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# **Analysis of the association of MPO and MMP-9 with stroke severity and outcome:**

## **Cohort study**

**Running head title:** plasma biomarkers and neutrophils in stroke

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## **Abstract**

**Objective:** In acute cerebral ischemia, circulating neutrophil count and neutrophil-to-lymphocyte ratio (NLR) are positively associated with stroke severity and worse outcomes. However, mediators of this effect are unknown. We aimed to investigate: (i) the relationship between plasmatic matrix metalloproteinase-9 (MMP-9) and myeloperoxidase (MPO) concentrations with stroke severity and outcome, and (ii) MMP-9 and MPO release after *ex-vivo* stimulation of neutrophils by recombinant tissue plasminogen activator (rt-PA).

**Methods:** We analyzed data collected in 255 patients with supratentorial cerebral infarcts recruited within 48 hours of symptoms onset irrespective of rt-PA treatment. The endpoints were excellent outcome (modified Rankin Scale score 0-1), symptomatic intra-cerebral hemorrhage (ECASS-II definition) and death at 3 months. The role of rt-PA treatment on peripheral neutrophil degranulation was investigated in 18 patients within 4.5 hours and after 72 hours.

**Results:** Neutrophil counts, NLR and plasmatic MPO concentrations, but not plasmatic MMP-9, were positively correlated with stroke severity. Higher neutrophil counts and NLR, but not plasmatic MMP-9 and MPO concentrations, were independently associated with worse outcomes and higher mortality rates at 3 months. Neutrophil-derived MMP-9, after *ex-vivo* rt-PA stimulation, but not MPO, were higher after 72 hours in patients treated by intravenous rt-PA although not associated with hemorrhagic transformation.

**Conclusion:** Unlike the neutrophil counts and NLR, plasmatic MMP-9 and MPO concentrations are not associated with worse outcomes in patients with acute cerebral ischemia. This suggests that MMP-9 and MPO are not the main mediators of the deleterious effects of neutrophils and cannot be considered as useful therapeutic targets.

## Introduction

A higher circulating neutrophil count in ischemic stroke (IS) is associated with higher stroke severity, larger infarct size, increased risk of hemorrhagic transformation (HT), and worse outcomes,<sup>1, 2</sup> regardless of intravenous (i.v.) recombinant tissue plasminogen activator (rt-PA) administration.<sup>3, 4</sup> Some observations suggest that plasma matrix metalloproteinase (MMP-9) can be responsible for the deleterious effect of neutrophils in acute IS: (i) there is an increase in leukocyte MMP-9 mRNA within the first 3 hours after stroke onset;<sup>5</sup> (ii) the plasmatic MMP-9 concentration is higher in IS patients compared to controls;<sup>6</sup> (iii) higher plasmatic MMP-9 concentrations are associated with larger infarct volume,<sup>7</sup> worse stroke severity,<sup>7, 8</sup> worse functional outcomes<sup>8-10</sup> and increased risk of HT, irrespective of treatment with i.v. rt-PA.<sup>11,9, 12-15</sup> Since MMP-9 is released from neutrophils, it is considered the main mediator of this effect.<sup>16</sup>

The plasmatic myeloperoxidase (MPO) concentration, an enzyme secreted during inflammation by activated neutrophils,<sup>17</sup> has been found increased in acute IS patients.<sup>18</sup> Inhibiting MPO activity led to a reduction in infarct size in animals.<sup>19</sup> Some data also suggest a detrimental effect of MPO activity on post-ischemic neurogenesis<sup>20</sup> and neuroprotection.<sup>21</sup> Furthermore, rt-PA can promote *in-vitro* and *in-vivo*<sup>16</sup> release of early degranulation products from neutrophils, including MMP-9 and MPO.<sup>22</sup>

The aim of this study was to investigate, in acute IS patients, the relationship between plasmatic MMP-9 and MPO concentrations and (i) baseline stroke severity; (ii) circulating neutrophils and neutrophil-to-lymphocyte ratio (NLR); (iii) outcome at month-3. We also investigated MMP-9 and MPO release after *ex-vivo* rt-PA stimulation of neutrophils.

## Methods

### *Inclusion and non-inclusion criteria*

We analyzed data collected in patients aged 40 years or more, with supratentorial **IS**, prospectively recruited within 48 hours of symptoms onset between June 2005 and May 2009 in the *Biostroke* study (NCT00763217), a study of biomarkers conducted in the stroke center of the Lille University hospital. A subgroup of patients was recruited within 4.5 hours of symptoms onset in order to evaluate MMP-9 and MPO release after *ex-vivo* rt-PA treatment of neutrophils. Patients included in the *Biostroke* cohort who had either transient ischemic attacks or intra-cerebral hemorrhages were excluded from the analysis. Patients were not consecutive, because of the need to obtain written informed consents for all patients and the ability to perform the *in-vitro* analysis only between 8 am and 2 pm 3 days per week for the subgroup.

### *Clinical assessment*

Stroke severity was assessed by the National Institutes of Health Stroke Scale (NIHSS)<sup>23</sup> at inclusion in the study, or just before the bolus of i.v. rt-PA in patients who received thrombolytic therapy. Definitions used for variables included in the medical history, vascular risk factors, and evaluation of the baseline clinical characteristics, were those used by our group in a previous study.<sup>24</sup> The outcome was assessed either at a face-to-face visit or by a telephone interview at 3 months using the modified Rankin Scale (mRS).<sup>25</sup>

### *Intravenous thrombolysis administration*

Intravenous rt-PA (Actilyse®, Boeringher, Ingelheim, Germany) was administered according to the recommendations of the European Stroke Organisation<sup>26</sup> (i.e. 0.9 mg/kg body weight, maximum 90 mg, 10% of the dose as a bolus, followed by a 60-min infusion).

## *Imaging*

All patients underwent at admission either a non-contrast computed tomographic (CT)-scan (Siemens, Somatom Sensation 16 detectors, Erlangen, Germany), or a magnetic resonance imaging (MRI)-scan (Achieva Philips, 1.5 T, with FLAIR, gradient echo T2\*, diffusion b1000 with ADC mapping, and TOF sequences) in both cohorts. All patients underwent a second neuroimaging investigation by CT or MRI 6 to 10 days later in *Biostroke* study and also 24 to 72 hours later in the subgroup, or at any time in case of clinical worsening. In patients who received i.v. rt-PA, the second imaging investigation was performed between 22 to 36 hours after treatment.

## *Evaluation of neutrophil counts, plasmatic MMP-9 and MPO concentrations*

Neutrophil and lymphocyte counts, plasmatic MMP-9 and MPO concentrations were determined on blood samples drawn at inclusion and after 72 hours in the subgroup. Blood samples from patients who received rt-PA were collected before thrombolysis.

Plasmatic MMP-9 and MPO concentrations were determined as follows: all samples were collected into Vacutainer tubes (Becton Dickinson France, Le Pont de Claix, France). Plasma samples were obtained from tubes containing heparin and were kept frozen until analysis. Activated MMP-9 was assessed with a commercially available kit (Fluorokine® E, Human Active MMP-9, R&D Systems, Inc., USA). Plasma MPO was measured by Magnetic Human High Sensitivity Luminex assay (Millipore, St Quentin en Yvelines, France), according to the manufacturers' instructions. These assays were performed according to the recommendations of the manufacturer in duplicate the same day on the same run to allow direct comparisons.

## *End points*

The 3 endpoints were (i) excellent outcome defined as a mRS<sup>25</sup> score 0 or 1 at 3 months, (ii) symptomatic intra-cerebral hemorrhage (s-ICH) according to the European Cooperative Acute Stroke Study (ECASS)-II definition,<sup>27</sup> and (iii) death within 3 months.

#### *Neutrophils isolation and ex-vivo stimulation*

Peripheral neutrophils degranulation was investigated at inclusion and before rt-PA treatment for patient who received i.v. rt-PA, and after 72 hours for all patients. According to our previously described method,<sup>28</sup> neutrophils were isolated from patients heparinized venous blood by centrifugation on Ficoll-Hypaque density gradient. Neutrophils resuspended in culture medium (Roswell Park Memorial Institute (RPMI) 1640 medium) were > 98% pure, as determined by hematology analyzer (XS800i, Sysmex Corporation, Kobe, Japan). Then, purified neutrophils ( $4 \times 10^5$  cells per well) were seeded into 24-well plates and cultured in RPMI-1640 medium with L-glutamine and sodium pyruvate (Gibco BRL) at 37°C, 5% CO<sub>2</sub>. Neutrophils were incubated with 0.1 mg/ml rt-PA for 30 min. Neutrophils supernatant was collected, centrifuged (12 minutes at 14000 rpm) and stored at -80°C until analysis. Nontreated cells were used as controls.

#### *Statistics*

Quantitative variables are expressed as mean (standard deviation) in case of normal distribution or median (interquartile range) otherwise. Categorical variables are expressed as percentage (count). We compared groups for categorical variables, with the Chi Square test with Yates' correction or Fisher exact test when appropriate, and for continuous variables with the Mann-Whitney U test. Normality of distributions was assessed using histograms and Shapiro-Wilk test. Log-transformed values for all biological parameters with skewed distributions were used in statistical analysis.

We performed a stepwise multiple logistic regression analysis with excellent outcome, s-ICH, or death within 3 months as dependent variables. Independent variables were selected from the bivariate analysis for the excellent outcome, with a 0.10 level as a screening criterion for the selection of candidate variables. The variables “age, NIHSS, neutrophil counts (model 1) or plasmatic MMP-9 (model 2) or MPO concentrations (model 3) or all (model 4), and thrombolysis” were forced into the model. Correlations between variables were checked for possible co-linearity, which was defined as  $r$  values  $>0.6$ . The same analysis was repeated using NLR instead of neutrophil counts (model 5). Data were analyzed using the SPSS 22.0 package for Windows and GraphPad Prism 6 for Windows.

### *Ethics*

*Biostroke* study was approved by the local ethical committee. Patients were managed according to local rules without any investigation or treatment specifically performed. The study was fully explained to the patients. All patients or a close relative gave a signed informed consent for the dosages of MMP-9 and MPO. Neutrophil and lymphocyte counts were considered as part of usual care.

### **Results**

Out of 322 patients meeting inclusion criteria, 67 (20.8%) could not be analyzed because of missing data for delay of sample collection for neutrophils (5 patients) or plasmatic MMP-9 and MPO concentrations (20 patients), neutrophil count (19 patients), mRS at 3 months (32 patients), or any combination in the same patients. Patients included in the analysis differed from patients excluded only for a lower proportion of female, a lower baseline stroke severity and no intra-hospital death occurrence (Table 1).



Out of 255 patients included in our analysis, 137 patients (53.7%) had a low stroke severity (admission NIHSS score  $\leq 5$ , Table 2). Among demographic characteristics and baseline clinical measures, only no history of congestive heart failure ( $9 \pm 7.6$  vs  $22 \pm 16.1$ ,  $p = 0.04$  for treated and not treated patients, respectively), no nitrate use ( $6 \pm 5.1$  vs  $17 \pm 12.5$ ,  $p = 0.04$ ) and lower admission glucose level ( $94.5$  [86.7-109.7] vs  $112$  [95-131.5],  $p < 0.001$ ) were associated with a lower stroke severity.

#### *Admission stroke severity and biological parameters*

Except plasmatic MMP-9 concentrations, all biological parameters differed significantly between patients with and without admission NIHSS  $\leq 5$  (Table 2). Patients with admission NIHSS  $\leq 5$  had lower neutrophil counts, NLR and plasmatic MPO concentrations than patients with admission NIHSS  $> 5$ . When the NIHSS was analyzed as a quantitative scale, NIHSS was positively correlated with neutrophils ( $r=0.49$ ,  $p<0.001$ ), NLR ( $r= 0.48$ ,  $p<0.001$ ) and plasmatic MPO ( $r=0.21$ ,  $p=0.001$ ) concentrations but the correlation was not statistically significant for plasmatic MMP-9 concentrations ( $r=0.06$ ,  $p=0.359$ ).

#### *Rt-PA and biological parameters*

Patients treated by rt-PA had higher neutrophil counts and higher NLR than patients not treated by i.v. rt-PA (Table 3). Conversely, plasmatic MMP-9 and MPO concentrations did not significantly differ between patients eligible or not to i.v. rt-PA.

#### *Adjusted and unadjusted correlation between neutrophil counts, NLR and plasmatic MPO and MMP-9 concentrations*

Neutrophil counts were significantly correlated with plasmatic MPO as well as MMP-9 concentrations in all patients (Table 4). Moreover, the NLR was correlated with plasmatic MPO concentrations in all patients.

After adjustment for NIHSS and onset to sample delay, circulating neutrophil counts were significantly correlated with plasmatic MMP-9 concentrations in all patients ( $\rho=0.215$ ,  $p=0.001$ ). The NLR was significantly correlated with plasmatic MMP-9 concentrations ( $\rho=0.146$ ,  $p=0.021$ ).

#### *Influence of neutrophils, NLR, plasmatic MMP-9 and MPO concentrations on outcome*

Higher neutrophil counts were independently associated with a lower probability to have an excellent outcome, and a higher mortality rate at month-3, but was not associated with the risk of s-ICH. Plasmatic MMP-9 and MPO concentrations were not associated with any of these outcome measures. These results remained similar in the 4 different models, with MMP-9 alone, MPO alone, neutrophils alone, and all combined (Table 5). These results were not modified by the replacement of neutrophil count by NLR (Table 5).

#### *Neutrophil isolation and degranulation*

To better understand if MMP-9 and MPO could be considered as mediators of the neutrophil effects, we recruited 18 patients out of 255 [8 (44.4%) women, median age 80 years (74-88) and 12 patients (66.7%) received i.v. rt-PA]. Biology characteristics of this subgroup at baseline and after 72 hours are described in Table 6. Patients who received i.v. rt-PA were similar in terms of age and pre-stroke functional status as well as for stroke severity to patients who did not receive i.v. rt-PA. HT was significantly more frequent in patients who received i.v. rt-PA. Additionally, plasmatic MPO concentrations were significantly higher at admission in patients eligible to i.v. rt-PA compared to patients not eligible, while any

difference was found at 72 hours between patients who received or not i.v. rt-PA treatment. Any difference was found for neutrophil counts, NLR and plasmatic MMP-9 concentrations at baseline and after 72 hours.

#### *Neutrophil-derived MMP-9 and MPO concentrations*

We therefore analyzed in this subgroup MMP-9 and MPO release after *ex-vivo* rt-PA stimulation of native neutrophils at admission and after 72 hours. At admission and before thrombolysis (H0), no differences were found in neutrophil-derived MMP-9 and MPO concentrations between patients treated or not by i.v. rt-PA (figure 1A and 1B). After seventy-two hours (H72), circulating neutrophils from patients treated by i.v. rt-PA release more MMP-9 after rt-PA stimulation than those not treated ( $568 \pm 119$  vs  $229.8 \pm 23.75$ ,  $p=0.03$ , figure 1A). No differences were found for neutrophil-derived MPO at H0 and H72 between patients treated or not by i.v. rt-PA (figure 1B). Since there were no s-ICH in this subgroup, we considered this time any HT found at the control neuroimaging investigation. However, no significant differences were found between neutrophil-derived MMP-9 (figure 1C) or MPO (figure 1D) concentrations and HT according to treatment.

Therefore, we explored the correlations between plasmatic MMP-9 and MPO concentrations, circulating neutrophil counts, NLR and neutrophil-derived MMP-9 and MPO concentrations at corresponding time in whole population (Table 7). Our results show that, at admission, neutrophil-derived MPO concentrations were negatively correlated with neutrophil-derived MMP-9. At H72, plasmatic MPO concentrations were positively correlated with NLR.

## **Discussion**

Our study has shown that, within the first 48 hours after symptoms onset in patients with supratentorial cerebral infarct: (i) circulating neutrophil counts, NLR, and plasmatic MPO

concentrations were correlated with stroke severity but not plasmatic MMP-9 concentrations; (ii) higher neutrophil counts were correlated with higher plasmatic MMP-9 concentrations in the whole cohort, even after adjustment for delay of sample collection and stroke severity; (iii) higher neutrophil counts were correlated with plasmatic MPO concentrations in all patients; (iv) neutrophil counts and NLR were higher in patients eligible to i.v. rt-PA treatment but not plasmatic MMP-9 and MPO concentrations; (v) higher neutrophil counts and NLR were independently associated with less excellent outcomes and higher mortality rate at 3 months, but not with s-ICH; (vi) plasmatic MMP-9 and MPO concentrations were not associated with any of the 3 endpoints.

To better understand if MMP-9 and MPO could be considered as mediators of the neutrophil effects<sup>5</sup>, we then focused our research on the neutrophil-derived MMP-9 and MPO. We found that: (i) at H0 plasmatic MPO concentrations after *ex-vivo* rt-PA stimulation were higher in patients eligible for i.v. rt-PA treatment but not associated with HT, but no differences were found concerning neutrophil-derived MPO concentrations in patients treated or not by i.v. rt-PA; (ii) after H72, circulating neutrophils from patients treated by i.v. rt-PA release more MMP-9, but not MPO, after stimulation than those from patients not treated; (iii) in the whole cohort, only neutrophil-derived MPO concentrations were found to be correlated negatively with neutrophil-derived MMP-9 at H0, and (iv) plasmatic MPO concentrations were found to be correlated with NLR at H72.

The strength of our study was to be the first to evaluate the correlation between plasmatic MMP-9 concentrations, neutrophil-derived MMP-9 and neutrophils in patients with acute cerebral ischemia within the first hours after symptoms onset.

The main limitations are the delay of several hours between symptoms onset and sample collection in *Biostroke* study, with a possible selection bias due to the need of written

informed consent. When analyses were adjusted on delays of sample collection concerning MMP-9, the results remained similar, but this may be insufficient to fully correct for the delay if the relationship is not perfectly linear. Given the relatively brief lifespan of neutrophils in culture, the delay following their isolation from blood was a practical difficulty that limits the number of patients in the subgroup recruited for studying the neutrophil degranulation.

In 2 previous studies, but not in ours, higher plasmatic MMP-9 concentrations were associated both in patients treated<sup>13</sup> and untreated by i.v. rt-PA,<sup>29</sup> with worse outcomes,<sup>29</sup> especially s-ICH.<sup>13</sup> A possible explanation for this discrepancy could be the delay of sample collection, i.e. less than 3<sup>29</sup> or 4.5 hours<sup>13</sup> vs. up to 48 hours in our study: MMP-9 concentrations increase within the first 2 to 6 hours after symptom onset,<sup>5, 30</sup> then remained stable over the first 48 hours after stroke,<sup>7</sup> as confirmed in our subgroup. However, it has also been reported that neither baseline MMP-9 concentrations measured within a few hours of IS onset nor the rate of MMP-9 at any point within 14 days had any association with the risk of HT in patients treated or not with lower dose of rt-PA.<sup>31</sup>

Our study showed an influence of rt-PA on neutrophil degranulation according to the kinetic and distribution pattern of the tertiary (MMP-9) and primary granules (MPO). This is consistent with evidences that rt-PA play a role on neutrophil degranulation and MMP-9 release as shown in some *in-vitro* experiments.<sup>7,22</sup>

Moreover, no correlation was found between plasmatic MMP-9 concentrations and stroke severity in the whole cohort of *Biostroke* study included within 48 hours. This finding could be connected to the short half-life (*i.e.* 1 to 2 hours) of rt-PA, so that rt-PA could influence more the MMP-9 up-regulation in the first hours following its administration.<sup>29</sup> We cannot exclude that the sample size was too small for a subgroup analysis in patients treated by rt-

PA. Nevertheless, worse outcomes were statistically associated with higher neutrophil counts and NLR in the same sample as previously described in our published study.<sup>4</sup>

The cellular source of MMP-9 is controversial. If we consider the whole cohort, plasmatic MMP-9 concentrations were correlated with neutrophil counts and NLR. This finding together with the results of our *in-vitro* experiments are consistent with the hypothesis that circulating neutrophils represent a major cellular source of circulating MMP-9.<sup>32</sup> Nevertheless the lack of correlation between plasmatic MMP-9 concentration and the outcome in our study could be explained by the presence of other cellular sources in the acute phase of cerebral ischemia as well as resident brain cells, cells of the vasculature or other circulating immune cells.<sup>32</sup>

Plasmatic MPO concentrations were associated with stroke severity, which agrees with earlier studies.<sup>18, 33</sup> Plasmatic MPO levels are elevated in stroke patients compared with normal subjects<sup>18</sup> and can predict future stroke and vasculopathic events in Fabry disease.<sup>34</sup> In our study, plasmatic MPO concentrations were correlated with neutrophil counts. MPO is the most abundant component in azurophilic (or primary) granules in neutrophils, which are the last to be released from the neutrophil by degranulation.<sup>16</sup> Indeed, in our study, the negative correlation between neutrophil-derived MMP-9 and MPO at admission could be due to the kinetics of activation of neutrophils in response to rt-PA.<sup>6</sup> In fact, MPO activity peaks later, around 3 to 7 days after stroke.<sup>17, 19</sup> Traditionally it has been used as a histopathological marker for neutrophils. Indeed, it is now accepted that MPO is not an exclusive marker of neutrophils, but it is also expressed in the myeloid line, especially in monocytes and macrophages/microglia.<sup>35</sup> A study demonstrates that circulating MPO concentration has no direct action on barrier disruption but increases expression of adhesion molecules in

endothelial cells.<sup>36</sup> Finally, all these data demonstrate that MPO may be not an interesting biomarker of the detrimental effect of neutrophils in acute cerebral ischemia.

Our study revealed a statistical association between neutrophil counts and NLR, but not plasmatic MMP-9 and MPO concentrations with stroke outcome, suggesting that MMP-9 and MPO are probably not the main mediators of the deleterious effects of neutrophils on stroke severity and outcome. Therefore, a selective inhibition of MMP-9<sup>32, 37</sup> and MPO may be less promising than a global inhibition of neutrophils to improve outcomes in patients with cerebral ischemia.<sup>38</sup> Other potential mediators, such as elastase, reactive oxygen species, and other MMPs, need to be evaluated.<sup>5</sup> The neutrophil is the key factor of post-thrombolysis hemorrhages.<sup>4, 38</sup> Among all factors released by neutrophil, MMP-9 and MPO do not appear as the main contributors although it remains to determine its place in addition or synergy with other released factors.<sup>39</sup>

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## Appendix 1 Authors

<b>Name</b>	<b>Location</b>	<b>Role</b>	<b>Contribution</b>
Ilaria Maestrini, MD, PhD	University of Lille, Inserm U1171, Lille,France  Sapienza University, Rome, Italy	Author	Collected data, analyzed and interpreted all data, performed the literature search, conceptualized the study and drafted the manuscript.
Madjid Tagzirt, PhD	University of Lille, Inserm U1011, Lille, France	Author	Collected data, analyzed and interpreted all data, performed the literature search, conceptualized the study and drafted the manuscript.
Sophie Gautier, PharmD, PhD	University of Lille, Inserm U1171, Lille, France	Author	Collected data and revised the manuscript.
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Anne-Marie Mendyk, RN	University of Lille, Inserm U1171, Lille,France	Author	Collected data, conceptualized the study and revised the manuscript.
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Didier Leys, MD, PhD, FAAN, FEAN	University of Lille, Inserm U1171, Lille,France	Author	Designed the study, analyzed and interpreted all data, drafted the manuscript and conceptualized the study.
Regis Bordet, MD, PhD.	University of Lille, Inserm U1171, Lille,France	Author	Designed the study, interpreted all data, conceptualized the study and drafted and revised the manuscript.



## **Disclosures**

**CC** reports receiving speaker fees from Boehringer-Ingelheim and Pfizer, is an investigator in clinical trials for Astra-Zeneca, Boehringer-Ingelheim, Daichii-Sankyo, Servier, and is a member of advisory boards for BMS.

**DL** reports grants from Ministry of Health- Grant PHRC, during the conduct of the study, receiving compensation to the research found of the hospital and Adrinord for advisory boards and symposia from BMS / Pfizer, from Boehringer-Ingelheim, and from Bayer, outside the submitted work, and is Vice-editor of the *European Stroke Journal*.

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The other authors have no conflict to report.

## References

1. Mo X, Li T, Ji G, et al. Peripheral polymorphonuclear leukocyte activation as a systemic inflammatory response in ischemic stroke. *Neurol Sci* 2013;34:1509-1516.
2. Buck BH, Liebeskind DS, Saver JL, et al. Early neutrophilia is associated with volume of ischemic tissue in acute stroke. *Stroke* 2008;39:355-360.
3. Kumar AD, Boehme AK, Siegler JE, et al. Leukocytosis in patients with neurologic deterioration after acute ischemic stroke is associated with poor outcomes. *J Stroke Cerebrovasc Dis* 2013;22:e111-117.
4. Maestrini I, Strbian D, Gautier S, et al. Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology* 2015;85:1408-1416.
5. Jickling GC, Liu D, Ander BP, et al. Targeting neutrophils in ischemic stroke: translational insights from experimental studies. *J Cereb Blood Flow Metab* 2015;35:888-901.
6. Morancho A, Rosell A, Garcia-Bonilla L, Montaner J. Metalloproteinase and stroke infarct size: role for anti-inflammatory treatment? *Ann N Y Acad Sci* 2010;1207:123-133.
7. Carbone F, Vuilleumier N, Bertolotto M, et al. Treatment with recombinant tissue plasminogen activator (r-TPA) induces neutrophil degranulation in vitro via defined pathways. *Vascul Pharmacol* 2015;64:16-27.
8. Jickling GC, Liu D, Stamova B, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab* 2014;34:185-199.

9. Montaner J, Alvarez-Sabin J, Molina C, et al. Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. *Stroke* 2001;32:1759-1766.
10. Inzitari D, Giusti B, Nencini P, et al. MMP9 variation after thrombolysis is associated with hemorrhagic transformation of lesion and death. *Stroke* 2013;44:2901-2903.
11. Ramos-Fernandez M, Bellolio MF, Stead LG. Matrix metalloproteinase-9 as a marker for acute ischemic stroke: a systematic review. *J Stroke Cerebrovasc Dis* 2011;20:47-54.
12. Rosell A, Cuadrado E, Ortega-Aznar A, et al. MMP-9-positive neutrophil infiltration is associated to blood-brain barrier breakdown and basal lamina type IV collagen degradation during hemorrhagic transformation after human ischemic stroke. *Stroke* 2008;39:1121-1126.
13. Castellanos M, Leira R, Serena J, et al. Plasma metalloproteinase-9 concentration predicts hemorrhagic transformation in acute ischemic stroke. *Stroke* 2003;34:40-46.
14. Montaner J, Molina CA, Monasterio J, et al. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. *Circulation* 2003;107:598-603.
15. Alvarez-Sabin J, Maisterra O, Santamarina E, Kase CS. Factors influencing haemorrhagic transformation in ischaemic stroke. *Lancet Neurol* 2013;12:689-705.
16. Castellanos M, Sobrino T, Millan M, et al. Serum cellular fibronectin and matrix metalloproteinase-9 as screening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: a multicenter confirmatory study. *Stroke* 2007;38:1855-1859.

17. Breckwoldt MO, Chen JW, Stangenberg L, et al. Tracking the inflammatory response in stroke in vivo by sensing the enzyme myeloperoxidase. *Proc Natl Acad Sci U S A* 2008;105:18584-18589.
18. Tay A, Tamam Y, Yokus B, et al. Serum myeloperoxidase levels in predicting the severity of stroke and mortality in acute ischemic stroke patients. *Eur Rev Med Pharmacol Sci* 2015;19:1983-1988.
19. Forghani R, Kim HJ, Wojtkiewicz GR, et al. Myeloperoxidase propagates damage and is a potential therapeutic target for subacute stroke. *J Cereb Blood Flow Metab* 2015;35:485-493.
20. Kim H, Wei Y, Lee JY, et al. Myeloperoxidase Inhibition Increases Neurogenesis after Ischemic Stroke. *J Pharmacol Exp Ther* 2016;359:262-272.
21. Kim HJ, Wei Y, Wojtkiewicz GR, et al. Reducing myeloperoxidase activity decreases inflammation and increases cellular protection in ischemic stroke. *J Cereb Blood Flow Metab* 2018:271678X18771978.
22. Cuadrado E, Ortega L, Hernandez-Guillamon M, et al. Tissue plasminogen activator (t-PA) promotes neutrophil degranulation and MMP-9 release. *J Leukoc Biol* 2008;84:207-214.
23. Lyden P, Brott T, Tilley B, et al. Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group. *Stroke* 1994;25:2220-2226.
24. Ducroquet A, Leys D, Al Saabi A, et al. Influence of chronic ethanol consumption on the neurological severity in patients with acute cerebral ischemia. *Stroke* 2013;44:2324-2326.

25. van Swieten JC, Koudstaal PJ, Visser MC, et al. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988;19:604-607.
26. European Stroke Organisation Executive C, Committee ESOW. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. *Cerebrovasc Dis* 2008;25:457-507.
27. Hacke W, Kaste M, Fieschi C, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Second European-Australasian Acute Stroke Study Investigators. *Lancet* 1998;352:1245-1251.
28. Gautier S, Ouk T, Tagzirt M, et al. Impact of the neutrophil response to granulocyte colony-stimulating factor on the risk of hemorrhage when used in combination with tissue plasminogen activator during the acute phase of experimental stroke. *J Neuroinflammation* 2014;11:96.
29. Ning M, Furie KL, Koroshetz WJ, et al. Association between tPA therapy and raised early matrix metalloproteinase-9 in acute stroke. *Neurology* 2006;66:1550-1555.
30. Kelly PJ, Morrow JD, Ning M, et al. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the Biomarker Evaluation for Antioxidant Therapies in Stroke (BEAT-Stroke) study. *Stroke* 2008;39:100-104.
31. Tsuruoka A, Atsumi C, Mizukami H, et al. Effects of edaravone, a free radical scavenger, on circulating levels of MMP-9 and hemorrhagic transformation in patients with intravenous thrombolysis using low-dose alteplase. *J Stroke Cerebrovasc Dis* 2014;23:2894-2899.

32. Turner RJ, Sharp FR. Implications of MMP9 for Blood Brain Barrier Disruption and Hemorrhagic Transformation Following Ischemic Stroke. *Front Cell Neurosci* 2016;10:56.
33. Palm F, Pussinen PJ, Safer A, et al. Serum matrix metalloproteinase-8, tissue inhibitor of metalloproteinase and myeloperoxidase in ischemic stroke. *Atherosclerosis* 2018;271:9-14.
34. Kaneski CR, Moore DF, Ries M, et al. Myeloperoxidase predicts risk of vasculopathic events in hemizygous males with Fabry disease. *Neurology* 2006;67:2045-2047.
35. Rausch PG, Pryzwansky KB, Spitznagel JK. Immunocytochemical identification of azurophilic and specific granule markers in the giant granules of Chediak-Higashi neutrophils. *N Engl J Med* 1978;298:693-698.
36. Chu Y, Wilson K, Gu H, et al. Myeloperoxidase is increased in human cerebral aneurysms and increases formation and rupture of cerebral aneurysms in mice. *Stroke* 2015;46:1651-1656.
37. Chaturvedi M, Kaczmarek L. Mmp-9 inhibition: a therapeutic strategy in ischemic stroke. *Mol Neurobiol* 2014;49:563-573.
38. Ouk T, Potey C, Maestrini I, et al. Neutrophils in tPA-induced hemorrhagic transformations: Main culprit, accomplice or innocent bystander? *Pharmacol Ther* 2018.
39. Roeber L, Levine SR. Cerebral hemorrhage following thrombolytic therapy for stroke: Are neutrophils really neutral? *Neurology* 2015;85:1360-1361.

**Figure 1. MMP-9 and MPO release from neutrophils after *ex-vivo* recombinant tissue plasminogen activator (rt-PA) stimulation in patients treated by rt-PA versus not treated (A and B) and in hemorrhagic (HT) group vs non-hemorrhagic (non-HT) group (C and D).**

**Table 1.** Baseline characteristics of the study population and bivariate comparison with patients excluded. Unless specified, values are number of patients (%). \*Value given as median (interquartile range). mRS: modified Rankin Scale; s-ICH: symptomatic intra-cerebral hemorrhage (ECASS2 definition); MMP-9: metalloproteinase-9; MPO: myeloperoxidase; rt-PA: recombinant tissue plasminogen activator.

	<b>Included (n=255)</b>	<b>Excluded (n=67)</b>	<b>p value</b>
<b>Demographic characteristics</b>			
Female sex	116 (45.5)	40 (59.7)	<b>0.038</b>
Age*	70 (57-79)	72 (61-82)	0.155
Body mass index (kg/m <sup>2</sup> )*	26.4 (23.1-29.4)	27.3 (23.7-30.1)	0.509
<b>Medical history</b>			
Arterial hypertension	160 (62.7)	41 (61.2)	0.816
Diabetes mellitus	51 (20.0)	11 (16.4)	0.508
Hypercholesterolemia	124 (48.6)	29 (43.3)	0.436
Coronary artery disease	53 (20.8)	17 (25.4)	0.418
Heart failure	31 (12.2)	7 (10.4)	0.700
History of atrial fibrillation	61 (23.9)	20 (30.3)	0.287
Recurrent stroke	28 (11.0)	9 (13.4)	0.575
Previous physical activity	133 (52.4)	29 (43.9)	0.223
Alcohol consumption	38 (15.0)	7 (10.6)	0.365
Smoking habit	73 (28.6)	20 (29.9)	0.844
Statins	80 (31.4)	24 (35.8)	0.488
Antithrombotic therapy	109 (50.6)	30 (44.8)	0.766
<b>Stroke characteristics and treatment</b>			
Baseline NIH Stroke Scale*	6 (2-13)	8 (2-19)	<b>0.026</b>
rt-PA treatment	71 (28.1)	23 (34.3)	0.317
Onset-to-treatment time (min)*	140 (106.2-180)	150 (86.2-187)	0.744
<b>Clinical and biological characteristics</b>			
Systolic blood pressure (mmHg)*	152 (137-170)	156 (136-170)	0.741
Blood glucose concentration (g/L)*	1.02 (0.90-1.25)	1.04 (0.90-1.29)	0.792
Platelet count (x 1,000/mm <sup>3</sup> )*	236 (197-278)	228 (189-261)	0.200
Temperature (°C)*	36.7 (36.2-37.0)	36.5 (36.3-37.1)	0.665
<b>Delay of sample collection</b>			
Onset-to-sample time (min)*	1440 (840-2100)	1200 (472-2100)	0.210
<b>Outcome measures</b>			
s-ICH	32 (12.6)	5 (8.5)	0.377
Death at day-8 (mRS = 6)	0 (0.0)	16 (23.9)	<b>&lt;0.001</b>
Excellent outcome at 3 months (mRS = 0-1)	130 (51.0)	20 (57.1)	0.494
Death at 3 months (mRS = 6)	25 (9.8)	4 (10.3)	1.000



**Table 2. Comparisons of biological parameters including neutrophil counts, neutrophil to lymphocyte ratio (NLR), plasmatic metalloproteinase-9 and myeloperoxidase concentrations between patients with and without lower stroke severity (NIHSS  $\leq$ 5) at admission.** Values are median (interquartile range). Comparisons were done using parametric tests (Student t test) for all parameters (using log-transformed values for skewed distribution).

Abbreviations: NLR = neutrophil to lymphocyte ratio, MMP-9 = matrix metalloproteinase 9, MPO = myeloperoxidase.

	<b>NIHSS <math>\leq</math>5 at admission</b>		
	Yes (n=118)	No (n=137)	p value
<b>Neutrophils</b> (x1000/mm <sup>3</sup> )	4.6 (3.6-6.0)	6.4 (5.2-8.5)	<b>&lt;0.001</b>
<b>NLR</b>	2.4 (1.8-3.6)	4.3 (2.8-6.6)	<b>&lt;0.001</b>
<b>MMP-9</b> (ng/ml)	116 (74.5-174)	125 (71-217)	0.19
<b>MPO</b> (x1000 ng/ml)	57.5 (42-115)	77.9 (50.9-120)	<b>0.009</b>

**Table 3. Comparisons of biological cascade including neutrophils, neutrophil to lymphocyte ratio, plasmatic metalloproteinase-9 and myeloperoxidase concentrations between patients eligible to intravenous recombinant tissue plasminogen activator (rt-PA) or not.** Values are median (interquartile range). Comparisons were done using parametric tests (Student t test) for all parameters.

Abbreviations: NLR = neutrophil to lymphocyte ratio, MMP-9 = matrix metalloproteinase, MPO = myeloperoxidase.

	<b>rt-PA treatment</b>		
	Yes (n=101)	No (n=257)	p value*
<b>Neutrophils</b> (x1000/mm <sup>3</sup> )	6.4 (4.9-8.5)	5.4 (3.9-6.9)	<b>&lt;0.001</b>
<b>NLR</b>	4.2 (2.6-7.2)	3.0 (2.0-4.8)	<b>0.001</b>
<b>MMP-9</b> (ng/ml)	140 (67-257)	116.5 (76.2-172.5)	0.24
<b>MPO</b> (ng/ml)	69.6 (48.1-112.2)	65.2 (46.9-117.6)	0.48

**Table 4. Bivariate correlation between neutrophils or neutrophil-to-lymphocyte ratio (NLR) and plasmatic MMP-9 or MPO concentrations in all patients.**

Abbreviations: MMP-9 = metalloproteinase-9; MPO = myeloperoxidase; NLR = neutrophil to lymphocyte ratio.

	Neutrophils		NLR	
	rho	p value	rho	p value
<i>All patients</i>				
<b>MMP-9</b>	0.157	<b>0.012</b>	0.108	0.088
<b>MPO</b>	0.242	<b>&lt;0.001</b>	0.217	<b>0.001</b>

**Table 5. Results of the logistic regression analyses with excellent outcome (modified Rankin Scale 0 or 1) at 3 months, death at month-3, and symptomatic intra-cerebral hemorrhage (s-ICH), as dependent variables in all patients.** Model 4: with neutrophils, plasmatic MMP-9 and MPO concentrations. Model 5: with NLR, plasmatic MMP-9 and MPO concentrations. \* for 1,000 neutrophils/mm<sup>3</sup> increase. Abbreviations: NIHSS = National Institutes of Health Stroke Scale; MMP-9 = metalloproteinase-9; MPO = myeloperoxidase; NLR = neutrophil-to-lymphocyte ratio; rt-PA: recombinant tissue plasminogen activator.

Dependent variables	Logistic regression analyses	Independent variables	adjOR (95%CI)	p value	Variables entered in the model but not selected
<b>Model 4 (with Neutrophils + MMP-9 + MPO)</b>					
<b>Excellent outcome</b>	Overall p value	Age	0.96 (0.93-0.99)	0.006	MMP-9, MPO, rt-PA, female, congestive heart failure, atrial fibrillation, diabetes, smoking
	<0.001	NIHSS ≤5	14.37 (6.47-	<0.001	
	R <sup>2</sup> = 0.536	Neutrophils*	31.95)	0.001	
<b>s-ICH</b>	Well classified =80.3%	Hypertension	0.76 (0.64-0.90)	0.026	Age, NIHSS ≤5, neutrophils, MMP-9, MPO
	Overall p value	Rt-PA	4.19 (1.81-9.71)	0.001	
	=0.001				
<b>Death at month-3</b>	R <sup>2</sup> = 0.163				MMP-9, MPO, rt-PA
	Well classified =87.4%				
	Overall p value	Age	1.10 (1.04-1.15)	<0.001	
	<0.001	NIHSS ≤5	0.13 (0.03-0.63)	0.012	
<b>Model 5 (with NLR + MMP-9 + MPO)</b>	R <sup>2</sup> = 0.395	Neutrophils*	1.48 (1.16-1.88)	0.002	MMP-9, MPO, rt-PA, female, congestive heart failure, atrial fibrillation, diabetes, smoking,
	Well classified =92.5%				
	Overall p value	Age	0.97 (0.94-0.99)	0.024	
<b>Excellent outcome</b>	<0.001	NIHSS ≤5	15.19 (6.85-	<0.001	MMP-9, MPO, rt-PA, female, congestive heart failure, atrial fibrillation, diabetes, smoking,
	R <sup>2</sup> = 0.526	NLR	33.67)	0.005	
	Well classified =79.8%	Hypertension	0.83 (0.72-0.94)	0.015	
<b>s-ICH</b>			0.36 (0.16-0.82)		Age, NIHSS ≤5, NLR, MMP-9, MPO,
	Overall p value	Rt-PA	4.26 (1.83-9.91)	0.001	
	=0.001				
<b>Model 5 (with NLR + MMP-9 + MPO)</b>	R <sup>2</sup> = 0.172				MMP-9, MPO, rt-PA, female, congestive heart failure, atrial fibrillation, diabetes, smoking,
	Well classified =87.4%				
	Overall p value	Age	0.97 (0.94-0.99)	0.024	
<b>Excellent outcome</b>	<0.001	NIHSS ≤5	15.19 (6.85-	<0.001	MMP-9, MPO, rt-PA, female, congestive heart failure, atrial fibrillation, diabetes, smoking,
	R <sup>2</sup> = 0.526	NLR	33.67)	0.005	
	Well classified =79.8%	Hypertension	0.83 (0.72-0.94)	0.015	
<b>s-ICH</b>			0.36 (0.16-0.82)		Age, NIHSS ≤5, NLR, MMP-9, MPO,
	Overall p value	Rt-PA	4.26 (1.83-9.91)	0.001	
	=0.001				

<b>Death at month-3</b>	Overall p value	Age	1.10 (1.04-1.16)	0.001	MMP-9, MPO, rt-PA,
	<0.001	NIHSS $\leq 5$	0.09 (0.02-0.49)	0.005	
	$R^2 = 0.405$	NLR	1.35 (1.14-1.61)	0.001	
	Well classified =91.1%				

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**Table 6. Biology characteristics at baseline and after 72 hours according to i.v. rt-PA treatment.**

Abbreviations: NLR = neutrophil-to-lymphocyte ratio; MMP-9 = matrix metalloproteinase-9; MPO = myeloperoxidase; NIHSS = National Stroke Health Stroke Score; HT = hemorrhagic transformation.

	H0			H72		
	r-tPA(n=12)	not r-tPA (n=6)	p value	r-tPA(n=12)	not r-tPA (n=6)	p value
<b>Leukocytes</b> (x1000/mm <sup>3</sup> )	9 (7.5-9.7)	7.6 (4.7-9.7)	0.2	8.8 (7-10.9)	6.7 (3.2-11.1)	0.2
<b>Neutrophils</b> (x1000/mm <sup>3</sup> )	6.8 (5.6-7.5)	5.6 (3.2-7.2)	0.2	6.5 (4.4-9.1)	3.2 (2.2-8.1)	0.1
<b>NLR</b>	6 (3-10)	5.3 (2.4-18.10)	0.6	5.9 (3.7-7)	3.2 (1.1-15.1)	0.2
<b>Plasma MMP-9</b> (ng/ml)	121.4 (84.9-168.4)	135.8 (11.5-199)	0.5	130 (93-220)	103 (70.4-199)	0.6
<b>Plasma MPO</b> (ng/ml)	<b>69.9 (44.2-99)</b>	<b>32,9 (27.3-69.9)</b>	<b>0.03</b>	77.2 (52 -103)	62 (52.6-103)	0.5
<b>NIHSS</b>	11 (6-16)	7 (1-16)	0,15	6 (2-15.5)	3 (0.7-9.7)	0,3
<b>HT</b>				<b>7 (58.3%)</b>	<b>1 (16%)</b>	<b>0.03</b>

**Table 7. Spearman's (Rho) correlation between neutrophil-derived MMP-9 and MPO release and plasmatic MMP-9 and MPO concentrations, neutrophils and NLR.**

\*: correlation is significant at the 0.05 level; \*\*: correlation is significant at the 0.01 level.

Abbreviations: NLR = neutrophil-to-lymphocyte ratio; MMP-9 = matrix metalloproteinase-9;

MPO = myeloperoxidase; HT = hemorrhagic transformation.

		Neutrophils	NLR	Plasma MMP-9	Neutrophil- derived MMP-9	Plasma MPO	Neutrophil- derived MPO
H0	NLR	0,46					
	Plasma MMP-9	-0,04	0,26				
	Neutrophil-derived MMP-9	0,26	0,3	-0,24			
	Plasma MPO	0,23	0,28	0,25	0,15		
	Neutrophil-derived MPO	0,02	-0,17	0,03	<b>-0,74**</b>	-0,04	
	HT	0,07	0,26	0,24	-0,24	0,05	0,31
H72	NLR	<b>0,64**</b>					
	Plasma MMP-9	0,16	0,23				
	Neutrophil-derived MMP-9	0,43	0,03	0,25			
	Plasma MPO	0,28	<b>0,55*</b>	0,43	0,14		
	Neutrophil-derived MPO	0,42	0,19	-0,39	-0,14	-0,17	
	HT	0,24	0,11	-0,31	-0,12	0,05	0,01