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1 **Neutrophil activation in patients treated with endovascular therapy is associated with**  
2 **unfavorable outcomes and mitigated by intravenous thrombolysis**

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10

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14 **Abbreviations:** AIS: acute ischemic stroke; EVT: endovascular therapy; IVT: intravenous  
15 thrombolysis; ICH: intracranial hemorrhage; LVO: large vessel occlusion; MPO:  
16 myeloperoxidase; NA: neutrophil activation; tPA: tissue-type plasminogen activator

17 **Cover title:** Neutrophil activation and ischemic stroke

18 **Keywords: Stroke, Thrombectomy, Inflammation, Disability, Hemorrhage, Activation,**  
19 **Neutrophil**

20

21

22 **Supplemental Results:**

23 - **Supplemental Table 1**

24

25

1 **Abstract**

2

3 **Background-** Accumulating evidence indicate that neutrophil activation (NA) contributes to  
4 microvascular thromboinflammation in acute ischemic stroke (AIS) due to a large vessel  
5 occlusion. Preclinical data have suggested that intravenous thrombolysis (IVT) before  
6 endovascular therapy (EVT) could dampen microvascular thromboinflammation. In this  
7 study, we investigated the association between NA dynamics and stroke outcome, and the  
8 impact of IVT on NA in AIS patients treated with EVT.

9 **Methods-** Monocentric prospective study, including patients treated with EVT, for whom 3  
10 blood samples (before, within 1-h, 24-h post-EVT) were drawn to measure plasma  
11 myeloperoxidase (MPO) concentration as a marker of NA. Unfavorable outcome was defined  
12 as a modified Rankin score between 3-6 at 3 months.

13 **Results-** Between 2016-2020, 179 patients were included. Plasma MPO concentration  
14 significantly peaked 1-h post-EVT (median increase: 21.0 ng/ml [IQR -2.1 to 150]), returned  
15 to pre-EVT baseline values 24 hours after EVT (median change from baseline, -0.8 ng/ml;  
16 [IQR -7.6 to 6.7]). This peak was strongly associated with unfavorable outcomes at 3 months  
17 (aOR=0.51, 95%CI=0.33-0.81, p=0.004). IVT before EVT abolished this 1-h post-EVT MPO  
18 peak. Changes in plasma MPO concentration (baseline-1-h post-EVT) were associated with  
19 unfavorable outcomes only in patients not treated with IVT before EVT (aOR=0.51,  
20 95%CI=0.32-0.83, p=0.006). However, we found no significant heterogeneity in the  
21 associations between changes in plasma MPO concentration and outcomes.

22 **Conclusions-** A peak in plasma MPO concentration occurs early after EVT and is associated  
23 with unfavorable outcomes. IVT abolished the post-EVT MPO peak and may modulate the  
24 association between NA and outcomes.

1 **What is already known on this topic:**

2 Recent randomized trials have suggested an added value of intravenous thrombolysis in  
3 patients treated with endovascular therapy, with a possible action on neutrophil action and the  
4 thrombo-inflammatory response.

5 **What this study adds:**

6 We describe for the first time the dynamic of neutrophil activation using myeloperoxidase and  
7 show a 1-hour myeloperoxidase peak after endovascular therapy which was strongly  
8 associated with unfavorable outcomes. Interestingly, our results also suggest that intravenous  
9 thrombolysis may abolish this peak. In patients successfully treated with endovascular  
10 therapy, we found an effect of intravenous thrombolysis in the association between neutrophil  
11 activation and main outcomes.

12 **How this study might affect research, practice or policy:**

13 **This study highlights the prognostic significance of myeloperoxidase dosage before and**  
14 **after endovascular therapy. Future studies are needed to understand the**  
15 **pathophysiological mechanisms underlying the association between myeloperoxidase,**  
16 **intravenous thrombolysis and main outcomes.**

## 1 **Introduction**

2

3 The treatment of patients suffering from an acute ischemic stroke (AIS) due to a large vessel  
4 occlusion (LVO) has been revolutionized by endovascular therapy (EVT).<sup>1</sup> Owing to the  
5 tremendous efficacy of EVT in terms of recanalization rates,<sup>2</sup> the relevance of combining it  
6 with intravenous thrombolysis (IVT) using tissue-type plasminogen activator (tPA) has been  
7 questioned in recent randomized trials that have yielded conflicting results.<sup>3-6</sup> The rationale of  
8 this ongoing debate is the supposedly poor added value of IVT in terms of recanalization,<sup>7</sup>  
9 and its increased risk of symptomatic intracranial hemorrhage.

10 Recent data are converging to indicate that the deleterious role of neutrophils in AIS goes  
11 beyond their interference with IVT via the release of extracellular traps.<sup>8, 9</sup> In fact, both high  
12 admission neutrophil count<sup>10-13</sup> and neutrophil to lymphocyte ratio (NLR)<sup>13-17</sup> have been  
13 shown to be associated with unfavorable outcome in AIS patients treated with IVT and EVT.  
14 Interestingly, higher baseline NLR is also associated with higher odds of symptomatic  
15 intracranial hemorrhage, in EVT-treated patients.<sup>18</sup> Circulating levels of neutrophil effectors  
16 like myeloperoxidase (MPO), which is released upon neutrophil activation (NA), is positively  
17 correlated with AIS severity at onset, and is independently associated with all-cause  
18 mortality.<sup>12, 19</sup> Whether dynamic changes in NA occur in the setting of a LVO is currently  
19 unknown, and so does the potential impact of recanalization therapies on NA in AIS patients.  
20 Yet, results from preclinical studies in animal models of AIS have indicated that LVO triggers  
21 a deleterious thrombo-inflammatory cascade involving NA,<sup>20-22</sup> which can be dampened by  
22 using IVT prior to mechanical recanalization<sup>23</sup>. In particular, IVT was shown to reduce the  
23 formation and accumulation of platelet/neutrophil aggregates in the microcirculation  
24 downstream of the initial arterial occlusion site, thus improving microvascular patency, and  
25 therefore the benefits of arterial recanalization.

1 In the present study, using MPO as a marker of NA, we investigated the longitudinal  
2 evolution of NA in EVT-treated AIS patient, and determined its relationships with major  
3 outcomes, as well as its modulation by IVT.

1 **Methods**

2

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

5 ***Standard Protocol Approvals, Registrations, and Patient Consents***

6 Patient information was collected prospectively using a standardized questionnaire. Patients  
7 or their legal surrogate provided informed consent for study participation. The local ethics  
8 committee approved this research protocol (CPP Ile de France VII, ID-RCB number: 2015-  
9 A01856-43). Clinical Trial Registration-URL: <http://www.clinicaltrials.gov>. Unique identifier:  
10 NCT02900833.

11 ***Patient data collection***

12 All consecutive patients treated by EVT for a LVO at Rothschild Foundation Hospital  
13 between April 2016 and April 2020, and for whom all 3 blood samples were available were  
14 included in this study. Patients' clinical, radiological, biological, and treatment characteristics  
15 were collected prospectively. Most patients preferentially underwent brain magnetic  
16 resonance imaging (MRI) using at least diffusion-weighted-imaging (DWI), T2 Fluid-  
17 Attenuation-Inversion-Recovery, 3D Time-of-Flight and susceptibility-weighted-imaging  
18 sequences. In cases of MRI contraindication, brain computed tomography (CT) scan with CT  
19 angiography was performed. The Alberta Stroke Program Early CT score (ASPECTS) was  
20 assessed on the baseline CT or diffusion weighted imaging. Patients were treated in a  
21 dedicated neuroangiography suite with up-to-date equipment under conscious sedation or  
22 general anesthesia, using last generation devices. Successful and near-to-complete  
23 recanalization were defined as a modified Thrombolysis In Cerebral Infarction (mTICI) score  
24 of 2b-3 and 2c-3, respectively. Times from symptoms onset to recanalization were recorded.  
25 CT scan or brain MRI were performed systematically 24 hours after EVT. Intracranial  
26 hemorrhages (ICH) were described as hemorrhagic infarction type 1 or 2 (HI-1, HI-2) and



1 parenchymal hematoma type 1 or 2 (PH-1, PH-2). All imaging data were analyzed by two  
2 senior neuroradiologists, who were not involved in the management of the patients.  
3 Functional outcome was assessed by neurologists with the modified Rankin Scale (mRS) at 3  
4 months, during face-to-face interviews or via telephone conversations with the patient or their  
5 relatives.

### 6 ***Plasma collection and myeloperoxidase quantification***

7 Blood was collected in citrate (BD Vacutainer 3ml, 0.129 M sodium citrate) and EDTA (BD  
8 Vacutainer 4ml, K3E 7.2mg) from the femoral artery for the first 2 samplings (at the  
9 beginning of EVT and within 1h after EVT), and from a venous brachial puncture at 24-hour  
10 post-EVT. In patients treated with IVT prior to EVT, the first sample was collected after IVT.  
11 Plasma was collected after two centrifugations (10 min x2000g at 20°C and 15 min x2500g at  
12 20°C) within 15 minutes after blood withdrawal and immediately frozen at -80°C for further  
13 analysis. We used free MPO as a marker of NA, given numerous published works in the  
14 stroke<sup>12</sup> and cardiological field showing that MPO reliably reflected NA and its strong  
15 prognostic significance.<sup>24-27</sup> Differences in MPO concentrations between 1 hour or 24 hours  
16 and baseline were then calculated. MPO was quantified using commercial ELISA kits (Hycult  
17 Biotech).

### 18 ***Clinical and radiological outcome definitions***

19 Favorable outcome and unfavorable outcomes were defined as a mRS between 0 and 2 and  
20 between 3 and 6 at 3 months, respectively. Early neurological improvement was defined as an  
21 improvement of  $\geq 8$  points on the NIHSS or an NIHSS between 0 and 1 at 24 hours. ICH  
22 were defined as any ICH (HI-1, HI-2, PH-1, PH-2) on the 24-hour brain control imaging.

### 23 ***Statistical analyses***

24 Categorical variables were expressed as frequencies and percentages. Quantitative variables  
25 were expressed as mean  $\pm$  standard deviation (SD), or median (interquartile range, IQR) for

1 non-normal distribution. Normality of distributions was assessed graphically and by using the  
2 Shapiro-Wilk test. We first assessed the intra-patient differences in MPO concentrations (after  
3 log-transformation to satisfy the normality of residuals) from pre-EVT to 1 and 24 hours after  
4 EVT using a linear mixed model (an unstructured covariance pattern model to consider the  
5 correlation between repeated measures within the same subjects) by treating time as fixed 3-  
6 level categorical variable.

7 We assessed the association of baseline and post EVT variations in MPO on favorable  
8 outcome, early neurological improvement, and any ICH by using penalized logistic regression  
9 models adjusted for pre-specified factors (age, initial ASPECT and NIHSS score, time from  
10 onset to puncture and neutrophil count for any outcomes and on IVT for ICH); all models on  
11 differences in MPO concentrations were adjusted for baseline values. We checked the log-  
12 linearity assumption for MPO variables by using restricted cubic spline function. In a second  
13 step, baseline characteristics were described according the IVT and non-IVT treated patients  
14 and the magnitude of the between-group differences was assessed by calculating the absolute  
15 standardized difference; an absolute standardized difference >10% was interpreted as a  
16 meaningful difference.

17 We further compared the intra-patient differences in MPO concentrations between IVT and  
18 non-IVT treated patients prior to EVT and post-EVT with a pre-specified adjustment on  
19 NIHSS, ASPECT score at baseline and time from onset to puncture by including time, IVT  
20 status,time\*IVT interaction terms and pre-specified covariates into the previous linear mixed  
21 model; in case of significant interaction term, post-hoc comparison in intra-patient differences  
22 in MPO concentrations between patients with and without IVT were done using linear  
23 contrasts. Finally, we investigated the heterogeneity in the associations of baseline and post  
24 EVT variations in MPO on favorable outcome, early neurological improvement, and any ICH  
25 according to IVT use in the overall study population and in patients with near-to-complete

1 recanalization (mTICI 2c-3), by introducing the corresponding interaction term into previous  
2 multivariable penalized logistic regression models. To avoid case deletion in analyses due to  
3 missing data on covariates and outcomes, missing data were imputed by multiple imputations  
4 using regression-switching approach (chained equations with  $m=10$ ). Imputation procedure  
5 was performed under the missing at random assumption using all baseline and procedural  
6 characteristics and study outcomes with a predictive mean matching method for continuous  
7 variables and multinomial or binary logistic regression model for categorical variables.  
8 Estimates obtained in the different imputed data sets were combined using the Rubin's rules.  
9 Statistical testing was done at the two-tailed  $\alpha$  level of 0.05. Data were analyzed using the  
10 SAS software package, release 9.4 (SAS Institute, Cary, NC).

## 1 **Results**

2 Between April 2016 and April 2020, 203 patients with AIS due to an anterior or posterior  
3 LVO and treated with EVT were included in the Neurostroke study. Among them, 179  
4 patients had all 3 blood samples available for the analysis and were included in the study  
5 (**Figure 1**). Main baseline and treatment characteristics are reported in **Table 1**. Overall, mean  
6 age was 68.8 years (SD, 16.3) and 54.7% (n=108) were men. Median admission NIHSS and  
7 ASPECTS were 17 (IQR, 10 to 21) and 7 (5 to 8), respectively. Ninety-one patients (50.8%)  
8 received IVT prior to EVT, and time from onset to needle for patients treated with IVT was  
9 145 min (115 to 190), Supplemental Table 1. Overall, 75 patients (44.4%) achieved favorable  
10 functional outcome at 3 months, 57 (35.6%) patients had an early neurological improvement,  
11 and 67 (38.3%) patients had an ICH on the 24-hour brain control imaging.

12

### 13 **Neutrophil activation and outcomes**

14 Overall, MPO plasma concentrations significantly raised from baseline to 1-hour after EVT,  
15 with a median increase of 21.0 ng/ml (IQR -2.1 to 150) and returned to pre-EVT baseline  
16 values 24 hours after EVT (median change from baseline, -0.8 ng/ml; IQR -7.6 to 6.7, **Figure**  
17 **2**). As shown in **Table 2**, the amplitude of the plasma MPO peak between baseline and 1 hour  
18 after EVT was associated with functional outcomes at 3 months (adjusted OR=0.53, 95% CI,  
19 0.34 to 0.84, p=0.007). Patients with unfavorable functional outcomes at 3 months had a  
20 median increase in plasma MPO concentrations between baseline and 1 hour after EVT of  
21 68.2 ng/ml (IQR, -1.4 to 180.7 ng/mL), compared to 1.9 ng/mL (IQR, -3.7 to 105.3 ng/mL)  
22 for patients with favorable functional outcomes at 3 months. There was no significant  
23 association between functional outcomes at 3 months and either baseline plasma MPO  
24 concentrations, or differences in plasma MPO concentrations between baseline and 24 hours  
25 after EVT.

1 Changes in plasma MPO concentrations between baseline and 1 hour after EVT were also  
2 associated with early neurological improvement (adjusted OR=0.66, 95% CI, 0.45 to 0.97,  
3  $p=0.032$ ). There was no association between occurrence of ICH and either baseline or 1-h and  
4 24-h post-EVT changes in plasma MPO concentrations (**Table 2**).

5

### 6 **Impact of intravenous thrombolysis on neutrophil activation and outcomes**

7 After adjustment on pre-specified covariates, plasma MPO concentrations before EVT were  
8 similar between patients with and without IVT (**Figure 3**). At 1 hour post-EVT, whereas  
9 MPO concentrations rose significantly and markedly from baseline in patients without IVT  
10 (median change in plasma MPO concentrations between baseline and 1 hour after EVT, 136  
11 ng/mL (IQR, 14.7 to 200 ng/mL), they remained stable in patients treated with IVT prior to  
12 EVT (median, 0.9 ng/mL, IQR -4.4 to 27.0 ng/L) (**Figure 3**). At 24 hours post-EVT, plasma  
13 MPO concentrations had returned to baseline levels in patients with IVT and did not differ  
14 with those of patients without IVT (**Figure 3**).

15 Regarding outcomes, the associations between changes in plasma MPO concentrations at 1  
16 hour post-EVT from pre-EVT levels with favorable and neurological improvements were  
17 only significant in patients without IVT prior to EVT, with an adjusted OR of 0.54 (95%CI  
18 0.33 to 0.88,  $p=0.013$ ) for favorable outcome and 0.65 (95%CI, 0.43 to 0.98,  $p=0.041$ ) for  
19 neurological improvement (**Figure 4**). However, we found no significant heterogeneity in the  
20 associations between differences in MPO concentrations (baseline and 1 hour after EVT) and  
21 favorable or neurological improvement with IVT use. Finally, longitudinal differences in  
22 MPO concentrations were not associated with ICH according to the use of IVT (**Figure 4**).

23

### 24 **Effect of intravenous thrombolysis on neutrophil activation in patients with near-to-** 25 **complete recanalization**

1 We then focused on patients with near-to-complete recanalization (mTICI 2c-3). In this  
2 subpopulation, plasma MPO concentrations before EVT were not significantly associated  
3 with functional outcome in patients treated or not with IVT, but there was a significant effect  
4 of IVT on the association between baseline MPO and functional outcomes ( $p_{\text{het}}=0.039$ ,  
5 **Figure 5**). Similarly, we found a heterogeneity in the association between baseline MPO  
6 concentrations and early neurological improvement according to IVT use ( $p_{\text{het}}=0.015$ , **Figure**  
7 **5**).

## 1 **Discussion**

2 Our present results show that NA continues to evolve after and despite successful  
3 recanalization with EVT. In particular, we found that plasma MPO concentration, a validated  
4 marker of NA, significantly and transiently rose 1 hour after recanalization in EVT-treated  
5 patients. This result led us to assess the association between dynamic changes in plasma MPO  
6 concentrations and clinical outcomes. We found that unfavorable functional outcome at 3  
7 months and lower rates of early neurological improvement were strongly associated with a  
8 marked elevation of plasma MPO between baseline and 1 hour post-EVT, thus indicating that  
9 NA following EVT-mediated recanalization is detrimental.

10 Several recent randomized trials have highlighted the possible added value of IVT in EVT-  
11 treated patients<sup>3-6</sup>. Yet, how IVT improves the benefits of EVT-mediated recanalization  
12 remains elusive. **In 2018, Desilles et al. found a marked adhesion of leukocytes in post-  
13 capillary venules in a preclinical model of AIS in rats occurring before recanalization  
14 (ie, during occlusion) and early after recanalization, increasing from 25 minutes to 1  
15 hour after recanalization (ref JAHA). The Neurostroke study specifically included a  
16 sample 1 hour post-EVT to compare preclinical results with clinical data, and also a  
17 sample at 24 hours as other groups demonstrated in seminal works an increase of  
18 microvascular thrombosis hours after recanalization.**

19 Interestingly, we observed that IVT prior to EVT resulted in a marked attenuation of the post-  
20 EVT MPO peak, suggesting that IVT may interfere with the detrimental thrombo-  
21 inflammatory response leading to NA in AIS. This hypothesis is supported by our previous  
22 results in a rat model of AIS, in which IVT prior to recanalization significantly reduced the  
23 thrombo-inflammatory response to ischemia.<sup>23</sup> **That said, the association between MPO  
24 and IVT is complex to understand, as several previous studies also supported that IVT  
25 could activate neutrophils and neutrophil degranulation, (PMID: 25530154, 32977685,**

1 **18390930) altogether leading to NETOsis and MMP-9 release. In addition, other factors**  
2 **could have an impact on MPO peak and concentration, such as the type and amount of**  
3 **contrast injection, the modality of anesthesia (general versus conscious sedation), blood**  
4 **loss during EVT or the effect of the device used for EVT, among others. Further studies**  
5 **(preclinical and clinical) will thus be needed to comprehensively delineate the effect of**  
6 **IVT on NA.**

7 These data are important as they may help to understand the results of recent randomized  
8 trials which evaluated the added value of IVT with EVT. Contrarily to previous retrospective  
9 studies, the recent SKIP (The Direct Mechanical Thrombectomy in Acute LVO Stroke),<sup>4</sup> MR-  
10 CLEAN-NO IV (Multicenter Randomized Clinical Trial of EVT for AIS in the Netherlands-  
11 NO IV)<sup>3</sup> and SWIFT-DIRECT (Solitaire with the intention for Thrombectomy plus  
12 intravenous tPA versus DIRECT Solitaire Stent Retriever thrombectomy in AIS)<sup>28</sup> failed to  
13 prove the superiority, or the non-inferiority of EVT alone versus EVT in association with IVT  
14 with tPA. Remarkably, these trials did not show any safety issues regarding IVT use, as the  
15 risk of ICH was not increased in the IVT+EVT group versus EVT alone.

16 Moreover, several studies have consistently shown the benefits of reperfusion over  
17 recanalization,<sup>29, 30</sup> and the recent CHOICE trial (Chemical Optimization of Cerebral  
18 Embolectomy in Patients With Acute Stroke Treated With Mechanical Thrombectomy)  
19 showed a strong benefit of targeting downstream microcirculatory thrombosis using intra-  
20 arterial thrombolysis following IVT and EVT in successfully recanalized patients with  
21 anterior or posterior LVO (expanded TICI 2b50-3).<sup>31</sup> Microvessel occlusions are well beyond  
22 EVT's scope of action (ie, recanalization), and may impair reperfusion despite arterial  
23 recanalization (currently known as the no-reflow phenomenon).<sup>32</sup> By highlighting the  
24 importance of microcirculatory impairments despite successful recanalization, CHOICE set a  
25 new paradigm in AIS management. Interestingly, in a subgroup analysis of patients with near-



1 to-perfect recanalization (mTICI 2c-3), we found a heterogeneity in the association between  
2 baseline MPO concentrations and functional outcome with IVT use. Overall, our results  
3 underscore the deleterious impact of NA and thrombo-inflammation within the early phase of  
4 AIS consecutive to LVO and provide further insights regarding the beneficial biological  
5 effects of IVT in addition to EVT, especially when a near-to-perfect recanalization is  
6 achieved.

7 This study has several strengths. Each patient included in the Neurostroke study had a  
8 detailed neuroradiological work-up at inclusion and at 24 hours, assessed by neuroradiologists  
9 blind to the clinical outcomes and not involved in the patient's management. Moreover, its  
10 design, based on longitudinal assessment of plasma MPO concentration (3 blood samples, 1  
11 before and 2 after recanalization), provides a unique opportunity to assess the dynamic of NA  
12 in a homogenous group of EVT-treated AIS patients with LVO. From a translational  
13 standpoint, it is worth noting that EVT-treated LVO AIS represents the situation modelled by  
14 the tMCAO model, which relies on the occlusion followed by mechanical recanalization of  
15 the middle cerebral artery origin. This model has been used in many studies, including the  
16 aforementioned study in which IVT prior to mechanical recanalization was shown to dampen  
17 microvascular thromboinflammation.<sup>23</sup> As illustrated by the present study, samples from the  
18 Neurostroke study may be precious to verify the validity and relevance of concepts derived  
19 from the tMCAO model.<sup>33</sup>

20 However, several limitations must be acknowledged. Despite its prospective design, the  
21 present study is observational, potentially leading to confounding biases in the analysis.  
22 Although the multivariate analysis included adjustments on the most relevant parameters (ie,  
23 age, initial stroke severity, neutrophil count, time from symptom onset to puncture), an impact  
24 of other confounding variables (ie, hypertension,<sup>34</sup> cancer or other inflammatory conditions)  
25 on plasma MPO concentration variations and their relationships with outcomes cannot be

1 excluded. Second, our results pertain only to a population of EVT-treated stroke patients, with  
2 or without IVT. In fact, because our study did not include control cohorts of non-stroke  
3 patients, no conclusion regarding the impact of AIS or EVT-treatment on NA can be drawn.  
4 In a similar manner, the absence of AIS patients having received no therapies or only IVT  
5 precludes conclusions on the effect of IVT on NA, irrespective of EVT. Third, another  
6 important unanswered question is whether, as suggested by preclinical studies in animal  
7 models of AIS,<sup>35</sup> plasma MPO concentration evolution or NA in general is related to or  
8 influences reperfusion. Indeed, besides the lack of non-reperfused AIS patients in our study,  
9 the reperfusion status at 24 hours was not systematically assessed by CT or MRI perfusion  
10 sequence in our patient cohort. Further and larger studies are therefore needed to fully  
11 understand the interrelationships between NA, IVT, reperfusion, and long-term outcomes in  
12 AIS. Fourth, this was a single-center study, with a moderate sample size, and these results  
13 should be externally validated in larger studies to fully understand the effect of NA on long-  
14 term outcomes. Fifth, the decision whether to use IVT prior to EVT was not randomized and  
15 depended upon the physician in charge and the patient's and stroke's characteristics (ie, no  
16 control group without IVT). Therefore, it is possible that cofounders (known or unknown)  
17 between these two groups partly explain our results.

18

1 **Conclusion**

2 We show that plasma MPO concentrations, a marker of NA, rise at 1 hour post-EVT in AIS  
3 patients with LVO, and that unfavorable functional outcome and lower rates of early  
4 neurological improvement are strongly associated with higher post-EVT increase in plasma  
5 MPO concentrations. The use of IVT before EVT drastically reduced the post-EVT peak in  
6 plasma MPO. Further studies will be needed with MPO assessment in control cohorts to  
7 comprehensively understand the impact of IVT on NA and its added value with EVT.

8

1 **Table 1. Patients and Treatment Characteristics in the study population (n=179)**

Characteristics	N=179
<b>Baseline demographics and medical history</b>	
Age, y, mean $\pm$ SD	68.8 $\pm$ 16.3
Men	98/179 (54.7)
Hypertension	109/178 (61.2)
Diabetes	34/178 (19.1)
Current smoking	44/178 (24.7)
Prior antithrombotic medications	
<i>Antiplatelet</i>	46/174 (26.4)
<i>Anticoagulant</i>	45/174 (25.9)
<b>Current stroke event</b>	
Pre-stroke mRS $\leq$ 2	159/178 (89.3)
Site of occlusion	
<i>M1-MCA</i>	76/179 (42.5)
<i>M2-M3-MCA</i>	46/179 (25.7)
<i>ICAT</i>	23/179 (12.8)
<i>Extracranial ICA or Tandem</i>	22/179 (12.3)
<i>Basilar artery</i>	9/179 (5.0)
<i>Multivessel occlusions</i>	3/179 (1.7)
Admission NIHSS score, median (IQR) <sup>a</sup>	17 (10 to 21)
ASPECTS score, median (IQR) <sup>b</sup>	7 (5 to 8)
MPO at baseline	27.9 (20.1 to 39.2)
<b>Etiology</b>	
<i>Cardioembolic</i>	85/172 (49.4)
<i>Others</i>	87/172 (50.6)
<b>Treatment characteristics</b>	
IVT	91/179 (50.8)
Number of devices passes, median (IQR) <sup>c</sup>	2 (1 to 3)
Procedural times, min, median (IQR)	
<i>Onset to puncture</i> <sup>c</sup>	225 (164 to 310)
<i>Imaging to groin puncture</i> <sup>d</sup>	111 (62 to 145)
<i>Onset to recanalization</i> <sup>a</sup>	275 (207 to 364)

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**Outcomes**

mTICI 2b-3	167/178 (93.8)
mTICI 2c-3	120/178 (67.4)
Favorable outcome at 3 months	75/169 (44.4)
Early neurological improvement	57/160 (35.6)
ICH	67/175 (38.3)

**Symptomatic ICH****15/157 (9.6%)**

- 
- 1 Values are expressed as number (%) unless otherwise indicated. <sup>a</sup>2 missing value, <sup>b</sup>13 missing values, <sup>c</sup>1 missing  
2 values, <sup>d</sup>4 missing values. Abbreviations: ASPECTS=Alberta Stroke Program Early Computed Tomography  
3 Score; EVT=endovascular therapy; ICA=intracranial carotid artery; ICH=intracranial hemorrhage;  
4 IQR=interquartile range; MCA=middle cerebral artery; MPO=myeloperoxidase; mRS=modified Ranking scale;  
5 mTICI=modified Thrombolysis in Cerebral Infarction; NIHSS=National Institutes of Health Stroke Scale;  
6 SD=standard deviation.

1 **Table 2. Associations between baseline and post-thrombectomy variations in myeloperoxidase plasma concentrations with outcomes.**

2

MPO concentrations	Functional outcome at 3 months		OR (95%CI)*	p-values	OR (95%CI)*†	P-Value†
	Poor outcome (n=99)	Favorable outcome (n=80)				
<b>MPO</b>						
Admission	30.5 (21.1 to 40.1)	25.9 (19.1 to 37.4)	0.94 (0.69 to 1.27)	0.90	1.03 (0.72 to 1.49)	0.85
Difference between baseline and 1 hour after EVT	68.2 (-1.4 to 180.7)	1.9 (-3.7 to 105.3)	0.54 (0.38 to 0.79)	0.002	0.53 (0.34 to 0.84)	0.007
Difference between baseline and 24 hours after EVT	0.7 (-7.6 to 10.2)	-1.7 (-8.3 to 2.7)	0.59 (0.32 to 1.08)	0.089	0.76 (0.45 to 1.28)	0.30
<b>Early neurological improvement</b>						
	No (n=114)	Yes (n=65)				
<b>MPO</b>						
Admission	29.3 (20.6 to 38.9)	27.1 (19.5 to 43.2)	1.02 (0.73 to 1.42)	0.93	0.99 (0.68 to 1.44)	0.95
Difference between baseline and 1 hour after EVT	65.9 (-0.3 to 169.5)	0.9 (-4.4 to 95.6)	0.76 (0.53 to 1.09)	0.14	0.66 (0.45 to 0.97)	0.032
Difference between baseline and 24 hours after EVT	-0.6 (-8.3 to 6.7)	-0.8 (-6.2 to 5.9)	0.85 (0.55 to 1.31)	0.46	0.88 (0.56 to 1.39)	0.59
<b>Intracranial hemorrhages</b>						
	No (n=110)	Yes (n=76)				
<b>MPO</b>						
Admission	27.2 (18.7 to 39.2)	28.4 (21.5 to 39.2)	1.04 (0.76 to 1.42)	0.82	0.97 (0.69 to 1.36)	0.85
Difference between baseline and 1 hour after EVT	11.6 (-2.4 to 121.4)	55.7 (-0.9 to 172.8)	1.29 (0.95 to 1.75)	0.22	1.38 (0.95 to 2.00)	0.087
Difference between baseline and 24 hours after EVT	-0.4 (-8.3 to 6.5)	-1.7 (-7.6 to 8.5)	1.01 (0.73 to 1.40)	0.96	0.89 (0.62 to 1.27)	0.52

3

1 Values (ng/mL) are expressed as median (IQR). \*OR expressed per one SD increase in MPO. † calculated using penalized logistic regression models adjusted for age, initial  
2 ASPECTS and NIHSS score, time from onset to puncture and neutrophil count (additional adjustment on IVT for ICH and additional adjustment on baseline MPO for  
3 association with MPO variations). Descriptive parameters, OR (95%CI) and P-values were calculated after handling missing values using multiple imputation procedure  
4 (m=10).  
5 Abbreviations: CI= confidence interval; EVT=endovascular treatment; ICH=intracranial hemorrhages; MPO= myeloperoxidase; mRS=modified ranking scale; OR=odds  
6 ratio.

## 1 **Figure legends**

### 2 3 **Figure 1. Study Flow Chart**

### 4 5 6 **Figure 2. Distribution (Tukey's Box plots) of plasma concentrations of myeloperoxidase** 7 **before endovascular thrombectomy, 1 hour, and 24 hours after endovascular** 8 **thrombectomy**

9 \* indicates P-value <0.05 from between time comparison in neutrophil biomarkers calculated using linear mixed  
10 model (an unstructured covariance pattern model) after Bonferroni correction. A log-transformation was applied  
11 to satisfy the normality of model residuals. Circle and cross indicate means values.

### 12 13 14 **Figure 3. Evolution of plasma myeloperoxidase concentrations according to IV tPA**

#### 15 **status**

16 \* indicates P-value <0.05 for post-hoc comparisons of variations in plasma myeloperoxidase (MPO)  
17 concentrations between IV tPA-treated and non-IV tPA-treated patients calculated using linear mixed model (an  
18 unstructured covariance pattern model) after Bonferroni correction and adjustment on NIHSS, ASPECT scores  
19 at baseline and time from onset to puncture. A log-transformation was applied to satisfy the normality of model  
20 residuals.

### 21 22 23 **Figure 4. Associations between baseline and variations in myeloperoxidase plasma** 24 **concentrations with outcomes according to the use of intravenous thrombolysis.**

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26  
27 Values (ng/mL) are expressed as median (IQR). Patients without IVT n=88 (n=33 with favorable outcomes and  
28 intracranial hemorrhages, n=25 with early neurological improvement); Patients with IVT n=91 (n=47 with  
29 favorable outcomes, n=36 with intracranial hemorrhages, n=40 with early neurological improvement).

30 \*OR expressed per one SD increase in neutrophil parameters calculated using penalized logistic regression  
31 models adjusted for age, initial ASPECTS and NIHSS score, time from onset to puncture and neutrophil count  
32 (additional adjustment on baseline myeloperoxidase (MPO) for associations with variations in plasma MPO  
33 levels). Descriptive parameters, OR (95%CI) and P-values were calculated after handling missing values using  
34 multiple imputation procedure (m=10). P-het represents the p-value of interaction between MPO and IVT.

35 Abbreviations: CI=confidence interval; EVT=endovascular treatment; ICH=intracranial hemorrhage;  
36 IVT=intravenous thrombolysis; MPO=myeloperoxidase; mRS=modified Ranking Scale; OR=odds ratio

### 37 38 39 **Figure 5. Associations between baseline and variations in myeloperoxidase plasma** 40 **concentrations with outcomes according to the use of intravenous thrombolysis in near-** 41 **to-complete recanalization (mTICI 2c-3)**

42  
43  
44 Values (ng/mL) are expressed as median (IQR). Patients without IVT n=56 (n=23 with favorable outcomes,  
45 n=22 with intracranial hemorrhages, n=17 with early neurological improvement); Patients with IVT n=64 (n=35  
46 with favorable outcomes, n=23 with intracranial hemorrhages, n=33 with early neurological improvement).

47 \*OR expressed per one SD increase in neutrophil parameters calculated using penalized logistic regression  
48 models adjusted for age, initial ASPECTS, NIHSS score and neutrophil count (additional adjustment on baseline  
49 myeloperoxidase (MPO) for associations with variations in plasma MPO concentrations). Descriptive  
50 parameters, OR (95%CI) and P-values were calculated after handling missing values using multiple imputation  
51 procedure (m=10). P-het represents the p-value of interaction between MPO and IVT.

52 Abbreviations: ICH=intracranial hemorrhage; EVT=endovascular treatment; IVT=intravenous thrombolysis;  
53 MPO=myeloperoxidase; mRS=modified Ranking Scale; OR=odds ratio.



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2

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6

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