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Original research article

IL-1α and IL-6 predict vascular events or death in patients with cerebral small vessel disease—Data from the SHEF-CSVD study

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ABSTRACT

Purpose: The natural clinical course of cerebral small vessel disease (CSVD) was not thoroughly described. The aim of this single center cohort study was to establish biochemical predictors of vascular events and death in CSVD patients during a 24-month follow-up.

Patients and methods: A total of 130 functionally independent patients with marked MRI features of CSVD and recent lacunar stroke (n = 52,LS), vascular Parkinsonism (n = 28,VaP) or dementia (n = 50,VaD) were prospectively recruited. Serum markers of endothelial dysfunction, inflammation and hemostasis were determined at baseline. The primary outcome was defined as occurrence of death or any vascular events during the observation.

Results: The mean age was 72 ± 8.1 years, and 37.6% of the patients were women. The mean follow-up time was 22.3 ± 4.3 months, and 84.6% of patients had extensive white matter lesions on baseline MRI. The overall mortality rate was 6.9%, and vascular events or death occurred in 27% of the patients. Kaplan-Meier survival curves revealed no significant differences between CSVD groups (log rank p = 0.49). Cox regression analysis revealed that IL-1α (HR 1.4; 95%CI 1.09–1.8), IL-6 (1.4;1.1–2.2), hs-CRP (1.1;1.06–1.9), homocysteine (1.4;1.1–1.8), fibrinogen (1.4;1.05–2), and D-dimer (2.7;1.6–4.5) were significantly associated with the primary outcome. IL-1α (1.3;1.07–1.8), IL-6 (1.4;1.02–2.2), D-dimer (2.8;1.6–5) and homocysteine (1.4;1.1–1.8) remained significant after adjusting for age, sex and CSVD radiological markers.

Conclusions: Our study demonstrated the important prognostic role of various circulation markers of inflammation in individuals with different clinical signs and radiological markers of CSVD. The strongest association occurred between IL-1α, IL-6 and recurrent stroke, other vascular events and death.

1. Introduction

Cerebral small vessel disease (CSVD) is a syndrome of clinical, cognitive, neuroimaging and pathological findings that predominantly arises from ischemic brain damage in cerebral white and deep gray matter [1]. The most common clinical manifestations are recurrent lacunar strokes (LS) and slowly progressive vascular dementia (VaD) or vascular Parkinsonism (VaP). The primary imaging features of CSVD are interrelated and visible on conventional magnetic resonance imaging (MRI). These features include: acute lacunar infarcts, lacunes (old infarcts), white matter hyperintensities (WMH) of presumed vascular origin, visible perivascular spaces (PVS) and microbleeds (MBs) [2]. Despite its clinical importance, no specific treatments are available for this disease. The cause of CSVD is largely unknown, but endothelial dysfunction is considered one of the pivotal mechanisms of structural and functional brain-vessel alterations [3]. Growing evidence supports associations of serum levels of biomarkers of inflammation, such as C-reactive protein (CRP), interleukin 6 (IL-6), and fibrinogen with myocardial infarction, stroke, cardiovascular death, and peripheral arterial disease, especially in patients with large artery disease [4,5]. However, the prognosis of CSVD, risk of vascular events and predictors of outcome remain controversial, especially in rarely studied VaP and VaD [6]. The SPS3 trial demonstrated that patients with recent LS experienced 8–10% of recurrent strokes and 6.6–7% died during 4 years of follow-up, depending on the blood pressure (BP) control [7]. We previously demonstrated that prognosis in CSVD was poor regardless of the clinical presentation, and compared with controls with high atherothrombotic risk and free of cerebrovascular disease, patients with CSVD

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exhibit greater than a four-fold risk of vascular events or death in 24 months of observation [8]. The broad spectrum of radiological features of CSVD and its pathological pathways, from endothelial dysfunction to microatherosclerosis, suggest that several components of the fibrinolytic or inflammatory-atherothrombotic cascade influence the prognosis. However, studies investigating the association between different serum biomarkers and risk of recurrent LS or WMH progression provide conflicting results [9]. Whether biomarkers add predictive capacity beyond vascular risk factors and predict outcome in patients with CSVD is not known because studies were impeded by between-study heterogeneity and differed in their conclusions [10]. Considerable evidence showed that interleukin-1 alpha (IL-1α), interleukin 1 beta, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), soluble intercellular cell adhesion molecule-1 (sICAM-1), and platelet selectin (sP-selectin) modulate tissue and endothelial injury, and facilitate leukocyte migration into the cerebral ischemic region. These factors were linked to greater infarct volumes, neurological deterioration, and poorer long-term outcome of ischemic stroke [11,12]. Elevations in serum IL-2, 4, 6 and TNF-α concentrations were also observed in VaP compared to those in idiopathic Parkinson's disease [13]. However, the prognostic significance of these factors in these patients was not assessed. Soluble CD40 ligand (sCD40 L) released from activated platelets plays a role in atherosclerotic plaque instability, and it is a predictor of first and subsequent cardio- and cerebrovascular events. However, sCD40 L was not examined in patients with CSVD [14,15]. We recently found a relationship between hemostatic markers, namely fibrinogen, tissue factor, and beta-thromboglobulin, and the risk of radiological progression in patients with LS, VaD and VaP [16]. Endothelial dysfunction is involved in CSVD pathogenesis. Therefore, we hypothesized that some blood-derived markers of endothelial dysfunction and the inflammatory cascade are also associated with the risk of vascular events and death in patients with CSVD, especially in those with extensive radiological markers of the disease.

2. Material and methods

A single-center, prospective cohort study was performed as a part of the 'Significance of hemodynamic and hemostatic factors in the course of different manifestations of cerebral small vessel disease' study (SHEF-SVD) to identify biochemical predictors of vascular incidents and all-cause mortality subsequent to a diagnosis of CSVD in a 24-month follow-up. [17]. The studied group consisted of consecutive patients with symptomatic CSVD: with first-ever recent LS, newly diagnosed VaP or VaD presumed to be caused by CSVD, with evidence of typical findings on neuroimaging (MRI). Clinical manifestations of chronic CSVD may overlap, especially at advanced disease stages, but to the best of our knowledge, no study evaluated the prognostic factors and course of VaP and VaD using similar methodologies. Therefore, we analyzed patients with VaD and VaP separately. The study protocol and methods have been thoroughly described elsewhere [17].

The CSVD group consisted of patients recruited from the Neurological Outpatient Department who were prospectively enrolled in the study and followed-up between December 2011 and September 2015. All patients were physically independent (Modified Rankin Scale, mRS ≤ 3 and total Barthel Index, BI ≥ 80 points) and without severe dementia (Mini-Mental State Examination, MMSE ≥ 12 points). Every effort was taken to perform differential diagnoses to include patients with sporadic CSVD only. Patients were diagnosed based on a complex evaluation of past medical history, clinical context, available diagnostic tests (e.g., carotid and transcranial duplex ultrasound, Holter ECG) and a typical radiological and clinical picture: LS - according to the Oxfordshire Community Stroke Project (OCSP) criteria; VaP and VaD - after exclusion of other neurodegenerative conditions and based on widely accepted criteria and guidelines: VaP clinical criteria and NINDS-AIREN criteria with Modified Hachinski Ischemic Scale ≥7 points, respectively [18–20]. All participants were aged between 60 and 90 years. Patients with recurrent LS, strategic single-infarct dementia, poststroke VaD or VaP, significant stenosis (≥50%) of a major extracranial or intracranial artery, atrial fibrillation, non-SVD related WMH (e.g., due to vasculitis, multiple sclerosis, CADASIL), acute or chronic infectious process, malignancy, rheumatological disease, life expectancy less than 6 months, and MRI contraindications were excluded.

2.1. Study procedures

2.1.1. Neurological examination and atherothrombotic risk evaluation

Detailed physical and neurological examinations were performed at baseline. Atherothrombotic risk factors were evaluated based on medical records, physical examination and available comprehensive history. Hypertension was defined as persistent elevation of systolic blood pressure (SBP) ≥140 mmHg, diastolic BP (DBP) ≥90 mmHg, or current antihypertensive drug treatment. Diabetes mellitus was defined as a previous diagnosis of type I or type II diabetes, at least two random glucose readings ≥200 mg/dL or fasting glucose (FG) readings of ≥126 mg/dL. Hypercholesterolemia was defined as serum total cholesterol ≥200 mg/dL or current treatment with statin. The following criteria were used to diagnose polymetabolic syndrome (PS): waist circumference ≥102 cm in men or ≥88 cm in women; HDL ≤ 40 mg/dL in men and ≤50 mg/dL in women; elevated SBP ≥ 130 mmHg or ≥85 mmHg DBP; elevated TG ≥ 150 mg/dL; and elevated FG ≥ 100 mg/dL or treatment for diabetes or hyperlipidemia. Coronary artery disease (CAD) was defined in patients with stable angina, prior myocardial infarction (MI), prior percutaneous revascularization, coronary artery bypass graft, angiographically proven coronary atherosclerosis, or reliable noninvasive evidence of myocardial ischemia. Ambulatory BP monitoring (ABPM) was performed using a portable noninvasive oscillometric and auscultatory device (Schiller MT-300). Measurements over a 24 h period were recorded every 15 min during daytime hours (07:00 to 23:00) and every 30 min during night-time hours (23:00 to 07:00). Mean 24 h arterial pressure (MAP), average SBP and DBP were calculated and recorded automatically. The type of device and time of application (± 2.8 h) was the same in all patients. Patients were instructed to attend to their usual day-to-day activities. All patients received optimal medical treatment, according to guidelines.

2.1.2. Laboratory examinations

We investigated sets of markers related to endothelial dysfunction and systemic inflammation (IL-1α, IL-6, hs-CRP, sICAM-1, sP-selectin, TNF-α, homocysteine), hemostasis (fibrinogen, D-dimer), and markers of increased cardiovascular risk (serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), eGFR, serum FG, HbA1c, albumin and uric acid (UA)) based on a priori knowledge of biological relevance. Plasma was isolated from overnight fasting blood samples for enzyme-linked immunosorbent assay (ELISA) to analyze the concentrations of ICAM-1, TNF-α, sP-selectin, IL-1α and IL-6, according to the manufacturer's protocol (Bio-Source, Europe, Nivelles, Belgium; eBioscience, Vienna, Austria). Duplicate readings were performed for each sample, and the intra-assay coefficient of variation was < 10%. Other biochemical markers were measured using an automatic biochemical analyzer. These assays had well-defined internal and external quality control via participation in national quality assurance schemes. All assays were performed blind to study status. Whenever a compound or metabolite is mentioned, we actually mean its serum or plasma concentration to improve the wording in manuscript. All LS patients underwent study procedures at least 2 weeks (mean 19.4 ± 4.1 days) after their index strokes to prevent confounding by hyperacute phase responses.

2.1.3. MRI evaluation

All patients underwent MRI (GE Healthcare 1.5 T scanner)

examination prior to entering the study. We categorized MRI findings according to STRIVE (Standards for Reporting Vascular Changes on Neuroimaging) guidelines as a reference standard [21]. The simple modified Fazekas rating scale was used to estimate the extent of periventricular WMH (pWMH) and deep WMH (dWMH) [22]. Imaging features of CSVD were used as radiological markers of the presence and severity of CSVD, and images were rated for the presence of lacunes (small ≤15 mm, subcortical lesions of similar signal to CSF; increased signal on T2-weighted, decreased signal on FLAIR and T1-weighted images), WMH (ill-defined hyperintensities ≥5 mm on T2 and PD/FLAIR images), MBs (small ≤5 mm, homogeneous, round foci of low signal intensity on T2*-weighted images in basal ganglia, thalamus, white matter, or cortico-subcortical junction), and enlarged PVS (small ≤3 mm, punctate (if perpendicular) and linear (if longitudinal to the plane of scan) hyperintensities on T2 images) according to a recommended visual SVD scale [23]. One point on the SVD scale was awarded if (early) extensive confluent dWMH (Fazekas score 2 and 3) or irregular pWMH extended into the deep white matter (Fazekas score 3) or when 1 or more lacunes or MBs were present. One point was awarded if moderate to extensive (10–25 or > 25) enlarged PVS were present. Finally, the presence of each of the above markers produced a minimum score of 0 and a maximum of 4, which represented the total MRI load of CSVD. All patients exhibited at least grade 1 dWMH or pWMH in Fazekas scale to become eligible.

2.2. Main outcome

Data on vascular events during the study and/or cause of death (classified according to the ICD-10) were obtained at a 24-month follow-up visit from the patients, treating physician, general practitioner or medical records. Patients with incomplete follow-up were censored at the last observation. The primary outcome event was time to any vascular event or death. Vascular events were further classified as cerebrovascular events (hemorrhagic or ischemic stroke or transient ischemic attack - TIA) or cardiovascular events (MI or coronary intervention for stable angina or other peripheral vascular intervention). Vascular death was defined as any cardiovascular or cerebrovascular death or sudden death. Noncardiovascular death was defined as any death with a specific cause that was not thought to be CVD in nature. Undetermined causes of death were death not attributable to one of the above categories. Ischemic and hemorrhagic strokes were defined based on typical clinical features associated with relevant findings on diagnostic brain imaging. Peripheral vascular intervention was defined as a catheter-based or open surgical procedure designed to improve arterial or venous blood flow or otherwise modify or revise vascular conduits.

2.3. Statistical analysis

Quantitative and qualitative demographic characteristics were summarized. Data were tabulated and tested for normality using the Shapiro-Wilk test. Categorical data are presented as frequencies, and continuous data are reported as the means ± SD. One-way ANOVA and chi-square test were used to assess significant differences between study groups using *post hoc* Tukey's HSD test for comparisons between CSVD subgroups. The Kaplan–Meier method was used to estimate event-free survival, and the Cox regression model was used to estimate the impact of possible determinants on main outcome. All variables that achieved a p-value ≤0.1 in any of the analyses were included in the multivariate regression model as candidate variables. Adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated with fixed entry of a predefined set of potential confounders (age, sex, CSVD group) that were selected on the basis of clinical plausibility and previous literature reviews and measured at the baseline visit. The HRs per 1-SD increase was used for continuous variables. The Spearman correlation coefficient was used to analyze the relationship between biomarkers, SVD scale, MAP and functional status at baseline (BI). A probability value of $p < 0.05$ was considered significant. All data are presented as the mean ± SD values. All analyses were performed using Statistica 12 software (StatSoft Inc, Tulsa, OK, USA).

2.4. Ethical issues

This study complied with the Declaration of Helsinki. All participants signed an informed consent form. Informed written consent for patients with VaD was obtained from their responsible caregiver, and only, if in the opinion of the investigator, the participant understood the nature of the research and his/her participation. The local Ethics Committee of the Military Institute of Medicine (Warsaw, Poland) approved this study (46/WIM/2010).

3. Results

3.1. Baseline patient characteristics

The study included 130 patients: 52 (40%) with first-ever recent LS, 28 (21%) with VaP and 50 (39%) with subcortical VaD. The estimated mean time from LS onset to enrollment was 17.5 ± 3.8 days and 25.5 ± 11 months in VaD and 27.5 ± 14 months in VaP. Table 1 shows the mean age, sex distribution and vascular risk factor profiles and other patient characteristics. ANOVA and chi-square tests revealed significant differences between groups in mean age, BMI, levels of sICAM-1, distribution of gender and frequency of hypertension. Post hoc analyses revealed that patients with VaD were older than patients with LS, and there were more females with VaD than with VaP and LS ($p < 0.05$). Hypertension was more prevalent, and mean BMI was higher in LS than in VaP. Patients with LS and VaD exhibited significantly lower mean concentration of sICAM-1 than did those with VaP. There was also a trend towards lower HDL-C levels in LS than in VaP, lower TG in VaD than in LS and higher homocysteine in VaP than in LS. There was no significant difference between CSVD groups in baseline control of BP, prevalence of other vascular risk factors or levels of other biochemical compounds. Over half of the patients (53%, n = 69) exhibited extensive dWMH and scored grade 2 (28%, n = 36) or 3 (25%, n = 33) on the dWMH scale, and 20.7% (n = 27) exhibited extensive pWMH and scored 3 on the pWMH scale. Most patients (84.6%, n = 110) exhibited extensive dWMH and/or pWMH, and 23% of patients (n = 26) exhibited extensive dWMH and pWMH. Patients with LS, VaD and VaP exhibited similar burdens of dWMH (grade 2 or 3 dWMH: 55.7% (n = 29), 54% (n = 27) and 46% (n = 13), respectively; p = 0.6) and pWMH (grade 3 pWMH: 9.1% (n = 9), 24% (n = 12), 21.5% (n = 6), respectively; p = 0.2).

3.2. All-cause mortality and vascular events

Information on the outcome was available from 98% of the enrolled patients. Two patients (both with VaD) were lost to follow-up. The mean follow-up time in the studied cohort was 22.3 ± 4.3 months (240.5 person-years). Vascular events or death occurred in 35 patients (27%) during this period, and none of the patients' experienced outcome event during baseline hospitalization. Outcome events occurred following the first 12 months of follow-up in most patients (n = 24; 68%), particularly patients with VaD (n = 15; 88%) and VaP (n = 5; 71%). The overall mortality rate was 6.9% (n = 9), and the difference between patients with LS (3.8%), VaP (14.3%) and VaD (6%) was not statistically significant (p = 0.2). The cause of death was vascular-related in 6 (67%) patients due to cardiovascular events (fatal MI, n = 3; sudden cardiac death, n = 3), nonvascular events in 2 patients (22%) (infection, n = 1; malignant neoplasm, n = 1) and undetermined in 1 patient. There were 26 nonfatal vascular events: 21 cerebrovascular events (all LS), and 5 cardiovascular events (3 MIs and 2 angioplasty for PAD) (Table 2). There were no hemorrhagic strokes during the follow-up. The frequency of primary outcome events was not different between

Table 1
Comparison of clinical, laboratory and radiological data of patients with CSVD.

n (%)	CSVD 130	LS 52 (40)	VaP 28 (21.5)	VaD 50 (38.5)	$p^{\#}$ –
Demographic data and vascular risk factors					
Age (y)	72.2 (8.1)	69.9 (8.7)	72.3 (6.24)	74.4 (7.9)	0.02
60–69 (%)	50 (38.5)	26 (50)	10 (35.7)	14 (28)	0.06
70–79	47 (36.2)	14 (26.9)	14 (50)	19 (38)	
80–90	33 (25.4)	12 (23.1)	4 (14.3)	17 (34)	
Female sex (%)	64 (49.2)	17 (32.6)	10 (35.7)	37 (74)	0.01
Hypertension (%)	113 (86.9)	49 (94)	20 (71.4)	44 (88)	0.01
CAD (%)	26 (20)	10 (19)	3 (11)	13 (26)	0.39
24 h MAP	94.4 (12)	95 (12.9)	91.2 (11.1)	95.2 (11.1)	0.5
24 h mean SBP (mmHg)	133.5 (16.7)	136.6 (18.3)	128.1 (13)	131.6 (15.3)	0.2
24 h mean DBP (mmHg)	74.8 (11.3)	74.2 (11.7)	72.8 (11.1)	77 (10.7)	0.4
Diabetes mellitus (%)	71 (54.6)	29 (55.7)	14 (50)	28 (56)	0.8
HbA1c %	6.37 (1)	6.57 (1.22)	6.3 (1)	6.2 (0.9)	0.2
FG (mg/dL)	121.9 (45)	132 (51.47)	113 (32.7)	116 (41.4)	0.11
Current smoking (%)	40 (30.7)	18 (34.6)	11 (39.3)	11 (22)	0.2
Hyperlipidemia (%)	96 (73.8)	39 (75)	20 (71.4)	37 (74)	0.37
LDL-C (mg/dL)	109.3 (37.5)	106.7 (39.7)	107.4 (34)	113.2 (37.2)	0.67
HDL-C (mg/dL)	49.9 (15.4)	46.1 (10)	54.9 (13.5)	49.8 (19.8)	0.06
TG (mg/dL)	130.8 (84.1)	150.7 (115)	126.3 (54.1)	111.8 (46.6)	0.08
TC (mg/dL)	183.3 (44.1)	176.3 (43.3)	187.5 (43.1)	188.5 (45.3)	0.34
BMI	27.2 (5.2)	28.5 (5.99)	25.4 (3.6)	27 (4.5)	0.04
Obesity (BMI > 30) (%)	35 (26.9)	18 (34.6)	5 (17.8)	12 (24)	0.12
PS (%)	56 (43.1)	25 (48)	9 (32)	22 (44)	0.4
CKD (%)	20 (15.3)	10 (19)	5 (17.8)	5 (10)	0.4
Antiplatelet treatment (%)	89 (68.4)	33 (63.4)	19 (67.9)	37 (74)	0.1
Statin treatment (%)	82 (63)	29 (55.7)	17 (60.7)	36 (72)	0.11
Radiological markers of CSVD					
CSVD Score	2.13 (0.6)	2.19 (0.6)	2.11 (0.7)	2.08(0.7)	0.6
Fazekas pWMH	1.6 (0.9)	1.52 (0.9)	1.52 (0.9)	1.68 (0.87)	0.7
Fazekas dWMH	1.7 (0.9)	1.68 (0.9)	1.64 (0.8)	1.7 (0.9)	0.9
Biochemical markers					
eGFR (ml/min)	77 (24.9)	78.2 (2.87)	70.2 (22.2)	79.8 (21.2)	0.25
Albumin (g/dL)	3.7 (0.8)	3.7 (0.7)	3.9 (0.6)	3.7 (0.9)	0.4
Fibrinogen (mg/dl)	351.2 (88.4)	349.7 (76.6)	343.4 (99)	357.7 (95)	0.8
D-dimer (mg/L)	0.9 (1.2)	0.73 (0.6)	0.95 (0.96)	1.27 (1.8)	0.17
Uric acid (mg/dl)	5.9 (2)	6.3 (2.4)	5.9 (1.4)	5.4 (1.6)	0.12
Homocysteine (mg/dl)	15.1 (6.5)	13.8 (4.6)	17.5 (7.5)	15.1 (7.5)	0.06
sICAM-1 (ng/mL)	312.1 (100)	297.8 (68.7)	365.6 (155.8)	293.1 (70.2)	0.01
PF-4 (ng/mL)	0.11 (0.2)	0.14 (0.3)	0.08 (0.11)	0.11 (0.24)	0.6
IL-1α (pg/mL)	0.42 (1)	0.3 (0.8)	0.42 (0.7)	0.52 (1.3)	0.6
IL-6 (pg/mL)	7.8 (14)	6.6 (7.5)	2.07 (1.7)	10.7 (20.2)	0.4
TNF-α (ng/mL)	5.3 (3.7)	4.3 (3.9)	4.5 (4.1)	6.7 (3)	0.12
sCD40 L (ng/mL)	7.8 (4.4)	7.7 (4.7)	5.7 (4.4)	8.4 (4)	0.5
hs-CRP (mg/dL)	0.73 (1.7)	0.78 (2.3)	0.74 (0.9)	0.68 (1.1)	0.9
sP-selectin (ng/mL)	158.1 (44.2)	167.9 (45.1)	154.2 (46.5)	151.3 (41.7)	0.3
Baseline BI	90.2 (12.7)	94.8 (11.4)	92.6 (11.9)	88.2 (20.2)	0.4

Values are means (± SD) for continuously distributed data or numbers (%) for categorical data; # ANOVA and $\chi 2$ difference between CSVD groups.
CSVD – cerebral small vessel disease; LS – lacunar stroke; VaD – vascular dementia; VaP – vascular parkinsonism; WMH – white matter hyperintensities; pWMH – periventricular WMH; dWMH – deep WMH; CAD – coronary artery disease; BMI – body mass index; FG – fasting glucose; PAD – peripheral artery disease; PS – polymetabolic syndrome; sICAM-1 – soluble intercellular adhesion molecule-1; IL-1α – interleukin 1 alpha; IL-6 – interleukin 6; TNF-α – tumor necrosis factor α; sCD40 L – soluble CD40 ligand; hs-CRP – high sensitivity CRP; CKD – chronic kidney disease; MAP – mean arterial pressure; SBP – systolic blood pressure; DBP – diastolic blood pressure.

Table 2
The cause and numbers of different outcome events in patients with CSVD.

	Number of patients	Non-fatal IS	Non-fatal MI or angioplasty for PAD	Vascular death	Non vascular or undetermined death	Main outcome
CSVD n (%)	130 (100)	21 (16)	5 (3.8)*	6 (4.6)	3 (2.3)	35 (26.9)
LS	52 (40)	5 (9.6)	4 (7.6) **	2 (3.8)	0	11 (21)
VaP	28 (21.5)	2 (7.1)	1 (3.6)	1 (3.6)	3 (10.7)	7 (25)
VaD	50 (38.5)	14 (28)	0	3 (6)	0	17 (34)

CSVD – cerebral small vessel disease; LS – lacunar stroke; VaP – vascular parkinsonism; VaD – vascular dementia; PAD – peripheral arterial disease; IS – ischemic stroke; MI – myocardial infarction.
* 3 MIs, 2 angioplasty for PAD.
** 2 MIs, 2 angioplasty for PAD.

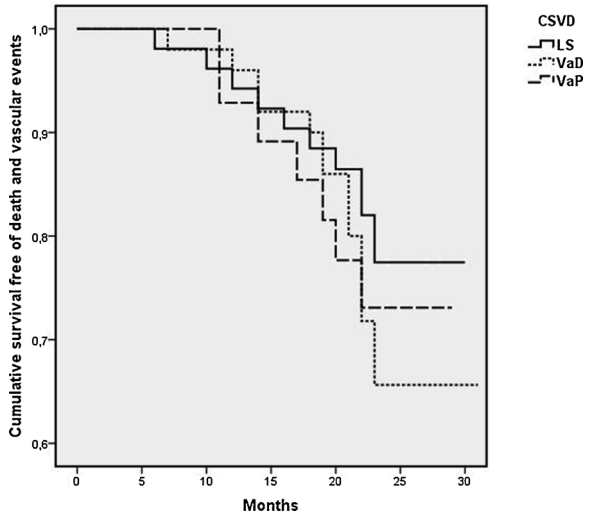

Fig. 1. Kaplan-Meier curves for main outcome events by grouped diagnostic category (log rank p = 0.49). LS – lacunar stroke; VaP – vascular parkinsonism; VaD – vascular dementia.

LS (21%), VaD (34%) and VaP (25%) (p = 0.4). Kaplan-Meier survival curves of the CSVD group revealed no significant differences in groups (log rank p = 0.49) (Fig. 1).

3.3. Univariate and multivariate analyses of associations between risk of primary outcome and analyzed biomarkers

The following factors were associated with the primary outcome in the Cox regression analysis: higher concentrations of inflammatory and hemostatic markers (IL-1α, IL-6, hs-CRP, homocysteine, fibrinogen, D-dimer), decreased levels of albumin and elevated 24 h MAP and DBP (Table 3). Older age, CKD, global SVD score and isolated dWMH marginally correlated with this risk. Baseline IL-1α, IL-6, homocysteine, albumin level, D-dimer, 24 h MAP and DBP were significantly associated with risk of main outcome in the multivariable analysis, after adjustment for age, sex and SVD score (Table 4). Hs-CRP, fibrinogen and 24 h SBP were marginally associated with that risk. Additional analyses after adjustment for CSVD group, CKD, other vascular risk factors or statin and antiplatelet treatment did not alter these results. The prognostic values of IL-1α (HR 1.4; 95%CI 1.08–1.8, p = 0.01), IL-6 (HR 1.4; 1.03–2.25, p = 0.03), homocysteine (HR 1.36; 1.04–1.77, p = 0.02), hs-CRP (HR 1.1; 0.98–1.38, p = 0.09) and albumin (HR 0.66; 0.48-0.91, p = 0.01) were similar after exclusion of patients with nonvascular death.

Table 4
Multivariable model adjusted for age, sex and SVD score.

Variable	HR (95%CI)*	p	Variable	HR (95%CI)*	p
IL-1α	1.3 (1.07-1.8)	0.01	sCD40 L	1.5 (0.8-2.9)	0.2
IL-6	1.4 (1.02-2.2)	0.04	Albumin	0.65 (0.48-0.89)	< 0.01
hs-CRP	1.02 (0.98-1.7)	0.06	MAP	1.7 (1.06-2.8)	0.02
Fibrinogen	1.4 (0.99-1.9)	0.05	DBP	1.65 (1.05-2.6)	0.02
D-dimer	2.8 (1.6-5)	< 0.01	SBP	1.6 (0.97-2.6)	0.08
Homocysteine	1. 4 (1.1-1.8)	< 0.01	CKD	1.9 (0.9-4.1)	0.1

IL-1α – interleukin 1 alpha; IL-6 – interleukin 6; sCD40 L – soluble CD40 ligand; hs-CRP – high sensitivity CRP; CKD – chronic kidney disease; MAP – mean arterial pressure; SBP – systolic blood pressure; DBP – diastolic blood pressure.
* Cox proportional hazards ratios for a 1-SD increase in continuous variables or from 1 level to another for categorical variables.

3.4. Correlations between different cytokines, adhesion molecules, and acute-phase reactants

Table 5 shows correlations between biomarkers. The only significant, good correlations occurred between sCD40 L and sP-selectin and IL-6 and IL-1α and sCD40 L. Only sICAM-1 and hs-CRP were significantly correlated with age, but the strength of the associations was weak. Hs-CRP was associated with all radiological markers of CSVD (pWMH and dWMH), sICAM-1 correlated with pWMH, and IL-6 was

Table 3
Characteristics of patients with main outcome according to Cox-proportional hazard analysis.

Co-variate	Dependent variable: main outcome (HR 95%CI)*	p	Co-variate	Dependent variable: main outcome (HR 95%CI)*	p
Age per decade	1.3 (0.93–2)	0.09	SVD score **	1.6 (0.98–2.7)	0.05
Female sex	0.9 (0.6–1.3)	0.7	Fazekas pWMH **	1.01 (0.7–1.7)	0.7
Hypertension	1.2 (0.8–1.9)	0.3	Fazekas dWMH**	1.5 (0.96–2.5)	0.07
CAD	1.2 (0.6–1.5)	0.3	sCD40L	1.5 (0.88–2.7)	0.1
Diabetes mellitus	1.1 (0.59–2.2)	0.6	Fibrinogen	1.4 (1.05–2)	0.02
HbA1c	0.8 (0.6–1.2)	0.3	D-dimer	2.7 (1.6–4.5)	< 0.001
FG	1.06 (0.8–1.4)	0.6	Uric acid	1.09 (0.8–1.4)	0.5
Current smoking	1.2 (0.6–2.7)	0.5	Homocysteine	1.4 (1.1–1.8)	< 0.01
Hyperlipidemia	1.2 (0.9–1.8)	0.15	sP-selectin	1.08 (0.7–1.7)	0.7
LDL-C	1.05 (0.7–1.4)	0.7	sICAM-1	0.99 (0.99–1.003)	0.2
HDL-C	0.8 (0.5–1.3)	0.5	PF-4	1.9 (0.5–8.2)	0.3
TG	0.6 (0.3–1.2)	0.18	IL-1α	1.4 (1.09–1.8)	< 0.01
TC	0.95 (0.68–1.3)	0.7	IL-6	1.4 (1.1–2.2)	0.04
BMI	1.2 (0.9–1.6)	0.2	TNF-α	1.3 (0.8–2.3)	0.2
Obesity (BMI > 30)	0.75 (0.36–1.5)	0.7	hs-CRP	1.1 (1.06–1.9)	0.01
PS	1.3 (0.67–2.6)	0.3	Albumin	0.6 (0.49–0.8)	< 0.01
CKD	2.1 (1.0–4.5)	0.05	No antiplatelet treatment	1.7 (0.8–3.6)	0.12
eGFR	0.7 (0.5–1.07)	0.11	Statin treatment	0.8 (0.3–1.7)	0.5
CSVD			24 h MAP	1.6 (1.04–2.7)	0.03
VaD vs LS	1.5 (0.7–3.3)	0.2	24 h SBP	1.5 (0.96–2.4)	0.07
VaP vs LS	2.3 (0.5–3.4)	0.5	24 h DBP	1.6 (1.03–2.5)	0.03

CSVD – cerebral small vessel disease; LS – lacunar stroke; VaD – vascular dementia; VaP – vascular parkinsonism; WMH – white matter hyperintensities; pWMH – periventricular WMH; dWMLs – deep WMH; CAD – coronary artery disease; BMI – body mass index; FG – fasting glucose; PAD – peripheral artery disease; PS – polymetabolic syndrome; sICAM-1 – soluble intercellular adhesion molecule-1; IL-1α – interleukin 1 alpha; IL-6 – interleukin 6; TNF-α – tumor necrosis factor α, sCD40 L – soluble CD40 ligand; CKD – chronic kidney disease; MAP – mean arterial pressure; SBP – systolic blood pressure; DBP – diastolic blood pressure.
* Cox proportional hazards ratios for a 1-SD increase in continuous variables or transfer from one level to another for categorical variables.
** Cox proportional hazards ratios for a 1 increase.

Table 5
Correlations between biochemical markers and age. radiological markers of CSVD. blood pressure and Barthel Index.

Values of Spearman rho correlations:	sP-selectin	sICAM	PF-4	IL-1	TNF-α	sCD40L	IL-6	SVD scale	pWMH	dWMH	hs-CRP
sP-selectin		−0.02	0.23*	−0.13	0.14	0.58*	−0.05	−0.07	−0.09	0.02	0.05
sICAM-1			0.20*	0.07	−0.01	−0.21	0.14	0.04	0.19*	−0.06	0.12
PF-4				−0.17*	−0.07	0.04	0.33	0.11	−0.10	0.06	−0.01
IL-1α					−0.11	0.34	0.52*	0.08	0.15	−0.01	0.12
TNF-α						0.17	0.01	0.15	0.21	0.09	0.07
sCD40L							0.4**	−0.16	0.17	0.14	0.05
IL-6								0.10	0.2	0.43*	0.29*
SVD scale									0.49**	0.76**	0.3**
pWMH										0.4**	0.20*
dWMH											0.27*
Age	−0.06	0.2*	−0.05	0.07	−0.06	0.04	0.11	0.2*	0.21*	0.27**	0.15*
Homocysteine	−0.04	0.23*	0.2*	0.13	0.5**	0.21	0.08	0.14*	0.04	0.14	0.23**
Uric acid	0.12	0.07	0.13	0.02	0.24	0.23	0.01	0.00	−0.09	−0.10	0.09
Albumin	0.13	−0.13	−0.23*	−0.1	−0.24	−0.15	−0.33*	−0.01	−0.01	−0.05	−0.36**
D-dimer	0.02	−0.05	0.17	0.09	0.07	−0.01	0.31	0.27**	0.22*	0.29*	0.35**
Fibrinogen	0.23*	0.14	.184*	0.00	.28*	0.23	0.25	0.25**	0.22*	0.32**	0.51**
LDL-C	0.04	−0.07	−0.32**	−0.06	0.07	−0.06	−0.08	−0.05	−0.08	−0.05	0.12
HDL-C	−0.12	−0.03	−0.16	−0.14	−0.24	−0.23	−0.21	−0.02	0.08	−0.03	−0.09
TG	0.24*	−0.11	0.05	−0.04	−0.04	0.09	−0.20	−0.25**	−0.36**	−0.11	−.197*
TC	0.07	−0.10	−0.31**	−0.05	−0.03	−0.08	−0.21	−0.12	−0.16	−0.08	0.04
FG	0.24*	0.17	0.12	0.01	0.04	.359*	0.09	−.144*	−0.24*	−0.13	0.08
eGFR	−0.02	−0.19*	−0.12	−0.02	−0.38*	0.00	0.02	0.03	−0.03	0.01	−0.10
24 h MAP	−0.02	−0.24	0.14	−0.17	0.13	−0.16	−0.49**	0.17	0.33*	0.11	0.14
24 h SBP	0.16	−0.17	0.12	−0.14	0.14	−0.22	−0.349*	0.10	0.25*	0.12	0.16
24 h DBP	−0.14	−0.29*	0.15	−0.18	0.19	−0.15	−0.53**	0.20	0.37**	0.14	0.12
Baseline BI	−0.10	0.09	−0.03	−0.06	−0.20	−0.22	−0.26	−0.27**	−0.04	−0.3**	−0.44**

SVD scale – small vessel disease radiological scale; WMH – white matter hyperintensities; pWMH – periventricular WMH; dWMH – deep WMH; FG–fasting glucose; sICAM-1 – soluble intercellular adhesion molecule-1; IL-1α – interleukin 1 alpha; IL-6 – interleukin 6; TNF α – tumor necrosis factor α. sCD40 L – soluble CD40 ligand; hs-CRP – high sensitivity CRP; MAP – mean arterial pressure; SBP – systolic blood pressure; DBP – diastolic blood pressure.

* $P < 0.05$.

** $P < 0.01$ (two-sided).

correlated with dWMH.

4. Discussion

The present study used a group of symptomatic patients with extensive WMH and demonstrated that the course of CSVD was poor. Over one-fourth of initially independently living elderly experienced vascular events or died during 2 years of follow-up. The principal finding is that among a panel of different biochemical markers, IL-1α and IL-6 were significantly related to a long-term risk of vascular events or death in patients with CSVD. This association was independent of age, sex and the radiological burden of CSVD. Homocysteine, albumin, MAP, DBP, and marginally fibrinogen and hs-CRP, were associated with unfavorable outcome. The prognostic value of inflammatory biomarkers was similar after exclusion of nonvascular death. Notably, there was no significant difference in basal levels of analyzed cytokines between patients with different clinical manifestation of CSVD. Hs-CRP emerged as the most closely related factor to clinical severity assessed in baseline BI, and sICAM-1, IL-6, fibrinogen and D-dimer correlated with the burden of radiological markers of CSVD.

The natural long-term course of different CSVD manifestations is poorly characterized, which makes our data more important. We demonstrated that 16% of patients with CSVD experienced recurrent symptomatic ischemic stroke, 10.7% had other fatal or nonfatal vascular events, and 7% of patients died due to vascular or nonvascular reasons during two years of observation. Ischemic strokes were responsible for most (60%) events in the CSVD group, and all of these events were LS. The risk of primary outcome was similar in LS, VaD and VaP patients. Stroke and mortality rates in the studied patients with LS, VaD or VaP were similar to previously reported values [24]. Another study reported, that the risk for stroke recurrence was 7.7% after 1 year in patients with LS and increased to 22.4% after 5 years [25]. Most deaths (52%) were cardiovascular, and 21% were due to ischemic stroke. Other analyses showed that older age, smoking, diabetes, male sex, non-use of antiplatelets, extensive WMH and elevated baseline BP were all associated with a worse prognosis in LS [26,27,28]. The high incidence of diabetes (55%) in our cohort may be surprising, but several studies showed that diabetes is an independent risk factor for LS and WMH [29,30]. Although some studies have not confirmed the relationship between diabetes and any of the MRI features, the recent ones using more advanced brain MRI techniques, demonstrated an association between diabetes and the presence and severity of WMH [31,32]. Very few reports have evaluated the influence of type 1 diabetes (T1D) on the course of CSVD. Cohort studies showed that total stroke mortality was significantly higher in patients with T1D than in those without diabetes, and T1D increased the risk of all stroke subtypes, which was attributable to longer duration of diabetes, insulin deficiency, and development of hypertension with diabetic nephropathy, disturbances of coagulation-fibrinolytic parameters, increased platelet adhesiveness, and episodes of hypoglycemia [33,34]. Notably, Brands et al. [35] reported that WMH in T1D patients was less severe than age- and education-matched type-2 diabetes patients.

We included only 28 patients with Parkinsonism, but this group is important because VaP accounts for approximately 10–20% of all cases of Parkinsonism. Previous studies also generally included low numbers of these patients because of problems with definition [36]. Our results are consistent with studies that revealed that subjects with VaP exhibited short survival (median 2 years) and a high rate of dependency or death (96%) after 3 years of observation [37,38]. The rate of stroke in VaP (7.1%) was similar to the rates of 5–11% reported in prior prospective and epidemiological studies [39,40], but the rate of strokes in VaD (28%) was higher than previously reported, which is likely due to the older mean age in this cohort. Mortality rate in patients with VaD (6%) was lower compared to a study in which 24% of individuals with VaD who were followed for 2 years died [41]. However, Brunnström et al. [42] demonstrated that cardiac diseases and pneumonia were the main causes of death in these patients, which is similar to our results. High risk of nonvascular death may be related to physical frailty or

falls, but we did not notice any injury-related death [36]. However, low incidence of cardioembolic- and large vessel-related vascular events in our cohort is explained by the exclusion of patients with atrial fibrillation and significant stenosis of extracranial or intracranial arteries.

The relationship between plasma markers and clinical progression in CSVD is not clear. The LIMITS study identified an association between IL-6 and TNF-α and recurrent cerebrovascular events following LS, but sCD40 L and serum amyloid A were unrelated to that risk [43]. In contrast, various studies suggested that sCD40 L and PF-4 were associated with a risk of vascular events, infarct size and worse clinical courses in stroke [44,45]. Elevations in inflammatory biomarkers, e.g., CRP, were correlated with an increased risk for ischemic stroke independent of other vascular risk factors in the Framingham Study [46]. Epidemiological studies have found increased cardio- and cerebrovascular risks in association with elevated cytokine levels (IL-6 and TNF-α), homocysteine, acute-phase reactants (CRP, fibrinogen, and serum amyloid A) and cell adhesion molecules (sICAM-1, P-selectin) [47]. Our study also demonstrated that IL-1α, IL-6, homocysteine and D-dimer considerably correlated with the risk of vascular events or death. Fibrinogen and hs-CRP negatively influenced outcome in univariate analyses, but lost statistical significance after adjustment for age, sex and SVD score, which likely resulted from the low number of analyzed patients. These data are consistent with some studies in different patient populations. The Northern Manhattan Study and Woman's Health Study demonstrated that hs-CRP, IL-6, sICAM-1 and serum amyloid A predicted incident stroke, MI or death, but hs-CRP remained the only inflammatory marker that independently predicted that risk in the multivariate models [48,49]. Studies also revealed that hs-CRP, IL-6, TNF-α and sICAM-1 levels were predictive of the risk of recurrent strokes, early neurological deterioration and poor outcome after 3 months and other major vascular events (MI and vascular death) [50,51]. An increase in fibrinogen, D-dimer levels and other markers of hemostasis, e.g., PF-4, are associated with infarct size, worse clinical course and increased mortality in stroke [52,53]. Soluble P-selectin was not associated with outcome in our study, which is consistent with one study but was not confirmed in the OXVASC study [54,55]. Little is known of the predictive value of IL-1α in CSVD. Macrophages, endothelial cells, and vascular smooth muscle cells primarily produce IL-1α, and it is an early and important regulator of brain inflammation and damage [56]. IL-1α directly inhibits insulin signaling, and it is implicated in the development of diabetes and exhibits overlapping biological activities with TNF-α and IL-6. Genetic association studies demonstrated an association of IL-1 gene polymorphisms with high risk of atherosclerosis and stroke, and patients with higher IL-1 levels exhibited a significantly increased risk of CAD [57]. We did not observe a relationship between TNF-α, sCD40 L, sICAM-1 and outcome, which is in opposition to the ASPS study that demonstrated that sICAM-1 levels were related to LS after 3 and 6 years and the CHANCE trial that suggested that baseline sCD40 L was a prognostic factor of future recurrent strokes [58,59]. In contrast, one neuropathological study concluded that elevated sICAM-1 levels originated from the peripheral, and not brain, vasculature [60].

Most of our patients exhibited extensive WMH at baseline. Cohort studies and meta-analysis demonstrated that the presence of severe WMH was an independent predictor of worse outcomes, including vascular death, stroke and MI [4,61]. The reason for this effect was not explained, but it may be related to the potential vulnerability of the brain, an augmented susceptibility to vascular brain damage from reductions in perfusion and gas transfer in deep brain structures or to bystander phenomena from shared vascular risk factors causing systemic micro- and macroangiopathies [62]. Notably, we demonstrated that baseline sICAM-1, hs-CRP, IL-6, fibrinogen and D-dimer correlated with markers of radiological damage (dWMH, pWMLS). Similar correlations and association between TNF-α, IL-6 and volume of hypoperfused tissue were also documented in other hospital- and population-based studies [63,64]. Our findings suggest that low-grade inflammation (as assessed by hs-CRP) with a prothrombotic profile (elevated levels of fibrinogen and D-dimer) is associated with the extent of WMH regardless of the different CSVD clinical manifestation. The hypothesized mechanism by which endothelial dysfunction may contribute to WMH is increased brain blood barrier permeability, with consequent leakage of plasma components into the vessel wall and surrounding parenchyma [65]. Some studies showed that higher serum fibrinogen levels were independently associated with WMH, lacunar lesions on MRI in asymptomatic and symptomatic CSVD patients, but other studies found no association between inflammatory biomarkers, fibrinogen and WMH [66,67,68].

Notably, we demonstrated similar concentrations of PF-4, IL-1α, IL-6, TNF-α, sCD40 L, hs-CRP, and sP-selectin in patients with diverse clinical manifestations of CSVD, which is a novel finding. This observation may result from comparable parenchyma damage and suggests that similar underlying pathophysiological mechanisms are involved in these different clinical entities. Endothelial dysfunction in CSVD may occur well before the radiological or clinical manifestation of overt small- or large-vessel disease and may represent an independent predictor of potential cardio- or cerebrovascular events.

Currently, there is no blood biomarker that fulfils the criteria of a surrogate endpoint in CSVD, but biomarkers representing endothelial dysfunction, especially in combination with neuroimaging markers, may exhibit a better correlation with clinical outcomes. It is of clinical importance because effective therapies to reduce the burden of CSVD are lacking. Management of traditional risk factors remains the primary approach for treating CSVD, despite the fact that most of these treatments exerted little or no effect in clinical trials [7]. However, how these markers should affect secondary CSVD prevention is not known. The prevention and treatment of CSVD should consider targeting microvascular function and brain endothelium, and more experimental studies are encouraged. Larger studies are required to determine predictive values within radiological or clinically specific CSVD groups of patients.

4.1. Limitations of the study

We acknowledge several limitations of this study. The major weakness is the small study size, lack of control group, and data-driven approaches to selecting variables with multiple comparisons, which may have produced biased estimates. Therefore, the results may not be generalizable to other population. However, this limitation is also a limiting factor in most published reports on the subject.

Another limitation is that there were differences between the mean age in patients with LS and VaD, which may impact inflammatory markers. However, the age distribution was not significantly different between groups, and we controlled for age in the multivariate analysis. The number of patients in each group was small, and statistical power was limited by the 35 outcome events making interpretation difficult. Therefore, we were unable to determine the predictive power stratified by CSVD subgroup. However, some correlations between biomarkers were highly powered.

The third limitation is that, we only measured markers reflecting systemic endothelial function, which does not necessarily correspond to brain endothelium. Anti-inflammatory and pro-endothelial actions of statins and antiplatelet effects of aspirin treatment may have affected the number of vascular events and the levels of cytokines. However, the high rate of compliance in our cohort prevented determinations of the predictive value of biomarkers in patients not on medication.

Another limitation is that we did not analyze the adequacy of other vascular risk factor management and control. Our findings also require confirmation because the results may simply reflect reverse-causation, especially when more severe strokes exhibit greater acute phase inflammatory responses. However, we included functionally non-dependent patients at least 2 weeks after index LS and most patients suffered chronic CSVD (VaP or VaD). Therefore, this risk is not very

likely. Patients with VaP and VaD were included in the present study immediately after diagnosis, but they were in an advanced stage of their disease. Therefore, whether our results are applicable to less severely affected patients is unknown. It is impossible to achieve 100% accuracy in the diagnosis of Parkinsonism without pathological confirmation. Therefore, our study may include some imprecision despite the application of strict diagnostic criteria based on all available clinical data.

Therefore, considering these limitations, our study is hypothesis-generating rather than definitive, and a larger study and replication are needed for more robust conclusions. Our study has several advantages notwithstanding these limitations. Previous studies investigating the predictive value of cytokines after stroke had a shorter duration of follow-up and included patients with major or undetermined stroke which might have influenced the results. We studied a group of clinically and radiologically well-characterized patients, including rarely studied patients with VaD and VaP. Moreover, our cohort had a similar functional status and large burden of WMH. Few previous studies distinguished between radiological markers of CSVD. Another advantage is the complete follow-up for mortality and single-center design, which allowed us to consistently collect all measures. Importantly, we avoided the pitfall of known causes of increased inflammatory biomarkers, e.g., vasculitis or malignancies.

5. Conclusions

In conclusion, our study demonstrated the important prognostic role of various circulation markers of inflammation in persons with different clinical manifestations of CSVD. The strongest association occurred between IL-1α, IL-6 and recurrent stroke, other vascular events and death. The mechanisms have not been determined, and our results should be interpreted with caution and require further validation in other populations. Our study further supports the view that inflammatory cytokines may be involved in the pathogenesis and course of CSVD.

Conflict of interest

The authors declare no conflict of interests.

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