedding was also observed *ex-vivo* and was unaffected by 1) red blood cells, 2) the
23 coagulation potential, 3) an antibody blocking VWF-GPIb α interaction, 4) an antibody limiting VWF
24 degradation and 5) supraphysiological VWF plasma concentrations. In contrast, GPIb α -shedding was
25 dependent on rheologic conditions, with a 2.8-fold increase at high- versus low flow rates. Platelets
26 perfused at high flow rates before being transfused to immunodeficient mice were eliminated faster *in*
27 *vivo* with an accelerated clearance of GPIb α -negative versus GPIb α -positive platelets.

1 **Conclusion**

2 ECMO-associated shear forces induce GPIb α -shedding and thrombocytopenia due to faster clearance
3 of GPIb α -negative platelets. Inhibiting GPIb α -shedding could represent an approach to reduce
4 thrombocytopenia during ECMO.

5
6 **Keywords:** Extracorporeal Membrane Oxygenation, thrombocytopenia, bleeding, platelet clearance,
7 glycoprotein Ib α , shedding, shear forces

8

9

- 1 **Non-standard abbreviations and acronyms**
- 2 ADAM: a desintegrin and metalloproteases
- 3 ECMO: extracorporeal membrane oxygenation
- 4 GPIb α : glycoprotein Ib α
- 5 GPVI: glycoprotein VI
- 6 GPIIb: glycoprotein IIb
- 7 HMWMs: high molecular weight multimers
- 8 MFI: mean fluorescence intensity
- 9 NPP: normal platelet count in plasma
- 10 PPP: platelet poor plasma
- 11 PRP: platelet rich plasma
- 12 RBC: red blood cells
- 13 Te: immediately before ECMO explantation
- 14 Ti: immediately before ECMO implantation
- 15 TRAP: thrombin receptor activating peptide
- 16 UFH: unfractionated heparin
- 17 VA: veno-arterial
- 18 VV: veno-venous
- 19 VWD: von Willebrand disease
- 20 VWF: von Willebrand factor
- 21

1 INTRODUCTION

2 The use of extracorporeal membrane oxygenation (ECMO) devices has largely increased over the last
3 two decades, and even more in recent years due to the COVID-19 pandemic. Despite major
4 improvements in the design and engineering of the devices and circuit components, severe hemostatic
5 complications (i.e. bleeding or thromboembolic events)^{1,2} often occur in patients undergoing ECMO
6 and are associated with increased morbidity, mortality and hospitalization costs.

7 Thrombocytopenia, a major risk-factor for bleeding, has been consistently described during ECMO^{2,3}
8 but its real prevalence is poorly established. In a recent meta-analysis, Jiritano F. et al analyzed
9 fourteen studies documenting thrombocytopenia in ECMO patients and reported high heterogeneity,
10 including prevalence as high as 80%². Importantly, only few studies until now have reported the
11 kinetics of platelet count changes after ECMO implantation and its progression while on ECMO³. The
12 origin of thrombocytopenia in ECMO patients is thought to be multifactorial, due to infections,
13 heparin treatment or specific patient-related factors, although its risk factors, prevalence and evolution
14 are yet to be determined. However, it is well established that thrombocytopenia exposes to life-
15 threatening bleeding, requiring blood transfusions, use of pro-hemostatics, and various invasive
16 procedures. Ultimately, these bleedings may also lead to interruption of some of the antithrombotic
17 medications, which is associated with an increased rate of thrombotic complications.

18 ECMO support is associated with multiple procedure-related modifications that can affect platelets,
19 for example, the contact with artificial surfaces, blood cell fragmentation or release of inflammatory
20 mediators^{4,5}. However, one of the major hemodynamic changes induced by the mechanical device is
21 an increase in the shear forces applied to the blood components.

22 Elevated shear stress induces von Willebrand factor (VWF) conformational changes and binding to
23 platelet glycoprotein Iba (GPIb α)⁶. Recently, GPIb α ectodomain shedding has been reported in
24 ECMO patients^{7,8} and it has been hypothesized that mechanical circulatory support enhances VWF-
25 GPIb α interaction⁹. These observations suggest that GPIb α shedding requires ligand-receptor
26 engagement. Although shear force-induced receptor shedding independent of agonist receptor

1 engagement has been described for GPVI¹⁰, to the best of our knowledge, this has not yet been
2 reported for GPIb α .

3 A limited number of studies have investigated the direct effects of ECMO and the mechanisms
4 underlying the onset and progression of the thrombocytopenia and the modifications of platelet
5 functions through a systematic temporal analysis of platelet counts and functions.

6 To address these questions, we prospectively studied platelet count, desialylation, activation and
7 GPIb α shedding in patients included in the WITECMO-H study. *Ex vivo*, we assessed platelet
8 responses to low- and high-flow rates in a mock circulatory ECMO loop and at pathologic shear rates
9 in a microfluidic device. The impact of red blood cells (RBCs), anticoagulation, VWF-GPIb α
10 interactions and VWF supraphysiological concentrations were also evaluated. Finally, we studied the
11 clearance of human platelets subjected or not to *ex vivo* low- and high-flow rates, using an *in vivo*
12 transfusion model in NOD/SCID mice.

13

14

15

16

1 MATERIAL AND METHODS

2 The data that support the findings of this study are available from the corresponding author upon
3 reasonable request.

4 Please see the Major Resources Table and the Supplemental Methods included in the Supplemental
5 Material.

6 Patients on ECMO

7 Consecutive adult patients on ECMO were prospectively included in the WITECMO-H trial
8 (<https://clinicaltrials.gov/study/NCT03070912>) from February 2018 to September 2020. Details of the
9 protocol are described in the Supplemental material and methods. All patients were sampled at least
10 three times: at admission immediately before ECMO implantation (Ti), 1-hour and 24-hours post-
11 implantation (Ti+1h and Ti+24h, respectively). All patients, except for ten who died early on ECMO,
12 were also sampled 48-hour post-implantation (Ti+48h). Survivors (73 patients) were subsequently
13 sampled immediately before ECMO explantation (Te) and 48-hour post-explantation (Te+48h).

14

15 Mock circulatory ECMO loop model and microfluidic device

16 We used a standardized mock circulatory ECMO loop in accordance with the American Society of the
17 international association for Testing and Materials guidelines¹¹. Perfusions with whole blood, plasma
18 with normal platelet concentration (NPP) or poor platelet plasma (PPP) were performed *ex vivo* for
19 180 minutes (min) at 37°C in a thermostated tubing system using the ECMO Rotaflow (Maquet
20 Cardiopulmonary AG, Hirrlingen, Germany). Experimental ECMO settings (see supplemental
21 material and methods) were applied to achieve a range of outflow rates close to those obtained in
22 ECMO patients¹². Time points for sample collection and analysis were chosen to mimic the number of
23 passages of the blood within ECMO *in vivo* (**Supplementary Figure 1**). Additional *ex vivo*
24 perfusions were performed in presence of unfractionated heparin (UFH), tirofiban, apyrase or EDTA.
25 Separate experiments were performed with inhibitors of VWF degradation, VWF-GPIIb/IIIa interaction
26 or after addition of recombinant VWF concentrate to mimic the supra-physiological VWF plasma

1 concentrations observed in ECMO-patients⁷. We also used a microfluidic device allowing to circulate
2 fluids at a constant and controlled pathologic shear rate (see supplemental material and methods).

3

4 **Human platelet transfusion in NOD/SCID mice**

5 To assess the impact of ECMO rheology on platelet survival *in vivo*, we used a NOD/SCID mouse
6 model infused with human platelets isolated from NPP exposed for 180 min to low- or high-flow rates
7 in the mock circulatory ECMO loop. Details are provided in Supplemental material and methods.

8

9 **Statistical analysis**

10 Quantitative variables are expressed as means (standard deviation), or medians (interquartile range) in
11 case of non-Gaussian distribution. Categorical variables are expressed as frequencies (percentages).
12 Normality of distribution was assessed graphically and by using the Shapiro-Wilk test. Between group
13 comparisons were performed using Student-test (or ANOVA) in case of normal distribution and
14 Mann-Whitney test or Kruskal-Wallis in case of non-normal distribution or in case of small sample
15 size (<10). Within group comparisons according to different time points were performed using the
16 paired Student-test given no deviation to normality was observed or Wilcoxon test in case of non-
17 normal distribution. No correction for multiplicity was done and results are considered as hypothesis
18 generating. Statistical testing was conducted at the two-tailed α -level of 0.05. All statistical analyses
19 were performed using GraphPad Prism software version 8.0 (GraphPad Software, CA, USA).

1 RESULTS

2 Patients

3 Patients' characteristics are summarized in **Table 1**. One hundred and seven consecutive patients were
4 recruited, 90 patients were treated with veno-arterial (VA)-ECMO and seventeen with veno-venous
5 (VV)-ECMO.

6

7 Thrombocytopenia occurs early after ECMO implantation

8 A time-dependent decrease in peripheral platelet count was observed after ECMO implantation
9 (**Figure 1A**). Platelet count dropped in 90% (96/107) of patients 1-hour post-ECMO initiation
10 (177 ± 97 at $Ti+1h$ versus 191 ± 104 G.L⁻¹ at Ti , $p=0.00013$). Platelet count continued to decrease with a
11 mean drop of 35% 24 hours later (125 ± 72 G.L⁻¹ at $Ti+24h$, $p<0.0001$) that further decreased to 41%
12 of the initial count two days post-ECMO implantation (112 ± 61 G.L⁻¹ at $Ti+48h$, $p<0.0001$). At this
13 later time point, 75% of survivors ($n=85$) still under ECMO presented thrombocytopenia (platelet
14 count <150 G.L⁻¹). As expected, thrombocytopenic patients received more units of platelet and packed
15 RBCs (1 versus 0, $p=0.00022$, 7 concentrates versus 4, $p=0.0084$, respectively) during ECMO
16 support, compared to patients with normal platelet count. No other outcomes (major bleeding,
17 thrombosis, death during ECMO or within 30 days after ECMO implantation) were associated with
18 platelet count in our study (data not shown). A similar time course of platelet drop was observed in
19 VA- and VV-ECMO patients (**Supplementary Figure 2**) suggesting that the drop in platelet count is
20 dependent on ECMO but independent of the type of support. Platelets were also quantified
21 immediately prior to ECMO explantation (Te ; time range 1 to 37 days post-ECMO initiation, $n=73$)
22 and two days later ($Te+48h$). Thrombocytopenia was present in 78% of patients before ECMO
23 removal ($Te=107\pm 48$ G.L⁻¹). A remarkable 51% increase in platelet count was observed two days
24 after device removal (162 ± 80 G.L⁻¹ at $Te+48h$, $p<0.0001$), resulting in 22% of patients that resolved
25 their thrombocytopenia at this early post-removal time point.

26 As a potential cause of thrombocytopenia, we evaluated platelet desialylation. β -galactose exposure

1 was only modestly increased on platelets from ECMO patients (and after *ex vivo* perfusion in the
2 mock circulatory ECMO loop) to levels largely below the threshold associated with
3 thrombocytopenia¹³ (**Supplementary Figures 3A and 3B**), indicating that platelet desialylation does
4 not explain ECMO-induced platelet count drop.

5 Overall, these data confirm that ECMO support is associated with a rapid drop in platelet count that
6 corrects quickly after device removal, suggesting a causal link between ECMO and
7 thrombocytopenia, independent of platelet desialylation and of the type of support.

8

9 **ECMO promotes platelet GPIb α shedding in patients**

10 We investigated platelet functions in the first 42 patients, representative of the entire cohort (**Table 1**).

11 Surface and soluble GPIb α levels were similar in patients before ECMO implantation (Ti) and in
12 healthy controls (47.8 \pm 9.1 at Ti vs 50.3 \pm 7.2 MFI in controls and 5.6 \pm 3.3 at Ti vs 3.4 \pm 0.8 μ g.mL⁻¹ in
13 controls respectively, not-significant), suggesting that pre-existing illness conditions do not
14 significantly affect platelet GPIb α expression.

15 However, ECMO implantation coincided with a significant: 1) reduction in platelet GPIb α surface
16 expression that decreased over time (47.8 \pm 9.1 at Ti versus 44.7 \pm 10.3 MFI at Ti+1h, p=0.0011 and
17 42.3 \pm 8.9 at Ti+24h, p=0.0020; **Figure 1B**) and 2) progressive increase in soluble GPIb α levels in
18 plasma (5.6 \pm 3.3 at Ti versus 6.7 \pm 4.7 μ g.mL⁻¹ at Ti+1h, p=0.083, and 10.8 \pm 4.1 at Ti+24h, p<0.0001,
19 **Figure 1C**), indicating that GPIb α shedding rather than receptor internalization occurs under ECMO.

20 Similar results were observed in platelets and plasma from VA- and VV-ECMO patients
21 (**Supplementary Figures 4A and 4B**) pointing to a mechanism of shedding independent of the type
22 of ECMO support. Interestingly, at Ti+24h we observed a negative correlation between sGPIb α levels
23 and platelet count (r=-0.29, p=0.044; **Figure 1D**) suggesting a link between GPIb α shedding and
24 platelet clearance.

25 We also monitored platelet GPVI surface expression (**Figure 1E**). In contrast to GPIb α and in
26 accordance with the literature¹⁴, GPVI expression was already lower in ECMO patients before support

1 initiation (Ti) compared to healthy controls (19.9±4.6 at Ti versus 26.0±2.7 MFI in controls,
2 p<0.0001), with only 3% additional reduction 24 hours post-ECMO onset (19.1±4.3 at Ti+24h,
3 p=0.00052) independently of the type of ECMO (**Supplementary Figure 4C**).

4

5 **ECMO promotes platelet GPIb α shedding *ex vivo***

6 To validate the results obtained in patients and exclude all confounding variables, except the direct
7 effect of ECMO on platelets, experiments were performed *ex vivo* in a mock circulatory ECMO loop
8 at different flow rates. Because VWF degradation is highly dependent on shear conditions and
9 happens rapidly¹⁵, HMWMs disappearance was evaluated in every *ex vivo* experiment, to validate the
10 perfusion conditions (i.e. elevated shear forces). HMWMs disappearance was almost identical in NPP
11 versus PPP and in NPP versus whole blood (**Supplementary Figure 5**), suggesting that very high
12 shear is generated within the ECMO loop model regardless of RBCs-induced changes in viscosity.

13 A time-dependent decrease of platelet GPIb α surface expression was observed at both low and high
14 flow rates (**Figure 2A**). At high flow rate, a loss of GPIb α expression rapidly occurred 30 min after
15 turning on the pump (p=0.0098), that further increased 60 (p=0.0019) and 180 min later (p=0.0019),
16 with a maximum drop of 77% at the latest time point. At low flow rate, the loss of GPIb α expression
17 was slower, with a drop of 35% at the latest time point. At the end of the *ex vivo* perfusions western
18 blot analysis of platelet lysates revealed a decrease in ectodomain levels of GPIb α post-perfusion
19 (**Supplementary Figure 6**) supporting that ECMO induces ectodomain shedding of GPIb α rather
20 than receptor internalization.

21 In accordance with time- and flow rate-dependent shedding of GPIb α from the platelet surface, we
22 observed, the appearance and expansion of a sub-population of GPIb α -negative platelets (**Figure 2B**).
23 Detection of GPIb α -negative platelets increased over-time at both flow rates but these platelets were
24 consistently 2.6- to 2.8—fold more abundant at high compared to low flow rates, at every time point
25 (30 min, 17.4±12.1 versus 6.2±3.3%, p=0.011, 60 min, 23.8±9.4 versus 8.6±4.1%, p=0.00025, 180
26 min, 37.2±7.4 versus 14.3±4.6%, p=0.00025), whereas almost no GPIb α -negative platelets (3%) were

1 detected after 3 hours in samples not exposed to shear (data not shown). Strikingly, in the subset of 42
2 patients analyzed by flow cytometry, we observed only few GPIb α -negative platelets ($3.1\pm 2.9\%$ at Ti
3 versus $3.9\pm 3.6\%$, at Ti+1h, $p=0.0069$ and $2.5\pm 2.3\%$ at Ti+24h, not-significant, **Supplementary**
4 **Figure 7A**). Similar data were observed for GPVI-negative platelets (**Supplementary Figure 7B**).
5 Therefore, we hypothesized that, while GPIb α -negative platelets accumulate in the closed circuit, they
6 are rapidly eliminated *in vivo*, which would explain why they are barely detectable in circulation in
7 ECMO patients.

8 GPVI shedding also occurred in the ECMO loop model (**Figure 2C**), was more pronounced at high
9 flow rate (maximum drop of 69%) and was associated with the appearance of GPVI negative platelets
10 (**Figure 2D**). The time course of GPIb α and GPVI shedding (**Figures 2A -2D**) was not modified by
11 the presence or absence of RBCs.

12 In a microfluidic device (supplemental material and methods and **Supplementary Figure 1**), GPIb α
13 and GPVI receptor cleavage similarly occurred at pathologic shear rates of $10\ 000\text{s}^{-1}$ (**Figure 2E**),
14 concomitant with the appearance of GPIb α - and GPVI negative platelets (**Figure 2F**).

15 Overall, these data suggest that GPIb α and GPVI shedding are dependent on blood flow rate *ex vivo*
16 and that this parameter can be used as proxy of more complex shear conditions *in vivo*.

17 We further hypothesized that GPIb α receptor shedding could be mediated by activation of the
18 ADAM10 and ADAM17 proteases and performed perfusion experiments in presence of EDTA, a
19 broad metalloprotease inhibitor. Pre-treatment with EDTA abrogated GPIb α shedding from the
20 platelet surface (**Figure 2G**) and the appearance of a GPIb α -negative subpopulation of platelets
21 (**Figure 2H**), providing evidence for the involvement of metalloproteases in ECMO-induced platelet
22 receptor shedding *ex vivo*.

23

24 **ECMO does not induce major platelet activation**

25 Because GPIb α shedding is often associated with platelet activation^{4,5,8}, we measured soluble P-
26 selectin (sP-selectin) and soluble CD40 ligand (sCD40L) in plasma from ECMO patients. Before

1 ECMO initiation, both markers were elevated compared to healthy controls (**Figures 3A and 3B**),
2 suggesting that the underlying diseases induce a mild platelet activation. However, when these
3 markers were evaluated 24 hours post-ECMO onset, discordant and minor changes were observed.
4 While sP-selectin levels modestly increased (42.6 ± 12.5 at Ti versus 49.7 ± 11.2 ng.ml⁻¹ at Ti+24h,
5 $p=0.035$, **Figure 3A**), sCD40L decreased (184 ± 36 at Ti versus 64 ± 13 ng.ml⁻¹ at Ti+24h, $p=0.0038$,
6 **Figure 3B**), suggesting that, if any, ECMO has only a minor effect on platelet activation.

7 To further evaluate the platelet activation state, TRAP-induced activation was assessed one day after
8 ECMO implantation in 5 patients (3 on, and 2 without aspirin, none on a P2Y12 inhibitor). Upon *in*
9 *vitro* stimulation, GPIIb and P-selectin surface expression significantly increased in all patients
10 (43.7 ± 4.2 versus 75.5 ± 9.9 , $p < 0.0001$, 0.6 ± 0.2 versus 9.5 ± 0.6 , $p < 0.0001$, respectively; **Figures 3C**
11 **and 3D**) and in the same range as platelets from healthy subjects (50.7 ± 7.3 versus 82.2 ± 11 $p < 0.0001$,
12 0.5 ± 0.2 versus 9.5 ± 3 , $p < 0.0001$, respectively). These observations support the hypothesis that
13 platelets remaining in circulation during ECMO are in a minor pre-activated state that does not
14 prevent a subsequent strong agonist-induced response.

15 Platelet morphology one day after ECMO implantation assessed in 6 patients appeared normal and
16 similar to that of platelets collected pre-ECMO implantation, except for a reduction in α -granule
17 content (2.2 ± 0.3 versus 1.8 ± 0.2 α -granules per μm^2 , $p=0.031$, Wilcoxon test) (**Figure 3E**). Although
18 statistically significant, the difference was only negligible, which is consistent with minor platelet pre-
19 activation results.

20 To exclude platelet activation-dependent shedding of GPIIb α (ADP-mediated), we next measured
21 platelet surface expression of GPIIb α in patients treated or not with a P2Y12 inhibitor and found no
22 significant difference (**Figure 3F**). Moreover, no associations were found between platelet activation
23 markers and GPIIb α shedding (sCD40L and GPIIb α MFI, $r=-0.12$ $p=0.73$, sP-selectin and GPIIb α
24 MFI, $r=-0.053$ $p=0.81$), further supporting that ECMO-associated receptor shedding occurs
25 independently of platelet activation.

1 When NPP was perfused *ex vivo* in the mock circulatory ECMO loop, platelet activation profiles were
2 similar to those obtained on patients' samples. Both P-selectin expression at the platelet surface and
3 sCD40L levels did not significantly change after 180 min (**Figures 4A and 4B**).

4
5 We also observed similar decreased GPIb α expression (and accumulation of GPIb α -negative
6 platelets) in NPP perfused at high flow rate, in presence or absence of the platelet inhibitors tirofiban
7 or apyrase (**Figures 4C-F**).

8 Overall, concordant *ex vivo* and *in vivo* results show that ECMO does not induce major platelet
9 activation nor is ECMO-dependent GPIb α -shedding affected by platelet activation.

10

11 **ECMO-induced GPIb α shedding is independent of anticoagulation regimen**

12 We investigated whether anticoagulation could affect receptor cleavage on ECMO. At 24 hours post-
13 ECMO implantation, three out of 36 alive patients still under ECMO had undetectable anti-FXa
14 activity levels. However, GPIb α expression, at the platelet surface and in plasma (sGPIb α), was
15 similar between patients with or without detectable anti-FXa activity levels (**Figure 5A and 5B**).
16 Moreover, thrombin generation was evaluated in 18 patients one day after ECMO implantation
17 (Ti+24h) and, as expected, very low thrombin generation was observed in patients with detectable
18 anticoagulation (data not shown).

19 Mirroring the results observed in patients, when *ex vivo* perfusions were performed in the mock
20 circulatory ECMO loop in presence of heparin (0.5 IU.ml⁻¹, mimicking the patients' regimen),
21 thrombin generation was nearly completely inhibited (not shown). An almost identical time course
22 was observed for both the loss of GPIb α ectodomain from the platelet surface (**Figure 5C**) and the
23 accumulation of GPIb α -negative platelets (**Figure 5D**) in presence or absence of heparin. These data
24 indicate that the ECMO effects on platelets are independent of coagulation activation both in patients
25 and *ex vivo*.

26

1 **ECMO-induced platelet GPIb α -shedding is independent of VWF-GPIb α interaction**

2 Because elevated shear forces under ECMO promote VWF conformational changes and
3 disappearance of HMWMs, we investigated whether ECMO-associated GPIb α shedding relies on
4 VWF multimerization or VWF-GPIb α binding. Inhibiting VWF degradation using an antibody
5 against the VWF-D4 domain prevented the loss of VWF HMWMs in the mock circulatory ECMO
6 loop (**Figure 6A**), but did not prevent GPIb α shedding at a high flow rate (**Figure 6B**). Inhibition of
7 VWF-GPIb α binding using an antibody against the VWF-A1 domain also failed to inhibit GPIb α
8 shedding (**Figure 6B**). These data indicate that GPIb α ectodomain release happens independently of
9 VWF degradation and VWF- GPIb α interaction. Furthermore, shedding was still observed after
10 perfusion of NPP from a von Willebrand disease-type 3 patient with undetectable VWF
11 (**Supplementary Figure 8A and 8B**). Finally, we found that GPIb α shedding over time was similar at
12 physiological or supra-physiological VWF concentrations (**Figure 6C and 6D**).

13 These data confirm that ECMO support promotes platelet-GPIb α shedding independently of ECMO-
14 associated effects on VWF.

15

16 **ECMO drives the clearance of human platelets transfused in NOD/SCID mice through a shear**
17 **forces dependent mechanism**

18 As GPIb α -shedding has been reported to be associated with increased platelet clearance from the
19 circulation, we aimed to evaluate the *in vivo* kinetics of platelets exposed to low or high flow rates in
20 the mock circulatory ECMO loop. First, NPP was perfused in the ECMO loop for 180 min and a
21 control aliquot was kept at 37°C for the same time. We subsequently transfused similar amounts of
22 human platelets into NOD/SCID immune deficient mice and measured human platelet counts over
23 time (**Figure 7A**). ECMO-exposed platelets were eliminated more rapidly than control platelets. At 6
24 hours (360 minutes), 81 \pm 13, 60 \pm 26 and 54 \pm 16% of platelets exposed to high-, low- or no flow,
25 respectively, had disappeared (**Figure 7B**).

1 Interestingly, we also observed that GPIb α -negative platelets disappeared more rapidly than platelets
2 still bearing intact receptors at their surface (**Figure 7C**) and thus had a shorter half-life (1.30 ± 0.2
3 hours for GPIb α -positive platelets versus 0.41 ± 0.1 hours for GPIb α -negative platelets, Mann Whitney
4 test: $p=0.028$), indicating that GPIb α -negative platelets are cleared more rapidly from the circulation.

5

6 **DISCUSSION**

7 Using a translational approach, we demonstrate that the thrombocytopenia observed during ECMO
8 support: 1) has an early onset and offset in close relation to ECMO implantation and withdrawal, 2) is
9 associated with shear-rate induced GPIb α shedding, and 3) is the result of faster clearance of GPIb α -
10 negative platelets. Importantly, our data also show that ECMO-associated decline in platelet count is
11 independent of platelet activation or desialylation, presence of RBCs, activation of coagulation and
12 VWF-GPIb α interaction.

13 Consistent with other studies^{3,4}, in all but eleven patients of the cohort (>90%), we observed a
14 progressive decrease in platelet count over time following ECMO initiation, which resolved rapidly
15 post-device explantation, suggesting a causal link between thrombocytopenia and ECMO. Previous
16 studies have reported that thrombocytopenia is not dependent on the type of ECMO, cannulation nor
17 on treatment regimen of the ECMO patients.¹⁶ Our data confirmed that platelet count drop was similar
18 in VA- and VV-ECMO patients, with/without antiplatelet therapy and despite anticoagulation
19 regimen. Overall, these data indicate an accelerated clearance of platelets due to the high shear
20 induced by the device, independent of underlying medical conditions and treatments, as a systematic
21 and specific mechanism of thrombocytopenia under ECMO support.

22 Thrombocytopenia and GPIb α -shedding have been both associated with desialylation¹⁷, therefore, we
23 investigated if ECMO-associated decrease in platelet count could be due to the loss of platelet sialic
24 acid content. In our previous study, we established a threshold above which thrombocytopenia is
25 likely to be desialylation-dependent¹³. Our results show that both in ECMO patients and in the *ex vivo*
26 ECMO model, platelet desialylation is significantly increased, but remains below this threshold

1 indicating that mechanisms other than platelet desialylation are probably responsible for the
2 thrombocytopenia.

3 VWF interaction with its GPIb α platelet receptor can trigger various platelet responses and mediate
4 GPIb α -ectodomain shedding, which has been reported to promote rapid platelet clearance, possibly
5 preventing aggregation and thrombus formation⁶. Lukito et al.⁷ reported that GPIb α and GPVI surface
6 levels are significantly reduced in patients undergoing ECMO- or ventricular assist device-support
7 compared to healthy controls, but the time-course of these events post-ECMO implantation was not
8 described in their paper. In our study, GPIb α ectodomain shedding occurs rapidly, within 24 hours
9 post-ECMO and concomitant with an increase of sGPIb α plasma levels, demonstrating that ECMO
10 favors rapid GPIb α proteolysis. At the same time, GPVI shedding was also observed. However, while
11 sGPIb α measured immediately before ECMO implantation was modestly increased compared to
12 levels in healthy individuals as previously reported¹⁴, GPVI surface expression was severely
13 decreased in patients before ECMO initiation compared to controls, suggesting that pulmonary and
14 cardiac distress are associated with substantial GPVI- and minor GPIb α shedding. Conversely,
15 shedding post-ECMO onset was pronounced for GPIb α but minor for GPVI. These results largely
16 confirm data from the literature showing that GPIb α and GPVI shedding can be regulated by
17 independent, simultaneously occurring mechanisms¹⁴.

18 Mock circulatory loop experiments were instrumental to better understand the relationship between
19 blood flow rates as proxy of shear stress and GPIb α shedding. We first carefully validated that flow
20 dynamics within the mock circulatory ECMO loop were consistent with blood hemodynamics in
21 ECMO patients by 1) using a microfluidic device allowing to circulate fluids at a constant and
22 controlled pathological shear rate; 2) assessing ADAMTS13-mediated VWF degradation as internal
23 control of shear conditions at every perfusion and 3) demonstrating that ECMO-associated effects on
24 platelets were independent of the presence of RBCs during *ex vivo* perfusions. While low flow rates
25 promoted significant but minor reduction of GPIb α surface levels of circulating platelets, a rapid
26 shedding of \approx 90% of the receptors from the platelet surface was observed within 3 hours at high flow
27 rates. At these high flow rates, two distinct platelet populations were present: one expressing variable

1 amounts of GPIb α , which decreased over time, and one without any detectable surface GPIb α .
2 Similarly, a small, but consistent increase in GPIb α shedding and accumulation of GPIb α -negative
3 platelets has been reported in platelet storage lesion studies¹⁸⁻²⁰. Therefore, it seems reasonable to
4 hypothesize that shear stress induced by high flow rates promotes efficient GPIb α shedding.

5 Since ECMO patients suffer from acquired von Willebrand syndrome and since all the
6 aforementioned platelet abnormalities can be associated with signaling defects downstream of VWF-
7 GPIb α engagement, a direct role of VWF in this clinical picture seemed possible. However, we
8 demonstrated that ECMO-associated platelet abnormalities are independent of either VWF proteolytic
9 degradation or its interaction with GPIb α and are, instead, directly triggered by the elevated shear
10 stress secondary to high blood flow rates.

11 We also demonstrated that shear-dependent ECMO-associated receptor shedding was independent of
12 platelet- or coagulation activation, while it was abrogated by EDTA, similarly to what was previously
13 demonstrated in similar conditions for VWF degradation by ADAMTS13²¹. Recently, Six et al.
14 reported that strong and sustained dual agonist-induced platelet activation induces ADAM17-
15 mediated shedding of an intracellular subpopulation of GPIb α receptors²². Interestingly, these authors
16 also demonstrated that the degree and kinetics of GPIb α cleavage depend on the agonist and
17 receptor(s) engaged and thus on the platelet activation response, further supporting the notion of
18 heterogeneity in platelet glycoprotein shedding that can happen in association with different events.

19 The putative mechanisms of release of platelet protein's ectodomains can differ significantly, as
20 shown by the results of perfusion experiments with EDTA in the ECMO loop model. A mild increase
21 in P-selectin expression was still observed in platelets exposed to high flow rate (data not shown)
22 whereas the loss of GPIb α from the platelet surface was completely inhibited.

23 Only few single-center observational studies have evaluated the impact of ECMO on platelet function
24 reporting discrepant results regarding platelet activation markers and agonist-induced platelet
25 aggregation^{23,24}. We observed only a minor platelet pre-activation before and during ECMO, which
26 does not prevent agonist-induced responses, nor corresponds to the strong activation evoked by Six
27 and colleagues²². We also demonstrated that receptor shedding was similar in patients with/without

1 platelet inhibitors, confirming that platelet activation plays a minor role in GPIb α shedding and
2 thrombocytopenia under ECMO, and that ECMO support does not induce major platelet activation.
3 However, while we don't observe major changes in platelet surface p-selectin, we observed a
4 moderate reduction in α -granule content in ECMO patient's platelets. This discrepancy could be due
5 to platelet microparticle release, known to occur with mechanical shear²⁵. Although parent platelets
6 may retain normal or slightly reduced reactivity, platelet microparticles could still promote
7 thrombosis in ECMO setting.

8

9 We performed *ex vivo* perfusions in presence of heparin to mimic full-dose anticoagulation. Receptor
10 shedding was similar in patients with normal or undetectable thrombin generation potential i.e.
11 inefficient or efficient anticoagulation at the time of blood collection, confirming that the
12 anticoagulation regimen does not affect shear-induced platelet shedding.

13 When human platelets perfused within the mock circulatory ECMO loop were infused into immune-
14 deficient mice, we noticed a rapid drop in platelet count in the first 6 hours post-transfusion, followed
15 by a more gradual disappearance at later time points, suggesting a two-phase elimination kinetic. It is
16 tempting to speculate that GPIb α -negative platelets are rapidly eliminated post-infusion in mice
17 (calculated half-life of 0.4 hours), while platelets bearing residual receptors at their surface are
18 eliminated more slowly (half-life of 1.30 hours). This hypothesis is supported by the finding that only
19 very small amounts of GPIb α -negative platelets are present in the circulation of the patients, without
20 any significant difference between levels measured before and 24h post-ECMO implantation. To the
21 best of our knowledge, this is the first study comparing clearance of GPIb α -positive and -negative
22 platelets following ECMO support. It remains to be determined whether GPIb α -negative platelets are
23 being sequestered in a specific organ such as the liver or the spleen and the molecular mechanism by
24 which GPIb α shedding leads to platelet clearance in the context of elevated shear forces.

25 We propose here a new mechanism of thrombocytopenia, in which elevated shear forces
26 directly induce only minor platelet activation, but a pronounced GPIb α -shedding resulting in a rapid
27 clearance of platelets expressing low or undetectable GPIb α levels at their surface. ADAM17 has

1 been shown to cleave the extracellular portion of GPIIb/IIIa in many different conditions secondary to
2 VWF-GPIIb/IIIa engagement²⁶ or profound platelet activation²². However, we have previously
3 demonstrated that ADAM17-independent GPIIb/IIIa-shedding can happen even in presence of active
4 GPIIb/IIIa-binding VWF bearing the p.V1316M/VWD-type 2B mutation²⁷, suggesting that several
5 proteases can proteolyze GPIIb/IIIa. Whether shear stress induced-, VWF independent- GPIIb/IIIa-shedding
6 is mediated by ADAM17 or by other EDTA-sensitive proteases require further investigation.

7 Our study has a number of limitations. First it is a single-center study that wasn't designed to assess
8 association between outcomes and platelet count. However, we found an association between early
9 decrease of platelet count and transfusion of RBCs suggesting an increased risk of bleeding.
10 Adherence to antiplatelet medications was not assessed. Thrombocytopenia is probably multifactorial
11 in heterogeneous and critically ill patients requiring ECMO. Although the ECMO loop model cannot
12 recapitulate all the aspects of ECMO support and patient heterogeneity, we aimed to model as
13 accurate as possible both the shear conditions and the anticoagulation regimen present *in vivo*.

14 Despite these limitations, our concordant results clearly indicate that platelet GPIIb/IIIa shedding is
15 directly dependent on elevated shear stress.

16 In conclusion, our results demonstrate that ECMO-associated GPIIb/IIIa shedding from the platelet
17 surface is directly dependent on blood flow rates but independent of VWF-GPIIb/IIIa interactions and
18 coagulation potential and promotes platelet clearance. We also demonstrate that the occurrence of
19 thrombocytopenia is inherent to ECMO support and to the high-shear induced by the device, which
20 should be taken into account for the management of these patients. Inhibiting platelet-GPIIb/IIIa shedding
21 could represent an innovative approach to reduce thrombocytopenia during ECMO, which could
22 potentially be more efficient than platelet transfusion which is the only current therapeutic option.
23 This unique and previously undescribed mechanism of shear-induced thrombocytopenia appears a key
24 feature of ECMO support. Whether a similar mechanism may happen in other conditions, is still
25 unknown and requires further investigations.

26

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8 **CONFLICT OF INTEREST DISCLOSURES**

9 None

10 **SUPPLEMENTAL MATERIALS**

11 Expanded Materials and Methods

12 Major Resources Table

13 Online Figure S1-S8

14 References 28-43

15

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- 21

1 **FIGURE LEGENDS**

2 **Figure 1: Platelet count timeline, GPIb α and GPVI levels in patients undergoing ECMO**
3 **support.**

4 A) Platelet count in patients (n=107) undergoing VV or VA ECMO at admission immediately before
5 ECMO implantation (Ti), 1-, 24- and 48-hour post-implantation (Ti+1h, Ti+24h, Ti+48h,
6 respectively), immediately before ECMO removal (Te) and 48-hour after ECMO removal (Te+48h)
7 in patients weaned alive (n=74). Red circles indicate platelet count in patients who received platelet
8 concentrates in the first day of ECMO support and grey circles indicate patients who died on ECMO.
9 Normal range of platelet count is indicated in grey. B) GPIb α expressed at the platelet surface
10 assessed by flow cytometry (MFI) and C) sGPIb α plasma levels in a group of healthy subjects (grey)
11 and in ECMO patients (blue) before, 1- and 24-hour after ECMO implantation. D) Correlation
12 between sGPIb α plasma levels and platelet count at Ti+24h (r=-0.29, p=0.04; Spearman correlation
13 test). E) GPVI expressed at the platelet surface assessed by flow cytometry (MFI) in healthy subjects
14 (grey) and in ECMO patients (blue). Time points as in panels B-C.
15 Panels B-C-D-E: n=42 ECMO patients. Panels B-C-E: n=11 healthy subjects. Panels A-B-C-E:
16 statistical analyses were performed using Wilcoxon test for paired comparisons and Mann-Whitney
17 test otherwise. The exact p values are specified. Error bars indicate standard deviation from the mean.
18 A.U: arbitrary units. MFI: mean fluorescent intensity. sGPIb α : soluble GPIb α . VA: veno-arterial,
19 VV: veno-venous

20
21 **Figure 2: ECMO promotes shear-induced platelet GPIb α and GPVI shedding in a mock**
22 **circulatory ECMO loop and a microfluidic device.**

23 A) Platelet GPIb α surface expression (MFI) in NPP perfused at high (3.7 L.min⁻¹, n=10, blue circles)
24 or low flow rates (1.5 L.min⁻¹, n=6, light grey circles) or in whole blood perfused *ex vivo* at high flow
25 rate (n=3, dark grey circles) in the mock circulatory ECMO loop B) GPIb α -negative platelets.
26 Perfusions as in A. C) Platelets GPVI surface expression and D) GPVI-negative platelets. Perfusions
27 as in A. E) Platelets GPIb α (grey) and GPVI (green) surface expression (MFI) and F) GPIb α - (grey)
28 and GPVI-negative (green) platelets in NPP perfused at a constant shear rate of 10 000s⁻¹ for 20 hours

1 in a microfluidic device (ibidi Pump System). G) GPIIb α surface expression and H) GPIIb α -negative
2 platelets from NPP perfused *ex vivo* at high flow rate (3.7 L.min⁻¹) in the mock circulatory ECMO
3 loop in presence (red) or absence (grey) of 10 mM EDTA (n=3).

4 Panels A-B-C-D: ξ =p-ANOVA (blue: NPP high flow, light grey: NPP low flow, dark grey: whole
5 blood high flow); ϕ =comparison at each time point versus T0=baseline (blue: NPP high flow, light
6 grey: NPP low flow, dark grey: whole blood high flow; Wilcoxon rank-test); *=comparison at a
7 specific time point between high- versus low flow rate condition). Panel E-F: Mann-Whitney test.
8 Panels G-H: ξ =p-ANOVA (grey: NPP high flow, red: NPP high flow + EDTA); *=comparison at a
9 specific time point between high- versus low flow rate condition (Mann-Whitney test).

10 The exact p values are specified.

11 A.U.: arbitrary units, EDTA: ethylenediaminetetraacetic acid, NPP: plasma with normal platelet
12 concentration, MFI: mean fluorescence intensity, WB: whole blood. Error bars indicate standard
13 deviation from the mean.

14

15 **Figure 3: Platelet activation and morphology prior- and post-ECMO implantation.**

16 A) Soluble P-selectin plasma levels in patients at Ti and Ti+24h (blue, n=27) and healthy controls
17 (grey, n=10). B) soluble CD40L plasma levels in patients (blue, n=15) and healthy controls (grey,
18 n=8). C) GPIIb and D) P-selectin quantification in resting (gray bars) and TRAP-activated (60 μ M,
19 blue bars) platelets, collected from ECMO patients at Ti+24h (n=4) or from healthy controls (n=9). E)
20 Representative transmission electron microscopy images showing morphology of platelets (left
21 images) collected from patients (n=6) at Ti (upper images) and Ti+24 after ECMO initiation (lower
22 images) and close-up views of individual platelets in cross-sections (right images). F) Platelet GPIIb α
23 surface expression (MFI) Ti+24 after ECMO initiation in patients without (n=5) and on P2Y12
24 inhibitor (n=31).

25 A.U.: arbitrary units, MFI: mean fluorescent intensity, sCD40L: soluble CD40 ligand, TRAP:
26 Thrombin Receptor Activator Peptide. Panel A: Wilcoxon test for paired comparisons and unpaired t-

1 test otherwise. Panels B-E: Wilcoxon test for paired comparisons and Mann-Whitney test otherwise.
2 The exact p values are specified. Error bars indicate standard deviation from the mean.

3

4 **Figure 4: ECMO does not induce major platelet activation *in vitro* and platelet GPIb α shedding**
5 **is independent of platelet activation**

6 Platelet activation markers, (A) P-selectin at the platelet surface and (B) soluble CD40L, before (black
7 bars) and after perfusion *ex vivo* of NPP at high flow rate (3.7 L.min⁻¹) in the mock circulatory ECMO
8 loop (blue bars) (n=3). C) Platelet GPIb α surface expression (MFI) and D) GPIb α -negative platelets
9 in NPP perfused *ex vivo* at high flow rate in presence (green circles) or absence (grey circles) of
10 tirofiban (150ng.ml⁻¹, n=3). E) GPIb α surface expression (MFI) and F) GPIb α -negative platelets in
11 NPP perfused at high flow rate in presence (red circles) or absence (grey circles) of apyrase (0.2
12 IU.ml⁻¹, n=3). Panels C, D, E and F: ξ = p-ANOVA (grey: NPP high flow, green: NPP high flow +
13 tirofiban (C and D), red: NPP high flow + apyrase (E and F)). Panels A-F: no significant differences
14 (Mann-Whitney test) between groups whatever the time.

15 A.U.: arbitrary units, MFI: mean fluorescent intensity, NPP: plasma with normal platelet
16 concentration, sCD40L: soluble CD40 ligand. Error bars indicate standard deviation from the mean.

17

18 **Figure 5: ECMO-associated platelet GPIb α shedding is independent of anticoagulation regimen**

19 A) Platelet GPIb α surface expression (MFI) and B) sGPIb α plasma levels in ECMO patients on
20 heparin with anti-FXa activity below (left, n=3) or above (right, n=24) of 0.1 IU.ml⁻¹ (detection limit
21 of the quantification method) Ti+24 after ECMO initiation. C) Platelet GPIb α surface expression
22 (MFI) and D) GPIb α -negative platelets in NPP perfused *ex vivo* at high flow rate (3.7 L.min⁻¹) in the
23 mock circulatory ECMO loop in presence (purple circles) or absence (grey circles) of heparin (0.5
24 IU.ml⁻¹, n=3). Panels C and D: ξ =p-ANOVA (grey: NPP high flow, purple: NPP high flow + heparin,
25 the exact p values are specified). Panels A-D: no significant differences (Mann-Whitney test) between
26 groups whatever the time.

1 A.U.: arbitrary units, MFI: mean fluorescent intensity, NPP: plasma with normal platelet
2 concentration, sGPIb α : soluble GPIb α . Error bars indicate standard deviation from the mean.

3

4 **Figure 6: GPIb α shedding observed during ECMO support is independent of VWF-GPIb α**
5 **interactions**

6 A) VWF HMWMs (>10mers) quantification in NPP perfused *ex vivo* at high flow rate (3.7 L.min⁻¹)
7 in the mock circulatory ECMO loop in absence (blue circles) or presence (grey circles) of a
8 monoclonal antibody anti-VWF D4 domain (50 μ g.ml⁻¹) (n=3). Ratio of HMWMs (>10 mers) of each
9 sample to HMWMs of standard human plasma (SHP) are indicated. B) Platelet GPIb α expression
10 (MFI). Perfusions as in A in absence (blue circles) or presence (dark grey circles) of a monoclonal
11 antibody anti-VWF D4 domain (50 μ g.ml⁻¹) or a nanobody anti-VWFA1 domain (KB-VWF-006bi, 5
12 μ g.ml⁻¹; light grey circles) (n=3). C) VWF levels in NPP in absence (blue bars) and presence (grey
13 bars) of fully multimerized recombinant VWF (Veyvondi® 5 IU.ml⁻¹). D) Platelet GPIb α expression
14 (MFI) in NPP, with normal (blue) or supraphysiological (grey, Veyvondi® 5 IU.ml⁻¹) VWF
15 concentrations, perfused *ex vivo* at high flow rate (n=3).

16 Panels A-B: ξ =p-ANOVA (blue: NPP, grey: NPP + anti-VWF D4, light grey: NPP + anti-VWF A1;);
17 ϕ =comparison at each time point versus T0=baseline (blue: NPP, grey: NPP + anti-VWF D4, light
18 grey: NPP + anti-VWF A1; Wilcoxon rank-test); *=comparison at a specific time point between high-
19 versus low flow rate condition (Mann-Whitney test). PanelC: Mann-Whitney test. Panel D: p-
20 ANOVA (blue: NPP, grey: NPP + rVWF). The exact p values are specified. Panels B and D: no
21 significant differences (Mann-Whitney test) between groups whatever the time.

22 A.U.: arbitrary units, HMWMs: high molecular weight multimers, MFI: mean fluorescent intensity,
23 NPP: normal platelet concentration plasma, r: recombinant, SHP: standard human plasma, VWF: von
24 Willebrand factor, VWF:Ag: Willebrand antigen. Error bars indicate standard deviation from the
25 mean.

26

27 **Figure 7: Platelet perfused in a mock circulatory loop model are rapidly eliminated *in vivo* in**
28 **NOD/SCID mice**

1 A) Protocol overview of the human platelet transfusion experiment. B) Survival of human platelets
2 (GPIIb-positive) transfused in NOD/SCID mice prepared after 180-minute of NPP not perfused
3 (white bars, n=4) or perfused *ex vivo* at high ($3.7 \text{ L}\cdot\text{min}^{-1}$, n=7, blue bars) or low ($1.5 \text{ L}\cdot\text{min}^{-1}$, n=7,
4 grey bars) flow rate conditions in the mock circulatory ECMO loop. C) Survival of human GPIb α -
5 positive/GPIIb-positive (solid blue bars) and GPIb α -negative/GPIIb-positive (pattern blue bars)
6 platelets transfused in NOD/SCID mice prepared after 180-minute of NPP perfusion at high flow rate
7 (n=4).
8 Human GPIIb+, GPIb+/GPIIb+ and GPIb-/GPIIb+ platelets present 3-minute post transfusion were
9 set at 100% and platelets quantified at different time points were expressed as relative percentage of
10 this baseline. Panel B: Kruskal-Wallis test. Panel C: Mann-Whitney test. The exact p values are
11 specified. Error bars indicate standard deviation from the mean.

1 **Table 1: Characteristics of the studied population**
 2

	ECMO patients (n=107)	ECMO patients analyzed for GPIIb/IIIa shedding (n=42)	P
Age (years), mean(SD)	55(14)	53(13)	0.68
Male gender, n(%)	69(64)	27(64)	>0.99
BMI (kg.m⁻²) mean(SD)	27.2(5.8)	26.4(5.9)	0.51
Cardiac risk factors, n(%)			
Hypertension	38(36)	14(33)	0.85
Dyslipidemia	25(23)	9(21)	>0.99
Smoking	37(36)	12(28)	0.56
Diabetes mellitus	33(31)	13(31)	>0.99
Platelet count at admission (G/L), mean(SD)	191(104)	205(110)	0.45
Type of ECMO, n(%)			
VA-ECMO	90(84)	32(77)	0.34
VV-ECMO	17(16)	10(23)	
ECMO indication, n(%)			
Post-cardiotomy shock	23(22)	5(12)	0.24
Cardiac arrest	17(16)	7(17)	>0.99
Pulmonary embolism	2(2)	1(2)	>0.99
Decompensated CHF	16(15)	7(17)	0.80
STEMI	26(24)	10(24)	>0.99
ARDS	13(12)	8(19)	0.3
Others	10(9)	4(9)	>0.99
Pump type, n(%)			
Rotaflo (Maquet)	56(52)	24(57)	0.72
Revolution (Livanova)	44(41)	16(38)	0.85
Centrimag (Thoratec)	7(7)	2(5)	>0.99
ECMO outflow 1h after implantation (L/min), mean(SD)	3.8(1.1)	3.9(1.1)	0.69
ECMO outflow 24h after implantation (L/min), mean(SD)	3.7(0.8)	3.8(0.8)	0.97
ECMO duration, days, median(IQR)	6[3-12]	5[2-9]	0.56
Antiplatelet drug before and/or during ECMO, n(%)			
Aspirin	61(57)	23(55)	>0.99
P2Y12 inhibitors	16(15)	6(14)	>0.99
Antifibrinolytic during ECMO, n(%)	37(35)	8(19)	0.08
Packed red blood cells during ECMO, units, median(IQR)	5[2-13]	4[0-10]	0.39
Fresh frozen plasma during ECMO, units, median(IQR)	0[0-4]	NA	NA
Platelet concentrates during ECMO, units, median(IQR)	0[0-3]	0[0-2]	0.29
Bleeding complication during ECMO, n(%)	75(70)	27(64)	0.56

ISTH major bleed complication during ECMO, n(%)	56(52)	20(48)	0.72
Thrombotic complication during ECMO, n(%)*	28(26)	8(19)	0.40
Extrarenal epuration requiring, n (%)	38(35)	15(35)	>0.99
Death under ECMO, n (%)	34(32)	17(40)	0.34
Death within 30 days after ECMO implantation, n (%)	43(40)	19(45)	0.58

1
2 Values are numbers(percentage), mean(standard deviation) or median[interquartile]. ARDS: acute
3 respiratory distress syndrome, BMI: body mass index, CHF: congestive heart failure, ECMO:
4 extracorporeal membrane oxygenation; NA: non applicable, STEMI: ST-Elevation Myocardial
5 Infarction, VA: veno-arterial, VV: veno-venous; *none associated with heparin-induced
6 thrombocytopenia. Categorical variables were compared using chi-squared test. Continuous variables
7 were compared using the Student-test in case of normal distribution or Mann-Whitney test otherwise.
8

1 **What is Known?**

- 2 - Thrombocytopenia is frequently observed in patients assisted with extracorporeal membrane
3 oxygenation (ECMO) for pulmonary and/or cardiac support.
4 - Thrombocytopenia is a major risk-factor for severe, life-threatening complications in ECMO
5 patients
6 - Shedding of the platelet receptor GPIIb/IIIa has been reported in ECMO patients and is
7 associated with increased platelet clearance, but the mechanisms contributing to receptor
8 cleavage are poorly understood.
9

10 **What New Information Does This Article Contribute**

- 11 - Thrombocytopenia appears rapidly after ECMO support initiation, corrects quickly after
12 support withdrawal and is associated with a parallel platelet GPIIb/IIIa shedding.
13 - Platelet count is inversely correlated with GPIIb/IIIa shedding.
14 - Rapid elimination of GPIIb/IIIa-negative platelets from the circulation is directly dependent on
15 shear forces pertinent to the mechanical support. Patients under mechanical pulmonary and/or
16 cardiac support often display low peripheral platelet count and hemostatic complications.
17 Thrombocytopenia could be due to platelet clearance secondary to GPIIb/IIIa shedding but the
18 mechanisms responsible for cleavage of the platelet receptor in ECMO settings are currently
19 unknown.
20

21 Patients under mechanical pulmonary and/or cardiac support often display low peripheral platelet
22 count and hemostatic complications. Thrombocytopenia could be due to platelet clearance secondary
23 to GPIIb/IIIa shedding but the mechanisms responsible for cleavage of the platelet receptor in ECMO
24 settings are currently unknown.
25

26 To investigate the causes of ECMO-associated thrombocytopenia we studied platelet count,
27 modifications and functions in patients, and developed in vitro & animal models mimicking those
28 conditions. Our study demonstrates that thrombocytopenia has a high prevalence and an early
29 onset/offset closely related to ECMO implantation and withdrawal in assisted patients. Our
30 translational models were instrumental to demonstrate that shear forces generated during ECMO
31 support directly induce GPIIb/IIIa shedding from the platelet surface but do not inhibit platelet functions.
32 GPIIb/IIIa-negative platelets are rapidly cleared from circulation in NOD/SCID mice.

33 Overall, we describe a new mechanism of thrombocytopenia pertinent to ECMO patients that could
34 be targeted by new therapeutic approaches aiming to decrease and counteract life-threatening
35 complications.
36
37