Monocyte count is an underlying marker of lacunar subtype of hypertensive small vessel disease

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\textbf{Background:} In the hypertensive small vessel disease (HSVD), it remains unclear why some patients develop lacunar infarcts (LIs) whilst others develop deep intracerebral hemorrhages (dIChs). Inflammation might be related to LI, and leukocyte and monocyte counts are regarded as an inflammatory marker of ischemic stroke. \textbf{Objective:} We investigated the relationship between leukocyte and monocyte counts determined in the first 24 h after stroke onset in HSVD patients. \textbf{Methods:} We prospectively studied 236 patients with first acute stroke because of HSVD (129 LI and 107 dICH). We analyzed demographic data, vascular risk factors, and white blood cell count subtypes obtained in the first 24 h after stroke. \textbf{Results:} The multivariate analysis showed that LI subtype of HSVD was correlated with hyperlipidemia ($P < 0.0001$), a higher monocyte count ($P = 0.002$), and showed a trend with current smoking ($P = 0.051$), whereas dICH subtype was correlated with low serum total cholesterol ($P = 0.003$), low serum triglycerides ($P < 0.0001$), and high neutrophil count ($P = 0.050$). \textbf{Conclusions:} In patients who developed HSVD-related stroke, high monocyte count, current smoking, and hyperlipidemia are prothrombotic factors related to LI, whereas low cholesterol and triglyceride values are related to dICH. Monocyte count might be an inflammatory risk marker for the occlusion of small vessels in hypertensive patients.

\section*{Introduction}

The term hypertensive small vessel disease (HSVD) includes two distinct entities that occur in the same structures, lacunar infarcts (LIs) and deep intracerebral hemorrhages (dIChs). Fisher [1] distinguished in autopsies two different underlying vascular pathologies for small vessel disease (SVD): lipohyalinosis and microatheromatosis. Lipohyalinosis was present mainly in patients with small, multiple and asymptomatic lacunae, and this type of vessel lesion is most commonly associated with dICH [2–5]. Microatheromatosis was found mainly in patients with single, large, and symptomatic lacunae, being the second most common cause of LI.

Hypertension is the major risk factor for LI and dICH but it fails to account for much of the risk. Inflammation was shown to play an important role in lacunar infarctions [6]. Several markers of inflammation, such as leukocyte and monocyte counts, have been identified as predictors of ischemic stroke [7]. Studies demonstrate that leukocyte count independently predicts ischemic risk [8–10], mainly in the atheroesclerotic group. Studies also demonstrated that monocyte count is a better marker for proinflammatory state in carotid atherosclerosis than leukocyte count [11–13].

Our aim was to analyze differences between LI and dICH patients in demographic data, vascular risk factors, and on leukocyte count determined in the first 24 h after the stroke onset. We sought to determine whether increase in leukocyte or monocyte count might be related to the development of clinical LI in patients with acute HSVD stroke.

\section*{Methods}

\textbf{Study population}

From January 2001 to February 2006, 2193 consecutive patients with acute stroke were admitted to our hospital and enrolled in the stroke register. We selected the consecutive patients with the diagnosis of primary intracerebral hemorrhages and with the diagnosis of LI. A total of 284 patients had primary intracerebral...
hemorrhages and 166 amongst them had dICH. From this subgroup of dICH patients, we excluded patients receiving anticoagulant treatment \((n = 13)\), patients without hypertension as a risk factor \((n = 9)\), patients with history of infection or fever the week before stroke onset \((n = 4)\), patients with large hemorrhages of unclear origin \((n = 6)\), patients with previous ischemic or hemorrhagic stroke \((n = 15)\). Patients with incomplete data \((n = 12)\), because they had died during the first 24 h \((n = 10)\) or because blood tests could not be obtained, were also excluded. A total of 443 patients had LIs, from this LI subgroup we excluded patients without history of hypertension \((n = 71)\), embolic heart disease \((n = 62)\), carotid atherosclerosis \((n = 61)\), current anticoagulant treatment \((n = 46)\), patients with history of infection or fever the week before stroke onset \((n = 5)\), previous ischemic or hemorrhagic stroke \((n = 54)\), and patients with incomplete data \((n = 15)\). Finally, the remaining 236 patients were analyzed: 129 fulfilled the TOAST criteria of LI \([14]\), and 107 had the diagnosis of dICH.

Classifications and variables

Lacunar infarct was defined according to the TOAST criteria \([14]\). The definition for intracerebral hemorrhage (ICH) is adapted from the Classification of Cerebrovascular Disease III (1989). ICH was defined as non-traumatic abrupt onset of severe headache, altered level of consciousness, and/or focal neurological deficit that is associated with a focal collection of blood within the brain parenchyma as observed on a head CT scan or at autopsy and is not because of hemorrhagic conversion of a cerebral infraction. Only the hemorrhages located at deep zone (involving predominately the basal ganglia, periventricular white matter or internal capsule) were considered eligible for the study.

A neurologist examined all patients during the first 24 h after the stroke onset. Standard blood tests, 12-lead electrocardiogram (ECG), chest X-ray, ultrasound studies, and head CT scan were obtained during the 12 h after stroke. Echocardiography, 24-h ECG (Holter) monitoring, and cerebral angiography were performed in selected patients.

All patients fulfilled a clinical protocol which included demographic data and the presence of the following vascular risk factors: arterial hypertension (defined as evidence of at least two blood pressure measurements \(> 140/90\) mmHg recorded on different days before the stroke onset), a physician’s diagnosis, or use of antihypertensive treatment), diabetes (fasting serum glucose level \(\geq 7.0\) mmol/l, a physician’s diagnosis, or use of diabetic medication), hyperlipidemia (serum cholesterol concentration \(> 12.2\) mmol/l or serum triglyceride concentration \(> 11.1\) mmol/l, a physician’s diagnosis, or use of medication), and current smoking status and alcohol overuse (\(> 60\) g/day).

Stroke severity was evaluated by the National Institute Health Stroke Scale (NIHSS) \([15]\) performed at admission. We also recorded the following basic biological markers obtained at admission: plasma glucose levels, white blood cell count (WCC) (leukocytes, neutrophils, lymphocytes, and monocytes), hemoglobin, hematocrit, mean corpuscular volume (MCV), platelet count, prothrombin, urea, and creatinin. Cholesterol and triglyceride levels were measured in blood samples taken from fasting patients 12–48 h after admission. All patients were evaluated with a minimum of two ECG procedures.

Statistical analysis

Demographic data, clinical characteristics, and vascular risk factors of LI and dICH patients were compared using t-tests, and chi-square tests, with the exception of NIHSS scores, where the analysis was performed using Mann–Whitney rank sum test, as the data distribution failed the normality requirement. We have also used t-tests to check correlations between alcohol overuse and cholesterol and triglyceride values. Variables with \(P < 0.15\) were included in the multivariate analysis.

A logistic regression model was used to analyze differences between LI and dICH patients after controlling for potentially confounding variables according to the results of the initial analysis: age, neutrophil count, monocyte count, NIHSS, prothrombin, cholesterol level, triglyceride level (independent continuous variables) and male gender, current smoking status, hyperlipidemia (dichotomous variables). In order to avoid a colinearity effect between cholesterol and triglyceride values, we chose the variable hyperlipidemia for our model.

In order to explore the association between monocyte count and stroke severity, we performed a Spearman’s rho analysis. In all tests, results with probability values \(< 0.05\) were considered statistically significant. Statistical analyses were performed with the spss 13.0 software package (SPSS Inc., Chicago, IL, USA).

Ethics

The data for the study were collected from our hospital’s prospective clinical protocols, which complied with the local ethical guidelines, and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.
Results

Baseline characteristics of patients and the results of univariate analyses are shown in Table 1. Patients with LI had significantly higher leukocyte and monocyte counts than patients with dICH. No differences were found between LI and dICH with regard to age and gender. Amongst the known vascular risk factors, current smoking and previous history of hyperlipidemia were more common in patients with LI, whereas no differences were found for diabetes and alcohol intake.

We checked for correlations between alcohol overuse and cholesterol and triglyceride values, and there were no differences between groups: cholesterol was 206.9 (57.1) for alcohol drinkers versus 199.6 (47.1) for non-drinkers ($P = 0.38$). Triglyceride mean was 134.7 (52.3) for alcohol drinkers versus 133.3 (68.8) for non-drinkers ($P = 0.75$). In order to explore other indication of alcohol overuse we compared the MCV values showing that alcoholic patients had higher MCV values [92.7 (5.1)] than non-drinkers [90.9 (5.5)], $P = 0.038$. There were no differences between MCV for dICH, [91.40 (5.52)] and LI [91.04 (5.38)], $P = 0.61$.

National Institute Health Stroke Scale and percentage of in-hospital deaths were higher in dICH than in LI patients. Likewise, dICH patients had lower triglycerides and cholesterol values, higher neutrophil count, and longer prothrombin time.

The logistic regression model with hyperlipidemia (which includes those patients with high cholesterol and/or triglyceride values) showed that the following variables independently distinguished LI from dICH: current smoking, neutrophil count, monocyte count, hyperlipidemia, and prothrombin time (Table 2). The logistic regression model with cholesterol or triglycerides instead of hyperlipidemia showed that cholesterol ($P = 0.003$) and triglyceride ($P < 0.0001$) values were significantly lower in dICH than in LI.

We explored the association between monocyte count and stroke severity. The Spearman's correlation coefficient was: $0.077$, $P = 0.237$, so we were unable to find

Table 1 Demographics, vascular risk factors, and clinical data of patients according to lacunar infarcts or deep intracerebral hemorrhage (univariate analysis)

<table>
<thead>
<tr>
<th>Total cases ($n = 236$)</th>
<th>LI ($n = 129$)</th>
<th>dICH ($n = 107$)</th>
<th>$P$-value (OR; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ($^a$) (years)</td>
<td>70.7 (12.5)</td>
<td>69.8 (12.8)</td>
<td>71.7 (11.9)</td>
</tr>
<tr>
<td>Male gender ($^a$) (%)</td>
<td>135 (57.2)</td>
<td>80 (62.1)</td>
<td>55 (51.4)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>69 (29.2)</td>
<td>44 (34.1)</td>
<td>25 (23.3)</td>
</tr>
<tr>
<td>Hyperlipidemia ($^a$) (%)</td>
<td>77 (32.6)</td>
<td>62 (48)</td>
<td>15 (14)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>63 (26.6)</td>
<td>44 (34.1)</td>
<td>19 (17.7)</td>
</tr>
<tr>
<td>Alcohol overuse (%)</td>
<td>44 (18.6)</td>
<td>25 (19.3)</td>
<td>19 (17.7)</td>
</tr>
<tr>
<td>NIHSS score</td>
<td>5.0 (4.4)</td>
<td>4.0 (3.6)</td>
<td>6.3 (4.9)</td>
</tr>
<tr>
<td>In-hospital death (%)</td>
<td>13.1</td>
<td>2.3</td>
<td>26.1</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>11.2 (2.7)</td>
<td>11.7 (2.7)</td>
<td>10.4 (2.5)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>7.4 (3.6)</td>
<td>8.5 (3.9)</td>
<td>6 (2.6)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>7.8 (3.7)</td>
<td>7.9 (3.3)</td>
<td>7.7 (4.2)</td>
</tr>
<tr>
<td>Leucocytes ($\times 10^9$/l)</td>
<td>9.213 (8.716)</td>
<td>9.177 (11.328)</td>
<td>9.257 (3.670)</td>
</tr>
<tr>
<td>Neutrophils ($^a$) ($\times 10^9$/l)</td>
<td>6.224 (2.833)</td>
<td>5.640 (2.309)</td>
<td>6.928 (3.231)</td>
</tr>
<tr>
<td>Lymphocytes ($\times 10^9$/l)</td>
<td>1.637 (972)</td>
<td>1.722 (710)</td>
<td>1.534 (1.210)</td>
</tr>
<tr>
<td>Monocytes ($^a$) ($\times 10^9$/l)</td>
<td>0.634 (0.369)</td>
<td>0.698 (0.383)</td>
<td>0.555 (0.336)</td>
</tr>
<tr>
<td>Hemoglobin (mmol/l)</td>
<td>9.2 (1.1)</td>
<td>9.3 (1.1)</td>
<td>9.1 (1.2)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>41.6 (5)</td>
<td>41.8 (4.6)</td>
<td>41.5 (5.4)</td>
</tr>
<tr>
<td>Platelets ($\times 10^{11}$/l)</td>
<td>218.7 (75.3)</td>
<td>224.6 (75.4)</td>
<td>211.7 (74.9)</td>
</tr>
<tr>
<td>Prothrombin ($^a$) (%)</td>
<td>92.6 (13.7)</td>
<td>94.8 (14.1)</td>
<td>89.9 (12.8)</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>2.6 (1.1)</td>
<td>2.5 (1.2)</td>
<td>2.7 (1.1)</td>
</tr>
<tr>
<td>Creatinin (mmol/l)</td>
<td>0.06 (0.03)</td>
<td>0.06 (0.02)</td>
<td>0.06 (0.04)</td>
</tr>
</tbody>
</table>

Values are given as mean (SD). NIHSS, National Institute Health Stroke Scale; LI, lacunar infarcts; dICH, deep intracerebral hemorrhages; OR, odds ratio.

$^a$Variables included in the logistic regression.

Table 2 Differences between lacunar and deep intracerebral hemorrhage

<table>
<thead>
<tr>
<th>Vascular risk factor</th>
<th>$P$</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.714</td>
<td>0.995</td>
<td>0.970–1.021</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.705</td>
<td>1.145</td>
<td>0.568–2.311</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.051</td>
<td>2.213</td>
<td>0.996–4.914</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.050</td>
<td>1.002</td>
<td>0.997–1.003</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.002</td>
<td>0.999</td>
<td>0.998–0.999</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0.0001</td>
<td>5.769</td>
<td>2.877–11.811</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>0.031</td>
<td>0.974</td>
<td>0.950–0.998</td>
</tr>
<tr>
<td>NIHSS</td>
<td>0.020</td>
<td>1.119</td>
<td>1.017–1.231</td>
</tr>
</tbody>
</table>

Logistic regression model including hyperlipidemia. OR, odds ratio; NIHSS, National Institute Health Stroke Scale.
Discussion

The main finding in this study is that monocyte count determined in the first 24 h after acute stroke is higher in LI than in dICH patients. The monocyte count has a predictive role independent from smoking. Previous studies [16,17] reported that the monocyte count rise appears between 3 and 7 days after the stroke onset, so the monocyte count difference we found may be more related to the pre-stroke inflammatory status rather than as a consequence of stroke. Neutrophils have been in evidence from day 1 after stroke, and appear to be most numerous on days 2 and 3 following the clinical onset [16,17]. It is probable that the relationship between the neutrophil count and dICH represents a marker for injury. In addition, we found that atherothrombotic-related factors such as the current smoking status and hyperlipidemia are related to LI, whilst low blood levels of cholesterol and triglycerides are related to dICH. Despite the fact that elevated monocyte count could be a reflection of the severity of the stroke, we were unable to find any correlation between the variables.

Amongst the non-traditional risk factors, only blood WCC is related to lacunar stroke [18]. Previous studies demonstrated that leukocyte count predicts ischemic risk for first cerebral infarction [8] as well as for recurrent ischemic events [9]. Relative elevation in baseline leukocyte count predicts first cerebral infarction, mainly not only in atherosclerotic subtype, but also in cardioembolic and cryptogenic stroke subtypes. The leukocyte count elevation is not clearly associated with the lacunar stroke. These findings are consistent with the hypothesis that leukocyte count, as a surrogate for inflammation, is primarily associated with atherosclerosis. Grau et al. [9] demonstrated that leukocyte count is a predictor of ischemic recurrent attacks, and specifically showed the neutrophil count to be the most important predictor of recurrent events, whilst that of monocytes plays a smaller role. Neutrophils are not an important factor in atherogenesis, so it is possible that atherogenesis causes an inflammatory response and the high leukocyte count serves only as a risk marker for the atherosclerotic process [8]. So, the mechanisms which link leukocyte counts to cardiovascular risks are insufficiently understood. Cellular inflammation and especially monocytes/macrophages ratio play an important role in atherogenesis [19]. Monocyte count is an independent inflammatory risk marker for subclinical carotid atherosclerosis [12] and for plaque development in subjects without pre-existing carotid atherosclerosis [13]. Also, the relationship between preoperative monocyte count and acute neurocognitive weakens after carotid endarterectomy for asymptomatic stenosis has been established [11]. So, on the basis of LI hypothesis, we think that the role of monocyte count in LI is related to the microatheromatosis mechanism.

Current smoking status is a known etiologic risk factor for ischemic stroke; we found it to be an independent risk factor that distinguished LI from dICH. Although the strongest association between ischemic stroke and current smoking status is with the atherothrombotic group, other studies [20] have also shown a relationship between smoking and small vessel cerebrovascular disease, specifically in the silent small vessel stroke.

Cholesterol and triglyceride effects as a risk factor are different in ischemic and hemorrhagic strokes: whereas high levels of cholesterol and triglycerides are related to ischemic stroke [21], low cholesterol serum concentrations can increase the risk of hemorrhagic stroke [22–26]. We found that hyperlipidemia was related to LI, whereas blood levels of cholesterol and triglycerides obtained in the acute phase of stroke distinguish LI from dICH. A relationship was seen to exist [27] between lipid profile and the severity of hypertension with the existence of microbleeds in the ICH. Some epidemiologic studies have concluded that low levels of total cholesterol are a risk factor for ICH [22–26] and that low levels of cholesterol and triglycerides are predictors of 30 days mortality in patients with supratentorial ICH [28]. As alcohol consumption lowers cholesterol level and it could be a likely explanation for the increased risk of bleeding, we have checked for correlations between alcohol overuse and cholesterol and triglyceride values, but we did not find differences between groups. In order to explore other effects of alcohol overuse we compared the MCV values in LI and dICH, but we did not find differences.

Labovitz et al. [29], comparing LI and dICH cases derived from a population-based incidence study, demonstrated higher cholesterol levels in LI patients compared with those with dICH, and proposed hypercholesterolemia as a risk factor for symptomatic LI and microatheromatosis mechanism. Our data indicate that high monocyte count, hyperlipidemia, and current smoking are also related to LI and, probably, to the mechanism of microatheromatosis.

The strength of this study is based on a consecutive and well documented sample of patients including both those that required hospitalization and those that did not.

Our study has limitations, as the study sample was derived from patients admitted to the hospital on
account of stroke, we have no information on the normal counts of leukocyte and their subtypes in our patients prior to the stroke, but we excluded the patients with history of infection or fever the week before the stroke onset.

In conclusion, we found that high monocyte count, current smoking status, and hyperlipidemia mark patients with hypertension as more vulnerable to an ischemic subtype of HSVD, whilst low levels of cholesterol and triglycerides are related to dICH development. Monocyte count might be an inflammatory risk marker for the occlusion of small vessels in hypertensive patients.

Disclosure

The author and all the co-authors report no conflicts of interest.

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