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Monocyte count at onset predicts post-stroke outcomes during a 90-day follow up

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Running title: Monocytes and stroke outcomes.

Abstract

Background: Acute ischemic stroke (AIS) triggers both systemic and neurovascular inflammation, influencing post-stroke recovery. In smokers with AIS inflammation might be further upregulated, increasing ischemia/reperfusion injury. Here, the predictive value of leukocyte and adhesion molecules levels on post-stroke outcomes was investigated.

Materials and methods: 89 patients with AIS (n=30 smokers and n=59 non-smokers) were recruited and evaluated 1, 7 and 90 days after the onset to assess stroke severity by the National Institute of Health Stroke Scale (NIHSS) score as well as clinical recovery at 90 day by the modified Rankin Scale (mRS). Lesion volume was assessed by non-contrast computed tomography. Hematological parameters, blood chemistry and soluble adhesion molecules were measured.

Results: Smokers experienced a more severe stroke and at a younger age with respect to non-smokers, moreover they had higher circulating levels of monocytes, neutrophils and soluble adhesion molecules. Baseline monocytes positively correlated with stroke severity and disability across all time points in the overall cohort. No correlation was shown between adhesion molecules and post-stroke outcomes. A monocyte count >$0.63x10^9$/L predicted worse stroke severity (defined as NIHSS≥5) at day 90 independently of age, hypertension, thrombolysis and active smoking in the overall cohort. Similarly, a monocyte count >$0.64x10^9$/L predicted poor neurological recovery at day 90 (defined as mRS>2).

Conclusions: Smoker had more severe AIS and higher leukocytes and adhesion molecule levels. In the overall cohort, monocyte count was an independent predictor of worse post-stroke outcome. Although larger trials are needed, monocyte count might be a cheap prognostic parameter in AIS.

Keywords: inflammation; ischemic stroke; monocytes; adhesion molecules.

Introduction

A pro-inflammatory cascade was described to regulate the ischemia/reperfusion (I/R) injury [1]. Resident macrophage (microglia) activation in response to neuronal death is strictly followed by releasing of cytokines and chemokines, such as interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, and monocyte chemoattractant protein (MCP)-1 [2]. Among soluble inflammation mediators, adhesion molecules released by endothelial cells or leukocytes might influence immune cell recruitment into injured brain [3]. As marker of endothelial dysfunction/activation, levels of adhesion molecules were previously associated with both endothelial injury and cerebrovascular risk [4-6]. The peak of monocyte/macrophage infiltration within ischemic areas is reached only 3-7 days after acute ischemic stroke (AIS) [7]. Conversely, cir-

culating monocyte count was earlier increased as a result of different mechanisms, such as the increase of granulocyte colony-stimulating factor serum levels [8] and the mobilization of the spleen reserve [9, 10]. Treatment targeting monocytes kinetics after cerebral ischemia only partially confirmed these results since they did not induce relevant benefit in term of parenchymal rescue [11-13]. Therefore, additional studies are needed to potentially clarify the pathophysiological relevance of monocytes in AIS [14]. On the other hand, patients with AIS are often exposed to cardiovascular (CV) risk factors that can interfere with immune cells and vascular inflammation. For instance, smoking is a well-known risk factor for AIS [15]. Several compounds from cigarette have shown the ability to increase endothelial cell and white blood cell (WBC) expression of inflammatory mediators [9, 16]. Nicotine itself can induce leukocyte rolling and adhesion in cerebral circulation [17], increase brain infarct size and worsen neurological deficit in mice undergoing transient middle cerebral artery occlusion [18]. In this study, we aimed at comparing the inflammatory status of patients with AIS depending on their active smoking habits. The potential association of circulating levels of leukocytes and soluble adhesion molecules with clinical and radiological disease severity after 1, 7, and 90 days from AIS will be also explored. Finally, the predictive value of leukocyte count and adhesion molecules on 90-day clinical outcomes will be assessed.

Methods

The study protocol was performed in accordance to the guidelines of the Declaration of Helsinki and approved by Ethics Committee of Ferrara University Hospital. All patients or their legal representative gave informed consent prior to entering in the study. Reporting of the study conforms to STROBE statement [19].

Patients and clinical assessment

As previously described [20], 90 consecutive patients with a first AIS were recruited from April 2009 to December 2011 at the Neurology Department of Ferrara University Hospital, Italy. A focal neurological deficit characterized by neuroimaging evidence of cerebral infarction defined the AIS [21]. All patients admitted within 6 hours from AIS onset were consecutively recruited, except those with previous AIS or a combination of primary hemorrhagic stroke, seizure, intracranial abscess or brain cancer, acute infection, recent (<30 days) myocardial infarction or surgery, malignancy or renal/hepatic failure. These patients were treated in accordance with recommended guidelines [22] and followed-up for 90 days. In the present observational sub-study, we analyzed 89 patients, based on the residual available serum samples. Furthermore, we distinguished between smoker and non-smoker patients at AIS onset. According to the American Centers for Disease Control and Prevention definition [23], current smokers were considered those who have smoked 100 cigarettes in his or her lifetime and currently smoking at the time of AIS onset. Infarcts were distinguished accordingly to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification into five subtypes: i) secondary to large-artery atherosclerosis; ii) cardio-embolic stroke, iii) lacunar stroke, iv) stroke of other determined aetiology or v) stroke of undetermined aetiology [24]. Stroke severity were assessed at different time points (AIS onset, day 1, 7, and 90 after AIS) by using the National Institute of Health Stroke Scale (NIHSS) [25]. Clinical recovery at 90 day from AIS onset was also measured by the modified Rankin Scale (mRS) [26]. Good and poor outcomes were identified by mRS $\leq$2 and mRS >2 [26] or NIHSS <5 and NIHSS $\geq$5 [20, 27], respectively.

Patient follow-up and study endpoint adjudication

90-day clinical follow-up was completed for all patients. The primary endpoint was to investigate the predictive value of monocyte number at onset considering the long-term neurological recovery in the overall cohort. Secondary endpoints included correlations between circulating monocyte and blood adhesion molecule levels at AIS onset and clinical and radiological stroke severity at different time points in the overall cohort and in smoker and non-smoker patients. Specifically, clinical assessment was done by NIHSS and mRS, while ischemic lesion volume assessment was based on non-contrast cranial computed tomography (NCCT). As previously described [28], two study investigators at the Ferrara University Hospital who were blinded to the results of biochemical analysis independently adjudicated study endpoints.

Power study calculation

Since no previous studies have investigated the predictive role of monocyte count in stroke, the power of this study was calculated taking into account a rise in the incidence of worse outcome associated with increased circulating levels of C-reactive protein (CRP). Based on a recent published study [29], the sample size requested to observe a 1.5-fold increase in the risk of worse outcome with a power of 80% and a 2-sided α <5% was 198. Therefore this study has to be considered as a pilot study.

Neuroimaging

As already reported [28], NCCT was performed at onset and then 1, 7, and 90 days after AIS on a 64-slice Lightspeed VCT (GEMedical System,Milwaukee, WI; USA) from the skull base to the vertex by using an axial technique with the following imaging parameters: 120 kVp, 350 mA, 512 × 512 matrix, 25 cm-DFOV, 4 × 5-mm collimation, 1 s/rotation and table speed

of 15 mm/rotation. All NCCT images were acquired along the orbitomeatal plane with 2.5 mm (8 images/rotation) and 5 mm (4 images/rotation) slice thickness reconstruction for the posterior fossa and supra-tentorial region, respectively [20]. At admission, we evaluated the extension of early ischemic changes (hypo-attenuation, loss of the grey–white matter boundary and effacement of cortical sulci) by using the Alberta Stroke Program Early CT Score (ASPECT), a 10-point scale that quantifies the presence or absence of ischemia in 10 cerebral regions assigning a score of 1 for normal and 0 for a region showing ischemic signs [30]. Moreover, as described by Brott and colleagues [25], ischemic volume changing overtime was calculated with a multislice planimetric method by summation of the hypodense areas, manually traced on each slides in which they were detectable, multiplied by slice thickness.

Blood collection and analysis

By using a butterfly to reduce membrane shear stress, blood samples were collected at different time points and then drawn in tubes to obtain serum. The first sample was collected at time 0 (in thrombolysed patients within 1 h from the beginning of thrombolysis and in non-thrombolysed ones within 1 h from hospital admission) and then at days 1, 7, and 90 after AIS onset. By using a routine auto-analyzer, hematology parameters and blood chemistry were measured at admission.

Serum biomarker measurement

The levels of intercellular adhesion molecule (ICAM)-1, E-selectin, and L-selectin in serum were measured by colorimetric enzyme-linked immunosorbent assay (ELISA) following the manufacturer instruction (R&D Systems, Minneapolis, MN for all). Limits detection were 15.625 pg/mL for ICAM-1, 93.750 pg/mL for E-selectin, and 78.125 pg/mL for L-selectin.

Mean intra- and inter-assay coefficients of variation were <8% for all markers measured by ELISA methods.

Statistical analysis

Analyses were performed with IBM SPSS Statistics for Windows, version 20.0 (Armonk, NY: IBM Corp.). Overall cohort, non-smoker and smoker characteristics were described at admission. Qualitative data were presented as absolute and relative frequencies and then compared with Pearson $\chi 2$ test. Continuous variables were presented as median (interquartile range) and their comparison was performed by non-parametric Mann-Whitney U test. Intergroup comparison of marker serum levels at different time points was analyzed by Wilcoxon text. The relationships of monocyte count and adhesion molecule serum levels at stroke onset with NIHSS and mRS at day 90 were assessed by the linear regression analysis in both univariate and multivariate models. The predictive accuracy towards a worse disability score was assessed by the receiver operating characteristic (ROC) analysis. The area under the curve (AUC) was given with 95% confidence interval (CI) obtained using MedCalc 12.5 (MedCalc Software, Ostend, Belgium), and the cut-off point of monocyte number was calculated by maximizing the sensitivity according to the Youden index. Univariate and adjusted for age, hypertension, thrombolysis, and smoking status at stroke onset risk analyses were performed using logistic regression models. Categorized monocyte number (based on the cut-off value) and risk factors were set as the dependent variable, whereas mRS at day 90 was consecutively set as the independent variable. Results are expressed as odds ratio (OR) with 95% CI. A two-sided P-value < 0.05 was considered statistically significant.

Results

Smokers have more severe strokes and at a younger age with respect to non-smokers

Supplementary table 1 shows baseline demographic and clinical characteristics of patients from the overall cohort and study subgroups. Among patients, 30 (33.7%) had smoking habit at the time of AIS onset. Median age was 67 (56-76) years in the entire cohort, in particular smokers showed a younger age with respect to non-smokers (59 [50-67] and 73 [65-59] years, respectively; $p<0.001$) (Supplementary table 1). No difference for gender distribution between two subgroups has been shown. Among classical risk factors for stroke, 59.6% of subjects were hypertensive, 30.3% had atrial fibrillation (AF), 13.5% had diabetes mellitus, and 23.9% had dyslipidemia (Supplementary table 1). Comorbidity distribution analysis among smokers and non-smokers showed significant difference in terms of hypertension and AF. Particularly, 36.7% of smokers and 71.2% of non-smokers were found hypertensive ($p=0.003$), while AF affected 39.0% of non-smokers and 13.3% of smokers ($p=0.025$). Considering clinical features of disease at admission, only cardio-embolic stroke subtype was different between smokers and non-smokers (4 vs. 23, $p=0.015$). No difference in any current medication at the time of AIS onset has been demonstrated between the two groups, except for renin–angiotensin–aldosterone system inhibitors that were more frequently administered in non-smokers patients (Supplementary table 1). Among smokers, patients with NIHSS score ≥ 5 at day 1 after AIS were 80.0%, while in non-smokers group they were only 45.8% ($p=0.003$) (Table 1). No additional difference was demonstrated between groups on NCCT lesion volume or mRS up to 90-day follow up (Table 1).

Circulating leukocyte count and serum adhesion molecules are increased in smokers

In the overall cohort, biochemical analysis showed normal levels of WBC, red blood cells, and platelets (Table 2). At AIS onset, smokers showed higher levels of total WBCs, neutro-

phils, and monocytes when compared to non-smokers (Table 2). Moreover, triglyceride levels were significantly higher in smokers as compared to non-smokers (p=0.041). Finally, serum levels of E-selectin, L-selectin, and ICAM-1 were significantly higher in smokers compared to non-smokers (Figure 1A-1C). At day 1 and 7 from AIS, levels of these molecules remained higher in the smoker group, excepted for L-selectin (Figure 1A-1C).

Circulating monocyte count, but not neutrophils nor adhesion molecules are associated with worse post-stroke outcomes

In the overall cohort, monocyte count at AIS onset was significantly and positively correlated with NIHSS, mRS and NCCT lesion volume, as assessed at all follow up time points (Supplementary table 2). Moreover, monocyte count showed positive correlation with NIHSS and mRS at day 90 also in multivariate models considering neutrophils and age (Table 3). In smokers, neutrophil and monocyte counts positively correlated with disease severity (β=0.473 and 0.635, p=0.008 and <0.001, respectively) and disability (β=0.416 and 0.595, p=0.022 and 0.001, respectively) (Table 3). However, both in the overall cohort and smokers, only monocyte count remained significantly associated with NIHSS and mRS at day 90 in a multivariate analysis including age and neutrophil levels. No significant association between serum levels of adhesion molecules at AIS onset and NIHSS or mRS at 90-day follow up was observed (Supplementary table 3).

Monocyte count at AIS onset has significant prognostic accuracy to predict 90-day post-stroke outcomes.

ROC curve analysis showed a significant prognostic accuracy for monocyte number at onset in predicting a higher stroke severity assessed by NIHSS>5 at day 90 (AUC 0.680, 95% CI 0.530-0.829, p=0.018) (Figure 2A). Moreover, monocyte prognostic accuracy concerned also

worse stroke disability defined as mRS >2 at day 90 (AUC 0.708,95%CI 0.549-0.866, p=0.010) (Figure 2B). According to Youden index, the cut-off for monocytes at onset higher than 0.63 x 10^9/L and higher than 0.64 x 10^9/L were identified as best predictors of worse NIHSS and mRS, respectively (Fig. 2A and 2B).

Monocyte count at onset <0.63 x 10^9/L implied a five-fold risk of worse NIHSS compared to lower cellular counts in logistic regression model (OR 5.89, 95%CI 1.93-17.98, p=0.002) (Table 4). This result remained statistically significant after adjustment for age, gender, smoke habit, hypertension, and thrombolytic treatment (OR 5.48, 95%CI 1.68-17.87, p=0.005) (Table 4). Risk analyses using logistic regression model showed that monocyte number exceeding the cut-off showed a six-fold risk of poor recovery from AIS (OR 6.71, 95%CI 2.02-22.32, p=0.002) (Table 4). Also multivariate model adjusted for age, gender, smoke habit, thrombolytic treatment, and hypertension confirmed the result (OR 6.42, 95%CI 1.77-23.28, p=0.005) (Table 4).

Discussion

An investigation about the role of leukocytes and adhesion molecules as potential predictors of AIS severity in smoker and non-smoker patients was performed. This research work was planned on the basis of the remarkable influence of smoking on systemic and vascular inflammation that might accelerate AIS [31]. Accordingly with previous results [18, 32], smokers were hit earlier by stroke as compared with non-smokers, also in the presence of less comorbidities. Moreover the clinical assessments of stroke severity showed a more serious disease in smokers as compared to non-smokers. This result confirm previous publications [33, 34], although some other contrasting evidence has been also reported, suggesting a "smoker's paradox" [35]. The effects of tobacco smoke in the brain are largely known and a huge amount of detrimental compounds have been already identified [36]. Some of them (e.g.

superoxide, hydroxil radical, and hydrogen peroxide) were shown to directly damage the arterial endothelium through oxidative pathways [37]. Other indirect effects of smoke derivatives were demonstrated to upregulate cytokines [16], matrix metalloproteinases [38], and adhesion molecules [39] which favor leukocyte-mediated inflammation [40]. In our study, at AIS onset, smokers had higher levels of circulating leukocytes and (i.e. monocytes and neutrophils) as well as adhesion molecules when compared to non-smokers. Although no association between soluble adhesion molecules and post-stroke severity or disability was demonstrated, our results showed some correlation between leukocyte counts and worse neurological outcomes. These associations were demonstrated both in the smoker group and the overall cohort. However, in the overall cohort, only monocyte count at onset was demonstrated to predict worse outcomes independently of known confounders, such as age, hypertension and thrombolysis. Even when corrected for smoking, monocyte count remained a predictor of worse neurological outcomes, indicating that this prognostic parameter might be particularly useful in both smokers and mixed cohort enrolling both smokers and non-smokers. Evidence from previous studies indicates that acute ischemic diseases and their prognosis were associated with higher leukocyte counts [41-43]. For instance, neutrophil to lymphocyte ratio was shown to predict mortality in patients suffering from acute myocardial infarction [44]. Human monocyte count was only partially investigated as a predictor of disease, whereas membrane activation of these cells as well as subset population has been mainly explored [45]. Our study suggested a previously unexplored prognostic role for an easy-to-assess biomarker (such as circulating monocytes) that might be particularly relevant in smokers. Contrarily to other known inflammatory-related biomarker potentially predicting AIS outcome (e.g. neuron-specific enolase, matrix metalloproteinase 9 and malondialdehyde) [46], blood monocyte count is a routine and cheap test that is universally available. Thanks to these features, circulating monocytes might guide the physicians' therapeutic choices even at the emergency department.

This study has some limitations. First, the small size (30 smokers and 59 non-smokers enrolled) of a single-center study may not represent the complexity of all AIS patients and, for this reason, the results cannot be extended to a general population. Therefore, the present work has to be considered as a pilot study conducted in order to evaluate the feasibility of a larger multicenter clinical trials. On the other hands, potential bias related to heterogeneity of AIS treatment has been reduced by enrolling patients in a single center. Moreover, the definition of cut-off points for monocytes by a post-hoc ROC analysis needs to be extensively validated since no previous study on AIS cohorts describing monocyte cut-off values are available. Finally, we did not explore monocyte subsets, which deserve future investigations. Indeed, Kaito et al. showed that different monocyte subsets can have different role in AIS pathophysiology [47].

In conclusion, smokers were affected by AIS at a younger age and in the presence of less comorbidities. At AIS onset, smokers had higher leukocyte counts and serum adhesion molecules as compared to non-smokers. Both neutrophil and monocyte counts, but not adhesion molecules, were associated with clinical and radiological severity of AIS at 90-day follow up. Monocyte count independently predicted worse outcomes and recovery, both in the overall cohort and in smokers. Larger studies are needed to corroborate this observation that highlights a cheap hematological parameter (monocyte count) as a potential useful prognostic tool particularly in active smokers.

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Contributions

FMo, FMa, EF and FD designed the study and analyzed the data. FC, FMo, IC, SS, AT, MP and EF acquired the data. LL and FC performed the statistical analyses. LL and AB wrote the manuscript. FMo, FC and EF revised the manuscript. All authors read and approved the final version of the manuscript

Conflict of interest

None

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Figure legends

Figure 1. Time course of serum soluble adhesion molecules levels after acute ischemic stroke in smoker and non-smokers groups. A. Serum L-selectin levels were significantly higher in smokers subgroup at onset and at day 7. **B.** Serum E-selectin levels at stroke onset, at days 1 and 7 were higher in smokers group. **C.** Lower levels of ICAM-1 characterized the non-smoker group at the first time-points. Data are presented as median (interquartile range).

Figure 2. Receiver operating characteristic (ROC) analysis for monocytes count at stroke onset and clinical scales evaluating disease severity and neurological disability at 90 days. A. Predictive values of monocytes concentration at onset for a worse severity of

stroke (defined as defined as a National Institute of Health Stroke Scale (NIHSS) ≥5 at day 90). **B.** Predictive values of monocytes concentration at onset for a poor disability score (defined as a modified Rankin Scale (mRS) >2) at day 90.

Table 1. Comparison of neurological and radiological outcomes at different time-points in the two study groups.

	Non-smokers (n=59)	**Smokers (n=30)**	*p-value*
day1			
NIHSS* ≥5	27 (45.8)	24 (80.0)	**0.003**
NCCT† lesion volume, mm^3	4.1 (0.7-29.4)	17.0 (1.4-116.6)	0.096
day 7			
NIHSS≥5	19 (32.2)	13 (43.3)	0.353
NCCT lesion volume, mm^3	6.1 (0.8-50.0)	20.2 (2.4-96.2)	0.121
day 90			
NIHSS≥5	10 (16.9)	8 (26.7)	0.403
NCCT lesion volume, mm^3	1.9 (0.5-26.9)	7.4 (0.7-85.6)	0.156
mRS#>2	8 (13.6)	7 (23.3)	0.250

Data are expressed as median (interquartile range [IQR]) or number [no.] percentages [%]). *p*-values were calculated according to Pearson $\chi 2$ test or Mann-Whitney U test when appropriate and referred to comparison between non-smokers and smokers

* NIHSS: National Institutes of Health Stroke Scale
† NCCT: non-contrast computed tomography
# mRS: modified Rankin Scale

Table 2. Biochemical characteristics of patients with ischaemic stroke in overall cohort and in the two study groups at admission.

	Overall cohort (n=89)	**Non-smokers (n=59)**	**Smokers (n=30)**	*p*-value
Biochemical				
Total WBC*, no. x 10^9 (IQR)	7.77 (6.55-9.10)	7.40 (6.16-8.69)	8.52 (7.20-11.08)	**0.003**
Neutrophil count, no. x 10^9 (IQR)	4.69 (3.72-6.55)	4.48 (3.43-5.75)	5.74 (3.94-8.38)	**0.014**
Lymphocyte count, no. x 10^9 (IQR)	1.99 (1.52-2.56)	1.94 (1.47-2.45)	2.17 (1.68-2.88)	0.140
Monocyte, no. x 10^9 (IQR)	0.57 (0.44-0.68)	0.55 (0.42-0.63)	0.65 (0.48-0.75)	**0.027**
Platelet count, no. x 10^9 (IQR)	214 (172-243)	213 (167-243)	214 (186-242)	0.924
RBC# count, no. x 10^{12} (IQR)	4.68 (4.39-5.03)	4.68 (4.41-4.97)	4.65 (4.29-5.19)	0.976
Total-c, mg/dL (IQR)	204 (164-227)	203 (163-223)	210 (164-235)	0.333
HDL-c†, mg/dL (IQR)	50 (41-62)	47 (41-61)	52 (38-62)	0.893
LDL-c‖, mg/dL (IQR)	126 (94-144)	126 (93-143)	123 (94-148)	0.768
Triglyceride, mg/dL (IQR)	122 (87-151)	100 (85-150)	134 (113-156)	**0.041**
Serum glycaemia, mg/dL (IQR)	111 (97-144)	109 (96-135)	116 (101-152)	0.300
INR‡, no. (IQR)	1.09 (1.02-1.15)	1.09 (1.02-1.19)	1.06 (1.00-1.14)	0.229
Fibrinogen, mg/dL (IQR)	274 (236-306)	275 (248-304)	271 (229-322)	0.860

Data are expressed as median (interquartile range [IQR]) or number [no.] (percentages [%]).

p-values were calculated according to Pearson χ^2 test or Mann-Whitney U test when appropriate and referred to comparison between non-smokers and smokers.

* WBC: white blood cells

RBC: red blood cells

† HDL-c: high density lipoprotein cholesterol

‖ LDL-c: low density lipoprotein cholesterol

‡ INR: international normalized ratio

Table 3. Correlation of serum monocyte and neutrophil counts with stroke outcomes at day 90.

	NIHSS* day 90											
	Overall cohort				**Non-smokers**				**Smokers**			
	Univariate model		Multivariate model		Univariate model		Multivariate model		Univariate model		Multivariate model	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Age, years	0.040	0.707	0.086	0.408	0.134	0.312	0.134	0.321	-0.072	0.706	-0.047	0.754
Neutrophils, no. x 10^9/L	0.208	0.051	0.088	0.456	-0.005	0.967	-0.010	0.941	0.473	**0.008**	0.122	0.533
Monocytes, no. x 10^9/L	0.313	**0.003**	0.281	**0.018**	0.061	0.648	0.065	0.639	0.635	**<0.001**	0.557	**0.008**
	mRS# day 90											
	Overall cohort				**Non-smokers**				**Smokers**			
	Univariate model		Multivariate model		Univariate model		Multivariate model		Univariate model		Multivariate model	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Age, years	0.084	0.434	0.133	0.201	0.197	0.135	0.202	0.131	0.007	0.971	0.029	0.855
Neutrophils, no. x 10^9/L	0.224	**0.035**	0.121	0.303	0.059	0.659	0.057	0.675	0.416	**0.022**	0.073	0.721
Monocytes, no. x 10^9/L	0.304	**0.004**	0.261	**0.027**	0.083	0.531	0.073	0.591	0.595	**0.001**	0.550	**0.011**

Correlations were performed using univariate and multivariate linear regression analysis.

* NIHSS: national institute of health stroke scale

mRS: modified Rankin scale

Table 4. Logistic regression showing the predictive value of categorized monocyte levels at onset towards worse neurological outcomes at day 90 in the overall cohort.

	Univariate model			Multivariate model		
	OR[#]	95% CI[*]	*p*-value	OR	95% CI	*p*-value
NIHSS[†] day 90						
Monocyte count >0.63 no. x 10^9/L	5.889	1.928-17.983	**0.002**	5.483	1.683-17.868	**0.005**
Age, years	0.998	0.959-1.038	0.906	0.999	0.945-1.055	0.960
Hypertension	1.463	0.493-4.341	0.492	1.555	0.384-6.295	0.536
Thrombolysis	1.021	0.341-3.057	0.970	1.176	0.356-3.881	0.790
Smoking	1.782	0.619-5.128	0.284	1.242	0.323-4.774	0.753
mRS[§] day 90						
Monocyte count >0.64 no. x 10^9/L	6.706	2.015-22.318	**0.002**	6.418	1.770-23.276	**0.005**
Age, years	0.996	0.954-1.039	0.838	0.995	0.937-1.057	0.879
Hypertension	1.442	0.448-4.639	0.539	1.701	0.376-7.696	0.491
Thrombolysis	1.490	0.431-5.147	0.529	1.841	0.474-7.149	0.378
Smoking	1.940	0.628-5.991	0.249	1.244	0.279-5.549	0.775

OR: odds ratio
* CI: confidence interval
† NIHSS: National Institute of Health Stroke Scale
§ mRS: modified Rankin scale

Figure 1

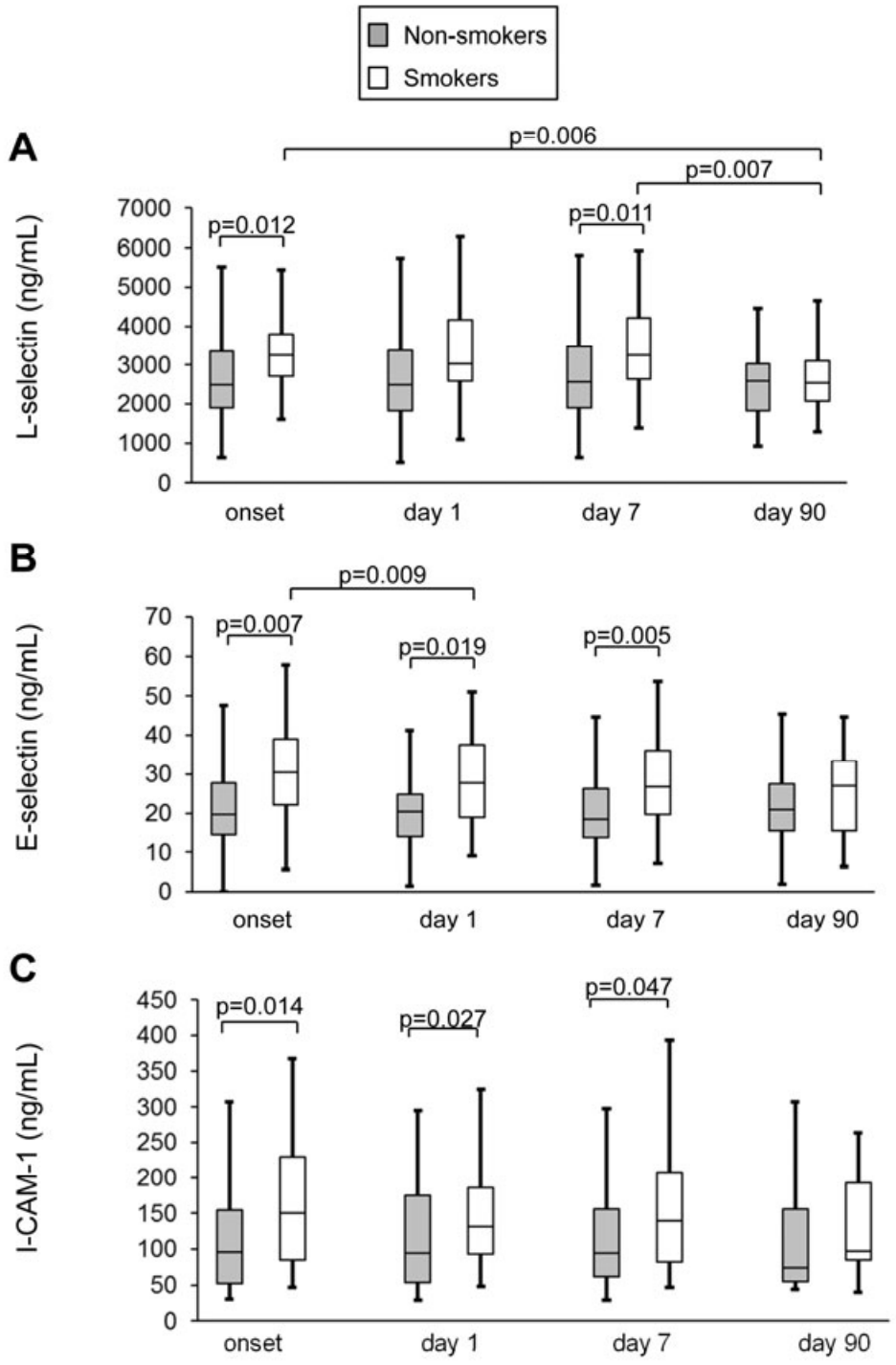

Figure 2