Clinical, radiological and pathological correlates of leukoaraiosis


Introduction - Leukoaraiosis is characterized by an abnormal appearance of the brain white matter on imaging. Its pathogenesis is still a matter of investigation. The purpose of this study was to investigate the radiological, clinical and pathological correlates of leukoaraiosis. Methods - The study population consisted of 93 deceased patients. The pre-mortem T2W magnetic resonance images were evaluated for the presence and grading of leukoaraiosis. The clinical and pathological characteristics based on the clinical charts and autopsy reports were evaluated. Tissue specimens of the blocks of 19 brains that demonstrated severe leukoaraiosis and those of five control brains were excised and stained.

Results - The variables found to be significantly associated with leukoaraiosis were age and a clinical history of Parkinson’s disease. Other risk factors and pathological markers of atherosclerosis were not significantly correlated with leukoaraiosis. No significant difference was found between the scoring of the myelin integrity, glial fibrillary acidic protein, cluster of differentiation 68 and smooth muscle actin. There was a significant difference with respect to thickening of vessels walls.

Conclusions - Our pathological results indicate that structural vascular abnormalities characterized by vessel wall thickening are associated with leukoaraiosis, supporting the assertion that vascular changes and ischemia generate leukoaraiosis. The relations between parkinsonism and leukoaraiosis may be explicable through vascular effects on the circuitry of the basal ganglia.

The prevalence of LA varies from 5% to 100% in different studies (3). The wide distribution in frequency is the result of different methodologies, differences in the size and selection of the study populations, variations in MRI specifications and scan quality, and the use of varied rating scales. The most common risk factor for LA has been found to be age, but other known vascular risk factors, such as hypertension, diabetes, obesity and metabolic syndrome also contribute to it as well (4–6). Although LA can be an incidental finding in subjects who are neurologically intact, the term has been strongly associated with cognitive impairment and dementia, including the vascular and neurodegenerative type, suggested to be a surrogate marker of brain dysfunction (7–10).
There is substantial evidence that LA is a predictor for ischemic stroke (11, 12), especially lacunar infarcts. LA was also found to predispose to intracerebral hemorrhage (ICH) especially when occurring in association with anticoagulation therapy (13). The strong epidemiological association between LA and cerebrovascular disease suggests that ischemia may be a contributing factor to the development of this pathology. Despite of several previous pathologic studies, the histopathologic correlates of LA remains unclear and no consensus has emerged so far. Proposed pathological correlates other than ischemia include breakdown of vascular and blood-brain barrier (BBB) integrity. This may result in plasma extravasation into the brain parenchyma and increased extracellular water condensation, leading to vasogenic edema and leakage of toxic components (14). Other suggested theories include dilated perivascular spaces ('Virchow-Robins' spaces) (15), demyelination, diffuse loss of glial cells and axonopathy culminating in spongiosis (16), impaired endothelial relaxation and reactivity of both cerebral and systemic vessels (17), disturbances of cerebrospinal fluid circulation (18), transient cerebral edema, Wallerian degeneration, impaired venous return in the deep white matter (16) and non-inflammatory collagenous thickening of the periventricular venous walls (19). In contrast, some studies failed to find any pathological lesion correlated with LA (20, 21). In the present study we aim to compare the severity of LA with clinical risk factors and to analyze the histological alterations in LA areas of the brain compared with corresponding areas of non-LA persons.

Materials and methods

Study population

The study population consisted of 93 deceased patients with no selection for sex or race who had undergone brain MRI (GE, Signa, LX 1.5 Tesla) with a protocol that included T2W and FLAIR sequences prior to their in-hospital deaths. The patients were autopsied between 1999–2006 at the Department of Neurology of the Medical and Health Science Center, Debrecen University (DEOEC, Hungary). All clinical charts were reviewed for demographic data, vascular risk factors, clinical diagnosis and pathological reports of autopsy. The brains obtained at autopsy were preserved in formalin. The subjects were included regardless of their clinical, radiological or pathological diagnoses. Subjects in whom brain disease contributed directly to death were included as well. The study was approved by the Ethical Committee of the DEOEC. The internal review board of the DEOEC does not require informed consent from the next of kin for the investigation of autopsy material.

Imaging

The pre-mortem T2W MRIs were evaluated by a neurologist and an experienced neuroradiologist for the presence and grading of white matter changes using the validated Wahlund scale (22). The MRI scans were assessed masked to any clinical or pathological information or other measurements. To be considered as an LA lesion, the area had to be hyperintense on T2W and FLAIR sequence MRI images and had to be located in the subcortical white matter. According to the Wahlund scale, images with no lesions were scored as grade 0, images with focal lesions were scored as grade 1, images with early signs of confluence as grade 2 and images showing involvement of an entire region in the white matter with or without an involvement of the sub- cortical U fibers, i.e. the myelinated fibers at the junction of the gray matter and the white matter, were scored as grade 3. Intermediate assessments were graded by the authors at intervals of one-half. The clinical and pathological characteristics, based on the hospital charts and general autopsy reports done in the DEOEC of the 32 patients found to demonstrate severe LA according to the Wahlund scale (i.e., a score of 2.5 or 3) were evaluated for demographic, clinical and pathological factors (Table 1).

Pathology

All autopsies were performed within 48 h after death. The brains were fixed in formalin and...
stored in the brain archives of the Department of Neuropathology in accordance with national law. Out of the 32 subjects diagnosed with severe LA based on ante mortem MRI images (Wahlund scale), 14 had available and intact brains (i.e., decomposed brains were excluded). These brains, as well as brains from five control subjects who died of non-vascular diseases were investigated. Post-mortem MRI of the brains were performed on controls and on those of the patients whose pre-mortem MRI had produced images of poor quality. The formalin-fixed brains were cut into slices and the areas affected by leukoaraiosis were identified using the pre- or post-mortem MRI. Brain tissue samples were excised from the areas with a Wahlund score of 2.5 or 3 indicative of severe LA.

**Histology**

The tissue samples were embedded in paraffin and cut into 5-micron slices. The following histological and immunohistochemical methods were used: hemotoxin and eosin (H&E) for the general architecture, tissue components and morphology, Luxol Fast Blue (LFB) for the integrity of myelin and immunohistochemistry with primary antibodies (manufactured by DAKO; Glostrup, Denmark) against cluster of differentiation 68 (CD68) stain for the presence of microglia cells, glial fibrillary acidic protein (GFAP) for the presence of astrocytes, smooth muscle actin (SMA) for smooth muscle, and Van Gieson, H&E and elastin for the vessels walls. Congo red staining of the white matter was used for the presence of cerebral amyloid angiopathy (CAA) in only three subjects. The slices were evaluated by a neuropathologist unaware of the MRI results. The slices were graded by a neuropathologist on a scale of 1–4. For myelin: grade 1 = appearance of mild pallor, grade 2 = swollen myelin sheaths, grade 3 = broken and laminated myelin, and grade 4 = absence of myelin stain. For thickening: grade 1 = partly thickened appearance, grades 2 = increased connective tissue in the wall of small arteries, grade 3 = marked fibrosis in the vessel wall with decreasing of smooth muscle indicates severe narrowing of the lumen and grade 4 = the most severe alteration with obliteration of blood vessels and absolute lacking of smooth muscle (Fig. 1). For the presence of astrocytes: grade 1 = few fibrous astrocytes, grades 2 and 3 = more abundant astrocytes in the LA focus or surrounding area, and grade 4 = concomitant presence of activated astrocytes and fibers. For the presence of microglia: grade 1 = few positive cells, grades 2 and 3 = numerous positive cells, and grade 4 = replete with microglia. For smooth muscle: grade 1 = presence of smooth muscle in all vessels (normal), grades 2 and 3 = lack of smooth muscle in some vessels, grade 4 = no smooth muscles in any of the vessels.

**Characterization of age related white matter lesions**

[Image of histology sections labeled A to D]

**Figure 1.** Histology. Demonstration of scoring of wall thickening vessels; grade 1 = partly thickened appearance (A), grade 2 = increased connective tissue in the wall of small arteries (B), grade 3 = marked fibrosis in the vessel wall with decreasing of smooth muscle indicates severe narrowing of the lumen (C) and grade 4 = the most severe alteration with obliteration of blood vessels and absolute lacking of smooth muscle (D).
Variables were described using standard statistics. Associations between factors and the presence of severe LA (grade 2.5–3) as a binary variable were evaluated using Fisher’s exact tests, logistic regression adjusted for age and gender only, and multiple logistic regression. Variables were included in the multiple model if they had a significant or sizeable odds ratio (>2 or <0.5), proving an active adjustment effect on other estimates, or if they were considered important on an a priori clinical basis. Continuous factors were categorized only if it meant significant improvement of model fit. Results were expressed as odds ratios, \( P \)-values and 95% confidence intervals. A \( P \)-value < 0.05 was considered as being significant. The final model was checked using the Hosmer–Lemeshow test. Student’s \( t \)-test was applied for the brain pathology results.

### Results

The 93 subjects were of mean age of 64 ± 13 years and included 45 (48%) females. Their clinical, demographic and general pathological autopsy characteristics are shown in Table 1. Thirty-two subjects (34.4%) had severe LA on pre- or post-mortem T2 MRI, which was defined as score of 2.5 or 3 points on the Wahlund scale. According to the general pathology autopsy reports, 73 (78.5%) subjects had pathological findings in their basal arteries, including sclerosis, plaques or hyalinization, 77 (82.8%) had plaques in the aorta and 88 (94.6%) had hypertrophic or dilated cardiomyopathy or signs of myocardial infarction.

The age at death was significantly and strongly associated with LA (\( P < 0.039 \)). The odds ratio for severe LA was 1.093 for every year of age. The only other factor significantly associated with severe LA in our cohort was the clinical diagnosis of Parkinson’s disease (PD) as was recorded in the medical charts (\( P < 0.036 \)).

Other clinical variables, including alcohol consumption, a clinical history of ischemic heart disease, the presence of dementia, atrial fibrillation, hypertension, diabetes, hyperlipidemia and smoking did not correlate significantly with the presence of LA. The atherosclerotic or hypertensive findings obtained at autopsy (including heart, aorta and basal arteries) did not correlate with the presence or severity of LA. The effects of various factors on LA (evaluated using multiple logistic regression model) are summarized in Table 2.

No significant difference was found between the scoring of the myelin integrity, GFAP, CD 68 and SMA in the study and control groups (3.4 ± 0.7 vs 3 ± 1 [\( P = 0.31 \)], 2.8 ± 1.1 vs 2.6 ± 1.1 [\( P = 0.7 \)], 2.4 ± 1.1 vs 1.5 ± 0.6 [\( P = 0.29 \)], and 2.8 ± 0.9 vs 3 ± 1 [\( P = 0.62 \)] respectively). There was a significant difference in the thickness of vessel walls between the two groups: the mean score for the vessels walls was 3.1 ± 0.66 in the study group compared to 2.2 ± 0.27 in the control group (\( P = 0.007 \)) (Fig. 2). Congo red staining was negative in all three cases of CAA.

![Figure 2. Scoring of stains for myelin, arteries (vessels walls), GFA, CD68, SMA.](image-url)
Discussion

Our aims were to compare the severity of LA with clinical risk factors and to analyze the histological alterations in LA areas compared with corresponding areas in brains lacking LA pathology. The advantage of our study over earlier reports lies in our comparison of MRI findings with general and brain autopsies. Patients’ age emerged as the single most important parameter significantly associated with LA in our cohort. This finding is strongly supported by previous studies (2–4). However, none of the traditional vascular risk factors, such as hypertension, diabetes, smoking and hyperlipidemia, was significantly correlated with LA, nor was there any association between arteriosclerotic findings of autopsies (heart, circle of Willis, aorta) and LA. Our small sample size may be a potential reason for this lack of association. Our findings are, however, supported by the Copenhagen stroke study (23): those authors demonstrated a significant correlation between the risk of LA and ageing, but there was no relationship emerged with a history of hypertension, diabetes, heart diseases or smoking. Other studies have found strong associations between LA and vascular risk factors (3–6, 24, 25). The inconsistency of all these results may be explained by the fact that most of the studies are cross-sectional and retrospective. Given that LA is a chronic and slowly progressive disease, longitudinal prospective studies would probably be more suitable to shed more light on the nature of the condition and the mechanisms that characterize its development. This kind of well-designed prospective study has been a rarity, not doubt due to practical and financial factors.

In the present study, we used the validated Wahlund scale for the assessment of LA severity (22, 26). By classifying only the severe cases (i.e., those with a Wahlund score of 2.5 or 3) as LA, we increased the sensitivity of the diagnosis by decreasing the subjectivity of our criteria. A possible limitation of this study is our sole use of a chart review for obtaining data on variables such as smoking and alcohol consumption in the absence of independent validation.

The diagnosis of Parkinson’s disease was the only parameter other than age that was independently and significantly associated with LA in a multivariable model ($P < 0.036$). The relations between LA and Parkinson’s disease and other extrapyramidal manifestations have been previously described but they remain incompletely understood. The extra-pyramidal symptoms may arise to interruption of pathways between the cortical motor strip, supplementary motor area (SMA), basal ganglia and cerebellum rather than from nigrostriatal degeneration per se. Cummings (27) proposed the hypothesis of parallel subcortical systems comprising the frontal cortex, striatum, pallidum, thalamus, caudate nucleus and white matter tracts and that interruption of these connections might result in motor impairment. In a recent study on 141 patients diagnosed with Parkinson’s disease, the presence and severity of LA correlated with the severity of the clinical symptoms of the former as measured by the United Parkinson’s disease rating scale (UPDRS) scores and the widely used Hoehn and Yahr (H+Y) stage (28). There were significant correlations observed between the LA grade and specific motor impairments, especially those manifesting in axial symptoms. Those authors assumed that their finding might be explained by the effects on non-dopaminergic subcortical pathways. LA was also associated with motor impairment in which gait disturbances were notably frequent. These disturbances include small steps, freezing of gait, turn and start hesitation and the presence of retropulsion, which may mimic parkinsonism or be mistakenly diagnosed as idiopathic Parkinson’s disease. Due to gait impairments, patients with LA are prone to fall (29–31). It should be re-emphasized that the diagnosis of Parkinson’s disease in our cohort was obtained from the chart review and not by using a specific diagnostic criteria, a feature that may have introduced a bias toward diagnostic vascular parkinsonism as idiopathic PD.

We found no significant relation between known pathological markers of systemic arteriosclerosis in the heart, aorta and basal arteries and LA. Previous studies have found correlations between LA and systemic arteriosclerosis assessed by non-invasive modalities such as image-based measurements of the thickness of the carotid intima extending to the medial wall and Q/QS abnormalities on the electrocardiogram (32, 33). Some other studies failed to find any association between stenosis of extra-cranial carotid vessels and the presence of LA (34, 35). The lack of any association between pathological markers of systemic arteriosclerosis and LA in the present study may also support our assumption that not only arteriosclerosis is responsible for white matter changes but rather that other factors may interact synergistically with an existing ischemia, contributing to the ultimate histopathological appearance of LA. These hitherto poorly described factors remain to be elucidated. Alternately, it may be that the angiopathy affecting the long and less branched penetrating vessels supplying the
deep white matter may be different than the pathology involving blood vessels in the other tissues.

By demonstrating significant wall size thickening of blood vessels that sometimes led to narrowing of the vessel lumen, the present histopathological observations indicate that vascular structural abnormalities are associated with LA. This finding, combined with a relatively impaired perfusion of the cerebral white matter by the terminal long penetrating arterioles supports the popular argument that vascular changes do, in fact, underlie the pathology of LA. Our pathological findings are consistent with the large study by Van Swieten et al. (36) who showed that white matter lesions as seen on MRI are correlated with increased cross-sectional thickness of the walls of small arterioles. Similar findings of increased vessels wall thickness in brains of patients with dementia and in nondemented subjects with white matter changes were also found by Fernando et al. who provided molecular biological support for the hypoxic origin of LA (37). In particular, immunoreactivity for inducible antigens indicative of hypoxia, i.e., HIF1alfa and HIF2alfa, was elevated in brain areas that demonstrated evidence of LA. Other hypoxia-regulated proteins, including matrix metalloproteinase-7 and neuroglobin-positive cells, were also increased in LA. Their data support the contention that a hypoxic environment contributes to white matter changes in the MRIs of patients with LA.

All of the reports cited above combined with the present pathological findings support the proposition that LA is probably a result of long-standing hemodynamic hypoxia, as opposed to vessel thrombosis or occlusion. This hemodynamic hypoxia is probably secondary to the occurrence of thickening of the vessels walls of the penetrating terminal arteries that supply the deep subcortical white matter. The resultant hypoxia is not severe enough to cause cerebral necrosis or damage to the gray matter while remaining sufficient to eventually produce the white matter changes.

In contrast to some earlier studies, we found no association between LA and loss of myelin as demonstrated with the LFB stain. Similar findings were found in a recently published study by Young et al. (14), who used an immunohistochemical stain for degraded myelin basic protein to investigate areas of myelin degeneration. The results of that study taken together with those of the present one suggest that myelin damage is not, as previously thought, the direct pathologic correlate of LA, and that demyelination alone does not underlie hyper intensities on T2W images. Notably, the myelin sheath comprises only about 50% of the dry weight of the cerebral white matter.

The issue of inflammation as a factor contributing to small vessel disease has gained considerable popularity in recent years. Inflammatory processes are implied in the pathogenesis of arteriosclerosis and were found to be an independent risk factor for stroke, LA, myocardial infarction and peripheral arterial disease as well (38–41). In contrast to the studies showing correlation between C-reactive protein as a systemic marker of inflammation and white matter changes, our results showed no significant relationships between LA and microglial and astrocytes density, suggesting no critical role for inflammation and gliosis in the mechanism of LA. Our findings are consistent and supported by previous pathological studies that found no evidence of inflammation in LA lesions (18, 42). This need not necessarily require conflicting interpretations if inflammation does not take a direct role in the pathogenesis of LA, but rather promotes arteriosclerosis and luminal narrowing in small cerebral vessels.

We conclude that there is a need for well-designed clinical and pathological studies on the progression of LA. A precise understanding of the contribution of small subcortical vessels to the onset of LA is essential before preventive and therapeutic approaches can be implemented.

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References

Characterization of age related white matter lesions


